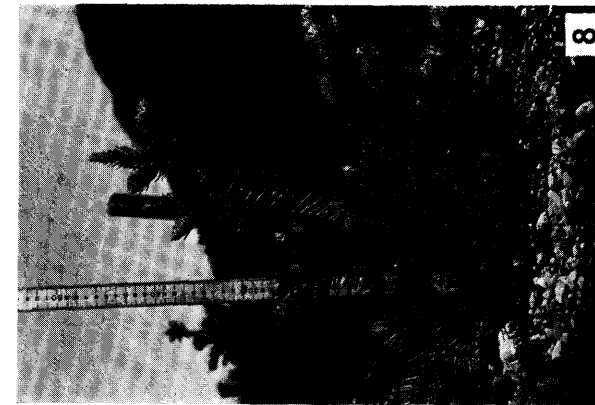
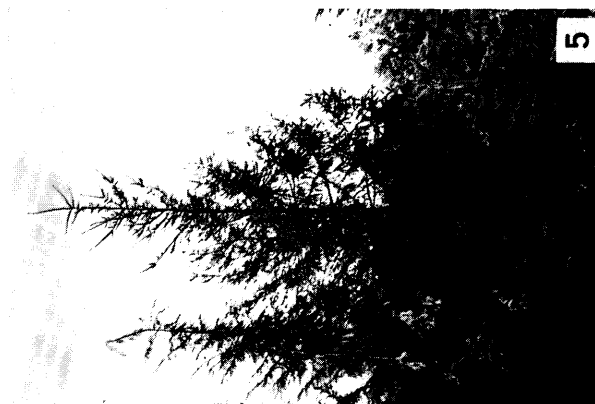


Figures 1—4. — (1) $S_{0,2}$, self-pollinated 1952. (2) $S_{1,2,2,1}$, self-pollinated 1966. (3) $S_{3,2,2,1,2,3}$, self-pollinated 1975. (4) S_3 inbreds, 1976.



Figures 5—8. — (1) $S_{0,1,1}$, self-pollinated 1954. (2) $S_{1,1,1,3,2}$, (centre tree) self-pollinated 1962. (3) $S_{3,1,1,3,2,6}$, self-pollinated 1971. (4) S_3 inbreds 1976.

It is evident from *Table 1* that S_0 11 was more self-fertile than S_0 2 with considerably more germinants being produced per cone. The two S_1 inbreds, 2.21 and 11.32 used in this study were the more vigorous trees in their respective selfed families and among the first to produce sufficient male and female strobili for self-pollinations to be possible in 1962, their heights at the time being 5.05 and 2.67 metres respectively. Only three of the S_2 progeny from 2.21 have survived, however, and two of these are dwarfs which have never produced any strobili. Subsequent pollinations to the S_3 generation were, therefore, delayed. Both trees have grown considerably since 1962, the 1976 heights being the same at 14.63 metres with diameters at 1.4 metres of 38.3 and 26.0 centimetres respectively. They have each been self-pollinated in different years and as is evident from *Table 1*, there was a striking variation from year to year in both the number of seeds and of germinants produced per cone. The causes for this are not completely known but cone and seed insects can drastically reduce the number of seed in some years as their attacks are cyclic. Again, the number of available male strobili and the weather at the time of pollination can greatly influence the number of germinants per cone. In 1966 and 1968, for example, there were large numbers of both male and female strobili on S_1 2.21. As many as possible of the former were included in the same pollination bags as the female strobili in both years so that no artificial pollination would be necessary, an added precaution against possible contamination. In 1966, the weather remained dry throughout the pollination period and the results were most satisfactory. In 1968, however, it was wet at the time of pollination, consequently there was inadequate pollination within the moist bags with the result that the number of germinants per cone was greatly reduced.

