

A STUDY OF THE PHENOTYPIC AND GENOTYPIC  
VARIATION OF 545 SINGLE TREE PROGENIES OF  
38 PROVENANCES OF THE 1970 I.U.F.R.O. SITKA  
SPRUCE (PICEA SITCHENSIS (BONG.) CARR.) COLLECTION

by

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## ABSTRACT

During the 1970 fall, the I.U.F.R.O. Section 22 "Working group on procurement of seed for provenance research" organized an expedition to collect Sitka spruce cones from British Columbia and Alaska. The locations of the 39 provenances range from  $48^{\circ} 38' 0''$  to  $58^{\circ} 37' 0''$  latitude N and from  $121^{\circ} 93' 0''$  to  $134^{\circ} 58' 0''$  longitude W. The elevation varies from 0 to 2,200 feet above sea level. In most cases, the collections were made from 15 trees in each location. The single tree progeny collection of 557 trees representing 39 locations constitute the material of this thesis.

In a first part, seed and cone morphology were studied on a single tree basis. Ten cones per progeny were randomly selected and the length of each cone measured to the nearest mm. Five randomly selected seeds from each tree were mounted on a special sheet, and seed length, seed width, wing length and wing width were measured to the nearest 0.01 mm. Nested analyses of variance and DUNCAN's multiple range tests for all the characteristics studied have been performed using five subregions. No definite classification of the provenances was possible by using univariate anova procedures. A simple correlation matrix has been calculated between all the traits studied and longitude, latitude and altitude of the place of origin of the provenances, using the provenance means. Multiple regression analyses have been used for investigating this correlation matrix. The percentage of variation

accounted for by the geographical co-ordinates varies between 10.2% and 43.6%.

Using the seed and cone traits studied, a comparison of several multivariate statistical analyses which could be used for classification purposes has been attempted. The so-called canonical analysis, discriminant function analysis and principal component analysis have been compared and applied for classifying the provenances. The sub-regions already used were analyzed separately. Dendrograms were also constructed and analyzed. Advantages and disadvantages of each multivariate method have been discussed. It was found that the discriminant function analysis, its associated generalized distances of MAHALANOBIS and dendrograms provided the most rational classification of the provenances.

In a second part, the genetic variability of 545 Sitka spruce single tree progenies was studied in a nursery test during 1971 and 1972.

A total of 545 single tree progenies grouped into 38 provenances was sown in April, 1971, using a randomized complete block design with four replications and 24 seedlings per replication or 96 seedlings per progeny. The seeds were placed in the cavities of styro-blocks using the method developed by the Pacific Forest Research Centre in co-operation with the B.C. Forest Service and they have been treated by the most recent nursery methods, in the new B.C.F.S. nursery at Surrey (B.C.). Germination rate, bud set, length of the epicotyl and survival after the first growing season were assessed in



1971. The seedlings were transplanted in plain soil seedbeds in May, 1972, to a distance of 6" to 6", each progeny being kept separate while respecting the same statistical design as in 1971. Bud burst, bud set, colour of the needles and total height after the second growing season, were assessed in 1972. There was a clinal variation in bud burst, bud set, colour of the needles and epicotyl length. Bud burst was negatively correlated with longitude ( $r = -0.50$ ) and positively correlated with altitude ( $r = 0.42$ ). Bud set appeared under strict genetic control as indicated by the second estimation of this trait, at the end of the second growing season (with latitude:  $r = 0.88$ ). Latitude and altitude of the seed sources explained 65% of the total variation in epicotyl length. Total height after the second growing season showed the same relationships as epicotyl length.

General equations for components of variance for unbalanced data were originally calculated for a nested-crossed model. Components of variance and their standard error were calculated for epicotyl length and total height after the second growing season. Depending on the subregions, the genetic variance among provenances is generally larger than the tree to tree genetic variation.

The narrow sense heritability, on an individual basis, and its standard error, for total height after the second growing season, were estimated on a subregion basis. Heritability was found generally to be close to 0.10, indicating low general combining ability.

The relationships between the seed, cone and seedling traits measured were studied. Multiple regression analysis showed that a higher proportion of the variation of the seedling traits was accounted for by the geographical coordinates of the provenances than by the cone and seed traits studied.

Variation in foliar macro- and micro- nutrients of 10 Sitka spruce provenances was studied, but no geographical pattern of variation detected in K, Ca, Mg, Fe, Mn, Zn, P, and N needle contents. Only K showed some provenance to provenance variation. Possible physiological explanations for this absence of variation are discussed.

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## INTRODUCTION

### 1. Methodological outline

Sitka spruce (Picea sitchensis (BONG.) CARR.) is the largest of all the spruces. In British Columbia, in volume cut, Sitka spruce ranks seventh in importance with more than 40,000,000 cubic feet cut in 1971, (British Columbia Forest Service Annual Report, 1971).

It is well known that the Queen Charlotte Islands of British Columbia are one of the areas where Sitka spruce yields its maximum production.

Commercial stands of Sitka spruce may occur at 1,000 feet elevation. However, 3/4 of commercial stands are estimated to be within 2-1/2 miles of tide water, a fact which would facilitate the commercial exploitation of this spruce. Single trees may contain up to 15,000 board feet of timber. Furthermore, Sitka spruce wood is the most valuable of all spruce woods and finds a multitude of uses (building construction, aircraft, pulp and paper, etc.).

Sitka spruce seems, therefore, to be a very valuable material to genetically improve for future extensive plantations in western North America.

It naturally grows in a narrow strip along the Pacific coast from northwestern California to northern Alaska. Its range is over 1,800 miles long and characterized by a pronounced oceanic climate.

As an exotic species, pure stands of Sitka spruce have proven to be very manageable and exceptionally productive, especially in Britain.

Recently, international interest in this tree species has increased and, during the fall of 1970, on behalf of 12 European countries, the I.U.F.R.O., Section 22 "Working group on procurement of seed for provenance research" organized an expedition to collect Sitka spruce cones from British Columbia and Alaska. Twenty cones from each of the 557 trees representing 39 locations were provided to Professor O. Sziklai, Faculty of Forestry, University of British Columbia. This single tree progeny collection constituted the material with which the author commenced the present study.

The objectives of the present study were to describe the variation pattern presented by the 557 parent trees and their progenies which were growing in a nursery environment, near Vancouver, B.C.

It was decided to keep the single tree progenies separate and to start a simultaneous provenance and progeny test in the nursery.

Many characters have been studied on both the parent trees and their progenies, in many tree species. Much discussion occurred on the possible patterns of variation of the traits studied (clinal, ecotypic or mixed), and how best to describe these patterns, but only resulted in very general explanations (GALOUX and FALKENHAGEN, 1965).

In provenance and open pollinated progeny research in tree species, many basic problems remain to be solved, both practical and theoretical.

Divisive techniques such as anovas and techniques of multiple correlation and regression analyses were considered complementary and it was decided to use both techniques to express in objective forms all the facets of the complex variability of the material studied. Beside univariate anova models and multiple regression and correlation techniques, multivariate statistical methods are also used; however, it was decided to solve some basic problems related to the choice of the best methods in multivariate statistics, prior to any definite classification of the biotas which might exist in the Sitka spruce natural populations.

Many techniques, more or less empirical, have been used to classify the natural populations of a tree species. For instance, once a great number of traits has been assessed on both the parent material and their progenies growing in a nursery test, correlation matrices are calculated and used to choose the best traits which could lead to the distinction of biotas (or races) through regrouping the provenances in larger entities. In a more rigorous way, these correlation matrices are also used in multivariate statistical analyses in order to find interpretable "components" or to cluster the provenances. The first method which leads to the "sum of differences method" developed by WRIGHT and BULL in 1962 has been criticized by STERN (1964). LANGLET (1959) has also pointed out the danger of following blindly a computer generated classification of provenances. A great number of sophisticated methods of numerical classification have been proposed

and applied without paying too much attention to their relative similarity, efficiency, mathematical theory, etc., (DAGNELIE, 1966). Thus, there is an urgent need to compare the techniques used in multivariate morphometrics. As part of the biosystematics of Sitka spruce, it was decided to measure 5 reproductive cone and seed characters, on a single tree basis, of the parent trees, in order to compare the following multivariate statistical methods which were available to us: principal component analysis, discriminant function analysis and the so-called canonical analysis. Multiple correlation analyses and anovas were extensively used in order to get an accurate picture of the variability of the 5 traits studied. This problem solving approach was chosen instead of assessing a number of characters and then to apply a given multivariate method.

Only 7 traits having adaptative values were studied on progenies growing in the nursery; among these traits, only height growth was measured on a single tree progeny basis. The genetic variation in foliar macro- and micro- nutrient contents was also studied because it was rarely attempted.

Genecology tells us that the natural populations of a tree species are locally adapted to their environment of origin, at least to some degree. It was decided to subdivide the material into 5 regions, at least fairly homogeneous ecologically and to analyze these 5 regions separately except for the correlation and regression analyses which were performed over all the provenances. Thus, the heterogeneity of



the variances is likely to be reduced and safe multiple comparison tests of the means can be performed on a reduced set of provenances: generally the number of provenances is less than 12 except for region 5 which contains a maximum of 16 provenances. Variance components estimation thus makes more sense on region basis than, on a global basis, by using 39 provenances and 10,000 degrees of freedom.

The objectives of the present study were thus two-pronged: on the one hand, a problem solving approach was used instead of blindly applying some given methods, a fresh look at the problems was attempted and many questions were raised; on the other hand, a large nursery test of more than 500 single tree progenies of Sitka spruce representing 39 provenances was initiated and taken care of, for three years. Basic measurements were made on the progenies and different hypotheses related to the variation pattern of Sitka spruce were expressed. In 1974, the nursery test yielded several field tests for long term research purposes which were established at strategic locations in B.C.

## 2. Autecology of Sitka spruce

Sitka spruce (Picea sitchensis (BONG.) CARR.) is a member of the Casicta group of the genus Picea. The Casicta group is characterized by unequally quadrangular flattened leaves, with wide upper and lower surfaces bearing the stomata. The cones scales are thin and flexible (DALLIMORE and JACKSON, 1966).

Its range (Fig. 1) is over 1,800 miles long and extends along the coast of Alaska, British Columbia, Washington, Oregon and southwards to northern California. The width of the range varies between a few miles in California to about 130 miles in Alaska. Sitka spruce reaches maximum development on the Olympic Peninsula of Washington and the Queen Charlotte Islands (British Columbia).

According to DAUBENMIRE (1957), Picea sitchensis would have roughly occupied its present range before the glaciation period, but the range formerly extended southward along the California coast, beyond the San Francisco bay. After the glaciations, it re-appeared nearly all over its present range. This would indicate a persistence on nunataks well scattered along the coast from Puget Sound to Juneau. However, it is difficult to justify this theory of refugia scattered from Alaska to Washington state, because Sitka spruce is presently adapted to a pronounced oceanic climate. Earlier authors (HULTEN, 1937; HALLIDAY and BROWN, 1943 in BURLEY, 1965) have besides proposed that Sitka spruce inhabited a southern "niche" during the glaciations and that it followed the retreating ice to Alaska.

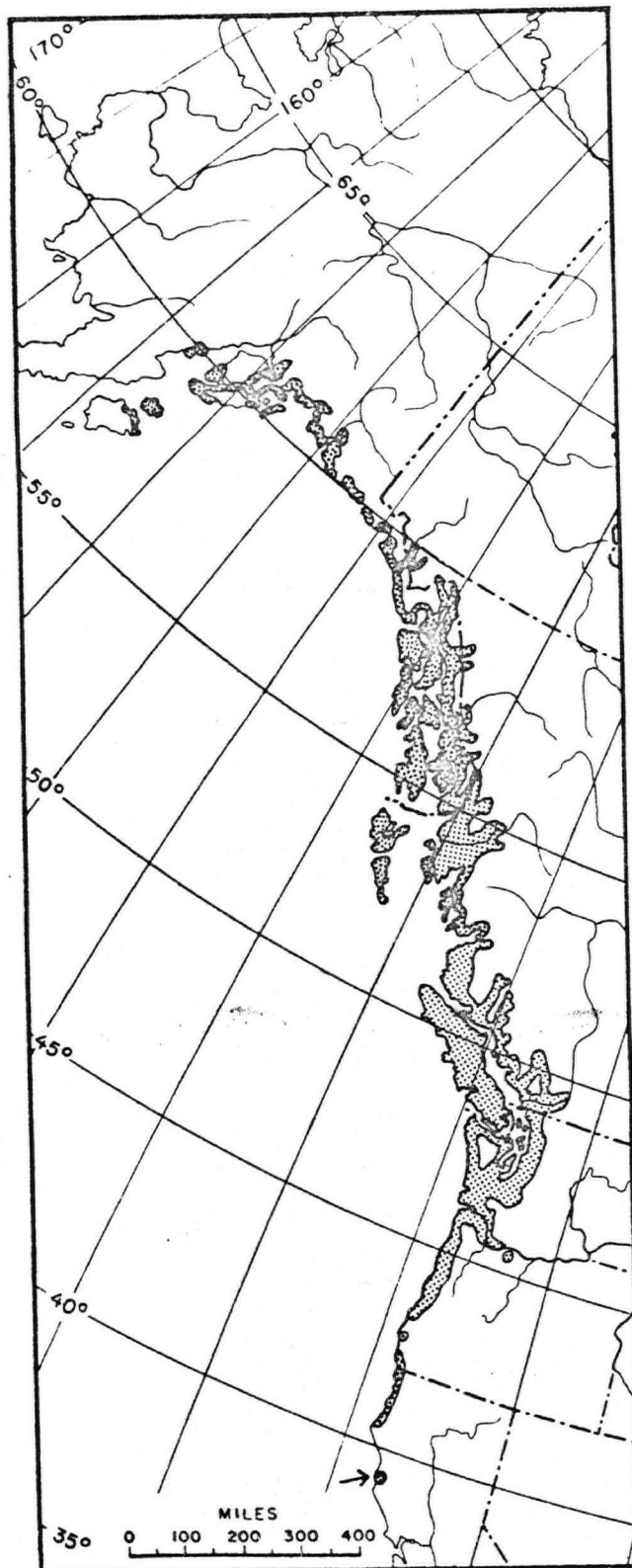


Fig. 1. The range of Sitka spruce  
( FOWELS, 1965 )

In slightly warmer pre-pleistocene times, Sitka spruce probably grew on the Aleutians and was linked to P. jezoensis, to which it is similar systematically. Sitka spruce is a member of the homogeneous northwest America group whose origin is recent (WRIGHT, 1955).

P. sitchensis is unique among the spruces in being a low-land conifer even in the southern portion of its range.

Sitka spruce is typically associated with the "fog belt" of the Pacific coast of North America. It grows in a climate characterized by equable temperature, high precipitation from 66 inches to 150 inches of annual precipitation, long vegetation period (at least 130 days), cloudiness averaging 200 days per year and mild winters (FOWELLS, 1965).

KRAJINA (1969) has defined the biogeoclimatic zones of British Columbia. In British Columbia, Sitka spruce thrives primarily in two biogeoclimatic zones: (1) The coastal Douglas-fir zone, the driest part of the mesothermal zone of British Columbia, which has been subdivided into two subzones, (2) The coastal western hemlock zone itself also subdivided into two subzones.

Soils are similar throughout the distribution area: they are high in organic matter with a pH of 4.0 to 5.7. DAY (1957) has

suggested that Sitka spruce is favoured, in comparison with its associated species, by soil conditions of high nutrient status and that it will assume a dominant position when supply of aerated water and nutrients is adequate. The frequent occurrence of Sitka spruce as a seashore species may have a nutritive reason, for such seaside situation must receive a relatively heavy deposit of sea spray. This idea has been recently defended again by KRAJINA (1969).

As noticed earlier, Sitka spruce is a low elevation species except in Southern Alaska. In British Columbia, it is seldom found at elevations exceeding 1,000 feet. It is a major species in only two forest types of the Pacific West according to the American classification of forest types used by FOWELLS (1965). It is a prolific seed producer with good cone crop, every three or four years and partial ones in between. Seed will germinate on almost any kind of seedbed if moisture is abundant. It is a fast growing tree that readily dominates its associates: western hemlock (Tsuga heterophylla (RAF.) SARG.) and western red cedar (Thuja plicata DONN.). However, Sitka spruce is less shade tolerant than western hemlock; and is able to take up a dominant position only when fertility of the site sufficiently favours its growth, (DAY, 1957). Pure Sitka spruce stands are essentially subclimax, the more tolerant western hemlock eventually replacing Sitka spruce.

Mature trees have straight boles with swollen and buttressed bases, are as high as 285 feet and possess diameter up to 10 feet or more. Two types have been distinguished: the bottomland type

and the slope type (CARY (1922) in KARLSBERG, 1961).

Sitka spruce is considered shallow-rooted and susceptible to wind throw. It is subject to decay after injury and is attacked by several insects that may kill or damage large volumes of timber. The most important pest hindering the extensive use of Sitka spruce, on the Pacific Coast, is the Sitka spruce weevil (Pissodes sitchensis). This beetle only attacks trees 2-8 inches in diameter and 5 to 25 feet tall, killing or injuring the terminal shoot. Forked or crooked stems often result from weeviling (WRIGHT, 1960). Only in Alaska and on the Queen Charlotte Islands, is Sitka spruce totally free from weevil attack.

In British Columbia, KRAJINA (1969) describes Sitka spruce as a "hygrohylophile to subhygrohylophile, submontane to montane, aciculilignous, subeutrophophytic, stenotrophophytic species" occurring in three biogeoclimatic zones. Its shade tolerance would be low to nil, depending on the habitat. It would require high quantities of available calcium and magnesium, requirements which would explain its tolerance to ocean spray. However, the seaside situation of Sitka spruce might also be explained by some resistance to sea salt deposit on its foliage as well as to wind. (Personal communication of Dr. Ph. HADDOCK).

PHELPS has recently reviewed the literature dealing with Sitka spruce; particularly interesting are the volume and yield tables which are basic instruments in managing Sitka spruce stands in the Pacific Northwest. Of interest to the geneticist, is the possibility of

propagating Sitka spruce by cuttings or by grafting (PHELPS, 1973).

According to FLETCHER and FAULKNER (1972), Sitka spruce is an excellent exotic species wherever introduced, provided its ecological preferences are respected. It has surpassed in volume production many of the native and introduced tree species in Belgium, Britain, Denmark, France, Ireland, New Zealand, Germany and Norway. The most noteworthy success is its introduction in Great Britain, where it can yield up to 25 cubic meters per ha per year. In that country, it already occupied 265,800 ha in 1970 and the proportion of Sitka spruce, according to the same authors, will probably continue increasing in Britain.

To summarize the most important ecological facts, Sitka spruce is the largest of all the spruces. It grows naturally in a narrow strip along the Pacific coast from northwestern California to northern Alaska, which is characterized by its pronounced oceanic climate. Sitka spruce is prolific, its seed germinating in almost any kind of seedbed, provided humidity is plentiful. It is shade tolerant to intolerant according to the habitat and grows rapidly, but mostly remains a secondary species mixed with western hemlock and western red cedar. Its regeneration is best after stand opening. It is, therefore, a sub-climax species, probably close to the "opportunistic" type (STERN, 1963), which is defined as having an irregular reproduction and random differentiation at the population level.

It is, however, difficult to forecast "a priori" the genetic

plasticity of Sitka spruce, because its ecological preferences are fairly narrow, at least in comparison with other western tree species such as Douglas-fir.

As an exotic species, pure stands of Sitka spruce have proven to be very manageable and exceptionally productive, notably in Britain, and there is no doubt that, in suitably managed plantations, Sitka spruce could become an important conifer in the Pacific Northwest if the weevil was under control.



PART I

SITKA SPRUCE AS GENECOLOGICAL MATERIAL

# CHAPTER I

## A REVIEW OF THE GENETIC LITERATURE PERTAINING TO SITKA SPRUCE

### 1.1 Silvicultural studies

BURLEY (1965<sub>b</sub>) has reviewed the scientific literature dealing with the genetic variation of Sitka spruce published before the early 1960's.

Most provenance research on Sitka spruce has been done in Europe. BURLEY (1965<sub>b</sub>) cites one North American report on plantations of P. sitchensis and the hybrid P. X lutzii from Alaska and Denmark on the east coast of Canada and one provenance trial in New Zealand.

CLARK (1965) has studied some cone and seed characteristics of 35 trees originating from seven different locations. He has found considerable within and between locations variability for all cone and seed traits studied. However, he has not related the variability observed to the place of origin of the provenances studied. The provenances have been transplanted on the Queen Charlotte Islands and are being studied by the Forest company RAYONIER.

In Europe, interest has centered on volume production, height growth and frost resistance, of generally a limited number of provenances. Results are more or less contradictory as far as what could be the best provenances for planting in given conditions is concerned.

ROBAK (1962) discussed the winter survival of 1+0 and 2+0 Sitka spruce provenances from Alaska and British Columbia in Norwegian nurseries.

KARLBERG (1961) reviewed the southern Scandinavian experiments with Sitka spruce provenances. Seed suitable for Danish conditions would have to be collected from areas as far south as British Columbia and Washington state. Superior results have been obtained from seed from Tongass (Lat.  $53^{\circ}33'$ ). In some experiments, however, northerly strains suffered extensive damage due to late spring frost; in other, the Washington origin was preferred. KARLBERG (1961) concludes his review by pointing out that the Pacific coast has a very complicated physiography and that it is important to know the precise location of the provenance and if it came from poor form trees, growing nearby shores and rivers.

SCHOBBER (1962) has presented extensive results of research on Sitka spruce provenances. He studied ten Sitka spruce provenances coming from close to the entire range: from  $56^{\circ}$  to  $41^{\circ}50'$  Lat. N, in two field tests near Hann. Münden (Germany) (Gahrenberg plantations, at the time of his study: 28 and 32 years old). Frost damage and frequency of forked stems increased with decreasing latitude of place of origin of the provenances. Flushing rate and late frost damage were clearly correlated. SCHOBBER used a 4 step scale to estimate bud burst every 3-4 days, during the flushing period. The Alaska provenance was the latest to flush, the Queen Charlotte one the

earliest. However, the difference in flushing date was small: 3 to 8 days. The length of the flushing period varied from 33 to 46 days between provenances. The provenances did not keep their rank during the process of bud burst and there was no unique relationship between latitude and bud burst. Tree to tree, within provenance, variation in bud burst was large. Wood production varied from provenance to provenance. Complete records of height, diameter and volume of wood above 7 cm. diameter showed that the Queen Charlotte Islands is best, the California one of the poorest, in one test; in the other test, the Quinault provenance proved the best. An optimum appears to be formed by the provenances from south British Columbia and Washington state; northward the yield decreased progressively, southward abruptly. The author concluded his studies by pointing out the necessity to match the climatic conditions of the place of origin with those of the place of introduction. His observations also suggested that the vegetation period and its number of accumulated day-degrees over 50°F would be important in forecasting the success of a given provenance.

ALDHOUS (1962) has briefly studied the early growth of 12 provenances of Sitka spruce collected between 61° and 47° Lat. N, growing in Britain. There was a clinal variation in height growth. Height growth decreased with latitude. The southern provenances were the latest to set their bud. The time of height growth cessation varied up to 3 months.

In general, therefore, the European provenance studies indicate

that southern sources of Sitka spruce prove more susceptible to frost damage than sources from British Columbia and Alaska and thus, unproductive.

BURLEY has studied many aspects of the genetic variation of Sitka spruce, using a material covering the entire range of the tree species, from Lat.  $41^{\circ}$  to  $60^{\circ}$  and from Long.  $122^{\circ}$  to  $152^{\circ}$ , and from altitude 0 to 1,300 feet (BURLEY, 1965<sub>a</sub>, 1965<sub>c</sub>, 1965<sub>d</sub>, 1966<sub>a</sub>, 1966<sub>b</sub>, 1966<sub>c</sub>).

Using bulk collection of 30 provenances, seed weight variability was studied. There was no significant relationship with latitude, but a trend for northern provenances to have heavier seeds. Different explanations were proposed. For the most heavy seed sources, there was a relationship between embryo length and seed dry weight, seed volume, etc. In the case of the lighter seed sources, none of these relationships were significant at the 5% probability level. There was no significant relationship with altitude or latitude. However, it was quite possible that northern provenances have a lower germination capacity. Rigid control of collection and storage treatment would be necessary for precise determination of these relationships. Cold, wet treatment, cold soaking, alternating temperatures, chemical treatment (gibberellic acid) have been compared. Germination rate was improved by cold soaking. Cotyledon number and length were studied in growth chamber: the cotyledon number varied between 5 and 7 and was not correlated with

latitude or seed weight. There was a significant relationship between cotyledon length and seed weight.

The genetic variation in seedling development of 47 provenances of Sitka spruce was studied in different environments: natural and artificial. The morphology of bud formation and flushing has been studied in a nursery at New Haven, Conn. (U.S.A.). Three stages in bud development have been distinguished. During the growing season, the apex of the terminal bud is not visible and is surrounded by needles in close spiral: this stage was called the type I bud by BURLEY. The type II bud was characterized by the fact that the last scales have been initiated and virtually no height growth occurs. The type III bud was characterized by the facts that the outer scales turn brown and resinous; all needle primordia were then formed, but in southern provenances, some needles could be initiated during the first flush of spring growth. No changes occur until spring when there is bud swelling and loss of the dark resinous colour of the bud scales and needle elongation. However, bud development in Sitka spruce, as in many tree species, is a continuous process with no well-defined stages. The types of bud distinguished by BURLEY are, therefore, somewhat arbitrary as will be discussed later. It is also important to examine if there is a relationship between the number of primordia in the terminal bud, and its diameter and height growth. These characters were investigated and it was found that the number of scales and primordia both contributed to bud diameter and that bud size and apex length or diameter were correlated. However,

bud diameter was not related significantly to latitude of seed origin, but increased with seedling height ( $r = 0.608$ ).

