

VARIATION AND INHERITANCE
OF SOME PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS IN
PSEUDOTSUGA MENZIESII (MIRB.) FRANCO VAR. MENZIESII

by

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Dipl. For.Eng., The University of Sopron, Hungary, 1946
M.F., The University of British Columbia, 1961

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ABSTRACT

The objectives of this study were to describe the variability, to evaluate the combining ability, and to calculate the heritability values for certain characteristics of Coastal Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco var. menziesii). Four trees (A, B, E, and 11) were selected on the University of British Columbia Campus. Three of them were selected from the local natural population while the fourth came from an unknown provenance. The investigation of variation included phenological observations on flushing and flowering times, and quantitative descriptions of pollen, seed and cone size.

A survey of Campus trees showed that Douglas-fir is extremely variable in the time of flushing and flowering, the size of pollen, seed and cones, and the total number of filled seeds. Times of flushing did not determine times of flowering. There was a strong negative correlation between pollen size and time of flowering. This suggests existence of adaptive significance to adverse climatic condition. Variation in width of the cone was greater than in cone length.

Seed germination percentage appeared to be inherited on a single factor basis, and the results from F₁ crosses substantiated the suggestion that tree E might possess a homozygous dominant state. Filled seeds have not been obtained from tree B when it was self-pollinated. This supports Orr-Ewing's theory, that self-sterility might be an inbreeding effect caused by the action of lethal genes, when brought together in a homozygous state.

Combining ability of the four study trees was tested by a polyallel cross with all sixteen possible combinations. The cross was completed in 1962, using three different pollination methods; dry, wet and dry-wet. Mortality of conelets was lowest in the case of wet pollination. Losses were doubled with dry pollination. Of 302 seed conelets pollinated, 201 were collected and 8,004 seeds were extracted from them. The number of

filled seeds per cone was lowest in the cases of self-pollination (1.91) and wind pollination (3.05). Cross pollination on the average surpassed wind pollination by 4.6 times, and the self-pollination by 7.3 times, producing 13.81. filled seeds per cone.

In order to minimize and test the variability due to environmental effects, the seedlings were grown under controlled environmental conditions. Two Percival (PGC-78) units were employed, one of them simulated long-day (15 hours illumination) and the other short-day (10 hours illumination) effects for 132 days.

Tree 11, which was different in origin from the local provenance trees, showed the best combining ability as a seed parent. Progeny from crosses between trees from the same populations showed smaller values compared to progenies from crosses between trees from different populations. Epicotyls, for example, were 73-78 per cent longer on seedlings from tree 11 compared to seedlings from trees B and E, when pollen from tree A was applied. Obviously, further investigation of intra-specific crosses has practical merit.

Heritability values in the narrow sense were calculated for twelve different juvenile seedling characteristics, and the practical application in relation to forest tree improvement was briefly discussed.

GRADUATE STUDIES

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Tree 11, which was different in origin from the local provenance trees, showed the best combining ability as a seed parent during short-day treatment, and also proved to be a good pollen parent. Progeny from crosses between trees from the same populations showed smaller values compared to progenies from crosses between trees from different populations. Shoots, for example, were 73-78 per cent longer on seedlings from

tree 11 compared to seedlings from trees B and E, when pollen from tree A was applied. Obviously, further investigation of intra-specific crosses has practical merit.

Heritability values in the narrow sense were calculated for twelve different juvenile seedlings (root-, hypocotyl-, epicotyl-, and branch-length, number of branches and number of cotyledons, diameter of root collar, green and dry weight of roots and shoots, and light transmittance of chlorophyll) characteristics, and the practical application in relation to forest tree improvement was briefly discussed.

P.G. Haddock

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1 INTRODUCTION

Forest genetics is the study of variation and heritability in forest trees. It is concerned with similarities and differences of various traits between related trees and their transmittance to the next generation.

Variation itself is a product of differences between individuals and the effects of environmental modifications, genetic recombinations and mutations (Stebbins, 1957). An understanding of the role of the above factors is essential in any genetic studies. The variation pattern from tree to tree and from stand to stand as well as the population composition throughout the range of the species must be known before any improvement work can be planned on a logical basis. The calculation of genetic gain requires an estimate of heritability which will express the probability that certain characteristics will appear in future generations. Only limited information is available at present on the inheritance of various traits of forest tree species. Consequently, most of the information on genetic gain is based on speculation rather than on actual calculation.

This investigation deals only with variation and heritability of certain morphological and physiological traits of Douglas-fir. The species range extends from central British Columbia into Northeastern Mexico, and from the Pacific Coast to the eastern slopes of the Rocky Mountains. The vegetation and phytogeography of the Douglas-fir region of the Pacific Northwest are described by Haddock and Schmidt (1957). Bio-

climatic zone descriptions for British Columbia have been made by Krajina (1959). The economic importance of Douglas-fir in British Columbia, especially within the Vancouver Forest District, was mentioned by Smith et al. (1961).

The species exhibits a high degree of genetic variability which has been noted by numerous authors, and has been expressed by Larsen (1937) in the following way:

"One has to travel very widely throughout the natural range of Douglas fir in order to get an impression of differences in geographical type, but standing on one place one can, without moving a foot, see many individuals differing widely in their structure; it is often more difficult to pick out those that resemble one another. Although they belong to the same species and the same geographical type, yet they can be widely divergent in respect of their economic value in forestry. This can be observed everywhere. It does not matter if one chooses in California a site in the Coast Range or in the Sierra Nevada, passes through Oregon and Washington, or in British Columbia selects a place on Vancouver Island or in the Rocky Mountains; everywhere one is bound to be impressed by the great individual variation of this tree-species."

The trees involved in this study were selected from Douglas-fir trees, previously used by Orr-Ewing (1956) and Allen (1961) in their experiments. The long life cycle in forest trees creates special problems in their breeding. Therefore complete and accurate record keeping on pertinent observations is absolutely essential. The data supplied by Orr-Ewing and Allen on phenology, combining ability and germination were the basis for the selection of four trees on the U.B.C. Campus. Further experiments are planned featuring these trees. Analysis of wood quality is underway on the four-year-old progenies (Kennedy, 1964) and field progeny tests are to be established at the University Research Forest, Haney, during the spring of

1964, using the seedlings grown from 1958 and 1962 crosses.

The objectives of this investigation of Douglas-fir may be stated as:

1. To determine the variation of some morphological and physiological traits at the inter- and intra-tree level.
2. To test the combining ability of four trees, using the complete polyallel cross and controlled environment.
3. To estimate heritability in the narrow sense for the twelve juvenile seedling characteristics studied and to use these estimations in planning a selection program.

2 REVIEW OF PAST WORK

21 Variation

There is a large literature on the variation of Douglas-fir. Little (1952) mentioned that Murray in 1869 was the first to realize the differences between Coastal and Inland Douglas-fir, 78 years after the discovery of this species by Menzies. The great geographic range and wide variation within the species account for the fact that the taxonomy of the species is still unsettled.

Flous' (1936) findings reflect the difficulties in classification when it is based only upon morphological and anatomical characteristics of herbarium specimens. She studied needle and cone characteristics on close to 150 specimens collected by different persons throughout the range of Douglas-fir. She described 11 species and one variety and could have described many more, if, using the same codes for classification, she had been in possession of a larger number of samples. Rehder (1947) mentioned three varieties and seven forms. Peace (1948) and Franco (1950) described three varieties. Recently Tusko (1963) recognized two subspecies Pseudotsuga menziesii (Mirb.) Franco ssp. menziesii, Coastal and Pseudotsuga menziesii (Mirb.) Franco ssp. glaucescens (Bailly) Schwein, Interior or Rocky Mountain Douglas-fir. Haddock (1962) stated:

"Older efforts to distinguish between a "glauca" and a "caesia" (gray) form within the interior

portion of the range should not be continued because they serve no useful purpose and only confuse the issue. Considerable variation in cone and leaf morphology, color of foliage etc. has been noted throughout the range of the species. The real differences between geographic provenances are much better measured by growth rates and other factors such as low temperature tolerance and length of growing period."

The literature citation in this review will be divided into broad categories somewhat similar to those used in the thesis.

211 Geographic variation

The importance of geographic variation of seed source was realized early, following Vilmorin's work from 1821-1850. Pinus sylvestris L. seeds were collected from 30 different geographic origins and were grown on his estate in France (Vilmorin 1862). Considerable deviations were observed in height and diameter growth, straightness of the stem, and needle length. His work clearly showed Pinus sylvestris L. varied considerably in its genetic constitution among different geographic origins. Further work by Cieslar, Dengler, Schott, Schwappach and Engler (Kalela, 1937) paved the way for Langlet's (1936) findings. In agreement with the previous investigators, he reached the conclusion that the local provenance is the best, and for planting Pinus sylvestris L. in southern Sweden, the seed should not be transferred more than 250 kilometers (approximately 150 miles) in a north-south direction, and 300 meters (approximately 900 feet) difference in elevation. His conclusion was widely accepted in

Europe and even in North America. Seed collection zones were worked out for the most important species in Europe and Isaac (1949) established similar rules for collecting and transferring Douglas-fir seeds in the Pacific Northwest.

Langlet's original statement was well-founded, but the supporting evidence for other species and other regions has come from low-precision, unreplicated experiments. There are several instances in which a non-local provenance has been proven definitely superior to the local seed source (Wright, 1962). Plantations with exotic species proved to be successful in many cases. Wright also mentioned an experiment on Douglas-fir from the Pacific Northwest Region. The plantation was initiated by Willis and Hoffman (1915), and reported by Munger and Morris (1936, 1942), which showed that the Granite Falls and Darrington trees are consistently among the leaders at each of five test localities, even after a quarter of a century.

The Research Branch of the Oregon State Board of Forestry at Corvallis initiated a cooperative provenance test in the Pacific Northwest in 1954. Sixteen provenances were included in the test, five from British Columbia, three from Washington and eight from Oregon. Ching and Bever (1960) reported on the performance of two-year-old nursery stock at Corvallis. The Vancouver Island and the Shelton provenances showed a better

growth than those of local origin. Significant variation was found in needle length and a correlation was detected between needle length and total height. Bud bursting was generally earliest in the southern provenances, but one northerly provenance (Courtenay) flushed just as early. Bud setting was earliest in the southern and Vancouver Island provenances, except Nimpkish. The results indicate that time of bud flushing and setting has no apparent effect on height growth. Similar observation was made by Griffith (1960) on radial growth on 68 Douglas-fir trees at the University Research Forest, Haney. Heaman (1963) reported on the same provenance test based on the first two-years field performance at the five test sites in British Columbia. Proper evaluation was possible only for two sites, Nimpkish and Courtenay. The yearly height growth and the total height were significantly different at both locations. The low-elevation provenances from Shelton and Sugar Loaf Mountain (Vancouver Island) showed better performance than the local provenances.

Revel (1960) evaluated one of the two provenance experiments of Dr. Haddock. Three sources each of Coast and Interior provenances were grown in soil in the greenhouse for seven months. Significant differences were found in height growth, vigour, needle color, and terminal bud condition, whereas no pronounced differences showed up in either mortality or number of Lammas shoots produced.

Hutchinson (1961) on the other hand mentioned "in Douglas-fir there are three growth cycles per year, under optimum

conditions, while in trees from mountain areas a single short period is noted". He also found significant differences in resistance to frost among the different provenances he worked with.

Morris et al. (1957) suggested that bud bursting is under strong genetic control. Carmichael (1961) observed polymorphism with respect to color of foliage.

Campbell (1958, 1963) studied the variations in crown form attributes. He sampled (1958) 300 trees from 10 areas throughout southwestern Washington, and found within-tree "variation in the stem interwhorl length, and in the ratio surface-area-of stem versus cross-sectional area of branch bases was very large". Between-tree variation was also large in the characteristics which make up the crown of a tree. Significant differences between area means were found for each attribute studied. Possible patterns of geographic variation for number of branches, length of branches, and cross-sectional area of branch bases were mentioned.

Irgens-Möller (1957) subjected seven Oregon provenances from elevations of 60 to 4000 feet to a short-day (9 hours light) and long-day (15 hours light) treatment. The date of bud bursting was not affected by the different treatments among the low-elevation provenances, but there was a significant difference for the high-elevation seedlings, in which long days hastened the bud bursting. Seven provenances and four light treatments were used in a further experiment by Irgens-Möller (1958). Seedlings from Interior British Columbia showed the

greatest differences in response to photoperiods. Again in a later experiment (Irgens-Möller, 1962) he found less variability in the Coast provenances than in seedlings of continental origin. He explained this as being perhaps the result of the wider range of environmental conditions under which the seedlings from continental origins grow.

Nicholson (1963), experimenting with 12 Interior and 16 Coastal provenances, using short day (6 hours light) and long day (18 hours light) for 52 days, was able to separate Coast and Interior provenances grown under short-day treatment more readily than those grown under long-day treatment. He also observed distinct groups within the Interior provenances, while no definite regional grouping was distinguishable within the Coastal provenances.

2111 Variation in cone characteristics

Peace (1948) personally covered the northern part of the Douglas-fir range and noted the large variation in cone characteristics within the species. He found the cone length varied between 3.4 and 8.4 centimeters (cm). Cones from the coast tend to occupy the upper end of the range. The reflexed bract characteristics were observed on the coast, while cones "without reflexed bracts" were found in the Rocky Mountain area; although the reverse situation had in the past been considered to prevail. Tusko (1963) collected samples from 43 provenances across British Columbia from East to West and found the average cone length to be 5.4 cm., ranging from 3.2 to 9.3 cm. The average

cone width was 2.1 cm. with a range between 1.6 and 2.7 cm. A certain overlap was observed in these characteristics so far as Coast and Interior origins were concerned, but generally the Coast provenances exhibited larger values in both characteristics. Willett (1963) measured the cone length and width from 22 Coast and 8 Interior provenances, from Nimpkish Lake (Vancouver Island) to Kananaskis (Alberta) on 348 trees. The average cone length for provenances was 6.0 cm. with a range from 5.1 cm. to 7.7 cm. and cone widths averaged 2.1 cm. with a minimum of 1.8 cm. and a maximum of 2.4 cm. for the different provenances. Longitude and latitude of the collection area, the height, diameter at breast height, crown width and age of the tree explained only 9.3 per cent for cone length, and 13.2 per cent for cone width of the total variation. This suggests that other environmental and probably genetical variables are also important.

2112 Variation in seed characteristics

Allen (1960/a, 1961) separated Coastal and Interior provenances on the basis of morphology of seeds. Seed from the Interior origin is "generally broader in proportion to its length; it has a shorter micropylar tip, a smoother and shinier outer surface, and fewer seed-coat wrinkles" than the Coastal seed. He noted that differences appeared to be under genetic control, assuming "that each came from a relatively confined refuge following the retreat of the glaciers, or that some of the characteristics have survival value or are linked with features that have".

Dunlap (1964) working on the same provenances as Robinson (1963) and Willett (1963) confirmed Allen's (1960/a) findings. The well-expressed contour ridges and the darker color on Coastal seed sources were the best characteristics to separate them from Interior sources. Robinson (1963) studying the variation in size of seed on 348 trees from 30 different provenances, found significant differences in both the length and width of seed and wing, among the different provenances. Generally, Coastal provenances exhibited larger values for the above-mentioned characteristics.

Identification of Coastal and Interior provenances during the germination period was worked out by Allen and Bientjes (1954), Allen (1958, 1960/b, 1961). Incubation temperature at 10° C or 15° C tended to separate these two provenances better than the more optimal 25° C temperature. The 10° C even separated the two geographic origins when seeds from the two areas were stratified for 40 days prior to germination. Days to reach 10 per cent of germinative capacity (R_{10}) were below 8 for Interior provenances whereas the values for Coastal seed were nearly always above 9, often above 12 and as high as 25 or even higher. Dry-belt Interior sources gave the lowest values, while the Prince George - Quesnel sources behaved more like the faster germinating Coast origins. Allen (1961) also mentioned the possibility of splitting the Interior provenances into two or more groups.

22 Heritability

The portion of the observed variance for which differences in heredity are responsible, called heritability (Knight, 1948), is a very valuable estimate in genetic improvement work. Estimates of heritability are necessary to express the reliability with which the phenotypic characteristics may be expected to appear in the future progeny. They can also be used to calculate the genetic gains that may be expected in the next generation.

Estimation of heritability can be carried out in various ways; Warner (1952) mentioned the following methods:

1. calculation of parent-offspring regression,
2. study of variance components from analysis of variance,
- and 3. approximation of nonheritable variance from genetically uniform populations.

Lerner (1958) described the methods somewhat differently:

1. comparisons of phenotypic traits displayed by monozygotic as against dizygotic twins,
2. calculations of parent-offspring regressions,
3. correlations between full-sibs and half-sibs, and
4. computations of differences between isogenic lines or lines selected in opposite directions.

In forest tree improvement programs, the parent-offspring regressions, correlations between full-sib and half-sib progenies, and the study of variance components from analysis of variance are the most important. Other methods may be used for those

tree species which can be propagated vegetatively, since the pure line and polyembryony have limited applications in forestry. Allard (1960) stated:

"The question of whether a characteristic is hereditary or environmental has no meaning. The genes cannot cause a character to develop unless they have the proper environment and conversely, no amount of manipulation of the environment will cause a characteristic to develop unless the necessary genes are present."

He also stated that it would be useful to have a quantitative statement of the relative importance of heredity and environment in determining the expression of characters. One way to express inheritance is to use the widely accepted term "heritability". Lush (1949) defined heritability as "the fraction of the observed or phenotypic variance which was caused by differences between the genes or genotypes of the individuals". This fraction can be expressed quantitatively as:

$$H \text{ or } h^2 = \frac{S_G^2}{S_P^2}$$

Where: H is heritability,
 h^2 is the characteristics of variance and is more appropriate than H ,
 S_G^2 is genetic variance, and
 S_P^2 is phenotypic variance.

Phenotypic variance (S_P^2) can be divided in accordance with the cause of variation as follows:

$$s_p^2 = s_g^2 + s_d^2 + s_i^2 + s_e^2 + s_j^2$$

where: s_g^2 is genic or additive genetic variance,

s_d^2 is dominant variance, and

s_i^2 is epistatic variance.

The total of these three variances (s_g^2 , s_d^2 , and s_i^2) is called genetic variance (s_G^2).

s_e^2 is variance due to linear effects of the environment,

s_j^2 is non-linear interactions between heredity and environment.

These two variances (s_e^2 and s_j^2) together are known as environmental variance (s_E^2).

When sexual reproduction takes place, only the additive or genetic effects are passed on to the progeny. The heritability value is expressed in this case in its (h_n^2) narrow sense only, and calculated by using the formula:

$$h_n^2 = \frac{s_g^2}{s_p^2}$$

On the other hand when vegetative propagation takes place the genotypes of the individuals are transferred unchanged. The heritability then is defined in its broad (h_b^2) sense by the formula:

$$h_b^2 = \frac{s_g^2 + s_d^2 + s_i^2}{s_p^2} = \frac{s_G^2}{s_p^2}$$

Broad heritability is also mentioned in the literature as gross heritability. Dadswell et al. (1961) estimated gross heritability for the various wood characteristics of Pinus radiata D. Don. by use of the following formula:

$$h^2 = \frac{\text{between-clone variance}}{\text{between-clone variance} + \text{within-clone variance}}$$

This estimation is based on the assumption that the proportion of the total variance of a given characteristic, which is due to hereditary differences among individuals, can be estimated by using the value of between-clone variance.

There is little information on heritability from studies of second and third generations of forest trees. Only a few observations have been reported on F_2 : Heribert-Nilsson (1918), Detlefsen and Ruth (1922), Roberts (1929), Yarnell (1933), Schreiner (1937), Larsen (1937), Graves (1924). The lack of information based on F_2 generations in forest trees may be attributed mainly to two facts. First, the long period between generations represents a time obstacle to genetic studies, and second, the great emphasis being placed on direct utilization of F_1 , diverts attention from F_2 and tends to reduce the practical need for genetic information that might be derived from this and later generations. Heritability estimations are more numerous in the F_1 than in F_2 generations and are based mostly on comparisons between parental and F_1 material: Johnson (1939), Richens (1945), Stern (1953), Mergen (1955, 1960), Toda (1958), Toda et al. (1959), Matthews et al. (1960), Dadswell and

Wardrop (1960), Pawsey (1960), Perry (1960), Callaham and Hasel (1961), Dadswell et al. (1961), Zobel (1961), Hanover and Barnes (1962), Goggans (1962), Squillace et al. (1962), Van Buijtenan (1962), Wilson (1962), Einspahr et al. (1963), Hattemer (1963), Trousdell et al. (1963), and Wright (1963).

Of the literature listed above, only that which has a definite bearing on the variables studied in this thesis will be discussed. Matthews et al. (1960) estimated the broad-sense heritability of the root-collar diameter on two-year-old Larix leptolepis (Sieb. and Zucc.) Gord. and Larix europea Mill. progenies. Broad-sense heritability estimations of 0.008 were produced in the intraspecific 7 x 7 incomplete diallel cross in the Larix leptolepis (Sieb. and Zucc.) Gord. 0.1 for the interspecific cross between 9 Larix leptolepis Mill. and 3 Larix europea Mill. and 0.01 for the reciprocal of the latter combination. In the same experiment the broad sense heritability estimations for height were 0.14, 0.14, and 0.08 respectively for the above mentioned crosses. Stern's (1962) estimations on height of individual trees from 16 half-sib families and from 8 full-sib families of Betula verrucosa Ehrh. gave broad-sense heritability values of zero for two-year-old, 0.25 for three, 0.53 for four, and 0.62 for five-year-old seedlings. In another experiment with the same species when a 3 x 9 diallel cross was applied between trees from the same region and the family means were used, the broad sense heritability estimations for height were 0.81 for two, 0.76 for three, and 0.78 for four-year old progenies. Wright (1963) estimated the broad sense herit-

ability for height as 0.82 for two-year-old progenies of Pinus sylvestris L. when the estimation was based on family means from 100 half-sib progenies of five stands of the same population. Wright also obtained a very similar estimation (0.8) for the same species at the same age, when he used 30 half-sib progenies of 3 Belgian stands. Squillace et al. (1960) calculated narrow-sense heritability for height on four-year-old Pinus monticola Dougl. seedlings, using the parent-offspring regression and obtained -0.08 to 0.21.