It was clear from the 1962 and subsequent pollinations that the S_2 inbreds from S_1 11.32 were not only the more vigorous but exhibited much less dwarfism than those from S_1 2.21. The dwarf S_2 inbreds from the latter were particularly striking and have been previously described and

illustrated. (ORR-EWING 1974). After the 1968 pollinations, X-ray photographs of the cleaned seed were taken to see whether continued inbreeding had had any effect on the size of the embryo. The results in *Table 2* showed that only a very low percentage of filled seeds had abnormal embryos that were half the normal size. More than 90 per cent of the filled S_2 seeds germinated.

These seeds were later sown, each one being identified so that they could be used for the subsequent data given in *Table 3* in which the seedlings have been classified by cotyledon number. It is obvious that the S_2 seedlings were extremely variable, the number of cotyledons, for example, appearing completely independent of the subsequent development of the seedling. Special attention was given to the familiar dwarfs from S_1 2.21 but it was found that their cotyledon numbers ranged from 6 to 9 and hypocotyl heights from 12 to 29 millimetres. The early vigour of the S_2 seedlings from S_1 11.32 is apparent from their heights at one year.

The more vigorous S_2 inbreds from S_1 11.32 produced male and female strobili several years earlier than those from S_1 2.21, the first pollinations to the S_3 generation being made in 1971 on S_2 11.32.6 shown in *Figure 7*. It is a vigorous tree with a height of 7.85 metres in 1976. It was evident that continued inbreeding has had no effect on either the size of the cone or of the number of seeds per cone. The number of germinants per cone, however, was extremely low but this could be partly due to shortage of pollen as the tree was producing many more female than male strobili, a feature common to young Douglas fir. The cleaned seed from both the 1971 and 1973 pollinations was photographed by X-ray and the results given in *Table 4* showed that there were very few abnormal embryos and that all the seed germinated.

The four S_3 germinants from the 1971 pollinations with cotyledon numbers varying from 5 to 8, have all survived and one is shown in *Figure 8* at 5 years of age. The seedlings are by no means lacking in vigour but are variable in both form and height, the latter ranging from 28 to 47

Table 2. — Results of X-ray photographs of S_2 seed.

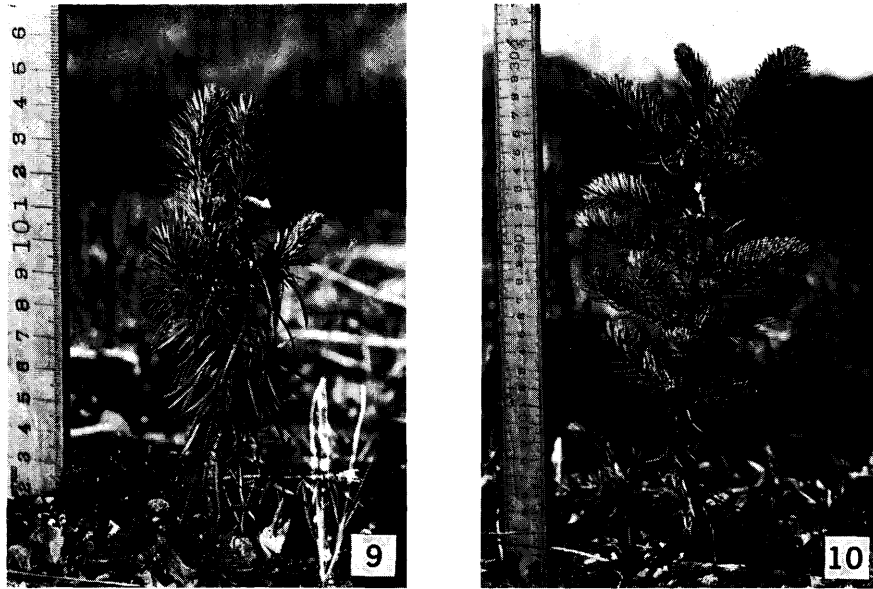
S_1 inbred	Pollination year	No. of filled seeds	Normal embryo	Abnormal embryo	Damaged seed	No. of germinants
2.21	1968	53	49	1	3	46
11.32	1968	97	89	5	3	88

Table 3. — Variability in the S_2 seedlings from the 1968 pollinations.