The number of days from July 1, 1963 to bud formation of type II decreased with increasing latitude of seed origin ( $r = -0.862$ ). The seedlings from northern sources thus formed their buds before the southern provenances (BURLEY, 1966<sub>a</sub>).

Sixteen provenances were studied under normal and extended photoperiod (20 hours). The author concluded that the time required to form buds (type II) decreased significantly with increasing latitude, but that under extended photoperiod, no buds developed until the artificial light was removed and then bud formation was much faster. The onset of dormancy was activated by an adequate photoperiod, but it could also be hastened by relatively high temperature. It is important to note that BURLEY has found a positive relationship between latitude and time to change from bud type II to type III. This latter relationship could, therefore, eliminate the previous negative relationship found on the basis of type II bud formation. Local temperature regimes of the place of origin were believed to explain the individual reaction of the provenances studied.

No relationship between flushing rate as measured by BURLEY and latitude of seed origin has been found, which confirms SCHOBES'S observations.

Seedling height decreased with increasing latitude of seed origin, but regression slopes varied substantially from one environment to the other, apparently indicating genotype by environment interactions.

The size of the first terminal bud was dependent on seedling height. Large buds produced greater shoots than small buds, but 8% only of the variation in extension growth was accounted for by previous seedling height, under natural photoperiod.

Both within and between provenances, the taller seedlings had more lateral branches than smaller seedlings and presumably would maintain or increase their height differential.

Daily growth rate was measured using a cathetometer on two seedlings per provenance. No relationship was found between the different growth rates and latitude or seed weight of the seed sources studied.

The author summarized his developmental studies on Sitka spruce by suggesting that flushing was controlled by temperature and that intraspecific variation in this trait occurred that was associated not with latitude, but with temperature regime. Two weeks separated the extreme provenances at any stage of flushing and three months in dates of bud formation. Height growth was a function of latitude and bud formation. Photoperiod and temperature modified this relationship with photoperiod increasing height growth by delaying bud formation. The pattern of adaptative variation was discontinuous. There was a strong interaction between genotype and environment.

Colours of the hypocotyl and needle glaucousness of 47 seed sources were qualitatively assessed in a controlled environmental room, during the first year of growth. Colour intensity increased with



latitude, the northern provenances developing red hypocotyl colourations before the southern ones. The time of appearance of blue-grey colour varied widely, the Alaska provenances showing it one month before the most southern seed sources.

DAUBENMIRE (1968) studied the morphological variability of adult trees in ten natural populations of Sitka spruce. The author claimed that there is a clinal variation in cone size and sterigina angle which decreased from south to north. Possible introgressions with P. glauca of some populations of the Skeena river watershed is discussed. Length width ratio of the cone scales could be used to differentiate insular from mainland populations. The very particular sampling procedure used by the author requires cautious interpretation of his data.

ROCHE (1969) has reported introgressive hybridization between the four species of spruce growing in British Columbia, on the basis of cone scale morphology. His investigation of 150 juvenile spruce populations genetic variability is not applicable for studying Sitka spruce genecology because his research was based on a pooled mixture of provenances originating from different spruce complexes.

LACAZE (1970) has studied the growth behaviour and flushing rate of 24 Sitka spruce commercial provenances, at the nursery stage, in France. The so-called Washington provenances were ranked the best in growth. They flushed the latest, too.

It is well known that Sitka spruce hybridizes, in natural conditions, with sympatric spruces such as P. glauca (yielding the hybrid P. X lutzii), (WRIGHT, 1955).

P. sitchensis has been successfully crossed with P. abies and P. omorika (WRIGHT, 1955). It has been implicated in a curious hybrid with Tsuga heterophylla: X Tsuga hookeriana (VAN CAMPO-DUPLAN and GAUSSEN, 1948).

FLETCHER and FAULKNER (1972) have published a detailed breeding and selection plan for the genetic improvement of Sitka spruce in Great Britain. This very ambitious plan covers every aspect of tree breeding: provenance studies, plus-tree selection, tree banks, cone and seed stimulation, etc.

SAMUEL et al. (1972) have successfully done a complete diallel cross among six Sitka spruce trees. The eight characters studied on one-year old seedlings were divided into two groups: those concerned with tree form (stem straightness, dormant bud number and branch angle), and those related to the vigour of the tree (height, dry weight, branch number and branch length). The tree form characteristics were predominantly inherited in an additive manner, but dominance effects were also operative. For the vigour traits, a complicated pattern of inheritance appeared: the additive effects were still large, but dominant and, to a lesser extent, maternal effects were present. Complicated gene interactions could be present, but the heterozygous nature of the material did not clarify the issues.

## 1.2 Physiological studies

ADDISON (1966) grew Sitka spruce seedlings in sand

cultures with nutrient deficient solutions. He studied six macro-elements in seedlings originating from seeds of four locations and from three trees per location. Seedlings were grown in a controlled environment. Significant differences existed between treatments and between provenances for all the variables studied: root length, shoot growth, root collar diameter, etc. In most cases, the response of the traits studied depended on the provenances. A lack of nitrogen was found to have the most detrimental effect. Sulfur, magnesium and calcium deficiencies had the least effect. Flushing rate was correlated with latitude of seed origin with the most southerly provenances flushing the earliest, an observation which contradicts BURLEY's findings.

Genetic variation in photoperiodic response has been demonstrated by VAARTAJA (1959) for Sitka spruce, for growth traits and bud set.

BRIX (1972) has studied the dry matter production, stem height, basal diameter, etc. of two seed sources of Sitka spruce, under different temperature regimes and light intensities, in comparison with white spruce. Sitka spruce was more shade tolerant than white spruce. Stem diameter growth was greatest in the 18° to 24°C range for Sitka spruce. Dry matter production was maximum with constant day-night temperatures. Responses varied with seed sources.

### 1.3 Cytogenetical studies

Ten Sitka spruce seed sources were used for chromosome study. Treatment with 1% colchicine for five hours was adopted. The

haploid number of chromosomes is 12. Most chromosomes were meta or sub-metacentric. Total haploid complement length increased with latitude ( $r = 0.576$ ). Nuclear volume was also correlated with latitude. These relationships could be interpreted as an indication of some variation in absolute karyotype structure or a variation in reaction to colchicine. The karyotype indicated three heterobrachial chromosomes and differed from the karyotype of other species of spruce by the arm lengths of two specific chromosomes (BURLEY, 1965<sub>c</sub>).

MIKSCH (1971) has used biochemical, Feulgen double wave-length cytophotometry and microdensitometry to study DNA per cell variation among seven seed sources of P. sitchensis. DNA estimates were related to latitude with the northern seed sources possessing more DNA per cell than the southern provenances. Changes in nuclear DNA levels due to external environmental stress (maternal effects) are discussed.

MOIR and FOX (1972) have described the presence of supernumerary chromosomes (also called B-chromosomes) in seedlings derived from eight provenances of Sitka spruce. They also refined the karyotype presented by BURLEY (1965<sub>d</sub>). New constrictions were observed and allowed a subdivision of the complement into five groups. The presence of B-chromosomes should be taken into account for interpreting the variation observed in growth, nuclear volume, etc.

#### 1.4 Variation in wood properties

Genetic variation in specific gravity, tracheid length, cell

wall thickness and other wood properties have been shown (BURLEY, 1965<sub>b</sub>; British Forest Product Research Organization (1966): different reports).

## CHAPTER 2

### GENECOLOGY DEFINED: ITS RELATIONSHIPS WITH TREE BREEDING

The study of forest tree provenances inescapably raises the question of the relevance of the science of genecology to provenance research. A number of provenance and progeny genetic variation studies of forest trees have been recently published as genecological studies, (GALOUX and FALKENHAGEN, 1965; GALOUX, 1966; FALKENHAGEN, 1968<sub>b</sub>, ROCHE, 1969; HAGNER, 1970).

Different authors have attributed different meanings to the term genecology (LANGLET, 1971). The term genecology was coined by TURESSON (1923), as synonym of race ecology or as the study of the species and its hereditary habitat types from an ecological point of view. Throughout his very interesting paper, LANGLET (op. cit.) has tried to restrict the use of the term genecology to the original Turessonian meaning. However, most foresters have adopted the broader meaning developed by HESLOP-HARRISON (1964) and STERN (1964). Genecology is then considered as a synthetic science which borrows its tools from a number of other scientific fields such as physiological ecology, quantitative genetics, etc. while also interacting with these sciences. Genecology is thus defined as the science whose subject matter is the study of the genetically based infra-specific variation of plant species in relation with the diversity of their habitat of origin (FALKENHAGEN, 1968<sub>a</sub>).

The reader interested in a very comprehensive review of forest genecology is referred to the paper by STERN (1964). It is sufficient to say that forest genecology forms the scientific bases of provenance research: a complete genecological study of a tree species should be a prerequisite to any selection of seed sources. When two tree species must be crossed, it is common sense to cross the races which will give the most productive and adapted progenies. Even the results of crossings in a seed orchard depend on a thorough knowledge of the genetic variability of the adaptative traits of the tree species (FALKENHAGEN, 1968).

One can ask why we did not name our study: genecology of Sitka spruce. The reasons are that we favoured LANGLET's concept of genecology and that we did not attempt to study experimentally the bases of adaptation of the genetic material that we were studying, to their habitat of origin. We did not use controlled environments to study the responses of the progenies to different ecological factors in the hope to explain their behaviour in the nursery or elsewhere. In this sense, we only realized a single tree progeny and provenance study in one set of growing conditions as an obligatory step to test this material in forest conditions.

### CHAPTER 3

#### ORIGIN AND CHARACTERISTICS OF THE MATERIAL STUDIED

During the 1970 fall, the I.U.F.R.O., Section 22, "WORKING GROUP ON PROCUREMENT OF SEED FOR PROVENANCE RESEARCH" organized an expedition to collect Sitka spruce cones from British Columbia and Alaska. In most cases, the collections were made from 15 trees in each location. The procedure of the collection has been outlined by the Working Group on Provenance Research and Testing Meeting" at Pont-à-Mousson, held on 6-9 September, 1965.

The locations of the 39 provenances range from  $48^{\circ} 38$  to  $58^{\circ} 37$  latitude N and from  $121^{\circ} 93$  to  $134^{\circ} 58$  longitude W. The elevation varies from 0 to 2,200 feet above sea level. See Table I and maps 1 and 2 for more details on the origins of the provenances studied.

Twenty cones from each of the 557 trees representing 39 locations were provided to Professor O. SZIKLAI. The seed was extracted by hand during the 1970-71 winter and stored at  $32^{\circ}\text{F}$ . This single tree progeny collection constitutes the material with which the author commenced the present study as his Ph.D. thesis.

It was decided to keep the single tree progenies separate and to study as much as possible the different seed or cone and seedling traits on a single tree basis so as to be able to compare the two hierarchical levels of variation: populations (= provenances) and between



TABLE I

GEOGRAPHICAL COORDINATES OF THE SITKA SPRUCE  
PROVENANCES STUDIED AND NUMBER OF SINGLE TREE  
PROGENIES AVAILABLE PER PROVENANCE

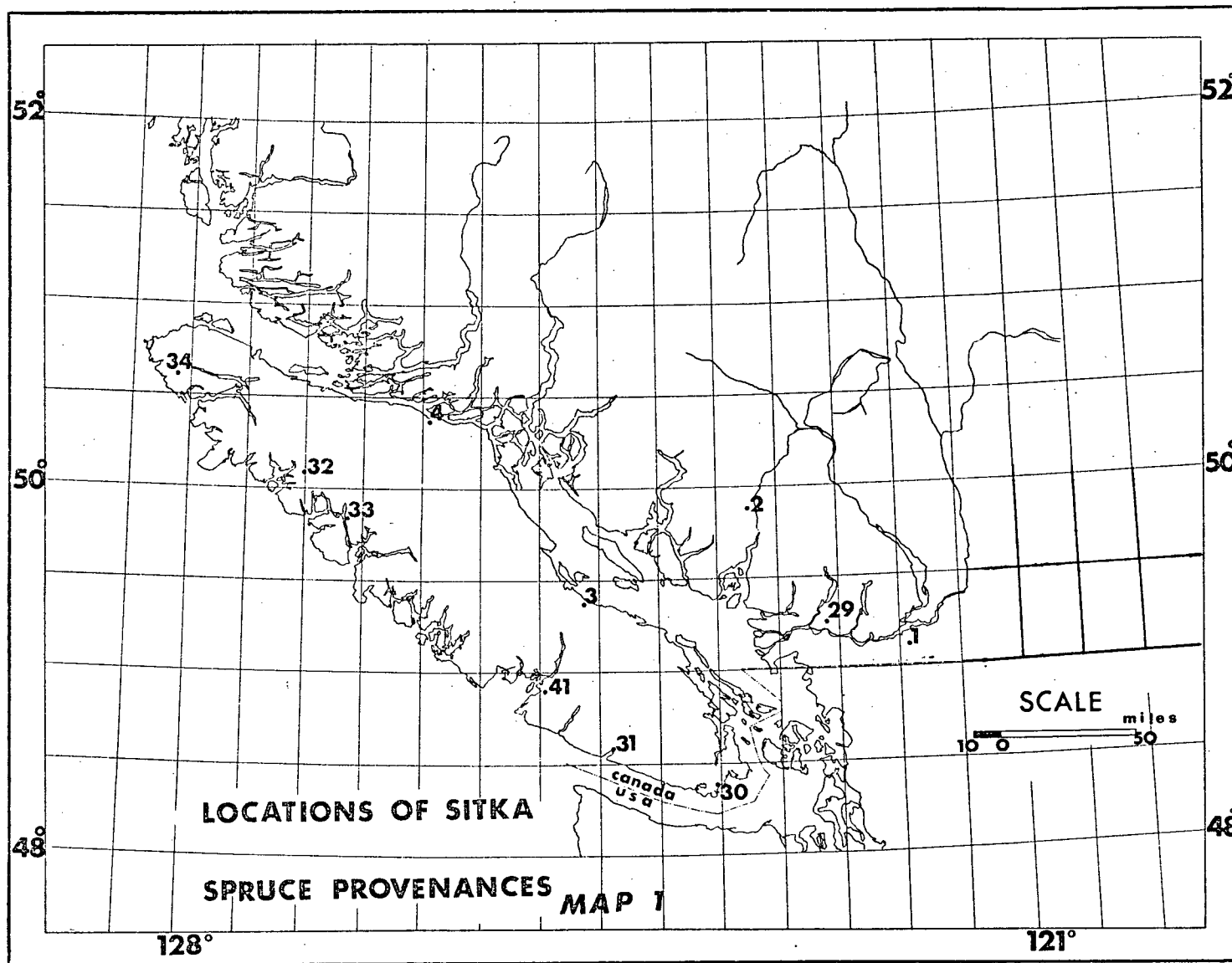
<u>No. of the</u> <u>provenance</u>	<u>Name</u>	<u>Lati-</u> <u>tude</u> <u>° tenth</u>	<u>Longi-</u> <u>tude</u> <u>° tenth</u>	<u>Eleva-</u> <u>tion</u> <u>feet</u>	<u>No. of</u> <u>single</u> <u>tree</u> <u>proge-</u> <u>nies</u>
1	VEDDER	49.12	121.93	100	10
2	SQUAMISH RIVER	49.92	123.25	100	15
3	BIG QUALICUM RIVER	49.38	124.62	0	15
4	SALMON BAY	50.38	125.95	0	15
5	CRANBERRY RIVER	55.47	128.23	1700	15
6	KITWANGA	55.17	127.87	2200	15
7	USK FERRY	54.63	128.40	450	15
8	SHAMES	54.40	128.95	100	5
9	WEDENE RIVER	54.13	128.62	550	15
10	KITSUMKALUM LAKE PARK	54.72	128.77	450	15
11	DERRICK LAKE	55.68	128.68	800	15
12	DRAGON LAKE	55.35	128.95	850	7
13	ZOLAP CREEK	55.15	129.22	50	15
14	FULMAR CREEK	55.15	128.97	1300	15
15	ABERDEEN CREEK	54.20	129.92	0	15
18	CEDARVALE	55.02	128.32	800	15
19	KASIKS RIVER	54.28	129.42	100	15
20	HUMPBACK CREEK-PORCHER ISLAND	54.03	130.37	1000	11
21	INVERNESS	54.20	130.25	50	15
22	HAYS MTN, PRINCE RUPERT	54.27	130.32	2100	15
23	MOSSPOINT, ANNETTE ISLAND	55.03	131.55	0	15
24	CRAIG	55.50	133.13	0	15
25	OLD HOLLIS	55.47	132.67	0	15
26	WARD LAKE	55.42	131.70	50	15
27	OHMER CREEK	56.58	132.73	25	15
28	DUCK CREEK	58.37	134.58	100	15
29	ALLOUETTE RIVER, HANEY	49.25	122.60	650	15
30	MUIR CREEK, SOOKE	48.38	123.87	0	15
31	PORT RENFREW	48.58	124.40	25	15
32	TAHSIS I	50.08	127.50	100	15
33	TAHSIS 2	49.83	126.67	10	15
34	HOLBERG	50.62	128.12	100	15
35	MORESBY IS, SKINCUTTLE INLET	52.28	131.22	50	8
36	MORESBY IS, SEWELL INLET	52.87	132.08	50	15
37	MORESBY IS, CUMSHEWA INLET	53.05	132.08	200	15

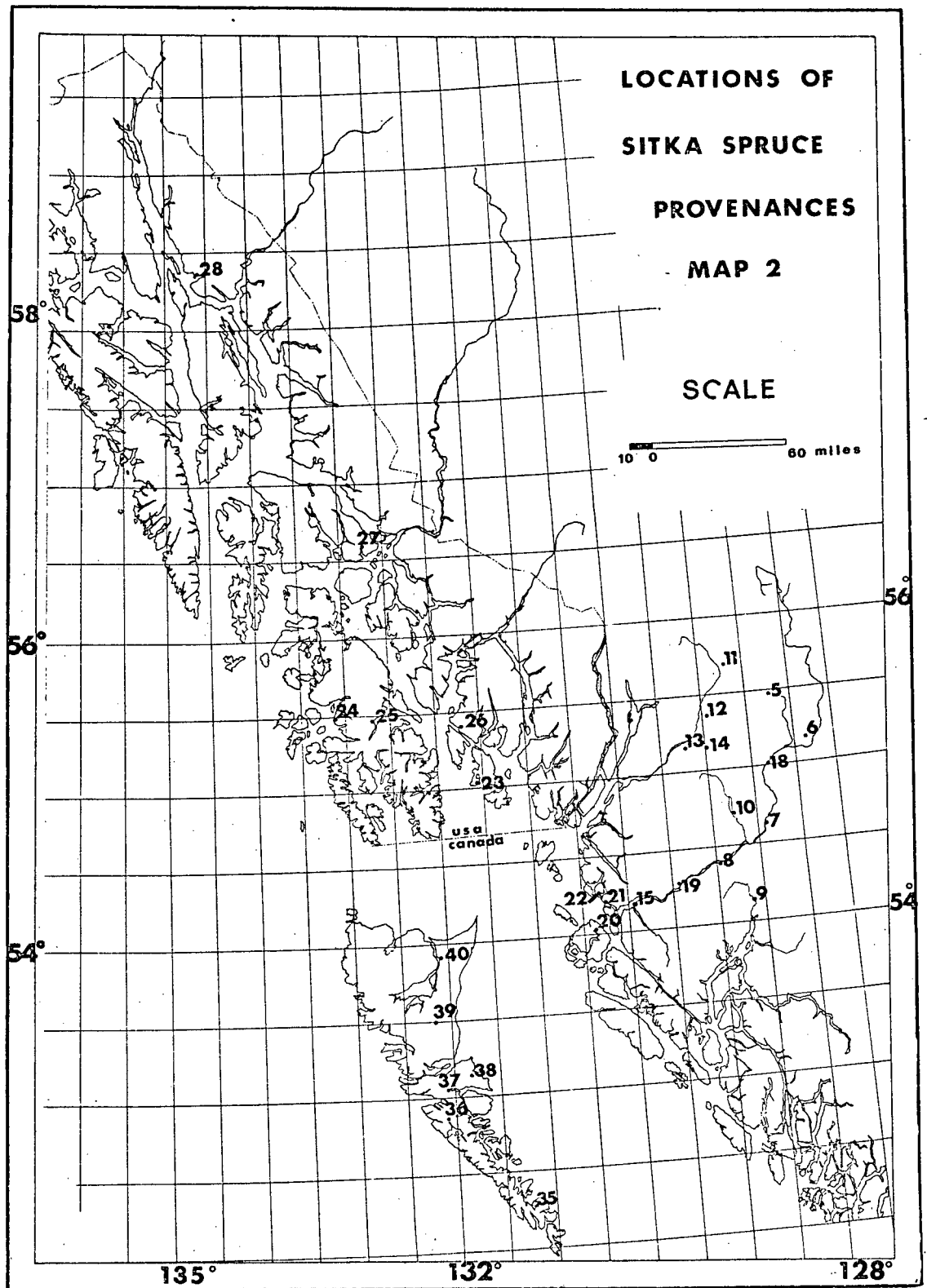
TABLE I - Continued

<u>No. of the provenances</u>	<u>Name</u>	<u>Lati- tude ° tenth</u>	<u>Longi- tude ° tenth</u>	<u>Eleva- tion feet</u>	<u>No. of single tree proge- nies</u>
38	SANDSPIT, QUEEN CHARLOTTE ISL.	53.13	131.80	250	15
39	TUSKATLA, QUEEN CHARLOTTE ISL.	53.50	132.17	300	15
40	MASSET INLET	53.92	132.08	0	15
41	BLENHEIM, SARITA	48.90	124.95	700	11

Total number of populations: 39

Total number of trees studied: 557





trees, within population variability. Such a study offers the advantages of a provenance test and those of a maternal test enabling, therefore, the comparisons of different nested levels of genetic variability. These comparisons are fundamental if we wish to rationally determine which type of selection is best for the material studied (mass selection, family selection, etc.).

Ten Sitka spruce provenances selected from the material collected in 1970, are being proposed for an international experiment (DRISCOLL, 1972). The twelve participating forest research organizations will provide the same treatments in order to study provenance by site interaction and genotype stability under different ecological conditions.

## CHAPTER 4

### CLIMATOLOGY OF THE AREA OF ORIGIN OF THE SITKA SPRUCE PROVENANCES

For forest tree species, it is generally admitted that the climatic differences between the places of origin of the provenances are mostly responsible for the genetically based adaptative variability that these provenances show in comparative field tests (STERN, 1964).

The extreme values taken by the ecological variables rather than the average values would explain the action of the environment in modifying the fine genetic make-up of the populations of trees. Thermal and pluviometric extremes, their frequencies and their dates of occurrence would act as selection factors. Biotic and edaphic factors might also intervene to modify the tree populations, but these ecological variables also depend on the climate. The forest tree species are perennial plants and, therefore, are most influenced by the climate and should be primarily adapted to the local climatic conditions (WRIGHT, 1963; FALKENHAGEN, 1968). Therefore, it is important to study the climate of the place of origin of the provenances of a tree species which is being studied, noting that the current climate may not be the climate of evolution.

Unfortunately, the network of the climatological stations in British Columbia and southern Alaska is too loose to allow an accurate description of the climatological conditions of the place of origin of our Sitka spruce provenances. Not only does the wild mountainous physio-

graphy of the region preclude any extrapolation, but some stations only provide precipitation records, while others have been established for too short a period of time. Most stations are along major rivers or near the sea and represent valley or sea level climates.

According to CHAPMAN (1952), two types of climate predominate in the area of origin of the Sitka spruce provenances studied: primarily the Marine West Coast climate and locally, the Cool Summer Mediterranean climate. These climates result from the prevailing west-east movement of air over the province which is due to several main pressure systems. During the winter, the winds are southeast along the coast, at the surface. However, the persistency of the winds is frequently interrupted by the passage of depressions and by the local topography. During the summer, the prevailing winds are northwest. The weather along the coast is most versatile as in all maritime climates, and this changeability is due to the passage of air masses of Pacific origin, over the coast. Altitude and topography, changing abruptly in the province, result in rapid modification of the local climate: temperature inversions, rain shadow effects are well known; the east coast of Vancouver Island is drier than the west coast. Naturally, aspect is very important. The following subdivisions may be used (CHAPMAN, 1952).

(1) The west coast climate:

The mean annual range of the Coastal stations is  $16^{\circ}\text{F}$  in the south to  $23^{\circ}\text{F}$  in the north and is evidence of the mild winters ( $38^{\circ}$ -  $41^{\circ}\text{F}$ ) and cool summers ( $56^{\circ}$  -  $58^{\circ}\text{F}$ ). The length of the frost-free period

Fig.2. Monthly variation in precipitation and temperature for two stations of the Lower mainland.