Hanover and Barnes (1962) estimated the narrow-sense heritability values on one-year-old Pinus monticola Dougl. seedlings, for total height and epicotyl length. The seedlings were obtained from an incomplete 4 x 7 diallel cross. The estimation was separated for male- and female-parent populations and the values were 0.314 in the male, and 0.054 in the female populations for epicotyl length, and for total height 0.197 and 0.084 respectively, for the different populations.

Campbell (1964) made tentative predictions of genetic gain per breeding generations. He indicated a low (1-3 per cent) gain in height growth and holocellulose per cent, a medium (4-7 per cent) gain in the majority of wood-quality traits, and a high (7-15 per cent) gain in branch angle, stem diameter and stem volume, when 1 per cent of a stand are selected as superior parents.

A total of 37 heritability estimations on tree height were found in the literature, ranging from minus 0.08 to plus 0.86,

which at first does not appear to provide a very solid foundation for selection based on height. It should be mentioned that because heritability is composed of two components, heredity and environment, it is always possible to change the numerical value of heritability by changing the environment. Therefore, without full description of the variation, it is of limited value to compare heritabilities for separate stands. Each heritability estimate is only applicable to a specific stand, which has similar environmental and genetical characteristics to that for which it was calculated. In general it could also be stated that the more uniform the environment, providing it is generally favourable to growth, the greater will be the value of the heritability estimates.

3 MATERIALS AND METHODS

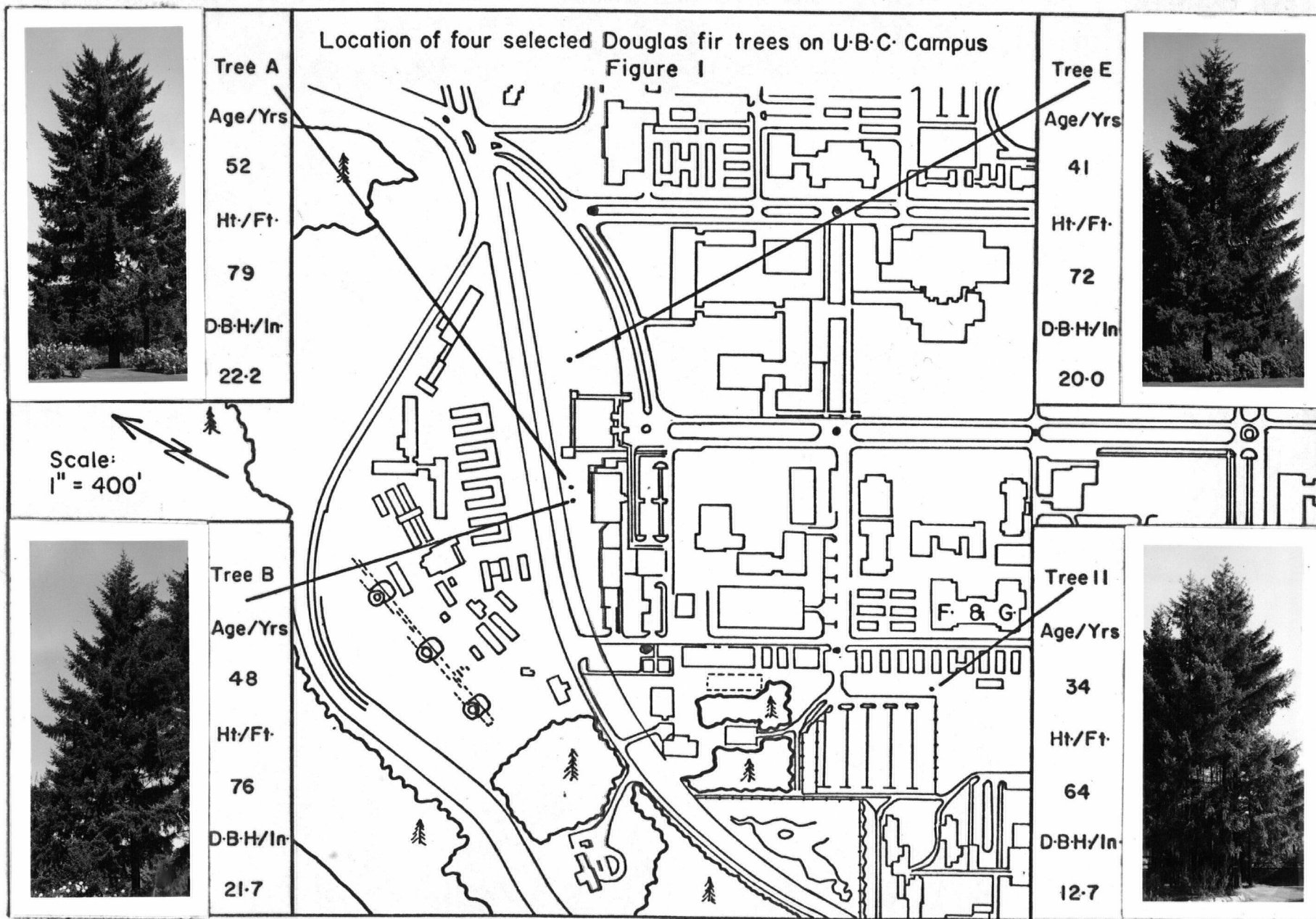
31 Description of Sample Trees on the U.B.C. Campus

For the study described in this thesis, four Pseudotsuga menziesii (Mirb.) Franco var. menziesii, Coastal Douglas-fir (subsequently called Douglas-fir) trees were selected on U.B.C. Campus and designated A, B, E, and 11, (Figure 1). Although a total of 24 Douglas-fir trees were used previously in various studies and experiments by Drs. Orr-Ewing (1956) and Allen (1961), these four were selected because they showed considerable variations in their characteristics. They produced cones frequently and were readily accessible. In 1963 the trees were measured for total height and diameter at breast height (d.b.h.) and their ages were determined. The crown radius was measured on all four main aspects and the number of branches counted, and averaged for the five whorls; 2nd, 4th, 6th, 8th and 10th from the tip of the tree.

Table 1. Age, height, diameter, crown radius, and number of branches in a whorl on four Douglas-fir trees studied, in 1963.

Tree	Age (years)	Total height (feet)	Diameter (d.b.h.) (inches)	Crown radius (feet)	Number of branches in a whorl
A	52	79	22.2	19.0	4.5
B	48	76	21.7	19.2	4.8
E	41	72	20.0	15.8	3.8
11	34	64	12.7	13.2	5.4

Trees A, B, and E, originated from natural regeneration and represent the local population of Douglas-fir. Tree 11 was



selected from a plantation established in 1934, and, although the origin of the seedlings is not known, its probable provenance is Coastal (Knapp, 1963).

32 Phenological Observations

Phenological observation on the flushing date of the vegetative bud of Douglas-fir was initiated by Dr. G. S. Allen in 1959. Observations were subsequently carried out in 1959, 1960, 1961 and 1963, and recorded as follows:

1. Dormant stage; the color of the terminal buds is dark brown.
2. Swollen stage; up to 50 per cent of the tip portion of the terminal buds is light.
3. Swollen stage; 50-75 per cent of the tip portion of the terminal buds is light.
4. Swollen stage; more than 75 per cent of the terminal buds are light.
5. Breaking stage; new needles are visible, bud scales cover only the basal part of the needles.
6. Opening stage 1; 10 per cent of the buds are open.
7. Opening stage 2; 10-50 per cent of the buds are open.
8. Opening stage 3; 50-90 per cent of the buds are open.
9. Opening stage 4; less than 10 per cent of the total buds are still not open.
10. Growing stage; all buds are open.

Observations were tabulated according to Lamb (1915). The days were numbered from a base date of January 1 as day number 1, which in this case facilitated comparison between the differ-

ent trees and years.

33 Controlled Pollination

By controlled pollination it is possible to combine characteristics of individuals which otherwise might not cross in nature, as in allopatric species, or where certain mechanical devices such as protogyny, protandry, dioecy or other floral mechanisms serve as a deterrent to natural crossing. Even within intraspecific crosses where "earliness" and "lateness" is expressed in flowering time, controlled pollination could produce progeny for further breeding work. Techniques of controlled pollination on forest trees have been extensively developed; Barnes (1928), Pjatnitsky (1934), Doyle and O'Leary (1935/a and 1935/b), Schreiner (1938), Johnson (1946), Cumming and Righter (1948), Reines and Greene (1956), Greene (1959), Rohmeder and Schönbach (1959), Pawsey (1961), Sarvas (1962), Wright (1962), and Matthews (1963). But only a few workers have used Douglas-fir; Duffield (1950), Orr-Ewing (1954 and 1956/b), Ching (1960), and Allen and Sziklai (1962).

Douglas-fir is monoecious, and microsporangiate strobili exist on the same branchlet with the megasporangiate strobili, subsequently and respectively referred to as pollen (staminate) conelets and seed (ovulate) conelets, before pollination, and seed cones after pollination.

331 Isolation of seed conelets

Seed conelets and pollen conelets are easily distinguishable from each other and from vegetative buds by the unaided eye by

the end of the previous growing season, as noted by Allen (1941), Finnis (1953), Orr-Ewing (1956/b) and Allen (1963). Isolation can be carried out several weeks prior to the opening of conelets, depending upon the "earliness" and "lateness" of the tree. In 1962 it was carried out at the end of March (Table 2). Originally it was planned to use 40 bags on each tree but the balanced design was eliminated at the early stage with unforeseen difficulties (such as damage by storm, humans, etc.).

Table 2. Dates of isolation, pollination, screening and cone collection, in 1962.

Designation of Tree	D a t e o f			
	Isolation	Pollination	Screening	Collection
A	March 27	April 16	May 29	Sept. 6
B	March 25	April 5-6	do	do
E	March 29	April 15-16	do	do
11	March 25	April 9-10	do	do

Before the isolation bag was placed over the seed conelets, the pollen conelets were removed from the branchlet. Usually two, but sometimes one or three seed conelets were included in one isolation bag, which was 14 centimeters wide and 30 centimeters long. Cotton was used as packing at the place of the tie. The tie was made with "twist-ties" and the branchlet was tagged for future reference. The bags were made from viscose casings as recommended by Duffield (1950) and used successfully by Orr-Ewing (1956/b) and by Allen and Sziklai (1962). The

The viscose bag is permeable to water vapour, retains its inflated size, allows flower development, is transparent, and can be used more than once. A difficulty encountered in using the viscose bag was the fact that the temperature increased within the bag. This was probably responsible for the loss of about half of the seed conelets in the dry pollination method in 1962. It should also be mentioned that on one of the earliest flowering trees, tree 1, (which was not included in the thesis) 54 conelets were isolated on north-east side of the middle portion of the crown 9 days earlier than those of tree B, the earliest flowering tree out of the four trees included in the experiment. At the end of the growing season, 47 cones were collected and only 7 were lost. On the other hand the loss was practically complete on the flowers which were not included in the bags. The beneficial effect of isolation bags is therefore apparent, but further investigation is necessary to answer the problem fully.

332 Obtaining of pollen

Branchlets 40-50 centimeters in length, with pollen conelets attached were collected 10-14 days prior to isolation. The pollen was extracted by a modification of the method described by Orr-Ewing (1954). Plastic sheets were used to cover the branchlets loosely in order to maintain the relative humidity at a high and constant level. The pollen grains as released were collected twice daily and were stored at 0°C. for two or three days. When a sufficient amount of pollen had been collected from one source, it was cleaned by passing the

pollen grains through three sizes of wire gauze, using 1.12, 0.62, and 0.11 millimeter openings. The cleaned pollen was then stored at 0° C., until required.

333 Pollination

Seed conelets were pollinated when they had completely emerged from the bud scales and had assumed an erect position on the branchlet. At this stage of the development (Figure 2) the bracts are greatly enlarged and nearly half the size in length of the fully developed cone bracts while the scales are not more than one-sixth as long as the fully developed cone scales (Jacques 1963).



Figure 2. The relative size of bract and scale of Douglas-fir at the time of pollination. Note the spherical tip of the integument, and the upcurving edge of the bract.

As a result of elongation of the central axis of the

strobilus the ovuliferous scales become separated allowing the pollen grains to roll along the channels formed by the upcurving of the lower edge of the bract to the unequal integuments. The nearly spherical tip of the integuments is well covered with unicellular hairs, and the cleft between the two unequal lips of the integuments is facing upward, thus allowing easy access to the micropyle chamber, (Allen 1963). Three different pollination methods were used, dry, wet and dry-wet. Dry pollination was carried out using a hypodermic syringe (Figure 3), as described by Cumming and Righter (1948), Duffield (1950), Orr-Ewing (1956/b) and Ching (1960). In wet pollination the pollen was diluted to about 100 times its volume with tap water and the suspension was applied to the seed conelets by means of a De Vilbiss atomizer (Allen and Sziklai, 1962) (Figure 3).

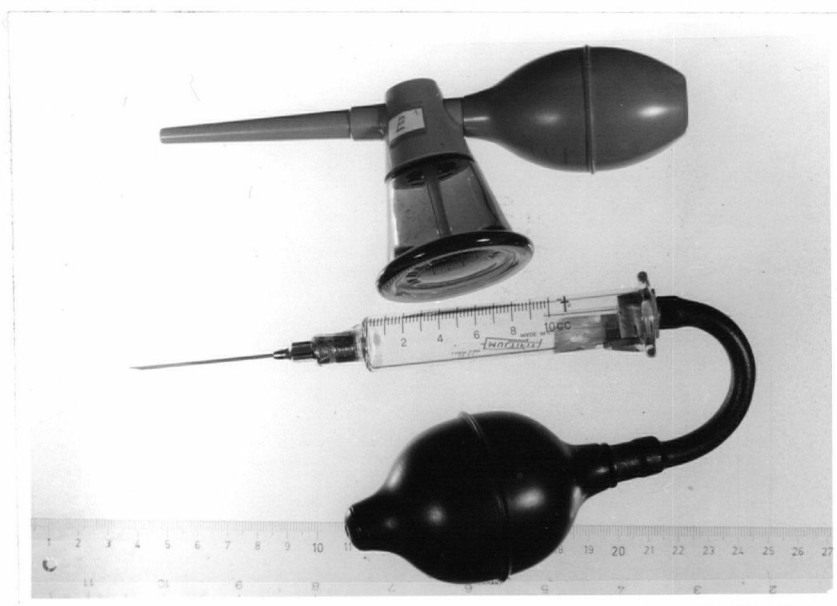


Figure 3. De Vilbiss atomizer for wet pollination (above) and hypodermic syringe for dry pollination (below).

The dry-wet pollination method consisted of distribution of the pollen on the seed conelets with a hypodermic needle and then spraying immediately with distilled water using three squirts from a De Vilbiss atomizer. When the dry and dry-wet pollination methods were used the density of pollen grains attained was 80-100 per square millimeter. The wet pollination technique resulted in the application of only about one-tenth as many pollen grains.

334 Protection of cones

Isolation bags were replaced by fiber-glass screens to protect the developing conelets from insect damage. In 1962 the exchange of bags was carried out on May 29. The screens were 16-17 centimeters wide and 30 centimeters long, with 1.5 millimeter openings. The screens prevented insect damage in almost every case, except when overwintering Dioryctria spp. eggs were enclosed in the isolation bags and later, in the screens. This was very rare, however.

335 Collection of cones and extraction of seeds

The branchlets with tags, screens, and cones, were cut off with clippers at the end of the growing season (September 6, 1962). At this time in some instances a few seeds had already been shed within the bag, and to prevent the loss of seeds, the screens were opened in the laboratory. Cones from each cross were stored in separate boxes and placed in a room having a southerly aspect. Within a few days the cones opened and all the seed was extracted, dewinged, and cleaned by hand. The seeds

were separated then into two classes by weight - filled seed and empty seed - using 7 milligrams as the lower limit for a "filled" seed.

34 Characteristics of Pollen Conelets

The variables of the pollen conelets on the four trees were assessed. These included the number of microsporophylls in a pollen conelet, the number of pollen grains in a microsporophyll and the diameter of individual pollen grains.

The samples for microsporophyll and pollen counts were taken in early March, in the crown on the south side of the trees. Five conelets were selected at random from each tree, and three microsporophylls were prepared for pollen counts. A stereomicroscope was used, with 10x oculars and 2x objectives.

For pollen grain measurements both fresh and stored pollen grains were used from eleven samples (Table 3).

Table 3. Pollen samples from different years (+ sampled, - not sampled).

Year of collection	T r e e s			
	A	B	E	ll
1958	+	+	-	-
1961	+	+	+	-
1962	+	+	+	+
1963	-	-	+	+

Permanent mounts of pollen grains were prepared, using cotton blue, as described by Cole (1958). The slides were used after 48 hours, by which time the penetration of stain was complete.

A random sample of 100 pollen grains was measured for each of the eleven samples. A compound binocular microscope equipped with 8x oculars, a 45x objective and an ocular micrometer was used to determine the measurements. Only completely circular pollen grains were selected and one measurement was recorded, including the entire exine.

35 Cone Measurements

To study intra-tree variation, 6 samples of 100 cones each were collected in 1963 from the top, middle, and lower portions of the crown, on the south and north sides of tree E. A total of 600 cones were included in the investigation. The length and width of the cones were recorded to 0.1 mm, using two calipers, one set for length and the other for width. The width was measured on the closed cone; to simulate the closed condition after extraction of the seeds, the cones were boiled in water for approximately one hour. The measurements were taken on the widest part of the cones, which is usually at one-third of the cone length from the base.

To study the inter-tree variation in length and width of the cones, cones were measured from 1959, 1961 and 1962 collections (Table 4).

Table 4. Cone and seed samples from different years. (+sampled, - not sampled).

Year of Collection	T r e e s			
	A	B	E	11
1959	-	+	+	+
1961	+	+	+	+
1962	+	+	+	+

The measurements were carried out as described under the intra-tree variation.

36 Seed Measurements

Only the intra-tree variation was studied on tree E in 1963. Fifty undamaged, winged seeds were selected randomly from cones collected from top, middle and lower portions of the south and north side of the crown. Measurements were taken from the abaxial surface, and included seed length and width, and wing length and width to 0.1 mm. (Figure 4).

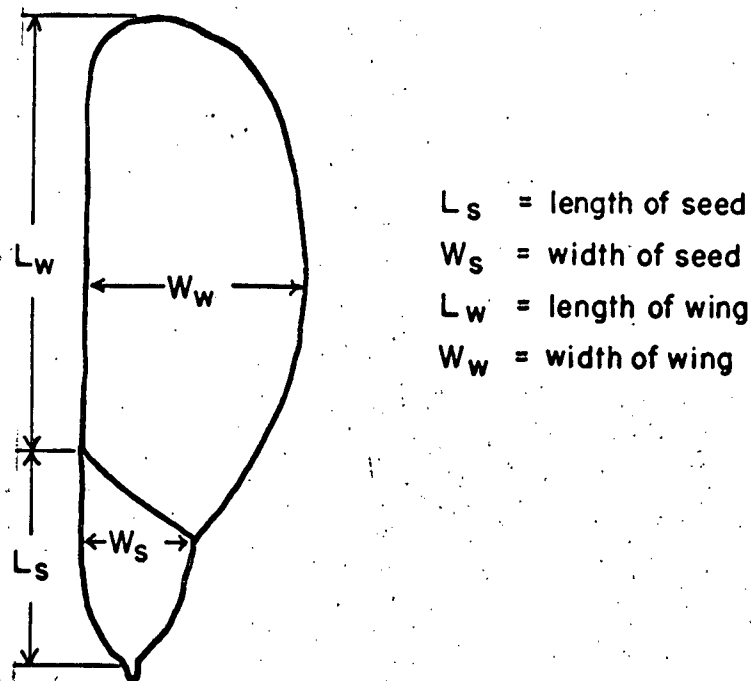


Figure 4. Definitions of seed and wing measurements taken on tree E in 1963.

The width measurements were taken at the widest part of the seed or wing. The tip of the seed was excluded from the length measurements since this part of the seed is very fragile and

frequently missing in cleaned seed.

To study the germination potential of seeds from the different portions of crown segments, 400 seeds were randomly selected from each area of crown. Indented germination pads were used as base sheets, and 50 seeds were mounted on each pad with a very light application of rubber cement (Figure 5). X-ray photographs were then made of these preparations.

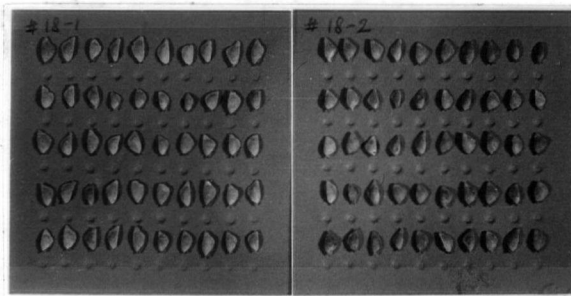


Figure 5. Seeds mounted on indented pads for X-ray photography.

A General Electric, rotating anode, medical diagnostic X-ray unit was used. This machine operates between 40 and 100 kilovolts and between 25 and 200 milliamperes. Using the lowest X-ray output of 40 kilovolts and 25 milliamperes and 1/20th of a second exposure from a distance of 18 inches on Kodak Industrial Type M film, the radiographic contrast was not good, due to the inability of the unit to operate below 40 kilovolts. However, it was still possible to distinguish the endosperm from the embryo, and thus sort the seed into classes, as was done by Simak and Gustafsson, (1953); Gustafsson and Simak (1956); Simak (1956); Klaehn and Wheeler (1961); Berryman and Stark (1962); Hagner (1963). The following classes were used:

Endosperm:

1. Seed completely empty.
2. Shrunken endosperm in horizontal or vertical position. Length less than 1/3 of the total seed length, or the

shape is rounded, occupying the middle part of seed cavity.

