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Effects of Inbreeding in Red Pine, Pinus resinosa Ait.

II. Poliination Studies[1])

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Introduction

The effect of inbreeding in normally cross-fertilized organisms is to increase the homozygosity of such organisms. Most organisms carry within their genomes recessive genes, many of which are deleterious when present in a homozygous condition. Inbreeding of such organisms considerably increases the frequency of individuals homozygous in respect to rare deleterious genes.

Recessive genes are, of course, not all deleterious although under "normal conditions" many of them are. These same genes are the building blocks of evolution. A deleterious gene may actually become advantageous in improving an organism's utilization of a changed environment. A cpecies is considered to have a better evolutionary potential when it carries a large amount of genetic variability. Such a species is plastic and able to survive with, or even to capitalize on environmental change.

The genus Pinus, in general, is composed of species depending on cross-fertilization for the production of normal progenies. Abundant support for this statement has been obtained from self-pollination studies with Pinus species (Austin, 1937; Bingham and Squillace, 1955; Dengler, 1932, 1939; Duffield and Stockwell, 1949; Ehrenberg and Simak, 1957; Johnson, 1945; Kolesnikoff, 1929; Magini, 1956; Mergen, 1954; Perry, 1960; Peters and Goddard, 1961; Plym Forshell, 1953; Righter, 1958; Sarvas, 1962; Squillace and Bingham, 1954; Toyama, 1950; Wettstein, 1940; and Wright and Gabriel, 1958). All of the Pinus species studied have proven to be partially self-fertile and self-compatible, although some authorities have reported instances of apparently completely self-sterile trees. Individual trees of several of the species studied have actually been found to be highly self-fertile and self-compatible (Bingham and Squillace, 1955; Dengler, 1932; Ehrenberg and Simak, 1957; Magini, 1956; Plym Forshell, 1953; Squillace and Bingham, 1954; Toyama, 1950).

Studies of the effects of inbreeding in red pine are almost non-existent. Johnson (1945), on the basis of self- and cross-pollination on a single tree, reported that self-pollination resulted in a reduced seed set. Seed lots from four isolated red pine trees, which probably resulted from natural self-pollination, were included among the 37 provenances studied by Rudolf (1947). Although Rudolf did not single out the "selfed" progenies for comparisons, it is quite evident from his data that these progenies did not differ appreciably from progenies of the other, presumably cross-pollinated trees. Two of the former seed lots were included among the 50 lots tested at Kane, Pennsylvania (Hough, 1952).

Table *II* — 1 shows the ranking of the four "selfed" progenies among the 37 provenances studied by Rudolf (1947) as well as the ranking of two of these seed sources in relation to the 46 to 49 seed sources studied by Hough (1952).

Inbreeding, especially self-pollination, is a useful method of determining the genetic variability of an individual and has been suggested as a method of testing selected trees (Austin, 1927, 1937; Heitmüller, 1957; Kolesnikoff, 1929; Langner, 1951; Mergen, 1954; Schreiner, 1953; and Schröck, 1957).

***Table** II — 1.* — Ranking of Red Pine Progenies. — Presumably Resulting from Self-pollination.

Collection Number	Climatic Region	Survival	Height	Diam. b. h.	Basal Area/ Acre	Unpeeled Volume/ Acre	Good Form	Better Dom. and Codom. Trees	Premium Trees
Rank out of 37 Progenies — Trees *16* years old — Rudolf *(1947)*									
24	Head of Lake	6	23	28	14	19	4	18	3
74	Brainerd Cameron	3	6	5	11	3 or 4	15	16	21
75	Brainerd Cameron	8	19	23	15	17	17	6	2
82	Lower Michigan	34	21	24	32	31	19	20	19

Rank out of *49* or 46 Progenies — Trees 10 years old — Hough (1952)

		Germination 49 Provenances	Survival 46 Provenances	Height 46 Provenances
74	Brainerd Cameron	6—8	38	25
75	Brainerd Cameron	19—20	31—34	8

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[1]) Contribution 64 — 6. Ontario Department of Lands and Forests. This paper is the second in a series based on a dissertation submitted to the Graduate School of Yale University as partial fulfillment of the requirements of the PhD degree in 1963.

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Studies of natural variation, with many *Pinus* species which are adversely affected by inbreeding, have also revealed these same species to be genetically quite variable. The one coniferous species which has been found to be highly self-fertile and self-compatible is *Picea Omórika* (PANČIĆ) PURKYNE (LANGNER, 1957, 1959). This species occupies a limited area in the Balkans and is morphologically uniform (LANGNER, 1959).

The apparent lack of widespread genetic variation, revealed by provenance tests (FOWLER, 1964), suggested an inbreeding study with red pine to determine the magnitude of its genetic variation. Self- and cross-pollinations were made on six trees in 1958 and on an additional four trees in 1959. In the fall of 1959, when cones and seeds became available from the 1958 pollinations, it was evident that these trees were highly self-fertile. Germination and early growth data of the seeds and seedlings from these pollinations revealed that the trees were self-compatible, contained few, if any, deleterious genes, and seedlings resulting from self-pollination suffered little or no inbreeding depression in growth (FOWLER, 1962).

On the basis of the results obtained from the 1958 pollinations and the fact that provenance studies with this species revealed the magnitude of genetic variation to be low, a working hypothesis was established. It was postulated that red pine, unlike other species of the genus *Pinus,* is homozygous in respect to most alleles, self-fertile, self-compatible, and that progenies resulting from self-pollination exhibit little inbreeding depression. To test this hypothesis, a pollination study, in which individuals from a large part of the species range were to be tested, was begun in 1961.

Genetic variation can be high even in a normally cross-pollinated species composed of self-fertile individuals. Within any one breeding population, the species could be uniform and yet different breeding populations could be genetically quite different. Self-pollination, cross-pollination with pollen from the same origin, and from a widely different origin were used to determine if there were genetic differences between individual trees of the same breeding population and between trees from different breeding populations.

Materials and Methods

The pollination techniques used throughout this study were similar to those described by MERGEN, ROSSOLL and POMEROY (1955).

An effort was made to avoid any possible effect of crown position on seed production, such as reported in *Pinus monticola* DOUGL. by SQUILLACE (1957). Approximately the same number of female conelets, on each whorl of the tree, were pollinated with each of the pollens being tested. The assignment of a specific pollen to a specific bag was done without regard to the position of that bag in the whorl of branches. Essentially, this resulted in a random distribution of pollens stratified by whorls.

In June of the year following pollination, the developing cones were sprayed with an aqueous solution of D. D. T. to control insect pests (primarily *Conophthorus resinosae* HOPK.). In August or early September, cloth bags which had been soaked in a D. D. T. solution were placed over the cones to protect them from rodents and catch any seed from early cone opening. In late September or early October, the cloth bags containing the mature cones were collected, tagged and transported to the Southern Research Station, Maple, Ontario.

The cones were dried individually in small paper boxes and were dissected to assure that all seeds had been removed. Full and empty seeds were separated with absolute ethyl alcohol. The floating seeds were cut open to determine if they contained any living gametophyte tissue. Cone length and number of full and empty seeds were recorded for up to ten undamaged cones from each pollination.

Each seed to be used in germination and growth tests was weighed and subsequent data were kept on an individual seed/seedling basis.

The seeds were germinated on moist granitic sand in Petri dishes. When a seed had produced a radicle of 2 mm in length, it was recorded as germinated. Germination was recorded daily over the germination period. Shortly after the seeds germinated, they were transplanted into a flat or bed, or plastic pot in a greenhouse, where they were grown under an 18 to 20-hour photoperiod.