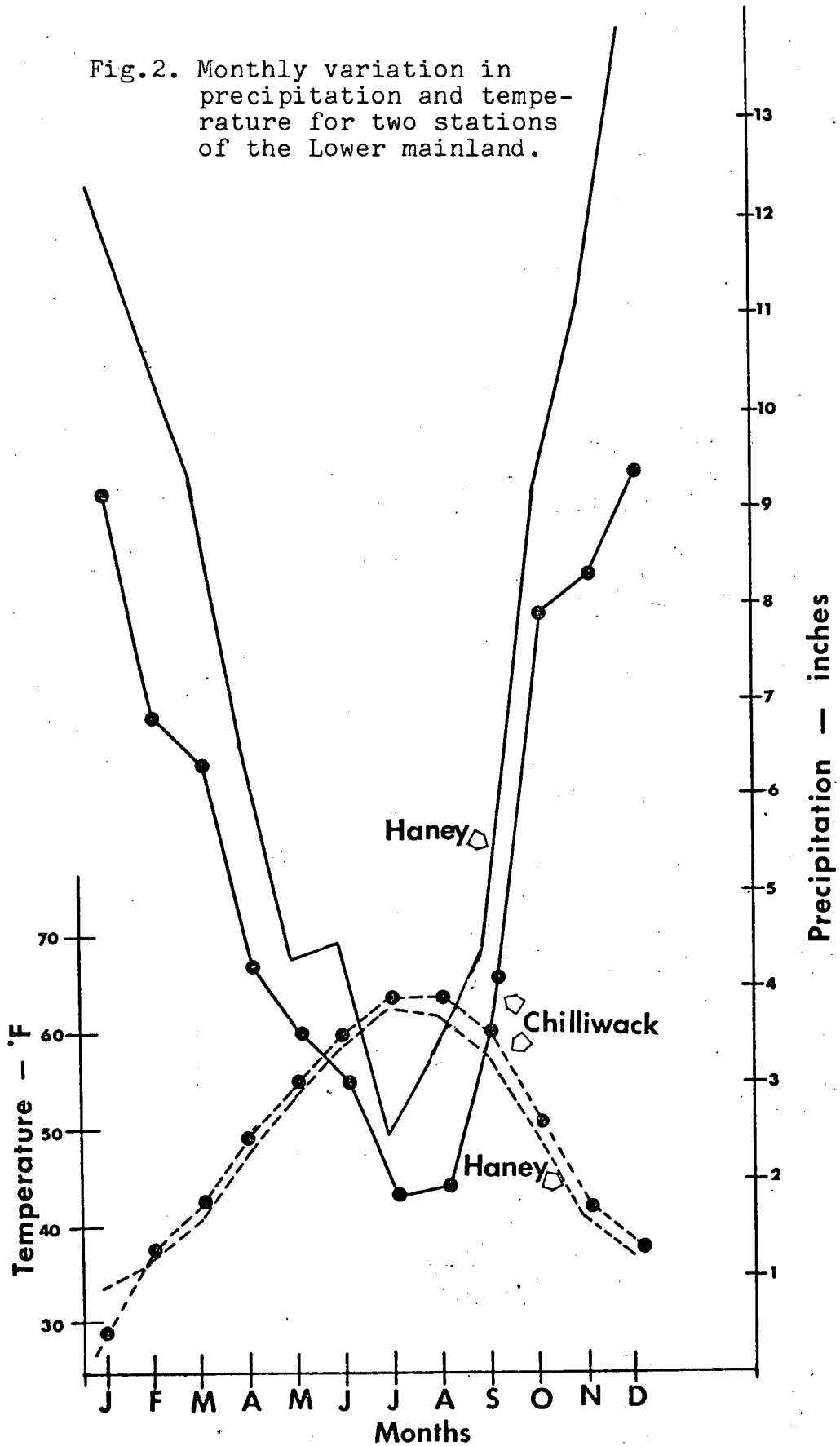
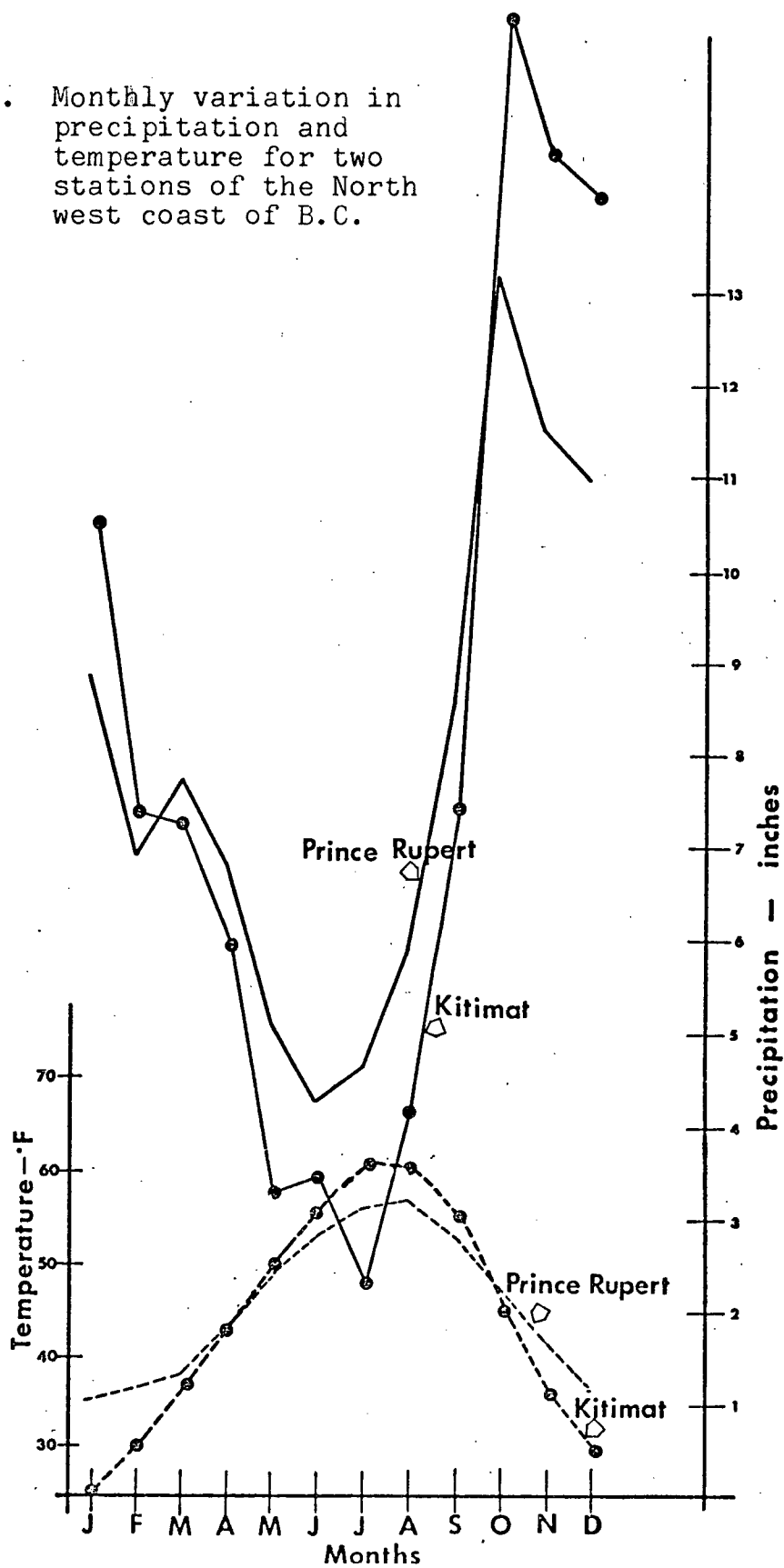




Fig.3. Monthly variation in precipitation and temperature for two stations of the North west coast of B.C.



varies from 150 to 250 days. Prince Rupert has the lowest annual total of sunshine hours in Canada (1053 hours). Annual precipitations are high (50 to 100 inches), mostly rain. The number of days of precipitation is very high (201 to 262). The seasonal rain distribution is maritime, but with a summer minimum well marked. Relative humidity is high. The coastal region may be subdivided in:

- (a) Outer division,
- (b) Inner division,
- (c) Fjords, and
- (d) Through valleys.

(2) The climate of southeast Vancouver Island, Georgia Strait and the Lower Fraser delta

The main characteristics are summer deficiencies of moisture, lower precipitations (20 to 40 inches) and particularly in the Victoria-Saanich Peninsula area, high totals of sunshine (2,207 hours at Victoria). The frost free period exceeds 200 days.

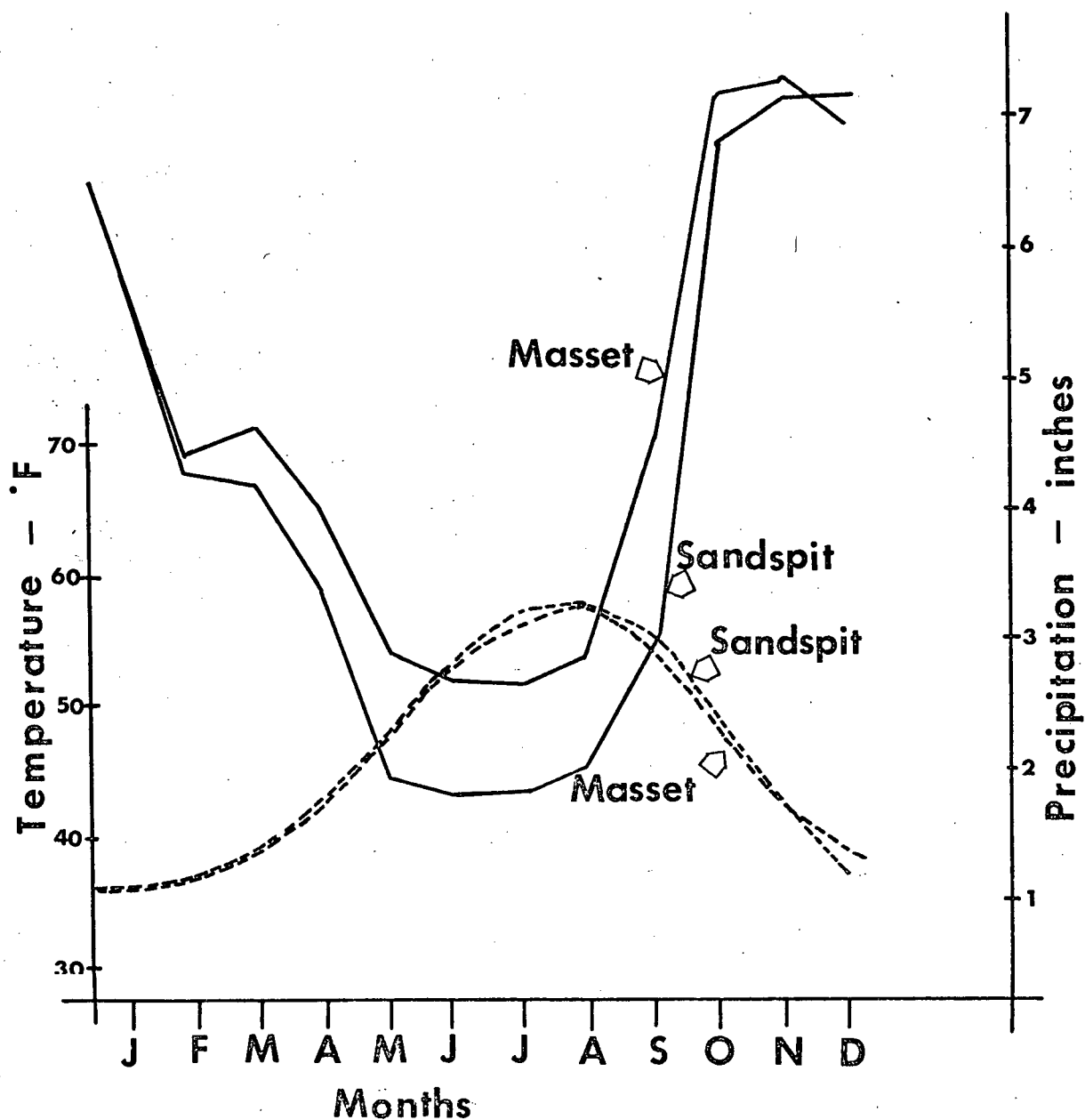
We have plotted the monthly averages for total precipitations (inches) and for temperature (degrees Fahrenheit) for some reliable climatological stations close enough to the locations of at least some of the provenances. (Temperature and precipitation tables for B.C., 1971).

(A) B.C. mainland (Fig. 2 and 3).

Four stations have been plotted: the stations of Chilliwack, Haney U.B.C. forest, Kitimat and Prince Rupert.

The station of Chilliwack is in the Lower Fraser valley while that of Haney is in the coastal range mountains. The temperature

Fig.4. Monthly variation in precipitation and temperature for two stations of the Queen Charlotte Islands.



curves are similar at both stations, but Chilliwack is a little warmer except in January. The precipitation is heavier at Haney and the minimum is less pronounced than at Chilliwack. It snows a little more at Haney. The climates of Kitimat and Prince Rupert are quite distinct in spite of their similar latitude. The climate of Kitimat is more continental with heavier precipitation during the winter months, a delayed and more pronounced rainfall minimum during the summer (in July instead of June). The seasonal temperature variation is also more pronounced. The average temperature at Prince Rupert remains above freezing point which is not the case at Kitimat.

(B) Queen Charlotte Islands stations (Fig. 4).

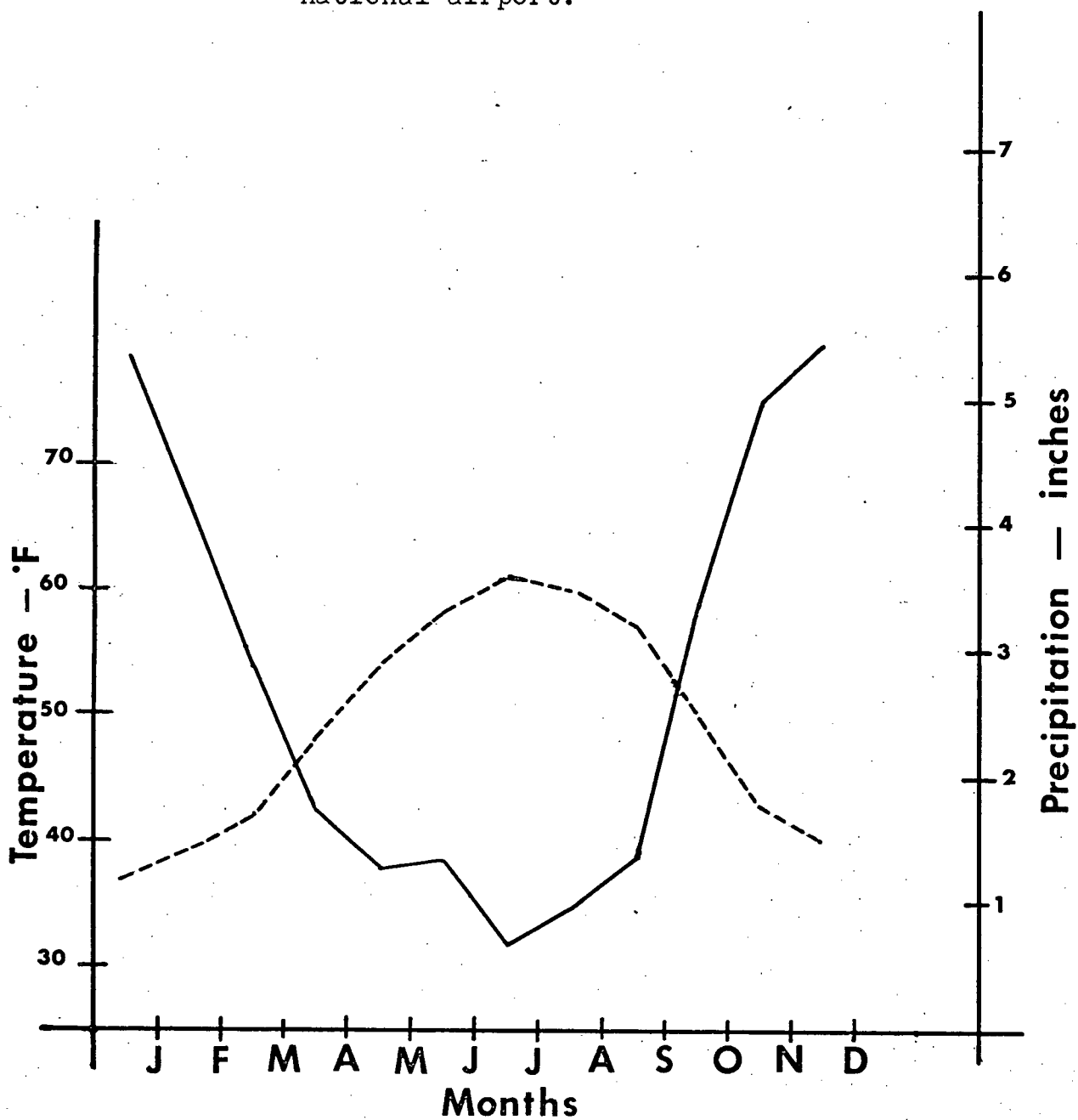
Two stations have been plotted: the stations of Sandspit and Masset. Only the latitude differentiates the two stations. The temperature curves are identical; only the precipitation curves are different with a more pronounced minimum at Sandspit. The annual total is 49.56 inches at Sandspit (Lat.  $53^{\circ} 15'$ ) and 56.27 at Masset (Lat.  $54^{\circ} 02'$ ).

(C) Vancouver Island (Fig. 5).

Victoria International Airport.

Some Sitka spruce provenances originate from islands in southeast Alaska. The topography of this region is rough and mountainous and is cut by numerous inland waterways. The climate is cool and moist with a relatively narrow range between summer and winter temperatures. The heavy precipitation is well distributed throughout

Fig.5. Monthly variation in precipitation and temperature at Victoria International airport.



the growing season. There is no pronounced summer drought (ANDERSEN, 1955). The extreme northern portion and along the mainland has a more continental climate. In winter, northerly winds from the Interior lower the temperature of the islands lying nearer the mainland, but the Pacific Ocean influences the climate of the outer islands. There are great variations in climate within short distances due to aspect, water mass and elevation effects as in British Columbia. The reader is referred to ANDERSEN (1955) who has given tables and isothermal or isohyetal maps for the following factors: precipitation, mean temperatures during different months, number of frost-free days and mean temperature during the three coldest months. Precipitation exceeds that necessary for optimum tree growth and low potential evapotranspiration results in a water surplus and makes soil drainage, together with summer temperatures, a critical factor in affecting tree productivity in southeast Alaska.

Tree growth ultimately depends primarily on the particular energy and water balances of a given region. A parameter is commonly used to describe the growth potential of an area when water supply is adequate: the number of growing degree-days which is defined as the sum of the differences between the daily mean temperature and  $42^{\circ}\text{F}$  or  $43^{\circ}\text{F}$  chosen as critical temperature for growth processes. BOUGHNER (1964) has reviewed the concept of growing degree-days and presented accumulated degree-days against time (Fig. 6) for Agassiz, Nanaimo and Smithers. The curves of Agassiz and Nanaimo are identical, but

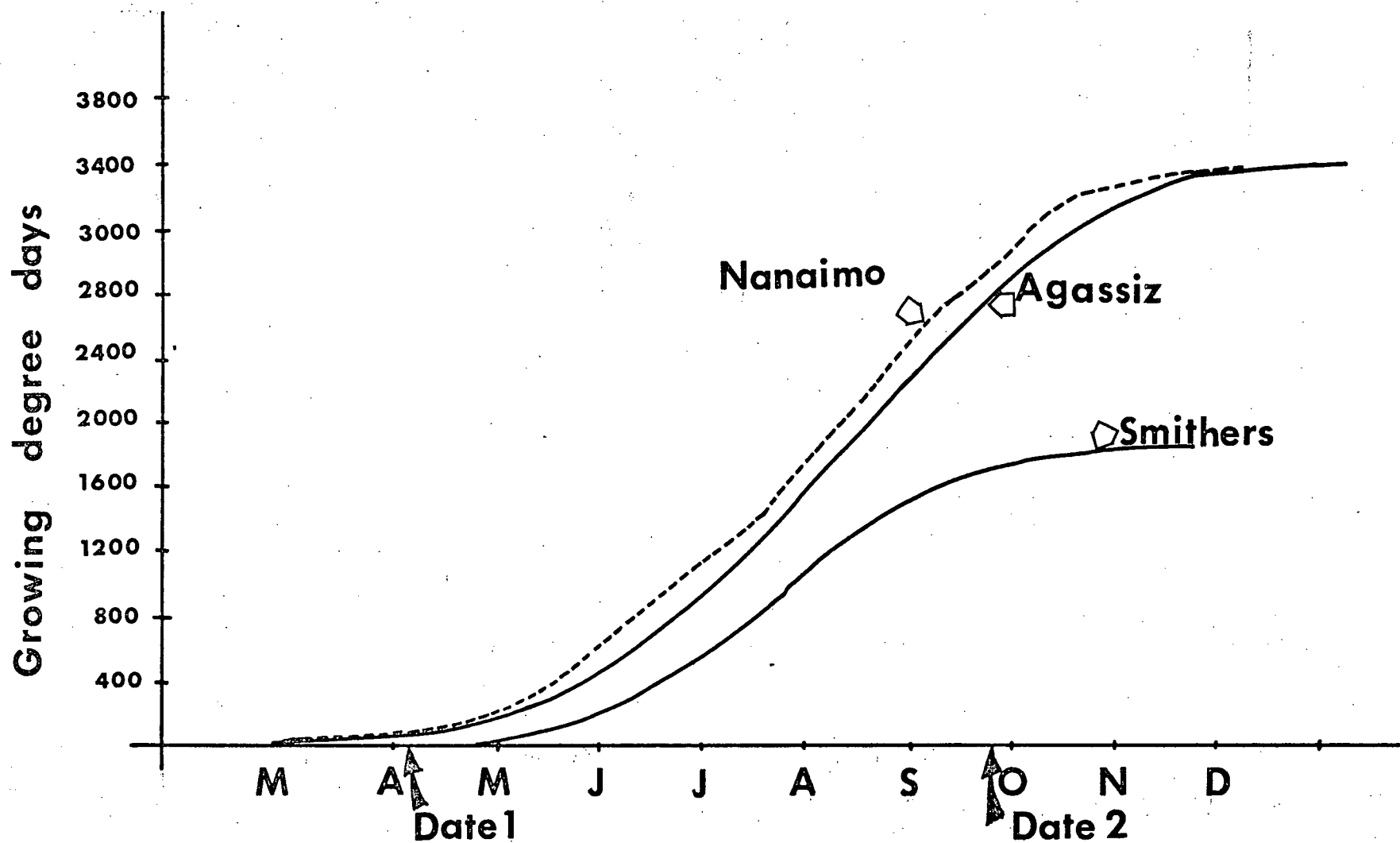


Fig.6. Growing degree-days accumulated for three stations of B.C. The dates when bud burst (Date 1) and bud set (Date 2) were estimated are shown.

differ sharply from the curve for Smithers, indicating that there is much less available radiation energy for growth here; thus, that latitude increases result in decreasing radiative energy usable by the plants.

The accumulated day-degrees over 43°F are 2,500 to 3,500 for the Coastal Douglas-fir zone and 1,500 to 2,500 for the Coastal Western Hemlock zone (KRAJINA, 1969).

The evaporative demand of the climate of British Columbia is little known. The potential evapo-transpiration, according to the well known THORNTHWAITE's method, of the Lower Fraser valley, during the period May - September, could be 20 inches, while the precipitation is only 14 (CHAPMAN and BROWN, 1966). The potential evapotranspiration (PET) has been calculated for a number of Alaskan and nearby Canadian stations (PATRIC and BLACK, 1968). A belt of heavy rainfall extends along the southern shoreline from British Columbia westward to the end of the Aleutian chain. Within this forest region, the average PET for 68 stations is 21.27 inches. The heavy precipitation along the coastal perhumid belt becomes much lighter within relatively short distances from the Pacific Ocean. Concurrently, there is a change from western hemlock-Sitka spruce to the spruce-aspen-birch forest of the interior. PET ranges in this region from 15.5 to 19.8 inches. However, commercial timbers may use moisture from water tables in addition to local precipitation for their water supply. Therefore, growth of the northern forest is primarily governed by temperature (PATRIC and BLACK, op. cit.).



PART II

SEED AND CONE MORPHOLOGY AS PART OF THE  
BIOSYSTEMATICS OF SITKA SPRUCE

## CHAPTER 1

### FOREST TREE BIOSYSTEMATICS DEFINED

Biosystematics has received different meanings varying with the authors who use this word. Originally created by CAMP and GILLY (1943) as the combination of taxonomy and evolution, biosystematics or biosystematy was considered by these authors as the classification of living organisms according to a taxonomic system based upon phylogenetic relationships, with emphasis upon the factors responsible for the segregation of the taxa involved. Biosystematics corresponds, therefore, to the "new systematics" as proposed by HUXLEY (1940). Biosystematics (*sensu stricto*) thus aims at:

- (1) delimiting the natural biotic units;
- (2) to apply to these units a system of nomenclature adequate to the task of conveying precise information regarding their defined limits, relationships, variability and dynamic structure, (CAMP and GILLY, 1943).

HESLOP-HARRISON (1964) has proposed a similar definition: biosystematics is the study of evolutionary processes in plants and the bearings of this study on their taxonomy. CALLAHAM (1963) has similarly defined biosystematy, but has also confounded it with genecology according to TURESSON, which needs further elaboration.

Most forest geneticists consider as part of biosystematics, the study of the morphological variability of the parent trees of the

progenies that they are studying. Biosystematical studies were to classify the parent trees into natural biotas, using different maternal adult characteristics such as cone, twig and leaf traits.

Despite the advocacy of parental morphology as useful or indispensable tools in provenance research by CALLAHAM (1963) and ROCHE (1968), many cone and seed morphological studies of forest trees have not given very useful results when the study was done at the infra-specific level. Some interesting results as an aid to classificatory purposes have been claimed for seed weight (more precisely 1,000 seed weight) for different species (for example, for Larix decidua MILL (SIMAK, 1967) and for Douglas-fir (BIROT, 1972); and for a set of seed characteristics (ALLEN, 1960). However, the use of these characteristics is limited to the distinction of a very broad geographical variation such as coastal and interior varieties of Douglas-fir.

Generally, there is a clinal variation for each characteristic studied, different for each region distinguished. A fact which does not simplify the delineation of workable units. An example of complicated pattern of variation, despite extensive measurements is given by YAO's study of seed weight and cone scale morphology of 124 provenances and 1,818 trees of Douglas-fir (YAO, 1971).