3. Megastigmus larva inside.
4. Endosperm fills out most of the seed cavity. A narrow but a well seen empty space exists between the endosperm and the seed coat.
5. Endosperm fully occupies the seed cavity.

Embryo:

1. Embryo absent.
2. Part of the embryo is visible. The total length of visible part is less than 50 per cent of the length of the seed.
3. Same as 2, but the visible part is between 50 and 75 per cent.
4. Same as 2, but the visible part is more than 75 per cent.

37 Germination Tests

As mentioned earlier, the seeds were separated into "filled" and "empty" classes by weight. Germination tests were based only on the "filled" seed class, using the method described by Allen and Bientjes (1954). Germination tests from the 1958 and 1959 crosses were made on both stratified and unstratified seeds but as it was observed that unstratified seeds revealed differences in germination behaviour more markedly, tests of stratified seeds were discontinued. The duration of the germination test was 40 days, the incubation temperature was a constant 25° C. Light was not applied, except for a short period every day, when the germinated seeds were removed.

The germination per cent was calculated by multiplying the number of germinated seeds during the 40-days period by one hundred and dividing by the total number of seeds. In calculat-

ing the germinative capacity in addition to germination per cent, the number of viable seeds still remaining at the end of the test period was included. The number of days required to reach 50 per cent of the germinative capacity was also recorded. This point is recorded as R_{50} in the tables.

38 Progeny Tests

381 Progeny tests in greenhouse and nursery

In 1959 the crosses of trees B x A, B x E, E x A, and E x B, produced larger numbers of seeds than resulted in 1958. Open-pollinated seeds were also available from trees B and E. After germination tests were completed the germinants were planted individually in paper cups containing sterilized sand and were placed in the greenhouse on February 6, 1960, (Figure 6).



Figure 6. Germinants from 1959 crosses planted individually in paper cups.

The seedlings were grown in these cups for approximately eight weeks. During that period the seedlings were watered to prevent drought. After one week of hardening in the cold frame, they were then transplanted into the nursery on April 11. The

seedlings were spaced 6 inches apart in rows 12 inches apart.

The following characteristics were determined for each seedling:

a) The color of hypocotyl (on March 2, 1960) using the following arbitrary classes, assuming multiple factor inheritance:

- 1 - green (GGG)
- 2 - green-red (GGR)
- 3 - red-green (GRR)
- 4 - red (RRR)

b) The number of cotyledons (on April 5, 1960).

c) Hypocotyl length to the nearest millimeter (on April 5, 1960).

d) The bud condition on July 9, July 30, and September 3, 1960, July 30, 1962, July 9, July 30 and September 3, 1963. The following classification was used:

- 1 - bud small, dark red, brown or light green,
- 2 - bud well developed, dark red or brown,
- 3 - bud swollen, tip light reddish,
- 4 - bud scales open partly, needles are visible,
- 5 - needles longer than the bud, and twisted,
- 6 - needles longer than the bud, and straight.

e) The current shoot growth in millimeters on June 5, 1960. June 7, July 23, August 30, 1962, June 7, July 23, August 30, 1963.

f) The total growth for each year was measured on September 30. for the years 1960-63 inclusive.

g) The total height in September 1963.

At the end of the 1963 growing season, three random samples of four seedlings each were selected out of the six progenies to test the correlations among the following nine independent variables (X_1 - X_9) and five dependent variables (Y_1 - Y_5):

Independent variables were:

- X₁ - color of hypocotyl,
- X₂ - length of hypocotyl,
- X₃ - bud condition on July 30, 1960,
- X₄ - " " " " " 1962,
- X₅ - " " " " " 1963,
- X₆ - current year shoot growth to June 6, 1962,
- X₇ - " " " " from June 6 to July 23, 1962,
- X₈ - " " " " to June 6, 1963,
- X₉ - " " " " from June 6, to July 23, 1963.

Dependent variables were:

- Y₁ - total height growth in 1960,
- Y₂ - " " " " 1961,
- Y₃ - " " " " 1962,
- Y₄ - " " " " 1963,
- Y₅ - total height on September 30, 1963.

382 Progeny test in growth chambers

Crosses had been carried out on the three Douglas-fir trees, A, B, and E, previously, but it was not until 1962 that a complete polyallel cross was attempted. In that year tree 11, a late flushing and late flowering tree was included in the polyallel cross (Table 5) to widen the range of characteristics. Tree 11 also represented a different, but unknown Coastal provenance from trees A, B, and E, and its effect was tested.

Table 5. Crosses required for complete polyallel crossing.

Seed parent	P o l l e n p a r e n t			
	A	B	E	ll
A	A x A	A x B	A x E	A x ll
B	B x A	B x B	B x E	B x ll
E	E x A	E x B	E x E	E x ll
ll	ll x A	ll x B	ll x E	ll x ll

Of the 16 possible combinations, many of the self-pollinations did not result in enough viable seed for complete testing of progeny, therefore, in order to keep the analysis balanced, all self-pollinations were eliminated from the analysis.

The seeds were germinated at 25° C. without stratification and light. A maximum of 20 seeds was germinated from each cross, but in four cases, where the number of filled seeds was less than 20, all available seeds were used. Six average germinants were later transplanted into a plastic container (100 x 100 x 135 mm.) filled with vermiculite to a depth of 130 mm. (Figure 7). Subirrigation with Hoagland's solution was provided daily. Two environmental conditions were established in Percival PGC-78 growth chambers. One chamber was set for 10 hours of continuous illumination (short day), and the other for 15 hours (long day). The light intensity was 3500 foot-candles. In both chambers a temperature of 25° C. was maintained during the illumination period and 15° C. was maintained during the dark period. Although the relative humidity was not controlled it was in close correlation with the temperature changes; 50-60 per cent



Figure 7. Germinants from 1962 crosses planted in plastic containers, filled with vermiculite.

relative humidity occurred during the illumination period and 80-100 per cent during the dark period.

A modified randomized-block design was used. Each cross was represented by 6 seedlings grown in one container in each chamber. The containers were placed randomly on the shelves of the growth chambers and every 10-14 days were rearranged randomly to further reduce biases that might have been caused by variations in the distance from lights, observation windows, or circulation fans.

The progeny test in the two growth chambers commenced on February 25, 1963, and terminated July 6, 1963, a period of 132 days. The seedlings were then examined for the following characteristics:

- a) length of root (LR) to 1.0 mm.,
- b) length of hypocotyl (LH) to 1.0 mm.,
- c) length of epicotyl (LE) to 1.0 mm.,
- d) length of branches (LB) to 1.0 mm.,
- e) number of branches (NB),
- f) number of cotyledons (NC),
- g) diameter of root collar (DRC) to 1.0 mm.,
- h) green weight of roots (GWR) to 0.1 g.,
- i) green weight of shoots (GWS) to 0.1 g.,
- j) dry weight of roots (DWR) to 0.001 g.,
- k) dry weight of shoots (DWS) to 0.001 g.,
- l) light transmittance of chlorophyll (TC).

The transmittance of chlorophyll was measured using the method described by Madison and Anderson (1963). One gram sample of needles was extracted in a 20 ml. methanol. The needles were collected from the middle portion of the shoot and were cut into approximately one mm. lengths to provide better penetration of methanol. A Beckman colorimeter was used, with a filter to pass wave lengths of about 440 millimicrons. An extract of needles of open-pollinated progeny from tree E was used as the standard. The readings were obtained in per cent and later on were transformed to arc. sine. values for analysis.

39 Statistical Analysis

The statistical analysis was completed in every case. When it was feasible the IBM 1620 electronic computer was used. The processing included analysis of variance, Duncan's multiple range test, derivation of correlation-coefficients and the

and the multiple regression analysis. Kempthorne (1957), Snedecor (1957), and Steel and Torrie (1960) were consulted concerning the statistical analysis, and the reader is referred to these sources for detailed discussion. Only one part of the statistical analysis will be discussed in more detail, namely that part which is connected with the heritability estimation.

The individual seedling records were used in the calculation, which is based on the following mathematical model:

$$x_{ijk} = m + a_i^s + b_j^p + c_{ij}^{sp} + e_{ijk}^l$$

¹ Owing to the difficulty of Greek letters the denotations were marked with Latin letters.

where: x_{ijk} is the observation of the k-th offspring of a cross, in which the seed (female) parent belonged to the i-th tree, and the pollen (male) parent belonged to the j-th tree.

i is the number of trees used as seed parent,

j is the number of trees used as pollen parent,

k is the number of seedlings from each cross,

l^1 is the number of selfings from polyallel cross,

m is the sample mean,

a_i^s denotes the effect of the i-th seed parent,

b_j^p denotes the effect of the j-th pollen parent,

c_{ij}^{sp} denotes the interaction of the i-th seed and the j-th pollen parent,

e_{ijk} is the error term.

The expected mean squares (MS) were derived from analysis

of variance (Table 6) and the component of variance was calculated using the following forms for each analysis.

Table 6. Analysis of variance and component of variance using individual seedlings from a polyallel cross of Douglas-fir.

Source of variation	DF	MS	Components of variance
Seed	$i-1$	MS_s	$S_e^2 + kS_{sp}^2 + kj S_s^2$
Pollen	$j-1$	MS_p	$S_e^2 + kS_{sp}^2 + ki S_p^2$
S x P	$(i-1)(j-1)-1'$	MS_{sp}	$S_e^2 + kS_{sp}^2$
Residual	$ijk-ij-1'(k-1)$	MS_e	S_e^2
Total			

where: DF refers to the degrees of freedom,
 MS denotes for mean squares,
 S_s^2 is the genetic component of the seed parents,
 S_p^2 is the genetic component of the pollen parents,
 S_{sp}^2 is the genetic component from interaction of seed and pollen parents,
 S_e^2 is the residual variance, which cannot be allocated to the other three terms.

When the mean square of the seed-pollen (S x P) interaction was less than the mean square of the residual variance its component of variance was considered as non-existent. To obtain more accurate estimates of the residual variance, their sums of squares and degrees of freedom were pooled and a new residual mean square was calculated. Consequently when the mean square

of the seed or pollen parent was less than the mean square of the residual and the interaction, it was considered to be non-existent. Instead of calculating negative heritability coefficients, which have no meaning, the heritability value was recorded as zero. The heritability coefficient was calculated for the seed and pollen parents separately, by dividing the genetic-variance components of each parent with the phenotypic variance, using Hattermer's (1963) formula:

for seed parents:

$$h^2_s = \frac{s^2_s}{s^2_s + \frac{s^2_{sp}}{i} + \frac{s^2_e}{ik}}$$

for pollen parents:

$$h^2_p = \frac{s^2_p}{s^2_p + \frac{s^2_{sp}}{j} + \frac{s^2_e}{jk}}$$

4 RESULTS AND DISCUSSION

41 Phenological Differences

Of the 24 Douglas-fir trees observed on Campus during the past four years, only the four included in the polyallel cross were analysed in detail. The relative consistency of vegetative bud flushing was observed (Table 7) and significant tree-to-tree differences were demonstrated (Table 8).

Table 7. The average date of vegetative bud-flushing in 1959, 1960, 1961 and 1963.

Year	D e s i g n a t i o n o f t r e e s				
	A	B	E	l1	Average
	in days from January 1, as day 1				
1959	150	143	148	157	149.5
1960	140	132	136	154	140.5
1961	148	141	144	155	147.0
1963	146	139	139	153	144.3
Average	146.0	138.7	141.7	154.7	

Table 8. Analysis of variance of the average time of vegetative bud-flushing, and Duncan's multiple range test.

Source of variance	df	MS	F	Signif.
Years	3	59.53	13.49	* *
Trees	3	193.70	43.92	***
Residual	9	4.41		

* * Significant at 1 per cent confidence level.

Duncan's multiple range test:

T r e e s				Y e a r s			
B	E	A	11	1960	1963	1961	1959
<u>138.7</u>	<u>141.7</u>	146.0	154.7	140.5	144.3	<u>147.0</u>	<u>149.5</u>

Flushing occurred earliest in tree B (May 15) and latest in tree 11 (May 31) during the four years. The difference was 16 days on the average. It should be mentioned that a few trees out of the 24 observed on the U.B.C. Campus, flushed earlier than tree B, and also a few of them were later flushing than tree 11. Griffith (1963) noted a maximum difference of 5 weeks between early- and late-flushing trees among the 154 trees observed at the University Research Forest, near Haney, B.C. Early-flushing tree B appears to be subject to greater variation ($SD = \pm 4.79$ days) than the late-flushing tree 11 ($SD = \pm 1.73$ days).

Although the years are also significantly different, the difference between the earliest (1960) and the latest (1959) flushing years on the average is only 9 days. Since the trees all stand within four hundred yards of each other in a very similar environment, it is safe to conclude that the consistency of vegetative bud flushing is under strong genetic control and most likely is highly heritable.

Although observation of flowering characteristics was not systematically carried out, notes on isolation and pollination (Table 9) reveal that the times of flowering and flushing are not necessarily correlated.

Table 9. Average data of pollination in 1961 and 1962.

Designation of trees	Days from January 1
A	101
B	96
E	99
11	98

Tree 11 is latest in flushing, 154.7 days after January 1st (Table 7), but earliest in flowering (Table 9).

Ripening of pollen flowers started a few days before the first ovulate flowers reached the fully receptive stage. This slight protandry was also observed by Orr-Ewing (1956/a) and Stoate et al. (1961). Simultaneous maturation of the male and female flowers (synacme) dominated the large part of the flowering period, and would allow self-pollination.

42 Results of Controlled Pollination

421 Number of cones obtained

Of 302 isolated ovulate conelets, 201 developed into cones (Table 10).

Table 10. Number of ovulate conelets isolated, pollinated and screened and the number of cones collected in 1962.

Mother tree	Number of conelets or cones				Lost cones	
	isolated	pollinated	screened	collected	lost	per cent
A	81	79	53	47	34	42
B	72	69	44	40	32	44
E	60	59	53	47	13	22
11	89	83	83	67	22	25
Total	302	290	233	201	101	33

101 conelets (33 per cent) were lost during the first part of the growing season. This is quite similar to the results that Orr-Ewing (1956) obtained in 1952 and 1954 when he recorded a 38 per cent loss. The losses were largest on tree B (44 per cent) and tree A (42 per cent). The other two trees, 11 and E, however exhibited only 25 and 22 per cent losses respectively, indicating the wide variation in this characteristic (Figure 8).

Among the different pollination methods, in the case of cross pollination (Table 11), the dry method resulted in the largest mortality of the cones (49 per cent), followed by the dry-wet method (34 per cent). The lowest mortality occurred in the wet method (19 per cent). Only the wet pollination method was used when selfing in which the loss of conelets was 24 per cent at the end of the growing season.

Figure 8. Changes in the number of ovulate conelets on four Douglas-fir trees at U.B.C. Campus in 1962.

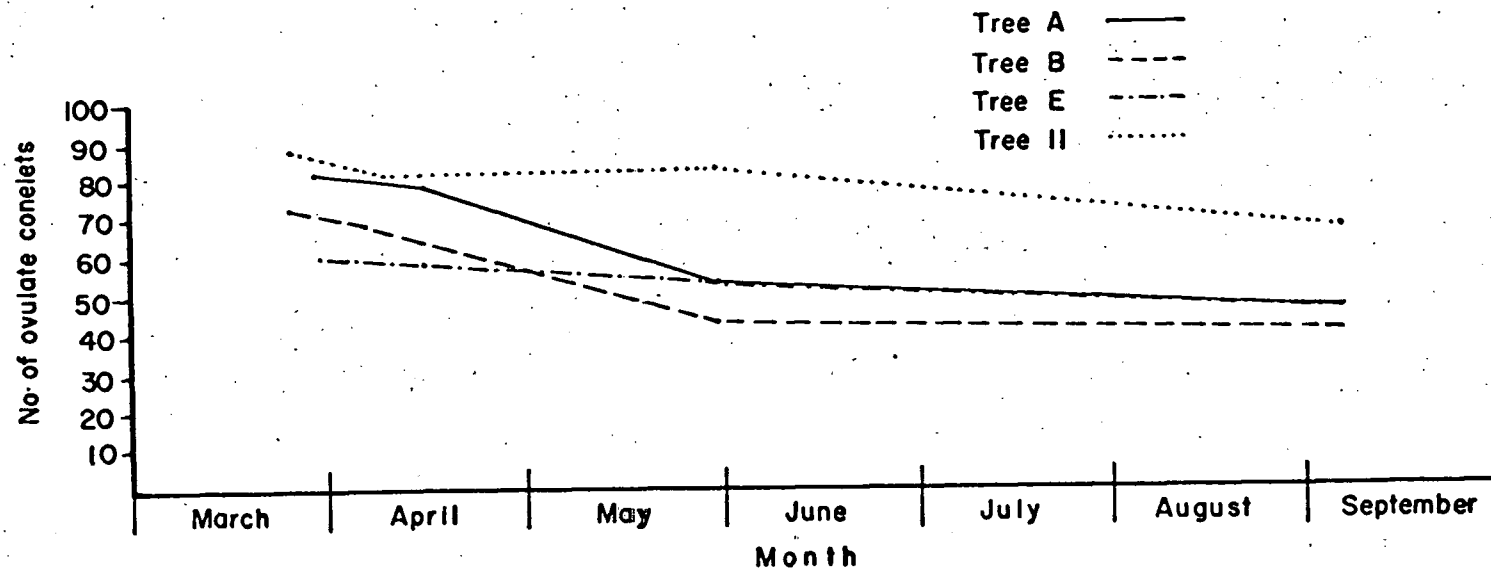


Table 11. Number of ovulate conelets isolated and cones collected using the various pollination methods in 1962.

Method of pollin- ation.	Number of										Lost cone-	
	conelets pollinated					cones collected						
	on trees											
	A	B	E	ll	Total	A	B	E	ll	Total	Lost	Per Cent
Cross												
Dry	15	17	12	20	65	2	7	6	18	33	32	49
Wet	22	19	11	21	73	20	16	9	14	59	14	19
Dry-Wet	18	15	19	15	67	7	7	17	13	44	23	34
Self												
Wet	23	18	17	27	85	18	10	15	22	65	20	24
Total	79	69	59	83	290	47	40	47	67	201	89	31

The result may emphasize the importance of relative humidity at the time of pollination and after. In the case of dry pollination, increased temperature inside the isolation bags, associated with decreased relative humidity may have imposed a strong moisture stress on the developing flowers which, in turn, may have resulted in abortion of the conelets. When the wet pollination method was used, the accumulated water within the bag may have decreased the number of aborted ovulate conelets by maintaining lower temperature and higher relative humidity within the bag. The high susceptibility to heat injury of Douglas-fir was also mentioned by Duffield (1950), who noted that the loss of cones was much higher than in most species of pines.

422 Number of seeds obtained

The controlled pollination resulted in 201 cones. After extraction, immature and shrivelled seeds were eliminated, and

8,004 seeds were obtained; 2,142 of these were filled (Table 12).

Table 12. Total number and filled seeds from artificial pollination in 1962.

Mother tree	N u m b e r o f				
	cones	total seeds	seeds/cone	filled	filled seeds/cone
A	47	1,823	38.78	288	6.13
B	40	1,660	41.50	630	15.75
E	47	2,392	50.89	100	2.12
11	67	2,129	31.77	1,124	16.77
Total	201	8,004	39.82	2,142	10.66

The total number of seeds per cone varied between 31.77 on tree 11 and 50.89 on tree E, with an average of 39.82. The number of filled seeds per cone was lowest on tree E with 2.12, and highest on tree 11 with 16.77, averaging 10.66.

The wet pollination method, using only one-tenth as many pollen grains as the dry pollination method, resulted in a lower number of filled seeds per cone on trees A, B and 11. The seed production per cone on tree B was 19.00 when dry, 19.38 when wet, and 26.71 when dry-wet pollination methods were used. This shows clearly that using water as a transferring agent will allow the pollen grains to come close to the lips of the integument with a larger chance of contact than in the case of dry pollination. (Figure 9).

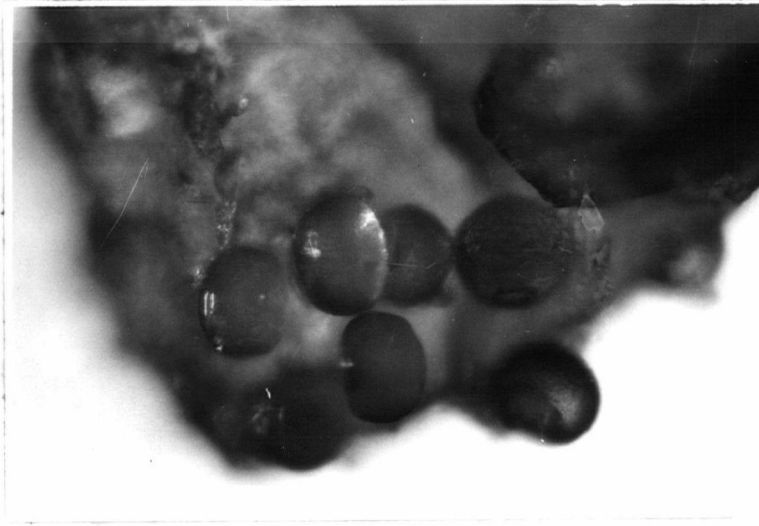


Figure 9. Position of pollen grains 24 hours after wet pollination.

Specific gravity of the pollen grains in Douglas-fir is larger than one, and the pollen could sink close to the integument of the ovule, when wet pollination is applied and water is accumulated between the ovuliferous scales and the bracts. That water also does not interfere with the stigmatic inner surface of integument during the pollination process was found by Allen and Sziklai (1962). Silen and Krueger (1962) indicated that rain during pollination period could not cause a major reduction in Douglas-fir seed set over a broad elevational zone. To further investigate seed production, artificial pollination was separated into cross- and self-pollination groups, and wind-pollination was also included (Table 13).

Table 13. Total number and filled seeds per cone from cross-, self- and wind- pollination in 1962.

