Embryo and seedling development in Picea glauca (Moench) Voss after self-, cross-, and wind-pollination

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Introduction

With few exceptions (e. g. Pinus resinosa; Fowler, 1962), self-pollination in gymnosperms results in inbreeding depressions. This can bring about a reduction in seed set, germination capacity and vigor, deformed growth, retarded flowering and varying degrees of albinism. However, the production of even a few viable seeds demonstrates that self-incompatibility is not completely established in the group, and several species of conifers include relatively self-compatible individuals (see Bingham and Squillace, 1955). A common feature is the great variation in self-compatibility among individuals of a species, e. g. Picea abies (Andersson, 1947 a, b).

Among spruce species, self-pollination, in comparison with out-crossings, resulted in depression in one or more of the following characters: cone and seed production, seed germination capacity, seedling vigor, and mortality (Picea abies [Sylvén, 1910; Langlet, 1940; Andersson, 1947 a, b; Wright, 1955; Klaehn and Wheeler, 1961; and Dieckert, 1964 a, b], Picea pungens [Cram, 1957; Cram, Lindquist and Martin, 1960], Picea asperata, Picea engelmannii, and Picea montigena [Wright, 19551). There was a slight increase in the number of seeds per cone in selfed Picea glauca and Picea omorika (Wright, 1955), and Langner (1959) reported improved seed viability in selfed Picea omorika, but inbreeding depressions were observed when the resulting seedlings were one or two years old.

In comparison with the (extensive research on incompatibility systems in angiosperms, there have been relatively few detailed studies of the causes of self-incompatibility and inbreeding depression in gymnosperms. The occurrence of empty seed has frequently been taken as an index of incompatibility, but Sarvas (1962) showed that in pine the percentage of empty seed varies with the abundance of pollen, so that the number of empty seed is not necessarily correlated with the number of aborted embryos. He used seed size classes as an index of the time when embryo failure occurred, either before fertilization, at fertilization, during the rosette stage, or later in embryo development.

An alternative method of determining the time of breakdown is the radiographic technique, which has been used for Picea abies and Picea glauca (Ehrenberg, Gustafsson, Forshell and Simak, 1955; Müller-Olsen, Simak and Gustafsson, 1956; and Klaehn and Wheeler, 1961). The different tissues within the seed absorb X-rays to varying degrees and it is possible to recognize on the radiograph the stages of embryo and endosperm development.

Possibly the most accurate method in identifying the stage at which failure occurs is the cytological technique, which has been used for Pinus peuce (Hagman and Mikkola, 1963) and Pseudotsuga menziesii (Orr-Ewing, 1957). The anatomical development of the seed is followed by microscopic examination of material collected at different stages, and, by this method, the stage of breakdown is determined.

The evidence obtained to date indicates that inbreeding depression is caused by the accumulation of homozygous lethal alleles in the progeny of heterozygous parents. Conclusive proof has not been presented for the stage at which incompatibility mechanisms operate, but absolute self-incompatibility is absent in many coniferous species. There appear to be few barriers to self-pollination or self-fertilization; failure occurs at the early embryo stage, presumably due to physiological incompatibility between embryo and gametophyte tissue.

The present study was initiated to examine the pattern of embryo development in self- and open-pollinated white spruce, Picea glauca (Moench) Voss.; to determine the stage at which incompatibility becomes apparent; and to investigate the interaction of known genotypes with specific environments in the control of development of resulting seedlings. Genotypic variation was ensured by the use of self- and open-pollinated materials from several parent trees, together with seedlings produced from controlled inter-specific hybridization.

Methods

Controlled pollination

Ten trees having sufficient male and female flower buds for wind and self-pollinations were selected in each of two separate plantations of Picea glauca in south central Connecticut.

During the first week of May, 1961, pollen was collected from the male flowers in both stands and stored at 6^0 C in desiccators. Female flowers were enclosed in Kraft paper bags, and they were hand-pollinated with a camel's hair brush; pollination was hampered by unseasonably wet and cold weather, and for this reason four of the trees in Stand 2 were probably unsatisfactorily pollinated.

Three trees in Stand 1 and one tree in Stand 2 were selected for cross-pollination in addition to self- and wind-pollination. To increase the heterogeneity of genetic material available for testing, the following inter-specific crosses were also attempted, using trees growing in the Yale Brett Pinetum at Fairfield, Connecticut:

> Picea glauca X Picea asperata Mast. (+ reciprocal); Picea glauca X Picea *orientalis* (L.) Link (+ reciprocal); Picea glauca X Picea smithiana Boiss.; Picea asperata X Picea orientalis (+ reciprocal); Picea asperata X Picea smithiana; and Picea orientalis X Picea smithiana.

