sources of variation each year which contributed heavily to among-plot and within-plot errors. The decrease in the plot error correlation indicates that such sources of variation are more or less random. In addition, the much slower decline in heritability experienced by the more carefully handled 1963 cross material indicates that these sources of error can be controlled by experimental procedures. Thus we conclude that selection can be carried out more effectively under controlled rather than common handling methods if genotype X handling interaction does not exist.

The breeding population from which the parent trees were drawn was limited in size and was itself derived from a very limited number of parent trees. Consequently, the parents are probably partly related and could be all half-sibs or even all full-sibs. If so, the additive genetic variance obtained in these analyses probably underestimates the actual parameter for the population (DICKERSON 1942). Within the conditions of the experiments, our heritability estimates are, therefore, probably conservative for the population sampled.

Summary

Diallel crosses with reciprocals were made in a native stand of Pinus strobus L. in Ohio, in two successive years. An analysis was also made of open-pollinated families from the same breeding population. Analyses of tree heights in each of the resulting incomplete sets of families were made at ages 1, 2 and 3, prior to field planting. A general least squares analysis was made for each set of crosses at each age.

Dominance genetic variance was small but reciprocal-maternal effects were moderately large. Heritability of height growth decreased with age. Heritability based on plot means was consistently higher than that based on individual trees.

Results indicated that the decline in heritability could be attributed to an increase in environmental variance rather than an actual reduction in genetic control of growth. Genetic correlation measured in trees from the 1963 crossing remained fairly constant with increasing age.

The careful experimental technique reduced environmental variation as compared to that expected in field tests. If no genotype X handling interaction existed, and if later analysis shows that strong juvenile-mature correlations exist in growth rate, the technique should be useful for juvenile selection of fast-growing families.

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Germination of Douglas-fir Pollen

By RONGHUI HO and OSCAR SZIKLAI[1])

Introduction

ALLEN and SZIKLAI (1962) reported that water suspensions of Pseudotsuga menziesii (Douglas-fir) pollen offered possibilities for obtaining satisfactory seed yields. This opens up the potentiality of stimulating the rate of pollen germination and tube elongation by adding nutrients to the suspensions. However, evidence is scanty on the beneficial effects of nutrients supplied to water suspensions to give high rates of pollination and fertilization of Douglas-fir. Therefore, the types of substances that stimulate pollen germination and elongation of the pollen tube should be studied, in vitro, for the practical value of recovering filled seed for Douglas-fir tree improvement programs.

LARUE (1953) put Douglas-fir pollen into a liquid form of WHITE's solution, but found no germination. ORR-EWING (1956) was the first to determine the viability of Douglas-fir pollen by incubating it on an agar medium. CHING and CHING (1959) introduced 10% sucrose and a series of gibberellic acid concentrations into the agar medium to culture Douglas-fir pollen and obtained a partial development of the male gametophyte to the three-celled stage. The complete development of the male gametophyte of Douglas-fir in vitro has not as yet been reported. BARNER and CHRISTIANSEN (1962) stated, "The actual germination of Douglas-fir pollen in vitro proved impossible." CHRISTIANSEN (1969) reported, "Pollen of Larix and Pseudotsuga cannot be germinated in vitro."

This study utilized a mineral ion solution, a long-term cultivation period, and methods different from those previously reported, and obtained the complete development of the male gametophyte of Douglas-fir.

Materials and Methods

Microsporangiate strobili of Douglas-fir were collected from 4-year-old grafted clones at Caycuse, Vancouver Island on May 2, 1967, and in 1968 from five trees on the University of British Columbia campus. Pollen was extracted from the strobili at room temperature (about 23° C) and stored in a closed vial at 0—2° C in the refrigerator.

Instruments were sterilized either with alcohol, then flamed, or by autoclaving at 15 psi for 20 minutes. In experiments in which boron was studied, all glassware was first soaked for 12 hours in *3N* HCl, rinsed 5 times in double-distilled water and covered.

A modified cotton blue staining technique (COLE, 1958), followed by destaining in distilled water, was used to test for pollen viability. Nuclei were stained by BELLING's iron-acetocarmin method (JOHANSEN, 1940). Identification of the separated cells of the male gametophyte was facilitated by smears and dehydration.

