On the Anatomy of Pollen Grains of Picea and Pinus

Starch-compartments built into cytoplasmic mantles

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Introduction

The vast quantities of starch filling the pollen grains and tubes of conifer pollen have in the course of time been the subject of numerous investigations and discussions, but tangible results are few. The reason for the meagre results is that the starch, although of paramount importance for pollen development and fecundation and thus deserving an intense research, in itself forms an almost impenetrable obstacle to the exploration of the interior of pollen grains and tubes.

The general assumption has always been that the masses of starch consist of loose starch grains, which during germination wander from pollen grain to pollen tube. The present investigation has, however, clearly documented, that this is not the case; on the contrary it appears, that each starch unit is contained in a fixed starch-compartment, built into the cytoplasmic mantles of pollen grains and tubes.

It seemed probable that if the starch could be removed at the most interesting stages of development, the way would be open for inspection of the interior of pollen grain and tube. So far this expectation has, however, been only partly fulfilled because the walls of the starch-compartments remained after dissolution of the starch, but further research and the improved technique will no doubt improve the results.

This new conception of the starch problem will no doubt accelerate the research in this field and the more so on account of the new and much improved method for pollen germination, worked out during this investigation and in viability tests and described under "Materials and Methods", which enables the investigator to produce, relatively easily, the large quantities of germinated pollen, needed for the work, be it further investigation of structure and connections of the starch-compartments or further attempts to reduce or remove the starch and find out how the starch mechanism works.

The main points of the investigation is outlined in the following.

Terminology

Generally the terminalogy used in (2) is followed in this work, but where new-found organs are involved we had to use provisional terms. The term "starch-compartment" was suggested by Professor, Dr. TH. BOECHER of the Institute of Plant Anatomy, University of Copenhagen.

Starch-Compartments

In order to be able to examine the interior of pollen and tubes obscured by the masses of starch, we tried — in cooperation with U. KAUFMANN — by means of treatment with *a*- and ß-amylases to decompose, or at least reduce, the starch, and we succeeded in so far that the starch was dissolved (controlled by iodine reaction), but the walls of the starch-compartments in the cytoplasmic mantles remained and still obscured the interior of pollen grains and tubes. Although the removal of the starch did not entirely solve the problem of visibility of the interior of pollen grains and tubes, it considerably increased the visibility and made it possible to see several of the cytoplasmic mantles.

During the attempts to solve these problems it became evident, that the starch-units were not, as hitherto assumed, loose grains, but complicated built-in starch-compartments mutually connected by tubuli and normally occupying and filling each of the cytoplasmic mantles of the pollen grain of Pinus and Picea, cf. (2), forming a compact, opaque layer (fig. 7). Fig. 5 shows an enlargement of a torn off piece of the pollen grain cytoplasm of Picea, marked "d" in fig. 3, with large starch-compartments. One of the compartments with connections to the adjoining compartments is in focus.

The starch-compartments are formed before dehiscence, no doubt at (or before) the tetrad stage. In the outer cytoplasm of the mature pollen grain of Picea abies the dimensions of the compartments are approximately: diameter 5—6p, height about 2μ; in Pinus the diameter is about 8μ. The shape of the compartments is often hexagonal, the surface of the layer resembling a honeycomb (fig. 1 and 7). The starch-compartments of the cytoplasmic nuclear mantles are smaller than those of the pollen grain mantle and gradually decreasing in size, the compartments of the innermost nuclear mantle being barely visible in phase contrast *(figs.* 1, 3 and *6).*

It is interesting to note, that the smaller the pollen grain of the investigated species, the larger their starch-compartments of the pollen grain mantle seem to be, and there is some evidence that *Cryptomeria* japonica, by a pollen diameter of only 30μ, has starch-compartments not far from the size of those in the pollen grain of Pinus (pollen diam. about 45μ).

When a pollen grain has germinated on artificial substrate, the volume of grain and tube (and consequently of the inner diameter of the latter) are often considerably increased. Now, if the tube is normal, only the unbroken layer of starch-compartments is seen, totally obscuring the interior. Fig. 7 shows an apparently normal pollen grain and tube of Pinus, germinated in a "horizontal drop" of medium "H" (see "Material and methods"). The layer of starch-compartments of the pollen grain cytoplasm and of the tube seems complete and it totally obscures the interior. The size of the compartments in pollen and tube is approximately the same, and this is well in accordance with the assumption that the pollen tube of Pinus, like that of Picea, cf. (2), is a bulge of the cytoplasms of the pollen grain.