The soil mixture used for all the growth experiments consisted of a 1 : 2 mixture of pulverized white pine humus and granitic sand. The soil surface was covered with a one-half inch layer of granitic sand.

1958 and 1959 Pollinations, Vivian, Ontario

Pollinations were made on six trees in 1958 and on nine trees in 1959. These trees were located in a plantation managed by the Ontario Department of Lands and Forests at Vivian, Ontario. They were 25 years old and had been sexually mature for at least ten years. The seed origin is not known. The only criterion for selecting the trees for the pollination studies was a sufficient number of ovulate strobili for the desired pollinations. The pollinations made in 1958 and 1959 are summarized in *Table II — 2.*

The pollen used for cross-pollination was obtained by collecting equal volumes of male catkins (microsporangiate strobili) from 20 trees in the same plantation. No catkins from any of the trees used as female parents were included in this mixture. Pollen ST 151 was a single tree collection made by Mr. MARK HOLST of the Canada Department of Forestry at Chalk River, Ontario. Pollen 622 was a single tree collection made at Vivian, Ontario.

Vivian, 1958 A: In February, 1960, 100 seeds from self- and cross-pollinations of two trees were divided into four lots of 25 seeds, weighed, and germinated. The germinated seeds were sown in flats containing a sandy loam soil. The design of the experiment consisted of ten blocks with randomized rows of ten seedlings from each of the four pollinations. The seedlings were grown in a greenhouse under the supervision of Dr. FRANÇOIS MERGEN, Yale School of Forestry, until December, 1960. At that time they were lifted and shipped to the Southern Research Station, Maple, Ontario where they were heeled-in in a cold frame for the winter.

Table II — 2. — 1958 and 1959 Pollinations — Vivian, Ontario.

Year	Number of Trees	Pollination	Conelets Pollinated Number
1958	6	Self	130
		Cross	126
1959	4	Self	84
		Cross	92
	5	622	123
		ST 151	121

On May 1st, 1961, the seedlings were lifted and the following measurements made: length of hypocotyl, epicotyl, root and longest needle. Oven-dry weight determinations were made of two seedlings from each pollination from each of the ten blocks. Six seedlings from each of the two kinds of pollinations, from each of the two trees, from each of the ten blocks were planted in a nursery bed. The original block design was maintained.

In July, 1961, after the seedlings had completed their second cycle of growth, the following measurements and observations were made: second cycle height growth, length of longest needle, stem diameter at two centimeters above the root collar, number of stems (main stem plus branches) per seedling, and survival.

Vivian, 1958 B: In June, 1960, 35 full seeds from the self- and cross-pollinations, of each of five of the six trees pollinated in 1958, were weighed. On June 30th these seeds were germinated. The germinated seeds were planted out in individual pots in a greenhouse, where they were raised under an 18-hour photoperiod. The design of this experiment consisted of three completely randomized blocks containing ten seedlings from each of the ten pollinations. Five seedlings from each pollination were planted as spares and used to replace seeds failing to germinate or dead seedlings.

In November, 1960, the seedlings were moved into a cool section of the greenhouse where they were subjected to normal photoperiods. All the seedlings had completed height growth and formed terminal buds at this time. After a two-week "hardening-up" period, the greenhouse windows were opened and the heat turned off. On January 15th, 1961, the seedlings were moved back into a warm section of the greenhouse and kept under an 18—20 hour photoperiod. Hypocotyl diameter and epicotyl length were measured in all the seedlings at this time.

The seedlings broke dormancy uniformly and produced a second cycle of growth. On July 16th to 17th, 1961, when all seedlings had ceased height growth and had formed terminal buds, the following measurements and observations were made: second cycle height growth, length of longest needle, stem diameter at one centimeter above the root collar, number of stems (main stem plus branches) per seedling, and survival. The seedlings were then transplanted into nursery beds for further observations.

Vivian, 1959: Fifty seeds from each of the 18 pollinations (*Table II — 2*) made in 1959 were weighed. On January 5th, 1961, these seeds were germinated in Petri dishes. Thirty germinated seeds from each pollination were planted out in six completely randomized blocks in greenhouse benches. The remaining germinated seeds were planted in randomized rows, five seedlings per row, in flats and were to be used to replace seeds that failed to germinate or seedlings that died during an early stage of development.

In November of 1961 the long daylight regime under which the plants had been growing, was terminated and the greenhouse heat was lowered for a period of two weeks. At the end of this period, the greenhouse windows were opened and the heat turned off.

On January 5th, 1962, the windows were closed, the heat and the auxiliary lighting turned on. Epicotyl length and hypocotyl diameter measurements were then made of the seedlings in the six beds.

The seedlings broke dormancy uniformly and went through a second cycle of growth. On June 18th to 21st, 1962, when height growth had ceased, total height, length of longest needle, stem diameter, root length and number of stems per seedling were recorded in all seedlings in the six beds. Survival was recorded of the seedlings in the six beds, as well as of those in the four flats. The seedlings were planted in a nursery bed for further observations.

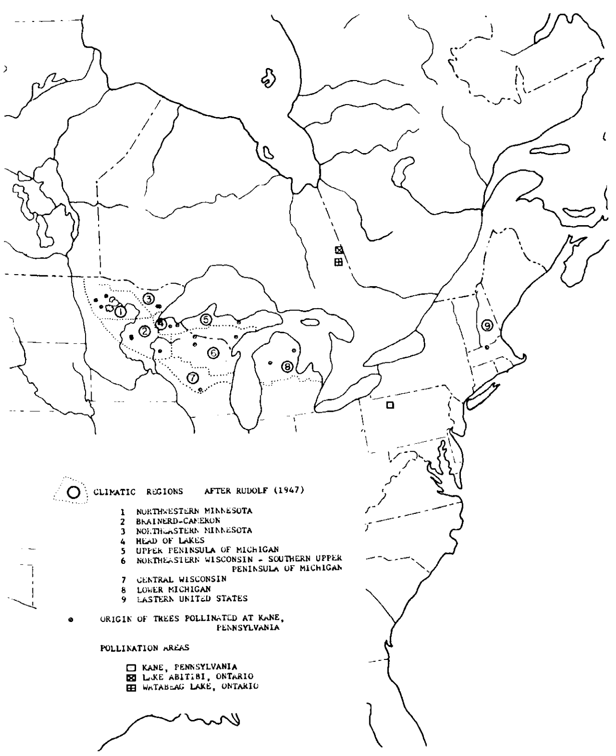

Figure II — 1. — Map of eastern North America showing locations of pollination areas and origins of trees pollinated in 1961.

1961 Pollinations — Kane, Pennsylvania; Lake Abitibi, Ontario and Watabeag Lake, Ontario

A map showing the areas where the pollinations were made, as well as the origins of the trees pollinated, is presented in *Figure II — 1.* Thirty-six red pine trees, representing nine climatic regions (Rudolf, 1947), were utilized for the controlled pollinations at Kane, Pennsylvania. These trees were part of a provenance test established in 1937 by the U. S. Forest Service to test red pine of 50 origins, representing eight climatic regions in the Lake States and one in the eastern United States (Hough, 1952). In 1961, ovulate strobili on the trees at Kane were restricted to edge trees and to a few of the larger dominant trees within the plantation. Because of the restricted number of flowering trees, it was not possible to sample the individual origins within the nine climatic regions. Four trees from each of the climatic regions described by Rudolf (1947) were selected for pollination work. In some instances all four trees were of the same origin within the climatic region; in other cases trees of two or three origins represented a climatic region. The only basis for selecting trees within each region was that they contained enough ovulate strobili to warrant pollination. Two of the trees selected from the Lower Michigan region were later found to be from the Northeastern Wisconsin-Southern Upper Peninsula of the Michigan region.

