Table 2. — *Percent germination and average length of elongated pollen grains (± one standard deviation).*

Solution	24 Hours		48 Hours	
	%	Length (μ)	%	Length (μ)
Stock Solution A[1])	60	184 ± 28	69	211 ± 25
Stock Solution B[2]) 5% sucrose	54	189 ± 28	68	219 ± 24
10 ppm IAA Stock B 8% sucrose	67	175 ± 26	67	205 ± 19
10 ppm IAA Stock B 10% sucrose	65	186 ± 23	68	208 ± 28
10 ppm IAA Stock B 15% sucrose	63	169 ± 18	71	218 ± 23
10 ppm IAA Stock B 20% sucrose	67	184 ± 18	71	209 ± 29
10 ppm IAA Stock B	70	173 ± 26	69	217 ± 20
Distilled water	40	168 ± 15	41	189 ± 20

[1]) *Stock Solution A:* H_3BO_3 0.1 *g*; $Ca(NO_3)_2 \cdot 4H_2O$ 0.3 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; KNO_3 0.1 g; *in 100 ml. distilled water.*

[2]) *Stock Solution B:* *Stock Solution A* 1 *ml.*; *distilled water* 9 *ml.*

taining boric acid, calcium nitrate, magnesium sulfate and potassium nitrate. The generative cell divided into the body cell and the stalk cell after two days of incubation, and three days later the body cell divided to form two sperm cells. Only the elongated pollen was observed after seven days of incubation.

Literature Cited

ALLEN, G. S. *The embryogeny of Pseudotsuga taxifolia* (LAMB.) BRITT. *Amer. Jour. Bot.* **30**, *655-661 (1943).* — ALLEN, G. S., and SZIKLAI, O.: *Pollination of Douglas-fir with water suspensions of pollen. For. Sci.* **8**, *64-65 (1962).* — BARNER, H., and CHRISTIANSEN, H.: *The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in Pseudotsuga menziesii. Silvae Genetica 11, 89–102 (1962).* — BREWBAKER, J. G., and KWACK, B. H.: *The essential role of calcium ion in pollen germination and pollen tube growth. Amer. Jour. Bot.* **50**, *859–865 (1963).* — CHING, K. K., and CHING, T. M.: *Extracting Douglas-fir pollen and effects of gibberellic acid on its germination. For. Sci. 5, 74–80 (1959).* — CHRISTIANSEN, H.: *On the pollen grain and the fertilization mechanism of Pseudotsuga menziesii (MIRB.) FRANCO var. viridis SCHWER. Silvae Genetica* **18**, *97–144 (1969).* — COLE, K.: *Turtox CMC-10 mounting medium used in pollen fertility Counts. Turtox News 36,* **240—241** *(1958).* — Ho, R. H. and Rouse, G. E.: *Pollen germination of Larix sibirica in vitro. Can. Jour. Bot.* **48**, *213–215 (1970).* — JOHANSEN, D. A.: *Plant microtechnique. McGraw-Hill Book Co. Inc., N.Y. and London (1940).* — RARUE, C. D.: *Studies on growth and regeneration in gametophytes and sporophytes of gymnosperms. Brookhaven Symp. Biol. 6,* **187—208** *(1953).* — LAWSON, A. A.: *The gametophyte and embryo of Pseudotsuga Douglasii. Ann. Bot.* **23**, *163–180 (1909).* — ORR-EWING, A. L.: *Controlled pollination techniques for the Douglas-fir. For. Sci.* **2**, *251–257* **(1956).** — SZIKLAI, O.: *Variation and inheritance of some physiological and morphological traits in Pseudotsuga menziesii (MIRB.) FRANCO var. menziesii. Ph. D. thesis. University of British Columbia (1964).* — TULECKE, W.: *The pollen of Ginkgo biloba : in vitro culture and tissue formation. Amer. Jour. Bot.* **44**, **602—608** *(1957).* — VAN CAMPO-DUPLAN, M.: *Recherches sur la Phylogénie des Abiétienées d'après leur grains de pollen. Trav. Lab. For. de Toulouse, t. 11, Sect. 1, Vol. 9, art. 1, 10–182 (1950).* — WODEHOUSE, R. P.: *Pollen grains. Hafner Pub. Co., N. Y. (1959).*

On the Development of Pollen and the Fertilization Mechanism of Picea abies (L.) Karst.

By H. CHRISTIANSEN

The Laboratory of Genetics of the Royal Veterinary and Agricultural University, Copenhagen, and the Danish State Forestry's Tree Improvement Station, Humlebaek, Denmark.

Introduction

CHRISTIANSEN (1), as a result of several years research showed, inter alia. that the transfer of the male nametes of Pseudotsuga menziesii, from the germinating pollen on the apex of the nucellus to the egg cell, is carried out by means of a kind of motile spermatozoids.

In a later article (1a) it was shown that the corresponding process in Larix decidua is probably a transitional stage between fertilization by means of spermatozoids and fertilization by pollen tube.

In respect of both species it was found:

1) that they produce no ordinary pollen tube and that all attempts to cultivate their pollen on artificial substrate till "gerrnination", i. e. till the male gametes leave the pollen grain, have proved unsuccessful.

2) that the classic formation of a "generative cell", a "tube cell" and a "sterile cell" could not be confirmed.

3) that a "tube-junction" is occupying the place where the "tube cell" = "vegetative cell" is reported to occur, and that this tube-junction evidently, inter alia, is governing the formation of neocytoplasm in the elongating pollen grains.

4) that, when not dividing, the chromosomes of pollen mother cells in early meiotic prophase, and of microspores and pollen cells are packed together inside a resting nucleus; they seem not to be despiralized but resemble metaphase chromosomes and are arranged in a pattern suggesting a sort of skeleton supporting the shape of the resting nucleus; they are embedded in nucleoplasm and surrounded and totally obscured by a thick mantle (Valves of the resting nucleus protruding through nucleoplasm and mantle may easily be confounded with nucleoli).

Considering that the above observations differ considerably from older reports, not only as regards Larix and Pseudotsuga, but also in respect of other conifecs, it would, of course, be of interest to check the pollen development (and structure) of pollen-tube-producing species, particularly Pinus, Picea and Abies. And the more so because Larix and Pseudotsuga have always been classified as Pinaceae.

These problems do not only present a theoretical interest. The very fact that pollen of *Larix* and *Pseudotsuga* produce no pollen tube and cannot be germinated on artificial substrate is remarkable and unique and of considerable interest for practice (f. inst. in connection with viability tests).

Climatic, technical and other difficulties are no doubt the main causes why these observations have not been made before, and we have, as yet, only been able to elucidate, *in outlines,* the principal parts of anatomy and tube formation in one species of pollentube-producing conifers, i. e. *Picea abies* (Norway spruce).

The main results are outlined in the following.

Terminology

Generally the terminology of STERLING (12) is used. The term "germination", when used in connection with the tubeless *Larix* and *Pseudotsuga* pollen, indicates the stage when the male gametes are discharged from the pollen grain. Terms like "tube- and valve-system", "special tube junction" etc. are used provisionally because no adequate description of the organelles in question could be found in literature.

There are certain difficulties as regards the definition of the word *nucleus* in the conifer pollen grains in question. This is mainly due to the exact periods of occurrence of *resting nuclei* as yet not having been exactly determined, but also to the fact that, at least in mature pollen grains, there is more than one cytoplasm. Therefore, after telophase II, when no *cell* divisions, but *nuclear* divisions only, occur, the term nucleus, *during the divisions,* covers the space inside membrane 4, *fig. 2,* but after the divisions, the actual nucleus is the *resting nucleus,* in which the chromosomes are concentrated *(fig. 2/1).*

Material and Methods

Good flowering in *Picea abies* is generally supposed to occur with intervals of 4—7 years, but the intervals may be longer, and the inflorescences may be damaged by frost during meiosis (probably after pachytene) and during the intranuclear mitotic divisions in the pollen grain. In the intervals single trees may have male inflorescences, and they may to some extent be used for studies of pollen development, but generally only at certain stages. Furthermore, when flowering occurs, it usually takes place in high trees, and the divisions procede only when the temperature is sufficiently high, often during a couple of hours when the sun is shining (in *Larix* the threshold temperature is about $+4^0$ C, cf. [1c]). It is, therefore, not possible to base a series-investigation on fixations directly from the trees.