A mixture of pollen from two male parent trees were used in each case with the exception of Picea glauca where five trees were used.

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Anatomical study

For the cytological investigation of selfing in *Picea glauca*, cones were collected every three to five days from four trees in Stand 2. The collections began on May 18 and involved trees having the greatest number of female flowers. However, cones from only one tree were used to follow the development from pollination to mature embryo. Ovules from this tree appeared to have a higher percentage of successful pollinations; i. e. more of these ovules sank when placed in fixative. Ovules from the other three trees were used, but only to follow certain stages of development.

Two cones were selected from each tree at each collection, measured for length and width, dissected, and their ovules placed in one of two fixatives — BELLING's modified NAWASHIN fluid, or NEWCOMER's Fixative. However, only those ovules fixed in NAWASHIN were used for all subsequent sectioning because preliminary tests showed that this fixative gave the best staining results. The ovules were embedded in 60—63^0 Tissuemat, sectioned serially on a microtome at 10 μ, and stained in Safranin and Hematoxylin (DELAFIELD's). Slides were dehydrated through alcohols and xylene, and made permanent in Permount.

From each collection three ovules that sank in the fixative were sectioned, except that, for those collections between May 24 and June 5, about nine ovules were sectioned to obtain a more complete picture of the events just prior to, and immediately following, fertilization. A similar number were analyzed for those collections between June 23 and July 6 when it appeared that a breakdown was occurring in the descending and developing embryo.

Morphological Development

To study seedling development, all available selfed cones were harvested, and fifteen wind-pollinated cones were collected for controls from all trees in Stand 2. Because Stand 1 was infested by squirrels, only three trees had any wind-pollinated cones. Check cones, i. e. unpollinated cones bagged to check the efficiency of the pollinated bags, were collected from several trees.

The seeds were extracted from the cones in the following manner:

a) Selfed seeds were extracted from 25 cones per tree, when available, and the seeds from each cone were stored in separate numbered vials. If there were more than 25 cones, all extra seeds were saved in a single vial.
b) Wind-pollinated seeds were extracted from 15 cones per tree, when available, and kept separately.
c) Seeds were extracted from all check cones and put in a separate vial for each tree.

The extracted seeds were counted and recorded for individual cones, and the seeds were separated into two size categories (large and small). It was assumed that the larger seeds had been successfully fertilized, whereas the smaller seeds had not, and were therefore shriveled and small. A wind-column apparatus was built to separate the filled and unfilled seed.

All filled seeds were stratified in sand for four weeks in a refrigerator and during December they were sown in rows of 50 seeds in the greenhouse. They were planted in paired rows, one row self- and one row wind-pollinated, and the paired rows were repeated several times. There were seeds available for 17 paired rows of seeds, and, when there were less than 50 selfed seeds available from a particular tree, wind- and selfed-seeds were still planted in adjacent rows for comparison. The total number of seeds sown were: 1,695 (selfed) and 1,200 (wind-pollinated).

After five days the seeds started to germinate, and the total number of germinated seeds was recorded daily for a week. During the second week of growth, counts were taken every two or three days, and, for the next five weeks, counts were taken once a week. The germination capacity was calculated on the basis of the number of seeds sown, and the height of each seedlings was measured to the nearest millimeter after six weeks, and at the end of the first growing season.

During July 1962, the seedlings were transplanted into plastic pots with three seedlings per pot, and moved to an outdoor nursery until the latter part of December when they were placed under various environmental conditions. To study the nature of the interaction between genotype and environment, seedlings from ten progeny groups were subjected to six sets of environmental conditions. The ten groups were composed of selfing and wind-pollination of each of two trees in both plantations, and one hybrid (*Picea glauca* × *Picea smithiana*) from each stand. A maximum of eight pots per group (24 seedlings) were placed in each of three controlled environment rooms and three greenhouse locations, providing the combinations of temperature and light treatments given in *Table 1*.

Table 1. — Conditions in the controlled environment rooms, and in the greenhouse.

Treatment	Location	Photoperiod (hours)	Temperature (°F) Light Per.	Dark Per.	Pots (number)
A	Controlled Environment Room	10	60	45	80
B	Controlled Environment Room	10	80	65	80
C	Controlled Environment Room	10	70	55	80
D	Greenhouse	16	70—75	60	49
E	Greenhouse	10	70—75	60	80
F	Greenhouse	Local	70—75	60	48

In March 1963 the length of the terminal flush and the preceding year's growth was measured to the nearest millimeter. The length of the terminal flush was adjusted by covariance with the length of the preceding year's growth. The logarithm of the adjusted variance for each progeny group was then subjected to analysis of variance (SCHEFFÉ, 1959; page 85) to test for differences in the variability of response.

