Chairman: A. D. Nutting

INITIAL STUDIES INDICATE PINUS RESINOSA LITTLE AFFECTED BY SELFING

D. P Fowler¹

INTRODUCTION

The genus <u>Pinus</u> is generally considered to be composed of species which are dependent upon cross fertilization for the production of normal progenies Information supplied by such workers as Bingham and Squillace (1955), Mergen (1959), Perry (1960), Righter (1958), and others supports this general conclusion.

Most of the <u>Pinus</u> species studied exhibit a considerable amount of morphological variation, at least some of which can be attributed to genetic vatiation Red pine, <u>Pinus resinosa</u> Ait would appear to differ from many other members of the genus in that it exhibits very little morphological variation. If this uniformity is the result of genetic uniformity, then one would expect that red pine would be relatively self-fertile and selfed progenies would not exhibit many phenodeviants or suffer serious inbreeding depression in growth.

To test this hypothesis, a series of experiments concerning the effects of inbreeding in red pine was initiated in 1958. This paper presents some of the preliminary findings of this work.

MATERIALS AND METHODS

The trees selected for the initial aspects of this study are located in a plantation managed by the Ontario Department of Lands and Forests at Vivian, Ontario. The exact origin of the trees in the plantation is not known, but it is suspected that the seeds originally came from the Ottawa valley. The only criterion for selection of trees within this plantation was that they had enough female flowers to produce the desired number of crosses.

Five trees were selected in 1958 and four more in 1959 for artificial pollination work. The pollination technique used throughout the work was similar to that described by Mergen et al (1955) for slash pine. The main difference from Mergen s method was that the upper portion of the sausage casing bags used for isolation of the female flowers was coated with aluminum paint and the bags were secured with twistems rather than staples.

¹Ontario Department of Lands and Forests. Maple, Ontario, Canada The material presented in this paper is a portion of a comprehensive study of the effects of inbreeding in red pine, undertaken in partial fulfillment of the requirements for the Ph. D. degree, Yale University.

Two different pollens were used on each tree. Approximately half of the isolated female flowers were pollinated with pollen from the same tree (self) and the remaining flowers received a mixed pollen (mixed). The mixed pollen consisted of pollens obtained from equal volumes of male flowers collected from twenty trees in the same plantation. No pollen from the trees selected for this study was included in the mixture.

Observations on time of pollen shedding and time of female flower receptivity as well as the position of male and female flowers in the tree crowns were made. Data on flower set were recorded approximately three weeks following pollination, when the isolation bags were removed from the flowers.

In the fall of 1959 and again in the fall of 1960, the cones resulting from the 1958 and 1959 pollinations respectively were collected and counted. The cones were dried and the seed extracted. Data on cone length, number of seeds per cone, and number of full seeds per cone were then obtained.

Experiment 1. In February of 1960, one hundred seeds from each of the self and mixed pollinations from each of two of the 1958 trees were germinated in Petri dishes and planted out in flats in an experiment at Yale University. The design of the experiment consisted of ten blocks with randomized rows of ten seedlings from each of the four crosses. As a few of the seeds failed to germinate, only nine of the ten blocks were complete. The seedlings were grown in a greenhouse until December of 1960 when they were lifted and shipped to Maple, Ontario, where they were heeled-in for the winter. In May of 1961 the seedlings were lifted and the following measurements made hypocotyl length, length of the longest needle, root length and top length. Two seedlings from each of the blocks were randomly selected for oven dry weight determinations. The remaining seedlings were planted out in a nursery for further study.

Experiment 2. In June of 1960 thirty seeds from each of the self and mixed pollinations, from each of five of the 1958 trees, were individually weighed and the seeds subsequently kept separate, so that seed weights could be correlated with seedling growth. The seeds were germinated in Petri dishes and planted out in three completely randomized blocks in a greenhouse, In January of 1961, the seedlings were scored for presence or absence of secondary needles, height above cotyledons and hypocotyl diameter.

Experiment 3. The cones and seeds of the 1959 crosses were subjected to the same measurements as the cones and seeds used in experiment 2. The seeds were germinated and planted in a randomized block design in a greenhouse in December of 1960. No further measurements have been made.

RESULTS

None of the red pine trees studied indicate any tendency toward dichogamy. The position of the male and female flowers in the crowns of the trees examined also offers no obstacle to selfing. The male flowers were most plentiful on the lower branches and the female flowers were concentrated on the upper branches but there was a considerable portion of the crown on which both male and female flowers occurred.

Of the nine trees studied in these experiments, only one carried a recessive mutant that was detectable among its progeny. The selfed progeny of this tree contained seedlings which exhibited what appeared to be a chlorophyll deficiency. The ratio of mutant to normal was found to be approximately 1:5. This is probably a simple single gene recessive mutation, in which the divergence from an expected 1:3 ratio can be explained by a lower survival of homozygous recessive seeds and seedlings.

The results of experiments 1, 2, and 3 are presented in figures 1, 2, and 3.