However, by forcing twigs with male inflorescences before and after meiosis it proved possible, during a couple of months, to produce in the laboratory the wanted stages of pollen development up to dehiscence. This solved to some extent an important problem, but not the equally important difficulty, that the intranuclear pollen divisions in a normal pollen grain are totally obscured under two cytoplasmic mantles, one inside the other, and at least six membranes plus intine and exine. During the divisions the *chromosomes* may sometimes be brought to light by feulgen hydrolysis and staining, but the visibility is obtained at the cost of dissolution of organelles and cytoplasm, and the method is unsuitable for the present purpose. — No usable cytotechnical method could be found, but it turned out, that a sufficient number of frostdamaged pollen were well suited for studies of anatomy, the cytoplasm being more or less transparent or undeveloped, but the organelles undamaged.

Technique

Twigs with male inflorescences were taken from the tree, placed in a closed plastic bag with water and taken to the laboratory. Here about 1 cm of the cut end of the twig was cut off *under water* to prevent formation of air bubbles in the vascular system, and the twigs were placed in water in glass containers in a Climatic Growth-Cabinet at approximately $+15^0$ C, 70—80% relative air humidity, by mercury light during the day and darkness during night. It proved necessary to spray the twigs twice daily by means of an "atomizer" to keep up the humidity; and with intervals of two days a fresh piece was cut off the cut ends *under water* and the water changed. By this regime the buds could usually be kept developing satisfactorily for a couple of weeks. — *Microtome sections* of coniferous pollen grains are usually of no value. Nuclei and pieces of membranes etc. drop off during treatment or they are carried away by the knife, and almost all sections are damaged; reliable reconstruction is not possible. *Whole pollen grains* were therefore used. 45% acetic acid (and stain-fixative: acetic-orcein) proved the only *fixative* not causing shrinkage, and it was also used as mounting medium in preparations. To prevent flattening of the pollen grains small glass splinters were placed under two edges of the cover slip.

To make preparations permanent without shrinkage, even after freezing, proved impossible by any of the known methods, wherefore semipermanent glycerine-preparations were used, starting with 10% glycerine in 45% acetic acid (or in 45% acetic-orcein) and gradually, during a couple of days, replacing the evaporating acetic acid, by adding 45% acetic acid containing 30% glycerine to the edge of the coverslip. Such preparations *may* keep for months, particularly if kept by low temperature, but a certain number deteriorate. Therefore, when possible, plates of interest for the investigation were photographed and recorded and later, together with slides, used for the survey. — About 250 microphotos were taken and about 3—4000 usable pollen examined. The number of usable pollen amounted to approximately 5% of the total number examined.

As regards the examinations of *germinating pollen and pollen tubes* the cytotechnical difficulties are here aggravated considerably on account of the pollen grains and the tubes at this stage usually being tightly stowed with starch grains; and the trouble is, that without the starch grains, no normal growth of the pollen tube takes place.

Up to now no reliable method has been found to remove the starch grains without, at the same time, damaging the organelles, but the attempts are continued.

For preparation and control of germination of pollen a *Wild dissecting microscope* (M.5) with an auxiliary lens was used. The examination of whole pollen grains was only possible by means of Phase-contrast microscopy, whereby staining could be avoided or reduced to a minimum. A Zeiss Phasecontrast microscope (WL) with "pony camera" (24 × 36), and illumination by means of a SCHOTT "cold light" unit from WILD, was used. The cold light prevents heating of the preparation and considerably reduces diffuse light in the otherwise, for the purpose of phase contrast, rather thick preparations.

Trees for examination were placed at our disposal by the Director of the Tree Improvement Station of the Danish State Forestry at Humlebaek, Mr. H. BARNER, who also arranged isolation and pollination for necessary experiments, and by Dr. agro. B. SOEGAARD of the Arboretum at Hoersholm, who kindly placed at our disposal a number of clones of *P. abies* of different origin.

The reason why we, in the first place, have investigated the *pollen development* in *Picea* and not in *Pinus* is, that fertilization in *Pinus* takes place about a year after pollination, while in *Picea* it takes place about 4 weeks after pollination. It cannot, therefore, be taken for granted, that the pollen development in the two species is identical.

The Pollen of P. abies

General information: It has not been possible to find in the literature an adequate description of the structure and development of pollen of *P. abies*. The most comprehensive ones are probably: MIYAKE (8), who inter alia cites STRASBURGER; FERGUSON (4) and WODEHOUSE (14), but the picture is not complete, and the interpretation sometimes doubtful. WODEHOUSE does not mention *P. abies* but describes the morphology of 5 other species of *Picea*. The pollen diameter is stated to be 68—91 my (about twice the diameter of pollen of *Pinus)* with relatively small air-bladders, which are more or less concave on the inside and, in dry pollen, fold inwards and cover the germinal furrow. The *dorsal* side is covered with a thick, rugged exine, the *ventral* side is mainly occupied by the two bladders (or wings). In his comments on the wings WODEHOUSE (p. 254) points out: ".... if these bladders are organs of flight, pollen grains are possibly the only flying organisms of which it can be said that they fold up their wings and fly away". We can confirm W's elegant thesis as regards pollen of *P. abies,* and we may add that according to our observations the pollen, when it leaves the anther, consist of a mixture of spherical and more or less expanded pollen. But only the spherical pollen grains reach the stigmatic flaps and the nucellus, the expanded pollen, evidently being too heavy, is sorted out during the flight. The proportion of spherical/non spherical pollen grains seems to correspond fairly well to the proportion of germinated/ungerminated pollen when subjected to the usual viability test, and this circumstance might probably be utilized for a rough estimate of the quality of pollen samples. — According to DOYLE (3) the airborne pollen of *P. abies* is caught on the stigmatic flaps and deposited by the fluid in the depression in the apex of the nucellus. Here, according to our observations, germination starts as soon as the pollen grains have absorbed fluid and expanded (by artificial germination this takes only a few minutes), but there is evidence suggesting that this is only a first phase of the germination and that the tube growth stops at 5—6 pollen

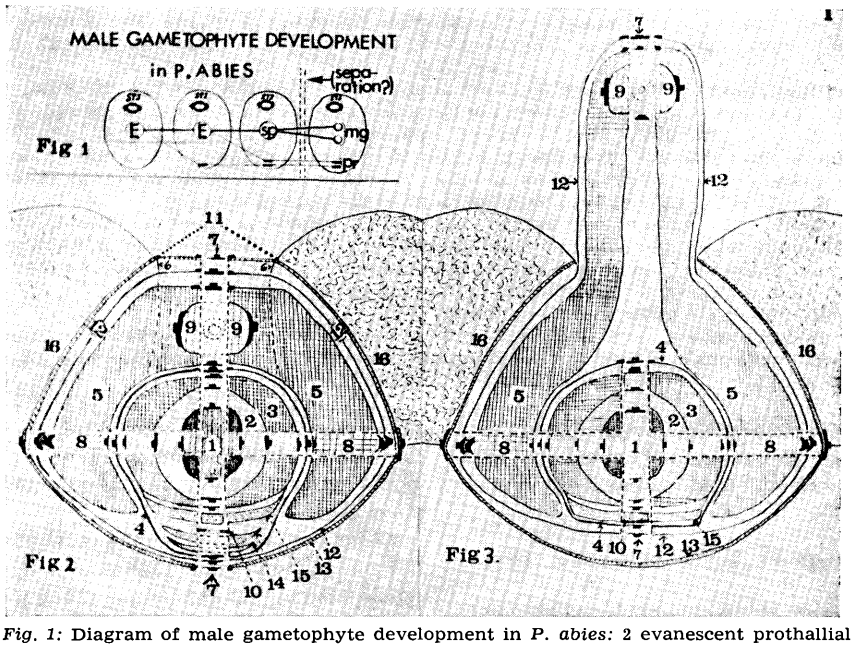

Fig. 1: Diagram of male gametophyte development in *P. abies:* 2 evanescent prothallial *nuclei* and 2 male gametes are formed; the latter are presumably formed before dehiscence, but they seem not to separate until maturity (cf. *Pseudotsuga* (1)). A special tube-junction (STJ) was observed already at meiotic prophase (cf. text).

Fig. 2: Approximate diagram of structure of pollen just before dehiscence (cf. text and explanation of numerals below). × ca 540.

Fig. 3: Similar diagram of same pollen as in *fig. 2* at the beginning of pollen-tube formation. *Note!* The STJ *(fig. 2/9)* is now at the tip of the tube. × ca 540.

Fig. 2 and 3: Explanation of numerals: 1: Resting nucleus; 2: inner nuclear cytoplasmic mantle; 3: outer nuclear cytoplasmic mantle; 4: antheridial/prothallial membrane; 5: outer cytoplasmic mantle of the spermatogeneous cell; 6: germinal duct; 7: vertical tube through the pollen from pore, 10, to exit of duct, 11; 8: transversal tube connecting with vertical tube, 7, in centre of nucleus, 1; 9: special tube-junction (STJ) active in formation of pollen tube (cf. *fig. 3* and text); 10: pore; 11: exit of germinal duct; 12: intine, forming later the outer wall of pollen-tube; 13: exine; 14: prothallial nuclei; 15: "stalk cell"; 16: proximal part of air-bladders; ▬ valves in vertical tube, 7, and transversal tube, 8; ⋘ double-valves near the ends of the transversal tube, 8, where it passes through the intine,12.

