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 examgle, 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 crass, 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.

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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 embrylogy 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.

PEIRCE (1934) studied a number of anatomical characters

which reveal that *Pseudolarix* occupies a high position within the Abietineae. In contrast to the other genera in this sub-family, *Pseudolarix* is the only genus without ray tracheids which is similar to members of Taxodineae where they are rarely found. KUPILA and GIFFORD (1963) noted that *Pseudolarix* exhibits the same type of bud periodicity and zonation within the shoot apex that characterizes many members of the *Pinaceae*. Another similarity is the occurrence of periclinal divisions found in the surface layer at the summit of the shoot apex.

Chromosome studies revealed a complement of n = 22, and 2n = 44 in *Pseudolarix amabilis* (SAX and SAX, 1933; MERGEN, 1961). These studies showed 22 chromosomes made up of 20 I shaped and 2 V shaped chromosomes, indicating that this condition arose from the breakage of 10 V shaped chromosomes at the centromere from an original complement of n = 12. This unusual karyotype is a deviant from the basic number of n = 12 that occurs in most species of the *Pinaceae*. One other exception is *Pseudotsuga menziesii* which has thirteen chromosomes (SAX and SAX, 1933).

Materials and Methods

To study floral phenology in *Pseudolarix*, branches were collected from a mature tree growing in the Yale University Nursery in Hamden, Connecticut. Collections were started in September and were made at two to three-week intervals until the following May. Branches that had borne male and female flowers the preceeding year were selected. They were placed in water and kept either in a greenhouse or in a controlled environment room, which had a 16-hour photoperiod and a temperature which alternated between 27° C (16 hours light) and 16° C (8 hours dark). A total of 180 buds were fixed in FAA, dehydrated and embedded in paraffin. Serial microtome sections, 8 μ thick, were stained with safranin and fast green. Observations on these buds showed no morphological or anatomical characteristics of floral primordia, even though the tree had produced a large number of microsporangiate and macrosporangiate strobili the previous year. There were also no "flowers" on the tree *in situ*.

Branch collection was resumed during the latter part of September at which time floral buds were discernible. The branches and the buds were treated and processed using the same schedule as during the previous collections. The sections from the female buds were stained with safranin and fast green, and those from the male buds were stained with haematoxylin and safranin. Because the buds collected in September were still in the process of differentiation, a second collection was made during the first week of November. The last collection was made during the first week of February at which time the buds started active normal growth after being placed in the controlled environment room. Buds from this collection were used to follow the phenology and the sequences of micro- and macrosporo-