Stock solutions A and B (Table 2) were adopted from BREWBAKER and KWACK (1963) and used for viability tests

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alone and in combination with sucrose and indoleacetic acid (IAA). The test media were adjusted to pH 7 with 1*N* HCl or 1*N* KOH and autoclaved for 20 minutes at 15 psi.

Two or three drops of solution were added to a two-cavity slide, which was then placed in the petri dish on filter paper and kept wet with sterile distilled water. Pollen grains were dusted onto the solution and left uncovered. The cultures were incubated at room temperature.

Over 1,000 pollen grains were observed, and 50 or more randomy selected grains were measured in microns under the microscope for width and length. Pollen was counted as having germinated if the exine was ruptured and the pollen cell had elongated to at least one-half of the pollen length.

Pollen was contaminated with bacteria and fungi, and both the fungicide (Arason) and bactericide (Fortimycin) either damaged or killed the pollen. All efforts to obtain bacteria- and fungi-free pollen failed. Attempts were also made to minimize contamination by sterilizing all instruments and media. It is possible that by-products of microorganisms may influence the pollen elongation and the development of the male gametophyte.

Results and Discussion

I. Pollen morphology and viability test:

Stained Douglas-fir pollen was reported by Wodehouse (1959) to average 140 μ in diameter while Van Campo-Duplan (1950) found fresh pollen to range from 90 to 100μ. Sziklai (1964) reported that the diameter of pollen grains varied considerably among four trees from 91.1 to 99.2 μ.

In this study, pollen grains in liquid were turgid but oval-shaped *(Figs. 1 and 2)* so that two measurements at right angles were necessary. The average dimensions of 240 grains from 8 trees were $94.3 \pm 5.7\ \mu$ in width and $128.9 \pm 16.3\ \mu$ in length.

Dry pollen grains of Douglas-fir appeared cup-shaped, while those in solutions were spherical or elliptical, without a trace of bladders or furrows. The exine was smooth and thin (about 2 μ thick), while the intine had a uniformly hyaline appearance and was about 8 μ thick. The mature pollen grain had two *(Fig. 5)* and occasionally three cells *(Fig. 6)*, in addition to the two prothallial cells.

For tests of viability, pollen was treated with 1% cotton blue in lactophenol, glycerol and water, giving the following four classes of stained grains:

1. Cytoplasm filled the pollen cell *(Fig. 1)*.
2. The exine split open at the distal pole to the pro- bubbles were present *(Fig. 2)*.
3. Pollen cell shrunken with thickened exine and intine *(Fig. 3)*.
4. Pollen cell shrunken with deteriorated cytoplasm *(Fig. 4)*.

For germination tests, pollen grains were incubated in stock solution B for 24 hours. The results of viability and germination tests showed that Class 1 and 2 pollen grains germinated, while Class 3 and 4 did not *(Table 1)*. In all cases, no significant differences (at the 5% level) were found between the viability of Classes 1 and 2 and the germination percentages. The presence of bubbles in the pollen may indicate the beginning of cytoplasmic deterioration. The germination tests are probably the most reliable method of testing pollen, but are time-consuming.

II. The dehiscence of pollen exine:

Following pollen germination, the exine dehisced in one of the following four ways:

1. The exine split in two *(Fig. 11)*.
2. The exine split open at the distal pole to the prothallial cells *(Fig. 7)*.
3. The exine at each end of the pollen grain was cut off and a ribbon-like strip of exine was left around the middle of the germinating pollen grain *(Fig. 8)*.
4. The exine at the distal pole was cut off and (a) the pollen appeared to be squeezed out of the exine *(Fig. 9)*, or (b) the remaining exine split open *(Fig. 10)*.

Approximately two-thirds of the pollen split in two as described in category one, and one-third split in a manner similar to category two; in only a few cases were other types of splitting observed. In the fourth case, germination aborted if the exine did not split open, suggesting that the exine may either be too thick for rupture to occur, or that a genetic lethal may cause abortion. The exine was always cast off by germinating Douglas-fir pollen. This differs from hemlock and pine in which the exine always persists.

III. The complete development of the male gametophyte:

Stock solutions A and B, as well as a combination stock B with IAA and sucrose *(Table 2)*, all stimulated germination and elongation of Douglas-fir pollen and effectively prevented bursting. Of all the solutions, B alone and B plus 10 ppm IAA and 10% sucrose appeared superior to the others after 48 hours of incubation, but the addition of sucrose accelerated contamination by fungi and bacteria.