Abrevv.: E = embryonal cell; mg = male gametes; pr = prothallial cell; sp = spermatogenous cell.

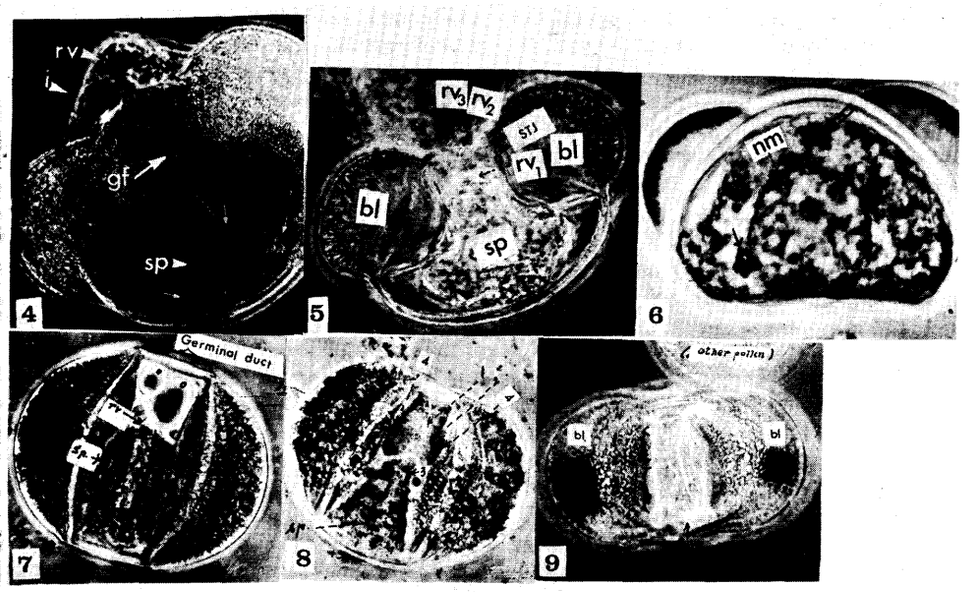

Fig. 4: Early stage of "germination" of pollen, ventral view. Tip of tube has broken out of germinal duct, note rv on outer membrane of outer cytoplasmic mantle; below, at three arrows, nucleus of spermatogenous cell.

Fig. 5: Ventral view of deteriorating mature pollen, showing bottle-shaped space occupied by spermatogeneous cell, and two air-bladders (bl); near top STJ (*Fig. 2/9*); rv_1, rv_2 and rv_3 are rosette-valves respectively on outer membrane of outer cytoplasmic mantle, on intine and (probably) on exine.

Fig. 6: Dorsal view of slightly damaged young pollen; resting nucleus and most of cytoplasm dissolved; nm: probably outer membrane of outer nuclear cytoplasmic mantle (*Fig. 2/3*); left, at arrow: valve where transversal tube (*Fig. 2/8*) passes through nm; at top (double arrows): STJ (*Fig. 2/9*).

Fig. 7: Damaged mature pollen ready for flight. Exine, outer cytoplasm and STJ dissolved; air bladders are closed, nucleus pressed together in germinal duct; bright stripe through middle of sp = lower part of vertical tube (*Fig. 2/7*). (cf. text).

Fig. 8: Stage as *Fig. 7* but grain more damaged. Most of nucleus (sp) dissolved; at 3 rosette-valve, probably on nm, *Fig. 6*; above: remnants of tubes: 1: probably that shown in *Fig. 9/1*; 2: STJ junction-box, with two sieve-plates marked 4.

Fig. 9: Damaged pollen, stage slightly earlier than *Fig. 7, 8*. Cytoplasm dissolved, showing tube-formed skeleton on which spermatogeneous cell is built up. 1 corresponds to 1 and 2 in *Fig. 8*; the constriction, 2, is probably where the skeleton-tube passes the antheridial/prothallial membrane; 3 is the skeleton-tube occupied by the nucleus sp in *fig. 8*. (cf. text).

Enlargements: Figs. 4, 6 × ca 425; *Figs. 7—9* × ca 350.

Abbrevv.: bl =air-bladders; gf = germinal furrow; i = intine; nm = outer membrane of outer nuclear cytoplasmic mantle; rv = rosette-valve; sp = nucleus of spermatogeneous cell; STJ = special tube-junction.

diameters. A second growth phase seems to take place about 3 weeks later i. e. just before fertilization, when the male gametes are making preparations for leaving the pollen grain.

The Structure of the Pollen Grain at Pollination Time

The large embryonal/spermatogenous cell is the all dominating organ in the pollen grain. The complicated structures and organelles of which it is built up: cytoplasmic mantles, tubes, valves, junctions and membranes, are all parts of an intricate complex intended to ensure the best possible conditions for growth and development of the male gametes.

The *nucleus* of the spermatogenous cell is occupying a strategic position in the centre of the cell, and, as far as it has been possible to find out, the intersection (or junction) of the vertical and horizontal tubes is situated inside the resting nucleus. There is no doubt that this is the vital unit of the pollen grain.

The structure of a pollen grain at dehiscence is diagrammed in *fig. 2;* and, *at germination,* in *fig. 3.* The diagrams are, as far as possible, drawn to scale, but in order to facilitate the survey the dimensions of the tubes are somewhat exaggerated and the sites of the membranes not always quite exact. — The details of the diagrams are as far as possible documented by means of microphotographs.

While the pollen is young, and the air-bladders small, the embryonal/spermatogenous cell occupies almost the whole interior of the pollen grain *(fig. 6).* But gradually, as the air-bladders develop, the space is reduced, and at dehiscence, when the pollen is spherical and ready for flight, the cell, i. e. the organelles inside membrane 12, *fig. 2,* is pressed together in a narrow canal between the pore and the outlet formed by the walls of the air-bladders. *Fig. 7* shows a damaged pollen at this stage. The outer cytoplasmic mantle and the STJ are dissolved, but the nucleus, corresponding to *fig. 2,* inside membrane 4, is clearly seen. *Fig. 8* shows a similar stage; here most of the nucleus is dissolved, but remnants of the STJ are seen.

Figs. 9, 10 and 11 are highly interesting. They show most of the anatomy of the same pollen grain at different foci. The pollen grain has, probably on account of frost-damage, no cytoplasm, but membranes, structures and valves seem to be preserved. *Fig. 9* shows the tubeformed "skeleton" on which the spermatogenous cell is built up: the nucleus on the space marked 3, the traverse of the prothallial mem-

brane marked 2, and the STJ on the space marked, 1—2. — *Fig. 10* and particularly *11*, at other foci, show rosette valves in the air-bladders which may, perhaps, have something to do with the development of the latter; they seem also to show (besides the valves in the vertical tube) that the spermatogenous cell on four sides has patterned membranes, of which two have extensions of the horizontal tube to the interior of the air-bladders. Nothing of this has, as far as known, been recorded before, and it shows very clearly, that the determination of stage and structure to a very high degree depends upon the position of the grain and on the focussing; the present interpretation is, of course, by no means exhaustive, but it should, now the outlines are known, not be difficult for the electrone microscope to clarify the details.

It is interesting to note, that although all organelles inside the pollen grain are parts of the spermatogenous cell, the prothallial membrane *(fig. 2/4)* forms a barrier between the nucleus with the inner cytoplasm and the outer cytoplasmic mantle with the STJ (cf. *fig. 12—14*). Evidently the reason for this is, that while the nucleus and the inner cytoplasmic mantle remain stationary till about fertilization, the outer cytoplasmic mantle with the STJ and the intine elongates and stretches and forms the pollen tube *(fig. 3* and *figs. 19—21)*. It is in this connection important to remember, that during the whole development, there is only one living and active cell in the pollen grain (the embryonal/spermatogenous), while hitherto the presence of several living and active cells, which change position and functions, has been taken for granted.

In the following the various organelles of the pollen grain are described in detail as far as the material permits:

THE GERMINAL DUCT (Fig. 2/6 & 11, figs. 7, 8, 15). — As mentioned before a part of this duct is formed by the walls of the air-bladders, but the details of the structure of the interior are unknown. It is however evident, inter alia from the two pollen in *fig. 15*, that several membranes are present and that the interior is more or less quadrangular. When germination starts the duct is ruptured.