Results and discussion

Anatomical development

Direct comparisons were made of material from self- and wind- pollinated white spruce at all stages from pollination through the development of the embryo. The stages are illustrated by photomicrographs in *Figures 1, 2, and 3*. Up to the two-tiered embryo stage the development was similar in both types of pollination *(Figure 1)*, but the subsequent development of the embryo differed between wind-pollination *(Figure 2)* and self-pollination *(Figure 3)*. Normal development was observed in some self-pollinated ovules, and no breakdown was observed in wind-pollinated ovules.

Pollinations were carried out between May 9 and 16, 1961. The pollen grains were rapidly drawn into the micropylar canal *(Fig. 1 A)* where they germinated *(Fig. 1 B)*, and within a few days the generative and stalk nucleus stage was reached *(Fig. 1 C)*. During the last week of May the

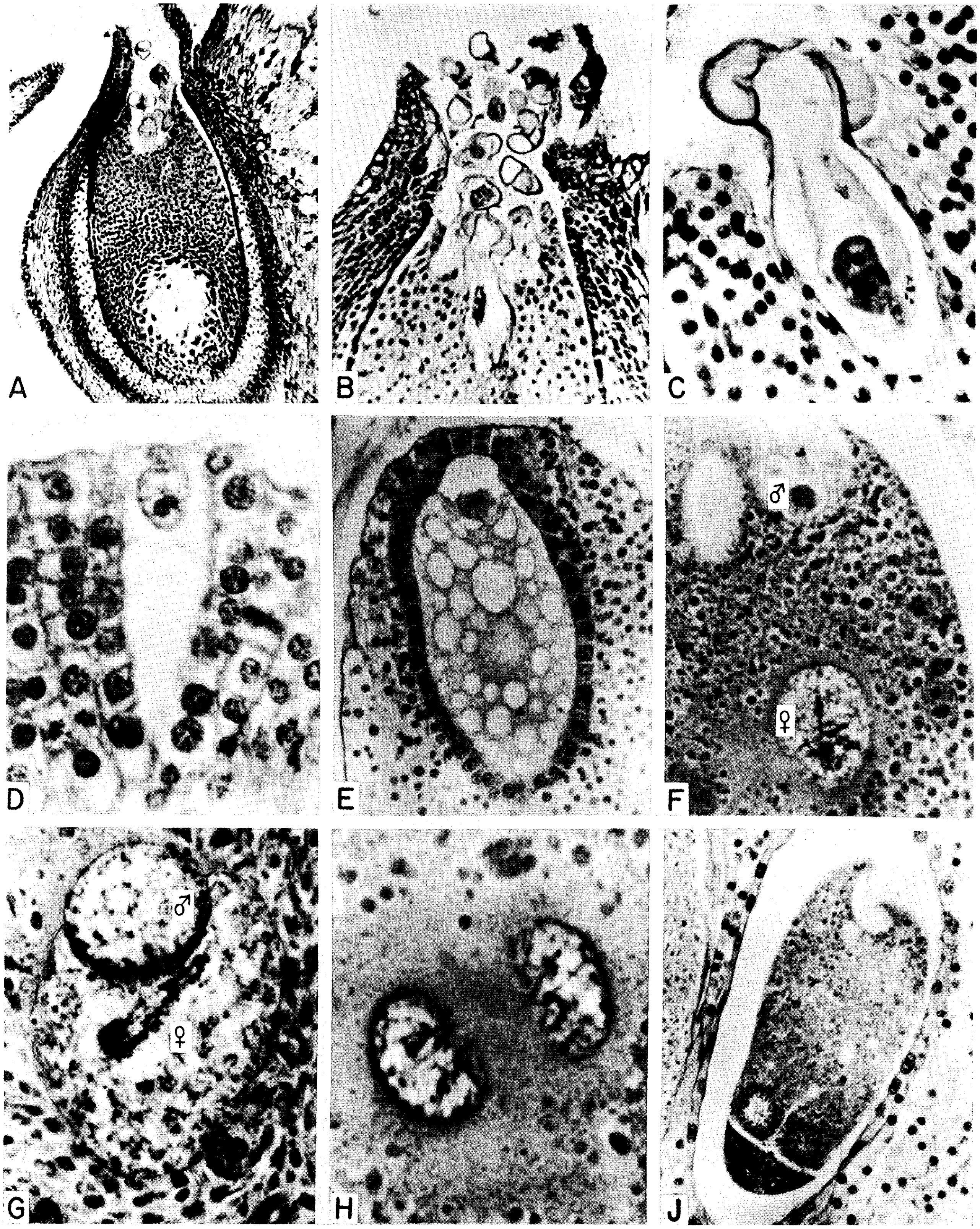

Figure 1. — Cytology of fertilization.