Ovulate strobili, on the 36 trees, were isolated during the period June 2nd to 5th, 1961. Male catkins were col-

Table II — 3. — 1961 Pollinations at Kane, Pennsylvania; Lake Abitibi, Ontario and Watabeag Lake, Ontario.

Tree Number	Location	Collection Number (Hough, 1952)	Origin	Climatic Region (Rudolf, 1947)	Number Conelets Pollinated			Date Pollinated June
					Self	Local	Maine	
4	Kane	179	Itasca State Park, Minn.	1	18	16	19	15
5	„	179	Itasca State Park, Minn.	1	8	7	9	15
12	„	188	Chippewa Nat. For., Minn.	1	19	19	19	15
24	„	181	Bagley, Minn.	1	13	16	15	16
15	„	75	Onamia, Minn.	2	12	16	16	15
23	„	74	Onamia, Minn.	2	7	9	7	15
27	„	159	Turtle Lake, Wisc.	2	11	12	8	15
36	„	159	Turtle Lake, Wisc.	2	9	9	9	15
13	„	36	Aurora, Minn.	3	16	19	17	13
14	„	36	Aurora, Minn.	3	13	16	16	13
16	„	36	Aurora, Minn.	3	9	12	10	13
19	„	38	Virginia, Minn.	3	6	9	8	13
7	„	165	Cedar, Wisc.	4	15	16	17	14
8	„	165	Cedar, Wisc.	4	7	5	3	14
32	„	165	Cedar, Wisc.	4	16	16	17	14
35	„	47	Iron River, Wisc.	4	10	9	10	15
20	„	246	Munising, Mich.	5	11	10	11	15
28	„	246	Munising, Mich.	5	12	10	9	15
30	„	246	Munising, Mich.	5	11	14	14	15
31	„	246	Munising, Mich.	5	9	13	11	15
6	„	10	Trout Lake, Wisc.	6	16	17	17	15
18	„	10	Trout Lake, Wisc.	6	7	9	9	15
21	„	298	Trout Lake, Wisc.	6	5	10	6	16
22	„	227	Irma, Wisc.	6	15	15	15	16
25	„	54	Rapid River, Mich.	6	9	5	7	16
26	„	54	Rapid River, Mich.	6	16	16	11	16
9	„	60	Kilbourn, Wisc.	7	15	18	18	13
10	„	60	Kilbourn, Wisc.	7	18	13	13	13
11	„	60	Kilbourn, Wisc.	7	19	15	15	13
17	„	60	Kilbourn, Wisc.	7	12	10	15	13
33	„	210	Cadillac, Mich.	8	14	14	15	15
34	„	213	Curran, Mich.	8	17	18	19	15
1	„	123	Winchendon, Mass.	9	17	20	20	13
2	„	123	Winchendon, Mass.	9	10	11	9	13
3	„	123	Winchendon, Mass.	9	20	20	21	13
29	„	123	Winchendon, Mass.	9	8	5	7	13
39	Abitibi	—	Lake Abitibi, Ont.	—	18	19	19	27
41	„	—	Lake Abitibi, Ont.	—	15	13	15	27
42	„	—	Lake Abitibi, Ont.	—	15	15	8	27
43	„	—	Lake Abitibi, Ont.	—	11	11	11	27
1358	Watabeag	—	Watabeag Lake, Ont.	—	17	12	20	29
1359	„	—	Watabeag Lake, Ont.	—	9	7	9	29
1360	„	—	Watabeag Lake, Ont.	—	14	13	16	29
1361	„	—	Watabeag Lake, Ont.	—	12	35	22	29
1362	„	—	Watabeag Lake, Ont.	—	14	17	24	29

lected during the period June 5th to 7th. Three kinds of pollen collection were made:

1. Self. — Pollen from each of the 36 trees at Kane, Penna. collected separately.
2. Local. — Pollen collected in approximately equal volumes from the ten trees of each of the provenances on which ovulate strobili had been isolated. These pollens were kept separate by provenances.
3. Maine. — Pollens collected from 20 individual trees of the Maine provenance (Hough, [1952] collection number 293) and kept separate.

Pollination of the ovulate strobili was accomplished during the period June 13th to 16th. The pollinations made during this period are listed in *Table II — 3.*

The local pollen from each provenance was extracted as a single pollen. The twenty individual tree collections from the Maine provenance were extracted separately and equal volumes of pollen from each of these trees mixed together.

Weather conditions during the pollination period were not favourable. Rain showers occurred on the night of June 13th to 14th and during the afternoon of June 14th. Pollinations made during the period from June 14th to 15th were made in damp isolation bags. Pollination in such bags is not completely satisfactory as much of the pollen adheres to the bags. In an effort to compensate for this, extra care was taken in aiming the pollen at the ovulate strobili and extra pollen was forced into the bags.

Two small red pine stands located on Long Point in Lake Abitibi, Ontario were chosen for pollination work. Six trees were selected, on the basis of number of female conelets available, in a stand in which the trees were 55 to 60 years old and 45 to 55 feet in height. Three additional trees were selected in a second stand in which the trees were 70 to 80 years old and 40 to 50 feet in height.

Mr. C. W. Yeatman of the Canada Department of Forestry selected 13 trees for pollination work on the east shore of Watabeag Lake in Sheba and Nordic Townships, approximately 45 miles west of Swastika, Ontario. The trees in this stand were 150 to 250 years old and 80 to 100 feet in height.

Four of the six trees located in the first stand at Lake Abitibi and five of the trees at Watabeag Lake were used for pollination studies similar to those made at Kane, Penna. The pollinations made on these trees are listed in *Table II — 3.* The remaining five trees at Lake Abitibi and the remaining eight trees at Watabeag Lake were used for long-distance pollination studies. These pollinations are

Table II — 4. — 1961 Pollinations at Lake Abitibi and Watabeag Lake, Ontario.

Tree Number	Location	Origin of Pollen	Collection Number (HOUGH, 1952)	Place Collected	Conelets Pollinated No.	Date Pollinated June
40	Abitibi	Pennsylvania	267	Kane, Pa.	29	27
44	„	Wisconsin	10	„ „	33	27
45	„	Chalk River, Ont.	—	Chalk River, Ont.	72	27
46	„	Maine	293	Kane, Pa.	59	27
47	„	New York	294	„ „	66	27
1350	Watabeag	Pennsylvania	267	„ „	20	29
1351	„	Minnesota	179	„ „	47	29
1352	„	Minnesota	179	„ „	25	29
1353	„	Wisconsin	10	„ „	42	29
1354	„	Maine	293	„ „	42	29
1355	„	Pennsylvania	267	„ „	45	29
1356	„	Maine	293	„ „	134	29
1357	„	New York	294	„ „	62	29

listed in *Table II — 4.* All the pollination work at Watabeag Lake was carried out under the direction of Mr. YEATMAN.

Female conelets were isolated during the period of June 22nd to 24th at Lake Abitibi and on June 22nd at Watabeag Lake. Male catkins were collected on June 24th at Lake Abitibi and on June 28th at Watabeag Lake.

Self and local pollen collections were made in the same manner as described for the Kane, Penna. pollination work. These pollens were extracted in sausage casing extractors similar to those described by MERGEN *et al* (1955). All other pollen collections, with the exception of one collected and extracted by Mr. MARK HOLST of the Canada Department of Forestry, were made at Kane, Penna. and the pollen was extracted at Maple, Ontario.