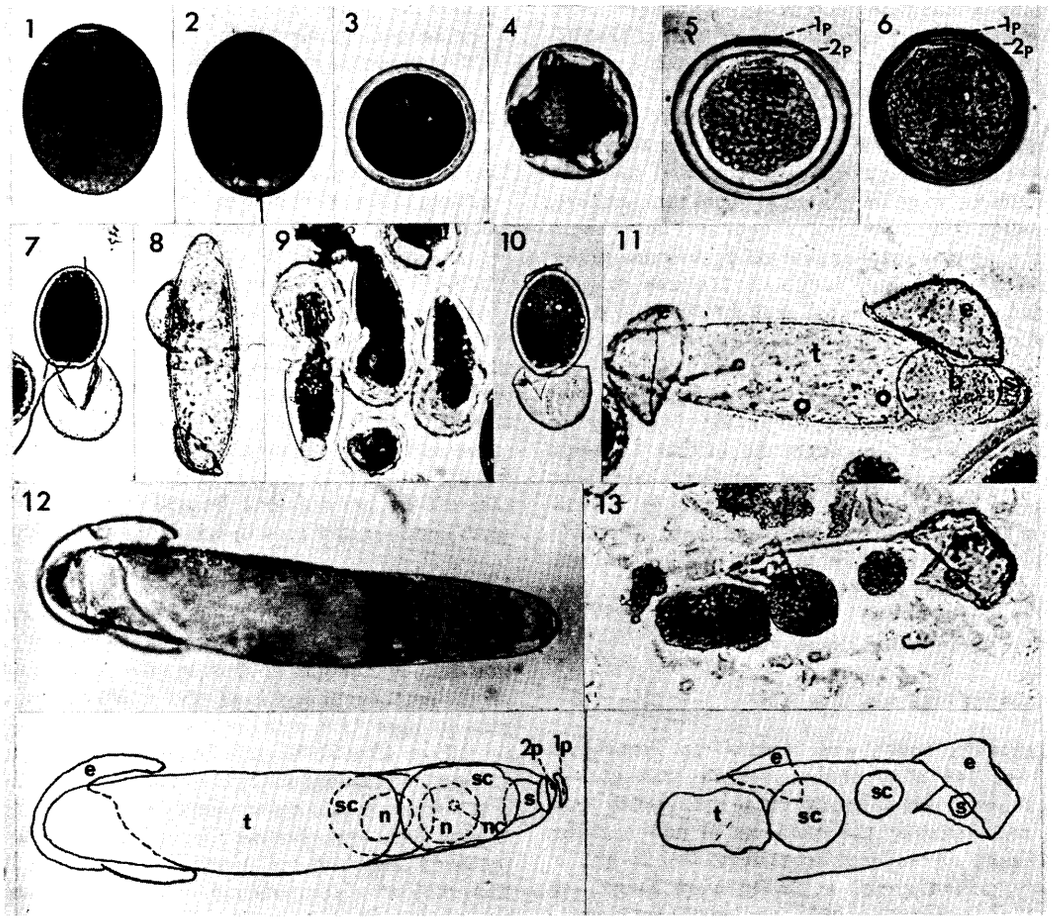
When the pollen grains were incubated in Solution B, or B plus IAA and sucrose, the generative cell divided into the body and stalk cell in two days *(Fig. 11)*. The body cell then divided within another three days to form two sperm cells of unequal size *(Figs. 12 and 13)*. A nucleolus was found in one of the male cells *(Fig. 12)*. After seven days of incubation only elongated pollen was observed, with protrusions always at the pole distal to the prothallial cells *(Fig. 12)*.

Mature pollen of Douglas-fir has one generative cell and one tube cell, in addition to two prothallial cells, as reported by Lawson (1909), Allen (1943), and Barner and Christiansen (1962), and verified by observations made in this study. Allen (1943) reported that in approximately one pollen grain in each one hundred examined, the generative nucleus divided to form the body and stalk nuclei. This is confirmed in this study *(Fig. 6)*. Christiansen (1969) did not observe the generative cell, and suggested that the formation of the two prothallial cells and the two male cells was by division of the embryonal nucleus, which originates from the divisions of the pollen mother cell. However, this study indicated that the embryonal cell does not form two prothallial cells and two male cells, but rather two prothallial cells and an antheridial cell. The latter divides into a generative and a tube cell, as shown by Barner and Christiansen (1962).