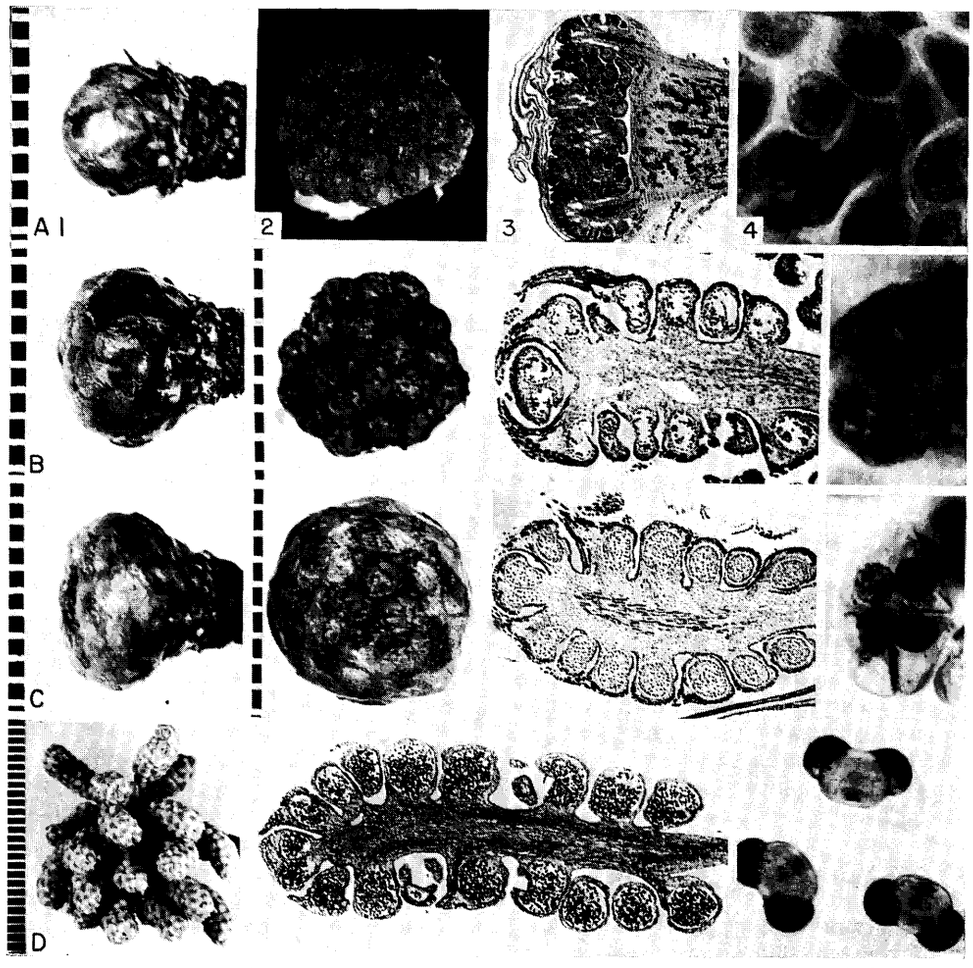

Figure 1. — Phenology of staminate strobilus: 1 morphology; 2 scales removed; 3 anatomical; 4 cytological. Collected September 29: A 1 scale in mm, A 2 0,5×, A 3 12×, A 4 390×, (archesporial); collected April 18: B 1 scale in mm, B 2 scale in mm, B 3 23,5×, B 4 360× (prophase I); collected April 27: C 1 scale in mm, C 2 scale in mm, C 3 15×, C 4 405× (tetrad); collected May 23: D 1 scale in mm, D 2 6×, D 3 145× (mature pollen).