In June, 1962, the red pine cones resulting from the pollinations at Kane, Penna. and at Lake Abitibi, Ontario, were sprayed with an aqueous solution of D. D. T. Similarly, cones at Watabeag Lake, Ontario were sprayed in May and again in June, 1962, with a D. D. T. oil emulsion by the Canada Department of Forestry.

Cloth bags were placed over the cones resulting from the pollinations at Kane, Penna. and Lake Abitibi, Ontario in August and September, 1962 respectively.

In late September and early October all cones resulting from the pollinations were collected. Cones resulting from open pollination were also collected from the trees at Lake Abitibi and Watabeag Lake. The cones were dried and the seed extracted at Maple.

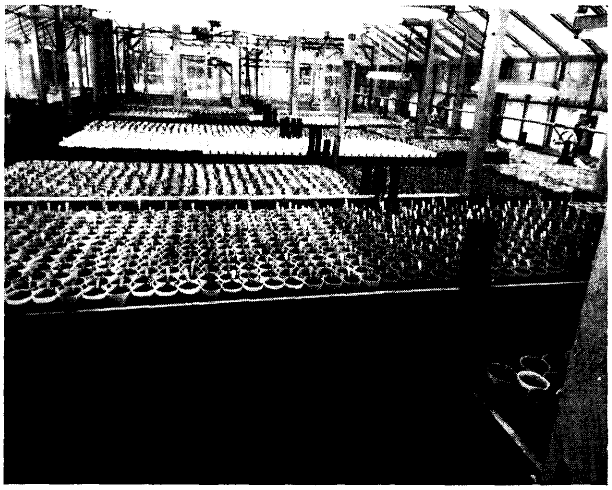

Figure II — 2. — View of Southern Research Station greenhouse showing pots in completely randomized experiment, December, 1962.

1961 Self, Local and Maine Pollinations: Up to thirty seeds from each pollination were individually weighed and kept separate. On December 7th, 1962, all seeds were sown individually in pots in a completely random design in a greenhouse. The seeds were placed at a depth of approximately $^3/_8$ of an inch in the granitic sand on the soil surface. *Figure II — 2* shows the layout of this experiment in the greenhouse. Seed germination was observed daily. A seed was considered to have germinated when the seed coat had been pushed above the soil surface. When a germinated seed was observed, a coloured toothpick, indicating the day of germination, was placed in the pot. During the second week of February, 1963, germination, hypocotyl length, and number of cotyledons were recorded for all seedlings which had germinated prior to January 23rd, 1963.

1961 Long-distance Pollination: Tree 46 at Lake Abitibi failed to produce any seeds from pollination with the Maine pollen. Seeds from the cross 42 × Maine and open-pollinated seed from this same tree were substituted for tree

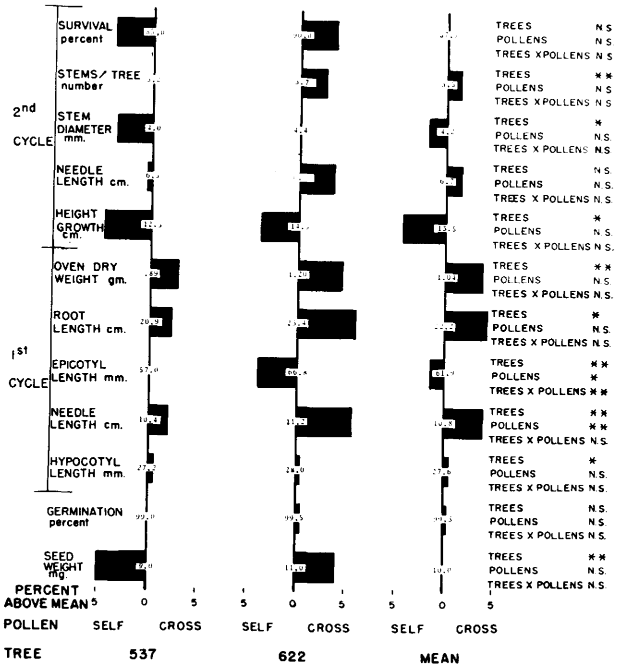

Figure II — 3. — Graphic presentation of data from Experiment Vivian 1958 A. The numbers centered on the vertical "tree" lines represent the mean values for the variables. N. S. = non-significant; * = significant at five percent level; ** = significant at one percent level.

46. Two replications, containing 25 seeds from each of the long-distance pollinations and the open pollinations from each of the 13 trees, were germinated in Petri dishes on December 9th, 1962. When the seeds had germinated, they were planted out in individual pots in a greenhouse. Thirteen seedlings per row were placed in a randomized row design in each of three blocks. In February, 1963, when the seedlings were approximately two months old, cotyledon numbers, hypocotyl length and survival were recorded for all seedlings in the three blocks.

Results

1958 and 1959 Pollinations

Cone mortality was high in the Vivian, Ontario area in the summer of 1959. A cone insect, *Conophtorus resinosae* Hopk. caused most of the losses. Eleven of the 12 pollinations made in 1958 on six red pine trees yielded cones which contained viable seed. Tree 1755 failed to produce any cones following cross-pollination, although two cones containing viable seed were produced after self-pollination

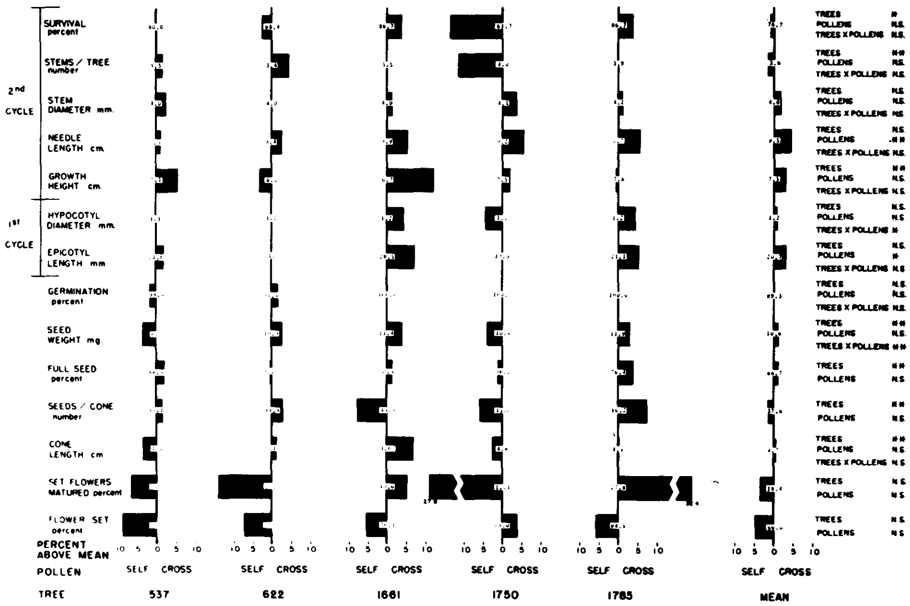

Figure II — 4. — Graphic presentation of data from Experiment Vivian 1958 B. The numbers centered on the vertical "tree" lines represent the mean values for the variables.