There are a number of possible reasons to explain the lack of power to classify the natural populations of a tree species into useful biotas:

1. A mixture of clinal variation and ecotypic variation may

complicate the distinction of workable units.

2. It is quite possible that the traits studied are not so stable as claimed by their proponents: cone and seed traits certainly foliage or twig characters might not be under strict genetic control: their heritability might be very low.
3. The choice of the characters to measure has been so far empirical or subjective: it was often decided to measure seed width, different angles on the cone bract, etc., without knowing the discriminatory power of the traits measured. One method would be to measure a great number of traits: 20 to 30 in an adequate sample of populations and then to perform a step-wise discriminant function analysis or a principal component analysis to eliminate any redundancy of information in the traits studied.
4. Most studies considered one trait at a time. A multivariate approach could be useful.
5. Another important problem generally neglected is the sampling problem: how many individuals should be measured? The problem is very complex as different nested levels of variation exist, and must be simultaneously taken into account. A stratified sampling procedure has shown that 15 bracts were necessary to estimate correctly the catkin means and that four catkins were necessary to estimate the tree means with a low error, prior to a morphological

study of the catkin of yellow birch (Betula alleghaniensis BRITT.). This sampling study is the only one ever mentioned in the forest genetics literature (FALKENHAGEN, 1968<sub>b</sub>).

6. Naturally, the reasons just mentioned can combine to blur the classification of the parent trees on the basis of their phenotypic morphology.

CHAPTER 2  
SEED AND CONE MORPHOLOGY  
UNIVARIATE ANALYSES OF VARIANCE AND MULTIPLE  
CORRELATION AND REGRESSION ANALYSES

The geographical co-ordinates of the provenances studied for cone and seed morphology and the number of progenies studied per provenance are indicated in Table I.

Five seeds were randomly collected from each tree progeny and stuck on a special sheet. The measurements, as shown in Fig. 7, were made using an AB ADDO's machine for measuring annual rings, to the nearest 0.01 mm.

Ten cones per progeny were randomly collected and the length of each cone was measured to the nearest mm.

### 2.1 Univariate analyses of variance

The variance and the mean, for each provenance, of the different characteristics measured on the seed and the cone in mm are shown in Table II.

Hierarchal analyses of variance have been performed, using the total material and considering three levels of variation: provenances, trees within provenances, seeds within trees. The large heterogeneity of the material results in the non-homogeneity of the variances, at the different levels of variation, and in modifying the level of significance, thus in increasing the type I and II error. However,

Fig.7. The four measurements taken for studying seed morphology of Sitka spruce.

**a: wing length**

**b: wing width**

**c: seed length**

**d: seed width**

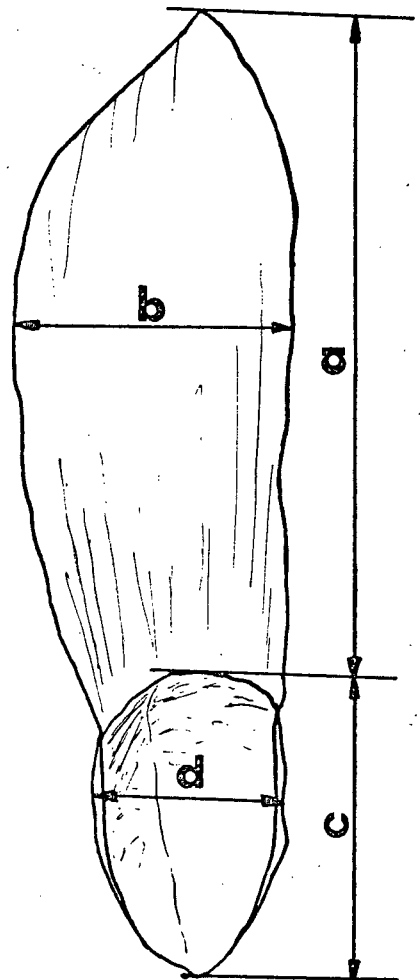


TABLE II  
VARIANCES AND MEANS OF THE SEED AND CONE  
CHARACTERISTICS MEASURED

Number of the <u>provenance</u>		<u>Seed Characteristics</u>				<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	
1	variance	0.84	0.07	0.75	0.03	70.58
	mean	7.73	3.38	2.93	1.74	54.26
2		0.50	0.11	0.10	0.02	112.68
		8.06	3.58	3.08	1.84	66.81
3		0.54	0.12	0.05	0.02	64.98
		6.96	3.41	2.95	1.77	64.90
4		0.43	0.22	0.07	0.03	77.02
		6.82	3.41	2.82	1.83	60.19
5		1.16	0.12	0.06	0.02	68.40
		7.50	3.50	2.73	1.68	40.65
6		1.19	0.23	0.05	0.04	53.44
		7.53	3.41	2.77	1.70	42.60
7		1.21	0.15	0.12	0.04	111.35
		8.01	3.62	2.89	1.81	59.59
8		0.71	0.14	0.06	0.02	66.85
		8.00	3.91	3.15	1.97	66.08
9		0.59	0.12	0.09	0.03	126.18
		7.18	3.58	2.74	1.77	54.71
10		0.96	0.14	0.06	0.05	102.64
		7.53	3.69	2.99	1.81	56.46
11		0.67	0.15	0.08	0.04	28.55
		6.54	3.29	2.48	1.74	38.82
12		1.82	0.12	0.10	0.02	117.29
		7.48	3.36	2.56	1.76	41.56
13		0.86	0.09	0.06	0.02	81.42
		7.08	3.39	2.55	1.88	51.30



TABLE II - Continued

Number of the provenance		Seed Characteristics				Cone Length
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	
14	variance	0.83	0.15	0.10	0.03	43.14
	mean	7.31	3.62	2.74	1.88	53.07
15		0.49	0.10	0.06	0.02	71.96
		7.30	3.71	2.83	1.99	62.12
18		1.41	0.32	0.11	0.05	54.53
		7.82	3.72	2.83	1.79	44.50
19		0.35	0.11	0.10	0.04	65.17
		7.38	3.72	2.86	1.86	60.39
20		1.22	0.11	0.08	0.03	155.54
		6.53	3.39	2.81	1.80	55.07
21		0.75	0.33	0.05	0.04	99.07
		7.45	3.95	2.97	2.09	59.97
22		0.29	0.15	0.07	0.05	67.98
		6.46	3.65	2.72	1.84	49.73
23		0.71	0.11	0.14	0.04	47.04
		6.34	3.45	2.50	1.76	44.52
24		0.73	0.13	0.05	0.03	116.05
		7.77	4.01	3.04	1.99	64.51
25		0.39	0.17	0.06	0.03	95.83
		7.13	3.81	2.81	2.01	62.62
26		0.37	0.13	0.06	0.03	143.97
		7.56	3.68	3.00	1.96	58.83
27		0.62	0.16	0.06	0.03	88.97
		6.84	3.60	2.86	1.90	55.57
28		0.83	0.19	0.03	0.02	34.90
		7.73	3.61	2.96	1.93	58.58
29		0.44	0.09	0.07	0.03	89.31
		7.24	3.52	2.99	1.78	53.35

TABLE II - Continued

Number of the <u>provenance</u>		<u>Seed Characteristics</u>				<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	
30	variance	0.44	0.10	0.12	0.04	130.78
	mean	7.04	3.41	3.04	1.75	59.31
31		0.68	0.14	0.11	0.03	117.30
		7.25	3.54	2.91	1.81	63.63
32		0.96	0.12	0.07	0.03	46.39
		6.35	3.22	2.58	1.75	52.51
33		0.93	0.23	0.09	0.06	72.19
		7.06	3.45	2.85	1.86	57.84
34		0.50	0.16	0.08	0.04	53.24
		6.70	3.31	2.79	1.84	56.28
35		0.95	0.26	0.15	0.05	201.54
		6.97	3.56	2.86	1.77	61.17
36		0.93	0.15	0.08	0.02	53.70
		6.51	3.51	2.86	1.86	49.20
37		0.64	0.20	0.10	0.05	141.07
		7.94	4.07	3.11	1.95	68.99
38		0.81	0.13	0.08	0.04	59.28
		6.70	3.41	2.75	1.77	49.33
39		1.07	0.15	0.06	0.04	80.27
		6.95	3.68	2.83	1.90	53.83
40		0.77	0.12	0.07	0.03	172.34
		6.96	3.61	2.70	1.77	66.26
41		0.77	0.13	0.13	0.03	64.23
		6.81	3.31	2.67	1.69	60.77

because the F values calculated are very high, the results of the Anova are, nevertheless presented here (Table III).

TABLE III  
F VALUES OF THE NESTED ANOVA PERFORMED  
ON THE DIFFERENT TRAITS MEASURED

<u>Sources of Variation</u>	<u>D.F.</u>	<u>Seed Traits</u>				<u>D.F.</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	38	58.8	44.5	30.24	24.6	38	231.51
Trees/Prov.	517	9.8	8.1	3.9	2.6	517	15.33
Error	2224					5004	

All the F values calculated are very highly significant. The heterogeneous pattern of variation of the total material precludes any estimation of variance components or multiple comparisons of the means of the provenances.

It should be noted that between provenances and between trees within provenance, variabilities do exist and that the cone length seems to be the most variable characteristic.

It is important to review the assumptions made in the analysis of variance:

- (1) The treatment effects and the environmental effects must be additive;
- (2) The experimental errors must all be independent;

- (3) The experimental errors must have a common variance;
  - (4) The experimental errors should be normally distributed
- (COCHRAN, 1947).

Generally, a small departure of the basic data from normality does not affect too much the significance level or the power of the F test, but the heterogeneity of the error variance does affect these properties. When the error variance changes with the treatment means according to a definite relationship, then some transformation of the data can be calculated (BARTLETT, 1947).

Naturally, the data should also respect the supplementary assumptions of the particular model used. If some level of variation suffers heterogeneous variances, then the assumptions are unwarranted and the estimation of variance components do not make much sense.

To test the homogeneity of variances, for each level of variation and for each variable while using the large number of measurements accumulated (more than 7,200 measurements) would have been a very expensive proposition. Therefore, we decided to test only the homogeneity of variances of the tree-progenies, for each provenance and for each trait separately. The following table summarizes the BARTLETT's tests performed (Table IV).

Thus, between 15% to 28% of the provenances have heterogeneous variances for the traits studied. On the basis of these low percentages, it was decided that it was not worth the work to find out which - if any - transformation will stabilize the variances. Furthermore, a

plotting (not shown) of the variances against their means for some characters did not show any definite relationship. An easier way to alleviate the heterogeneity of variance problems is to subdivide the material and analyze each subset separately.

TABLE IV  
NUMBER OF PROVENANCES OUT OF A TOTAL OF 39  
WITH HETEROGENEITY OF VARIANCES FOR FOUR  
SEED CHARACTERISTICS AND CONE LENGTH  
 (Significance level .005)

	<u>Seed Characteristics</u>				<u>Cone Length</u>
	<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	
Number	7	7	10	6	11

It was decided, for the anovas and the multivariate statistical analyses, to analyze separately 5 ecological subregions. Thus, the large area of origin of the Sitka spruce provenances was subdivided on the basis of the bioclimatic and physiographic data available. Naturally, within each region, climatic gradients exist because of their size.

These regions are working units which have the following advantages: these regions are fairly ecologically different from each other, the heterogeneity of variance problem is likely to be limited and safe multiple comparison tests of the provenance means are possible because the number of treatments (here provenances) is never greater than 12 (except for region 5 represented by 16 provenances).

- Region 1: is formed by a climatically homogeneous region - the eastern coast of Vancouver Island and the Lower Fraser Valley have similar climatic characteristics, at least in comparison with the western coast of Vancouver Island which is perhumid.
- Region 2: is formed by the western coast of Vancouver Island, perhumid and influenced by the Pacific Ocean.
- Region 3: is formed by the Queen Charlotte Islands whose oceanic climate is accentuated.
- Region 4: is less well defined and very broad: it is formed by the Alaska panhandle.
- Region 5: is formed by two major river basins, however close enough to be pooled as the Prince Rupert region or the Skeena River region. It represents provenances scattered along an altitudinal gradient in two major valleys of central coastal B.C. Provenance 9 is isolated and was arbitrarily placed in region 5.

Some provenances were arbitrarily placed in some regions: for instance, provenance 4 was placed in region 1 despite its northern position. Provenance 30 has been placed in region 2 because the Sooke area belongs to the western-humid coast of Vancouver Island according to the map of the biogeoclimatic subzones of Vancouver Island published by the forest company MacMillan Bloedel Ltd. in 1971.

For each of the five regions, separate nested analyses of

variance were performed as well as DUNCAN's multiple range test of the provenance means, for each trait studied. See Tables V, VI, VII, VIII and IX.

The DUNCAN's tests clearly show that no coherent classification of the provenances is possible on the basis of the different traits studied if they are considered independently. This erratic variation results from the fact that the characteristics studied are weakly correlated (see Table XI), disregarding the regions.

TABLE V

NESTED ANALYSES OF VARIANCE FOR REGION 1

<u>Source of Variation</u>	<u>DF</u>	F Values <u>Seed Characteristics</u>				<u>DF</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	3	34.28 ***	4.73 **	2.83 *	2.93 *	3	98.02 ***
Trees/Prov.	50	6.21 ***	10.07 ***	3.59 ***	2.65 ***	50	10.03 ***
Error	216					486	
TOTAL	269					539	

DUNCAN's multiple range test (\*) ( $\alpha = 0.05$ )

Seed characteristics      Code number of the provenances ranked in decreasing order

a	<u>1</u>	<u>29</u>	<u>3</u>	<u>4</u>
b	<u>29</u>	<u>3</u>	<u>4</u>	<u>1</u>
c	<u>29</u>	<u>3</u>	<u>1</u>	<u>4</u>
d	<u>4</u>	<u>29</u>	<u>3</u>	<u>1</u>
Cone Length	<u>3</u>	<u>4</u>	<u>1</u>	<u>29</u>

(\*) Underlined provenances are not statistically different at the error level of 5%.



TABLE VI  
NESTED ANALYSES OF VARIANCE FOR REGION 2

<u>Source of Variation</u>	<u>DF</u>	F Values <u>Seed Characteristics</u>				<u>DF</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	5	25.95	12.08	28.09	8.53	5	57.69
Tree/Prov.	80	8.53	5.39	3.25	2.52	80	13.66
Error	344					774	
TOTAL	429					859	

All the F values are significant at the level 0.001.

DUNCAN's multiple range test ( $\alpha = 0.05$ )

Seed characteristics      Code number of the provenance ranked in decreasing order

a	31	33	30	41	34	32
b	31	33	30	34	41	32
c	30	31	33	34	41	32
d	33	34	31	30	32	41
Cone Length	31	41	30	33	34	32

TABLE VII  
NESTED ANALYSES OF VARIANCE FOR REGION 3

<u>Source of Variation</u>	<u>DF</u>	F Values Seed Characteristics				<u>DF</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	5	68.96	68.89	31.42	16.29	5	255.85
Tree/Prov.	77	12.82	11.12	4.90	2.95	77	18.76
Error	332					747	
TOTAL	414					829	

All the F values are significant at the level 0.001.

DUNCAN's multiple range test ( $\alpha = 0.05$ )

<u>Seed characteristics</u>	Code number of the provenances ranked in decreasing order					
a	37	35	40	39	38	36
b	37	39	40	35	36	38
c	37	35	36	39	38	40
d	37	39	36	35	40	38
Cone Length	37	40	35	39	38	36

TABLE VIII  
NESTED ANALYSES OF VARIANCE FOR REGION 4

<u>Source of Variation</u>	<u>DF</u>	F Values <u>Seed Characteristics</u>				<u>DF</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	5	102.52	50.78	80.41	27.66	5	242.61
Tree/Prov.	84	9.26	9.50	5.57	3.12	84	20.63
Error	360					810	
TOTAL	449					899	

All the F values are significant at the level  $\alpha = 0.001$ .

DUNCAN's multiple range test ( $\alpha = 0.05$ )

Seed characteristics      Code number of the provenances ranked in decreasing order

a	24	28	26	25	27	23
b	24	28	26	25	27	23
c	24	26	28	27	25	23
d	25	24	26	28	27	23
Cone Length	24	25	26	28	27	23

TABLE IX  
NESTED ANALYSES OF VARIANCE FOR REGION 5

<u>Source of Variation</u>	<u>DF</u>	F Values <u>Seed Characteristics</u>				<u>DF</u>	<u>Cone Length</u>
		<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>		
Provenances	15	62.03 ***	38.49 ***	44.81 ***	30.84 ***	15	313.16 ***
Tree/Prov.	220	11.71 ***	9.04 ***	3.89 ***	2.45 ***	220	14.29 ***
Error	952					2167	
TOTAL	1187					2402	

DUNCAN's multiple range test (  $\alpha = 0.05$  )

Seed characteristics      Code number of the provenances ranked in decreasing order

a	7	8	18	6	10	5	12	21	19	14	15	9	13	11	20	22
b	21	8	19	18	15	10	22	14	7	9	5	6	13	20	12	11
c	8	10	21	7	19	15	18	20	6	14	9	5	22	12	13	11
d	21	15	8	13	14	19	22	10	7	20	18	9	12	11	6	5
Cone Length:	8	15	19	21	7	10	20	9	14	13	22	18	6	12	5	11

If the tree to tree variation in seed and cone characteristics is large enough in comparison with the provenance to provenance variation, it is likely that a coherent classification of the stands on the basis of the characters studied would be difficult. Components of variance were, therefore, calculated assuming a completely random model (Model II of Eisenhart) and eliminating the provenances not represented by 15 tree progenies. We so insured that the model was balanced which enables easy and unbiased estimations of the components of variances.

Table X summarizes the components of variance in % for the different seed and cone characters studied and for each region separately. T symbolizes the tree to tree variance, S the provenance to provenance variance. T and S are expressed as % of T + S.

A close examination of Table X shows that the variability is mostly confined to the between tree variability, or within provenance variability, except for seed length and width and for cone length; in this case, for some regions only. A hypothesis would be that seed length, seed width and cone length could be good discriminatory variates for classifying the provenances.

## 2.2 Multiple correlation and regression analyses. Relationships with the geographical coordinates of the place of origin of the provenances studied

A simple correlation matrix has been calculated between the characteristics a, b, c, d, cone length and longitude, latitude and altitude of the place of origin of the provenances, using the provenance means (Table XI), ignoring regional groupings.

TABLE X  
COMPONENTS OF VARIANCE IN PERCENTAGES FOR  
THE SEED TRAITS STUDIED AND CONE LENGTH

Region No.	Seed Traits								Cone Length	
	a		b		c		d		T	S
1	71.35	28.64	100	-	100	-	100	-	57.89	42.10
2	86.13	13.86	90.90	9.09	55.35	44.64	75.00	25.00	80.43	19.56
3	74.31	25.68	70.44	29.55	66.66	33.33	68.75	31.25	50.70	49.29
4	57.08	42.91	75.78	24.21	47.82	52.17	58.82	41.17	56.88	43.11
5	75.25	24.74	79.52	20.47	50.00	50.00	42.10	57.80	38.82	61.17

T: tree variance;      S: provenance variance

A close examination of this correlation matrix shows that all traits are correlated to some extent with each other, with the exception of the wing length (a) which is not correlated with seed width (d). Cone length is correlated with seed characteristics, but the relationships are not too strong, the correlation coefficients varying between 0.32 and 0.66. Only wing width and seed width are significantly correlated with the geographical coordinates. The percentage of variation accounted for oscillates around 16%.

There is a tendency for all the traits to decrease when the elevation increases. Cone length decreases significantly with elevation ( $r = - 0.57$ ).

This correlation matrix has been further investigated by

TABLE XI

CORRELATION MATRIX BETWEEN THE SEED CHARACTERISTICS AND  
CONE LENGTH AND THE GEOGRAPHICAL COORDINATES OF THE PLACE OF  
ORIGIN OF THE 39 PROVENANCES STUDIED

	<u>a</u>	<u>b</u>	<u>c</u>	<u>d</u>	<u>Cone</u>	<u>Long.</u>	<u>Lat.</u>	<u>Alt.</u>
a	1.00							
b	0.57***	1.00						
c	0.62***	0.60***	1.00					
d	0.30NS	0.75***	0.45**	1.00				
Cone	0.32*	0.48**	0.66***	0.49**	1.00			
Long.	-0.099NS	0.45**	-0.10NS	0.49**	-0.033NS	1.00		
Lat.	0.17NS	0.39*	-0.19NS	0.34*	-0.31NS	0.78***	1.00	
Alt.	0.0054NS	-0.15NS	-0.28NS	-0.40*	-0.57***	-0.09NS	0.24NS	1.00

NS Not significant at 5% level

\* significant at 5%

\*\* significant at 1%

\*\*\* significant at 0.1%

means of the multiple regression analysis which allows the study of different influences independently of each other. A "backward step-wise" technique programmed by Dr. A. KOZAK (U.B.C. Faculty of Forestry) has been used. This technique consists in calculating a multiple regression equation between the dependent variable and all the variables chosen as independent; and then, in dropping successively the independent variable with the least partial F test corresponding to its partial regression coefficient from all the remaining independent variables. Then to calculate and test a new multiple regression equation with the remaining variables. The partial F test is defined in standard textbooks on multiple regression such as DRAPER and SMITH's book (1966, p. 71) which also discusses the advantages and disadvantages of the different methods existing for selecting the best regression equation. The procedure is repeated until there remains only one independent variable. The variables were considered as contributing significantly to the variation of the dependent variable when rejecting one of them significantly lowered the multiple correlation coefficient.

1) Wing Length (a)

The altitude does not contribute significantly to wing length variability and the prediction equation becomes:

$$a = 7.29 - 0.0099 (\text{Long.} - 128.87) + 0.12 (\text{Lat.} - 53.21)$$

$$r^2 = 0.187$$

$$r = 0.433^*$$



2) Wing Width (b)

Only the longitude contributes significantly to b variability and the prediction equation becomes:

$$b = 3.56 + 0.028 (\text{Long.} - 128.87)$$

$$r^2 = 0.209$$

$$r = 0.457^{***}$$

3) Seed Length (c)

No relationship whatever has been demonstrated.

4) Seed Width (d)

The prediction equation reduces to:

$$d = 1.83 + 0.014 (\text{Long.} - 128.87) - 0.000060 (\text{Alt.} - 400.25).$$

$$r^2 = 0.377$$

$$r = 0.614^{***}$$

5) Cone Length

The prediction equation reduces to

$$\text{cone} = 55.90 - 0.0077 (\text{Alt.} - 400.25)$$

$$r^2 = 0.333$$

$$r = 0.577^{***}$$

To summarize the equations obtained, one can say that the pattern of variation differs from one character to another. The percentage of variation accounted for by the geographical coordinates is not too high, leaving much of the variation not explained. The explanations are difficult: lack of clinal variation, precision too low, etc. Wing length is a multiple function of longitude and latitude, wing width primarily

increases with longitude, cone length primarily increases when elevation decreases. Seed width is related to longitude and altitude and is independently related to longitude, latitude and altitude. The regression equations for seed width are:

$$d = 1.83 + 0.015 (\text{Long.} - 128.87); r = 0.497^{**}$$

$$d = 1.83 + 0.012 (\text{Lat.} - 53.21); r = 0.347^*$$

$$d = 1.83 + 0.000076 (\text{Alt.} - 400.25); r = 0.406^*$$

The provenance means have been plotted against longitude, latitude and elevation of the place of origin of the provenances neglecting the regions. A close examination of the graphs obtained shows that two groups of provenances could exist overlapping in about the same range of latitude or longitude, for each trait. Therefore, a separate regression line has been adjusted for each group and for each characteristic studied. No significant regression has been found except that two regression lines exist for cone length on longitude. The corresponding equations are:

$$\text{cone} = 54.84 - 0.018 (\text{Long.} - 126.21); r = 0.535^*$$

$$\text{cone} = 54.71 + 0.019 (\text{Long.} - 130.38); r = 0.423^*$$

These two regression lines correspond to two groups of provenances of longitudes east of  $127^{\circ}$  and west of  $130^{\circ}$ .

In spite of the fact that no statistical adjustment has been possible, it would be interesting to further test the hypothesis of a large change in variation pattern in seed characteristics between the latitudes  $52^{\circ}$  and  $54^{\circ}$  and the longitude  $127^{\circ}$  and  $130^{\circ}$ . Two large groups of provenances could be distinguished: one group with latitudes below  $52^{\circ}$  and

one group with latitudes larger than  $54^{\circ}$ ; with none in between. Note also that the latitude and longitude of the places of origin are correlated to some extent ( $r = 0.78$ ).

Table XI shows the correlation coefficients of the seed and cone traits studied calculated on the basis of the provenance means. These correlations are naturally different from those calculated on the basis of tree means. Thirty-nine correlation coefficients have been calculated between the traits c and d, one for each provenance, and as an example plotted against latitude of the place of origin (Fig. 8).

On the basis of latitude of origin, two groups of provenances seem to be distinct. The Vancouver Island region and the more northern provenances. It seems as if, within each group, the correlation coefficients increase with latitude, the slopes being similar.