A, B. Germination of pollen on nucellus (A = 45×; B = 95×)
C. Pollen tube with nuclei (250×)
D. Formation of archegonium (420×)
E. Mature archegonium (155×)
F. Sperm nucleus approaching egg cell (350×)
G. Entrance of sperm nucleus into egg cell (550×)
H. First mitotic division of zygote (720×)
J. Two-tiered pro-embryo stage (180×)

first archegonium appeared *(Fig. 1 D)* and mature archegonia were present after a few days *(Fig. 1 E)*. The egg and sperm nuclei approached each other *(Fig. 1 F)* and fertilization took place early in June *(Fig. 1 G)*. The first zygotic mitosis occurred almost immediately *(Fig. 1 H)* and the two-tiered stage was observed in mid-June *(Fig. 1 J)*.

The total time occupied by these stages was approximately five weeks; this is obviously less than that required in pines (see FERGUSON, 1904; or MCWILLIAM and MERGEN, 1959), and it is slightly less than that reported in Douglas-fir growing in British Columbia (ORR-EWING, 1957). No differences were observed between the effects of the two types of pollination up to the stage of the two-tiered embryo. Similar conclusions were reached for Douglas-fir (ORR-EWING, 1957) and Macedonian pine (HAGMAN and MIKKOLA, 1963).

In the normal course of development of wind-pollinated ovules, elongation of the suspensors began in the latter half of June *(Fig. 2 A)*, and the cleavage pro-embryos were pushed down into the female gametophyte tissue *(Fig. 2 B)*. An enlarged view of a single, descending pro-embryo is illustrated in *Figure 2 C*. Embryo development began in the last few days of June and cotyledons appeared during the first week of July *(Fig. 2 D)*. Rapid tissue differentiation occurred in July *(Fig. 2 E)*, and by mid-August the embryo was mature *(Fig. 2 F)*.

This pattern of development was followed by less than half the embryos studied in self-pollinated ovules. In the majority, the suspensors began to push the pro-embryo down into the female gametophyte tissue *(Fig. 3 A and B)* but, as the suspensors reached their maximum elongation, the tissues of the embryo began to collapse *(Fig. 3 C)*. The suspensors themselves disintegrated *(Fig. 3 D)* and finally all the tissue of the ovule collapsed *(Fig. 3 E)*. By the beginning of July, when cotyledons appeared on normal embryos, the self-pollinated ovule contained only a mass of undifferentiated, structureless tissues *(Fig. 3 F)*.

The total number of selfed ovules examined was about 300, and of these some 180 had broken down. This high proportion of empty seed and the low vigor of self-pollinated progenies have commonly been attributed to an overall system of genetic incompatibility. However, there are various stages at which genetic barriers to inbreeding might be set up, from the time of flowering to the time of seedling competition and growth. The results of this anatomical investigation indicate that when white spruce trees are selfed there is no barrier to pollen germination and self-fertilization. The barrier occurs in some form of physiological incompatibility between the early embryo and female gametophyte tissue. Embryo collapse presumably results from increased homozygosity of deleterious recessive genes, and it probably accounts for the reduced yields of filled seeds reported after self-pollination in conifers.

Morphological development

There were no significant differences in rate of cone elongation and in final cone length among pollinations in which *Picea glauca* was the female parent. However, cone lengths in reciprocal hybrids with *Picea asperata* and *Picea orientalis* were significantly greater.

A total of 319 self-pollinated cones yielded 13,175 large seeds, and 1695 (13%) of these fell in the heavy or full class. The average number of filled seeds in self-pollinated cones varied with individual trees from 0 to 21.9, with a mean of 5.3. Only two trees failed to produce filled seeds after selfing. From 177 wind-pollinated cones, 6351 large seeds were extracted, of which 3792 (60%) were full. The average number of filled seeds in wind-pollinated cones ranged from 3.9 to 42.5, with a mean of 21.4. No full seeds were found in the 99 unpollinated cones collected. Twelve seeds were obtained from 45 cones of *Picea glauca* × *Picea asperata,* and 7 seeds from 33 cones of *Picea glauca* × *Picea orientalis.* These were omitted from further analysis. A total of 852 seeds were collected from 32 cones of *Picea glauca* × *Picea smithiana,* and all the other crosses failed to yield seed.