If, however, the layer of starch-compartments is defective (and no inner layers present), with patches of compartments missing (a frequent occurrence in our climate), it is aften possible to study, through the transparent spaces of the upper tube wall, both outside of the layer near the upper tube wall, and the inside of this layer near the nethermost wall. Fig. 4 shows a piece of a defective pollen-

tube of *Picea abies* with patches of starch-compartments and transparent spaces in between. The tube is somewhat flattened, and it is therefore possible, at the same focus, to see the outside of the compartments near the upper wall (f. inst. at "a") and the inside of the compartments of the same layer near the nethermost wall at "b"; no visible difference could be ascertained between the upside and downside of the starch-compartments. It is therefore probable, that each compartment forms a channel through the cytoplasm and that these channels are part of the endoplasmic reticulum, (cf. (2), p. 56); it is evident that the compartments and channels form an exellent system for quickly filling the pollen grain with fluid and for activating the starchproduction, inter alia when the pollen grain overnight must produce a tube in the micropyle.

As regards the size of the large starch-compartments of *Pinus* (and also of *Cryptomeria japonica)* it seems probable that it may have something to do with the fact, that in both species there is a winter resting period between pollination and fertilization, while in *Picea abies,* this period is only 3—4 weeks.

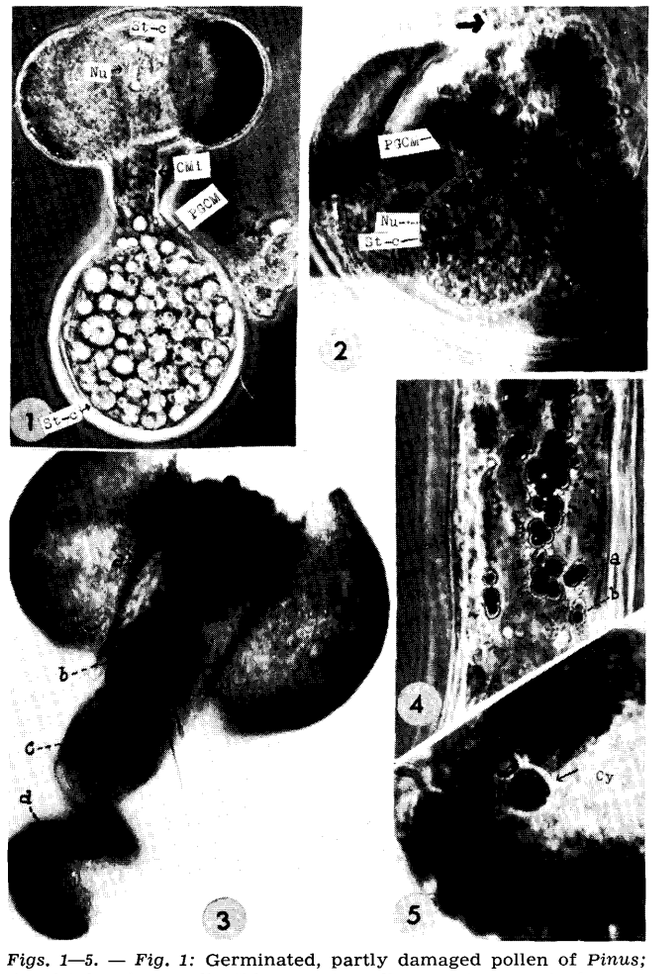
The Cytoplasmic Mantles Carrying the Starch-Compartments

The number of cytoplasmic mantles were, as regards the pollen grain of *Picea abies* (2), stated as 3. This number seems too low, but exact evidence and new details are difficult to get. *Figs. 1 and 6* are of interest in this connection; *fig. 6* shows an abnormal and partly degenerated pollen grain of *Pinus* with two short tubes. The number and size of the starch-compartments of the longer tube are about normal, but several compartments are empty (compare with *fig. 7).* The interior of the short tube is disorganized, but 4—5 membranes are seen in optical section; different interference colours are often seen, cf. text to *fig. 6.* The wall of the longer tube consists of 4, perhaps 5 membranes, but 1 or 2 may be double membranes. The pollen grain seems almost devoid of cytoplasm and starch, and also the starch-compartments of the outer mantle, except one compartment partly covering the resting nucleus, are missing. 4—5 more or less empty cytoplasmic mantles can be distinguished. *Fig. 2* shows a germinated pollen grain of *Picea abies* damaged by preparation, exit of tube at top (arrow). Part of the outer mantle with large starch-compartments is missing, exposing, as far as it could be ascertained, the resting nucleus with the nuclear cytoplasms and small starch-compartments; 3—4 nuclear mantles may be distinguished. *Fig. 3* shows an apparently normal pollen grain of *Picea abies* just before dehiscence. It has swelled in a drop of medium "H", and it has deliberately been pressed to force out the contents; „a" and "b" are, respectively, the upper and the nethermost part of the pollen grain cytoplasm with large compartments, "c" one of the nuclear cytoplasms with smaller compartments.