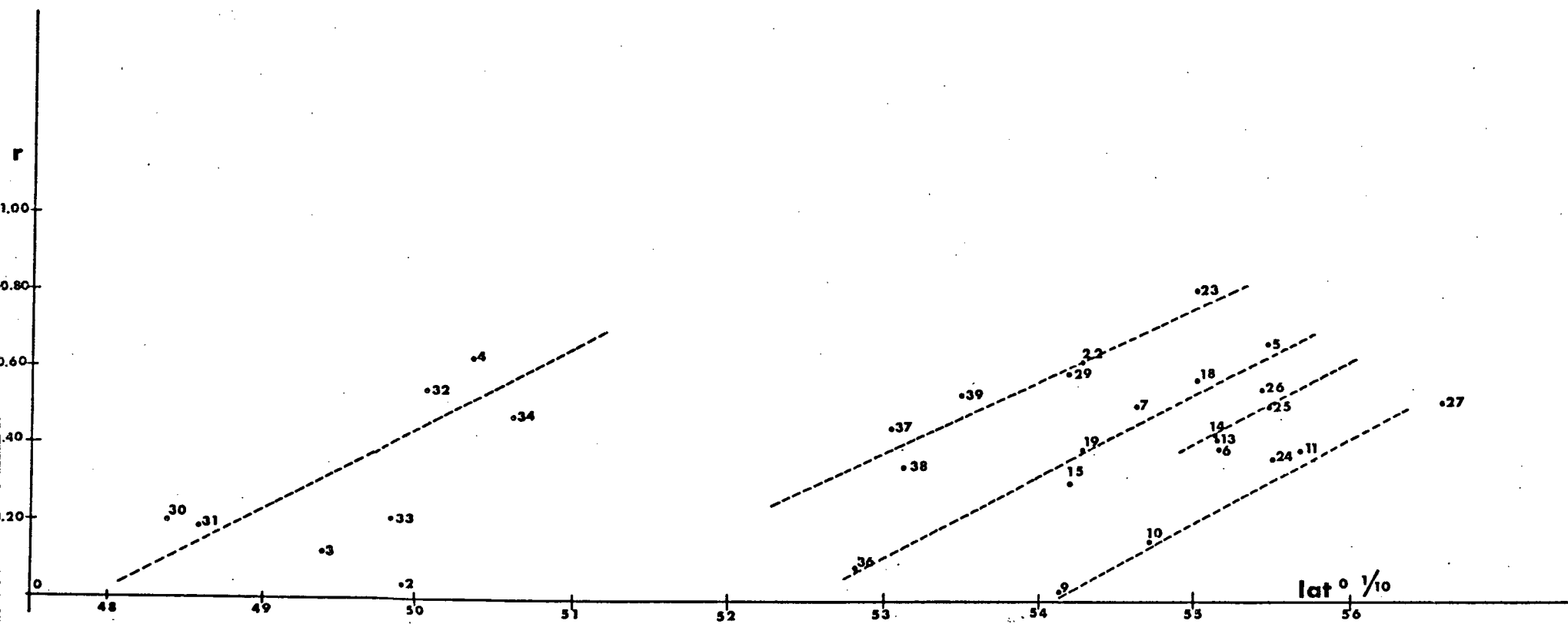


Fig.8. Relationships between the correlations of seed length with seed width and latitude of the place of origin.

CHAPTER 3  
MULTIVARIATE STATISTICAL METHODS  
A COMPARATIVE REVIEW OF THE MATHEMATICAL  
THEORIES BEHIND SOME TECHNIQUES FROM A  
BIOLOGIST'S POINT OF VIEW

Introduction

The five traits studied did not result in a clear classification of the parent stands of the Sitka spruce provenances and it was, therefore, decided to use a multivariate approach as the simultaneous consideration of several characters may result in a meaningful classification (COOLEY and LOHNES, 1966; HORTON, et al., 1968).

Classification should be distinguished from discrimination or classing techniques and from dissection. The latter techniques are concerned with comparing the profile of an individual with that of groups already defined. The former techniques aim at classifying individuals into different groups in the form of a significant pattern or a few group constellations. The "groups" within a constellation must necessarily be closer to one another, in some sense, than those belonging to different constellations.

It is not possible to cover the numerous techniques designed for classifying individuals, groups or entities of higher hierarchal rank.

So many techniques designed for classifying individuals, groups, etc. have been proposed that confusion is widespread in the field

of numerical classification as to the names, validity, usefulness, similarities, efficiency, statistical or mathematical theories of these methods. The opinions of the authors often diverge. BLACKITH and REYMENT's opinion (1971) on factor analysis is in opposition to that of CATTELL (1965). The former authors, furthermore, propose in their book a biological interpretation of multivariate statistics by rejecting the limits which could be imposed by the statistical theory.

DAGNELIE (1966) has outlined the characters of several methods of numerical classification, the needs and the possible research approaches in clearing the field. Two approaches are basically possible: on the one hand, the existing techniques can be compared; on the other, an optimum solution can be worked out. In both cases, some "optimum" criteria must be decided upon. An optimum classification (DAGNELIE, 1966) could be defined as the one which results in, for a given number of classes, one of the following - not exclusive - characteristics: the generalized distances between individuals within classes are minimum; the generalized distances between classes are maximum; the average generalized distance within classes is minimum; the average generalized distance between classes is maximum. Ideally, the comparison of the methods should include the cost in dollars of running these techniques on a computer and should be applied on as much data as possible.

A sound interpretation of the results of the use of a given method is only possible when one completely understands the theoretical and statistical background of the techniques used. We shall, therefore,

review and compare the mathematical theory buried in the methods that were available to us.

To avoid a lengthy exposition, we will suppose that the reader is familiar with multivariate statistics. For a complete exposition of the mathematical theories of the methods used, the reader is referred to the books of MORRISON (1967), KENDALL (1966) and ANDERSON (1958).

The capital letters A, B, C, etc., will be used for matrix and small letters x, y, z, etc., for vector notation; furthermore, the letters will be underlined A, x, etc.

### 3.1 Multivariate Anova (MANOVA)

#### 3.1.1 Multivariate generalization of a one-way univariate ANOVA

The steps are the following (after COOLEY and LOHNES, 1966):

- (1) Test of homogeneity of the dispersions;
- (2) Test of homogeneity of the population centroids by using

WILKS' Lambda criterion ( $\lambda$ ):

$$\lambda = |WS| / |TS|$$

$| |$  : determinant notation

WS : pooled within group sums of squares and cross products matrix

TS : total deviation sums of squares and cross products matrix

The test of significance uses a Chi-square approximation of

BARTLETT or a F approximation of RAO.

No real grouping of the basic populations is possible without a multiple comparison test of the centroids.

### 3.1.2 General case

The general procedure for any MANOVA is given by SEAL (1964). The basic linear model is:

$$\underline{X} = \underline{Z}' \cdot \underline{B} + \underline{E}$$

Thus, there are N p-variate observations and Q error free observations (= treatments, etc.).

It involves the computation of a "reduced model" and of a variate approximately distributed as Chi-square which is used as a basis for a test of significance. SEAL gives an extension of the SCHEFFE's S method of multiple comparison of the mean vectors. Therefore, it is possible to find out group constellations by calculating contrasts between all the pairs of centroids. The S test is very robust and might be used more frequently.

## 3.2 Principal Component Analysis (PCA)

PCA is the technique most used and most known from the mathematical point of view. This method should be distinguished from factor analysis (FA), (SEAL, 1964 and CATTELL, 1965).

PCA has been interpreted by SEAL (op. cit) as a procedure for applying a linear transformation to the original variates represented by the vector  $\underline{x}$ . This transformation is:



$$\underline{y} = \underline{A} \cdot \underline{x}$$

The transformation is orthogonal and the new variates are mutually independent and form new axes of coordinates of maximum variation, called principal components. A sample of  $N$  observations of  $p$  variates ( $N$   $p$ -variate observations) is summarized in a few variates which are not correlated and account for a maximum of the total primitive variation.

CATTELL (op. cit.) has interpreted PCA as a very particular case of FA where the simple correlation matrix  $R$  calculated from the  $p$  variates is equated as follows:

$$\underline{R} = \underline{V} \cdot \underline{V}'$$

$$\underline{V} = \text{factor matrix and } \underline{V} = \underline{M} \cdot \underline{L}^{\delta/2} \text{ where}$$

$\underline{M}$  is the matrix of latent vectors of  $\underline{R}$

$\underline{L}^{\delta}$  is the diagonal matrix of latent roots of  $\underline{R}$

PCA is based on the derivation of  $\underline{V}$  from a so-called "non reduced" matrix  $\underline{R}$ , i.e., a matrix  $\underline{R}$  where the diagonal coefficients have all been equated to 1. This approach is similar to that of DAGNELIE (1960) where the original variates  $z_j$  are explained by means of  $r$  factors; also called components common to all the variates  $z_j$  and a specific variate or factor  $v_j$ . The model is:

$$z_j = a_{j1} x_1 + \dots + a_{jr} x_r + u_j v_j$$

In matrix notation, the model can be expressed as:

$$\underline{Z} = \underline{A} \cdot \underline{X} + \underline{U} \cdot \underline{V}$$

It should be noted that PCA can be carried out on the variance - covariance matrix of the observations or on the correlation matrix obtained when the observations have been standardized. The components obtained from these two different matrices are not the same and it is not possible to pass from one set of components to the other by scaling the coefficients of the components. The covariance matrix should be preferred when the measurements were made in similar units and when tests are forecast.

It is also important to note that the  $j$ th principal component of the sample of  $p$  variate observations is the linear component:

$$y_j = a_{ij} x_i + \dots + a_{pj} x_p$$

where the coefficients are the elements of the characteristic vector of the sample covariance matrix  $\underline{S}$  corresponding to the  $j$ th largest characteristic root  $l_j$ . This is, of course, equivalent to the DAGNELIE's model of above, where the specific factor is not considered. The sample variance of the  $j$ th component is  $l_j$ , and the total system variance is thus  $l_1 + \dots + l_p = \text{tr } S$ . The importance of the  $j$ th component in a more parsimonious description of the system is thus measured by:

$$l_j / \text{tr } S$$

If the components have been extracted from the correlation matrix  $\underline{R}$  rather than  $\underline{S}$ , the sum of the characteristic roots will be

tr  $R = p$ , and the proportion of the total variance in the scatter of dimensionless standard scores attributable to the  $j$ th component will be  $l_j/p$ . The sum of the squared correlations  $a_{ij} \sqrt{l_j}$  of the variables on that component will, of course, be the component variance  $l_j$  (after MORRISON, 1967).

Finally, it must be remembered that as the transformation matrix is orthogonal, the transformation is distance preserving between the points in the new space. Since the angles of a triangle are determined by the length of its sides, the principal component transformation also preserves the angles (ANDERSON, 1958).

The disadvantages of PCA are numerous:

\* Strictly, PCA should only be used to summarize a mass of  $p$ -variate observations resulting from the sampling of one multivariate normal universe. Many authors have applied PCA to a set of observations already structured in groups or for testing the hypothesis that groups do exist. It is questionable if PCA is the adequate tool for such endeavours. If we want to construct tests of hypotheses and confidence intervals for the population roots, the direction cosines, etc., it is necessary to suppose that the sample be drawn from a multivariate normal population whose covariance matrix has a specified covariance structure (MORRISON, 1967).

- The forms of the components are not invariant under changes in the scales of the responses. Thus, it is doubtful whether PCA should be applied to variates measuring different entities, e.g., a combination of

lengths, weights and dichotomous variates (variates taking the values 0 or 1), (MORRISON, op. cit.; SEAL, op. cit.). An attempt to avoid this difficulty is to standardize all the variates by dividing each by its estimated standard deviation. However, there are two main disadvantages in using standardized variates:

(a) the standardized observations are only approximately normal, and (b) the new variates are put on an equal footing, thus resulting in a distortion of the measurements (SEAL, op. cit.).

- No rational criteria exist for deciding when a sufficient proportion of the variance of all the observations has been accounted for by the components, nor can provision be made for components that are attributable only to the sampling variation of the individual responses. PCA is thus merely an orthogonal transformation rather than a model for studying covariance structure (MORRISON, 1967). Furthermore, if the off diagonal elements of a correlation matrix are approximately equal, the interpretive value of PCA is dubious (SEAL, op. cit.).

- Finally, the common factors are distorted because of the artificial conditions imposed of being non-correlated (CATTELL, op. cit.).

- It is often difficult or subjective to interpret biologically the new factors found, as a look at the numerous applications of PCA can show:

The advantages are the following:

- Simplicity of the mathematics involved.
- Orthogonality of the new space.
- Distance preserving transformation.

- Rotation of the factors possible.
- Test of the latent roots possible.
- Possibility of calculating the centroids in the new coordinates

and to get a visual representation of the possible grouping of the populations to be classified. For an example, see JEFFERS and BLACK (1963).

### 3.3 Factor Analysis (FA)

According to CATTELL (1965), FA is performed when the latent vectors and latent roots of a so-called reduced matrix  $\underline{R}_1$  are estimated.  $\underline{R}_1$  is reduced when the so-called "communalities", estimated in some way, replace the diagonal coefficients of the original matrix  $\underline{R}$ . Using this "open model" is more logical, but results in many more difficulties than with PCA. Such difficulties are: the estimation of the communalities; the estimation of the number of factors to be used; the indetermination of the specific factors and of the errors. CATTELL (op. cit.) has given some rules for determining the number of factors to start with and the criteria for deciding which rotation should be used. He has proposed to first decide upon the number of factors, then to adjust the communalities. As there is an infinity of equivalent factor matrices, the next problem is to fix up his mind on a meaningful rotation in order to get a meaningful factor matrix. The criteria given have their own defects and difficulties. CATTELL believes that oblique factors are the rule in nature.

FA is an interesting tool, a hypothesis generating method.

It is more a method for classifying the variables but not, as such, a tool for generating clusters of individuals. Cluster search or classification of entities could be done after an analysis of the factors responsible for the structure of the universe under study (CATTELL, op. cit.).

### 3.4 Discriminant Function Analysis (DF) and Canonical Analysis (CA)

The concept of linear discriminant function was originally introduced by FISHER in 1936 as a solution to the problem of classifying an observation into one of two predetermined groups. In the case of two populations with a common covariance matrix, but different mean vectors if  $\underline{x}_1$  and  $\underline{x}_2$  are the sample mean vectors and  $\underline{W}$  the pooled estimate of the covariance matrix, the linear discriminant function is

$$y = (\underline{x}_1 - \underline{x}_2)' \underline{W}^{-1} \underline{x}$$

$\underline{x}$  being the vector of variates

The linear discriminant function corresponds to the linear combinations of the variables which assures the maximum value to the ratio between the "between" sub-population sample variance of the new variate  $y$  and the "within" sub-population sample variance. Actually, this ratio corresponds to the HOTELLING's  $T^2$  statistic (MORRISON, 1967).

The linear discriminant function is connected with the MAHALANOBIS' generalized distance  $D^2$  by the relationship

$$D^2 = \underline{d}' \underline{a}$$

where  $\underline{d}$  is the difference between the two sample mean vectors and  $\underline{a}$  the vector of coefficients of the discriminant function.

For a number  $h$  of  $p$ -variate groups,  $D^2$  is defined as

$$D^2 = \sum_{i=1}^p \sum_{j=1}^p \omega^{ij} (\bar{x}_{ik} - \bar{x}_{il}) (\bar{x}_{jk} - \bar{x}_{jl})$$

$$\text{or } D^2 = \sum_i \sum_j \omega^{ij} d_i d_j$$

$\omega^{ij}$  is the adequate element of the inverted sample within covariance matrix.

$D^2$  is not very sensitive to the non-normality of the original variates, but the within covariance matrices (= dispersions) should be homogeneous (MILLIER and TOMASSONE, 1969).

The discriminant function is intimately linked with the problem of classifying an individual into one of two normal multivariate populations provided that some "a priori" probabilities, cost of misclassification, etc. are known. For a complete coverage of the problems of classing individuals in one of several groups, see ANDERSON (1958), (Chapter 6).

The multiple discriminant functions are computed as latent vectors associated with the latent roots of the determinantal equation  $|D|$  with:

$$|\underline{D}| = |\underline{W}^{-1} \underline{B} - \lambda \underline{I}| = 0 \quad (1)$$

This equation can also be written as:

$$| \underline{D} | = | \underline{B} - \lambda \underline{W} | = 0$$

$\underline{B}$  is the matrix of variance - covariances between the groups or universes

$\underline{W}$  is the matrix of variance-covariances within the universes

$\lambda$  is a variable representing the latent roots of the dissymmetric matrix  $\underline{W}^{-1} \underline{B}$

$\underline{I}$  is the usual identity matrix

The word canonical analysis has been used in connection with quite different techniques, adding to the confusion of the user of multivariate techniques. For KENDALL and STUART (1966), it includes PCA, canonical correlations and FA. BLACKITH and REYMENT (1971) separates the canonical variates from discriminant function analysis (their Chapters 7 and 8) and distinguish (p. 49) between discriminant analysis as described by ANDERSON (1958) and canonical discriminant functions. In fact, their "canonical variates" (Chapter 8, op. cit.) are only the usual discriminant variates corresponding to the usual discriminant axes generated by the equation

$$(\underline{B} - \lambda \underline{W}) \underline{U} = \underline{0}$$

in our notation,

which corresponds to their equation (8.2), p. 89:

$$(\underline{B} - \lambda \underline{W}) \underline{t} = \underline{0}$$



SEAL (op. cit.) has proposed his canonical analysis by criticizing the generalized distance technique which is a by-product of the discriminant function analysis. He contends that:

- (1) the comparisons of  $h$   $p$ -variate universes where  $h < p$  should be made in a space of  $h-1$  dimensions rather than in a space of  $p$  dimensions, and
- (2) the distance technique depends on all  $p$  variates being measured in the same units.

This last criticism does not hold as the MAHALANOBIS' generalized distance is independent of the scale of the variables (COOPER, 1963). The MAHALANOBIS' distance remains unchanged no matter what (non-singular) change of variables one makes. SEAL (op. cit.) proceeds as follows:

Suppose that we have  $h$   $p$ -variate normal universes represented by samples. Each universe is characterized by its centroid and its variance-covariance matrix. Two steps are then distinguished:

- test for the equality of variance-covariance matrices
- comparison of the  $h$  universes

It is possible to find a transformation such that the first axis is inclined in the direction of the greatest variability between the mean vectors, then that the second axis, at right angle to the first, is to be inclined in the direction of the next greatest variability, and so on.

This transformation, is, let us say:

$$\underline{y} = \underline{U}' \underline{x}$$

$\underline{x}$  = vector of the original measurements

$\underline{y}$  = vector of the canonical variates

$\underline{U}$  = matrix of the transformation

Because the elements of the transformation matrix are indeterminate, two conditions are imposed: the new canonical variates should be uncorrelated (i.e., with zero covariances) and of unit variances.

CA thus reduces itself to finding the latent roots  $\lambda$  and the associated eigenvectors of the following determinantal equation:

$$| \underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2} - \lambda \underline{I} | = 0 \quad (2)$$

$\underline{B}$  is the matrix of variance-covariances between the universes or the groups

$\underline{W}$  is the matrix of variance-covariances within the universes

$\lambda$  is a variable representing the latent roots

$\underline{I}$  is the usual identity matrix

There are a maximum of  $p$  roots and the  $p$  roots are distinguishable when  $p \leq h - 1$ . When  $p > h - 1$ , there are  $p - h + 1$  zero roots and  $h - 1$  distinguishable roots, i.e., different from zero (BLACK-ITH and REYMENT, 1971).

If  $\underline{V}$  is the matrix of eigenvectors corresponding to equation (2), then  $\underline{V}$  is generated by the equation

$$(\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2} - \lambda \underline{I}) \underline{V} = \underline{0}$$

$\underline{\Lambda}$  is the diagonal matrix of the characteristic roots of (2)

Then the transformation matrix  $\underline{U}$  is obtained, according to SEAL (op. cit.) by the equation

$$\underline{W}^{1/2} \underline{U} = \underline{V} \quad (3)$$

The centroids of the different universes can be expressed in canonical form and their mutual position visualized in a diagram. Confidence circles can be drawn around the points representing the universes in the different graphs. A visual classification is, therefore, possible. Circles are appropriate instead of ellipses because CA transforms the within sample ellipsoids of scatter into spheres, more precisely hyperspheres.

According to SEAL, in CA, the  $p$  variates may be different types of measurements. The new dimensions required would be less than those required by PCA.

CA is thus based on the calculation of the latent roots and the associated latent vectors of the matrix  $\underline{C}$  with

$$\underline{C} = \underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2}$$

while DF is based on the estimation of the eigenvalues and eigenvectors of the matrix  $\underline{D}$  with

$$\underline{D} = \underline{W}^{-1} \underline{B}$$

$\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2}$  is generally different from  $\underline{W}^{-1} \underline{B}$ . However, they have the same characteristic roots  $\lambda$ .

Proof:

The non-zero characteristic roots of the product  $\underline{A} \underline{B}$  are equal to the non-zero roots of  $\underline{B} \underline{A}$  (MORRISSON, 1967, p. 62); but the following matrices have the same characteristic roots:  $\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2}$ ,  $\underline{B} \underline{W}^{-1/2} \underline{W}^{-1/2} = \underline{B} \underline{W}^{-1}$ ; therefore  $\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2}$  and  $\underline{B} \underline{W}^{-1}$  or  $\underline{W}^{-1} \underline{B}$  have the same eigenvalues. However, the associated eigenvectors are generally different.

Let  $\underline{U} = \begin{bmatrix} \underline{u}_1 & \dots & \underline{u}_p \end{bmatrix}$  be a matrix of eigenvectors corresponding to DF.  $\underline{U}$  is generated by the following set of equations:

$$(\underline{W}^{-1} \underline{B} - \underline{\Lambda} \underline{I}) \underline{U} = \underline{O}$$

Similarly,  $\underline{V}$  is generated by the set of equations:

$$(\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2} - \underline{\Lambda} \underline{I}) \underline{V} = \underline{O}$$

corresponding to CA.

Therefore,  $\underline{W}^{-1} \underline{B} \underline{U} = \underline{U} \underline{\Lambda}$  and  $\underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2} \underline{V} = \underline{V} \underline{\Lambda}$ . But  $\underline{W}^{-1/2} \underline{B} (\underline{W}^{-1/2} \underline{V}) = \underline{V} \underline{\Lambda}$ , thus  $\underline{B} (\underline{W}^{-1/2} \underline{V}) = (\underline{W}^{1/2} \underline{V}) \underline{\Lambda} = \underline{W} (\underline{W}^{-1/2} \underline{V}) \underline{\Lambda}$  and  $\underline{W}^{-1} \underline{B} (\underline{W}^{-1/2} \underline{V}) = \underline{W}^{-1/2} \underline{V} \underline{\Lambda}$ ; therefore,  $\underline{U} = \underline{W}^{-1/2} \underline{V}$  or  $\underline{V} = \underline{W}^{1/2} \underline{U}$ .

$$\underline{V} = \underline{W}^{1/2} \underline{U}$$

But this equation is identical to equation (3). Consequently, CA, according to SEAL and DF in the usual meaning perform exactly the same transformation and this transformation is not orthogonal, in general.

$\underline{U}$  is not orthogonal, in general.

Proof:

If  $\underline{U}$  was orthogonal, then  $|\underline{U}| = \pm 1$  and  $\underline{U}'\underline{U} = \underline{I}$ ,  $\underline{I}$  being the identity matrix as usual; furthermore  $|\underline{U}'\underline{U}| = |\underline{I}| = 1$  (MORRISON, 1967, p. 59). If  $|\underline{U}| = \pm 1$  and  $\underline{U}'\underline{U} = \underline{I}$ , then  $|\underline{U}'\underline{W}\underline{U}| = |\underline{U}'||\underline{W}||\underline{U}| = |\underline{W}|$  but  $|\underline{W}| \neq \pm 1$ . But  $\underline{U}'\underline{W}\underline{U} = \underline{I}$ , then  $|\underline{U}'\underline{W}\underline{U}| = 1$  which is contradictory. Thus, in spite of the fact that  $\underline{U}'\underline{W}\underline{U}$  is equal to  $\underline{I}$ ,  $\underline{U}$  is not generally orthogonal.

But even if  $\underline{W}$  and  $\underline{U}$  are not orthogonal,  $\underline{V} = \underline{W}^{1/2}$ .  $\underline{U}$  is an orthogonal matrix.

The transformed values have thus the following covariance matrices, as it is easy to demonstrate:

$$\text{Within:} \quad \underline{U}'\underline{W}\underline{U} = \underline{I}$$

$$\text{Between:} \quad \underline{U}'\underline{B}\underline{U} = \underline{\Lambda}$$

The new variables are thus uncorrelated and of variance 1. Each group has been so transformed as to take the shape of an hypersphere.

One obvious consequence of this non-orthogonal transformation is that, unlike in PCA, the distances and angles in the new space are changed. Therefore, the graphs representing the individuals in the new space are pure conventions as the new axes are not perpendicular.

Canonical analysis, according to SEAL, is simply another interpretation of DF. CA or DF take into account the nested levels of variation which might exist: between groups and within groups. Furthermore, it can be used to calculate the so-called MAHALANOBIS' distances. This fact seems to have been overlooked by SEAL who introduces his CA

by criticizing the generalized distances.

An important characteristic of DF or CA is that:

$$\begin{aligned} \text{trace of } \underline{W}^{-1} \underline{B} &= \text{trace of } \underline{W}^{-1/2} \underline{W}^{-1/2} \underline{B} = \\ \text{trace of } \underline{W}^{-1/2} \underline{B} \underline{W}^{-1/2} &= \text{trace of } \underline{B} \underline{W}^{-1} = \text{trace of } \underline{\Lambda} \end{aligned}$$

Consequently, the trace of  $\underline{\Lambda}$  is the sum of the variances between groups of the different variables studied and the percent trace of a characteristic root can be interpreted as the fraction of the total variation between groups explained by that root.

BLACKITH and REYMENT (1971) contend that it is not because some roots do not contribute significantly to the total sum of squares in the analysis of dispersion that they must be disregarded. The largest of the canonical roots would generate a vector which often represents size variation in morphometric analyses, but size variation would seldom be an object of study in itself. However, their argumentation is not convincing. If some roots are not statistically significant, it is difficult to understand how one could possibly base reliable biological interpretation on the variability associated with the corresponding eigenvectors.

### 3.5 Results and Conclusions

It is obvious that there is a pressing need to compare the classification techniques under all possible aspects, even those techniques which are supported by a substantial body of statistical theory.