VALVES (Fig. 2 in tubes *7 & 8, figs. 10—11).* — The organelles, which in the present work are provisionally denominated valves, seem to belong to two categories viz. those situated in the outer walls of the pollen grain and those in the interior, where a tube traverses a membrane or the wall of a tube. They were observed by phase-contrast microscopy in frost-damaged pollen more or less lacking cytoplasm. According to Rieger, Michaelis and Green (10) those of the former category may, perhaps, be *Plasmodesmata*, (after Strasburger, 1882), while those of the latter may belong to the *endoplasmic reticulum*, a designation covering a wide field as yet not thoroughly investigated.

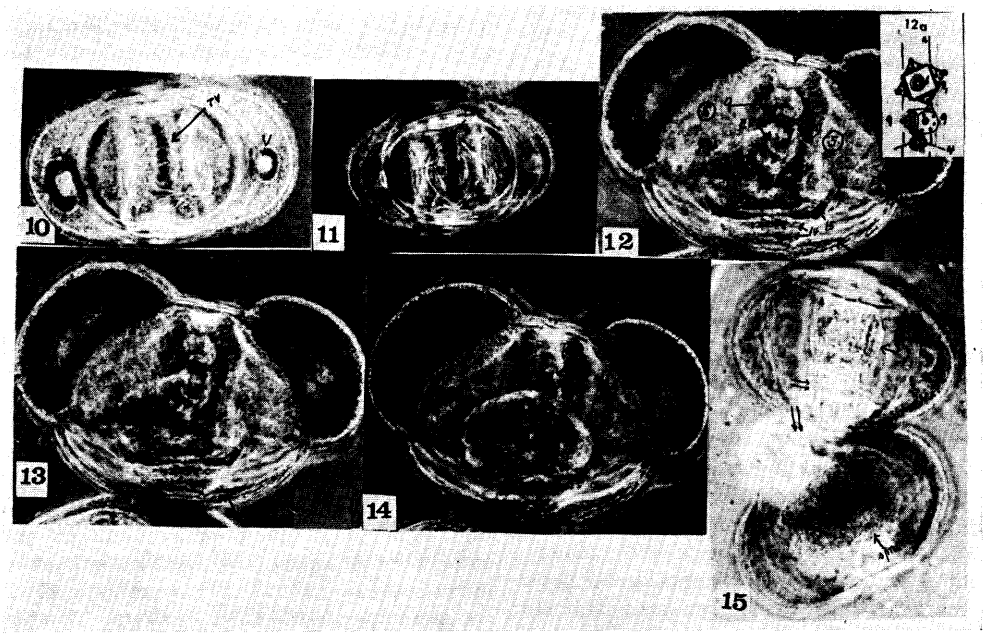

Fig. 10 and 11: Same pollen as in *Fig. 9* but other focus, showing the vertical tube *(fig. 2/7)* with its row of valves (rv, arrow) corresponding in the main to diagram *Fig. 2*. V: valves in air-bladders.
Fig. 12: Dorsal view of damaged mature pollen showing STJ and other organelles of the pollen. Most of exine and part of cytoplasm missing. Nucleus is slightly displaced making the vertical tube (the bright stripe) curve somewhat to the left. — Numerals are the ones used in *Fig. 2*: 1: damaged resting nucleus; 4: antheridial/prothallial membrane; 5: outer cytoplasmic mantle; 9: STJ, left sieve-plate particularly clear; 11: exit of germinal duct and vertical tube; 13: thick exine cap; 14: 2 degenerated prothallial nuclei; 15: "stalk cell", see also *Fig. 2* and text. —
Fig. 12a: (Inset) partly hypothetic diagram of STJ and the rosette valve beneath. a: vertical tube; 9_1: hypothetical view gf top of STJ; b: probably valve; 9: corner-view of STJ with 2 sieve-plates; 4: rosette-valve rv on antheridial/prothallial membrane (see also *Fig. 14*).
Fig. 13: Same pollen as *Fig. 12* but slightly different focus. Note valves in bl (double arrows), and exine cap (arrow).
Fig. 14: Same pollen as *Fig. 12 and 13* but different focus. Valve on 4 particularly conspicuous (arrow). Note valves in bl (double arrows).
Fig. 15: 2 degenerated pollen showing germinal duct. The upper pollen shows the angular shape of the duct, which is confirmed by the piece of the duct pressed out of the nethermost pollen (double arrows).
Enlargements: Fig. 10, 11, 15 × ca 350.
Fig. 12, 13, 14 × ca 425.
Abrevv.: bl = air-bladders; rv = rosette valve; spn = nucleus of spermatogenous cell.

Exact classification will probably not be possible until the anatomy of the valves has been clarified in detail, and valves will no doubt be found under the cytoplasm in many other places than those shown in *fig. 2.* — It seems however evident that some valves consist of a cylindrical cone containing a complicated laminar system and that they are situated in the center of a circular plate of tissue with circular grooves ("rosette"-valves). In other valves the circular plate seems to be absent, and f. inst. at the end of the transverse tube shown in *fig. 2/8* the valves are double, but a more exact determination has not been possible.

Sometimes such valves have no doubt caused misinterpretations. For instance has the valve at the point where the vertical tube traverses the prothallial membrane *(fig. 12)* been taken for a decaying tube nucleus, and this also applies to the STJ *(Fig. 2/9, 22a and 26—29).*

THE TUBE SYSTEM (fig. 2/7 and 8) interweaves all parts of the pollen grain. — In broad outlines *one part* of the system consists of the tubes observable by phase-contrast microscopy and shown in *fig. 2.* The main components are: The vertical tube from pore to exit *(fig. 2/7)* and a transversal tube *(fig. 2/8),* and there is evidence that tube connections also exist between the STJ *(fig. 2/9)* and a point near the double-valves at the ends of the transverse tube *(fig. 2/8).* Similar tube systems were found in *Pseudotsuga* (1) and *Larix* (1a). — *Another part* of the tube system is on the electrone microscopic level and belongs perhaps to the *endoplasmic reticulum,* which, generally, according to (10) "is a hollow system which interlaces the whole cell and delimits "reaction rooms" or "compartments". To this part the tubes possibly belong, which could sometimes be faintly distinguished for instance in the cytoplasms of the nucleus. However, in view of the fact that even the part of the tube system outlined in the present work could not be investigated in detail, more exact classification does not seem possible at present.

SPECIAL TUBE-JUNCTION (abbrev. STJ). *(Fig. 2/9, 12, 23—29).* — This organelle, provisionally denominated STJ because at present no better name could be found, has actually been seen by several authors, but recorded as tube nucleus or vegetative cell. It is normally hidden under the cytoplasm and difficult to examine, but it is no doubt a junction-box for the main circulatory tubes of the outer cytoplasmic mantle and their connections to the vertical tube *fig. 2/7;* it may also (as in *Larix* and *Pseudotsuga)* have something to do with the formation of neocytoplasm during the growth of the pollen grain and, in *P. abies* with the growth of the pollen tube. In the main the STJ has the shape of a box, probably with six sides (or perhaps two boxes, one inside the other as faintly seen f. inst. in *fig. 26—29);* it is built up around the vertical tube, where the latter traverses the outer cytoplasmic mantle *(fig. 2/9).* It has sieve-plates, probably on four sides, and there is evidence that tubes connect these plates with similar plates situated near the double valves at the ends of the transversal tube, or tubes *(fig. 2/8),* evidently forming circulatory systems for the nutritive fluid in the cytoplasmic mantle. — The STJ has been observed already at meiotic prophase *(fig. 23, 24)* and, incidentally, its presence already at this early stage would seem to prevent the formation of a tube cell at this place and, consequently, also the formation of a traditional "generative cell".

THE AIR-BLADDERS (fig. 2, 3, 10, 11). — In view of the fact that at earlier stages the *interiors* of the pollen grains of *Larix, Pseudotsuga* and *P. abies* are rather similar, the question arises whether it could be the development later on of air-bladders in *P. abies,* which has caused the male gametes of the latter to leave the pollen grain through a pollen tube and not, as in *Larix* and *Pseudotsuga,* more or less at random from elongated pollen grains. A precise answer cannot be given at present, but the possibility seems to exist, that something to that effect may have happened. — If it is assumed that the documented bulges of intine, made by the spermatozoids of *Ginkgo* and *Pseudotsuga* and pushed into the apex of nucellus, are pollen tube initials, it might be, that in *P. abies,* during the gradual evolutionary development of the air-bladders, the male gametes have been forced to break out at the only place, where this was possible, that is through the *germinal duct,* and there to form the pollen tube. The question is then, how the air-bladders came into existence; it may perhaps have happened as follows: Facing the ends of the transversal tubes there are in the exine of pollen of *Larix* (which have no bladders) and of pollen of *P. abies* (which have bladders, cf. *figs. 10, 11* and others) complicated, no doubt lamellated, "rosette"-valves. The "rosette" in *Larix* are flat, those in the corresponding place in *P. abies* are voluminous, but the reasons for this could not be determined by phase-contrast. However, if for some reason (mutations and selection or otherwise) a lamella has become loosened and gradually expanded, thereby blowing up a layer of exine, it would not seem unlikely that a bladder might be formed. To clarify the structural differences between the flat rosettes in *Larix* and the voluminous ones in *P. abies* is, as mentioned above, hardly possible by phase contrast; it is no doubt a case for the electrone microscope.