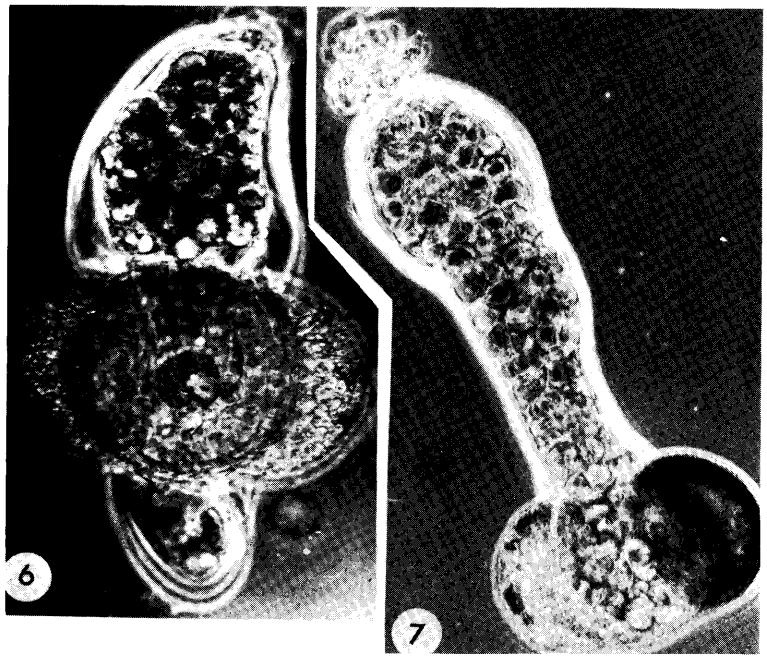
The Pollen Tube

There is some evidence, that the "wall" of a normal pollen tube in *Pinus* and *Abies* consists not only of the visible outer cytoplasmic mantle as shown in respect of *Picea abies* in ((2), *fig. 3/5),* but also includes extensions of the nuclear cytoplasms with their layers of starch-compartments. *Fig. 1* shows an abnormal pollen grain of *Pinus;* most of the contents are missing, and the tube is deformed. It is, however, fairly evident that a nuclear mantle with very small starch-compartments connects the disintegrating nucleus with the outer part of the "blown up" tube; this mantle no doubt surrounds, first the vertical tube, thereafter the lumen of the pollen tube.

If thus the "wall" of the pollen tube already at germination consists of layers of cytoplasm with their layers of starch-compartments, the size of which is decreasing in the direction of the lumen, it would be interesting to know, if



Figs. 1—5. — Fig. 1: Germinated, partly damaged pollen of *Pinus;* tip of tube abnormally "blown up"; pollen empty except of membranes, nucleus (Nu), (probably) male gametes and almost empty starch-compartments (St-c). Innermost cytoplasmic mantle (CMi) with very small st-c leads from nucleus to blown up part of tube with intact but almost empty st-c in pollen grain cytoplasmic mantle (PGCM). The inner cytoplasmic connection (CMi) no doubt contains the vertical tube (cf. (2)) and the lumen of the pollen tube. *Note:* many of the st-c in the blown up part show structure like that in *fig. 5.* (× c. 600.). — *Fig. 2:* Damaged, germinated pollen grain of *Picea abies,* focus on nucleus with resting nucleus and about 3 CMi with *small* st-c; above: rest of the pollen grain cytoplasmic mantle (PGCM) with *large* st-c's and above, at arrow, rest of broken tube. (× c. 750.). — *Fig. 3:* Normal pollen grain of *Picea abies* swelled in drop of medium "H" and pressed to show contents; "a" is the upper, "b" the nethermost part of the pollen grain cytoplasmic mantle with *large* st-c; "c" is one of the nuclear mantles with *smaller* st-c; "d" is a piece of "b" pushed down and turned about 90°; it is out of focus, but it is the same piece as shown enlarged in *fig. 5.* (× c. 600.). — *Fig. 4:* Section of pollen tube of *Picea abies* with patches of starch-compartments in pollen grain cytoplasmic mantle; at "a" are 2 st-c near the uppermost, at "b" the interior side of 2 st-c near the nethermost tube wall in a place where the tube is empty, and "a" and "b" can be clearly seen at the same time. For further particulars see text. (× c. 550.). — *Fig. 5:* Piece of pollen grain cytoplasmic mantle "d" of *Picea abies* in *fig. 3,* enlarged showing structural details of starch-compartment (arrow), built into cytoplasm with connections to neighbouring compartments. The starch-compartments of the empty space of cytoplasm (cy) have fallen off exposing the mantle. (× c. 1250.).