Lack of time and of money have resulted in our restricting

the comparison to the following techniques:

CA and its derivatives, the MAHALANOBIS' distances  
and the dendrograms;  
Stepwise Discriminant Function Analysis;  
PCA.

The five cone and seed characteristics measured have been used to do this comparison. These traits are supposed to be normally distributed and the relationships between their means and variances are not so strict as to impose some transformation of the original variates (see Section 2.1).

The "operational taxonomic units" ("OTU") in the sense of SOKAL and SNEATH (1963) are the provenances. Only the provenances with 15 trees will be considered, unless otherwise mentioned. The trees are considered as repetitions.

In order to be able to use CA as proposed by SEAL (1964), an original computer programme has been developed and tested. Furthermore, a computer programme developed at the Biometric Station of the National Centre for Forest Research at Champenoux, France, under the direction of Dr. C. MILLIER, has been adapted to the U.B.C.'s Michigan Terminal System. Both programmes required painstaking efforts during several months and further programming on the other programmes of multivariate analyses existing in the U.B.C.'s Computing Centre was precluded. The MANOVA has not been included in the comparison as the available program would have to be completed with a dispersion test and a multiple comparison test of the centroids in order to

become meaningful. FA has not been tried as such because there is no specific program to use this method and because this method is delicate to use and should be the subject of a thesis in itself.

### 3.5.1 CA according to SEAL (1964)

The programme written in FORTRAN IV for the I.B.M. System 360 - Model 67 computer of the U.B.C. computing centre follows exactly the procedure outlined by SEAL (1964, Chapter 7). It gives the following output:

- (1) the covariance and correlation matrices and their determinant for each group of individuals;
- (2) the T, B, W matrices;
- (3) test of the homogeneity of the within covariance matrices;
- (4) test of the overall differences of the different groups  
MANOVA's WILKS Lambda criterion tested by RAO's F approximation;
- (5) BARTLETT's test of the latent roots (SEAL, op. cit., p. 135);
- (6) the canonical functions and the centroid vectors in the new canonical space for each group;
- (7) the plotting of the groups in a chart formed by the axes with the greatest significant roots.

The provenances have been grouped into zones which have been analyzed separately in order to respect as much as possible the basic hypothesis of homogeneity of dispersion matrices. These are the same regions but with some deletions because only the



TABLE XII

CANONICAL FUNCTIONS FOR THE FOUR REGIONS STUDIED  
COEFFICIENTS OF THE ORIGINAL VARIABLES

The canonical functions are ranked according to the decreasing order of magnitude of their root						
Region	Number of the canonical functions	Variables				
		1	2	3	4	5
2	1	0.0306	0.668	3.518	-4.641	0.663
	2	0.331	-0.557	3.712	2.789	-0.140
	3	0.801	-0.135	-1.563	6.850	0.0286
	4	0.936	1.539	-1.511	-3.939	-0.0450
	5	-1.222	3.331	0.783	-0.155	-0.0191
3	1	-0.00502	1.477	-2.285	-1.996	0.120
	2	0.151	-0.112	3.799	4.079	-0.0230
	3	-0.513	1.012	-3.432	6.332	0.0109
	4	1.227	0.548	-3.056	0.209	-5.0990
4	1	0.577	-0.565	2.345	1.915	0.0488
	2	-1.051	1.754	-3.220	4.805	-8.038
	3	0.936	-3.020	-3.761	7.312	-0.00724
	4	-1.222	-1.313	3.257	1.595	0.141
	5	0.0849	0.777	0.399	5.440	-0.104
5	1	-0.366	-0.0783	0.243	4.591	0.120
	2	-0.386	-0.276	-4.405	6.330	-0.0111
	3	-0.997	2.262	2.849	0.670	-0.0720
	4	1.000	-0.966	-0.0140	5.555	-0.0652
	5	0.282	2.538	-2.790	-1.835	0.0115

populations with 15 trees have been included. As unit of observations, the means of each tree were considered. These means were calculated from the samples of five seeds and ten cones.

The canonical functions for the four regions studied are shown in Table XII.

Vancouver Island Zone (= Region 1 + 2)

= provenances 3, 4, 30, 31, 32, 33, 34.

Number of groups: 7.

Number of p-variate observations: 105.

Test of equality of the group dispersions:  $U = 3.93$ . This is very highly significant and the group dispersions are not equal, nor are the correlation matrices. There are thus, differences in size and/or orientation of the density ellipsoids for the different groups.

Test of the overall significance of the differences between groups:

$F(30, 378) = 2.84^{***}$

BARTLETT's Test of the Latent Roots:

Roots Removed	Roots	Chi-Square	D.F.	Significance	Percent- age trace
0	0.65	79.79	30	***	65.97
1	0.16	30.73	20	NS	16.51
2	0.14	15.96	12	NS	14.53
3	0.027	2.85	6	NS	2.78
4	0.002	0.20	2	NS	0.21

Only one root, the greatest, is significantly different from zero. The canonical vectors associated with the other roots do not

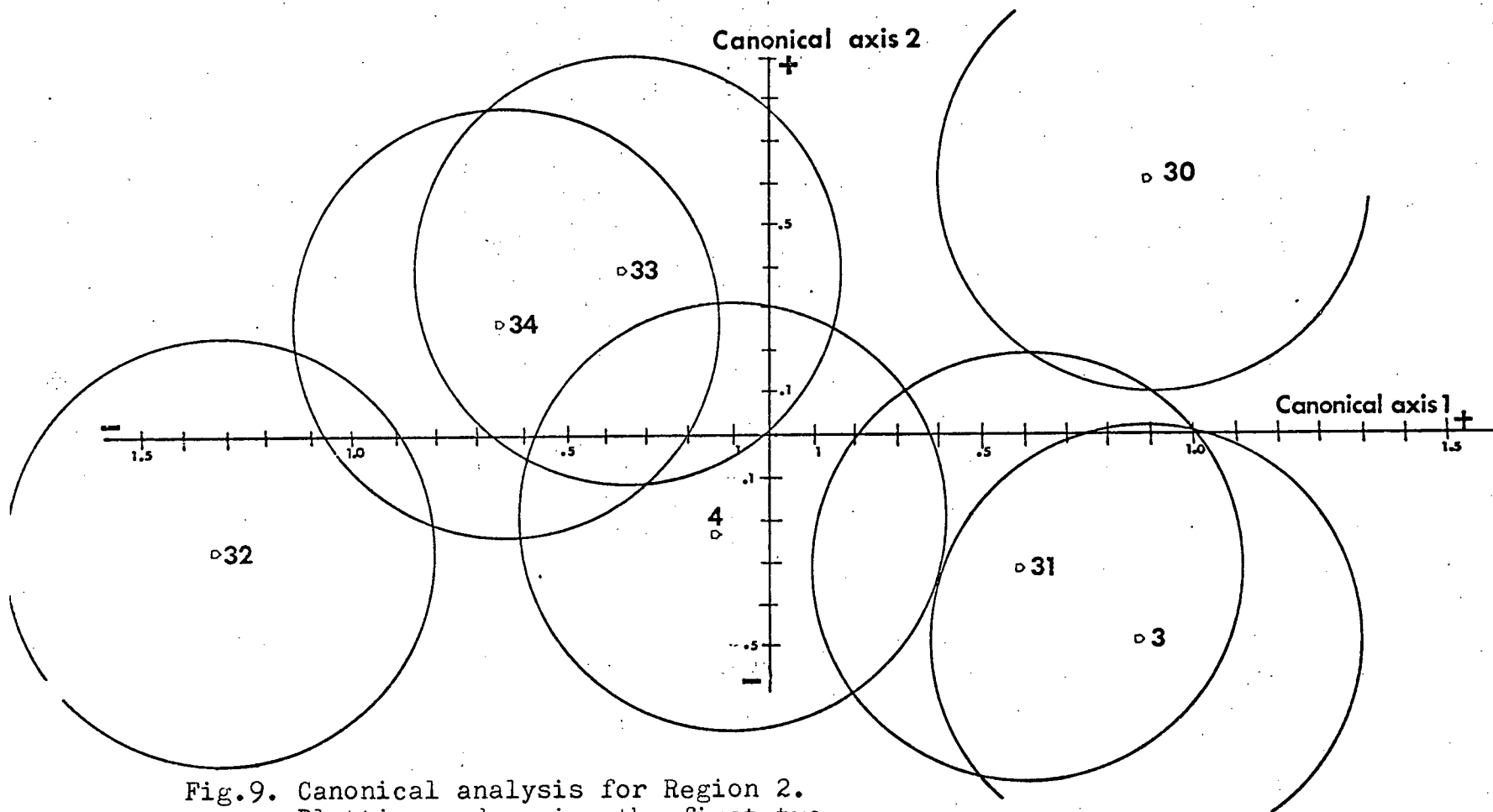


Fig.9. Canonical analysis for Region 2. Plotting made using the first two canonical axes. The numbers are the provenance numbers. 95 % confidence circles are shown.

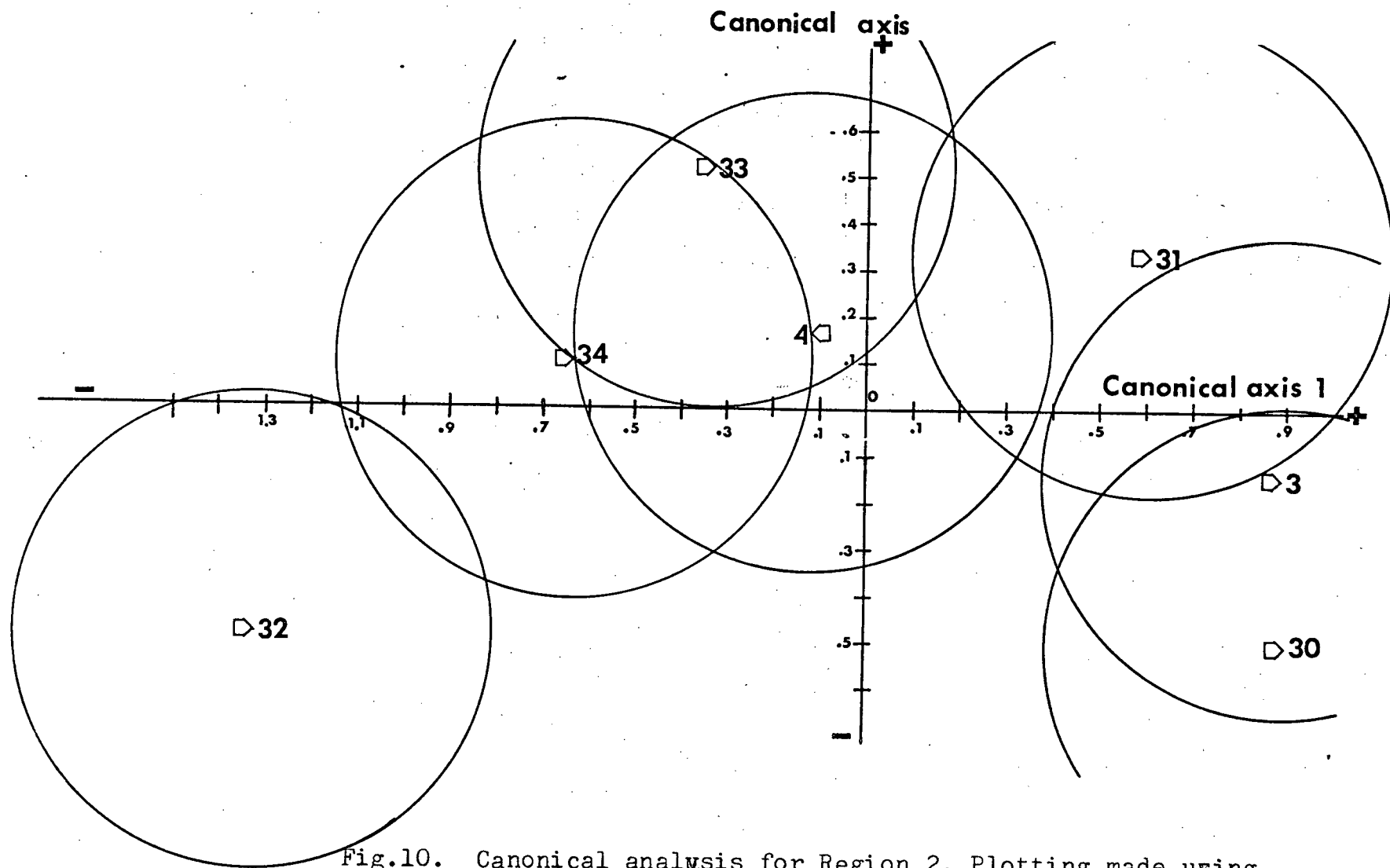


Fig.10. Canonical analysis for Region 2. Plotting made using the first canonical axis and the third axis.

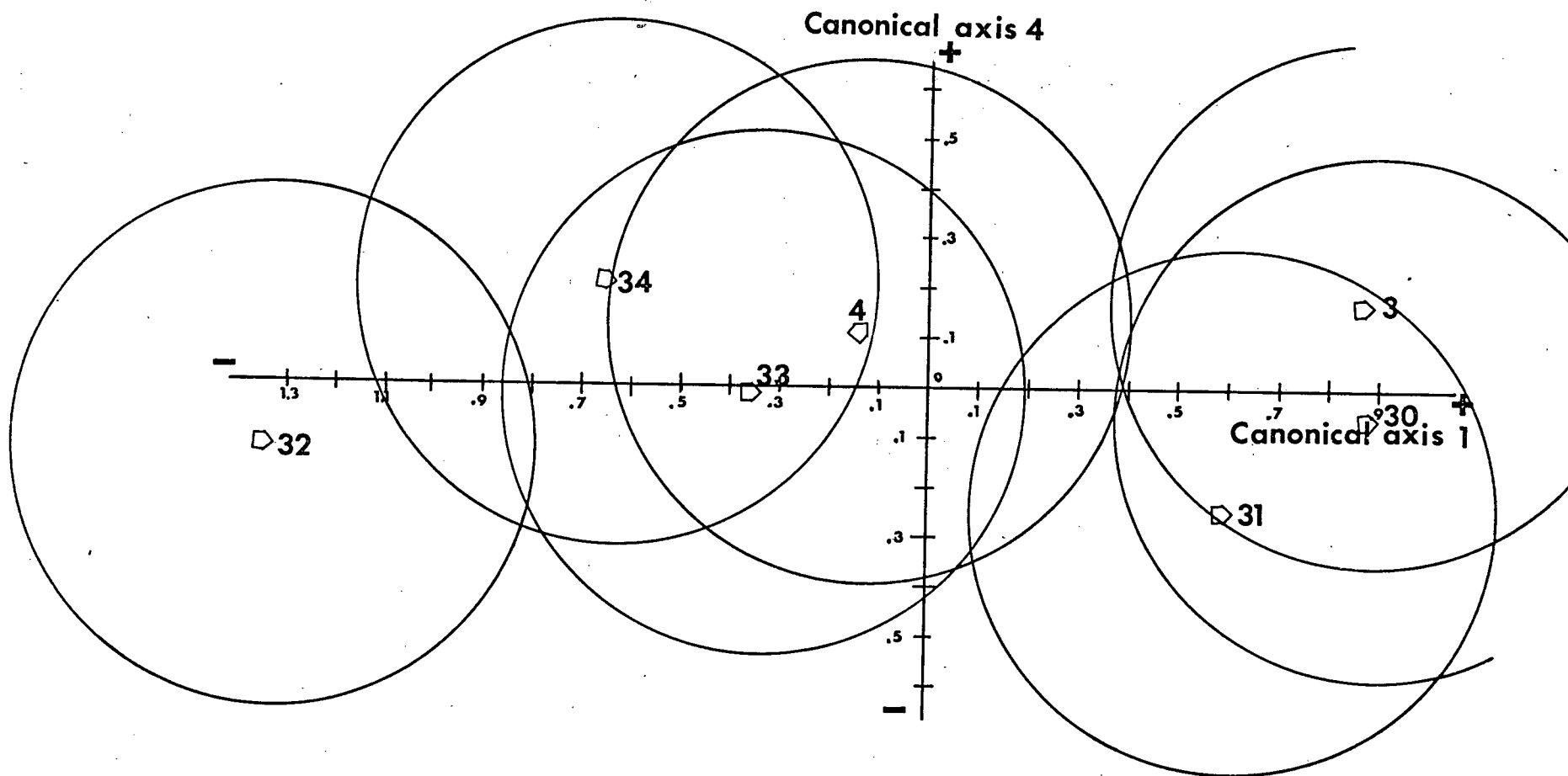


Fig.11. Canonical analysis for Region 2. Plotting made using the first and the fourth axes.

contribute significantly to the total variation. Sixty-six percent of the variation between groups is explained by the first canonical root.

Fig. 9 shows the ordination realized by the first axes and 95% "confidence circles"<sup>(\*)</sup>. When the circles do not overlap, the provenances are distinct. A look at Fig. 9 shows that the:

provenances 3 and 31 form one group;

provenances 33 and 34 form another group;

provenance 4 is intermediate between the two groups.

The provenances 32 and 30 are distinct from all the other provenances. However, if the first canonical axis is only considered, this axis ordinales the provenances of Vancouver Island along a latitudinal gradient contrasting the southern provenances (3, 30 and 31) and the northern provenances (4, 32, 33 and 34) without differentiating the west coast from the east coast. Fig. 10 and 11 show that the provenances overlap much along the other axes and the latter have not much meaning.

#### Queen Charlotte Islands Zone = Region 3

= provenances 36, 37, 38, 39, 40.

Number of groups: 5.

Number of p-variate observations: 75.

Test of equality of group dispersions:  $U = 1.218$  NS. The hypothesis of equality of group dispersions is not rejected.

Test of overall significance of the differences between group centroids:  $F(20, 219) = 6.08^{***}$ . There are differences between the group mean vectors.

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(\*) Strictly, the 95% confidence circles should vary in size from one provenance to another as the dispersions are not all identical.

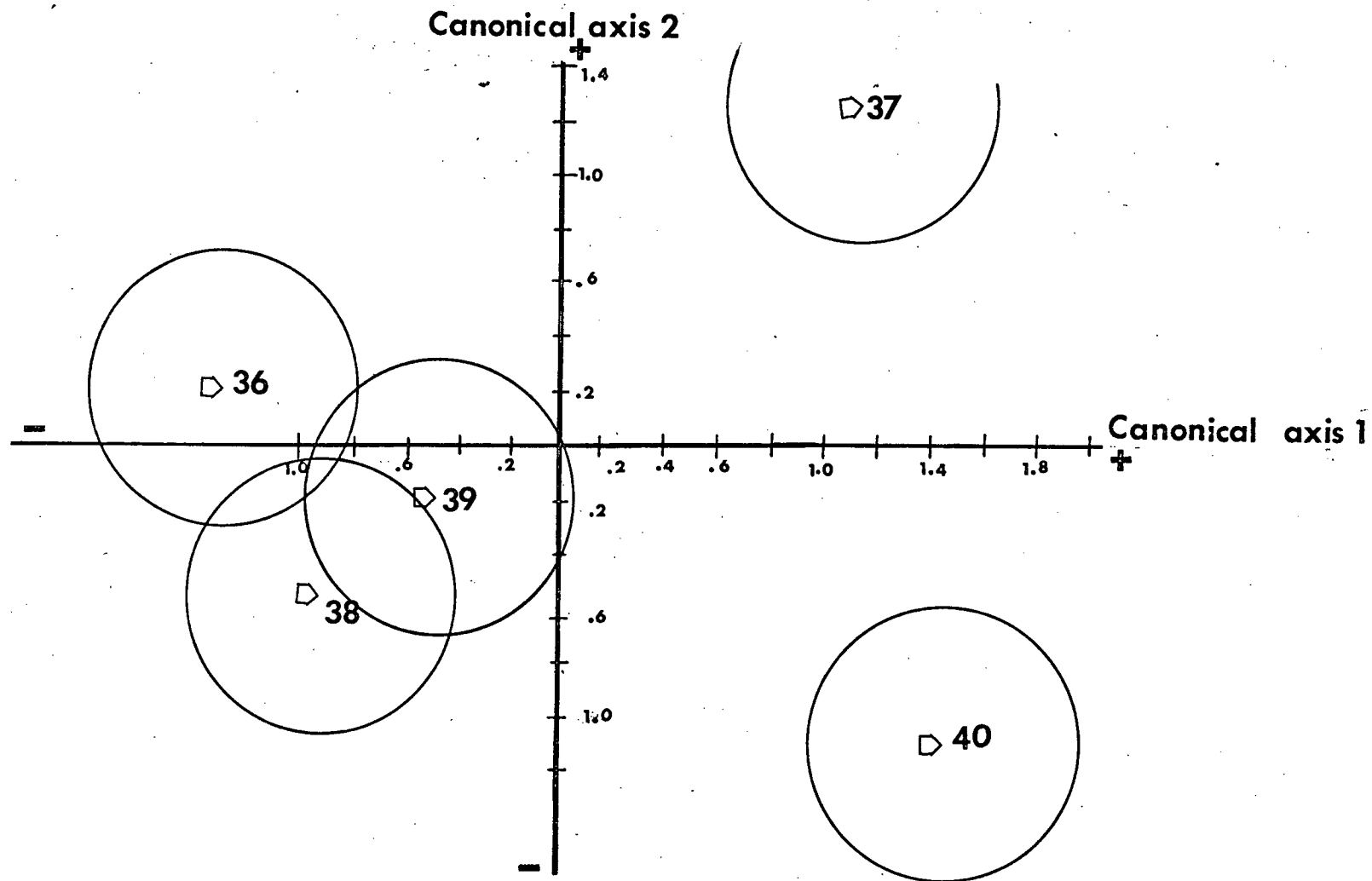


Fig.12. Canonical analysis for Region 3. Plotting made using the first two canonical axes.

BARTLETT's Test of the Latent Roots:

Roots Removed	Roots	Chi-Square	D.F.	Significance	Percent- age trace
0	1.317	100.60	20	***	62.71
1	0.681	42.72	12	***	32.40
2	0.069	6.90	6	NS	3.27
3	0.034	2.32	2	NS	1.62

Only the two greatest roots are significantly different from zero. Most of the variation in the new canonical space is confined to the canonical vectors associated with these roots as the plotting using the combination of the axes could show it.

A look at Fig. 12 shows that the provenances 36, 38 and 39 form one cluster. Provenance 37 is quite distinct from all the other provenances despite its geographical proximity from the provenances 36, 38 and 39. The provenance 40 is well isolated and different from all the provenances.

Alaska Zone = Region 4

= provenances 23, 24, 25, 26, 27, 28.

Number of groups: 6.

Number of p-variate observations: 90.

Test of equality of the group dispersions:  $U = 2.88^{**}$ . The null-hypothesis of equality of the dispersions is rejected.

Test of the overall significance of the differences between group centroids:  $F(25, 298): 5.56^{***}$ .



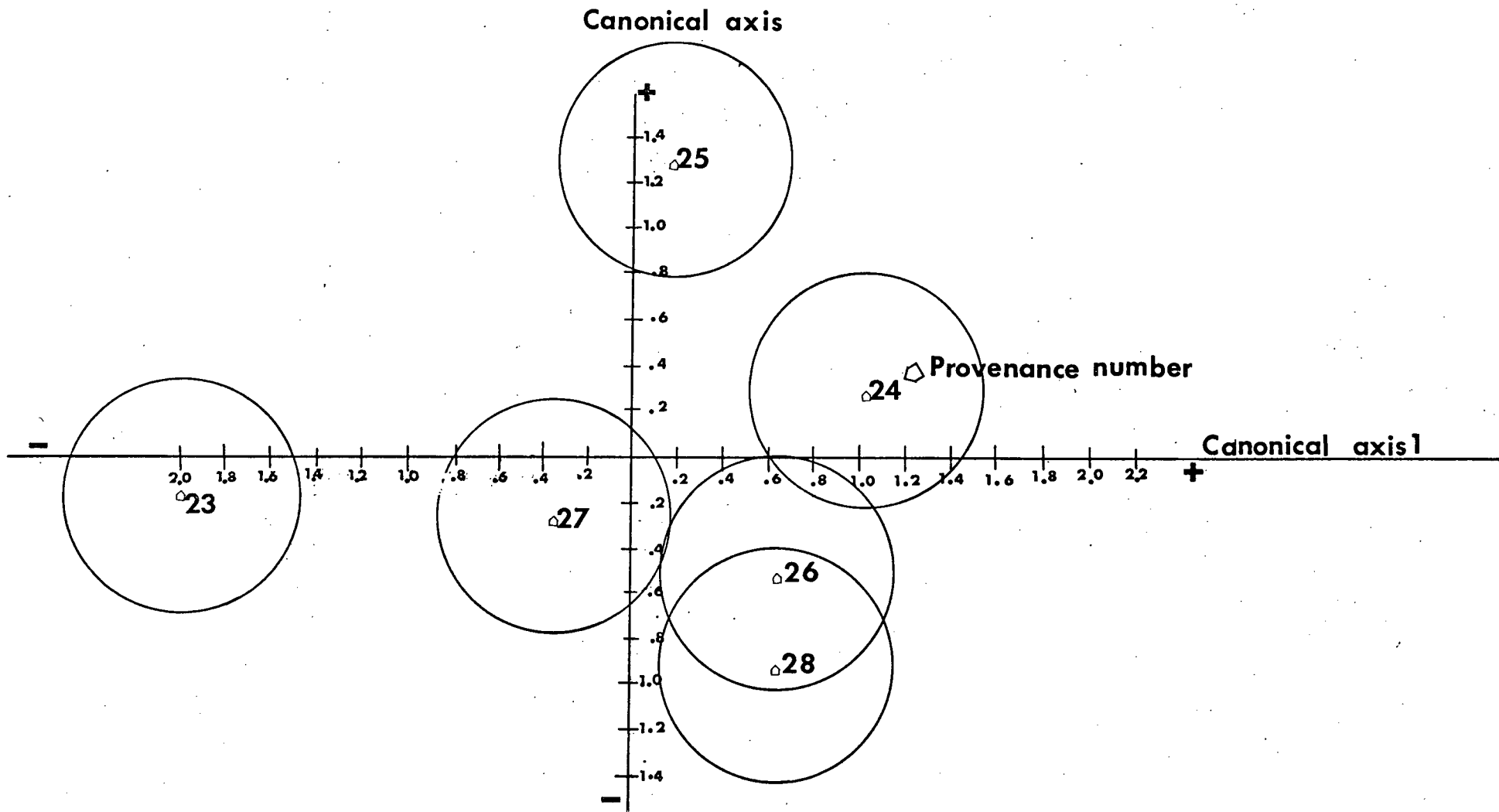


Fig.13. Canonical analysis for Region 4. Plotting made using the first two canonical axes.