MITOTIC DIVISIONS AND CELL FORMATION IN POLLEN (fig. 17, 18). — The mitotic divisions in the pollen grain are, as mentioned before, *nuclear* divisions, and tubes and valves in and outside the nucleus are, as far as it could be determined, not dissolved during the divisions.

Three mitotic divisions evidently take place forming 2 evanescent prothallial nuclei and 2 male gametes. At metaphase and anaphase the mitotic spindle is oriented along (or perhaps around) the vertical tube, and two rosette-valves, one near the pore, the other near the top of the nucleus, are evidently spindel poles *(fig. 18).* Wall formation takes place along (or around) the transversal tube, and the length of the wall equals the diameter of the newly formed nuclei *(fig. 17).* No other type of division (except the formation of the male gametes), by which a viable cell or nucleus could be produced, was found, and there seems no possibility that the traditional tube cell, generative cell and stalk cell are formed in *P. abies.* How the two male gametes survive is as yet undocumented, but the explanation is probably the same as regards the gametes of *Ginkgo* and *Pseudotsuga* i. e. that during the development from formation to maturity the gametes are lying close together connected by tubules. As regards the spermatogenous cell of *P. abies* it seems quite clear that, by through-going tubes and connecting valves it is securely locked in a certain fixed position, and also, that it is unable to produce viable sister cells or nuclei without destroying its own nutrimental system. As mentioned above this prevents the formation of the traditional pollen cells, but there is also important circumstantial evidence pointing in the same direction. It would for instance seem unlikely that a tube cell or vegetative cell could be formed in the place already

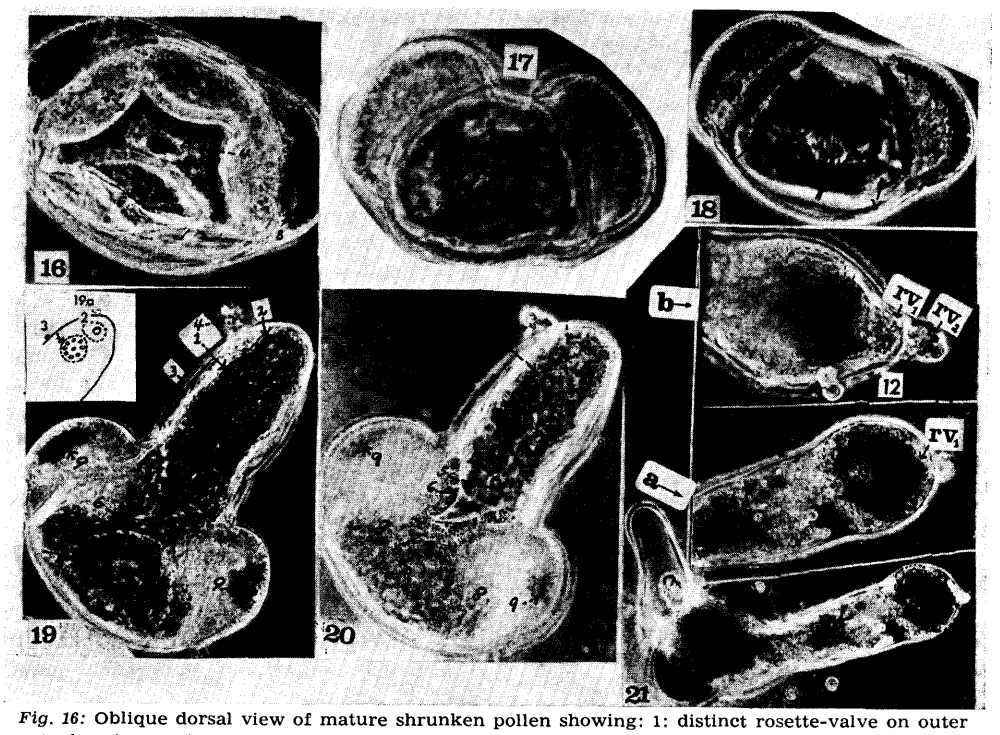

Fig. 16: Oblique dorsal view of mature shrunken pollen showing: 1: distinct rosette-valve on outer cytoplasmic membrane; 2: antheridial/prothallial membrane; 3: nucleus of spermatogenous cell; 4: resting nucleus with two valves (v); 5 and 6: evanescent prothallial nuclei; "stalk cell" not visible; 8: rosette-valves in air-bladder.

Fig. 17: First mitotic nuclear division. 1: spermatogenous nucleus; 2: wall formation in nucleus only; 3: first vanishing prothallial nucleus. Cytoplasmic mantles and valves seem not to be dissolved during mitosis. *The rosette-valves of the nucleus seem to act as spindle poles* (see also *Fig. 18*).

Fig. 18: Anaphase of first mitotic nuclear division. *Rosette-valves seem to act as spindle poles* (double arrows); valves (v) not dissolved (see also *Fig. 17*).

Fig. 19 and 20: Slightly damaged pollen with short tube, different foci, showing: 1: sieve-plate of STJ-complex (cf. *Fig. 12a and 2/9*); 2: rosette-valve on outer membrane of outer cytoplasmic mantle; 3: intine; 4: dislocated outer rosette-valve from tip of tube; 5: broken hole in outer cytoplasmic mantle, through which fluid-filled interior is visible; 6: opening ventral end of germinal forrow; 7: nucleus of spermatogeneous cell with inner cytoplasm; 8: outer cytoplasmic mantle; 9: valves in air-bladders.

Fig. 19a: (Inset): diagrammatic sketch of interior of tip *Fig. 19: 1:* sieve-plate of STJ; 2: rosette valve; 3:intine.

Fig. 21: Degenerated pollen with two "tubes". Right tube contains cytoplasm and STJ-complex, 9, but cytoplasmic mantle broken (at arrow); left tube, consisting of intine only, is probably caused by blockade of germinal duct on opposite side (frequently seen by germination of poor pollen samples); *a:* enlargement of tip of right tube shows: two sieve-plates of STJ (dotted circles); rosette-valve on outer membrane of outer cytoplasmic mantle (rv_1), and, at arrow, probably dislocated sieve-plate from lower part of STJ; *b:* shows tip of similar (more distinct) tube with STJ-complex, 9, rv_1 as in "a" and oblique view of loose rv_2 on outer side of intine; 12: intine; (see text).

Enlargements: × ca 350.

occupied by the STJ. It is unlikely that *newly-formed* tube cells and generative cells should never have been documented, if they existed, and also that these stages could have been overlooked by us.

The conclusion seems to be that the embryonal/spermatogenous cell, during its whole existence, is the only living and active cell in the pollen grain of *P. abies*.

THE POLLEN TUBE FORMATION (fig. 3, 4, 19, 20, 21, 21b). — As mentioned above the pollen tube in *P. abies* is an elongation of the outer cytoplasmic mantle including the STJ and covered by a bulge of the intine of the pollen grain. The mechanism of pollen tube formation in conifers is as yet not quite clear, and only few investigations are reported in literature (1b), (4), (5), (6), (7), (9), (12), (13), (13a). It seems, however, that in *P. abies* several factors are involved and that the tube formation takes place during two growth periods interrupted by a resting period. The first growth period, during which the tube attains a length of about 3—5 pollen diameters, takes place immediately after pollination when the grain has taken up fluid and expanded, the second occurs immediately before fertilization. (Also by germination on artificial substrate as a rule the pollen tubes stop growth at 3—5 pollen diameters, which seems to be about half the total length they reach in nature). The first growth period lasts about 24 hours, the resting period probably 3—4 weeks.

The main factors initiating the first period of tube growth (by normal germination) seem to be: 1) The absorption of fluid and expansion of the contents of the grain (particularly the starch), 2) The chemical composition of the fluid absorbed and 3) The presence and formation of starch in pollen and tube.