Figs. 6—7. — Fig. 6: Partly degenerated *Pinus* pollen with one long and one short tube, most of starch and cytoplasm missing; in pollen is seen: resting nucleus and optical sections of 4—5 cytoplasmic mantles with faint starch compartments;; on top of resting nucleus one or two large st-c; around tubes, particularly the short one, 4—5 membranes are seen (phase contrast). These membranes often show different interference colours (cf. (6)). (× c. 650.). —*Fig. 7:* Apparently normal germinated pollen of *Pinus;* in pollen and tube only the layer of starch-compartments of the pollen grain cytoplasmic mantle is seen, the interior is completely hidden. The size of the starch-compartments is the same, and they are sitting in the same cytoplasmic mantle, in pollen and tube. (× c. 500.). — *Abbreviations (figs. 1—7):* CMi = inner cytoplasmic mantle; Cy = cytoplasmic mantle; Nu = nucleus; PGCM = pollen grain cytoplasmic mantle; St-c = starch-compartment.

all compartments, irrespective of size, are starting starch production simultaneously, or if the innermost ones are resting until the second phase of germination begins, i. e. when the male gametes are leaving the pollen grain for fertilization (cf. (2), p. 53—54). On account of lack of sound pollen the question must, however, be left unanswered.

Material and Methods

For studies of germination of conifer pollen traditional microtome sections are practically of no use (cf. (2), p. 60, 3). Traditional squash is not much better because, if the outer membrane is stained, it obscures the interior. The only usable method has proved to be phasecontrast microscopy, microphotography and piecing together the results from pollen grains damaged in various ways and revealing different parts of their structure. Obviously, to get reliable information by this method, large numbers of pollen must be scrutinized, but this is not as time consuming as it may seem, and the repeated scrutiny offers valuable opportunities of checking previous observations and getting familiar with the material.

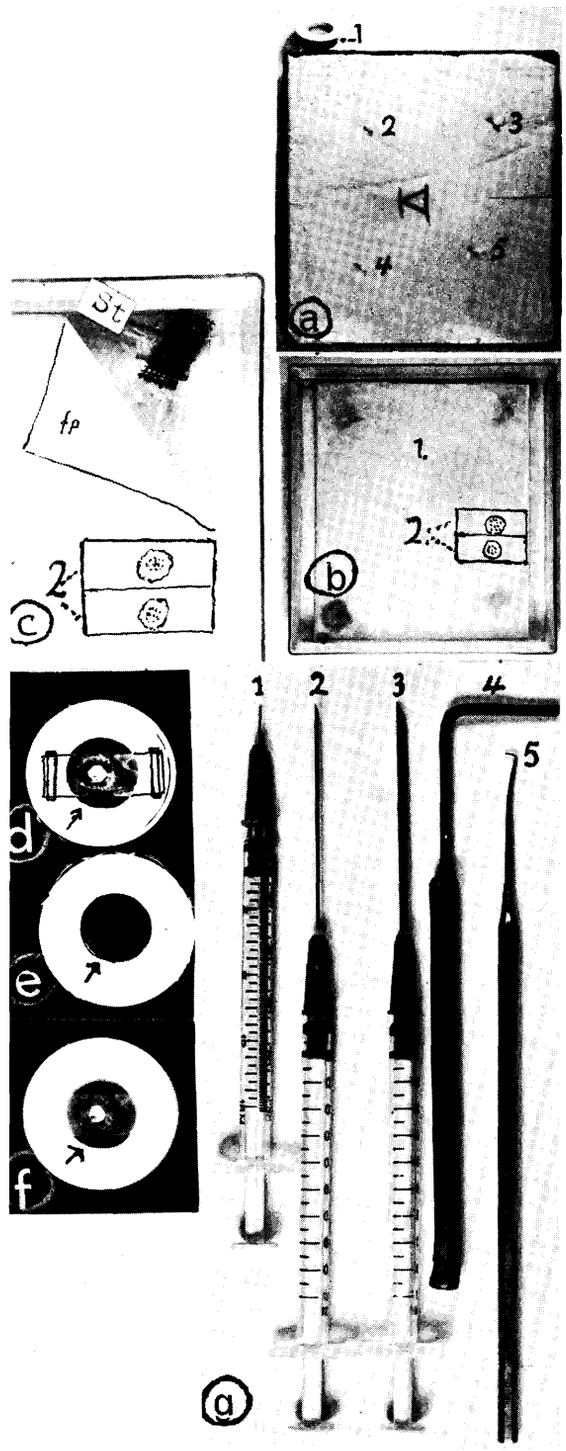
The usual methods for germinating pollen viz. sugar solutions, hanging drops etc. are of course not suitable for germination of large numbers of pollen, and we have therefore worked out a new method, based on germination in a special medium "H" (cf. text and (5)) forming a drop within a silicone circle on a horizontal slide, placed in a hermetically sealed humidity chamber with saturated humidity and temperature about 28° C (cf. text). The humidity chamber holds about 20 slides, each with one drop, which holds about 4—500 germinating pollen, and this makes it possible simultaneously, in the course of 2—3 days, to germinate, *under exact identical conditions,* up to 10.000 pollen grains from several different samples, according to the safety degree wanted. This method has been tested on roughly 250.000 pollen of *Picea* and *Pinus* but, on account of the poor quality of available pollen, so far only for tube lengths up to 3—5 pollen diameters, i. e. the first germination phase (cf. (2), p. 59).