No significant differences appeared between self- and wind-pollinated seeds in rate of germination, germination capacity, and survival; an average of 84.5% germinated, and 97% of these survived at six weeks. Further, there were no differences between parent trees. These similarities were the result of the seed selection method that was used, as only filled seeds were planted, and any inbreeding effects during embryo formation had been removed. If all the seeds present in the cones had been sown, differences in germination capacity would have been present. In Norway spruce significant differences were observed in germination characteristics (DIECKERT, 1964 a), presumably because filled and empty seeds were not separated.

The genetic effect of selfing on later development is represented by variation in such characters as height growth. At six weeks, the average height of wind-pollinated plants (27.2 mm) was significantly greater (at the 5% level) than the height of selfed seedlings (24.7 mm), with no differences between parent trees. The corresponding heights at the end of the first growing season (37.7 and 38.6 mm), based on 1050 and 602 individuals respectively, were not significantly different. However, the ranges of the mean values for the progeny groups were considerably wider with selfing (27.8—44.0 mm) than with wind-pollination (36.9—44.5 mm), indicating that the increased homozogosity extended the total range encountered. The values for hybrid seedlings (*Picea glauca* × *Picea smithiana*) were intermediate between the values obtained for the two types of pollination.

Within a progeny group, however, inbreeding generally decreased the variability, and this was demonstrated by the analysis of height growth of seedlings growing under six different environments. The analysis of variance of the within-progeny variability in the length of the terminal shoot (expressed as [log mean square-1] × 1000 after adjustment for the preceding year's growth) showed a significant difference in variance between self- and wind-pollinated groups. There was also a highly significant difference in variance between the three growth chamber treatments as a group and the three greenhouse treatments, but no significant differences were found within the growth chamber or within the greenhouse treatments. The results are illustrated in *Figure 4;* wind-pollinated progenies were more variable than selfings, and the increased variability measured in the greenhouse reflects the more variable conditions in comparison with those in the controlled environment rooms. There were no significant effects attributed to individual parent trees, nor to interactions between any major factors.

The seedlings not required for growth chamber experiments were maintained in the outdoor nursery from December, 1961. In August, 1964, the total crown lengths (height above cotyledons) of 80 seedlings, representing wind- and self-pollinated progeny of one tree in each stand,