S_1 inbred	No. S_2 seedlings represented	No. of cotyledons	Hypocotyl ht. in mm.			Total ht. at 1 year in mm.		
			min.	max.	mean	min.	max.	mean
2.21	6	6	18	27	23.2	25	113	73.2
2.21	19	7	12	29	21.5	25	111	66.3
2.21	12	8	15	30	23.2	26	107	53.4
2.21	6	9	22	31	26.7	51	122	81.8
2.21	1	10			26.0			102.0
11.32	3	6	22	24	22.0	115	150	131.7
11.32	40	7	15	30	21.6	67	165	133.2
11.32	34	8	16	27	22.3	99	190	136.3
11.32	10	9	19	27	22.5	72	155	128.4

Table 4. — Results of X-ray photographs of S_3 seed.

S_2 inbred	Pollination year	No. of filled seeds	Normal embryos	Abnormal embryos	Damaged seed	No. of germinants
11.32.6	1971	4	3	1	—	4
11.32.6	1973	18	17	1	—	18



Figures 9—10. — Variation in size and form of S_3 inbreds from S_2 11.32.6, self-pollinated 1973.

centimetres in 1976. Eleven of the 18 germinants from the 1973 pollinations on the same S_2 inbred have since been planted and are also variable in form and size with heights ranging from 14 to 35 centimetres in 1976. Two of these seedlings photographed that same year are shown in Figures 9 and 10. It was not until 1975 that any pollinations could be made on the other inbred line and the results of these on S_2 .2.21.23 are given in Table 1. Inbreeding again had no effect on either the size of cone or the number of seeds per cone but the number of germinants was also extremely low, the two surviving seedlings are shown in Figure 4. The S_2 parent shown in Figure 3 was 3.70 metres tall in 1976 and has yet to produce an adequate number of male strobili so that some subsequent improvement in the yield of viable seed can be anticipated from later pollinations.

Discussion

In the author's opinion, there are two main objectives for an inbreeding program with Douglas fir, the first should

be to produce as many inbred lines as possible from selected trees and to inbreed them to successive generations. A great deal of information would be obtained and uniform inbred lines would be of immense value for many lines of research. This would be no easy task and it is, therefore, essential that such a program be located in an environment where regular cone and pollen production can be anticipated. Under the unfavourable climatic conditions at the Cowichan Lake Experiment Station in Southern Vancouver Island, it has already taken from 17 to 23 years to produce a few S_3 seedlings. It, moreover, will take at least eight to ten years longer if clones from the selected trees have to be used as the S_0 generation since the first pollinations will be inevitably delayed. The time element is most important as from the limited evidence available, several more generations of inbreeding beyond the S_3 will be required if uniform lines are to be produced. It is also evident that even under the most favourable conditions more than one generation of tree breeder will be needed



Figures 11—12. — (11) S_1 single crosses at 14 years. (12) S_2 single crosses at 6 years.

so it is essential that the sparse number of inbred lines already established in the Douglas fir region be carefully maintained and their identities ensured.

The second objective should be further studies into the possibilities of single crossing where different inbred lines are crossed to produce improved strains. The first single crosses with Douglas fir were made in 1962 (ORR-EWING 1965) but they were only on a small scale without adequate controls as at that time, it was not possible to develop a single crossing and a racial crossing program simultaneously. This early study, however, is of interest as some of the best single crosses have shown considerably vigour. The two fourteen year old trees in the foreground in *Figure 11*, for example, are the result of a cross between S_1 .2.12 and S_1 .11.40. Their heights when photographed in 1976 were 8.66 and 8.84 metres with diameters at 1.4 metres of 15 and 15.1 centimetres respectively. Subsequent single crosses between S_1 lines have also shown that yields as high as 60.4 germinants per cone can be obtained. *Figure 12* shows a six year tree from a second generation cross between S_2 .2.26.4 and S_2 .11.32.6. This cross, however, took 18 years to make and unless some techniques can be developed to drastically reduce the time element between generations, single crossing would have to be confined to the S_1 generation. The decision as to whether an extensive single crossing program would ever be implemented will ultimately depend on the outcome of the large tree improvement programs now being initiated in the Douglas fir region. In the meantime, however, it is important that more inbred lines be established so that they can be available if required.