A *dissecting microscope* with high magnification was used for current control of germination of pollen, a *phase contrast microscope* for examination of closed preparations, both with cold light illumination (cf. (2), p. 52); ordinary light can however be used, although the unavoidable heating may be detrimental, particularly as regards the dissecting microscope, where the apertures of the humidity chamber may be getting steamy.

Many of the implements needed for practizing the *"horizontal drop method"* are already at hand in a cytological laboratory, others may easily be made of household utensils at low costs. Most important is the *Humidity Chamber.* There are two types:

a) (see *fig. 8a)* holding up to 20 slides with horizontal drops. It is a transparent box of *hard* nontoxic plastic, 26 × 29 × 6 cm., inside of lid and sides are covered with moist filter paper; a glass plate to hold the slides is raised about 3 cm above bottom on legs of bent pieces of stainless steel-netting glued to the plate by "Araldite" *(fig. 8 b, c),* or f. inst., supported under each corner by a small glass jar of 3 cm height. When in use the bottom of the chamber is covered by tap water (boiled) to a height of 1 cm. A piece of filter paper covering the glass plate with one side hanging down into the bottom-water helps to keep humidity near saturation.

b) (see *fig. 8d—f)* holding one slide with horizontal drop. It is used for scanning under dissection microscope, when the scanning takes more than about 2 min., or for germination of single or few samples (one chamber for each sample). The chamber consists of a transparent petri dish of *hard* nontoxic plastic, c. 87 × 15 mm; the bottom and lid are lined with rings of moist filter paper, leaving circular apertures of c.



Figs. 8a—g. — *Fig. 8a:* Humidity-chamber 26 × 29 × 6 cm of *hard,* nontoxic plastic, top view, with lid on, hermetically sealed; 1: tape bobbin used for sealing; 2—5: U-formed small pieces of stainless steel wire, each put through two fine holes in lid and bent on inside to keep moist filter paper lining from falling down on drops if accidentally dried up; inside of sides of chamber are also lined with moist filter paper. (× c. 1/7.). — *Fig. 8b:* Same as *8a,* but with lid off; 1: glass plate covered with moist filter paper, nethermost side hanging down in bottom water, which must always be kept 1 cm high; 2: two slides with silicone circle and drops with pollen are drawn in schematically, actually the chamber holds two rows of slides with a total of c. 20 slides. — *Fig. 8c:* Section of *8b* enlarged, corner of filter paper bent back (fp) showing one glued-on leg (st) made of stainless steel netting keeping glass plate raised c. 3 cm above bottom. (× c. 1/4.). — *Fig. 8d:* Humidity-chamber for *1 slide,* top view, with lid off; slide with silicone circle and drop with pollen; it consists of a petri dish of *hard,* nontoxic plastic 40 mm diam.; the slide is resting on two loose pieces of nontoxic plastic, one at each end and thus raised c. 5 mm above the bottom with the drop in the middle of the aperture; it can thus be examined under a dissecting microscope without drying up. If used for germination the chamber can be sealed hermetically by tape, or a few chambers may be placed in a pile, wrapped in "glad pack" and the pile placed in a hermetically sealed small polyetylene bag.