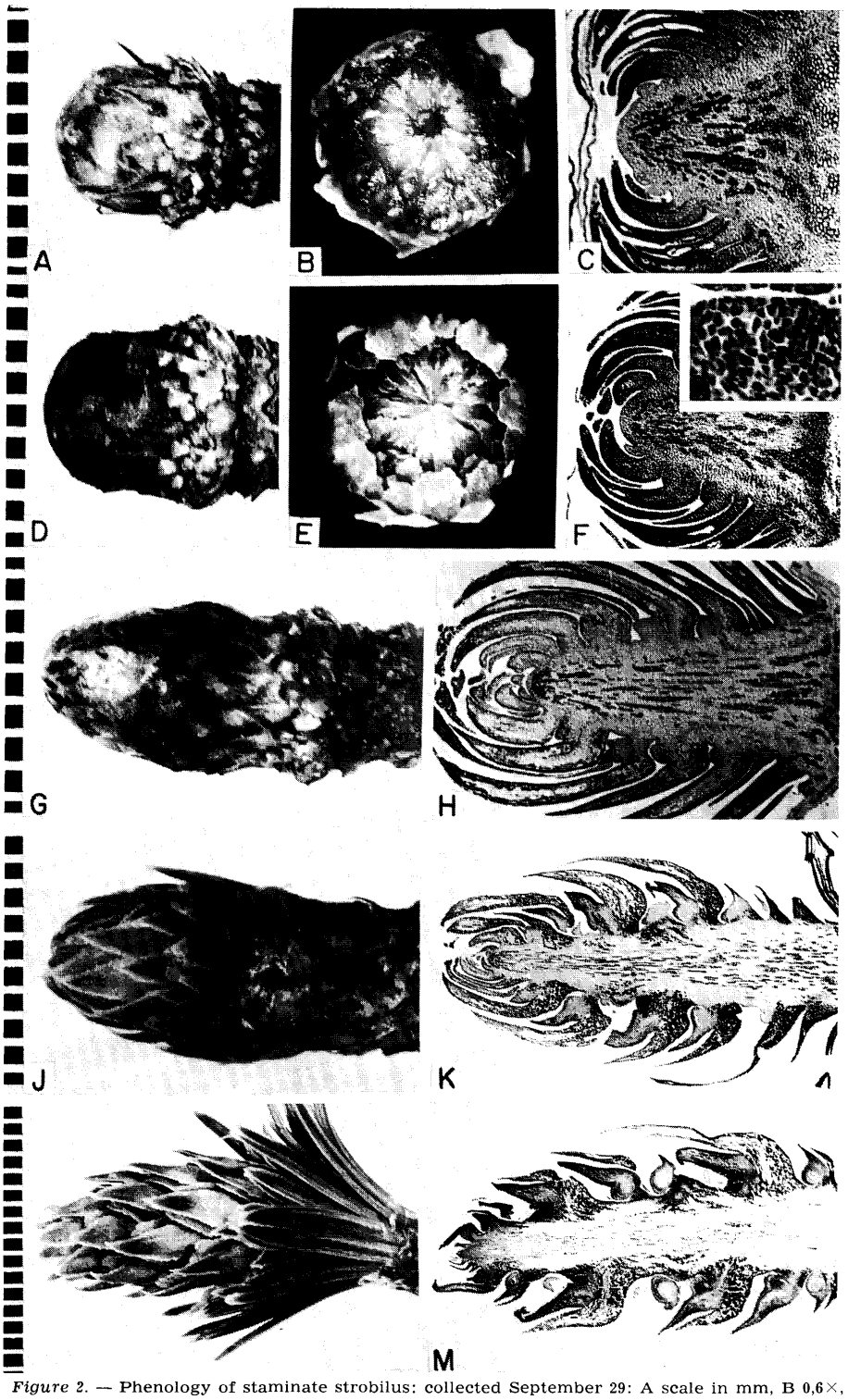

Figure 2. — Phenology of staminate strobilus: collected September 29: A scale in mm, B 0,6×, C 14,4×; collected November 30: D scale in mm, E 0,6×; collected November 3: F 13,2×, F (insert) 72×; collected April 18: G scale in mm, H 9×, J scale in mm, K 6×, L scale in mm, M 4,2×.

genesis. Observations were made in the field to determine the time of meiosis and pollination for this species in this location.

For the cytological observations on the developing microspores, microsporophylls from fresh field collections were fixed in 3 : 1 acetic acic-alcohol and stained with aceto-carmine.

Results

Phenology of staminate strobilus. Because no floral buds were encountered in the first year's collection, it can be assumed that the bud primordia are laid down sometime between the end of May and the end of September in the year preceeding flowering. Male buds are found in the middle and upper portion of the crown and counts on 50 buds showed that the short shoots bearing these buds were from 6—11 years old. The average number of strobili per 100 buds was 22.4 with a standard deviation of ±1.7.

Male buds could be identified by the end of September *(Fig. 1, A1)*, and when the external scales were removed the developing strobilate primordia were in evidence *(A2)*.

Figure 1, A3 is a longitudinal section through a strobilus showing primitive archesporial cells within the microsporangia *(A4)*. There were mitotic divisions in the sporogenous tissue.

During the later part of November the buds had enlarged slightly and there was a further development of the microsporangia. Mitotic divisions, although still present were less frequent than in previous collections. The developing cells were still in the primitive archesporial stage.

Throughout the winter there was no change until March when the sporogenous tissue was in the MMC stage. In April the strobili elongated rapidly *(B1, B2, B3)*, with meiosis occurring during the middle of April. *Figure 1, B4* illustrates cells in prophase I of meiosis; the nuclei having a reticulated appearance.