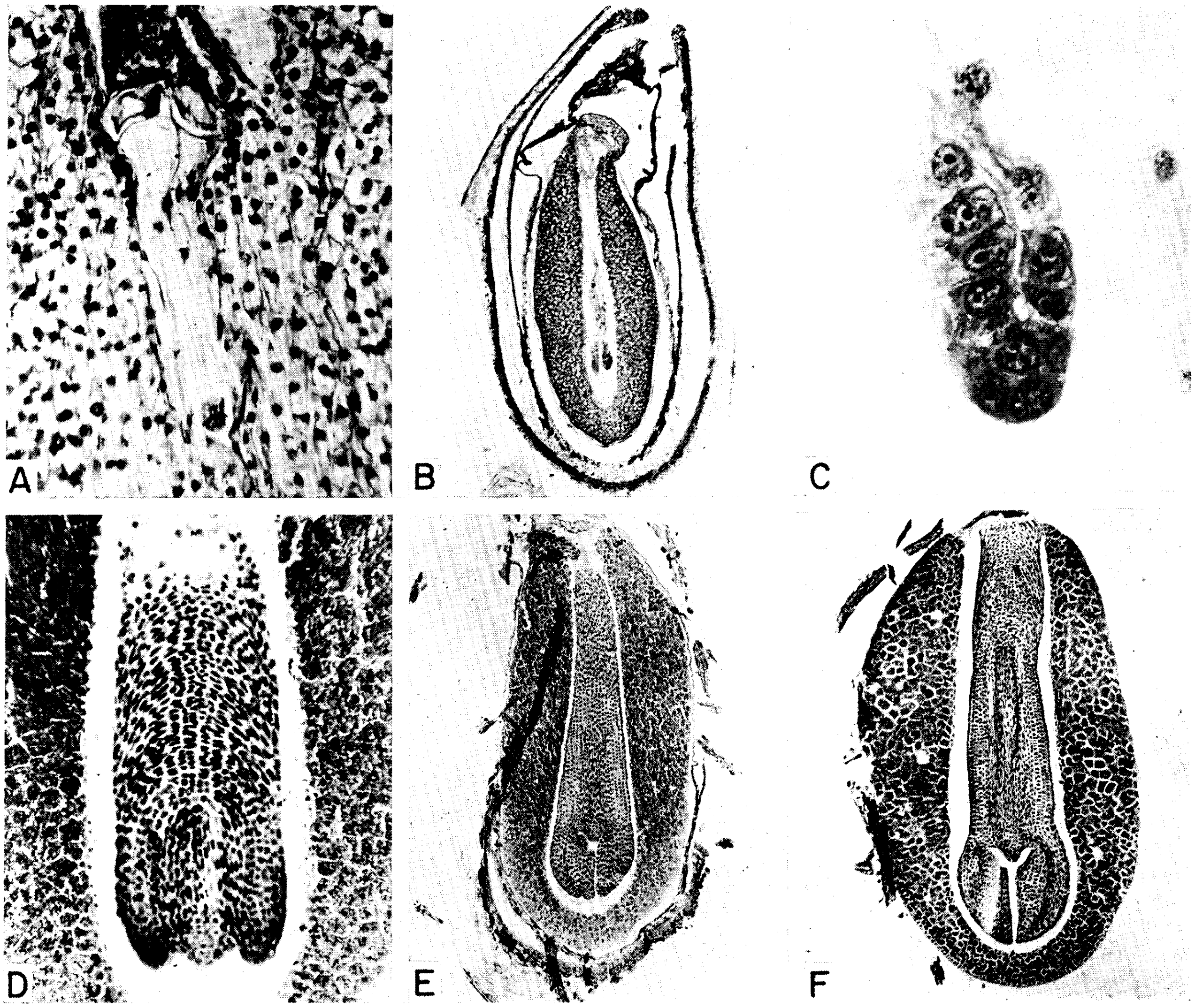

Figure 2. — Normal embryogenesis.

A. Suspensor cells of pro-embryos (135×)
B. Cleavage pro-embryos in embryo cavity (23×)
C. Enlarged section through pro-embryo (340×)
D. Appearance of cotyledon primordia on embryo (75×)
E. Immature embryo, July (23×)
F. Mature embryo at time of seed collection, August (27×)

were measured, in addition to 40 seedlings of the hybrid *Picea glauca* × *Picea smithiana.*

Mean values for the progenies of the two parent trees differed significantly (14.6 and 12.4 cm), and selfed progeny were significantly taller (14.3 cm) than wind-pollinated progeny (12.7 cm). The mean value for hybrid seedlings of *Picea glauca* × *Picea smithiana* was intermediate (13.4 cm).

Conclusions

In the white spruce trees investigated there appeared to be no barriers to self-pollination or self-fertilization. Breakdown began in the pro-embryo stage and during early embryonic differentiation. A high proportion of failures were observed in selfed seeds but none in seeds produced by wind-pollination. No viable seeds were produced in 2 out of 20 trees that were self-pollinated.

Several earlier workers have reported decreased germination rate, germination capacity, and seedling survival as a result of selfing. Here, however, such effects of selfing on subsequent development varied at different times. At six weeks, after the first growing season and after three years, the heights of self-pollinated seedlings were respectively less than, equal to, and greater than wind-pollinated seedlings. However, the mean values for three years were based on 80 trees only, and the difference may be fortuitous. There was a significant difference between the progenies of the two parent trees at three years, but at six weeks and six months, there were no differences among the 20 parent trees.

Selfed seedlings were less variable than wind-pollinated progenies; the difference would probably have been greater if controlled cross-pollination had been used instead of wind-pollination. Some self-pollination undoubtedly occurred in the wind-pollinated cones, and this decreased the variability of the resulting wind-pollinated progeny.

Seedlings of three interspecific crosses were surviving at the end of three growing seasons. One of these hybridizations has not been reported previously *(Picea glauca* × *Picea smithiana),* and the other two *(Picea glauca* × *Picea*