Other important items are:

1. *Culture medium H.* This medium is identical with the medium used by NITSCH, J. P. & NITSCH, C. (5) as a substrate for production of haploid Nicotiana plants; the only difference is, that our medium H contains no agar. It was suggested by KAUFMANN for trial as a medium for germination of conifer pollen, and the results were so satisfactory, that we continued to use it without further attempts at modification. It is, however probable that the formula may be simplified, but we have at present no means to undertake this time-consuming task.
   For use in the "horizontal drop" scheme "medium H" is autoclaved in 100 ml Erlenmeyer flasks with wide orifice covered with aluminium foil, through which the medium later may be sucked up sterilly with a long syringe needle *(fig. 8g/2).* The flask must be kept in dark at a temperature near zero, but without freezing. Before use, the flask is slowly heated to about room temperature, preferably in water bath at c. 28° C; the temperature must at no time exceed 30° C. If the removal of medium is done reasonably sterilly, and the flask after use is sealed hermetically with a piece of adhesive paper and cooled down immediately after use, it may keep for weeks.
2. *Assortment of hypodermic syringes (fig. 8g/1—3),* "Plastipak B.D." (Becton, Dickinson & Co, Drogheda, Ireland), mostly used mark: "tuberkulin", 1 ml with different lengths and lumens of needles. Most important are finest needles (B—D Yale 26 G 3/8) for substitution of fluids in drops, and a large size B—D Yale 14c 2 for piercing rubber lid and taking samples from hermetically cold-stored pollen containers. All syringes are nontoxic and pyrogen free.
3. *Slides.* Usual *well cleaned* slides could be used for carrying the "horizontal drops", but considerably better for holding the drops are slides cleaned with sulfo, on which a silicone circle, diam. c. 12 mm *(fig. 8d)* is made with a fine, pointed camel-hair brush; the slide is air dried and thereafter heated to 200° C for c. 2 hours. The slides can be cleaned (but *not* in strong *alkaline* solutions) and used again.
4. *Markers.* Diamond or carborundum are safest for numbering slides.
5. *Adhesive plastic tape* in different colours as used by electricians is used for sealing the slit between lid and box in humidity chambers *(fig. 8a).* The different colours may be useful if several chambers are used at the same time. Rolls of adhesive, *transparent, household plastic-foil* ("Glad-Pak") are useful for hermetically sealing of humidity chambers.

Directions for Practising "Horizontal Drop" Method

General remarks: At first sight it might seem difficult to move and handle the humidity chamber, and also single slides with free drops, without the drops rolling off the slides. Of course a certain measure of cautiousness is needed, but it is easily acquired, and on account of the silicone circles the slides may be tilted much more than expected without damage.

No disinfectants are used either of pollen or implements because no disinfectant is known, which does not involve risk of damage to germination.

As regards the *inevitable contamination* by fungi and bacteria practice seems to show, that until a *safe* disinfectant is found, the only rational modus operandi is always to start with a sufficiently large number of drops with pollen of the same sample and hope that enough drops for investigation will remain uncontaminated.

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87 × 15 mm; the sides, top and bottom are lined inside with moist filter paper; the linings of top and bottom have apertures (arrows) of c. 40 mm diam. for through-light; the slide is resting on two loose pices of nontoxic material, diam. c. 5 mm, one at each end, raising the slide about 5 mm above bottom (cf. text). (× c. 1/4). — *Fig. 8e:* Lid of *8d.* — *Fig. 8f:* Humidity chamber *8d* with lid on, ready for use; same slide as in *8d.* — *Fig. 8g:* Implements used in "horizontal drop" work (cf. text): 1—3: syringes for different purposes (cf. text); 4 rimming rod for sealing preparations with paraffin; 5: pin with hook for fishing small fungi colonies out of drops with germinating pollen (cf. text). (× c. 1/2.).

In our experience the occurrence of uncontaminated drops may — with luck — be expected to be about 10—15% when ordinary precautions for avoiding contamination are taken. This may seem a meagre result of much trouble, but as a matter of fact 5 humidity chambers with a total of 100 drops (each with 4—500 pollen) could easily be filled in a days work, and the scanning for fungi etc. takes only a few hours.

For *cleaning of implements and humidity chamber,* and for water in the bottom of the latter, boiled tap water is used. Only vessels of *glass* or *hard,* nontoxic plastic and metal-implements of *stainless steel* should be used.