In this investigation, the body cell divided into two male cells, one of which included a nucleolus *(Fig. 12)*. The

Table 1. — Viability and germination percentage of Douglas-fir pollen.

	Class	Tree				
		A	B	E	1	2
Viability %	1 & 2	56.5	54.7	58.7	65.0	65.1
	3 & 4	43.4	45.3	41.3	35.0	34.9
Germination %		65.9	60.7	52.5	59.5	67.7



Figs. 1—4: Four classes of Douglas-fir pollen stained with cotton blue. (120×). — *Fig. 1:* Cytoplasm fills the pollen cell. — *Fig. 2:* Cytoplasm almost fills the pollen cell, but some bubbles (arrow) are present. — *Fig. 3:* Shrunken pollen cell with thickened exine and intine. — *Fig. 4:* Shrunken pollen cell and deterioration of cytoplasm. — *Fig. 5:* A mature pollen grain at the two-celled stage with two prothallial cells. (g) generative cell, (1p) first prothallial cell, (2p) second prothallial cell, (t) tube cell. (150×). — *Fig. 6:* A mature pollen grain at the three-celled stage with two prothallial cells. (b) body cell, (1p) first prothallial cell, (2p) second prothallial cell, (s) stalk cell, (tn) tube nucleus. (150×). — *Figs. 7—10:* Breakage of pollen exine (80×). — *Fig. 7:* Exine split open at one end. — *Fig. 8:* Two ends of the exine cut off and a ribbon-ike exine left around the middle of the germinating pollen. — *Fig. 9:* Exine at the distal end cut off and the germinating pollen squeezed out of the exine. — *Fig. 10:* Exine at the distal end cut off and the remaining exine split open. — *Fig. 11:* Germinating pollen at the three-celled stage. (b) body cell, (e) exine, (s)stalk cell, (t) tube cell. (120×). — *Fig. 12:* Germinating pollen at the four celled stage with two prothallial cells (1p, 2p). One sperm cell (sc) overlaps the other sperm cell and the tube cell (t) as shown in the drawing. (n) sperm nucleus, (nc) sperm nucleolus, (s) stalk cell, (t) tube cell. (120×). — *Fig. 13:* Burst pollen and drawing showing (e) exine, (s) stalk cell, (sc) sperm cell, and (t) tube cell. (120×).

formation of spermatozoids, as reported by Christiansen (1969), was not observed in this study. Tulecke (1957) reported that a nucleolus was found in the body nucleus in cultured pollen of *Ginkgo biloba*. Christiansen (1969) suggested that the nucleolus was a "cap of nucleus of spermatozoid". Barner and Christiansen (1962) reported the formation of male cells *in vivo*. Lawson (1909) and Allen (1943) reported that two male nuclei were found inside the archegonium. LaRue (1953) and Tulecke (1957) successfully grew *in vivo, Zamia* and *Ginkgo* pollen, respectively, to the immature sperm cell stage, but not to the formation of spermatozoids.

Only elongated pollen (without ordinary pollen tubes) was observed after seven days of incubation. Christiansen (1969) also concluded that Douglas-fir pollen produces no ordinary pollen tube as is common with most other genera of *Pinaceae*. Ho and Rouse (1970) did report the absence of pollen tube formation during the development of the male gametophyte of *Larix sibirica in vitro*, but Barner and Christiansen (1962) and Christiansen (1969) reported that elongated pollen grains, germinated *in vivo*, had short pollen tubes. Allen (1943) and Lawson (1909) reported the same. This may support the observation of previous studies *in vivo* that Douglas-fir does not grow a pollen tube before the pollen reaches the nucellus of the ovule.

Summary

Douglas-fir pollen viability was assessed by both germination and staining techniques.

Breakage of the exine occurred in one of four ways, but predominantly the exine split into two halves in two-thirds of the pollen while in about one-third the exine split wide open.

The development of the male gametophyte in Douglas-fir pollen was complete after five days in a solution con-

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.

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

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Introduction

Christiansen (1), as a result of several years research showed, inter alia, that the transfer of the male gametes 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 (1 a) 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 "germination", 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 conifers, 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*.