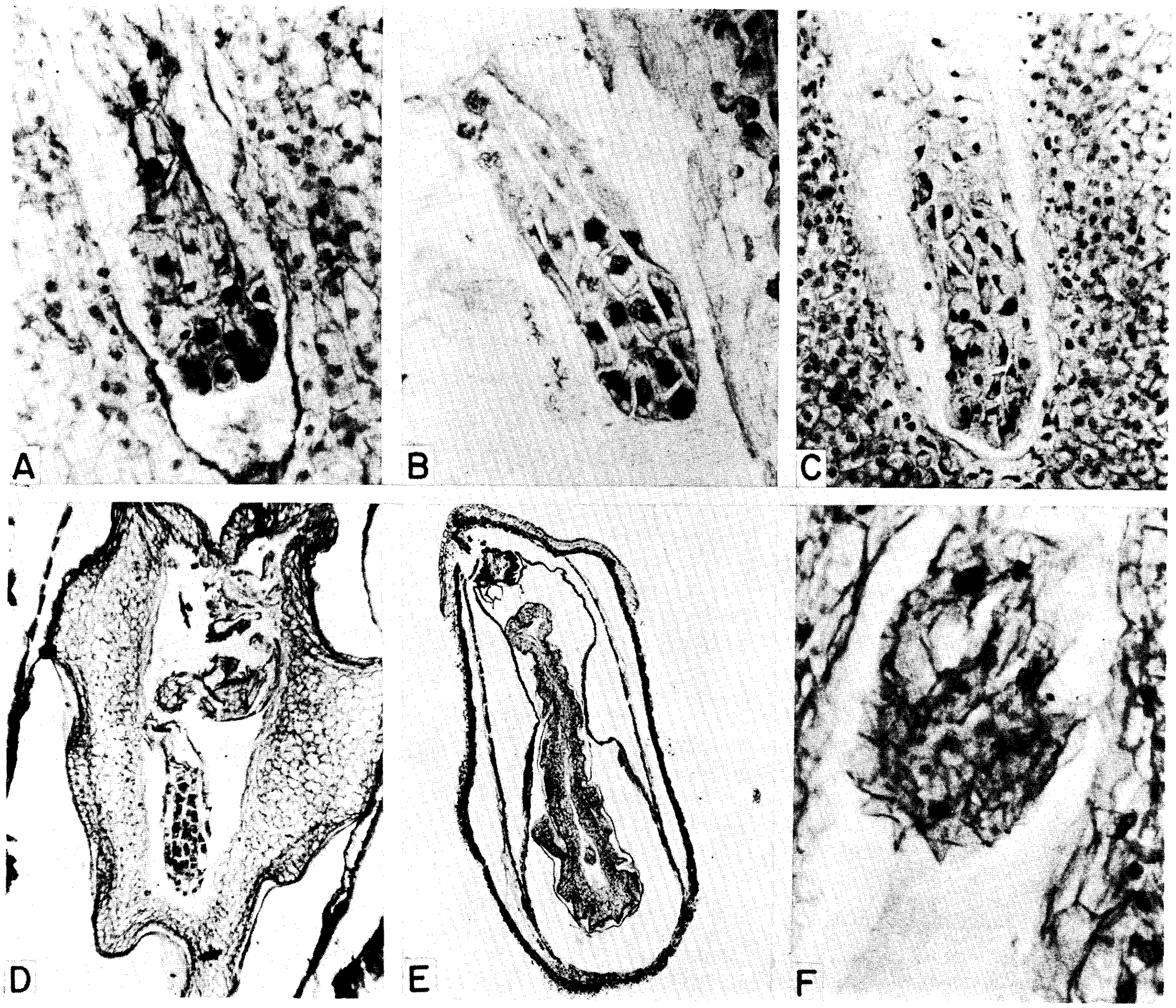

Figure 3. — *Failure of embryogenesis after selfing.*

A, B. Apparently normal pro-embryos in embryo cavity (A = 155×; B = 155×)
C. Collapse of tissue in pro-embryo (110×)
D. Disintegration of suspensor cells (38×)
E. Collapse of pro-embryo and female gametophyte tissue (20×)
F. Collapsed pro-embryo tissue, July (340×)

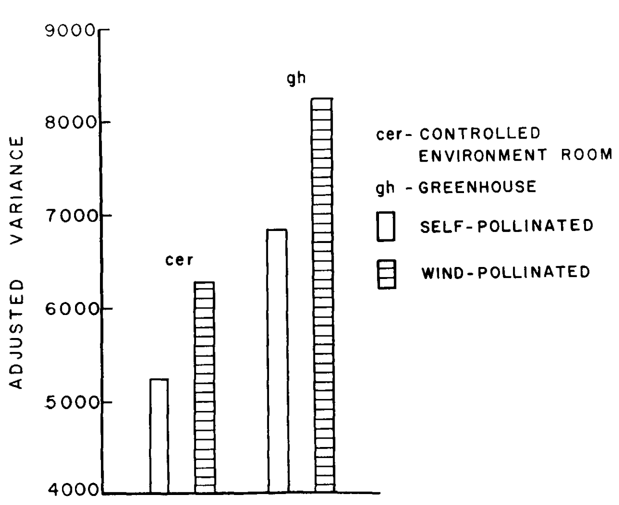

Figure 4. — Bar-graph showing adjusted variances for self- and wind-pollinated progeny when grown in controlled environment rooms and in a greenhouse.

asperata and *Picea glauca* × *Picea orientalis*) had been attempted unsuccessfully (Wright, 1955).