Filling a humidity chamber for germination must be done as quickly as consistent with accuracy, otherwise too much of the drops may evaporate. It is therefore important that before the drops are placed on the slides all preliminaries have been made, such as: arranging the *pollen samples* in correct and convenient order; *numbering or marking* the slides, and arranging them in the order, they are to occupy in the chamber, on a clean glass plate outside the chamber; *making up a list* showing which pollen sample is to be used for each slide number and placing it handy; taking out and placing conveniently and in the right order the *syringes* to be used for pollination (if the pollen is stored in vacuum) and those used for placing the drops of medium "H" on the slides; lighting a Bunsen burner for flambing syringe needles.

"Pollination" of drops with vacuum stored pollen is done by means of a syringe with a large needle *(fig. 8g/3),* with which the rubber lid of the pollen container is pierced and pollen sucked up.

Pollen stored in unsealed phials over calcium chloride may be removed to the drops as follows: Nip the sulphurized end off an ordinary match, draw the nipped off end quickly through a small flame, dip the flambed matchend in the pollen and deposit the adhering pollen on the drop by a light tap on the match. *Caution:* 1) only 4—500 pollen per drop should be used, otherwise the pollen tubes will be intertwined and their development hampered. 2) keep the match-end as close as possible to the drop during "pollination" and watch the pollen descending, if there is a draught at the working place, the pollen may blow away. 3) If more than one species-sample is used in one humidity chamber, the slides with drops belonging to each species must, during "pollination", be removed to a place a few meters away from the other slides, and the latter covered, so as to avoid mixing of pollen, 4) when the pollination is done, place the slides immediately in the humidity chamber and put the lid on without sealing. In this position, i. e. unsealed, the evaporation from the drops is negligible even for one or two hours, and unsealed lid is therefore also used during the daily inspection of germinating pollen. Only when work is finished the chamber is sealed with tape and placed at a temperature of about 28° C (+ — 2—3°) in the dark. 5) When at some stage the drops are getting too small on account of evaporation, fill *slowly* up with medium "H" to original size with a fine syringe (B—D Yale 26G 3/8, *fig. 8g/1)* under dissecting microscope *and try not to alter the position of germinated pollen* in the drop.

If, during germination in a drop, a *beginning concentration of hyphae of fungi* is detected, it may often be fished out by means of a needle of *hard* steel with a very fine hook *(fig. 8g/5),* and the drop saved for at least a couple of days. The fishing must be done at highest magnification on the dissecting microscope so that even the smallest hyphae are visible. When a load of hyphae are fished out on the hook, stick hook and load into a flame and burn away the hyphae, the hook is thus disinfected and ready for a new fishing expedition.

"Horizontal drops" are, as mentioned above, actually "hanging drops" turned upwards and enclosed in a common humidity chamber, whereby each drop is easily made, easily accessible and utilizable for many purposes. A few examples are mentioned in the following: By means of suitable syringes part of a drop can at any time be removed under a dissecting microscope and new chemicals introduced. If investigation by phase contrast or ordinary light microscope is wanted, the contents of the drop may be fixed (best in 45% acetic acid or carnoy) by, in the course of c. 30 min., gradually substituting most of the substrate by the fixative; after fixation place a clean cover glass, vertically, without sidewards movements, on the drop, the glass must swim freely; place a drop of molten paraffin on each corner of the swimming cover glass, so that part of the paraffin runs under the corner and forms a foot, on which the glass rests; place a rim of molten paraffin around the coverglass sealing it hermetically (use a rimming rod), *(fig. 8g/4).* If this operation is properly carried out, the pollen and tubes are in very near the original position and condition and could be examined and photographed by ordinary light and phase contrast (although the latter is actually on account of the thick preparation a combination of phase contrast and ordinary light). If the slide is stored in a freezer, it may keep for a week or more. A fixed drop may be made permanent by gradually substituting the fixative by glycerine and putting on a coverglass as described above. Such slides have remained in good condition for several months. The described coverglass-technique may also be used for making a hermetically closed substrate chamber, in which f. inst. pollen grain divisions currently could be studied by high magnification. A chamber like this could also be used for studying the action of chemicals, enzymes, or the iodine reaction for starch; at any time: remove about 2 mm of the paraffin rim on two sides of the cover glass, suck out fluid with a pointed piece of filter paper until air begins to show, place a drop of the new fluid at one or both openings of the rim and let the fluid flow in, close the openings with drops of molten paraffin, and continue observations.

Discussion

Although the difficulties caused by the starch by exploration of the interior of the pollen grains of conifers have often been mentioned in the literature during the last hundred years and still exist, very little is recorded as regards the production of starch, and practically nothing could be found about "starch-compartments".