As regards 3) KÜHLWEIN (6) states, p. 67: „Zusammenfassend kann gesagt werden: Tempo der Stärkebildung und Menge der gebildeten Stärke wirken auf Keimung und Schlauchwachstum wie Antagonisten. Es scheint zum normalen Schlauchwachstum ein bestimmtes Gleichgewicht bestehen zu müssen zwischen gelösten und festen

Kohlehydraten, dessen Regulator offenbar die vorhandene Diastase ist. Die Aktivierung der Diastase wiederum ist abhängig von einem optimalen pH-Wert des Keimungsmediums". Our results point in the same direction, but we have as yet not been able to find a method for dissolving the starch without loss of structural details.

There is no doubt an active, purely mechanical, factor involved (the rupture of exine and of the germinal duct), and short tubes may probably be formed by stretchings of the cytoplasm, but for normal growth the chemical composition of the fluid and the starch formation seem decisive.

In nature the fluid absorbed by the pollen is of course the fluid exudated into the micropyle at pollination and before fertilization, but little is known about the chemistry of this fluid.

Judging from similar processes in *Larix* and *Pseudotsuga* it is probable that the STJ is active as regards the growth of the tube, and this is probably the reason why pollen without nucleus sometimes may form tubes.

Investigations of these problems continue.

Fig. 4 shows a pollen grain at the first stage of tube formation, the germinal duct is ruptured, the germinal furrow of the exine is opening, the nucleus of the spermatogenous cell is faintly seen at the pore. *Fig. 19* shows a pollen with a short tube; it is somewhat damaged by frost and lacking starch grains, whereby its structure is revealed. It shows the outer cytoplasmic mantle, which serves as tube, and through a hole in the mantle (at the triangular opening of the germinal furrow) the interior of the tube is visible. Near the tube end one of the sieve-plates of the STJ is seen. Between the STJ and the intine the rosette-valve on the cytoplasmic membrane can be distinguished, and to the left, on the outside, the rosette-valve of the intine is lying, torn off from the tip of the tube. *Fig. 20* shows the pollen in *fig. 19* at a higher focus. *Fig. 21b* shows the tip of an almost normal tube with STJ and rosette-valves. — *Fig. 21* shows a pollen with 2 tubes. The tube to the right contains the STJ and valves, the one to the left is only a bulge of intine, probably pressed out on account of a blockade somewhere in the germinal duct; such pollen grains are often found by viability tests of poor pollen samples.

The rosette-valves and the STJ, among other functions, probably also help to ensure quick absorbtion of fluid from the outside. A spherical pollen grain can absorb fluid and expand in the course of a few minutes.

The "Tüpfel" at the end of the pollen tube described by Strasburger ((13), Tafel II, fig. 4, 5 and p. 13) and (13a) is probably identical with the STJ-complex.

THE PROTHALLIAL/ANTHERIDIAL MEMBRANE (fig. 2/4, 16). — It has not been possible to determine exactly when and how the "antheridium" is formed, and this also applies to the "prothallial/antheridial" membrane, which separates the outer cytoplasmic mantle from the cytoplasmic mantle of the nucleus. — The variable terminology in earlier records concerning the *male gametangium* in gymnosperms makes a survey difficult, but the membrane in question seems not to be recorded. — The existence of the membrane has been documented in *Larix* and *Pseudotsuga,* and its existence in *P. abies* appears from *fig. 16,* but details cannot be determined by phase contrast.

However, Müller-Stoll ((9), p. 629, 637) found that in *Larix* and *Pseudotsuga* each deteriorating prothallial cell is separated from the nucleus and from each other by layers of intine. This is in accordance with our observations, and the possibility seems to exist that the *prothallial/antheridial membrane* may be composed of these layers of intine.

RESTING NUCLEUS = INTERPHASE NUCLEUS (fig. 2/1, 3/1, 23—25). — Resting nuclei of the type recorded in the pollen of *Larix* and *Pseudotsuga,* cf. (1a), seem not to have been observed before in *P. abies,* and on account of the absence of sufficient quantities of normal pollen in 1970—71 only scant information could be derived as regards their structure and behaviour. It seems, however, that the mantle of the resting nucleus in *P. abies* is less solid than in *Larix* and *Pseudotsuga.* In all three species the resting nucleus seems to be present in early meiotic prophase, in the periods between meiotic telophase II and the mitotic divisions of the nucleus of the embryonal/spermatogenous cell, and, again, after the formation of the male gametes.

LIFE FUNCTIONS OF A POLLEN OF P. ABIES. — The type of pollen tube now revealed in *P. abies* seems to be at a transitional stage between fertilization by spermatozoids and fertilization by ordinary pollen tubes as in the angiosperms, and very little is known about its structure and organisation.

It might, therefore, be worth while to try to visualize, how a large pollen grain of this type functions without self-transporting spermatozoids and without the more or less self-contained pollen tubes of the angiosperms.

It is evident that this large pollen grain with: 1) its extension of cytoplasm serving as pollen tube, 2) its vast circulatory network of tubes and tubules interweaving the interior of the whole pollen grain and carrying a continuous flow of nutrimental fluid and 3) the task of nourishing through several weeks of two rather large male gametes, must consume much more energy than a usual pollen grain of an angiosperm, and, it must, as it would seem, have a relatively high rate of circulation of fluid.

To secure a satisfactory circulation of fluid a pump at a strategical point is necessary. The strategical point is evidently the (resting) nucleus *fig. 2—3/1,* where the main tubes of the grain converge, and as regards the pump, the male gametes seem to be the only possibility, a possibility, which is not as remote as it might seem at a first glance.

The literature contains very little as regards the circulation of fluid in the pollen and tubes of coniferous, but Hartmann-Dick and Müller-Stoll ((5), p. 288) observed directed currents in pollen grains of *Vinca;* and in *Narcissus* (p. 291) they observed many small vacuoles being pushed out into the tube in groups and they comment: "Dieser Übertritt ist von einem deutlichen Ruck begleitet und stellt sich eindrucksvoll als ein Hineinpumpen der Vacuolen in den Schlauch dar". Coulter and Chamberlain ((2), p. 148) state as regards the spermatozoids of the Cycads: "The cilia begin to move while the sperms are still within the mother cells, and their movement is accompanied by pulsating and amoeboid movements of the cytoplasm and nucleus ... the movements may continue for several hours before the sperms are discharged from the tube". Shimamura (11) has in *Ginkgo,* and Christiansen (1) in *Pseudotsuga,* recorded, that shortly before the discharge from the grain spermatozoids begin to move their cilia giving the impression that they are rotating.

It appears from the above that certain spermatozoids, at least shortly before their discharge, are able to cause pulsating and amoeboid movements of cytoplasm and nucleus. Whether such manifestations of life occur also at earlier stages of the development is as yet unknown, but considering that the life and development of a spermatozoid is quite different from that of a gamete of an angiosperm, it would at least not seem unlikely if they, also early stages, could make amoeboid movements.

Now, traditionally, the male gametes of coniferous pollen are not motile, but already HOFMEISTER expressed his doubts, and a glance for instance at the male gametes of *Pinus* shown by FERGUSON ((4), fig. 26, 49, 50) will confirm this doubt: they are much more like the spermatozoid-capsules in *Pseudotsuga* than gametes of an angiosperm.

It would, therefore, not seem unlikely, that the male gametes of *P. abies*, which are evidently at a stage between fertilization by spermatozoids and fertilization by pollen tube as in angiosperms, have retained some spermatozoid traits, inter alia a certain motility, in which case they may be able to act as pumping centre.

It is true that this conclusion is mainly based on circumstantial evidence, but it has a certain real background and deserves no doubt a closer investigation.

GERMINATION OF POLLEN. — As mentioned above experiments and observations in nature seem to show, that tube formation in *P. abies* is divided on two periods, one immediately after pollination when the tube grows to a length of 3—5 pollen diameters, and a second immediately before fertilization, when tube growth is resumed, and the male gametes leave the pollen grain. The resting period is probably necessary for the development of the male gametes.

The above germination experiments, as regards the chemical and biochemical part, have been carried out in close cooperation with cand. scient., amanuensis U. KAUFMANN of The Laboratory of Genetics of The Royal Veterinary and Agricultural University of Copenhagen, and it is intended, as far as the necessary material is obtainable, to continue this experimental work, the results of which may be of considerable importance, not only for the clarification of the mechanism of pollen tube formation, but also for the improvement of the viability tests.

SIMILARITIES AND DIFFERENCES IN THE DEVELOPMENT OF POLLEN OF PICEA, LARIX AND PSEUDOTSUGA. — A survey like this cannot, at our present stage of knowledge, be exact, but it may nevertheless, be of interest, particularly for the research.