FIGURE I. - EXPERIMENT I.



* DIFFERENCE SIGNIFICANT AT 5% LEVEL

** DIFFERENCE SIGNIFICANT AT 1% LEVEL

1/ INTERACTION BETWEEN TREES SIGNIFICANT

105 PERCENT 100 OF 95 AVERAGE 90 0 E PERCENT FLOWER NO. FULL SEED PERCENT NO. CONE SEED CONES LENGTH SET PER MATURED PER CM CONE CONE 91.8 SELF 20.9 38.1 34.3 4.65 MIXED 84.3 21.3 37.0 33.4 4,70 105 * PERCENT 100 OF 95 AVERAGE 90 SEED' WEIGHT GERM PERCENT HEIGHT DIAM. AT 14 WITH ABOVE COTYLEDONS MM. SECONDARY .000GM DAYS NEEDLES 10.5 68.7 SELF 1.14 96.0 20.1 MIXED 10.7 96.0 74.7 21.3 1.16

FIGURE 2. - EXPERIMENT 2.

* DIFFERENCE SIGNIFICANT AT 5% LEVEL



Effect on Flowers, Cones and Seeds

From the information obtained from these crosses, it would appear that the proportion of flowers set, proportion of cones matured and the size of the cones is not dependent upon the kind of pollen used. Selfing had no effect on these characters. This result is not unexpected for red pine, in that it would appear that pollination is not necessary for flower set and that, very likely, any type of pollen would be satisfactory to stimulate normal cone development.

The data obtained from both experiments 2 and 3 Indicate that self pollinations may result in a greater number of seeds per cone. The difference between self and mixed pollens was significant only in experiment 3 and then only at the 5 percent level. When number of seeds per cone was reduced to number of full seeds per cone, this difference was not as great and no significance was found. Though the evidence is very weak, it would appear that the self pollination resulted in a slightly higher seed set than mixed pollination, but that a slightly higher proportion of the seed was empty. If this is the case, then an explanation of the phenomenon evades the writer.

The effect of self or mixed pollination on seed weight is also obscure. Some trees produced heavier seed from self pollination, whereas on other trees the heaviest seed resulted from mixed pollination. This is indicated by the highly significant interaction found In the data from both experiments 2 and 3. Even if an actual difference is present, the effect of type of pollen used is very slight.

Germination from all the crosses was very good and no evidence was found to indicate that the type of pollen used had any effect.

Effect on Seedling Growth

Analysis of covariance was used to determine if seed weight was correlated with height above cotyledons or with hypocotyl diameter. In neither instance was the regression significant.

No evidence was found which suggests that hypocotyl size was effected by the male parent of the progeny.

Data from experiment 1 indicate that the progenies resulting from mixed pollinations produced longer needles. The difference between selfed and mixed progenies was highly significant. There is also some evidence from experiment 2 that the out-pollinated (mixed) seedlings produced secondary needles earlier than the selfed seedlings, though the difference was not significant. Root length of out-pollinated (mixed) seedlings was slightly greater than that of selfed seed-lings, but again this difference was not significant.

The seedlings of the two trees used in experiment 1 indicated a significantly greater top growth of the selfed seedlings from one of the trees and no difference in top growth between selfed and out pollinated seedlings of the other tree In experiment 2 in which seedlings of these same two trees were also grown, out- pollinated seedlings showed a greater height growth above cotyledons than did selfed seedlings. This difference was significant at the five percent level.

The out pollinated seedlings of experiment 1, had a slightly, but not significantly greater, oven-dry weight than the selfed seedlings.

The data on seedling growth seem to indicate that seedlings resulting from mixed pollen may be slightly more vigorous than selfed seedlings but it would certainly appear that any depression in growth, resulting from self pollination, is very small.

CONCLUSIONS

The effects of inbreeding upon flower set, cone maturity cone length, number of seeds per cone, seed weight and germination of seed are negligible for the nine trees studied The effects of inbreeding on early seedling growth, if any, are very small.

It can be concluded that these nine red pine trees differ from many other species of <u>Pinus</u> in that they are relatively homozygous, contain very few deleterious recessive genes, are self-compatible and self--fertile, and produce normal progenies following self pollination.

Further objectives of the inbreeding study are to determine if the ability to produce normal progenies following self-pollination is a characteristic of red pine regardless of its origin and, if this is found to be the case, to determine if differences exist between subpopulations of this species.

If red pine in general should prove to be as uniform as these experiments indicate for the nine trees thus far studied, it will be necessary to revamp completely our present ideas on selective breeding, seed production, etc, if we expect to make any progress. If it is found that red pine is uniform but that it exists in discontinuous uniform subpopulations, then possibly the best approach for improvement work will be in hybridization between different relatively inbred subpopulations. The interspecific approach to breeding red pine has thus far not been very promising, but it may not be impossible as Duffield and Snyder (1958) have reported a successful cross of red pine and <u>Pinus nigra</u> Arnold at Placerville.

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