However, Strasburger (3) in 1884 described a "starch-cover" enveloping the fertilized nucleus of the egg cell of certain gymnosperms while the nucleus was moving in the direction of the lower end of the egg cell, whereafter the "starch-cover" was dissolved. According to Strasburger this "starch-cover" consisted of "a layer of starch grains of almost equal size". Although Strasburger does not directly say so, it stands to reason that this layer actually consists of *"starch-compartments"* built-into a membrane (otherwise loose starch-grains could hardly keep together in one layer).

Otto Schmeil (4), p. 416, describes and depicts a "Starch-*producer*" i. e. a container enveloping the "starch-grain" and mounted on a foot. In this "starch-producer" starch reserves, f. inst. in potatos, grain etc., are converted to glucose. The shape of the "starch-*producer*" suggests that it is stationary and probably fixed to a membrane, but it seems quite different from the "starch-*compartments*" described above.

On the basis of Strasburger's observations as regards the zygote, and ours concerning the male gametes, it seems reasonably clear, that both have mantles with built-in starch-compartments. Whether or not these compartments are identical is an open question, but in view of the fact, that the male gametes and the female frequently are so much alike, that the former may be made to produce (haploid) plants, it seems not unlikely that their mantles may be identical.

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Summary

A hitherto — as far as we know — unknown organ, tentatively denoted "starch-compartment", which sur-

rounds each starch grain in the cytoplasmic mantles of the pollen and pollen tubes of *Picea abies, Pinus silvestris, Pinus nigra* (and *Cryptomeria japonica),* is described.

In order to be able to examine the rather complicated starch-compartments and their connections *(fig. 5),* which are built into the (probably 4—5) cytoplasmic mantles of pollen grain and pollen tube but normally hidden under the vast quantities of starch filling the last mentioned organs, it proved necessary to work out a new and more effective method for germination of pollen instead of the generally used toilsome, unstable and fungi- and bacteria-friendly sugar solutions. This method is called the "horizontal drop method" and it consists of application of "hanging drops" turned upwards and enclosed in a common, hermetically sealed humidity chamber, whereby each drop is easily made, easily accessible and utilizable for many purposes, f. inst. viability tests.

It has become possible by means of the "horizontal drop method" to produce, relatively easily, the large number of germinated pollen needed for the important current research as well as regards the structure of the starch-compartments and their connections as for further attempts to remove or reduce the masses of starch obscuring the interior of pollen and tube.

To facilitate this research detailed information as regards the applied technique is supplied.

Key words: Picea, Pinus, pollen grains, pollen tubes, starch compartments.

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Note

Richard T. "Dick" Bingham, Principal Plant Geneticist of the Forestry Sciences Laboratory, Moscow, retired January 18 after 31 years of service.

Director Robert W. Harris noted, "Bingham's research program for development of rust resistant pines has served as a model for similar and proposed projects throughout the world. Many American and foreign geneticists have visited the headquarters of the project for consultation on problems of rust resistance breeding, or for training in techniques, experimental designs, and progeny test analysis as developed there."

Mr. Bingham's career in forest disease control through genetic selection began in 1946. After service with the U.S. Marines during World War II, he was assigned to the Office of Blister Rust Control, Bureau of Entomology and Plant Quarantine, Spokane. His research unit was later transferred to the Administrative Branch of the Forest Service and in 1960 the unit became a part of Intermountain Station.

During the 1950's the genetics research of Bingham and his associates had shown that some western white pines were genetically resistant to blister rust; that resistance could be transmitted; and that a breeding program to produce a rust-resistant planting stock was feasible.

Mr. Bingham received the U.S.D.A. Superior Service Award in 1954, "for leadership and exceptional accomplishments in conducting a cooperative project on ... rust-resistant white pines." He has either authored or co-authored 39 professional articles and has presented invited papers both in this country and abroad.

Largely through Bingham's efforts and the cooperation of Dean Wohletz of the College of Forestry, University of Idaho, the Northern Idaho Forest Genetics Center was established and buildings constructed in 1957.

Mr. Bingham was scientific Director of the NATO-IUFRO Advanced Study Institute, Basic Biology and International Aspects of Rust Resistance in Forest Trees, which met in 1969 at the University of Idaho in Moscow. This Institute was a recognition of Bingham's worldwide leadership in research disease control in forest trees by genetic selection.

He received his B.S. and M.S. degrees in forestry from the University of Idaho, where he was elected to Xi Sigma Pi, National Forestry Honorary Society. He is a member of several professional societies, including Society of American Foresters and the Northwest Scientific Association.

Mr. Bingham and his wife Elizabeth will continue to reside at their home at 612 N. Moore in Moscow.

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