Mother tree	Number of seeds per cone from					
	cross		self		wind	
	p o l l i n a t i o n					
	total	filled	total	filled	total	filled
A	38.76	9.65	38.83	.44	37.13	2.02
B	42.17	21.00	39.50	.00	23.00	1.83
E	53.44	2.69	45.47	.93	32.74	4.07
11	35.31	21.91	24.55	6.27	30.61	4.26
Average	42.42	13.81	37.09	1.91	30.87	3.05

Since the development of seed does not depend on pollination, the total number of seeds per cone should not differ greatly on the same tree as a result of different pollination methods. Although the different average values among the total number of seeds did not differ significantly, wind-pollination resulted in only 30.87 seeds per cone, compared to self- (37.09) and to cross-pollination (42.42).

Considering that coastal Douglas-fir cones contain 35-40 cone scales, the maximum number of seeds per cone could have been 70-80 seeds. From various pollinations on six trees Orr-Ewing (1956/a) obtained from 27.6 to 60.7 seeds per cone with an average of 47. He excluded "the flat and misshapen seeds, which are often found at the tip and base of the cone". Garman (1951) reported 44 seeds per cone from natural seed production on Vancouver Island. Robinson (1963) studying the variation in seed characteristics of 183 individual trees of

coastal origins, found an average of 19.3 with a maximum of 63.5 seeds per cone. Kozak (1963) calculated the average number of seeds per cone on 97 trees on the U.B.C. Research Forest at 17.8.

The low number of seeds per cone in the case of wind pollination may be attributed to the damage caused by Contarinia oregonensis, Foote. The seeds become fused with the scale and are not released from the cone (Hedlin 1958). This may explain the low number of seeds in cones collected from open pollinated trees during years when cones were scarce and insect damage was extensive.

The number of filled seeds per cone from cross pollination (13.81) is significantly different from self- (1.91) and from wind-pollination (3.05) (Table 13). Compared with wind-pollination, cross-pollination increased the number of filled seeds on tree A 4.8 times, whereas on tree 11 and tree B, filled seeds were increased 5.1 times, 11.4 times respectively. However, the wind-pollination method produced more filled seeds than cross-pollination on tree E, probably because the controlled pollination took place too late in 1962. In 1961, when this was the only tree to produce flowers, it produced 32.0 filled seeds per cone in the case of wet cross-pollination, in comparison to 5.4 seeds per cone with wind-pollination. Application of artificial pollination in seed production areas, and especially in seed orchards, has obvious potential value in tree improvement programs.

Self-pollination gave the lowest average number of filled

seed per cone, (1.91), and the range varied between zero and 6.27. Orr-Ewing (1956/a) indicated a wider range, between 0.37 to 13.82, and pointed out that self-pollination might result in the same number of filled seeds as in cross-pollination. On the other hand, certain self combinations might produce only empty seeds, as on tree B. Self-pollinations were attempted in previous years on tree B, but filled seeds were never obtained. It is logical to assume that deleterious mutant recessive genes have accumulated in Pseudotsuga. Since this is an outcrossing species, the appearance of these genes is hidden by the corresponding dominant alleles. In the case of self-pollination (inbreeding) these genes could arrive at the homozygous condition, and their effect be clearly expressed in the seeds or progeny. It is also possible to assume that some of these recessive genes are lethal and are able to block fertilization, or destroy the embryo shortly after fertilization takes place.

43 Variation in Pollen Conelet Characteristics

The number of microsporophylls in a pollen conelet from all four trees varied between 61 and 96, with an average of 77. The number of pollen grains per microsporophyll averaged 306 and varied between 254 and 336 (Table 14).

Table 14. Average number of microsporophylls in a pollen conelet and average number of pollen grains in a microsporophyll in 1962.

Designation of mother tree	A v e r a g e n u m b e r o f		
	microsporophylls in a conelet	pollen grains in a micro- sporophyll	pollen grains in a conelet
A	96	336	32,256
B	75	254	19,050
E	76	301	22,876
11	61	333	20,313
Average	77	306	23,624

Table 15. Pollen size frequencies by years.

Tree	Year	S p a c e s o n t h e f i l a r m i c r o m e t e r ¹																			Total
		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47		
		n u m b e r o f p o l l e n g r a i n s																			
A	1958				14	12	26	19	17	9	3									100	
A	1961			11	16	21	27	16	5	2	0	1								100	
A	1962	1	1	11	21	27	25	10	3	1										100	
B	1958			1	4	6	17	16	18	19	9	8	2							100	
B	1961			1	2	5	9	22	18	23	13	6	1							100	
B	1962						2	2	6	8	22	12	13	9	11	8	4	2	1	100	
E	1961			2	0	6	8	22	18	18	13	9	3	1						100	
E	1962		4	9	16	15	20	17	5	7	1	1	5							100	
E	1963		1	5	17	13	19	15	11	4	3	3	3	3	2	1				100	
11	1962			4	10	25	18	16	10	15	2									100	
11	1963		1	1	7	18	18	17	9	10	6	9	3	1						100	
Total		1	7	45	107	148	190	172	120	116	172	49	30	14	13	9	4	2	1	1100	

¹ One space on the micrometer equals 2.6 microns.

Tree B, which yielded the lowest number of pollen grains (Table 14), produced large pollen (Table 16). On the other hand, tree A, with the smallest pollen produced the largest number of pollen grains in a male conelet.

Frequency distributions (Table 15) were worked out and frequency polygons were drawn (Figures 10-13) from the pollen measurement data. The most probable distribution was tested by using the I.B.M. 1620 computer program of Kozak and Munro (1963) (Table 16).

Generally the frequency distributions of pollen diameter were best fitted to the normal curve. The negative binomial and the Poisson curves were also significantly different at the 5 per cent probability level in the same number of cases as the normal curve. For example, the frequency distribution of tree A in 1961 was significantly different from the normal curve but fitted the negative binomial and Poisson curves. In this sample the smaller pollen grains lost viability during the storage and were not measured. The skewed frequency distribution (Table 15 and Figure 10) fitted better the negative binomial and the Poisson curves.

The diameter of pollen grains varied considerably between trees, from 91.08 microns (tree A) to 99.19 microns (tree B). Since tree B was also the earliest flowering of the four trees investigated (Table 9), the correlation between the days to flowering from January 1, and diameter of pollen grains was tested. The correlation coefficient was $r = -0.916$, which is significant at the 5 per cent probability level. To test the correlation

Figure 10. Frequency polygons of pollen diameters on tree A.

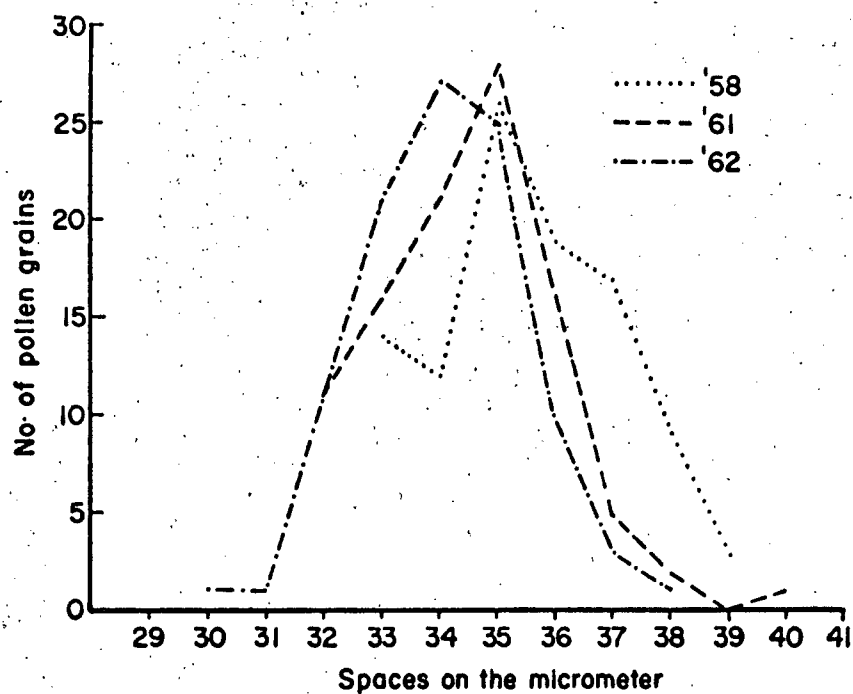


Figure 11. Frequency polygons of pollen diameters on tree B.

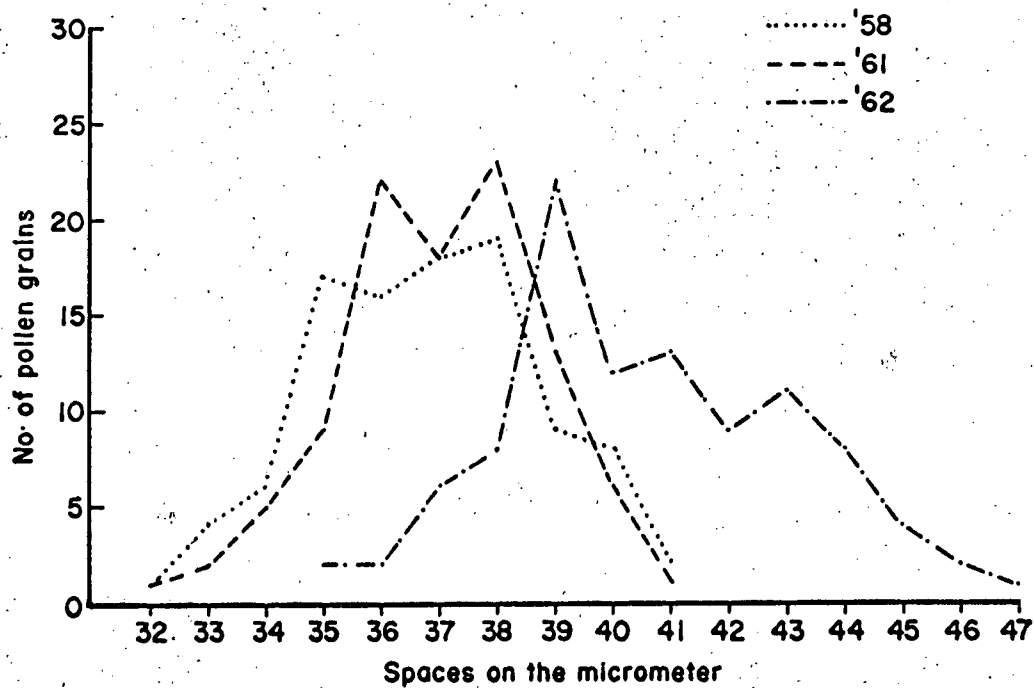


Figure 12: Frequency polygons of pollen diameters on tree E.

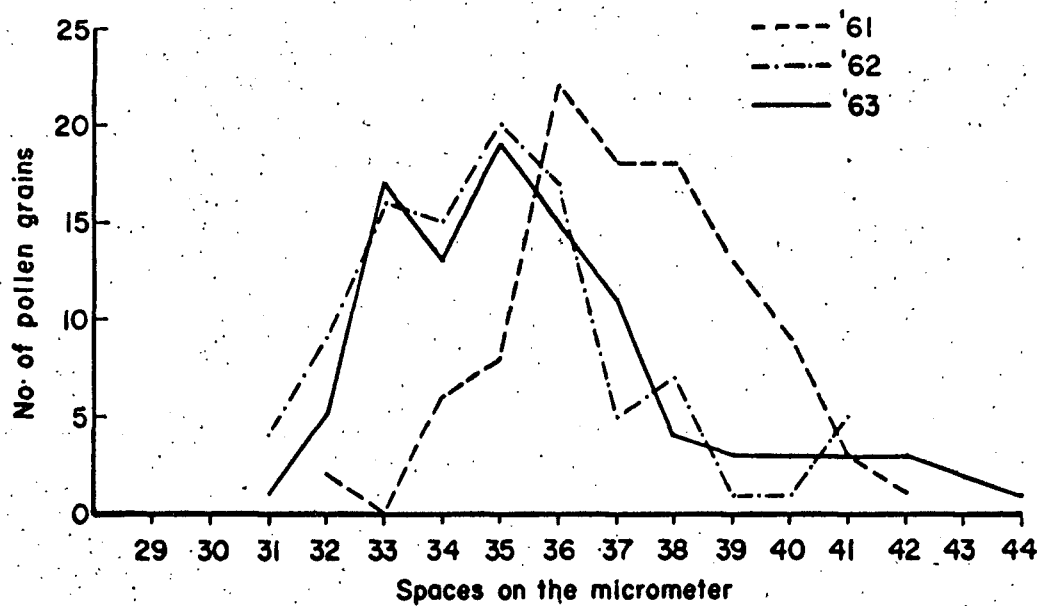


Figure 13: Frequency polygons of pollen diameters on tree II.

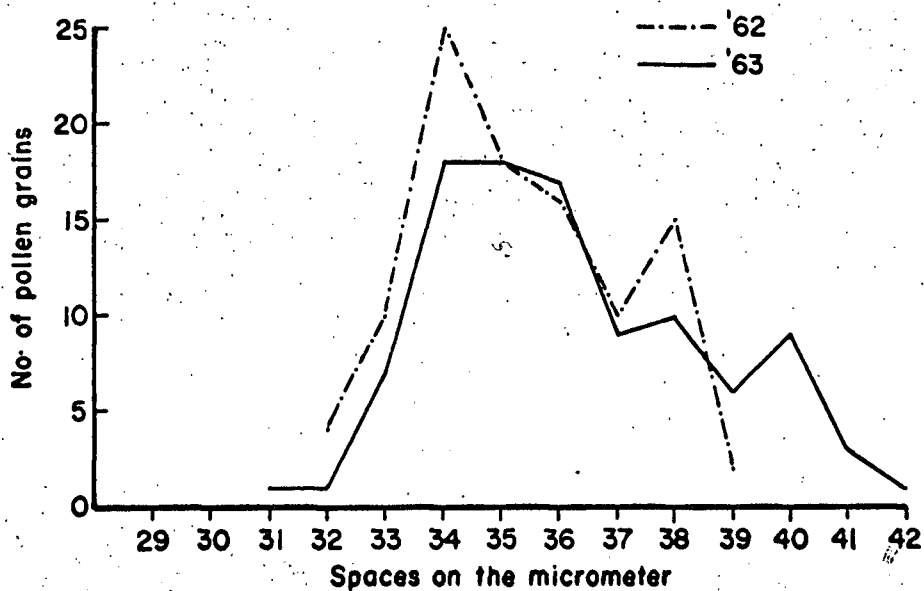


Table 16. Mean values, standard deviations and chi square values of pollen measurements.

Mother tree	Year	Values in spaces on micrometer ¹		Chi square values				df
		Mean	SD	Normal	Binomial	Negative binomial	Poisson ²	
A	1958	35.52	1.61	7	48**	8	9	4
A	1961	34.51	1.55	15*	115**	8	8	6
A	1962	35.06	1.47	8	17**	60**	19**	6
Average (spaces)		35.03						
Average (microns)		91.08						
B	1958	36.80	1.96	3	39**	4	4	7
B	1961	37.03	1.75	4	21**	7	10	7
B	1962	40.61	2.56	10	224**	11	15	10
Average (spaces)		38.15						
Average (microns)		99.19						
E	1961	37.22	1.95	8	75**	17*	11	8
E	1962	34.97	2.37	31**	2,598**	19*	46*	8
E	1963	35.74	2.79	26**	6,103**	11	45*	11
Average (spaces)		33.98						
Average (microns)		93.54						
11	1962	35.32	1.78	14*	51**	13*	13*	5
11	1963	36.20	2.35	15	114**	11	11	9
Average (spaces)		35.76						
Average (microns)		92.97						

¹ One space equals 2.6 microns

² Degrees of freedom (df) for Poisson distribution are one more than the given value.

* Significant at 0.05 probability.

** Significant at 0.01 probability.

further, tree 1, which is the earliest flowering tree on U.B.C. Campus and was not used in the polyallel cross, was included in the calculation. This tree flowered 89 days after January 1, and showed an average pollen diameter of 104.88 microns, based on two years' (1961 and 1963) observations. The correlation coefficient was $r = -0.967$, significant at the 1 per cent probability level. The early-flowering trees exhibited a larger pollen size and consequently a larger volume, which in turn may withstand adverse climatic conditions better than the smaller pollen. The adaptive significance of such correlation may seem evident and coincides with a somewhat similar adaptation observed by Covas and Schnack (1945). They found in the genus Glandularia of the family Verbenaceae, that the size of pollen grain is closely related to the distance which the pollen tube must pass through from the stigma to the ovule. A longer style was correlated with a larger pollen grain, and they suggested the existence of adaptation in providing the pollen tube with sufficient food to reach its destination. The correlation between pollen size and flowering time, although based only on three years' data, deserves further investigation. A larger number of trees representing a wider range of phenological differences would reveal more about such assumed correlation.

44 Variation in Cone Characteristics441 Intra-tree variation

4411 Cone length

Cone length of tree E from the 1963 collection shows a significant difference between aspects (Table 17). Cones on the south side of the tree were longer (69.59 millimeters) compared with those on the north side (65.56 mm). The three positions (top, middle, and lower) did not significantly affect cone length, although an explicit trend was observable (Figure 14). The length of the cone tended to decrease from the lower portion toward the top portion of the crown.

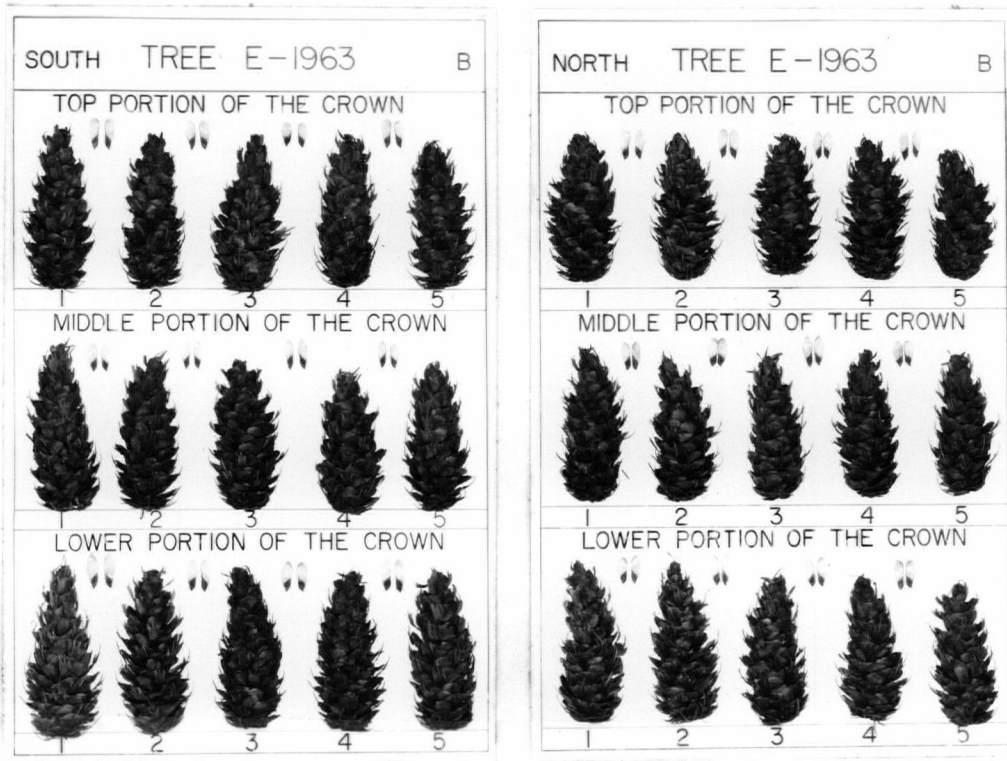


Figure 14. Five selected cones from the south and north sides and from the top, middle and lower portions of the crown of tree E, in 1963.

Table 17. Average cone lengths on tree E in 1963.

Portion of the crown	Cone length in millimeters	
	Side of tree	
	South	North
Top	68.82	62.47
Middle	69.15	66.83
Lower	70.80	67.37
Average	69.59	65.56

4412 Cone width

The width of the cone showed a larger variation at the three crown positions and also on the two aspects than did the length, being highly significantly different in both cases (Table 18). A trend exists similar to that observed in respect to lengths of cones. The average width of the cone on the north side of the tree (16.56 mm.), was smaller than on the south side, (18.07 mm.). The width also decreased from the lower portion of the crown towards the top portion.

Table 18. Average cone widths on tree E in 1963.

Portion of the crown	Cone length in millimeters	
	Side of Tree	
	North	South
Top	16.95	15.88
Middle	18.32	16.67
Lower	18.94	17.14
Average	18.07	16.56

442 Inter-tree variation

4421 Cone length

The lengths of the cone did not differ significantly over the years or between trees. Differences between trees however were close to the 5 per cent level of significance. Tree 11 produced the longest cones with an average of 70.2 mm. The shortest cones were measured on tree A (Figure 15), with an average of 63.9 mm. Trees B and E were represented with 66.7 and 67.9 mm. average cone lengths, respectively. The year-to-year variation was negligible: 68.5, 67.5, and 66.0 mm. in 1959, 1961, and 1962 respectively.

4422 Cone width

There was greater variation in cone width than in the length (Table 19). Highly significant variations were found for years and also for trees.

Table 19. Analysis of variance of the cone width and Duncan's multiple range test.