At this time the strobili ruptured the protective scales *(C1, C2)*, and the microsporangia in the longitudinal section *(C3)* reveal the developing pollen in the dyad or tetrad stages. *C4* illustrates a tetrad just prior to the time when the young microspores break away. By the end of April, young microspores with wings were present. Until the later part of May at which time pollen started to dehisce freely, the strobili continued to enlarge *(D1, D2)*. The divisions occurring within the microspore resulted in two prothallial cells, the tube cell, the body cell, and the stalk cell or the fully formed pollen grains, which are shown in *D3*.

Phenology of pistillate strobilus. Generally the female buds were located in the upper part of the crown, and they were externally distinguishable from the male buds by their slightly larger size and larger diameter at the base. Observation of 50 buds from the collection revealed that the spur shoots bearing the female bud were from 2—5 years old, but in previous years, cones had been produced on this tree on shoots up to 11 years old.

The megasporangiate bud was recognizable by the end of September *(Fig. 2A)*. When the scales were dissected

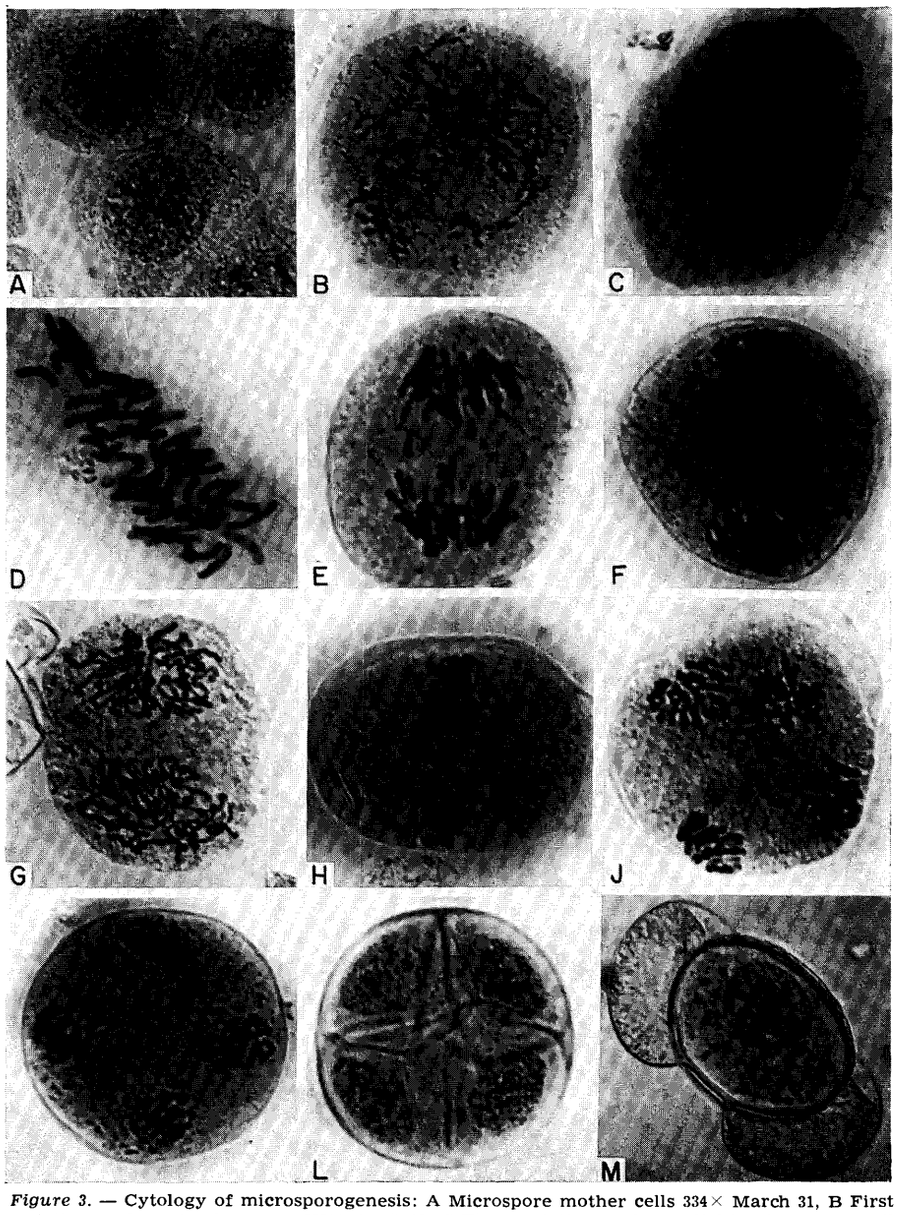

Figure 3. — Cytology of microsporogenesis: A Microspore mother cells 334× March 31, B First meiotic prophase (pachynema) 355× April 19, C First meiotic prophase (diakinesis) 472× April 19, D Metaphase I 631× April 19, E Anaphase I 477× April 19, F Telophase I 546× April 19, G Interphase of Prophase II 450× April 19, H Metaphase II 429× April 19, J Anaphase II 477× April 19, K Telophase II 477× April 19, L Tetrad 475× April 19, M Yung microspore with wings 429× May 11.

away, an enlarged floral apex, which was not yet overarched by the bract scales, was visible *(Fig. 2B)*. The longitudinal section *(Fig. 2C)* shows the developing bract scales which were initiated first at the base of the cone and then acropetally thereafter. At this time there was no evidence of ovular tissue and mitotic figures in the meristem and bract primordia indicated that differentiation was still in progress.