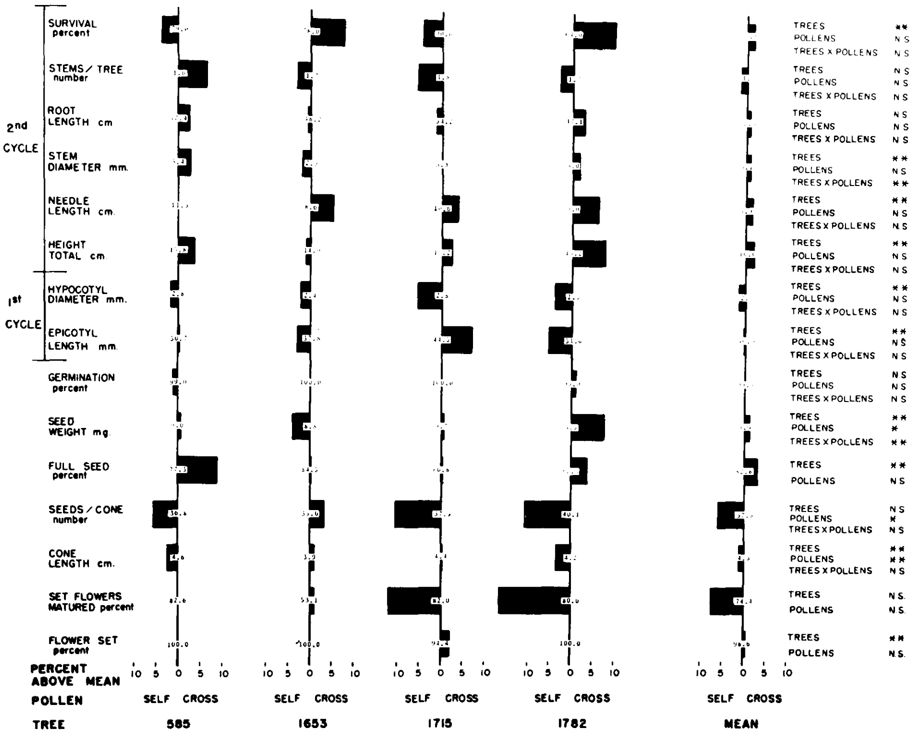

Figure II — 5. — Graphic presentation of data from Experiment Vivian 1959, Self vs. Cross. The numbers centered on the vertical "tree" lines represent the mean values for the variables.

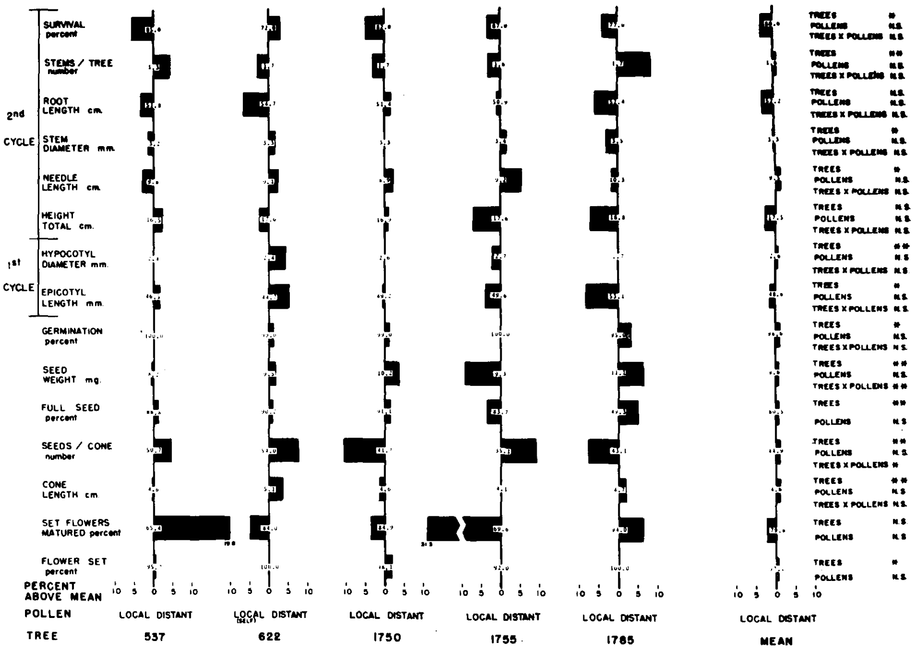

Figure II — 6. — Graphic presentation of data from Experiment Vivian 1959, Local vs. Distant. The numbers centered on the vertical "tree" lines represent the mean values for the variables.

on this tree. This failure of cross-pollination was undoubtedly due to chance and does not indicate that the tree is cross-sterile.

The averages, relative differences between averages and the significance of the differences caused by trees and pollens are presented in *Figures II — 3* to *II — 6*. The significance of differences was determined by analysis of variance.

Vivian 1958 A: Differences caused by trees (maternal effects) were significant in respect to nine of the twelve variables examined. Seedlings, resulting from cross-pollination, had significantly longer needles in the first growing cycle than those from self-pollination. The converse was true for epicotyl length but here a highly significant interaction among trees and pollens was also evidenced.

Vivian 1958 B: Seven of the 14 variables examined were found to differ significantly in respect to trees, while only two indicated that significant differences were caused by pollens (paternal effects). Epicotyl length and second-cycle needle length were significantly larger in progenies from cross-pollination. The interaction among trees and pollens was significant in respect to hypocotyl diameter and highly significant in respect to seed weight. Analysis of covariance failed to reveal any relationship between seed weight and epicotyl length ($F = 1.01$) or between seed weight and hypocotyl diameter ($F = .84$). Tree 537 yielded the lightest seed which produced seedlings with the longest hypocotyls. No phenodeviants were observed among any of the progenies produced by the 1958 pollinations.

All of the 1959 pollinations were successful and yielded cones with viable seeds in the fall of 1960. Seed germination averaged over 99 percent and early seedling mortality was only two percent. During the second growth cycle seedlings in two of the six beds were damaged by what appeared to be a root disease. Analysis of second-cycle growth data was made on the basis of both four and six blocks. The analysis, showing the greater significant differences, is presented in *Figure II — 4.* This is justified by the nature of the hypothesis being tested, that is, that red pine seedlings will not show differences derived from their male parents. Analysis of the survival data was made on the basis of all germinated seedlings (six beds plus four flats).

Vivian 1959 — Self vs. Cross-pollination: Of the 15 variables studied, for the self- and cross-pollinations on four trees, nine were found to differ significantly in respect to trees. Self-pollination gave significantly longer cones and more seeds per cone than cross-pollination. Cross-pollination gave significantly heavier seeds but the interaction between the influence of trees and pollens was highly significant. The interaction among trees and pollens was highly significant in respect to seedling stem diameter. Seedlings, homozygous for a deleterious recessive gene, were found among the "self" progeny of tree 585 (Fowler, 1962). No other pollinations produced abnormal seedlings.

Vivian, 1959 — Local vs. Long-distance pollinations: Significant maternal effects were found in respect to 12 of the 15 variables examined. Paternal effects were not significant in respect to any of the 15 variables, although the interaction between the effects of trees and pollens was significant in respect to number of seeds per cone and seed weight.

1961 Pollinations

Two insects, identified as *Paralobesia piceana* Free. and *Eucosma* sp. (possibly *monitrana* Heinr.) by Miss M. MacKay of the Canada Department of Agriculture, Systematics Unit, Ottawa, caused some mortality of ovulate strobili in the isolation bags at Kane, Pennsylvania.

1961, Self vs. Local vs. Maine: Pollinations with self, local and Maine pollens were made on 45 trees in the spring of 1961. Of these, 9 of the self-pollinations, 11 of the local pollinations and 14 of the Maine pollinations failed to produce any cones. The average number of seeds produced per cone was only 19.7 after the pollinations made at Kane. Intermittent precipitation encountered during the period when the ovulate strobili were receptive necessitated pollination in damp isolation bags. The large number of pollinations made at Kane made it impossible to synchronize these with the time of maximum receptivity of the ovulate strobili. These two factors probably account for the failure of some of the pollinations and the generally low number of seed in the Kane cones.