Summary

Two Douglas fir were inbred to the third generation over a period of from 17 to 23 years. There was considerable variation in both the number of seed and germinants per cone in different pollination years of the same two S_1 inbreds. No reduction in either size or number of seeds per cone was found in the two S_1 inbreds and X-ray photographs of the seed of both the S_1 and S_2 generations showed very few seeds with abnormal embryos. Inbreeding to the third generation resulted in few viable seeds being produced but this could have been partly due to shortage of pollen. The S_3 seedlings were variable in both size and form but vigorous. The objectives and the importance of further inbreeding and single crossing are discussed.

Key words: Racial crossing, inbreeding, S_0 , S_1 , S_2 , S_3 generation self-pollination, single crossing.

Zusammenfassung

Im Verlauf von 17 bis 23 Jahren konnten zwei Douglasien von der S_0 bis zur S_3 mit jeweils eigenem Pollen bestäubt werden. Bei den S_1 -Selbstungen wurden im Vergleich der Jahre lediglich Unterschiede in der Samenzahl und in der Anzahl der keimfähigen Samen pro Zapfen gefunden. Die Samen zeigten im Röntgentest eine geringe Anzahl abnormer Embryos, ebenso diese aus den S_2 -Selbstungen. Aus den S_3 -Selbstungen ging eine geringe Anzahl Samen und Pflanzen hervor, was aber auch andere Gründe gehabt haben könnte (Pollenangebot). Die S_3 -Pflanzen waren kräftig, zeigten jedoch größere Unterschiede in der Pflanzenhöhe und in der Wuchsform.

Literature Cited

ORR-EWING, A. L.: Inbreeding and single crossing in Douglas fir. *For. Sci.* 11: 279–290 (1965). — ORR-EWING, A. L.: The incidence of dwarfing in Douglas fir. *B.C. For. Serv. Res. Note* 64, 1–25 (1974).

Microsporogenesis and macrosporogenesis in *Pseudolarix amabilis*

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Introduction

Golden larch, *Pseudolarix amabilis* (NELS.) REHD., occurs as a monotypic species and its natural range is restricted to a limited area in the mountainous region of Eastern China. This species has been introduced successfully as an ornamental tree in various parts of the United States. It is a member of the Pinaceae, and is classified in REHDER'S Manual (1954) under the sub-family *Abietineae*.

Several studies have been concerned with the relationship of *Pseudolarix* to the other members of the *Pinaceae*. Gametophyte development, embryology, and some anatomical characteristics suggest that this genus occupies a relatively high evolutionary position within the *Abietineae*. Bud periodicity and zonation in the shoot apex also resemble that of most genera within the *Pinaceae*. However, *Pseudolarix*, with a chromosome complement of $n = 22$, and $2n = 44$, is a deviation from the basic number of $n = 12$ that is common in the *Pinaceae* (MERGEN, 1961).

This morphological, anatomical and cytological study of the chronological development of microsporogenesis and macrosporogenesis might aid future workers in elucidating the evolutionary history of this monotypic species.

Literature Review

MIYAKE and YASUI (1911) stated that the structure and development of the gametophytes and the embryology of *Pseudolarix* are similar to those found in *Abietineae*. Later studies also indicated that the early embryogeny of *Pseudolarix amabilis* was not unlike that of *Pinus*, except that the rosette tier does not form embryos and perishes undivided (JOHANSEN, 1950). This condition constitutes a unique and extreme condition of cleavage polyembryony. The lowest tier of four embryonal initials which becomes detached in *Pinus* to form separate embryos, remains united in *Pseudolarix* and becomes combined to produce a single embryo.

PERCE (1934) studied a number of anatomical characters