At the beginning of November *(Fig. 2D)* the bract scales have overarched the floral meristem *(Fig. 2E)*, and the axis of the cone has elongated *(Fig. 2F)*. Cellular organization has occurred in the upper and inward part of the bract scales as evidency by a rounded protrusion in this area (F insert) which exhibited many mitotic figures.

There were both anticlinal and periclinal divisions in the meristem and in the ovular area. Procambial cells were observed in the midrib area and strands of this vascular tissue appeared in the basal bract scales. Resin ducts with epithelial cells were also observed at the tips of the bracts, and the epidermal cells of the bract had begun to cutinize.

By the middle of April when the spring collection was made *(Fig. 2G)* the tip of the bud has emerged through the scales. The cone has approximately doubled in length and more bracts were initiated. The ovuliferous scale primordia have also enlarged, but distinct organization within the ovule is not yet evident *(Fig. 2H)*.

Figure 2J shows a bud collected during the first week of May. The scales have been shed and the bracts are apparent above the needle which have formed at the base of the bud. The ovuliferous scales have elongated but are not visible externally, and the ovule has enlarged in size *(Fig. 2K)*.

At the time of pollen shedding *(Fig. 2L)* the sides of the ovuliferous scales extent beyond the bract and can be observed externally. The ovule has assumed a distinct cellular organization where the megaspore mother cell is discernible in the center of differentiated spongy tissue *(Fig. 2M)*.

Cytology of microsporogenesis. During September the sporogenous tissue within the male buds is composed of angular primitive archesporial cells, which are shown in *Fig. 1, A4*. Between September and March, the sporogenous tissue differentiates to form the microspore mother cells, which are present during the latter part of March *(Fig. 3 A)*. Meiosis occurres during the middle of April, and the stages represented in *Fig. 3, B* through L were all from the collection of strobili made on April 19. *Figures 3B* and *3C* show nuclei in the first meiotic prophase. The chromosomes have shortened and have become thicker (pachynema) as can be seen in *B;* and in late diakinesis (C) the bivalents have contractred and chiasmata are visible for the bivalents which were evenly distributed throughout the nucleus.