Source of variation	df	MS	F	Signif.
Trees	3	128.71	43.48	* *
Years	2	33.56	11.34	* *
Residual	94	2.96		
Total	99			

* * Significant at 1 per cent probability

Duncan's multiple range test:

<u>Trees</u>				<u>Years</u>		
E	A	B	11	1961	1959	1962
18.3	<u>20.1</u>	<u>20.1</u>	24.0	19.2	20.3	21.2

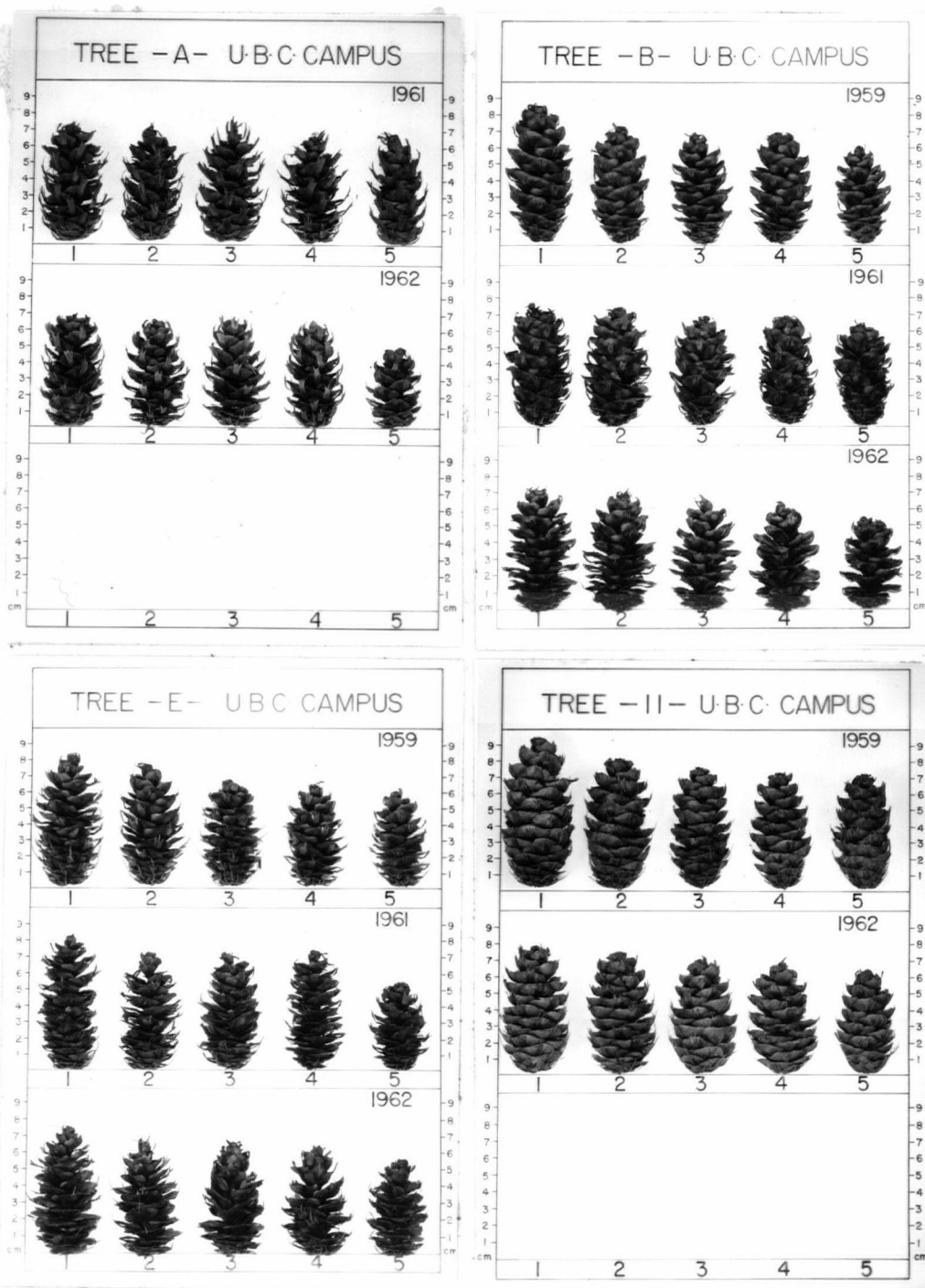


Figure 15. Photographs of five selected cones from the four Douglas-fir trees for the years of 1959, 1961, and 1962. Cone on the left represents the maximum size of the sample, on the right the minimum, and the three cones at the middle of each row the average sized cones.

The widest cone was on tree 11, and the narrowest on tree E. Trees A and B showed the same width (20.1 millimeters), and were significantly different from tree E and from tree 11.

The values for different years show significant differences. The widest (21.2 mm.) cone was produced in 1962, and the narrowest in 1961 (19.2 mm.). Tusko (1963) reported an average cone width of 21.02 millimeters, based on a collection of 264 trees from 28 coast and interior provenances in 1959. Willet (1963), reported an average cone width of 21.6 millimeters based on measurements from 348 trees collected from 30 provenances in 1962.

The strong effect of years on the width of the cone, and the lesser effect on the length of the cone may indicate that the "range of reaction" (Pauley 1954) may vary considerably even in the cone characteristics. The width of cone appears to be more plastic or genetically "loosely controlled" than the length of the cone. In other words, the environmental factors affect the width of the cone more noticeably than they do the length of the cone. Similarly the width and length of seeds seem to be under loose genetic control, as they showed variations according to aspect and position in the crown. Tusko (1963) supported the present findings by describing a strong correlation between the width of the cones and certain environmental factors such as annual precipitation, mean annual temperature, elevation and site class.

45 Variation in Seed Characteristics451 Intra-tree variation

4511 Total number of seeds

The total number of seeds differs significantly between south and north sides of the crown (Table 20).

Table 20. Total number of seeds from top, middle and lower parts of the crown, on the south and on the north side of tree E, in 1963.

Portion of the crown	<u>Number of seeds from 100 cones</u>		Total
	South	North	
Top	3,805	2,888	6,693
Middle	3,578	2,482	6,060
Lower	3,630	2,008	5,638
Total	11,013	7,738	18,391
Total number of seeds per cone	36.71	25.20	

The top portion of the crown produced the largest number of seeds (6,693) and the number decreased towards the lower portion, where the total number of seeds was 5,638.

4512 Number of filled seeds

To evaluate the number of filled seeds, 2,400 seeds were X-rayed, half of them from the south and the other half from the north side of the crown. Each portion of the crown (top, middle and lower) was represented by 400 seeds. Empty seeds were more numerous on the south side than on the north, the remaining endosperm and embryo classes on the north were repre-

sented with larger numbers (Table 21-22).

Table 21. Number of seeds in endosperm and embryo classes, on the south side of tree E, in 1963.

Portion of the crown	<u>Endosperm and embryo classes</u>					Total
	1	2	3	4	5*	
Top	322	2	62	11	3	400
Middle	311	2	71	5	11	400
Lower	304	3	89	1	3	400
Total	937	7	222	17	17	1,200

Table 22. Number of seeds in endosperm and embryo classes, on the north side of tree E, in 1963.

Portion of the crown	<u>Endosperm and embryo classes</u>					Total
	1	2	3	4	5	
Top	271	6	108	4	11	400
Middle	284	-	102	7	7	400
Lower	284	-	104	2	10	400
Total	839	6	314	13	28	1,200

*For explanation of endosperm and embryo classes see pages 31 and 32.

4513 Length and width of seeds and seed wings

Of the four seed characteristics measured, only two, the length of seeds and the width of seeds, varied according to their aspect on the crown and position in the crown (Table 23). The seed width was more strongly affected than seed length. The seed wing characteristics (length and width) did not show significant differences with respect to position in crown.

The values from the north side were generally larger than on the south side, with three exceptions; the lengths of the wing at both lower and middle portions of the crown, and the widths of the wing at the middle portion of the crown.

Table 23. Average values of seed characteristics on tree E, in 1963 (in mm.).

Aspect	P o r t i o n o f t h e c r o w n											
	T o p				M i d d l e				L o w e r			
	L _W	W _W	L _S	W _S	L _W	W _W	L _S	W _S	L _W	W _W	L _S	W _S
South	8.30	4.90	7.45	3.75	9.15	5.15	7.65	3.95	8.80	4.90	7.55	3.90
North	8.65	5.15	7.55	3.95	9.00	5.00	7.70	4.15	8.70	5.00	7.85	4.15
Average	8.47	5.02	7.50	3.85	9.07	5.07	7.67	4.05	8.75	4.95	7.70	4.02

The longer wing length of the seed on the south side may be an expression of a correlation with the scale length. Although scale length was not investigated, it could safely be assumed that the wider cones have longer cone scales, and it will be recalled that the cones are significantly wider on the south side (Table 18).

The average values of seed characteristics were lowest at the top part of the crown, which parallels cone characteristics. The largest values were observed at the middle part of the crown with one exception. The length of the seed was largest in the lower portion of the crown.

46 Germination Tests

Allen (1958, 1960, 1961 and 1962) showed that germination behaviour of Douglas-fir seed differs greatly from lot to lot, from tree to tree, and even within a tree. Specific causes of all the variation have not been determined, but effects of seed maturity, handling, processing, moisture content during stratification, stratification period, and incubation temperature, as well as origin (provenance) and inheritance in general, have been demonstrated.

In the germination tests on seeds from controlled crosses all of the above-mentioned variations in environmental factors were eliminated or reduced and this helped to bring to light the heritability variances. The R_{50} values exhibited a large range of variation (Table 24). Tree A had seeds with slow germination, taking an average of 21.64 days to reach 50 per cent of the germinative capacity, while seeds from tree E germinated faster and reached R_{50} value on the average after only 10.9 days. Trees B and 11 were intermediate with R_{50} values of 15.81 and 18.80 days respectively. The R_{50} values from selfing were also useful, - since the expression of the inherited characteristics is more clearly observable when the heterozygosity is reduced. The values from self-pollination were always the largest or the smallest, depending on the "slow" or "fast" seed germinating characteristics of the tree. Tree A, for example, showed 31 days, the highest average value, while tree E gave the lowest average value, 9.6 days.

The importance of the pollen parent is also evident from the crosses. When tree A, which displays "slow" germination was crossed with tree E, which has "fast" germination, the seeds from this cross showed the fastest germination of all the crosses on tree A. Conversely, when pollination was carried out on tree E, using pollen from tree A, seeds from this cross germinated slower than seeds from any crosses from tree E. The average number of days required to reach 50 per cent of the germinative capacity from reciprocal crosses were also very close to each other; A x B (20.03) and B x A (19.54), A x E (14.43) and E x A (12.56), B x E (12.35) and E x B (10.40). These values may indicate that the two parents play a very similar role in the inheritance of the rate of germination.

Germination per cent was highest in the seeds from tree E (Table 25) with an average of 91.26 per cent, followed by 76.20 per cent for tree A, 74.08 per cent for tree B and 42.00 per cent for tree 11. The inheritance pattern of germination per cent was entirely different from the inheritance pattern of the rate of germination. When tree E pollen was applied, a high germination value was observed in every cross. On tree E no change in germination percentage was evident in any cross, when a different pollen source was used. Considering the results of four years, although number of samples is limited, one can assume that the germination per cent is inherited on a single factor basis. Tree E can be assumed to possess the homozygous dominant condition because a high germination per cent is expressed in any cross, when this factor is transferred.

Table 24. Average number of days to reach 50 per cent of germinative capacity (R_{50})¹ from various crosses, during 1958, 1959, 1961 and 1962 years. Untreated seeds incubated at 25° C.

Cross	1958	1959	1961/1	1961/2	1962	Average	± SD	± CV
R_{50} v a l u e s								
A x A				31.0		31.00		
A x B			17.6	23.5	19.0	20.03	4.36	21.76
A x E			13.5	14.8	15.0	14.43	1.18	8.18
A x ll					25.0	25.00		
A x open		16.7		41.0	21.0	26.23	18.33	69.88
Average						21.64		
B x B								
B x A	30.0	13.7	14.0	22.0	18.0	19.54	9.14	46.77
B x E	13.9	12.0		10.5	13.0	12.35	2.52	20.40
B x ll					17.0	17.00		
B x open		12.6			13.0	12.80	.28	2.18
Average						15.81		
E x E				9.6		9.60		
E x A	17.4	9.2	11.8	11.4	13.0	12.56	6.07	48.32
E x B	10.0	7.6	10.2	11.2	13.0	10.40	2.33	22.40
E x ll					12.0	12.00		
E x open	12.0	5.2	9.7	10.0	12.0	9.78	6.99	71.47
Average						10.90		
ll x ll					23.0	23.00		
ll x A					20.0	20.00		
ll x B					22.0	22.00		
ll x E					12.0	12.00		
ll x open					17.0	17.00		
Average						18.80		

¹ R_{50} values based usually on one 40 seed sample. Because the uneven number of filled seeds obtained from the different crosses in a few cases samples with 10 seeds as minimum were also included.

Values for 1958 and 1959 crosses obtained from Allen's (1963) publication.

Table 25. Germination per cent¹ of seeds from various crosses, during 1958, 1959, 1961 and 1962 years. Untreated seeds, incubated at 25° C.

Cross	Year of cross					Average	± SD	± CV
	1958	1959	1961/1	1961/2	1962			
	Percentage							
A x A				50		50.00		
A x B			65	92	85	80.67	19.82	24.57
A x E			85	95	90	90.00	7.07	7.85
A x ll					65	65.00		
A x open				60	75	67.50	10.61	15.75
Average						76.20		
B x B				0		0.00		
B x A	90	90	54	25	90	69.80	58.99	84.51
B x E	90	100		85	90	91.25	10.89	11.93
B x ll					85	85.00		
B x open					90	90.00		
Average						74.08		
E x E				92		92.00		
E x A	88	95	90	95	91	91.80	6.23	6.78
E x B	90	98	100	93	71	90.40	23.11	25.56
E x ll					95	95.00		
E x open			76	100	95	90.33	17.89	19.81
Average						91.26		
ll x ll					10	10.00		
ll x A					35	35.00		
ll x B					25	25.00		
ll x E					75	75.00		
ll x open					65	65.00		
Average						42.00		

¹ Germination per cent average values based usually on one 40 seed sample. Because the uneven number of filled seeds obtained from the different crosses, in a few cases samples with 10 seeds as minimum were also included.

Values for 1958 and 1959 crosses obtained from Allen's (1963) publication.

47 Progeny Tests471 Progeny test in greenhouse and nursery

Survival of seedlings grown in the U.B.C. greenhouse from six different families established by the 1959 crosses showed a very interesting pattern (Table 26).

Table 26. Mortality of four crosses and two open pollinated families grown in greenhouse in 1960.

Cross	No. of germinants	Number of seedlings		
		planted	lost	per cent lost
	Feb. 6		April 5	
B x A	60	45	15	25.0
B x E	30	18	12	40.0
E x A	60	52	8	13.3
E x B	47	46	1	0.2
B open	50	38	12	24.0
E open	40	30	10	20.3
Total	287	229	58	20.3

The mortality was highest in B x E cross (40 per cent) and lowest in the reciprocal cross, E x B (0.2 per cent). Generally the crosses on tree B gave higher mortality than on tree E, although mortality in the open-pollinated seedlings from these two trees did not differ significantly.

The same pattern of loss was observed later in the nursery during the years from 1960 to 1963 (Table 27). The difference was even expressed in the open-pollinated progeny. Tree B mortality was 34.3 per cent, more than twice as large as mortality on tree E (16.7 per cent). The mortality was heaviest

Table 27. Mortality of seedlings from four crosses and two open pollinated families in the nursery from 1960- to 1963.

Cross	Planted April 11	Number of seedlings died during					Mortality percent
		1960	1961	1962	1963	total	
B x A	45	10	-	-	-	10	22.2
B x E	18	4	-	-	-	4	27.7
E x A	52	4	-	-	-	4	0.8
E x B	46	4	-	-	-	4	0.9
B open	38	10	-	3	-	13	34.3
E open	30	5	-	-	-	5	16.7
Total	229	37	-	4	-	41	18.6

during the first year (1960). In the following years the mortality was zero or negligible. Only four seedlings died in 1962. All four were of the progeny of tree B, suggesting that the mortality was under a certain maternal control, and that the pollen parent may not have played an important role in the early survival.

The total growth (in millimeters) was analysed for the six progenies based on the three samples described above and observed for four years (See appendices I-VII and Table 28).

During the first four years the cross E x B produced progeny with the longest average yearly height growth (183.1 mm.). Progeny from the reciprocal cross, B x E only grew 163.6 mm. (although this is not significantly different). The further sequence was: B x A (137.7 mm.). The total height showed exactly the same sequence (Table 29) for the progenies, although they were not significantly different from each other. Although the

Table 28. Analysis of variance for height growth and Duncan's multiple range test of six progenies from 1959 crosses.

Source of variance	df	MS	F	Signif
Progenies	5	12,241.3	3.68	**
Years	3	648,607.0	195.51	**
Blocks	2	3,216.7	0.96	NS
P x Y	15	2,007.0	0.60	NS
P x B	10	11,421.9	3.44	**
B x Y	6	2,087.8	0.62	NS
P x Y x B	30	1,556.3	0.46	NS
Error	216	3,317.4		
Total	287			

** significant at 1 per cent probability level.

Duncan's multiple range test.

Height growth of progenies					
E x A	B open	E open	B x A	B x E	E x B
137.7	146.1	149.3	156.2	<u>163.6</u>	<u>183.1</u>

total height of seedlings from E x B cross was 32 per cent higher than the total height from E x A cross, the differences between progenies were better revealed when the yearly height growth was compared. The absolute size of the seedlings is easily modified, while hardly less plastic is the amount of elongation of the stem.

Table 29. Total height of six progenies from 1959 crosses on September 30, 1963.

Progenies	Height in millimeters
E x B	746.9
B x E	661.2
B x A	648.3
E open	629.0
B open	600.8
E x A	565.2

4711 Correlation analysis

The coefficients of correlation were calculated for every pair of the fourteen variables (Appendix I). These coefficients of correlation are given in Appendices VIII-XIV in a diagrammatic presentation. The closely related variables were assorted into four groups such as hypocotyl, bud, current shoot growth and yearly growth (including total height).

The hypocotyl ($X_1 - X_2$) and bud characteristics ($X_3 - X_5$) were not intercorrelated and were not correlated at the 1 per cent probability level to any other variables (Appendices VIII-XIV). The bud condition in 1960 (X_3) was correlated twice, in 1962 (X_4) 6 times, and in 1963 (X_5) 12 times to the different current shoot growth and yearly growth. The hypocotyl color (X_1) and length (X_2) did not show any important correlations. The current shoot growth during the first part of the growing season in the third (X_6-X_7) and fourth (X_8-X_9) years were also correlated in many cases with the total height

and yearly growth. The first year growth (Y_1) was in every case correlated with the total height at the end of the fourth year (Y_5). The correlation was significant at 1 per cent level between the progenies from B x A, E x A, B x E, E x B, and B open that were tested.

4712 Regression analysis

From the regression analysis computed for the nine (X_1 - X_9) independent variables, against the first year growth (Y_1), it was observed that the nine variables account for 99 per cent of the total variation in B x A, 93 per cent in B x E, and 96 per cent in E open. The same nine independent variables accounted for 99 per cent of the variation in total height (Y_5) in the case of B x A, B x E, E x A, E x B, and E open, and for 98 per cent of the total variation of the total height in the case of B open. For study of the total height, the independent variables were eliminated one by one, in order of the least significance, until one or two variables remained. At least 90 per cent of the variation was still accounted for by these remaining variables. The equations indicating the relationship between the total height and the variables are:

$$\text{Cross B x A: } Y_5 = -76.25 + 2.49 X_7 + 5.22 X_8 \quad R^2 = 0.922$$

Residual variance 3,142.79.

$$\text{Cross B x E: } Y_5 = 42.45 + 96.96 X_5 + 3.77 X_6 \quad R^2 = 0.961$$

Residual variance 1,086.51.

Cross E x A: $Y_5 = 42.45 + 96.96 X_5 + 3.77 X_6$ $R^2 = 0.908$
 Residual variance 2,743.81.

Cross E x B: $Y_5 = 347.22 + 6.44 X_7$ $R^2 = 0.941$
 Residual variance 2,601.91

B open: $Y_5 = 15.64 + 1.96 X_8 + 3.47 X_9$ $R^2 = 0.929$
 Residual variance 3,142.79.

E open: $Y_5 = 239.25 + 5.88 X_7$ $R^2 = 0.921$
 Residual variance 6,552.65.

where: Y_5 total height on September 30, 1963,
 X_5 bud condition on July 30, 1963,
 X_6 current year shoot growth to June 6, 1962,
 X_7 " " " " from June 6 to July 23 1962,
 X_8 " " " " to June 6, 1963,
 X_9 " " " " to July 23, 1963,
 and $N = 12$.

It is evident from the multiple regression analyses, that more than 90 per cent of the variation in the total height at the end of the fourth year is determined by the shoot growth in the first part of the growing season during the third and the fourth year. The shoot growth, between June 6 and July 23, during the third year (X_7), alone explained 94.1 per cent of the variation in the total height in the progeny of E x B cross, and 92.1 per cent in the progeny of E open. This variable also

is important in B x A and E x A crosses, where in connection with X₈ in the first, and with X₉ in the latter it accounted for 92.2 per cent and 90.8 per cent of the variation in the total height.

472 Progeny test in the growth chambers

When assessing the combining ability and calculating the heritability estimates of different parental lines, the poly-allel cross is often of value. Every possible reciprocal combination will allow identification of those parents which combined well with the others in the production of progenies exhibiting desirable characteristics and calculation of the genetic gain achieved by cross breeding. The combining ability of the parental line can be assessed after analysing the data by using analysis of variance, and completing with the Duncan's multiple range test. The heritability estimation could be calculated by estimating the components of variance, as mentioned earlier.

Assessments of the combining ability and calculations of the heritability values were carried out separately on the progenies from the long-day and from the short-day treatments. The following characteristics of the 132-day-old progenies, from 1962 crosses were evaluated: length of root, hypocotyl, epicotyl and branches, number of branches and cotyledons, diameter of root collar, green and dry weight of roots and shoots and finally the light transmittance of chlorophyll extraction. These are presented in this sequence on the following pages.