BARTLETT's Test of the Latent Roots:

Removed	Roots	Chi-Square	D. F.	Significance	Percent- age trace
0	1.20	118.54	25	***	61.85
1	0.53	52.52	16	***	27.03
2	0.13	17.19	9	*	6.48
3	0.08	7.26	4	NS	4.35
4	0.01	0.41	1	NS	0.29

The first three greatest roots are significantly different from zero and their associated canonical vectors condense all the variation in the new space. Fig. 13 shows the ordination realized by the first axes. Provenances 23 and 25 are quite different from the provenances 24, 26, 27 and 28 which overlap more or less. Provenance 27 is contiguous to a group formed by the provenances 26 and 28.

Skeena River Zone = Region 5

= provenances 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 18, 19, 21, 22.

Number of groups: 14.

Number of p-variate observations: 210.

Test of equality of group dispersions:  $U = 3.86^{***}$ . The null

hypothesis of equality of dispersions is rejected.

Test of overall significance of the differences between the group

centroids:  $F(65, 911) = 8.21^{***}$ . There are differences

between the mean vectors of the groups.

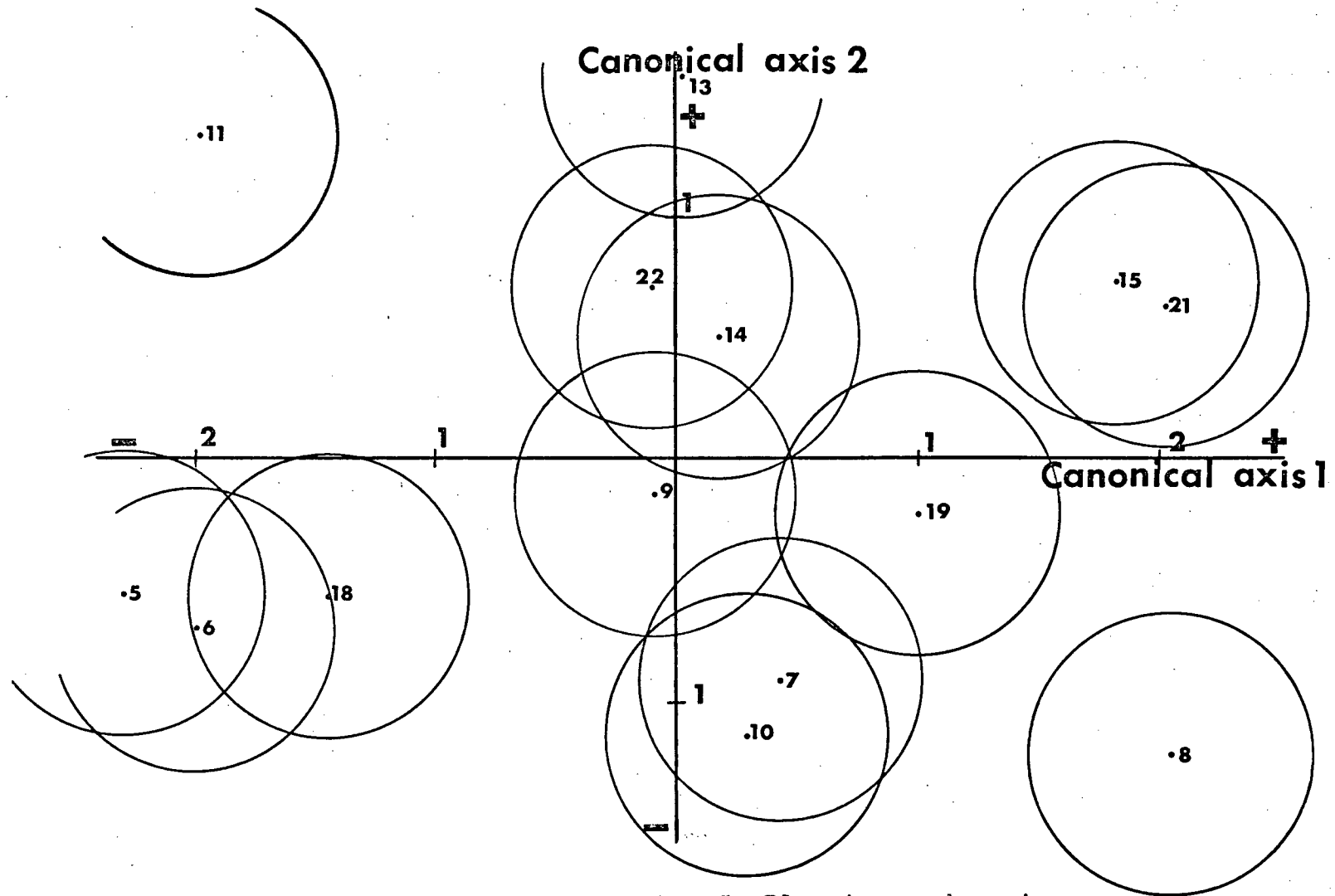


Fig.14. Canonical analysis for Region 5. Plotting made using the first two canonical axes.

BARTLETT's Test of the Latent Roots:

Roots Removed	Roots	Chi-Square	D.F.	Significance	Percent- age trace
0	2.15	434.51	65	***	62.39
1	0.82	205.46	48	***	23.81
2	0.27	85.83	33	***	7.75
3	0.18	38.56	20	**	5.33
4	0.02	4.90	9	NS	0.72

The first four roots are significantly different from zero. Their associated canonical axes condense the variability in the new space. Fig. 14 shows that the provenances, on the basis of the traits studied, form different clusters of geographical proximity origin. Provenances 7 and 10 form one group; 14 and 22 another; 15 and 21 another; 5, 6 and 18 still another group. The provenances 11, 13, 9, 19 and 8 are quite distinct, however some overlapping may occur. Provenances 11 and 9 are interior high valley provenances and belong to other river basins than the Skeena River basin.

### 3.5.2 Dendrogram analysis

The program developed by Dr. C. MILLIER in France performs a canonical analysis similar to that performed above, but also calculates, among many other things, the generalized distances of MAHALANOBIS between the groups and classifies the groups according to two types of dendrogram: one based on the "VAN DEN DRIESSE's classification", the other on the "ultrametric classification" developed by ROUX (1968). ROUX's thesis work was not available and the ultra-

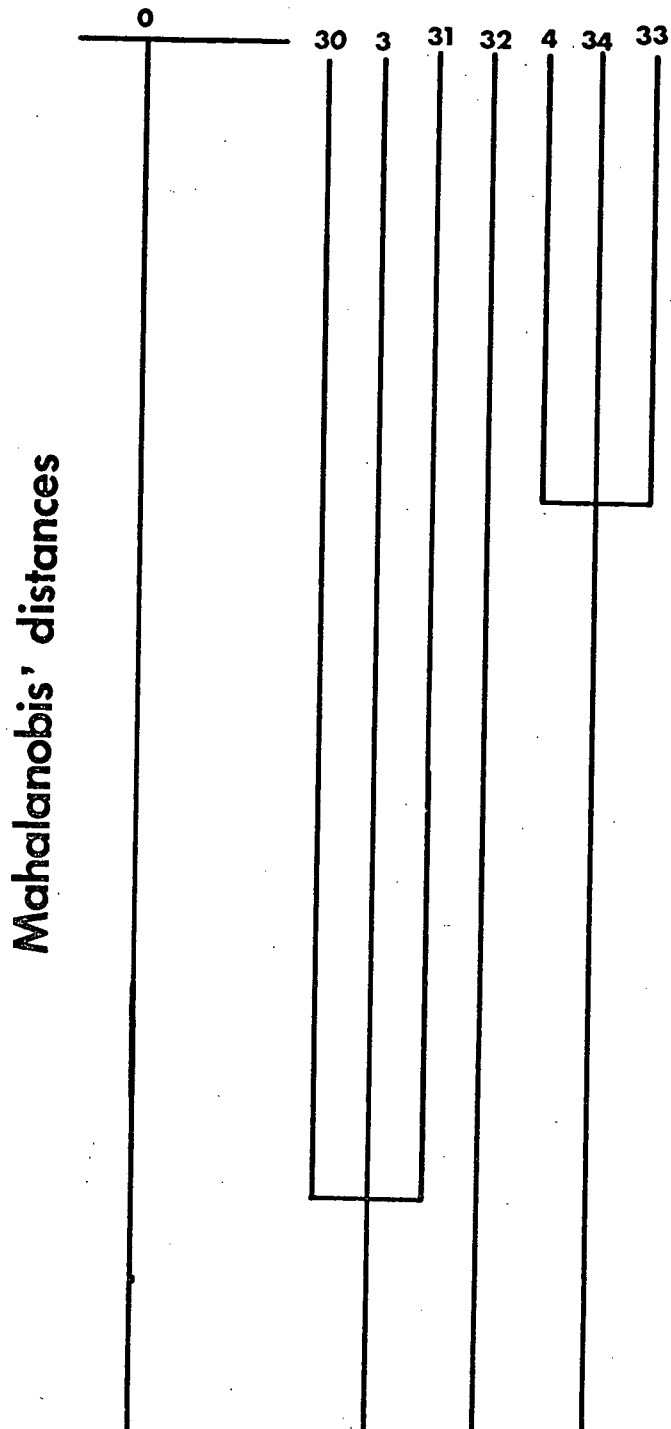
metric classification will not be considered. The dendrograms given by the two methods are quite different and the comparison of dendrograms is particularly complex (MILLIER and TOMASSONE, op. cit.) and beyond the scope of the present study.

Dendrograms are based on "phenetic resemblance" and should not imply descent. The abscissa of such a dendrogram has no special meaning and is indifferent; it serves only to separate the OTU's while the ordinate is in some similarity coefficient (SOKAL and SNEATH, 1963). The dendrograms presented here have an ordinate in a scale proportional to the MAHALANOBIS' distances between the populations of trees. The clustering procedure is based on common sense: the principle is that any two groups belonging to the same cluster should at least on the average, have a smaller  $D^2$  than those belonging to two different clusters (VAN DEN DRIESCHE, 1965). Points of junction between stems along the ordinate mean that the two connected populations form a cluster at the value shown on the ordinate. The dendrogram does not, however, represent the distances between the groups because it is unidimensional. The disadvantages of the dendrogram are that it has an imposed hierarchal structure and that consequently there is no provision for overlap between the entities classified (BLACKITH and REYMENT, 1971).

The distances of MAHALANOBIS between the provenances and for the four regions studied are shown in Tables XIII, XIV, XV and XVI.

The dendrogram of the following regions are represented

Fig.15. Dendrogram of the provenances of Region 2. The numbers are the provenance numbers.



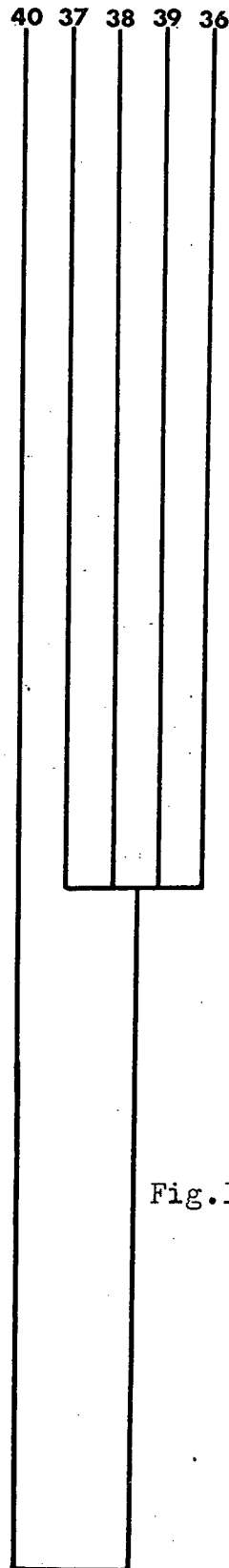


Fig.16. Dendrogram of the provenances of Region 3. The numbers are the provenance numbers.

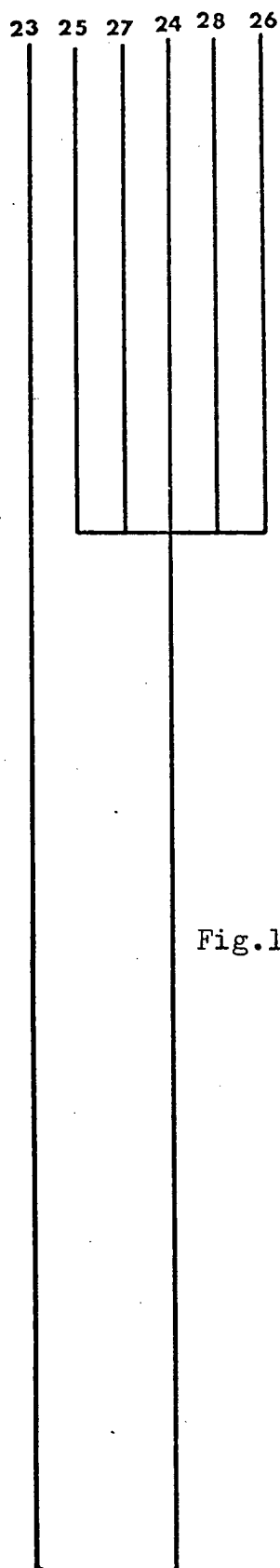
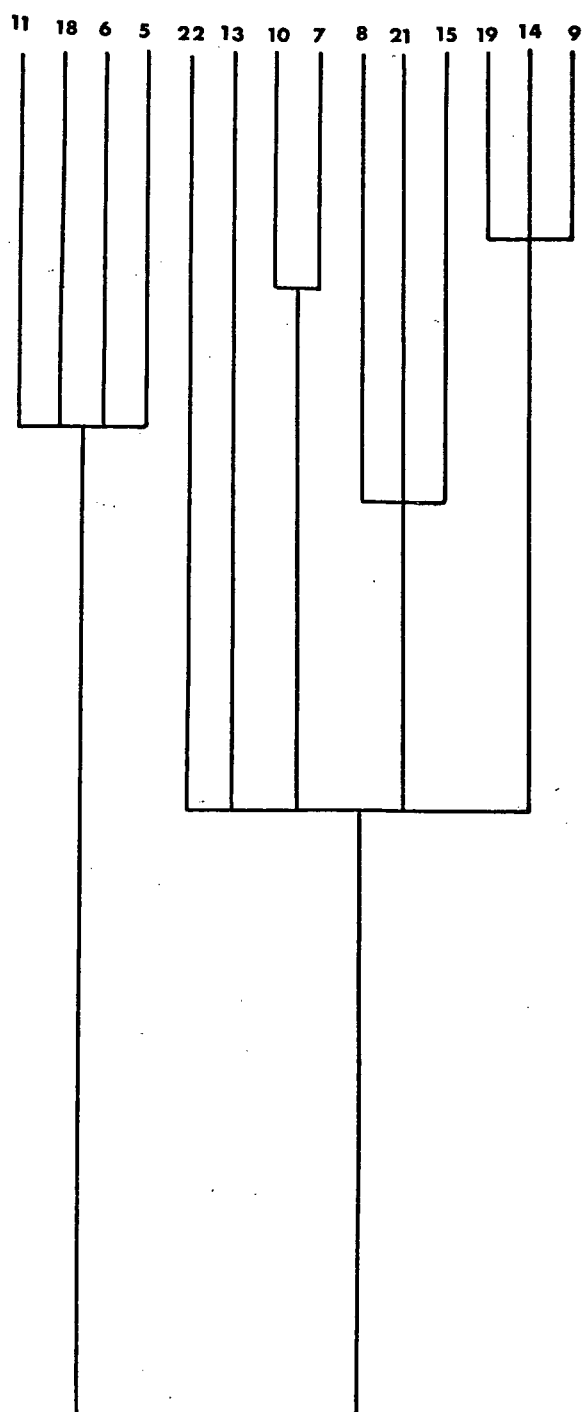


Fig.17. Dendrogram of the provenances of Region 4. The numbers are the provenance numbers.



Fig.18. Dendrogram of the provenances of Region 5. The numbers are the provenance numbers.



(Fig. 15, 16, 17 and 18). Only the provenances with 15 trees were classified.

Region 2: Vancouver Island; provenances 3, 4, 30,  
31, 32, 33 and 34.

Region 3: 36, 37, 38, 39 and 40.

Region 4: provenances 23, 24, 25, 26, 27, 28.

Region 5: provenances 5, 6, 7, 8, 9, 10, 11, 13,  
14, 15, 18, 19, 21, 22.

The dendrogram for Vancouver Island confirms that, on the basis of the traits studied, two groups of provenances appear: one group widely distinct formed by the provenances 30, 31 and 3 (southern part) and another group, with the provenance 32 well distinct from this group, formed by the provenances 32, 33, 34 and 4 (northern part of the Island).

In region 3, the provenances 36, 37, 38 and 39 cluster in one group, the provenance 40 being distinct.

In region 4, the provenances 24, 25, 26, 27 and 28 cluster in one group, the provenances 23 being distinct.

In region 5, the clusters are of geographic origin: the provenances geographically close tend to cluster. One group diverge: the upper valley provenances 5, 6, 11 and 18.

Generally, the dendrograms established according to the VAN DEN DRIESSCHE method tend to cluster the provenances according to their geographical origin. The clusters generally differ from the

TABLE XIII

MATRIX OF THE DISTANCES OF MAHALANOBIS  
BETWEEN THE PROVENANCES OF REGION 2

Provenances	3	4	30	31	32	33	34
3	0	123	140	51	512	279	295
4	123	0	220	73	195	56	49
30	140	220	0	168	574	264	293
31	51	73	168	0	439	147	213
32	512	195	574	439	0	243	123
33	279	56	264	147	243	0	33
34	295	49	293	213	123	33	0

TABLE XIV

MATRIX OF THE DISTANCES OF MAHALANOBIS  
BETWEEN THE PROVENANCES OF REGION 3

Provenances	36	37	38	39	40
36	0	714	108	101	923
37	714	0	760	412	572
38	108	760	0	131	618
39	101	412	131	0	557
40	923	572	618	557	0

TABLE XV

MATRIX OF THE DISTANCES OF MAHALANOBIS  
BETWEEN THE PROVENANCES OF REGION 4

Provenances	23	24	25	26	27	28
23	0	1068	799	835	409	866
24	1068	0	276	146	269	252
25	799	276	0	373	279	519
26	835	146	373	0	153	36
27	409	269	279	153	0	258
28	866	252	519	36	258	0

TABLE XVI

MATRIX OF THE DISTANCES OF MAHALANOBIS BETWEEN THE PROVENANCES OF REGION 5

Proven- ances	5	6	7	8	9	10	11	13	14	15	18	19	21	22
5	0	26	872	1927	599	741	391	1090	731	1881	110	1153	2088	799
6	26	0	684	1663	503	586	434	984	627	1673	105	991	1902	766
7	872	684	0	381	177	163	1201	646	269	508	571	159	828	697
8	1927	1663	381	0	657	325	2270	1276	646	389	1278	262	409	974
9	599	503	177	657	0	170	636	430	116	496	409	123	806	223
10	741	586	163	325	170	0	1139	897	307	614	437	168	738	430
11	391	434	1201	2270	636	1139	0	506	551	1498	483	1163	1770	505
13	1090	984	646	1276	430	897	506	0	174	442	840	529	770	499
14	731	627	269	646	116	307	551	174	0	280	420	158	458	202
15	1881	1673	508	389	496	614	1498	442	280	0	1295	189	146	570
18	110	105	571	1278	409	437	483	840	420	1295	0	749	1352	556
19	1153	991	159	262	123	168	1163	529	158	189	749	0	408	343
21	2088	1902	828	409	806	738	1770	770	458	146	1352	408	0	659
22	799	766	697	974	223	430	505	499	202	570	556	343	659	0

clusters constructed from the canonical analyses which spread the provenances according to several dimensions.

### 3.5.3 Stepwise discriminant analysis

The program used was the one available at the U.B.C. computing centre library under the name BMDO7M. It performs basically a discriminant function analysis as explained in Section 3.4, but in a stepwise manner.

At each step, one variable is entered into the set of discriminating variables. The variable is entered which gives the greatest decrease in the ratio of "within" to "total" generalized variances. A variable is deleted if the reverse occurs. When all the variables have been tested, a set of discriminant functions is obtained and used to calculate the MAHALANOBIS ( $D^2$ ) distance of an individual from one group to another group and the chi-square and its associated "a posteriori" probability for testing if a given individual of a given group could come from another group. These statistics are interesting to evaluate the discriminatory value of the variables used and the differences between the groups. The results already obtained by CA will not be repeated and only the new relevant results will be presented here for two sets of provenances: region 2 and region 5.

#### Region 2

= provenances 3, 4, 30, 31, 32, 33, 34.

Number of groups: 7.

Number of trees per groups: 15.

TABLE XVII

NUMBER OF TREES CLASSIFIED INTO THE DIFFERENT  
PROVENANCES ACCORDING TO THEIR PROVENANCE  
OF ORIGIN

Original Provenance	Classified Into the Provenances						
	3	4	30	31	32	33	34
3	5	4	4	2	0	0	0
4	1	3	1	3	3	3	1
30	3	2	7	0	0	1	2
31	7	1	2	2	2	0	1
32	0	1	0	0	10	1	3
33	1	2	3	1	1	3	4
34	0	1	1	0	4	4	4

Only three variables were successively entered: in decreasing order of discrimination; seed length, cone length, and finally seed width. Wing width and length did not significantly contribute to the discriminatory power of the calculated functions.

The F tests of the differences between each pair of groups showed that 5 pairs out of 21 were not significantly different from zero.

Provenance 3 was not different from provenance 31.

" 4 was not different from provenances 31, 33, 34.

" 33 was not different from provenance 34.

Generally, the "posteriori" probability of group appartenance was quite variable and generally low. The number of trees classified into the group to whom they belong ranges from 1 to 10, i. e., between 7% and 66% of the trees were classified back into their own population attesting the weak population discrimination of the five variables studied, or that the groups so overlap that they might be clustered as one large group (Table XVII).

#### Region 5

Number of groups: 14.

Number of trees per group: 15.

Number of p-variate observations: 210.

In this case, the five variables were successively entered in the discriminant function. In decreasing order of discrimination: cone length, seed width, seed length, wing length and finally, wing width. The F test of the differences between each pair of groups showed that six pairs out of 91 pairs were not significantly different from zero. Generally, again the "a posteriori" probability of group appartenance

was quite variable and generally low. The number of trees classified into their own group ranges from 1 to 9, i.e., from 7% to 60% attesting the weak discriminatory value of the 5 variables studied.

These results seem to confirm what we found in analyzing the components of variance for the traits studied: the best characters were supposed the ones with the smallest within provenance variability (see Section 2.1, Part II).

#### 3.5.4 Principal component analysis (PCA)

The U.B.C. Computing centre program UBC FACTO was tried on region 5. For a definition of Region 5, see Section 3.5.2.

The name of the program is misleading because it performs a PCA followed by a so-called VARIMAX rotation. PCA is a very peculiar case of FA and should be separated from the numerous techniques of FA (DAGNIELIE, 1960; CATTELL, 1964; SEAL, 1964 and MORRISON, 1967). The VARIMAX procedure used for rotating the principal component axes is explained by COOLEY and LOHNES (1966). According to CATTELL (1965), this method is inadequate because it imposes the orthogonality of the new axes and their rigid rotation.

The eigenvalues are 3.47, 1.01; 0.27, 0.20 and 0.04. Their corresponding cumulative proportions of the total variance are 69.5%, 89.8%, 95.2%, 99.1% and 100%. Thus, 90% of the total variation is explained by the first two eigenvectors.

