Germination of Douglas-fir Pollen

By RONGHUI HO and OSCAR SZIKLAI

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.

Domiance 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.

Literature Cited


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 Whitten’s solution, but found no germination. Obre-Ewing (1956) was the first to determine the viability of Douglas-fir pollen by incubating it on an agar medium. Ching and Cheng (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. Barrer and

1) Graduate student and Professor, respectively. Faculty of Forestry, The University of British Columbia, Vancouver, British Columbia, Canada. The research was supported by Grant NRC 87-0595, and Forest Genetics Scholarship by British Columbia Forest Products Limited.
alone and in combination with sucrose and indoleacetic acid (IAA). The test media were adjusted to pH 7 with 1N HCl or 1N 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 randomly 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 (Arazone) 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 hy-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 Woonhouse (1959) to average 140 μ in diameter while Van Camp-Duplan (1950) found fresh pollen to range from 90 to 100 μ. Sekla (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 ± 5.7 μ in width and 128.9 ± 16.3 μ 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 slightly 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 pro-thallial 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 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 (1945) 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).

| Table 1. — Viability and germination percentage of Douglas-fir pollen. |
|------------------|---|---|---|---|---|
| Class | A | B | E | 1 & 2 |
| Viability % | 56.5 | 54.7 | 58.7 | 65.0 | 65.1 |
| Germination % | 65.9 | 60.7 | 52.5 | 59.3 | 67.7 |
formation of spermatozooids, as reported by Christiansen (1969), was not observed in this study. Tulcke (1957) reported that a nucelus was found in the body nucleus in cultured pollen of Ginkgo biloba. Christiansen (1969) suggested that the nucleolus was a "cap of nucleus of spermatozooid". Banner 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 Tulcke (1957) successfully grew in vivo, Zamia and Ginkgo pollen, respectively, to the immature sperm cell stage, but not to the formation of spermatozooids.

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 Roux (1970) did report the absence of pollen tube formation during the development of the male gametophyte of Larix sibirica in vitro, but Banner 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 nucelus 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).

<table>
<thead>
<tr>
<th>Solution</th>
<th>24 Hours (%)</th>
<th>48 Hours (%)</th>
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<tbody>
<tr>
<td></td>
<td>Length (μ)</td>
<td>Length (μ)</td>
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<tr>
<td>Stock</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Solution A)</td>
<td>184 ± 28</td>
<td>211 ± 25</td>
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<tr>
<td>Stock</td>
<td>54</td>
<td>69</td>
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<tr>
<td>Solution B)</td>
<td>189 ± 28</td>
<td>219 ± 24</td>
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<td>5% sucrose</td>
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<td>10 ppm IAA</td>
<td>175 ± 26</td>
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<td>Stock B</td>
<td>65</td>
<td>68</td>
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<tr>
<td>8% sucrose</td>
<td>186 ± 23</td>
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<tr>
<td>10 ppm IAA</td>
<td>63</td>
<td>71</td>
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<tr>
<td>Stock B</td>
<td>169 ± 18</td>
<td>218 ± 23</td>
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<tr>
<td>10% sucrose</td>
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<td>71</td>
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<td>15% sucrose</td>
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<tr>
<td>Stock B</td>
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<td>20% sucrose</td>
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<tr>
<td>10 ppm IAA</td>
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<td>41</td>
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<tr>
<td>Stock B</td>
<td>168 ± 15</td>
<td>189 ± 20</td>
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<tr>
<td>Distilled water</td>
<td>1 ml</td>
<td>9 ml</td>
</tr>
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1) Stock Solution A: H₂H₃O₃ 0.1 g
Ca(NO₃)₂ · 4H₂O 0.5 g
MgSO₄ · 7H₂O 0.3 g
KNO₃ 0.1 g
in 100 ml distilled water.

2) Stock Solution B: Stock Solution A 1 ml
distilled water 9 ml

Table 2 shows the percent germination and average length of elongated pollen grains (± one standard deviation) for different solutions. The solutions were tested for their effect on germination, with the highest germination seen in Stock Solution A at 90% after 48 hours, and the lowest in Stock B at 69%. The average length of the pollen grains also varied significantly, with Stock Solution A showing the longest average length of 211 ± 25 μm.

### Literature Cited


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

By H. Christiansen

**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.