4721 Length of root

Tree 11 showed the best combining ability as a seed parent and as a pollen parent tree B was the best. However, there was no significant difference between the trees in the seed or in the pollen line. The heritability estimation calculated as 12 per cent was assessable only for the pollen parents.

a) Average values (in millimeters)

Seed parents	<u>Pollen parents</u>				Averages
	A	B	E	11	
A		348.3	291.3	292.0	310.5
B	297.7		318.0	283.3	299.7
E	263.7	306.7		299.0	289.8
11	329.0	316.7	297.3		
Average	296.8	323.9	302.2	291.4	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seeds	3	1,110.3	0.46	NS
Pollen	3	1,823.4	0.76	NS
S x P	5	1,491.5	0.62	NS
Residual	24	2,373.7		
Total	35			

c) Components of variance

$$s_s^2 = -32.36, \quad s_p^2 = 27.66, \quad s_{sp}^2 = -294.05, \quad s_r^2 = 2373.73$$

d) Heritability estimates

$$h_s^2 = \emptyset \quad h_p^2 = 0.12$$

4722 Length of hypocotyl

The values were not significantly different from each other. Tree 11 as a seed parent and tree B as a pollen parent produced the longest hypocotyl. The heritability value for the seed was 23 per cent. Since the components of variance were negative for the pollen parents, zero heritability was shown.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		30.0	25.0	27.7	27.5
B	21.7		26.7	25.0	24.4
E	24.7	30.0		26.3	27.0
11	32.0	26.3	29.0		29.1
Average	26.1	28.8	26.9	26.3	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seeds	3	33.8	2.78	NS
Pollen	3	13.2	1.08	NS
S x P	5	25.9	2.13	NS
Residual	24	12.1		
Total	35			

c) Components of variance

$$s_s^2 = 0.662, \quad s_p^2 = -1.061, \quad s_{sp}^2 = 4.591, \quad s_r^2 = 12.166$$

d) Heritability estimates

$$h_s^2 = 0.23 \quad h_p^2 = \emptyset$$

4723 Length of epicotyl

Tree 11 exhibited the best combining ability as seed and pollen parent. Tree B produced seedlings with the second longest epicotyl length, while the progenies from trees A and E were in both instances the shortest. The heritability value was 36 per cent for the seed parents and zero for the pollen parents.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		159.0	114.3	135.3	136.2
B	129.3		147.3	225.3	167.3
E	126.0	158.3		186.7	157.0
11	224.3	209.3	165.7		199.8
Averages	159.8	175.5	142.4	182.4	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	6203.8	4.33	*
Pollen	3	2782.5	1.94	NS
S x P	5	3992.7	2.79	NS
Residual	24	1430.7		
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
A	E	B	11
136.2	157.0	167.3	189.8

c) Components of variance

$$s_s^2 = 184.25, \quad s_p^2 = -100.85, \quad s_{sp}^2 = 854.01, \quad s_r^2 = 1430.72$$

d) Heritability estimates

$$h_s^2 = 0.36 \quad h_p^2 = \emptyset$$

4724 Length of branches

As seed parent tree produced seedlings with the longest branches when as a pollen parent gave seedlings with shortest branches. The inheritance seems to be under strong genetic control with the seed parent playing a more important role in the inheritance of the branch length than the pollen parent. The values were 72 per cent for seed and 58 per cent for male parents.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	ll	
A		100.0	53.3	106.3	86.5
B	82.7		107.7	120.0	103.4
E	112.7	161.3		126.7	133.5
ll	130.7	121.7	109.3		120.5
Averages	108.7	127.7	90.1	117.7	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seeds	3	3764.0	9.45	**
Pollen	3	2292.0	5.75	**
S x P	5	955.2	2.39	NS
Residual	24			
Total	35			

c) Duncan's multiple range test

S e e d p a r e n t s			
A	B	ll	E
86.5	103.4	120.5	133.5

P o l l e n p a r e n t s			
E	A	ll	B
90.1	108.7	117.7	127.7

c) Components of variance

$$s_s^2 = 209.06, \quad s_p^2 = 111.40, \quad s_{sp}^2 = 185.73, \quad s_r^2 = 398.03$$

d) Heritability estimates

$$h_s^2 = 0.72 \quad h_p^2 = 0.58$$

4725 Number of branches

Trees B and 11 as seed parents produced progenies with the largest number of branches but trees A and E did not differ from them significantly. As pollen parents, progenies from tree 11 produced the largest, while seedling from tree E had the lowest values. The heritability values were 23 per cent for pollen parents and zero for seed parents.

a) Average values

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		18.3	11.7	18.6	16.2
B	14.6		16.7	19.3	16.9
E	17.3	16.7		16.0	16.7
11	19.7	16.7	14.3		
Averages	17.2	17.2	14.2	18.0	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.88	0.15	NS
Pollen	3	25.11	4.38	*
S x P	5	19.33	3.37	NS
Residual	24	5.72		
Total	35			

Duncan's multiple range test

P o l l e n p a r e n t s		
E	A-B	11
14.2	17.2	18.0

c) Components of variance

$$s_s^2 = -1.537, \quad s_p^2 = 0.481, \quad s_{sp}^2 = 4.537, \quad s_r^2 = 5.722.$$

d) Heritability estimates

$$h_s^2 = \emptyset \quad h_p^2 = 0.23$$

4726 Number of cotyledons

Tree B as a seed and pollen parent produced seedlings with the largest number of cotyledons, while A and E gave seedlings with the lowest number. The heritability value is 42 per cent for pollen and zero for seed parents.

a) Average values

Seed parents	<u>P o l l e n p a r e n t s</u>				Average
	A	B	E	11	
A		7.0	5.7	6.7	6.4
B	6.7		6.3	7.7	6.8
E	6.7	7.0		6.0	6.5
11	6.3	7.3	6.3		6.6
Averages	6.5	7.1	6.1	6.8	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.324	0.64	NS
Pollen	3	1.583	3.16	*
S x P	5	0.916	1.83	NS
Residual	24	0.500		
Total	35			

Duncan's multiple range test

<u>P o l l e n p a r e n t s</u>			
E	A	B	11
6.1	6.5	6.8	7.1

c) Components of variance

$$s_s^2 = -0.049, \quad s_p^2 = 0.055, \quad s_{sp}^2 = 0.139, \quad s_r^2 = 0.5$$

d) Heritability estimates

$$h_p^2 = \emptyset \quad h_p^2 = 0.42$$

4727 Diameter of root collar

Tree 11 produced seedlings with the largest values for root collar diameter both as seed and as pollen parents. Tree B as a seed parent and tree E as a pollen parent produced seedlings with the smallest diameter of the root collar. The heritability estimations in both cases were negative and were recorded as zero.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		2.6	2.3	2.9	2.6
B	1.9		2.3	2.6	2.2
E	2.1	2.5		2.6	2.4
11	3.2	2.8	2.2		2.8
Averages	2.5	2.6	2.2	2.7	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.529	1.78	NS
Pollen	3	0.347	1.17	NS
S x P	5	0.537	1.81	NS
Residual	24	0.296		
Total	35			

c) Components of variance

$$s_s^2 = -0.00069, \quad s_p^2 = -0.01587, \quad s_{sp}^2 = 0.08038, \quad s_r^2 = 0.29641$$

d) Heritability estimates

$$h_s^2 = \emptyset, \quad h_p^2 = \emptyset$$

4728 Green weight of shoots

Tree 11 produced the largest values in both instances, not being significantly different from the other three trees, when the values of seed parents were compared, but when the averages of the pollen parents were tested, tree 11 significantly differed from A and E. The heritability values are in general agreement with number of branches and branch length characteristics, showing only the pollen effect of the parent with 22 per cent value, and no effect of the seed parents.

a) Average values (in grams)

Seed parents	<u>P o l l e n p a r e n t s</u>				Average
	A	B	E	11	
A		5.1	2.8	5.5	4.47
B	2.4		4.8	6.2	4.48
E	3.9	4.5		6.8	5.08
11	6.6	6.0	4.7		5.75
Averages	4.31	5.20	4.13	6.16	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	3.43	2.14	NS
Pollen	3	7.64	4.76	**
S x P	5	5.97	3.72	*
Residual	24	1.60		

Duncan's multiple range test

<u>P o l l e n p a r e n t s</u>			
E	A	B	11
4.13	4.31	5.20	6.16

c) Components of variance

$$s_s^2 = -0.2117, \quad s_p^2 = 0.1389, \quad s_{sp}^2 = 1.456, \quad s_r^2 = 1.602$$

d) Heritability estimates

$$h_p^2 = \emptyset, \quad h_p^2 = 0.22$$

4729 Dry weight of roots

Tree 11 as seed parent and tree B as pollen parent showed the best combining ability. The heritability values were calculated as 35 per cent for seed and 60 per cent for pollen parents.

a) Average values (in grams)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A	0.242	0.451	0.293	0.399	0.381
B	0.242		0.335	0.369	0.315
E	0.258	0.411		0.477	0.382
11	0.435	0.440	0.393		0.422
Averages	0.312	0.434	0.340	0.415	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.017	1.29	NS
Pollen	3	0.031	2.24	NS
S x P	5	0.010	0.75	NS
Residual	24	0.013		
Total	35			

c) Components of variance

$$s_s^2 = 0.00062, \quad s_p^2 = 0.00171, \quad s_{sp}^2 = -0.00113, \quad s_r^2 = 0.01371$$

d) Heritability estimates

$$h_s^2 = 0.35, \quad h_p^2 = 0.60$$

47210 Dry weight of shoots

Tree 11 produced the largest dry-weight values in both instances, while tree B as a seed parent and tree E as a pollen parent were represented with the lowest values. The heritability estimations were 9 per cent for seed and zero for pollen parent.

a) Average values (in grams)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		1.171	0.709	1.256	1.045
B	0.549		1.158	1.125	0.944
E	0.904	1.055		1.526	1.162
11	1.707	1.406	1.152		1.421
Averages	1.053	1.210	1.006	1.302	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seeds	3	0.38	2.79	NS
Pollen	3	0.17	1.24	NS
S x P	5	0.36	2.65	*
Residual	24	0.13		
Total	35			

c) Components of variance

$$s_S^2 = 0.0015, \quad s_P^2 = -0.0159, \quad s_{Sp}^2 = 0.0152, \quad s_F^2 = 0.1362$$

d) Heritability estimates

$$h_S^2 = 0.09, \quad h_P^2 = \emptyset$$

47211 Light transmittance of chlorophyll

Extract from tree 11 transmitted more light, having a lighter color than tree E, which gave the lowest transmitted light value when seed parents were compared. When the pollen parents were evaluated, the sequence was exactly reversed; the solution from tree E was lighter than solution from tree 11. The heritability values were the highest and very similar to each other as 98 per cent for seed and 97 per cent for pollen parents.

a) Average values (in arc. sine)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		49.603	48.646	47.103	48.451
B	49.213		48.430	45.763	47.935
E	46.166	47.293		46.720	46.726
11	49.413	49.550	49.556		49.800
Averages	48.264	48.962	49.157	46.528	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	14.58	10.81	* *
Pollen	3	12.87	9.54	* *
S x P	5	-1.00	-0.74	NS
Residual	24	1.34		
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
E	B	A	11
46.726	47.935	48.451	49.800

P o l l e n p a r e n t s			
11	A	B	E
46.528	48.264	48.962	49.157

c) Components of variance

$$s_s^2 = 3.41, \quad s_p^2 = 2.98, \quad s_{r^2} = 0.94$$

d) Heritability estimates

$$h_s^2 = 0.98, \quad h_p^2 = 0.97$$

473 Progeny tests in the growth chambers. Short day treatment

4731 Length of root

Tree 11 as a seed, and tree B as a pollen parent produced the longest root, while the shortest averages were calculated on tree B as seed and Tree A as pollen parents. The heritability values were zero for seed and 8 per cent for pollen parents.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		342.0	268.0	323.3	311.2
B	246.3		328.0	353.3	309.4
E	332.3	347.6		359.3	346.4
11	295.0	365.0	417.6		359.2
Averages	291.2	351.6	338.1	346.3	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	5668.4	10.54	**
Pollen	3	6792.16	12.63	**
S x P	5	6245.78	11.61	**
Residual	24	537.59		
Total	35			

Duncan's multiple range test

S e e d p a r e n t			
B	A	E	11
309.2	311.2	346.4	359.2
P o l l e n p a r e n t			
A	E	11	B
291.2	338.1	345.3	351.6

c) Components of variance

$$S_S^2 = -48.11, \quad S_P^2 = 45.53, \quad S_{Sp}^2 = 1902.73, \quad S_r^2 = 537.59$$

d) Heritability estimates

$$h_S^2 = \emptyset, \quad h_P^2 = 0.08$$

4732 Length of hypocotyl

Tree A as a seed parent produced the longest hypocotyl length and the value was not significantly different from the value obtained from tree 11, but differed significantly from Tree B and E. As pollen parent B & E gave the highest values. The heritability values were very similar, 77 per cent for seed and 74 per cent for pollen parents.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		44.0	34.3	32.0	36.8
B	30.7		32.3	33.0	32.0
E	30.0	32.3		25.7	29.3
11	32.7	33.7	36.3		34.2
Averages	31.1	36.6	34.3	30.2	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	90.54	8.31	**
Pollen	3	79.43	7.29	**
S x P	5	20.69	1.90	NS
Residual	24	10.88		
Total	35			

Duncan's Multiple Range Test

S e e d p a r e n t s			
E	B	11	A
29.3	32.0	34.2	36.8

P o l l e n p a r e n t s			
11	A	E	B
30.2	31.1	34.3	36.6

c) Components of variance

$$S_s^2 = 5.82 \quad S_p^2 = 4.89 \quad S_{sp}^2 = 3.27 \quad S_r^2 = 10.89$$

d) Heritability Estimates

$$h_s^2 = 0.77 \quad h_p^2 = 0.74$$

4733 Length of epicotyl

Trees A and B produced the longest epicotyl length and the values were significantly different from values observed on trees E and 11, when seed parents were compared. The average values did not differ significantly when pollen parents were compared. The heritability values were calculated as 71 per cent for seed and zero for pollen parents.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		91.7	107.3	54.0	88.3
B	86.3		65.3	65.7	72.4
E	38.0	51.0		55.7	48.2
11	42.7	51.0	41.3		45.0
Averages	56.7	64.6	71.3	58.4	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	3257.07	14.04	**
Pollen	3	436.18	1.88	NS
S x P	5	947.18	4.08	NS
Residual	24			
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
11	E	B	A
45.0	48.2	72.4	84.3

c) Components of variance

$$S_S^2 = 192.49, \quad S_P^2 = -42.58, \quad S_{Sp}^2 = 238.40, \quad S_F^2 = 231.97$$

d) Heritability estimates

$$h_S^2 = 0.71, \quad h_P^2 = \emptyset$$

4734 Length of branches

Although the length of the branch was not significant in any instances, the longest branches were produced on tree B as pollen and tree A as seed parent, while the shortest length was observed on tree E as seed and tree 11 as pollen parent. The heritability estimations were 57 per cent for seed and 19 per cent for pollen parents.

a) Average values

Seed parents	P o l l e n p a r e n t s				Average
	A	B	E	11	
A		42.3	42.0	38.3	40.9
B	49.7		15.0	44.7	36.4
E	23.3	46.0		25.0	31.4
11	26.0	20.0	0.0		15.3
Averages	33.0	36.1	19.0	36.0	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	1119.21	0.89	NS
Pollen	3	597.36	0.47	NS
S x P	5	482.78	0.38	NS
Residual	24	1249.63		
Total	35			

c) Components of variance

$$s_s^2 = 53.03, \quad s_p^2 = 9.55, \quad s_{sp}^2 = 119.27, \quad s_r^2 = 124.96$$

d) Heritability estimates

$$h_s^2 = 0.57, \quad h_p^2 = 0.19$$

4735 Number of branches

Tree A as a seed, and tree B as a pollen parent gave the highest number of branches. The heritability values were 60 per cent for seed and zero for pollen parents.

a) Average values

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	ll	
A		3.7	3.0	0.7	2.4
B	1.7		0.7	1.3	1.2
E	1.0	1.3		0.7	1.0
ll	0.7	0.3	0.0		0.3
Averages	1.1	1.8	1.2	0.9	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	6.99	3.99	*
Pollen	3	1.28	0.73	NS
S x P	5	2.78	1.59	NS
Residual	24	1.75		
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
ll	E	B	A
0.3	1.0	1.2	2.4

c) Components of variance

$$s_s^2 = 0.35, \quad s_p^2 = -0.12, \quad s_{sp}^2 = 0.34, \quad s_r^2 = 1.75$$

d) Heritability estimates

$$h_s^2 = 0.60, \quad h_p^2 = \emptyset$$

4736 Number of cotyledons

Tree B, both as a seed and pollen parent, produced the largest average number of cotyledons, while A as a seed parent and E as a pollen parent exhibited the lowest average values. The heritability values were 8 per cent for seed and 57 per cent for pollen parents.

a) Average values

Seed parents	<u>P o l l e n p a r e n t s</u>				Averages
	A	B	E	ll	
A		7.0	5.7	6.6	6.4
B	7.3		6.7	7.3	7.1
E	6.3	7.3		6.3	6.7
ll	6.3	7.3	6.3		6.7
Averages	6.7	7.2	6.2	6.8	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.70	1.79	NS
Pollen	3	1.51	3.87	*
S x P	5	0.64	1.64	NS
Residual	24	0.39		
Total	35			

Duncan's multiple range test

<u>P o l l e n p a r e n t s</u>			
E	A	ll	B
6.2	6.7	6.8	7.2

c) Components of variance

$$s_s^2 = 0.005, \quad s_p^2 = 0.073, \quad s_{sp}^2 = 0.08, \quad s_r^2 = 0.39$$

d) Heritability estimates

$$h_s^2 = 0.08, \quad h_p^2 = 0.57$$

4737 Diameter of root collar

Tree A as a seed parent, and tree 11 as a pollen parent produced the largest values. The values from seed parents only were significant since the diameter of root collar on tree A was much larger than on the other three trees, which were very similar to each other. The heritability values were calculated as 71 per cent for seed and 58 per cent for pollen parent.

a) Average values (in millimeters)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		2.2	2.0	2.4	2.24
B	1.9		1.8	2.1	1.94
E	1.8	1.8		1.9	1.85
11	2.1	2.1	1.7		1.95
Averages	1.94	2.05	1.84	2.15	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.257	3.35	*
Pollen	3	0.163	2.13	NS
S x P	5	0.055	0.72	NS
Residual	24	0.076		
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
E	B	11	A
1.85	1.94	1.95	2.24

c) Components of variance

$$S_s^2 = 0.106, \quad S_p^2 = 0.009, \quad S_{sp}^2 = -0.007, \quad S_r^2 = 0.077$$

d) Heritability estimates

$$h_s^2 = 0.71, \quad h_p^2 = 0.58$$

4338 Green weight of roots

Tree 11 as seed and tree E as pollen parents exhibited the highest values in green weight of root. The heritability values were similar to each other, 28 per cent for seed and 30 per cent for pollen parents.

a) Average values (in grams)

Seed parents	<u>P o l l e n p a r e n t s</u>				Averages
	A	B	E	11	
A		0.7	0.7	1.0	0.78
B	0.7		0.9	1.0	0.87
E	0.7	0.8		0.8	0.76
11	0.8	1.0	1.4		1.04
Averages	0.72	0.80	0.99	0.94	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.150	2.18	NS
Pollen	3	0.153	2.23	NS
S x P	5	0.108	1.56	NS
Residual	24	0.068		
Total	35			

c) Components of variance

$$s_s^2 = -.0035, \quad s_p^2 = 0.0038, \quad s_{sp}^2 = 0.0131, \quad s_r^2 = 0.0689$$

d) Heritability estimates

$$h_s^2 = 0.28, \quad h_p^2 = 0.30$$

4739 Green weight of shoots

Trees A and B produced the largest values as seed parents and were significantly different from tree 11, which gave the lowest green weight of shoot. The pollen parents were not significantly different from each other. The heritability values were 79 per cent for seed and zero for pollen parents.

a) Average values (in grams)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		1.8	1.6	1.3	1.58
B	1.4		1.0	1.5	1.32
E	1.0	1.3		1.1	1.16
11	1.0	0.9	0.8		0.88
Averages	1.13	1.34	1.14	1.33	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.759	4.55	*
Pollen	3	0.119	0.71	NS
S x P	5	0.120	0.71	NS
Residual	24			
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
11	E	B	A
0.88	1.16	1.32	1.58

c) Components of variance

$$S_S^2 = 0.0533, \quad S_P^2 = -0.00001, \quad S_{Sp}^2 = -0.0156, \quad S_F^2 = 0.1667$$

d) Heritability estimates

$$h_S^2 = 0.79, \quad h_P^2 = \emptyset$$

47310 Dry weight of roots

Tree 11 as seed and tree E as pollen parents represented the highest values in dry weight of root. No significant difference was found between trees, when they were compared as seed and pollen parents. The heritability values were 62 per cent for seed and zero for pollen parents.

a) Average values (in grams)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		0.332	0.285	0.349	0.322
B	0.283		0.296	0.308	0.296
E	0.292	0.316		0.270	0.292
11	0.321	0.368	0.492		0.394
Averages	0.299	0.338	0.358	0.309	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	0.019	2.55	NS
Pollen	3	0.006	0.84	NS
S x P	5	0.007	0.96	NS
Residual	24	0.007		
Total	35			

c) Components of variance

$$s_s^2 = 0.00104, \quad s_p^2 = -0.00007, \quad s_{sp}^2 = -0.00009, \quad s_r^2 = 0.00783$$

d) Heritability estimates

$$h_s^2 = 0.62, \quad h_p^2 = \emptyset$$

47311 Dry weight of shoots

Trees A, B, and E produced the largest values in the dry weight of shoot, while tree 11 was represented with the lowest value, when the trees were compared as seed parents. The pollen parents were not significantly different from each other. The heritability values were calculated as 76 per cent for seed and 37 per cent for pollen parents.

a) Average values (in grams)

Seed parents	<u>P o l l e n p a r e n t s</u>				Averages
	A	B	E	11	
A		0.574	0.467	0.480	0.507
B	0.417		0.335	0.453	0.402
E	0.346	0.422		0.353	0.374
11	0.313	0.285	0.257		0.285
Averages	0.359	0.427	0.353	0.428	

b) Analysis of variation

Source	DF	MS	F	Signif.
Seed	3	0.075	3.35	*
Pollen	3	0.015	0.69	NS
S x P	5	0.002	0.10	NS
Residual	24	0.022		
Total	35			

Duncan's multiple range test

<u>S e e d p a r e n t s</u>			
11	E	B	A
0.285	0.374	0.402	0.507

c) Components of variance

$$s_s^2 = 0.0061, \quad s_p^2 = -0.0011, \quad s_{sp}^2 = -0.0067, \quad s_r^2 = 0.0224$$

d) Heritability estimates

$$h_s^2 = 0.76, \quad h_p^2 = 0.37$$

47312 Light transmittance of chlorophyll

Tree 11 in both cases was represented with the lowest transmittance value, while trees A, B, and E reversed positions when they were compared as seed and as pollen parents. The heritability values were 65 per cent for seed and 78 per cent for pollen parents.

a) Average values (in arc. sine)

Seed parents	P o l l e n p a r e n t s				Averages
	A	B	E	11	
A		49.606	51.550	46.530	49.228
B	47.490		49.060	46.533	47.694
E	46.036	48.836		45.383	46.752
11	45.763	46.913	47.490		46.722
Averages	46.763	48.452	49.366	46.148	

b) Analysis of variance

Source	DF	MS	F	Signif.
Seed	3	12.45	3.38	*
Pollen	3	21.96	5.96	**
S x P	5	1.42	0.38	NS
Residual	24	3.68		
Total	35			

Duncan's multiple range test

S e e d p a r e n t s			
11	E	B	A
46.722	46.752	47.694	49.228

P o l l e n p a r e n t s			
11	A	B	E
46.148	46.430	48.452	49.366

c) Components of variance

$$s_s^2 = 0.919, \quad s_p^2 = 1.712, \quad s_{sp}^2 = 0.751, \quad s_r^2 = 3.682$$

d) Heritability estimates

$$h_s^2 = 0.65, \quad h_p^2 = 0.78$$

48 Combining Ability of Four Trees as Seed and Pollen Parents

Data available from the polyallel crosses permitted an assessment of the combining ability of the four Douglas-fir trees, both as seed and as pollen parents. After ranking the parents for each characteristic evaluated from one, as the largest to four as the smallest, for long-and for short-day treatment separately (Table 30) it appears that certain trees exceed others in many characteristics. Tree 11 appears to be the best seed parent in long day treatment, since it exhibited the largest values for the characteristics, followed by trees E, B, and A. As pollen partner, tree B proved to be the best, followed by trees 11, A, and E. Tree 11 as a pollen parent was not far behind tree B. In a few important characteristics, such as length of epicotyl, diameter of root collar, and green and dry weight of shoot, the crosses with pollen from tree 11 surpassed the crosses with pollen from tree B.