At metaphase the chromosomes were distinct, and it was possible to discern most of the 44 chromosomes found in *Pseudolarix (D)*. Anaphase and telophase of the first meiotic division are shown in *Fig. 3, E* and F. Cytokinesis after the first division was not observed; the nuclei enters an interphase stage in which the chromosomal material was dispersed throughout the nucleus *(G)*. *Figure 3, H, J,* and *K,* represent metaphase II, anaphase II, and telophase II. The tetrad shown in *L* has walls formed between the four microspores, which will eventually break away and develop wings. The young microspore with wings, picture in *M,* was from a collection made on May 11. Visible in *Figure 4A* is the prothallial cell resulting from the first vegetative division and also a metaphase of the second division, which will produce the second prothallial cell and the antheridial initial *(B)*. This pollen was collected May 18. *Figure 4C* shows the mature pollen grain at time of shedding, which occurred about the third week of May. The two prothallial cells are still visible, and the nuclei of the tube cell, body cell, and stalk cell are seen as darkly stained areas in the pollen grain. Fresh pollen was germinated in distilled water in hanging drop preparations made in Van Tiegham cells. An average germination capacity of 79.5% was observed and *Figure 3D* illustrates the pollen tube growth.

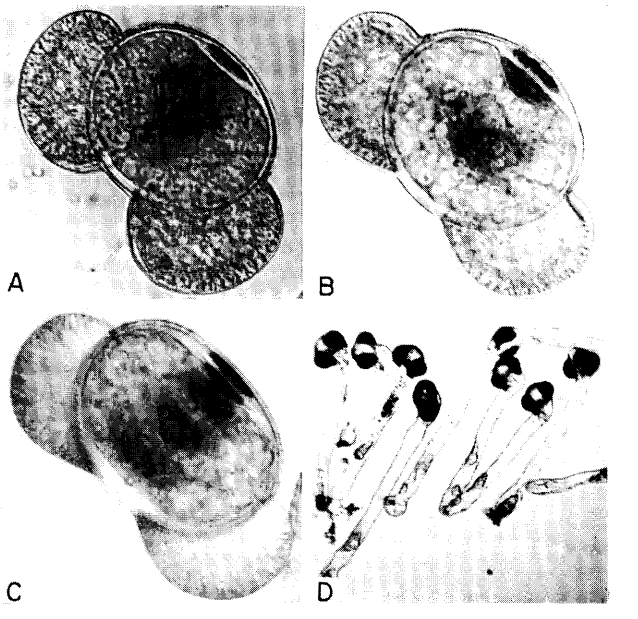

Figure 4. — Pollen formation and germination: A Pollen with first prothallial cell and metaphase of second division 384× May 18, B Second prothallial cell and antheridial initial 384× May 18, C Mature pollen grain 437× May 23, D Pollen germinating in distilled water 187× May 31.

Summary

A pictorial presentation of the phenology of the microsporangiate strobilus, pollen development as well as the phenology of the macrosporangiate strobilus of *Pseudolarix amabilis* has been given. For this species, in the Connecticut area, the flower bud primidia are laid down in the summer preceeding flowering. Both male and female buds can be recognized externally by September and the male buds overwinter in the primitive archesporial stage. Meiosis occurs during the middle of April, and the pollen is shed in the latter part of May. The cytology of microsporogenesis is illustrated with photomicrographs.

In the fall, the female cone shows differentiation of the ovuliferous scales, but organization within the ovule is not evident until November. Throughout the winter the bud is inactive until April, when it starts to elongate. More bracts and ovuliferous scales are initiated in the spring. In May, at time of pollination, the megaspore mother cell is in the center of differentiated spongy tissue. Although the strobili were followed in detail on one tree, sporadic observations over a period of several years indicate that this tree is representative for this area.

Key words: Pseudolarix amabilis, microsporogenesis, macrosporogenesis.

Zusammenfassung

Bei *Pseudolarix amabilis* (NELS.) REHD. wurde die Entwicklung der weiblichen und männlichen Blütenanlagen bis zum Zeitpunkt der Blüte untersucht. Im Raum Connecticut waren sowohl die weiblichen als auch die männlichen Blütenanlagen bereits im September des der Blüte vorausgehenden Jahres zu erkennen. Die Meiose fand dann etwa Mitte April statt. Der Pollen wurde etwa in der zweiten Maihälfte entlassen. Die weiblichen Blütenanlagen zeigten bis zum November nur eine geringe Entwicklung und blieben danach bis zum April inaktiv. Sie waren dann wenige Wochen später voll entwickelt.