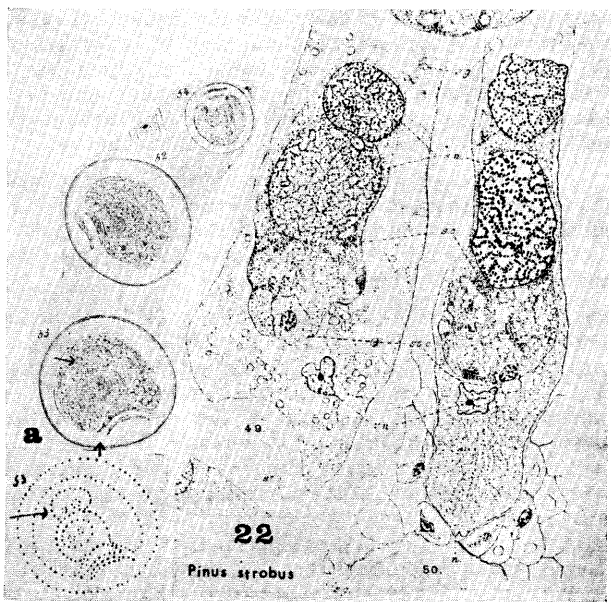

Fig. 22: Two pollen tubes from an older description of fertilization in *Pinus strobus* here used for demonstration of different interpretations; see text. — Note conformity of st.c and v.n with *fig. 21*, rv_1 and rv_2. —

Fig. 22a: Three pollen from an older description of the development of pollen of *Larix* here used for demonstration of different interpretations. Particularly pollen numbered 53 shows strong resemblance to our *fig. 27, 29;* see text.

Enlargements: × ca 300.

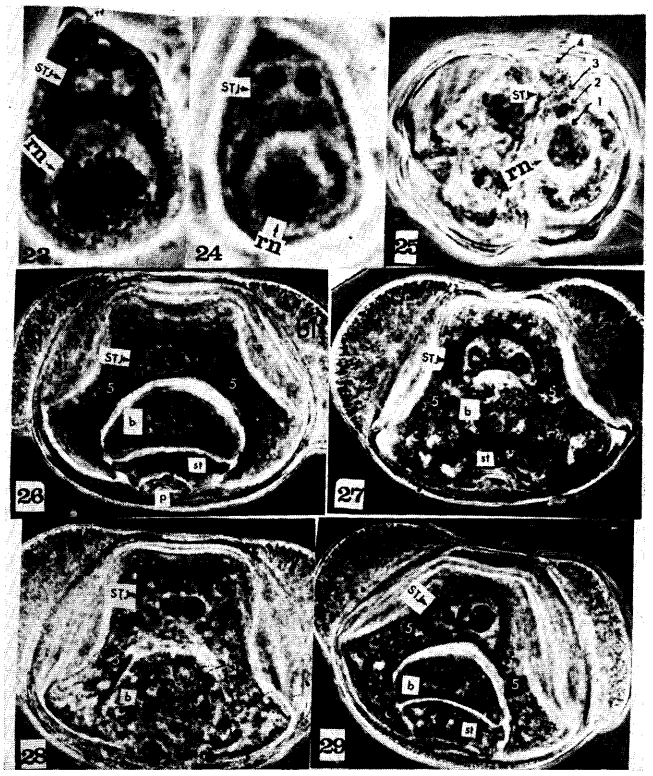

Fig. 23: Early meiotic prophase showing STJ (corner-view, see *fig. 12* and text) and resting nuclus (rn), on top rosette-valve (rv).

Fig. 24: Same cell as *Fig. 23*, but higher focus.

Fig. 25: Damaged tetrade: left side: cells almost dissolved; right side: one usable cell visible: rn: resting nucleus; 1: rv above rn; 2 and 3: two rosette-valves probably on nuclear membrane; STJ with two sieve-plates (dotted circles); 4: rv, probably on intine. *The organelles recorded above are evidently persisting in mature pollen* (confer text and *Fig. 26—29*).

Fig. 26—29: (To facilitate survey usual pollen-cell — terminologi is used): Four pollen approximately at same stage of development (near dehiscence) show the STJ-complex at different foci; 5 = outer cytoplasmic mantle of embryonal/spermatogeneous cell. The different appearances of the STJ are due to focussing, pressure or small differences in position of grains. *Note! The STJ seems present at all stages from meiotic prophase to mature pollen* (cf. text).

Enlargements: Fig. 23—25 × ca 700.
Fig. 26—29 × ca 425.

Abrevv.: b = "body cell"; bl = air-bladders; i = intine; p = pore; rn = resting nucleus; rv = rosette-valve; St = "stalk cell"; STJ = special tube-junction.

Until pollination the structure of the pollen of the three species is rather uniform, the main difference being that *P. abies* at pollination time has air-bladders, while *Larix* and *Pseudotsuga* have none. The mitotic nuclear divisions seem to procede along the same lines, and in all three species two evanescent prothallial nuclei and two male gametes are produced; no other pollen cells were observed. — After pollination considerable differences in the development of the pollen occur: The pollen grain of *Larix* changes its shape slightly, from spherical to ovate, that of *Pseudotsuga* elongates 4—5 pollen diameters, while the pollen of *Picea abies* keeps its height but expands sidewards. In all three species germination, i. e. discharge of the male gametes from the grain, takes place on the apex of the nucellus; in *Larix* and *Pseudotsuga* the male gametes (being spermatozoid-like organisms) leave the pollen grain through a bulge of intine, formed by themselves and resembling a pollen tube, while the male gametes of *P. abies* leave the pollen grain through the bulge of intine and cytoplasm, which serves as pollen tube.

There seems no doubt that all three species, as regards pollen development, are at different transitional stages between fertilization by spermatozoids and fertilization by pollen tube of angiosperm type. As fertilization by spermatozoides is considered more primitive than fertilization by pollen tube, *Pseudotsuga* would seem to have the most primitive system, *Larix* a slightly less primitive, while the system of *Picea abies* may signify a further advance in the direction of the modern pollen tube of the angiosperms.

Discussion

Part of the results reported above are not in accordance with the existing conceptions, particularly as regards the mitotic nuclear divisions in the pollen grain, and the formation of pollen cells: generative cell, tube cell and stalk cell.

The discrepancies are not caused by the published documentation; the exact and painstaking work of most of the pioneers in this field is on a level rarely seen today and deserves the highest appreciation. The discrepancies are due to differences in interpretation, and that such may arise is quite natural on account of the difficult material and the different aids available to science today and yesterday.

An example (from the beginning of the century) may elucidate the situation: *Fig. 22* shows two pollen tubes (of *Pinus strobus)* with their contents. The tip of the left tube is missing, probably cut off by the knife of the microtome, the tip of the tube to the right is near the membrane of the egg cell; each tube contains two male gametes, the foremost parts of the largest gametes are buried in a lump of tissue of peculiar shape, marked s.c = sperm cell. Actually s.c. is no doubt the STJ *(fig. 2—3/9),* and probably the two gametes are beginning to force their way through this organ, which is now superfluous. The organs marked st.c = stalk cell are no doubt rosette-valves on the top of the outer cytoplasmic mantle (cf. *fig. 16/1, 21b,* rv_1), and the organs marked v.n = vegetative nucleus are no doubt the shrunken rosette-valves on the outside of the tube-tips *(fig. 2—3/12 and 21b,* rv_2).

Even these interpretations are subject to some uncertainty on account of the lack of material and the general state of affairs as inter alia: 1) It has evidently never proved possible to carry out a fundamental, continuous and, from a scientific point of view, satisfactory investigation of the development of pollen even of a single coniferous tree. Even MARGARET FERGUSON (4) who, as told, devoted most of her life to the study of the *Pinaceae* and wrote the most exhaustive survey existing of "The Development of the Pollen-Tube and the Division of the Generative Nucleus in certain Species of *Pinus*" was quite aware of the incompleteness of her investigation, for the same reasons and on account of the same difficulties, which exist today. STERLING ((12), p. 188) states: ".... it would appear that a closer investigation of gametophyte development in this group (Coniferales and Taxales) could provide further clues to their phylogenetic development and their interrelationships. Nevertheless, there are many gaps in the knowledge of the different Genera".

2) The main causes for the stagnation of investigation in this field are no doubt the irregular flowering, cytotechnical difficulties, and the considerable expenses necessarily involved through several years if a satisfactory investigation of even a few trees were to be carried out.