When the performance of progenies from the four different seed trees were compared after the short-day treatment, the best combining ability was observed on tree A, (Figure 16), followed by B, 11, and E. Tree 11, which was on the top of the list after long-day progenies were evaluated, ranked last on the following characteristics; length of epicotyl and branches, number of branches, green and dry weight of shoot and transmittance of chlorophyll. As pollen parent, tree B proved to be the best, E second, 11 third and A fourth. Tree 11 was consequently ranked at the lower end of the scale following the short-day treatment, while it was on the upper end of the scale

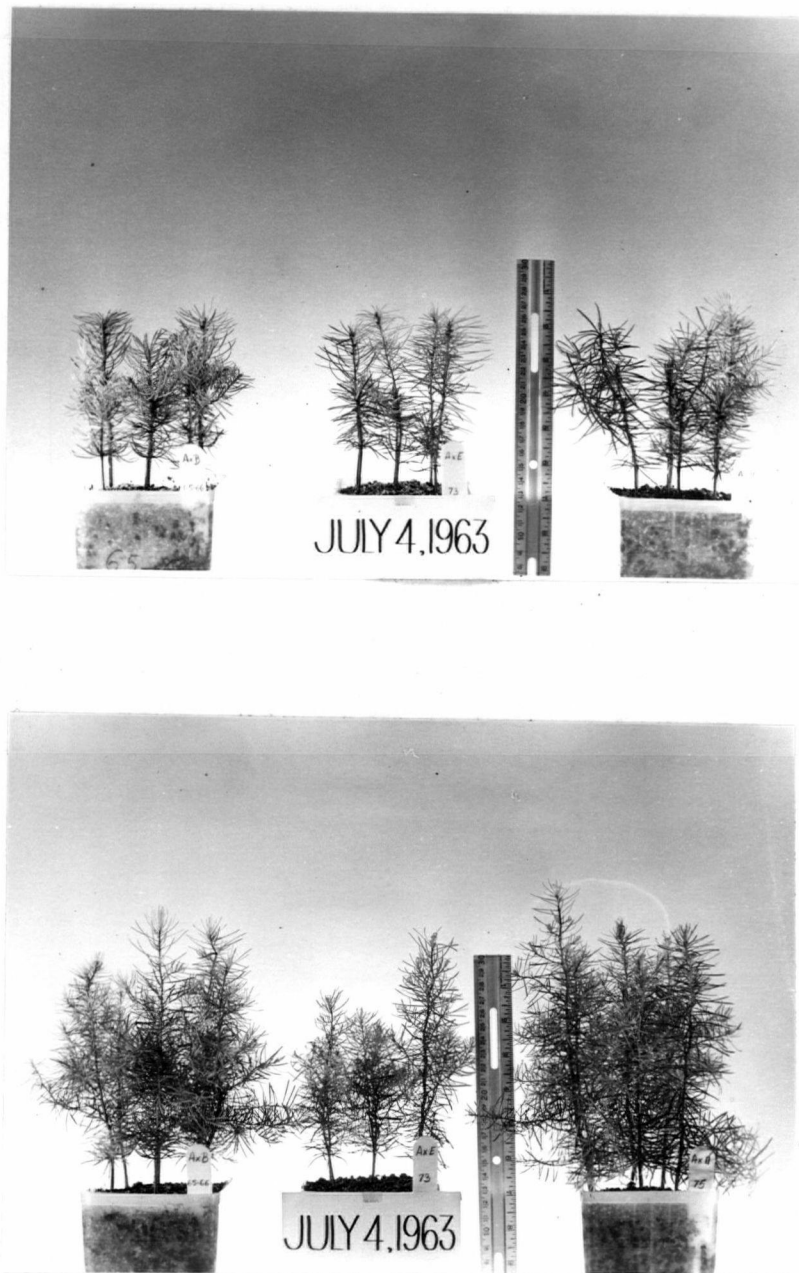


Figure 16. Height of seedlings from tree A after 132 days, short-day (above), and long-day (below) treatment. Left to right; progenies from A x B, A x E, and A x 11 crosses.

Table 30. Combining abilities of four Douglas-fir trees. The numbers illustrate the rank of the parents. Number one represents the largest, and number four the smallest value.

Long day treatment

Characteristics	T r e e s							
	A		B		E		11	
	Seed	Pollen	Seed	Pollen	Seed	Pollen	Seed	Pollen
1. Length of roots	2	3	3	1	4	2	1	4
2. Length of hypocotyl	2	4	4	1	3	2	1	3
3. Length of epicotyl	4	3	2	2	3	4	1	1
4. Length of branch	4	3	3	1	1	4	2	2
5. Number of branches	4	3	2	2	3	4	1	1
6. Number of cotyledons	4	3	1	1	3	4	2	2
7. Diameter of root collar	2	3	4	2	3	4	1	1
8. Green weight of roots	-	-	-	-	-	-	-	-
9. Green weight of shoots	3	3	4	2	2	4	1	1
10. Dry weight of roots	3	4	4	1	2	3	1	2
11. Dry weight of shoots	3	3	4	2	2	4	1	1
12. Transm. of chlorophyll	2	3	3	2	4	1	1	4
Total	34	35	33	17	30	36	13	22

Short day treatment

Characteristics	T r e e s							
	A		B		E		11	
	Seed	Pollen	Seed	Pollen	Seed	Pollen	Seed	Pollen
1. Length of roots	3	4	4	1	2	3	1	2
2. Length of hypocotyl	1	3	3	1	4	2	2	4
3. Length of epicotyl	1	4	2	2	3	1	4	3
4. Length of branch	1	3	2	1	3	4	4	2
5. Number of branches	1	3	2	1	3	2	4	4
6. Number of cotyledons	4	2	1	1	2	4	3	3
7. Diameter of root collar	1	3	3	2	4	4	2	1
8. Green weight of roots	3	4	2	3	4	1	1	2
9. Green weight of shoots	1	4	2	1	3	3	4	2
10. Dry weight of roots	2	4	4	2	3	1	1	3
11. Dry weight of shoots	1	3	2	2	3	4	4	1
12. Transm. of chlorophyll	1	3	2	2	3	1	4	4
Total	20	40	29	19	37	30	34	31

under long-day treatment. Such a contrast in performance between the two treatments supplies evidence to support the assumed difference in provenance of tree 11 from that of the other three trees. This result confirms Leibundgut's (1963) statement that different provenances will vary widely under adverse conditions; whereas if conditions are close to the optimum, differences will not show up. It also confirms Nicholson's (1963) separation of 28 Douglas-fir provenances using epicotyl lengths and number of branches under short-day, but not under long-day treatment. Tree A exhibited a good combining ability as seed parent, and the progeny appeared to produce highest values in length of hypocotyl and epicotyl, number of branches, diameter of root collar, green and dry weights of shoot and transmittance of chlorophyll. The best pollen parent under short-day as well as long-day treatment was tree B. The combining ability of the parents provides evidence that certain combinations of a polyallel cross even in four conceivably closely related trees can yield significantly different positive results in respect to 132-days-old progenies. The polyallel cross in connection with a short testing method, using growth chambers, seems very useful, but will have to be modified to best fulfil the requirements of forest tree improvement.

49. Heritability Values

The narrow-sense heritability values calculated for certain characteristics studied, indicate that they are under various degrees of genetic control (Table 31).

Table 31. Narrow-sense heritability values for twelve characteristics studied.

No.	Characteristics	H e r i t a b i l i t y v a l u e s							
		1962				1961			
		Long day		Short day		Long day		Short day	
		Seed	Pollen	Seed	Pollen	Seed	Pollen	Seed	Pollen
1.	Length of roots	-	0.12	-	<u>0.08</u>	-	-	-	-
2.	Length of hypocotyl	0.23	-	<u>0.77</u>	0.74	0.09	-	-	-
3.	Length of epicotyl	<u>0.36</u>	-	<u>0.71</u>	-	<u>0.87</u>	0.08	x	x
4.	Length of branch	<u>0.72</u>	<u>0.58</u>	0.57	0.19	x	x	x	x
5.	Number of branches	-	<u>0.23</u>	<u>0.60</u>	-	x	x	x	x
6.	Number of cotyledons	-	<u>0.42</u>	0.08	<u>0.57</u>	x	x	x	x
7.	Diameter of root collar	-	-	<u>0.71</u>	0.58	x	x	x	x
8.	Green weight of roots	x	x	0.28	0.30	x	x	x	x
9.	Green weight of shoots	-	<u>0.22</u>	<u>0.79</u>	-	x	x	x	x
10.	Dry weight of roots	0.35	0.60	0.62	-	x	x	x	x
11.	Dry weight of shoots	0.09	-	<u>0.76</u>	0.37	x	x	x	x
12.	Light Transm. of chlor.	<u>0.98</u>	<u>0.97</u>	<u>0.65</u>	<u>0.78</u>	x	x	x	x

- the heritability value was zero or negative

x no measurements were taken

 underlined values were significant in analysis of variance

Although the environment differed in the case of short- and long-day treatment, the length of root, length of epicotyl, length of branch, transmittance of chlorophyll and the number of cotyledons showed high consistency in the mode of inheritance during the 1962 experiment. The length of root is under a slight genetic control. The seed parent contribution was nil when compared to the pollen parent, which results heritability values 0.12 after long-day and 0.08 after short-day treatment in 1962.

The length of epicotyl appears to be under the control of the seed parent, while the pollen parent did not play an important part.

It is recognized that heritability estimations may not necessarily be reliably made from the first year's growth data, since the measurements are also affected at that age by the size of seeds, germination characteristics, and embryo development. After the third or fourth year, these effects are likely to be partly or entirely eliminated.

When the correlation coefficients between total height of four-year-old and the shoot lengths of 132-days-old progenies, using the four available crosses of B x A, B x E, E x A, and E x B, were calculated, a significant correlation was found with a value of 0.895 ($r_{.05} = 0.879$ and $r_{.01} = 0.959$, $df = 3$). The first year's total height growth also showed significant or highly significant correlation between total heights after one year and after four years (Appendices VIII - XIV), of the same crosses. This may mean that the effect of the seed

is not well pronounced in the case of crosses, or diminishes at a very early stage of development. On the other hand, the correlation between shoot (epicotyl) length at 132 days and the total height after 4 years is a useful one, and promises a certain time reduction in progeny testing in forest tree breeding, where time is one of the greatest obstacles.

The role of seed parents in the pattern of inheritance in shoot length is also important in forestry practice. Seedlings with rapid juvenile growth may overcome brush competition earlier and the new forest stand could be established more quickly and surely by using such seedlings. For instance, seedlings from $11 \times A$ crosses had grown to a height of 224.3 mm. at the end of the 132 day experimental period, while during the same period seedlings from $E \times A$ cross, reached 126.0 mm., and those from $B \times A$ cross, 129.3 mm. In the above-mentioned crosses the pollen parent was the same; only the seed parents were different. By selecting seedlings from $11 \times A$ crosses, the increase could be between 73 and 78 per cent in comparison to seedlings from $B \times A$ or $E \times A$ crosses respectively. Such trees should be propagated in seed orchards to give a quick increase in seedling height. This might be worthwhile even if we are taking a "calculated risk" in not knowing the correlation between seedling and mature tree height. The length of branch was under strong genetic control, and the seed parent effect was larger than that of the pollen parent.

The heritability value in the number of cotyledons

indicated the effect of the pollen parents. This effect is clearly shown in Table 32, where tree B as pollen parent, having the highest average cotyledon numbers in every cross with trees A, E, and 11, increased the average cotyledon number. But it is also obvious from the table, that although trees A, E, and 11 have very similar average cotyledon numbers when crossed with tree B as the pollen parents, the effect on the average cotyledon number is different, varying from 6.88 (B x E), to 7.43 (B x A). This may suggest that besides the additive genetic variance other genetic variance due to dominance or epistasis is also important.

The light transmittance of chlorophyll showed the highest heritability values in the long-day treatment, for instance 0.98 for seed and 0.97 for pollen parent, revealing that the trait is under strong additive genetic control.

The heritability values for the other characteristics did not show consistency and varied from zero to 0.76. The values were higher in the short- than in the long-day treatments, probably because the residual component of variance was smaller with smaller seedlings. To improve the precision of the estimation, a larger number of seedlings would be needed, mostly from the long-day seedlings. Generally, with the exception of the light transmittance of chlorophyll, the heritability estimates are moderate, which confirms the assumption of Toda et al. (1959), that in many characteristics the additive genetic variance is small compared to other variances, due to dominance or epistasis. The moderate heritability

Table 32. Average cotyledon numbers from different years of the same crosses.

	1959				1962				1963						
Cross	1	1	2	3	4	1	2	3	4	Av.	± SD	± CV			
A x A				6.0						6.00					
A x B		7.1	7.0	7.0	7.1	6.7	7.0	7.0	6.7	6.95	0.42	6.04			
A x E		6.6	6.5	5.7		6.2	6.3	5.7	6.2	6.19	0.86	13.89			
A x ll						7.0	6.6	6.6	6.7	6.73	0.96	14.26			
A x ?		6.2	6.4		6.2	6.8	6.5			6.42	0.50	7.79			
Total										<u>6.56</u>					
B x B															
B x A	7.6	7.8			7.8	7.8	6.7	7.3	7.0	7.43	1.08	14.53			
B x E	7.0	6.3	7.2			7.0	6.7	6.7	7.3	6.88	0.84	12.21			
B x ll						7.2	7.3	7.3	7.7	7.37	0.34	4.61			
B x ?	7.5					7.0	7.2			7.23	0.36	4.97			
Total										<u>7.21</u>					
E x E		6.3	6.0							6.15	0.22	3.58			
E x A	6.6	6.4	6.4	6.5	6.4	6.6	6.7	6.3	6.7	6.51	0.31	6.30			
E x B	6.7	6.6	7.3	6.5	7.0	6.7	7.0	6.6	6.9	6.81	0.73	10.72			
E x ll						6.3	6.0	6.3	6.0	6.15	0.30	4.87			
E x ?	6.6	6.1	6.4	6.4	6.0	6.3	6.0			6.26	1.82	29.07			
Total										<u>6.47</u>					
ll x ll															
ll x A						6.0	6.3	6.3	6.3	6.22	0.26	4.18			
ll x B						7.3	7.3	6.6	7.3	7.12	0.61	8.57			
ll x E						6.7	6.3	6.3	6.3	6.40	0.34	5.31			
ll x ?						6.3	6.0								
Total										<u>6.52</u>					

values also indicate that selection based on phenotypic values may not be a reliable indication of the success of such a breeding program. The writer wholeheartedly agrees with the statement of Wright et al. (1958): "most significant improvement will come from programs in which every parent is carefully selected and carefully progeny tested and in which controlled pollination plays a major part".

5 SUMMARY

The results of a study of variation and inheritance in Douglas-fir have been reported. The work was based on four trees located on the University of British Columbia Campus in Vancouver, B. C. All four trees represented the coastal variety of Douglas-fir. Three of them (trees A, B, and E) resulted from natural regeneration and represent the local population, while the fourth (tree 11) was chosen from a stand planted in 1934. The seed origin of the stock used for the plantation is not known, but represents a different coastal origin than the previous three trees. Observations on phenological characteristics were carried out in 1959, 1960, 1961 and 1963.

Variation in pollen size and number was investigated. Cone and seed characteristics were studied within and between trees, and germination tests were completed on the seeds from various crosses.

A progeny test in the nursery included four full-sib, and two half-sib progenies from 1959 crosses.

Appollyalel cross was completed successfully in 1962 on the four trees. All sixteen combinations were attempted, using three different pollination methods (dry, wet, and dry-wet).

Yield of cones, number of filled seeds and total number of seeds were obtained.

Seedlings from the various crosses were grown under controlled environment for 132 days. The combining ability of the four trees, both as seed and as pollen parents, as well as the

individual values of the 12 traits studied for crosses were investigated. The method of analysis used for computing heritability based on the polyallel cross was described in detail using the following twelve traits: lengths of roots, hypocotyl, epicotyl, and branch, number of branches and cotyledons, diameter of root collar, green and dry weight of roots and shoots, and finally the light transmittance of chlorophyll. The data were used to analyze components of variance and obtain estimates of narrow-sense heritability for all twelve traits. Some of the results are summarized as follows:

1. Vegetative bud flushing appeared to be under strong genetic control. Tree B flushed its vegetative buds earliest and tree 11 latest during the four years observed. The average difference was 16 days. Earliest date of flushing of the four trees was in 1960 and the latest in 1959, the average difference being 9 days.
2. Order of flowering is not correlated with the order of vegetative bud flushing.
3. The diameter of pollen grains varied from 91.08 microns (tree A) to 99.19 microns (tree B). A highly significant negative correlation was found between diameter of pollen grain and date of flowering. Early-flowering trees possessed larger pollen grains and possible adaptive significance to adverse climatic conditions was suggested.

4. Cone length varied significantly on tree E from south (69.59 mm.) to north (65.56 mm.). Although the difference was not significant the cone length decreased with height in tree on both aspects. Cone width decreased similarly with height in tree crown.
5. Variation was less in cone length than in cone width when the cones from the four trees were compared. The cone length also varied less than the cone width from year to year.
6. The total number of seeds per cone was significantly larger on the south than on the north aspect. The top portion of the crown produced the largest total number of seeds per cone.
7. Germination per cent was relatively high when crosses were carried out using various pollen parents with tree E. Tree E as a pollen parent also transmitted this characteristic to the progeny of every cross. It was suggested that the genetic control of germination per cent in this instance seemed to indicate control on a single factor basis and that tree E was homozygous dominant for this characteristic.
8. The first year's growth was a good indication of the total height growth at the end of the four years. The shoot length of the 132-days-old progenies also showed significant positive correlation with the total height at the end of the fourth year. The total height of the seedlings from

the E x B cross surpassed the total height of seedlings from E x A cross, by 32 per cent.

9. Of 302 seed conelets bagged and pollinated, 101 were lost. The loss was lowest on tree E (22 per cent of the isolated conelets), and highest (44 per cent) on tree B. With one-tenth of the pollen grains used in dry pollination, the wet cross-pollination method proved to be effective. Only 19 per cent of the isolated cones were lost using the wet, compared to 49 per cent in the case of dry pollination method.
10. From the 201 cones collected, 8,004 seeds were extracted. Of these, 2,142 seeds were defined as "filled", giving an average yield of 10.66 filled seeds per cone. From cross-pollination the number of filled seeds per cone averaged 13.81, from wind-pollination 3.05. The lowest number was obtained from self-pollination with only 1.91 filled seeds per cone.
11. Progeny from a polyallel cross provided evidence of the combining ability of the four trees. Tree 11 appeared to be the best seed parent, and the second best pollen parent in a long-day treatment provided for 132 days. The shoot length of the 132-days-old seedlings averaged 224.3 millimeters from the cross of trees 11 x A. When the same pollen parent (A) was used, the shoot length was 126.0 millimeters from trees E x A, and 129.3 millimeters from

the B x A cross. If the best parent combinations were selected the increase could be between 73-78 per cent in shoot growth at the end of the 132 days.

12. The narrow-sense heritability values appeared to be moderate in every trait studied with one exception, the light transmittance of chlorophyll. This trait showed strong additive genetic control, with heritability values of 0.98 for the seed and 0.97 for the pollen parent populations grown under long days, and 0.65 for seed and 0.78 for pollen parent populations under short days.