On the basis of a maximum of 30 seeds per pollination per tree, 2430 full seeds were sown in the completely randomized greenhouse experiment. The germination of seeds sown in individual pots in the greenhouse was slower than the germination in Petri dishes used for the other experiments. Final germination counts were made 60 days after the seed had been sown. At this time 2209 or 90.9% of the seeds had germinated. Of the germinated seeds, 2078 had completed hypocotyl growth and had shed their seed coats. Mortality during the period from onset of germination to seedling measurement was less than .5% and was not associated with any one pollen source. Five cases of reverse germination, where the embryo was reversed in relation to the micropyle, were observed. All of these occurred among the progenies of trees from Watabeag Lake. Four occurred among progenies resulting from Maine pollen and one from self-pollen.

The summarized data from the 1961 pollinations, averaged by climatic regions, are presented in *Table II — 5.* Because of missing values, two comparisons were made among the three kinds of pollen used, in order to utilize as much of the available data as possible. The comparisons were between the effects of self-pollination vs. cross-pollination (self vs. local and Maine) and of local pollination vs. Maine pollination. Again, because of missing data, the ten variables examined were divided into two groups in these tests. The first group of variables consisted of percent conelet set and percent of set conelets matured while the second group comprised cone length, number of seed per cone, percent full seed, seed weight, percent germination, number of days to germinate, seedlings cotyledon number and hypocotyl length. Arcsin transformation was performed on all percentages used in the analyses.

The vector of means for each contrast of each group of variables was tested for significance, by means of HOTELLING'S T test (multivariance), as described by RAO (1952). In addition, multiple correlations with latitude of origin were computed for each contrast and for each group of variables (canonical correlation). Computations were done with an IBM 709 computer. The summarized analyses are presented in *Table II — 6.*

No significant differences attributed to kinds of pollen used were found in any of the four groups of data tested. Three of the four groups of data were not significantly correlated with latitude of origin. The correlation of latitude of origin with the eight variables, pertaining to cone and seedling characters for self- vs. cross-pollination was significant at the five percent level $F = 3.10$ ($F_{.05} = 2.42$). The coefficient of determination (R^2) for this correlation was .542. The adjusted coefficient was .367, indicating that

Table II — 5. — 1961 Pollinations, Averages by Climatic Regions.

Climatic Region	Pollen	Conelet Set, Percent	Set Conelets Matured, Percent	Cone Length, mm.	Seed per cone, number	Full seed, Percent	Seed Weight, mg.	Germination, Percent	Days to Germinate, Number	Cotyledon, Number	Hypocotyl Length, mm.
1	Self	90	10	30	11	66	8	96	22	6.3	28
	Local	76	7	28	10	76	8	100	26	6.4	29
	Maine	74	9	32	18	63	8	93	24	6.1	29
2	Self	91	12	38	25	89	9	92	23	6.4	31
	Local	94	26	35	29	65	8	92	26	6.3	28
	Maine	92	24	35	17	84	9	97	22	6.4	31
3	Self	94	19	37	32	54	9	88	28	6.2	32
	Local	92	24	37	20	46	9	66	30	6.2	33
	Maine	83	13	37	16	52	11	93	23	6.3	30
4	Self	86	18	39	17	67	10	91	25	6.5	33
	Local	88	15	36	11	78	9	85	24	6.2	31
	Maine	89	8	32	10	55	7	85	36	6.2	26
5	Self	86	16	34	21	77	10	87	28	6.5	30
	Local	89	13	37	32	86	9	97	26	6.4	31
	Maine	78	14	36	21	86	9	90	26	6.1	31
6	Self	88	14	40	24	73	11	89	23	6.6	33
	Local	80	16	37	20	71	9	77	31	6.1	31
	Maine	91	22	38	21	83	10	95	25	6.5	33
7	Self	87	39	41	11	81	11	97	24	7.0	32
	Local	87	31	39	19	84	10	90	22	6.7	31
	Maine	77	27	39	15	76	10	98	25	6.5	32
8	Self	96	43	37	20	69	10	95	26	6.6	33
	Local	97	24	38	18	55	11	57	36	6.3	32
	Maine	93	21	43	27	65	10	98	25	6.3	33
9	Self	66	25	32	18	62	10	70	29	6.6	32
	Local	71	24	34	19	76	9	97	23	6.5	31
	Maine	75	33	37	37	75	9	93	23	6.5	31
Watabeag	Self	100	26	33	24	73	6	92	24	5.9	25
	Local	97	22	31	31	79	5	83	26	5.9	25
	Maine	100	49	31	23	57	6	74	26	5.9	24
Abitibi	Self	100	51	37	22	74	7	93	25	6.1	29
	Local	96	36	36	15	71	8	95	26	6.1	31
	Maine	98	59	37	24	89	7	96	24	6.2	30
Mean	Self	89	25	36	20	71	9	90	25	6.4	31
	Local	88	22	35	20	72	9	85	27	6.3	30
	Maine	86	25	36	21	71	9	92	25	6.3	30

Table II — 6. — Summarized Multivariance and Canonical Correlations Analyses.

Comparison	Grouping	Analysis	df	F	
Self vs. cross	Conelet	Multivariance	2,43	1.10	NS
		Canonical Correlation	2,42	.630	NS
	Cone-Seedling	Multivariance	8,22	.604	NS
		Canonical Correlation	8,21	3.10	*
Local vs. Maine	Conelet	Multivariance	2,43	.338	NS
		Canonical Correlation	2,42	.620	NS
	Cone-Seedling	Multivariance	8,20	.534	NS
		Canonical Correlation	8,19	1.243	NS

approximately 37 percent of the variation in latitude of origin was accounted for by the variation in the eight variables.

All simple and multiple coefficients of determination between latitude of origin and these eight variables were calculated to determine which of the variables and combinations of variables were important.

Differences in cotyledon number between seedlings resulting from self-pollination and those resulting from cross-pollination account for approximately 30 percent of the total multiple correlation with latitude of origin. Four variables (number of cotyledons, hypocotyl length, days to germinate and cone length) account for about 83 percent ($R^2 = .449$) of the total multiple correlation. The simple and multiple coefficients of determination of the variables with latitude of origin are presented in *Table II — 7*.

Table II — 7. — Self-pollination vs. cross-pollination. Simple and multiple correlation coefficients between latitude of origin and eight cone-seedling variables.

Variable	Correlation Coefficients
Cotyledon number	.163
Seed weight	.069
Hypocotyl length	.059
Days to germinate	.041
Seeds per cone	.031
Cone length	.014
Germination, percent	.009
Full seeds, percent	.000
Cotyledon number Hypocotyl length Days to germinate Cone length	.449
All variables	.542

The only statistically significant simple correlation was between seedling cotyledon numbers and latitude of origin (*Figure II — 7*). Seedlings resulting from self-pollination of trees from southern latitudes had more numerous cotyledons than seedlings resulting from cross-pollination. The converse was true of trees from northern latitudes. A similar, although weaker relationship, existed between seedling hypocotyl length and latitude of origin. The relationships of days to germinate and cone length to latitude of origin were obscure.

None of the progenies resulting from the 1961 pollinations contained abnormal seedlings.

1961, Long-distance Pollinations: The results of the long-distance pollinations vs. open-pollinations are summarized in *Table II — 8*. For purposes of analysis, no distinction was made between origins of mother trees (Lake Abitibi or Watabeag Lake) and only the general comparison between long-distance pollination and open-pollination was tested.