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Interspecific Hybridization in Pines with the special Reference to Pinus rigida X taeda[1])

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Introduction

In order to improve pitch pine (*Pinus rigida* MILL.) which has long been extensively used for reforestation in Korea by combining the cold hardiness of pitch pine and the rapid growth rate and better timber quality of loblolly pine (*P. taeda* L.), hybridization program has been carried out using pitch pine as seed parent and loblolly pine as pollen parent for nearly twenty years since 1954.

F_1-hybrid seeds were mass produced by large scale controlled pollination, test plantations of pitch-loblolly hybrid pine were then laid out with pitch pine check, and from these test plantations the F_1 wind pollinated seeds were produced in a commercial scale. And through controlled pollinations, F_2-hybrid seeds and *P. rigida* × F_1 back cross hybrid seeds were also produced.

The growth performances of these hybrids have been examined at different geographic localities. From the results obtained so far, the strategy for increasing the superiority of × *Pinus rigitaeda* hybrid in the advanced generations and the means of mass production of hybrid seeds were discussed.

Selection of The Parent

As it has been clearly demonstrated that the hybrid performance of × *Pinus rigitaeda* differ distinctly due to the seed source of the parent, it is essential to select the best seed source of the parent in the pitch-loblolly hybridization program. On an average, the pollen parent of the best seed source (New Jersey) gave 24.4 percent more growth than the pollen parent of the poorest seed source (Florida) in the hight growth of the F_1-hybrid mainly due to the better cold hardiness of the New Jersey source *(Fig. 1)*.

And, as it is also proven that the combining ability of parental species differ due to the individuals of the parent even within the same seed source, it is also needed to select the best parent individuals giving the highest combining ability within the selected seed source at the outset of utilizing desirable F_1-hybrid.

In the mass production program of pitch-loblolly hybrid pine, young commercial pitch pine plantations, 8 to 10 years in age, were used as maternal stands flowered when small

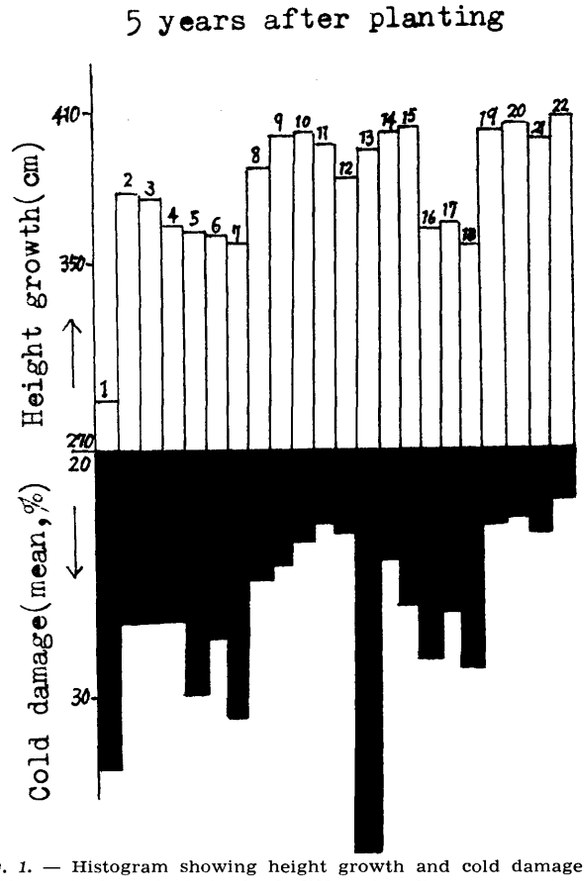

Fig. 1. — Histogram showing height growth and cold damage of × *Pinus rigida.taeda* hybrid of different pollen sources in the field at five years after planting. 1—3: Fla., 4—7: Tex., 8—9: Miss., 10—15: N.C., 16—18: Arkansas, 19: Va., 20—22: N.J.

[1]) A dedication article to the anniversary issue of Silvae Genetica in honour of professor LANGNER.