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Variation in Needle Cast Susceptibility among 29 Jack Pine Seed Sources

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Jack pine needle cast, caused by the fungus *Hypodermella ampla* Dearn., is one of a group of needle cast diseases that causes defoliation of pines. This parasite is distributed from Wisconsin to Nova Scotia (Darker, 1932) and attacks only jack pine (*Pinus banksiana* Lamb.). An infected tree may lose all but the current year's needles. The effect of *H. ampla* on growth has not been determined, but a closely related fungus (*H. medusa* Dearn.) reduced growth of ponderosa pine (*P. ponderosa* Laws.) by 40 percent on poor sites (Wagener, 1959).

A study of the effect of jack pine needle cast on 29 jack pine seed sources began in 1961 when a severe outbreak of this disease occurred in a test plantation at Watersmeet, Michigan. This plantation is part of a jack pine geographic variation study begun in 1952 by the Lake States Forest Experiment Station and the University of Minnesota in cooperation with various university, state, and private agencies[2]).

Materials and Methods

Twenty-nine seed collections were made from several average trees of stands in Minnesota, Wisconsin, and Michigan. Seeds were sown in the Hugo Sauer Nursery at Rhinelander, Wisconsin, and the General Andrews State Nursery at Willow River, Minnesota, in 1952. Seedlings were field planted in 1954 at 17 locations within the 3 states. At each location a 4-replication, randomized, complete block design was used. Each replication consists of one square 64-tree plot of each experimental origin and one "local" seedlot. The locations of the seed sources are shown in *Figure 1*.

Two plantations, consisting of stock grown at Rhinelander, were used in this study[3]). The Watersmeet plantation, located 7 miles north of Watersmeet, Michigan, is in the western portion of Michigan's Upper Peninsula. Stumps indicate that the area originally supported a cover of red pine (*P. resinosa* Ait.) and eastern white pine (*P. strobus* L.).

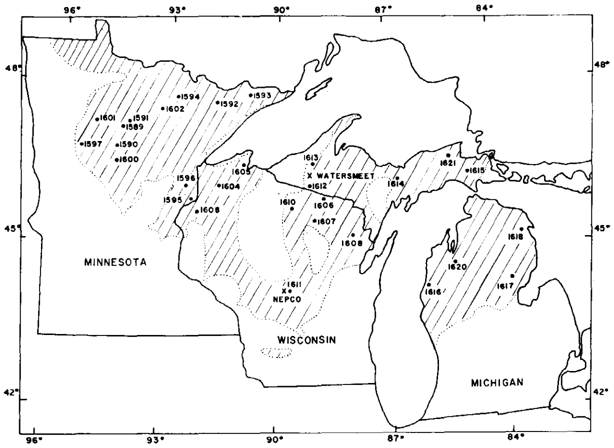

Figure 1. — Location of 29 seed source areas and 2 study plantations. The shaded area is the natural range of jack pine (from Rudolf and Schoenike, 1963).

Dense natural stands of jack pine now surround the plantation.

The Nepco plantation is on the Nepco Industrial Forest, Port Edwards, Wisconsin. The Nekoosa-Edwards Paper Company owns this industrial forest and maintains the jack pine test planting. This planting is bordered on two sides by thrifty red pine plantations. Dense natural stands of jack pine also occur nearby.

The 16 center trees on each plot were scored for needle infection in 1961, and the 10 center trees on each plot in 1962. Three replications were used in 1961, and four in 1962. If a center tree was missing, the one nearest was substituted.

As previously noted, the fungus does not attack current year's growth. Therefore, the 1961 scoring applied to needles formed in 1960 or before; the 1962 scoring applied only to needles formed in 1961.

The percent of infected needles was scored as a grade. Grades eliminated the need for a subsequent transformation. The grades are as follows:

Grade	Needles Infected
	Percent
1	1—10
2	11—33
3	34—66
4	67—90
5	91—99
6	100

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[1]) The authors are respectively, Associate Plant Geneticist and Principal Plant Geneticist, Institute of Forest Genetics, Lake States Forest Experiment Station, Forest Service, U. S. Dept. of Agriculture, Rhinelander, Wisconsin. Grateful acknowledgment is made to Dr. J. W. Wright for review of the manuscript.

[2]) P. O. Rudolf of the Lake States Forest Experiment Station and T. Schantz-Hansen of the University of Minnesota planned and initiated the overall study. P. O. Rudolf has also issued several mimeographed reports. The 1958 height data used in the analyses were available in the Lake States Forest Experiment Station files.

[3]) A plantation on the Argonne Experimental Forest, Three Lakes, Wisconsin, was also examined in 1962. However, 1961 frost damage and the needle cast measurements were confounded to such an extent that this plantation was eliminated from the analysis.