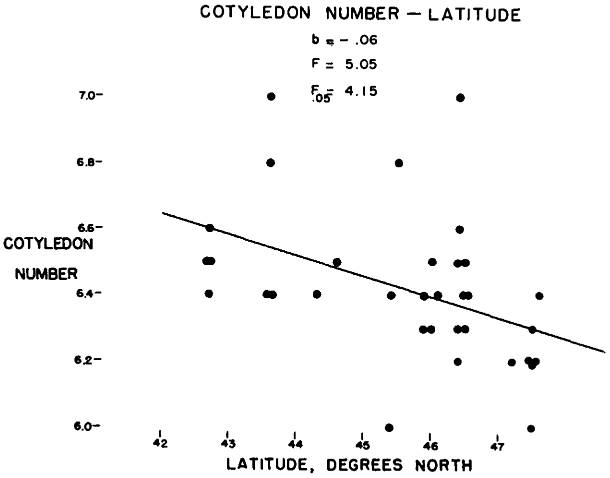

Figure II — 7. — Differences in cotyledon number in seedlings resulting from self- or cross-pollination of trees of different latitudinal origins

The averages of the four variables studied were higher for long-distance pollinations than for open-pollinations. Only in the case of seed germination was this difference significant. The apparent superiority of long-distance pollination is almost entirely due to the fact that the open-pollinated progenies of trees 44 and 47 were inferior. If these progenies are omitted from the averages, the difference between the effects of pollens is no longer significant except in respect to seed germination.

The average germination of full seed resulting from long-distance pollination was 98.3 percent compared with 91.8 percent of seeds from open-pollination. This difference

Table II — 8. — 1961 Pollinations, Lake Abitibi and Watabeag Lake. — Long distance Pollinations — Averages.

Tree	Pollen	Seed Germination, Percent	Seedling		
			Cotyledon Number	Hypocotyl Length, mm.	Survival Percent
40	Pa.	96	6.2	26.9	87
	Open	92	5.7	25.8	87
42	Maine	98	6.0	28.6	85
	Open	98	5.8	27.9	85
44	Wisc.	96	6.1	28.9	82
	Open	58	6.6	25.9	74
45	Ont.	100	5.9	28.8	95
	Open	92	5.8	26.4	75
47	N.Y.	98	5.9	28.0	90
	Open	74	5.7	25.7	54
1350	Pa.	94	6.3	28.6	90
	Open	90	6.1	30.0	85
1351	Minn.	100	5.9	27.6	92
	Open	100	6.2	29.3	92
1352	Minn.	100	6.0	28.3	82
	Open	96	6.1	29.5	92
1353	Wis.	98	6.3	28.4	92
	Open	100	6.1	29.9	92
1354	Maine	100	5.8	26.8	90
	Open	98	6.1	27.1	92
1355	Pa.	100	6.0	26.6	87
	Open	96	6.1	27.2	82
1356	Maine	100	6.1	28.3	87
	Open	100	5.8	27.7	87
1357	N.Y.	98	5.8	27.1	90
	Open	100	6.0	29.3	100
$\bar{x}$	Distant	98.3	6.05	28.03	88.4
	Open	91.8	5.99	27.71	84.4
Averages omitting Trees 44 and 47					
	Distant	98.5	6.06	27.96	88.8
	Open	96.5	5.97	28.06	88.1

was highly significant, as was the interaction between trees and pollens. Analysis of the data, omitting trees 44 and 47, reduced the difference to just above the five percent level ($F = 4.47$, $F_{.05} = 4.32$) and the interaction was no longer significant. Differences between trees and the interaction between trees and pollens were highly significant in respect to all variables except seedling survival.

Discussion and Conclusions

In all, 56 analyses were made testing maternal effects and paternal effects in the pollinations made in 1958 and 1959 at Vivian, Ontario. The main conclusion that can be drawn from these studies is that, while the female parent has a considerable effect on the variables examined, the effect of the male parent is slight. The magnitude of the maternal effects was not large but it was consistent, so that 38 of the 56 analyses revealed the difference among the effects of trees to be significant at the five percent level and that 27 of these differences were significant at the one percent level.

Significant paternal effects were found in seven of the 56 tests. Purely as a matter of chance about three of these tests would be expected to show significance at the five percent level. Of the seven significant differences, three indicate that self-pollination has a superior effect while four indicate the effect of cross-pollination to be superior. With the possible exception of seedling needle length, which was consistently greater after cross-pollination, there is a lack of consistency in the significance of differences among the variables studied. Tree 622 is a case in point. Self- and cross-pollinations were made on this tree in both 1958 and 1959 and seedling growth data are available from two experiments testing the effects of the 1958 pollinations. The relative maternal effect of this tree, in respect to other trees, is fairly consistent in the two years and the two "1958" experiments, while the paternal effect exhibits an almost complete lack of consistency. In experiment 1958 A seedlings resulting from self-pollination on tree 622 had significantly longer epicotyls, while in experiment 1958 B, seedlings from this same pollination had shorter epicotyls than seedlings from cross-pollination and a significant difference, favouring cross-pollination, is indicated.

The analysis of the data obtained from the pollinations on 45 trees in 1961 revealed that the pollen used had little or no effect on the conelet, cone, seed and seedling variables studied. The significant over-all correlation between the different effects of self- and cross-pollination and latitude is difficult to understand and even more difficult to interpret. The main contribution to this correlation is seedling cotyledon number. At first glance, it would appear that self-pollination of trees from southern latitudes was superior to cross-pollination, as the seedlings produced had more numerous cotyledons. This is not neceessarily true. The effects of self- vs. cross-pollination on cotyledon number was studied in *Pinus banksiana* LAMB. (FOWLER, 1965 c). Self-pollination in this species had a definite deleterious effect on most of the variables studied, but it yielded seedlings with significantly higher cotyledon numbers than cross-pollination. It is probable that an optimum number of cotyledons exists for the species as a whole and any deviation from this optimum number is detrimental.

If an increase in the number of cotyledons above a certain optimum value can be considered as detrimental in red pine, a plausible explanation of the correlation between latitude of origin and the difference in cotyledon number, resulting from self- and cross-pollination, is possible. Red pine trees from southern latitudes are closer to the gene centre of this species and can thus be expected to be genetically more variable than trees from northern latitudes. Self-pollination of trees with a relatively high degree of genetic variability would be expected to exhibit a greater inbreeding effect than of trees carrying a light load. Thus self-pollination of trees from southern latitudes would produce seedlings with higher cotyledon numbers, while the converse might be true for trees from northern latitudes.

In species that are heterozygous for a number of recessive genes, individuals of the same origin are likely to carry greater numbers of the same recessive genes than those from widely differing origins. Pollination between trees of the same origin would produce offspring more homozygous in respect to these genes than long-distance pollinations. As individuals homozygous in respect to recessive genes are often inferior to normal individuals, progenies resulting from pollination between trees of different origins can be expected to be superior to progenies resulting from pollinations between trees of the same origin.

The effects of long-distance pollinations were not found to differ from those of local pollination in the 1959 tests, where the effects of local (Vivian) pollen were compared with those of a central Ontario pollen. This was also true of the 1961 tests with local vs. Maine pollens on trees of widely different origins. Two trees among the 13, where the effects of open-pollination and long-distance pollination were compared, exhibited a definite inferiority of the effects of open-pollination. The differences between the two kinds of pollination on the other trees were negligible. It is possible that these two trees are heterozygous in respect to deleterious recessive genes and that open-pollination actually includes a high proportion of self-pollination.

The effect of the female parent on such characters as conelet set, cones matured, cone length, seed weight and possibly germination, is to be expected and does not require any genetic explanation. These characters are related to the nutritional status of the mother trees and can be modified by the environment in which the trees grow. If the correlation between seed weight and seedling epicotyl length and seed weight and seedling hypocotyl diameter had been strong, the effect of the mother tree through the second growth cycle might be explained. No correlation between these characters was detected.