3) For these reasons it has, more often than not, been necessary to base conclusions on scattered stages of development of single trees available by chance, and on a, often very small, number of pollen sectioned on microtome, and a still smaller number of usuable sections. Moreover, the use of microtome sections for pollen studies of conifer pollen (which are sometimes, even now, the only possibility) involves grave risks not always realized. Here should be mentioned: misinterpretations: 1) on account of undetectable losses of cells, or pieces of cells and nuclei carried away by the knife of the microtome or washed away during treatment or 2) on account of the frequently occurring impossibility of determining whether or not a section is oblique, or which part of an oblique cell (or nucleus) the seen organelle is actually representing.

In view of the above it is not surprising that many stages of development are still insufficiently explored.

Closer investigation in this field will no doubt throw more light on the interesting question how and when the conifers have changed from fertilization by spermatozoids to fertilization by pollen tube, and it is our hope, that other workers will assist in finding the "missing links".

Acknowledgements

The investigations described in the present work were carried out by means of a grant from the CARLSBERG FOUNDATION, Copenhagen, and by joint cooperation of the DANISH STATE FORESTRY'S TREE IMPROVEMENT STATION, Humlebaek, and THE LABORATORY OF GENETICS OF THE ROYAL VETERINARY AND AGRICULTURAL UNIVERSITY, Copenhagen, and I wish to express my sincere thanks to the Director of the Tree Improvement Station, Mr. H. BARNER, for inspiration, support and for his never failing interest in the work, and to Professor, fil. Dr. A. LUNDQUIST for the granting of working facilities and for highly appreciated aid and advice.

Summary

1) It is shown that after telophase II the embryonal *cell* of the pollen grain of *P. abies* does not divide but persists as spermatogenous (or generative) cell. It is furthermore shown that the embryonal cell, being the only living cell in the pollen grain during the whole development of the male gametophyte, remains firmly fixed in a certain position in the pollen grain, from which it cannot move.

2) No pollen *cell* divisions were observed, which means that the formation of a "generative cell", "tube cell" and "sterile cell" could not be confirmed. But 3 *nuclear* divisions take place, by which 2 evanescent prothallial nuclei and 2 male gametes are produced *(fig. 1).*

3) A "special tube-junction" (abbrev. STJ), observable from early meiosis *(fig. 12 and 23—29),* is occupying the place where the "tube cell" (tube nucleus) = vegetative cell is reported to occur. The main function of STJ is no doubt to govern the nutrition of the outer cytoplasmic mantle, but it seems also (in *Larix* and *Pseudotsuga)* to participate in the formation of neocytoplasm in elongating pollen grains and, in *P. abies,* perhaps also in the pollen tube.

4) A bulge of intine and cytoplasm from the outer cytoplasmic mantle, including the STJ, serves as pollen tube *(fig. 2—3/9).* — This type of pollen tube is provisionally and tentatively called „Picea-type". It may be a transitional stage to more advanced types of pollen tube.

5) The development of the pollen tube seems to take place during two periods: one immediately after pollination, when the tube grows to a length of 3—5 pollen diameters and thereafter stops, and another, immediately before fertilization, when tube-growth is resumed, and the male gametes leave the pollen grain. Also by germination on

artificial substrate the pollen tubes, as a rule, stop growth at 3—5 pollen diameters.

6) The *resting nuclei, membranes, tube- and valve-systems* and the anatomy of the pollen grain of *P. abies* are, until dehiscence, in the main very much like those in *Larix* and *Pseudotsuga.*

At least in *P. abies* and *Larix* (but probably also in *Pseudotsuga)* the embryonal/spermatogenous cell is built up on a tubeformed skeleton around the vertical tube (cf. *fig. 9).*

A conspicuous *difference* is the presence of *air bladders* in the pollen of *P. abies;* such bladders are absent in the pollen of *Larix* and *Pseudotsuga.*

The functions of the *air-bladders* and the possibility of their participation in the development of the pollen tube is discussed.

7) It is suggested that the pollen of *P. abies, Larix* and *Pseudotsuga,* as regards structure and functions, may be different transitional forms between fertilization by means of spermatozoids and fertilization by pollen tube.

8) Under heading *"LIFE FUNCTIONS IN A POLLEN GRAIN OF P. ABIES"* it has been tried to visualize the mechanism enabling the complicated, self-contained pollen grain to live, develop and produce gametes.

Literature Cited

(1) Christiansen, H.: On the Pollen Grain and the Fertilization Mechanism of *Pseudotsuga menziesii.* Silvae Genetica **18**, 97—104 (1969). — (1a) Christiansen, H.: On the Development of Pollen and the Fertilization Mechanisms of *Larix* and *Pseudotsuga menziesii.* Silvae Genetica, in press 1970/72. — (1b) Christiansen, H.: On the Germination of Pollen of *Larix* and *Pseudotsuga* on Artificial Substrate, and on Viability Tests of Pollen of Coniferous Forest Trees. Silvae Genetica **18**, 104—107 (1969). — (1c) Christiansen, H.: On the Effect of low Temperature on Meiosis and Pollen Fertility in *Larix decidua.* Silvae Genetica **9**, 65—92 (1960). — (2) Coulter, J. M., and Chamberlain, C. J.: Morphology of Gymnosperms. The University of Chicago Press, Chic. III, 1917, p. 148. — (3) Doyle, J., and Kane, Ann: Pollination in *Tsuga pattonia* and in species of *Abies* and *Picea.* Sci. Proc. Roy. Dub. Soc. Vol. **23**, N.S. 7, 1943. — (4) Ferguson, M. C.: The Development of the Pollen Tube and the Division of the Generative Nucleus in certain Species of Pines. Ann. of Bot., Vol. XV, 1901. — (5) Hartmann-Dick, U., und Müller-Stoll, W.: Zytomorphologische Studien über das normale und pathologische Verhalten von Pollenschläuchen in künstlicher Kultur. Österr. Bot. Zeitschr. **102**, 273—300 (1955). — (6) Kühlwein, H.: Zur Physiologie der Pollenkeimung insbes. der Frage nach dem Befruchtungsverzug bei Gymnospermen. Beih. zum Bot. Centralblatt, Bd. LVII, Abt. A, 1937. — (7) Linskens, H. F.: Pollen Physiology and Fertilization. North-Holland Publishing Company, Amsterdam, 1964. — (8) Miyake, K.: On the Development of the Sexual Organs and Fertilization in *Picea excelsa.* Ann. of Bot., Vol. XVII, No. LXVI, 1903. — (9) Müller-Stoll, W. R.: Zytomorphologische Studien am Pollen von *Taxus baccata* und anderen Koniferen. Planta **35**, 1948. — (10) Rieger, R., Michaelis, A., Green, M. M.: A Glossary of Genetics and Cytogenetics. Springer Verlag Berlin, Heidelberg, New York, 1968. — (11) Shimamura, T.: On the Spermatozoid of *Ginkgo biloba.* Cytologia, Fuji jub. Vol. 1937. — (12) Sterling, C.: Structure of the Male Gametophyte in Gymnosperms. Biol. Rev. **38**, 167—203 (1963). — (13) Strasburger, E.: Die Befruchtung bei den Coniferen. Jena, Hermann Dabis, 1869. — (13a) Strasburger, E.: Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen. Verlag G .Fischer, Jena, 1884. — (14) Wodehouse, R. P.: Pollen Grains. McGraw-Hill Book Company, New York and London, 1935.

Short Note

Labeling of seeds for seed-dispersal studies in conifers

The genetic structure of natural populations is mainly determined by migration of pollen and seeds. Several methods have been used to label both pollen and seeds of individual trees for dispersal studies. Indicator-activation methods with manganese, as the indicator, have given promising results when used for the labeling of pollen (Fendrik 1967, Schmidt 1970, Stern 1972). A similar method has been applied in a seed dispersal study in Scotch pine.

The maganese content of some trees has been shown to increase after inoculation in spring from 2—300 ppm to about 1000 ppm in the needles, when treated with 100 l solution of manganese sulphate containing 150 g of manganese. Seeds were collected from treated and non-treated trees in the following autumn and brought in a flow of $1.5—2.0 \times 10^{13}$ thermic neutrons for four minutes. The average of β-counts amounted to 4.012×10^6 and $2{,}619 \times 10^6$ imp/min resp. for a single seed from the treated trees and to 1.413×10^6 and 1.489×10^6 imp/min resp. for a single seed from the untreated trees in the surrounding stands. The distribution curves of β-counts from treated and untreated trees did not overlap.

Literature:

Fendrik, I., Diss. TU Hannover 1967. — Schmidt, H., Diss. Univ. Göttingen 1970. — Stern, K., Ztschr. f. Pflanzenzüchtung 1972 (in press).

K. Stern
Lehrstuhl für Forstgenetik und
Forstpflanzenzüchtung der
Forstlichen Fakultät der Universität
Göttingen
34 Göttingen-Weende
Büsgenweg 2

Received January 1972.