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Appendix I. Definition of characteristics used in Appendices II to VII inclusive.

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- X_1 - color of hypocotyl, (in four classes, see page 34),
 X_2 - length of hypocotyl, in millimeters,
 X_3 - bud condition on July 30, 1960, (in six classes, see page 34),
 X_4 - " " " " 30, 1962,
 X_5 - " " " " 30, 1963,
 X_6 - current year shoot growth to June 6, 1962 in millimeters,
 X_7 - " " " " June 6 to July 23, 1962, in millimeters,
 X_8 - current year shoot growth to June 6, 1963 in millimeters,
 X_9 - " " " " June 6 to July 23, 1963, in millimeters,

 Y_1 - total height growth in 1960, in millimeters,
 Y_2 - " " " " 1961, " " ,
 Y_3 - " " " " 1962, " " ,
 Y_4 - " " " " 1963, " " ,
 Y_5 - total height on September 30, 1963, in millimeters.
-

APPENDIX II. Values of the selected characteristics of four-year-old progenies from B x A cross pollination.

No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₈	Y ₁	Y ₂	Y ₃	Y ₄ ¹	Y ₅ ¹
1	3	12	4	1	2	165	63	274	42	36	196	230	334	808
2	3	21	6	6	2	75	60	226	62	19	177	145	302	664
3	3	12	4	2	2	140	67	167	65	81	176	210	247	729
4	3	18	5	2	1	111	40	154	56	47	155	155	222	597
1	3	15	5	5	2	109	66	184	63	35	140	185	263	638
2	2	13	6	1	2	96	56	209	28	39	80	172	249	553
3	2	13	6	3	1	185	135	245	209	115	256	330	470	1184
4	2	14	5	2	2	89	16	130	21	56	76	108	163	517
1	2	17	3	1	1	76	21	165	14	25	73	101	194	410
2	1	17	3	1	1	75	28	189	61	20	48	103	262	450
3	2	14	5	2	2	118	62	228	68	65	200	186	314	779
4	2	12	5	1	1	100	58	123	45	30	63	170	176	451
$\bar{X}$	2.33	14.83	4.74	2.24	1.58	111.58	55.99	191.16	61.16	47.33	136.66	174.58	26.33	648.33
$\pm SD$	.65	2.85	1.05	1.65	0.51	35.58	30.79	46.46	90.01	28.28	67.10	63.94	83.30	213.28
$\pm CV$	27.9	19.2	22.2	73.7	32.3	31.9	55.0	24.3	81.8	59.8	49.1	36.6	31.3	32.9

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¹ For explanation of characteristics see Appendix I.

Appendix III. Values of the selected characteristics of four-year-old progenies from B x E cross pollination.

No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	4	22	6	2	2	198	99	214	81	112	200	302	305	941
2	3	14	5	2	1	155	72	198	98	50	154	232	313	763
3	3	12	5	1	3	99	74	246	106	31	95	208	368	714
4	3	17	5	5	2	95	45	208	45	39	115	150	270	591
1	3	9	6	1	1	98	29	191	80	48	86	130	287	560
2	2	22	5	2	2	150	62	233	59	47	224	215	308	816
3	2	16	5	1	1	120	41	123	54	69	150	168	183	586
4	2	18	5	2	1	112	29	199	37	49	73	147	250	537
1	2	13	5	1	1	76	26	131	40	22	54	106	180	375
2	2	11	5	2	2	133	28	255	67	67	142	185	341	746
3	1	11	6	3	2	122	85	173	91	71	143	208	280	713
4	1	15	6	1	2	96	45	158	81	60	120	143	255	593
$\bar{X}$	2.33	14.99	5.24	1.91	1.66	121.16	54.58	194.08	69.91	55.41	129.66	191.16	278.33	661.24
$\pm$ SD	0.88	4.19	0.45	1.16	0.65	33.69	23.68	41.92	23.08	23.28	50.03	58.76	56.41	150.17
$\pm$ SV	37.8	28.0	8.6	60.1	39.2	27.8	43.4	21.6	33.0	42.0	38.6	30.7	20.3	22.7

Appendix IV. Values of selected characteristics of four-year-old progenies from E x A cross pollination.

No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	4	12	4	1	1	76	14	125	16	18	112	95	148	385
2	4	17	3	2	1	125	60	164	30	15	72	186	206	496
3	3	14	5	1	2	73	24	124	52	30	75	99	186	404
4	3	12	4	6	1	85	18	178	57	21	76	113	245	467
1	2	12	4	1	2	95	21	143	32	17	80	117	189	415
2	4	14	5	2	1	163	62	154	134	91	175	229	300	809
3	3	14	5	6	2	113	83	214	86	33	132	291	314	788
4	3	12	5	1	2	85	41	161	63	27	167	128	238	572
1	3	14	3	5	2	104	90	305	108	59	102	205	426	806
2	2	18	5	1	1	91	45	222	62	29	84	150	295	576
3	2	15	4	1	2	120	77	124	82	24	122	202	218	581
4	2	10	1	1	1	105	57	179	25	18	75	167	213	483
$\bar{X}$	2.91	13.66	3.99	2.33	1.49	120.91	49.33	174.41	62.24	31.83	105.99	164.66	248.16	565.16
$\pm$ SD	7.9	2.26	1.20	2.05	.52	25.15	26.36	52.34	35.43	22.03	36.43	59.56	75.24	156.57
$\pm$ CV	27.1	16.5	30.0	88.0	34.9	20.8	53.4	30.0	56.9	69.2	34.3	36.2	30.3	27.7

Appendix V. Values of selected characteristics of four-year-old progenies from E x A pollination.

No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	3	14	6	3	3	1700	109	245	135	130	230	289	392	1055
2	3	13	3	1	1	108	32	167	59	42	121	157	238	609
3	2	12	6	2	3	107	33	212	39	82	124	146	265	629
4	1	12	5	2	1	103	49	145	80	58	144	157	238	609
1	4	20	6	2	3	194	112	293	162	137	168	308	469	1102
2	3	19	5	5	2	110	54	245	86	61	149	181	347	757
3	3	14	5	1	1	95	40	261	53	45	75	139	329	602
4	3	14	6	2	1	115	45	140	70	40	106	166	221	547
1	2	18	5	1	3	108	42	205	71	35	150	156	290	649
2	2	13	5	2	2	110	48	205	92	35	132	163	311	654
3	2	14	5	2	3	110	78	287	101	50	133	190	398	785
4	1	9	6	2	1	166	103	289	189	35	175	272	522	1013
$\bar{X}$	2.41	14.33	5.24	2.08	1.99	124.66	62.08	224.50	94.75	62.49	142.24	192.66	335.16	746.91
± SD	0.90	3.17	0.86	1.08	0.95	32.37	30.13	54.45	45.40	35.88	38.56	60.72	94.98	200.00
± CV	37.3	22.1	16.4	5.19	47.7	26.0	48.5	24.3	47.9	57.4	27.1	31.5	28.3	26.8

Appendix VI. Values of selected characteristics of four-year-old progenies from B open pollination.

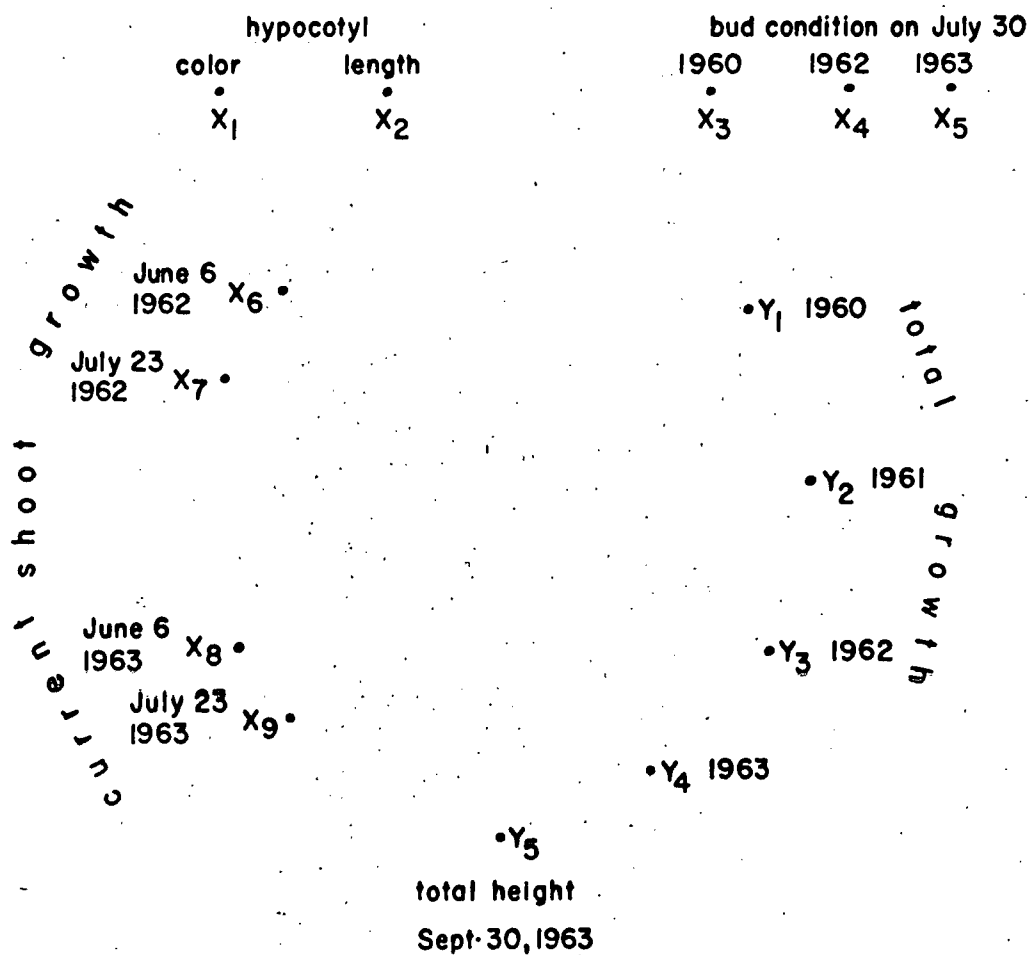
No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	4	17	3	2	2	153	82	300	80	24	208	250	385	884
2	4	22	4	1	2	90	22	180	25	24	177	115	216	554
3	3	11	5	2	2	129	37	180	63	50	113	169	256	599
4	3	16	6	6	2	84	20	189	36	43	56	128	237	480
1	3	15	5	1	1	64	9	161	15	18	51	75	187	346
2	3	15	5	1	2	50	26	195	33	38	80	77	240	450
3	3	20	3	1	2	76	66	174	79	60	76	163	266	585
4	3	12	3	2	2	88	22	215	36	32	96	110	261	511
1	3	9	3	1	2	92	26	190	22	35	108	122	220	494
2	2	16	3	6	1	102	53	183	42	84	96	165	236	599
3	2	16	6	6	3	93	32	266	139	151	223	230	418	1038
4	1	16	5	1	2	99	116	250	47	45	62	232	315	670
$\bar{X}$	2.83	15.41	4.24	2.49	1.91	93.33	50.91	206.91	51.41	50.33	112.16	152.99	268.83	600.83
$\pm$ SD	.83	3.57	1.21	2.15	.51	27.14	40.25	42.68	34.63	36.43	58.75	59.40	69.47	190.76
$\pm$ CV	29.3	23.2	28.5	86.3	26.7	29.1	79.1	20.6	67.4	72.4	52.4	38.8	25.8	31.7

Appendix VII. Values of selected characteristics of four-year-old progenies from E open pollination.

No.	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅
1	4	15	6	6	3	178	162	328	4	135	195	349	345	1239
2	3	17	5	6	2	176	139	310	31	72	154	320	352	1013
3	3	20	6	1	1	103	32	126	15	71	59	140	148	438
4	3	14	5	1	1	93	32	110	44	28	148	133	245	568
1	3	14	4	6	2	129	68	177	65	29	102	210	252	607
2	3	15	6	6	1	46	21	101	17	28	56	69	178	298
3	3	11	5	1	1	61	20	134	26	31	65	85	166	358
4	3	12	3	2	1	104	68	212	68	12	117	179	292	612
1	2	9	5	3	3	121	55	103	94	43	126	180	206	564
2	2	14	6	6	1	128	72	247	128	69	142	210	382	813
3	1	12	5	3	2	103	52	201	69	77	112	158	280	399
4	1	10	5	2	1	68	24	144	42	21	76	96	196	399
$\bar{X}$	2.58	13.58	5.08	3.58	1.58	109.17	66.24	182.75	50.25	51.33	109.67	176.58	256.50	629.00
± SD	.90	3.06	.90	2.23	.79	40.97	44.95	78.67	36.17	34.80	37.99	85.18	74.15	275.45
± CV	34.9	22.5	17.7	62.3	50.0	37.5	67.9	43.0	72.0	67.8	34.6	48.2	28.9	43.8

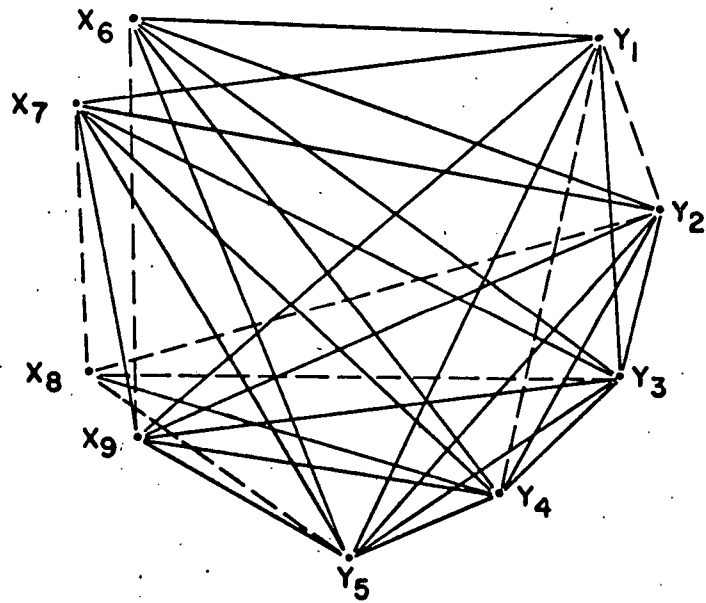
Appendix VIII

System of diagrammatic representation of correlations among characteristics in the progeny of certain crosses as shown in appendices IX to XIV inclusive.



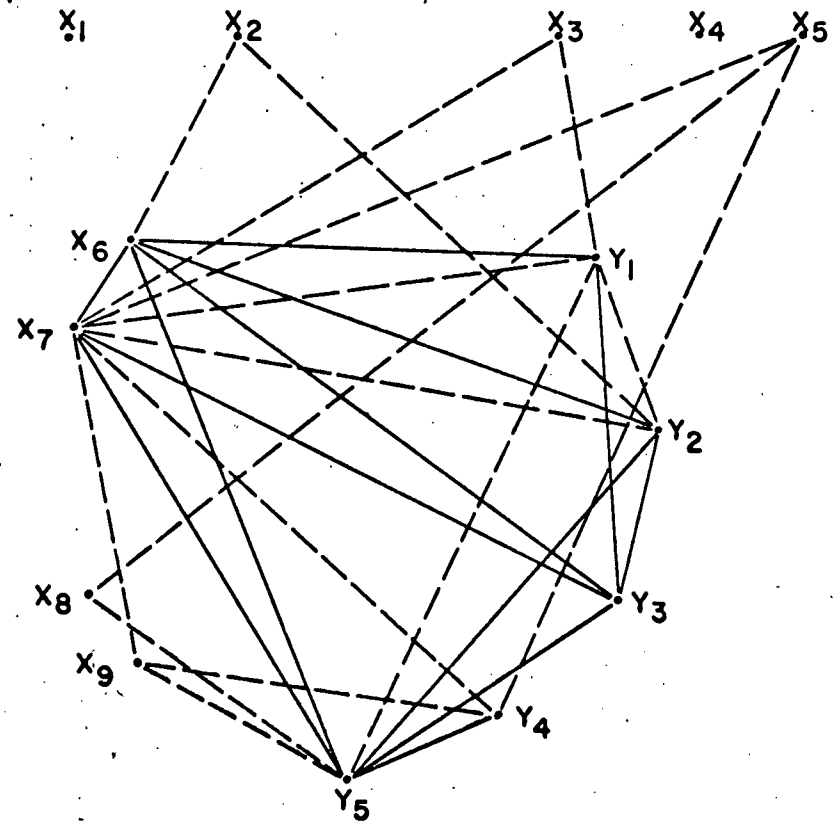
Appendix IX

B x A

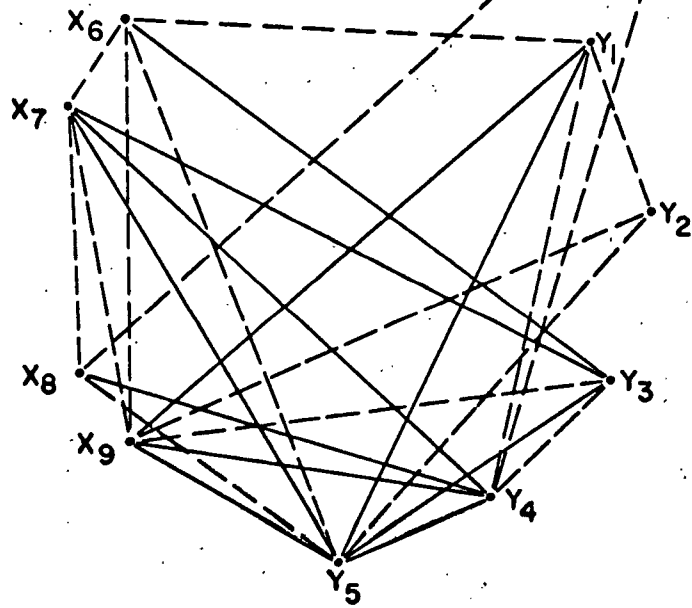
 $\dot{x}_1$ $\dot{x}_2$ $\dot{x}_3$ $\dot{x}_4$ $\dot{x}_5$ 

Appendix X

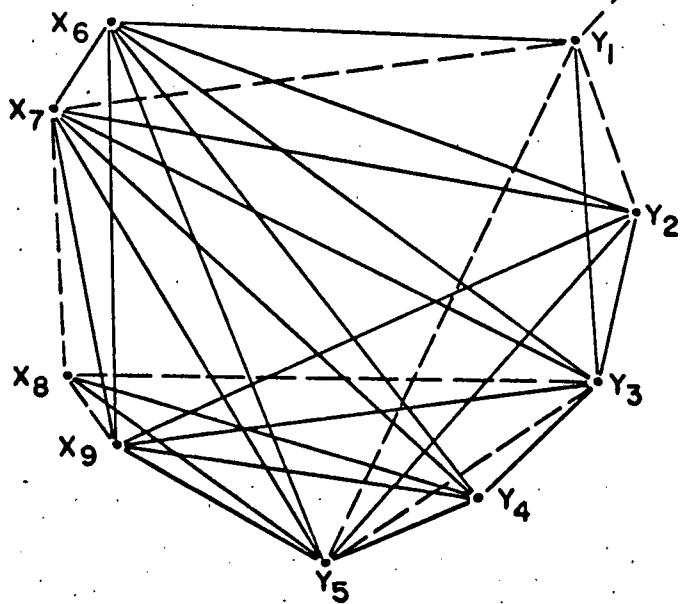
B x E

 $\dot{x}_1$ $\dot{x}_2$ $\dot{x}_3$ $\dot{x}_4$ $\dot{x}_5$ 

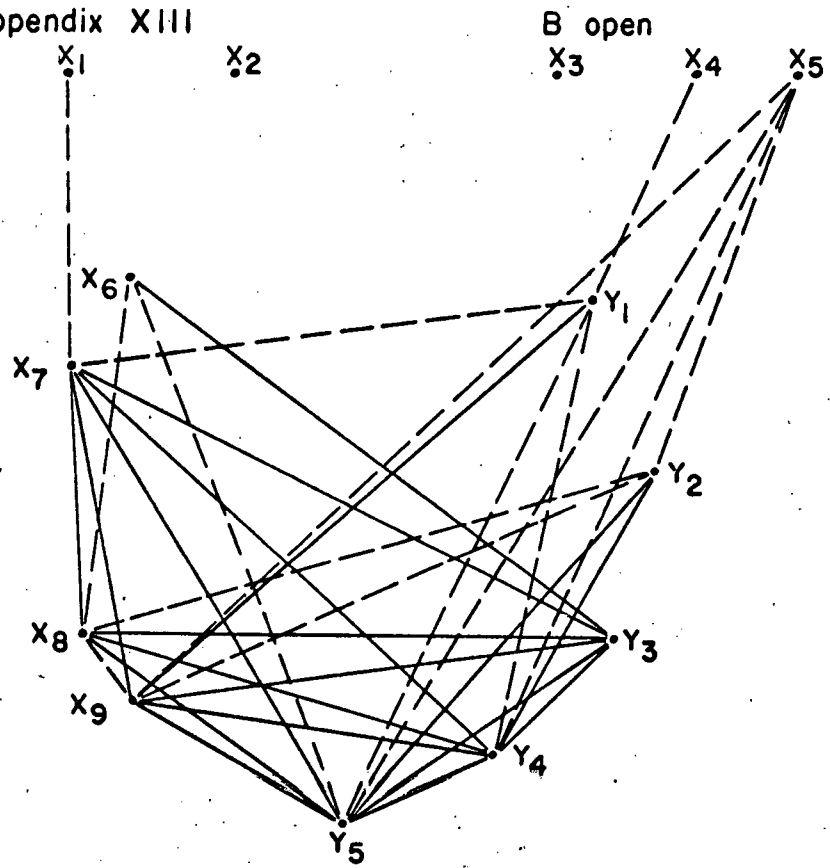
Appendix XI

 x_1 x_2 E x A
 x_3 x_4 x_5 

Appendix XII

 x_1 x_2 E x B
 x_3 x_4 x_5 

Appendix XIII



Appendix XIV