The strong effect of the female parent and the negligible effect of the male parent on several characters of the progenies, resulting from controlled pollinations, suggest that parthenogenesis might be important in this species. The question of parthenogenic seed development is examined in more detail in Section III (FOWLER, 1965 b). No evidence suggesting that parthenogenesis occurs in red pine was found. This is also supported by the fact that tree 585, the carrier of a recessive marker gene, produced no seedlings homozygous for this gene when cross-pollinated.

It is doubtful if the presence of dominant genes in this species could account for the significant effects of the female parent on its progeny. This explanation would be plausible if only a few pollinations were made using pollens from single trees but, as pollinations were made on many trees and as pollen mixtures were used in most instances, this explanation is unsatisfactory.

There are two possible explanations for the relatively large maternal effects on progenies. The first is that minor differences in the environment of a tree may have a measurable effect on its progeny or, second, that some factors affecting growth are transmitted through the cytoplasm.

The gametophyte tissue which provides the food supply for the developing embryo, the germinating seed, and the very young seedling is maternal and is produced before fertilization takes place. It is a product of only the female parent and, as such, it might be expected to exert a stronger effect on the developing seedling than the endosperm of angiosperms, which is a product of both parents. Either quantity or quality of the gametophyte tissue may affect the developing seedling. Minor differences in environment could conceivably affect either the quantity or the quality of this tissue.

Extranuclear inheritance, the transmission of hereditary characters by means of the cytoplasm rather than by the conventional gene-chromosome system, has been demonstrated for several species of plants. It is not inconceivable that it could also be important in red pine.

The writer favours the first explanation in accounting for the effect of the female parent on the resulting progeny, however, should the differences in mother trees persist over a period of several growth cycles, it will be necessary to reconsider the possibilities of cytoplasmic inheritance as an explanation of this phenomenon.

During the period of 1958 to 1961, self-pollinations were made on 55 trees; of these, 46 were successful and produced seedlings that were evaluated as normal or as homozygous for detectable recessive genes. The progeny of only one of these trees contained aberrant seedlings.

Why should red pine differ so markedly in these respects from other *Pinus* species which have been studied? There is evidence that red pine or a red pine-like species was present in North America during the Cretaceous period (Pierce, 1957; Chaney, 1954). Assuming that this was red pine, or a close relative, this species must have existed in an almost unchanged form for at least 100 million years. Such a species would have to possess a very stable genotype and yet it would have to be capable of surviving under widely different environmental conditions. To maintain such a stable genotype, the mutation rate of the species should be extremely low and, or, a system to keep mutant genes from becoming established should exist.

Red pine is an extremely uniform species both morphologically and genetically and yet it is capable of surviving and reproducing over a wide range of climatic conditions. Nothing is known of the mutation rate in red pine, but a mechanism does exist for controlling the number of mutant genes it carries. This mechanism is its method of natural reproduction. Under natural conditions, red pine reproduces after stand disturbances, such as fire. These disturbances often reduce the breeding population to a few trees which, in turn, supply the seedlings for the disturbed area. As the breeding population is greatly reduced in comparison with the actual population, a high degree of inbreeding is to be expected. Inbreeding in successive generations would result in the rapid elimination of deleterious mutant genes and tend to maintain the homozygosity of the species.

It is concluded that red pine, both as individual trees, and as a species, is homozygous in respect to most alleles, self-fertile, self-compatible and that seedlings resulting from self-pollination exhibit little or no inbreeding depression in respect to growth.

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Summary

The effects of self-pollination, in comparison with cross-pollination, on conelet, cone, seed and seedling characters were examined in 55 red pine trees. The effects of cross-pollination with widely different sources, in comparison with pollination with pollen from a local source, were examined in 63 trees.

The effect of the male parent was negligible on the characters studied, whereas the influence of the female parent was strong. The effect of the female parent persisted through the second growth cycle. This maternal effect is attributed to environmental factors rather than to genetic effects, although the possibility still exists that extranuclear inheritance may be important.

Of the 55 trees on which self-pollinations were attempted, 46 produced seedlings which were evaluated as normal or mutant. The progeny of one of these trees contained chlorotic seedlings homozygous in respect to a recessive gene.

It is concluded that red pine, both as individual trees and as a species, is homozygous for a large number of alleles, self-fertile, self-compatible and that seedlings resulting from self-pollination exhibit little or no inbreeding depression.

Zusammenfassung

Titel der Arbeit: *Effekte der Inzüchtung bei Pinus resinosa. II. Bestäubungsuntersuchungen.*

Die Auswirkungen von Selbstbestäubungen im Vergleich mit Fremdbestäubungen wurden bei Blüten, Zapfen, Samen und Sämlingsmerkmalen von 55 Kiefern untersucht. Bei 63 Bäumen wurde ferner Fremdbestäubung mit Pollen sehr unterschiedlicher Herkünfte mit Fremdbestäubung mit Pollen örtlicher Quellen verglichen.

Die Einwirkung des männlichen Elters auf diese Merkmale konnte vernachlässigt werden; der Einfluß des weiblichen Elters war dagegen stark. Er überdauerte den „Second Growth"-Zyklus. Dieser mütterliche Effekt wird vorwiegend Umweltfaktoren zugeschrieben und nicht so sehr genetischen Einflüssen, obwohl noch die Möglichkeit besteht, daß eine nicht-kerngebundene Vererbung von Bedeutung sein kann.

Von 55 Bäumen, auf denen Selbstungen versucht worden waren, produzierten 46 Sämlinge, die sich normal oder abgeändert entwickelt hatten. Die Nachkommenschaft eines dieser Bäume enthielt für ein rezessives Gen homozygote chlorotische Sämlinge.

Man kann aus den Untersuchungen schließen, daß *Pinus resinosa*, sowohl Einzelbäume wie die ganze Art, für eine große Anzahl Allele homozygot ist, daß sie selbstfertil und selbst-compatibel ist und daß die Sämlinge aus Selbstungen nur geringe oder gar keine Inzuchtdepression zeigen.

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Effect of the Chemical Mutagen Ethyl Methanesulfonate on Western White Pine

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Introduction

Induced mutation as a method of increasing genetic variability in trees has so far been neither profitable nor practical. This is largely because of the length of time required to obtain segregating second and later generation (M_2, M_3, etc.) progenies and to screen for genetic mutants. Also, the availability of large, virtually untapped pools of variation obtainable from natural populations and hybridization has inhibited research in use of induced mutation.

Disease resistance is one characteristic that holds considerable promise for improvement by induced mutation (Bhatia *et al.*, 1961; Konzak, 1956; Sparrow and Konzak, 1958). In systems involving hosts and obligate parasites, such as the rust fungi, a delicate physiological relationship exists. Mutations causing a subtle alteration in the host's physiology could be potential sources of genetic resistance. In addition, rust resistance is often a relatively simply inherited character (Stevenson and Jones, 1953). Therefore, it may be amenable to improvement through selection of resistant mutants.

The U. S. Forest Service has a long-range program aimed at genetic control of the blister rust fungus, *Cronartium ribicola* A. Fisch. infecting western white pine (*Pinus monticola* Dougl.). Progress has been made towards production of resistant varieties by family selection (Bingham *et al.*, 1953; Bingham *et al.*, 1960). The number of selected trees that have been progeny-tested and reselected on the basis of general combining ability for a fair level of resistance is now small. Researchers expect that about 25 reselected parents will be available in each of three seed orchards. In addition to this within-species breeding program, work has begun to transfer the inherent resistance of certain species to western white pine through interspecific hybridization. The present study is the first effort to use in-

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