A look at Table XVIII shows that the first component is positively and strongly correlated with the five measurements: it could



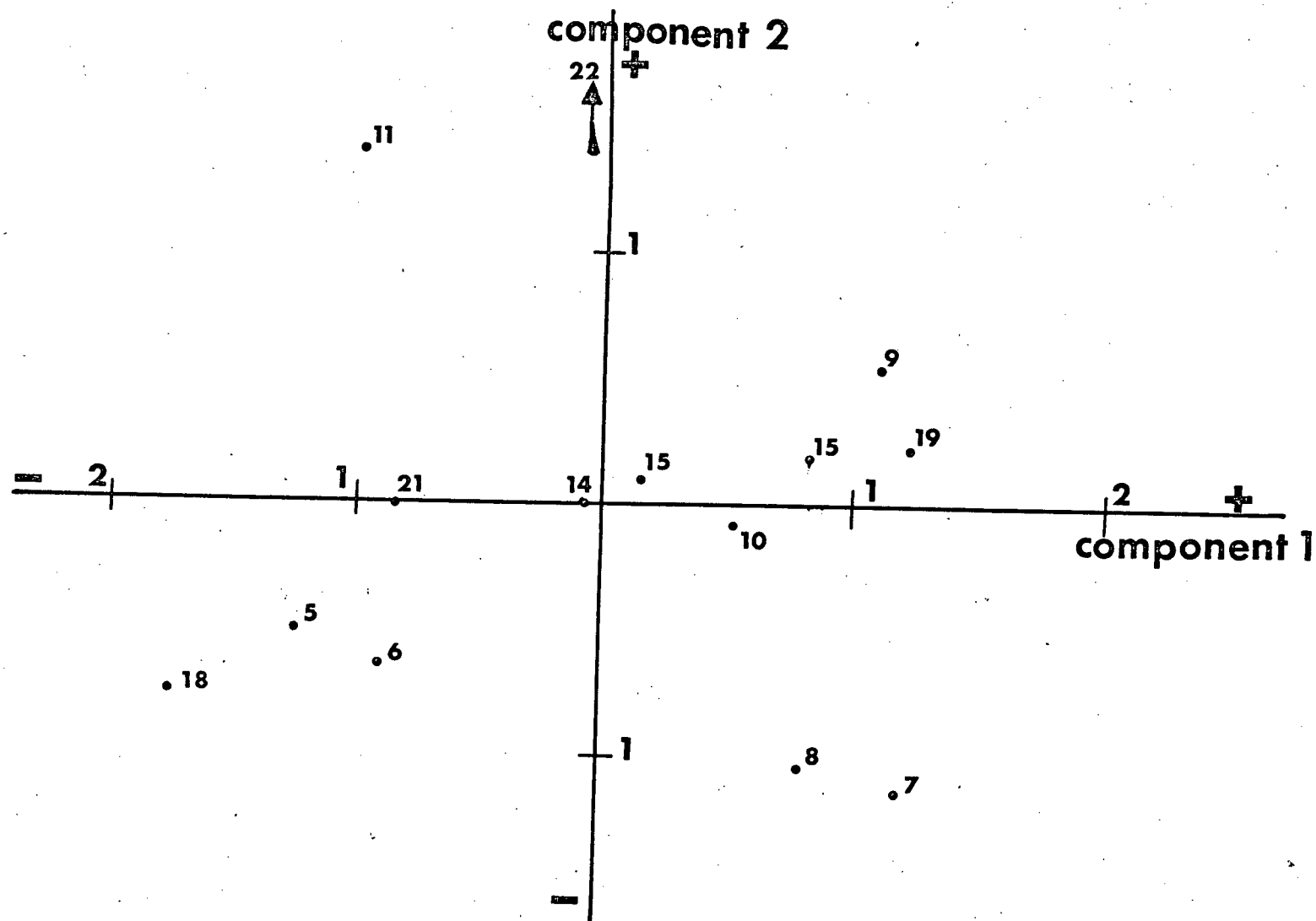


Fig.19. Principal component analysis of the provenances of Region 5. Plotting made using the first two principal components, The numbers are the provenance numbers.

TABLE XVIII  
LOADING OF THE COMPONENTS ON THE  
VARIABLES STUDIED

Variable	Components				
	1	2	3	4	5
1	0.63	-0.72	-0.20	-0.20	0.03
2	0.94	0.09	0.01	-0.03	0.13
3	0.91	-0.30	0.18	0.14	-0.14
4	0.76	0.58	-0.08	-0.27	-0.07
5	0.89	0.23	-0.31	0.25	0.04

be interpreted as a size component. The other components cannot be objectively interpreted.

The plotting of the populations in the new orthogonal space is shown in Fig. 19. The shuffling of the populations is quite different from the one performed by CA or DF. While a group of provenances (5, 6, 18) is maintained, the other provenances are widely mixed up. No geographic proximity can explain the scattering of the provenances performed by the PCA.

### 3.5.5 Conclusions

Before answering the question of what is the best multivariate statistical technique for classifying OTU's, of these we have tried, we must define what is best, what is classification, its aims, etc.

Classification is the basic method which man employs to

come to grips with and organize the external world (DAVIS and HEYWOOD, 1965), but any classification of living organisms must also have some biological meaning. It did happen that the methods used by the systematists in the past naturally resulted in coherent biological classifications where, if a class of plants shares many characters (morphological or physiological) in common, when some new trait is found in one member then the other members of the class also possess it.

Beyond the theoretical studies of classificatory schemes (See COLE, 1969 and JARDINE and SIBSON, 1971), there is only one ultimate criterion for judging a classification method: its relevance as an information storage and retrieval system. This criterion cannot be used in multivariate statistical analyses of some morphological characters which vary within a species. Even if a great number of traits could be included in the statistical analysis, which is rarely the case in practice, the adequacy of the method should be tested by using criteria outside the method itself. Mathematical artifacts are easily produced by blindly following the results of a computer generated analysis. LANGLET (1959) has demonstrated it by pointing out the inadequacies of analyses of variance of Scots pine (Pinus sylvestris L.) provenances as a "proof" of the existence of ecotypes.

It is, therefore, particularly important to understand the mechanics of a method as to its possible "distortion" effects, not to mention the consequences of disrespecting the hypotheses underlying a particular model.

PCA is an orthogonal transformation aiming at reducing the dimensionality of the original hyperspace into a few linear combinations of the original variates. PCA should only be applied to a sample whose origin can be safely attributed to one multivariate normal population.

CA is identical to DF. It results in a new non-orthogonal space. It can be used when two hierarchal levels of variation must be distinguished and when a visual representation in a few charts based on the most important canonical axes is contemplated.

CA and DF essentially lead to the same plotting into the new space. They both lead to the calculation of discriminant functions and the associated generalized distances  $D^2$ .

A simple plotting in the canonical space even with confidence circles, is not enough to classify the provenances into clusters as some criteria must be decided upon in order to define what is a cluster. The calculation of the generalized distances between all pairs of provenances result in a matrix of distances which can be used for constructing two or three dimension representations of the relative positions of these provenances. To cluster "OTU's" using the distances  $D^2$  between them, different methods have been proposed. One method has been tried: the method of VAN DEN DRIESCHE (1965) which resulted in dendrograms for which an interpretation has been possible. However, the clusters shown by the plottings of the provenances in the canonical space are generally different from the clusters displayed by the dendrograms.<sup>(\*)</sup>

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(\*) The canonical space shows the multi-dimensional aspects of the possible groupings with the amount of overlapping.

The discriminant functions are useful as a classification means when a new individual is measured.

If a stepwise discriminant analysis is used, DF could be the most efficient technique by:

- (1) reducing the original number (if possible very large) of variables to a set of discriminatory variables, thus eliminating any redundancy of information;
- (2) by calculating the discriminant functions and testing their classificatory power using the reduced set of variables;
- (3) by calculating the generalized distances  $D^2$  between all the OTUS, thus permitting the construction of three dimension or two dimension representations of the relative positions of these OTUS or permitting their clustering and their representation as a dendrogram. However, the dendrogram has the disadvantage of truncating the similarity relationships of the elements to be classified.

It must be noted that the plotting of the provenances in the different spaces generated by the techniques used (CA, DF and PCA) shows that when the axis used corresponded to a root which is not significantly different from zero, no meaningful variation could be tied up to it. This finding contradicts the contention of BLACKITH and REYMENT (1971), that the variation associated with the roots not significantly different from zero can be more important in clustering the OTUS than the significant roots. (See Fig. 10 and 11).

Another approach less efficient and above all more subjective than CA or DF, would be to use PCA on a few selected provenances, each provenance being analyzed separately. A great number of variables should be measured. Then the loadings of the variables on the principal components could be used as an indication of the importance of the variables in explaining the multidimension variability of these provenances. With the few variables selected, CA or DF could be used to ordinate the populations.

## PART III

### STUDY OF THE SEEDLING STAGE

## CHAPTER 1

## THE PHILOSOPHY OF THE NURSERY TEST

The nursery test design of tree genetic experiments is derived from the statistical theory of experimental design which has become classical since the books of FISHER (1947, 1954) have been published. For a complete exposition of the theory and application of experimental design, see the books of COCHRAN and COX, 1957 or KEMPTHORNE, 1952). The usual practice is to use some randomized complete block design. At the Michigan State University, the standard nursery experiment consists of four repetitions used for the measurements and of a fifth replication, from which most or all of the seedlings for field planting are derived (WRIGHT, 1970).

However, each forest geneticist has his own preferences: some use six replications, others use plots of linear shape (the so-called row or line plot), some of quadrat shape. The sizes vary from one author to the other. Recently, the problem of optimum size plot has been theoretically attacked (HUHN, 1970<sub>a</sub>, 1970<sub>b</sub>). Only WRIGHT (1963) has tried to give some indications as how to determine the best plot and sample sizes.

Little is known in practice about the possible effects of genotype by genotype interactions, as well as genotype by environment interactions on the differences expressed in the nursery. Only recently, the spacing effect has been studied in a progeny test of Douglas-fir



(CAMPBELL and WILSON, 1973). It seems that spacing-genotype interaction in Douglas-fir is not likely to appreciably affect the selection results. However, genotype by nursery interaction may be possible (VAN DEN DRIESCHE, 1973). The possible long term effects of these interactions are not known for sure.

Little is known about the possible maternal effects or pre-conditioning effect (ROWE, 1964). In provenance testing, this effect - if any - is susceptible to be repeated when collections are made from the same trees. The problem could be more serious as the results of a provenance test could be different from those obtained from a cross realized in a seed orchard composed of scions of the mother trees living in a new environment.

The genetic interpretation of a statistical model in terms of gene effects (additive, dominant, etc.) and the possible gains under different types of selection should be cautiously accepted.

The statistical model used to estimate and test the differences expressed in a nursery or field test has essentially statistical aims. A good experiment ensures:

(1) The absence of systematic errors. This is achieved by randomization. The estimate of a treatment contrast will only differ from its true value by random errors. The covariance between treatments and microenvironments is on the average, zero.

(2) The standard error of a treatment should be small enough to be able to draw reliable conclusions. The precision of an experiment

- will depend on: (a) the intrinsic variability of the experimental material and the accuracy of the experimental work;
- (b) the number of experimental units and the number of repetitions;
- (c) the design of the experiment.

(3) The range of validity of the test should be large enough: i.e., the conditions not too far from the practice.

(4) The design is simple.

(5) An adequate statistical analysis be possible, i.e., the observations respect the basic assumptions of the model used (normality, additivity, etc.), (after COX, 1958).

Therefore, it is not because the test used has been so analyzed as to eliminate the covariance between the genetic elements measured (provenances or progenies) and the environmental variation which could possibly occur in a nursery, that there is no physiological interaction between the genotypes and their micro-environment.

In the classical additive model, the phenotype of an individual is considered as the sum of two components:

$$P = G + E$$

P = phenotypic value

G = genotypic value

E = environmental deviation

Or, in terms of variances:

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + 2 \text{cov}(G, E).$$
 Generally, the design is such that  $\text{cov}(G, E) = 0$ . But even if  $\text{cov}(G, E) = 0$ , the environmental component of variance cannot be directly estimated unless the other component is eliminated. If vegetative propagation or highly inbred lines are used, the phenotypic variance provides an estimate of the environmental variance. In any case, the estimation of the environmental variance rests on the assumption that this variance is the same in all species genotypes. The environmental variance measured in one inbred line or in one vegetatively reproduced individual is that shown by that particular genotype (FALCONER, 1964). Therefore, the partition into genotypic and environmental variances depends on the genotypes studied and of the environments in which they grow. The broad sense heritability calculated for nursery or field test, thus, is but a measure of the precision of the test; it depends on the experimenter's choice of the design or care in seeding, seedbed preparation, etc. It is a repeatability of the test as defined, by FALCONER, (1964). It seems, therefore, misleading to speak of "genotypic heritability" (NANSON, 1970). The usefulness of the "genotypic" gains for different "selection intensities" by using such "genotypic heritability" is therefore dubious. (\*)

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(\*) That the genotypic heritability is a measure of repeatability of a progeny or provenance test and has no or little genetic significance comes from the fact that it is calculated from the means of the populations, the means of the progenies whatever the populations and the means of the progenies for one population (see the formulas given by NANSON, 1970, p. 117).

We can also question the necessity of uniform test conditions. REUTER (1971) has recently analyzed the results of a provenance test established in the forest conditions of a very mountainous region: coastal British Columbia. Provenance by block interactions were noted for total height. No correlation was found with height measured earlier. It does not seem, however, that the conclusions of the author are self-evident: more repetitions, more uniform test site, etc. In fact, it is quite possible that genotype by environment interaction undetected at the nursery stage (HADDOCK, et al. 1967) is so important in Douglas-fir and in coastal British Columbia conditions that, in practice, several coastal provenances could grow as well as any other, on a given site. This result is as important as finding the "best" provenance for a given station as it would relax the rules of seed transfer and allow freer plus tree selection.

In provenance or progeny tests, it is thus important, because so many factors are in interplay, to use as much as possible the nursery methods of the time, as well as the usual silvicultural methods in plain ordinary forest conditions because there is some evidence in the recent forest genetic literature, that different kinds of genotype by environment interactions, do exist (SILEN, 1966; MERGEN, et al. 1967; VAN DEN DRIESSCHE, 1973). (\*\*) However, many European foresters believe that

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(\*\*)

An interesting review of the possible genotype by environment interactions in forest trees has been done by SQUILLACE (1970). WRIGHT (1973), has also reviewed the provenance tests established in North Central United States, in this respect.

test site-genotype interactions are not important enough to preclude the choice of the best provenance for a large region. (See, among others, NANSON, 1970). The reasons for these differences of opinion between

the American forest geneticists and the European ones are not known for sure. The environment could be different as well as the tree species.

Norway spruce (Picea abies (L) KARST.) provenance performances in North East America are similar to the performances showed in European field tests, pointing to low or weak genotype by environment interactions in this tree species (BALDWIN, et al. 1973).

## CHAPTER 2

## MATERIAL AND METHODS USED IN THE NURSERY

2.1 Seedling characteristics studied in the nursery. Why those particular characters?

Much factual information on the infraspecific genetically based variation correlated with the place of origin of the genetic elements tested has been accumulated in the past decades for many forest tree species.

It is not possible to review here all the facts accumulated in the specialized literature. Since LANGLET (36/37) pioneered in this field, a great number of morphological and physiological characters have been studied: from dry matter, nitrogen content to height, bud set, length of the greatest internode, etc. for a large number of tree species. The matrices of correlations between the phenotypical traits studied and between these traits and the characteristics of the place of origin of the provenances (geographical coordinates, climatic variables such as vegetation period, mean annual temperature, etc.) can be explained by the selective action of the environment of origin of the provenances studied. Other factors of differentiation of the populations of trees: random drift, isolation (geographic or reproductive), introgression between species, etc. have been so far considered as of minor importance (GALOUX and FALKENHAGEN, 1965).

The problem of the clinal or ecotypic variation of adaptative traits in forest trees has been much discussed in the past. As LANGLET

(1959) showed it, the existence of significant correlations between traits and habitat of origin do not preclude the possibility of showing a discontinuous variation by using analyses of variances. The two techniques do not oppose themselves. This fact does not mean that how a forest tree is adapted to its environment is not important in terms of phenotypic flexibility (STERN, 1964) and its implications so far as the "genetic system" of the tree species is concerned. For a discussion of these important problems, see STERN (1964) or FALKENHAGEN (1968).

Very general explanations are only available to explain the correlations observed. STERN (1964) has extensively discussed the factors responsible for the variation observed in forest trees. The correlations of the traits with the habitats of origin could be explained by some clinal variation in gene frequencies controlling these traits which would parallel the variation in the environment.

The genetic correlations between traits could be attributed to pleiotropism and/or linkage. Moreover, as pointed out by GALOUX and FALKENHAGEN (1965) an organism grows and prospers only if adequate correlations between its organs, structures and functions are maintained. There is quite a redundancy of information in measuring a great number of traits due to the highly integrated aspect of all the functions of a living organism. Therefore, all the variation detected in a provenance test is not necessarily of adaptative nature. The relative importance and role of a trait is often a matter of hypothesis as only correlations with other traits are available most of the time in a study not accompanied with physiological investigations.

Recently, some authors have seriously misunderstood and misused the correlations calculated from provenance tests (FALKENHAGEN, 1972). The correlations and the regression coefficients which can be derived from them statistically relate the provenance or progeny character means to some characteristics of the place of origin of these genetic elements. They are pure statistics. The precise ecological law of variation of the regression or correlation coefficients for a given species, in relation with the test site has never been established. These statistics will vary from one test site to another according to the age and according to the genotype by environment interaction characteristic of the species. Inversion of the sign or disappearance of the regression coefficients are well known. See among others, KING (1965) and WAKELEY (1961).

The following table summarizes WAKELEY's fundamental findings (Table XIX). The variation presented by these coefficients is not regular or predictable. WAKELEY's useful planting sites (2 or 3) are not numerous enough to enable a precise interpretation. Furthermore, there seems to be an age effect. WAKELEY's ideas (1961, p. 23) are worth quoting:

"A clinal relationship of height growth to latitude proved common to loblolly at 5 years and to separate plantings of shortleaf at 5 and 3 years. When like sets of stocks were compared, curves of height over latitude of seed source had nearly significant to highly significant negative slopes in southern plantations and highly significant positive slopes in northern plantations. In plantations at intermediate latitudes the slopes were variable in direction, and less significant or non-significant. The



dominant characteristic of this clinal relationship is the reversal of the slope of the curve when planting of the same stocks is replicated at the opposite end of the species 'north and south' range."

TABLE XIX  
COEFFICIENTS FOR HEIGHT REGRESSED  
ON LATITUDE OF ORIGIN

Tree Species	Northern Planting Site	Southern Planting Site
Loblolly pine	0.93	- 0.48 NS
Shortleaf pine (1st test, 5 years old)	0.34 NS	- 0.97
Idem (2nd test, 3 years old)	0.94	-0.88 and 0.82

After WAKELEY (1961). Southern Conf. on Forest  
Tree Improv. Proceed. 6: 10-24.

KING's ideas (KING, 1965, p. 146) based on Scots pine studies are also worth quoting because the issue seems to be confusing to some:

"Thus, studies intended to determine the pattern of genetic variation within a species must be carried out in more than a single environment in order to accurately assess the variation pattern. Furthermore, correlations between seed source growth characters and seed source climate characters (mean temperature, length of growing season, latitude, etc.) must be carefully interpreted. It is quite conceivable that correlations between, for example, height growth and seed source latitude could be positive, negative or non significant depending upon the test environment."

It seems that, when one has only limited time and resources, some choice should be made so far as the traits to study are concerned in provenance testing.

We decided to measure only the traits which have proven to contain no redundant information and to have important adaptive values, on the basis of the available forest genetics literature. The following characters were thus estimated: germination rate, bud set, epicotyl length and survival at the end of the first growing season; bud burst, bud set and total height at the end of the second growing season and a striking character: colour of the needles, which is probably linked with frost and drought resistance.

## 2.2 Nursery treatments

A randomized complete block design with four replications was chosen. Each provenance was randomized within each block and within each provenance plot, the single tree progenies kept separate and identified by a number, were randomly placed in two lines (see Fig. 20 and photo 1). The seeds were placed in the cavities of styro-foam blocks and the styro-blocks so assembled as to group all the progenies pertaining to one provenance in one plot. Each progeny, for a given repetition, occupied 24 cavities, thus a theoretical number of 24 seedlings per replication or a total of 96 seedlings per progeny was aimed at.

We did not determine the germination capacity of the progenies because of the difficulties involved - and the length of time - in purifying and testing 557 seed lots of Sitka spruce. X-ray techniques available did not enable us to accurately separate empty from filled seed and it was decided to sow 2 or 3 seeds in each cavity.

No pretreatment was applied to the seeds.

The preparation of the containers has been described by



Photo 1. View of the container stage. Partial view of one row of containers. There were two rows along each side of a sprinkler line. Each row contained two repetitions. Photo by the author

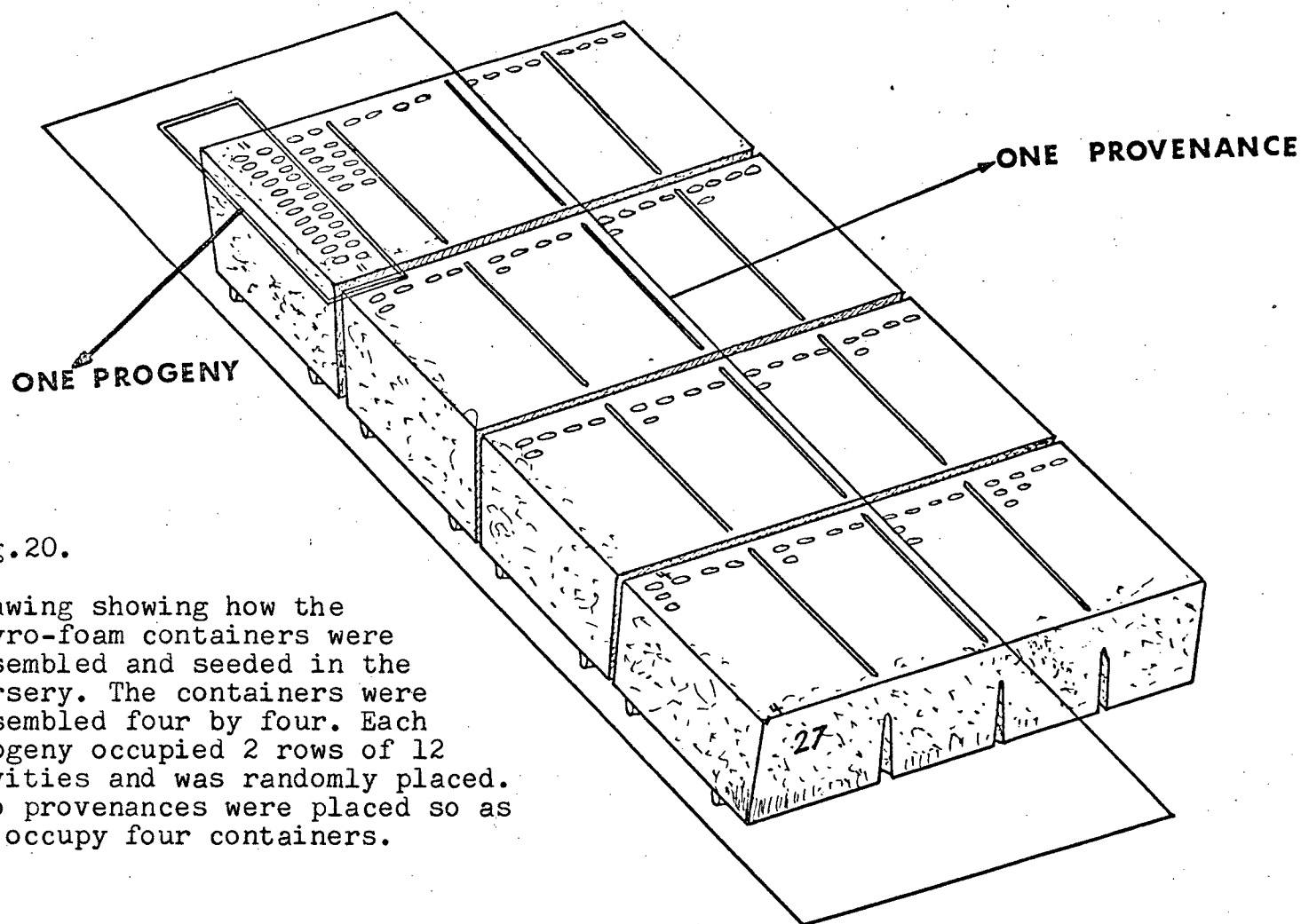


Fig.20.

Drawing showing how the styro-foam containers were assembled and seeded in the nursery. The containers were assembled four by four. Each progeny occupied 2 rows of 12 cavities and was randomly placed. Two provenances were placed so as to occupy four containers.

MATTHEWS (1971). The soil medium used in the containers consisted of a mix of three parts of commercial peat moss and one part of horticultural grade vermiculite. During mixing, dolomite was added to bring the pH of the mix to a value of about 5. The seed was covered with Granite grit.

Block A was seeded at the University of British Columbia. The containers of block A were labelled, seeded, covered with grit, wetted and immediately put in a cold room ( $t^{\circ} = 30 - 32^{\circ}\text{F}$ ). The lack of facilities resulted in lengthening the seeding process of block A and four days were necessary to seed this block which was completed on the 28th of April, 1971. The containers of block A were then moved to the B.C.-F.S. Surrey nursery and stored outside.

Block B was seeded using the Surrey nursery facilities and stored outside, on the 30th of April, 1971.

Block C was seeded on the 1st of May, 1971.

Block D was seeded on the 2nd of May, 1971.

The four blocks were then uniformly treated. They remained for several days outside until they were moved into the germination room where a humid and hot atmosphere ( $70^{\circ}\text{F}$ ) was maintained for about two weeks.

Then the containers were moved outside and were so assembled as to respect the statistical design (photo 1) with the four blocks in two long rows. The containers were placed on pallets to ensure adequate drainage and ventilation.



Photo 2. View of the transplantation of the (1 + 0) Sitka spruce seedlings. Five workers worked for five days to transplant the progenies. Photo by the author.

Early signs of germination were slow to occur and the counting of the germinants could only begin in June.

The containers were then treated according to MATTHEWS' (1971) provisions, so far as shade, irrigation and fertilization are concerned.

The containers with the seedlings overwintered outside. In April, 1972, the seedlings were thinned so as to leave only one healthy seedling per cavity.

In May, 1972, they were transplanted to a spacing of 6" to 6" in plain soil, in a nearby area, in the same nursery. The seedbeds were carefully prepared and the soil top-dressed with mixing of 100 lbs/acre of fertilizer (21 - 0 - 0). No soil analysis of the site has been done so far. The soil level is mostly horizontal with a slope in the western part (aspect: west). The soil is loamier in the upper part, but mostly loamy sand. Five workers worked for five days to transplant the 1-0 seedlings (photo 2) while the same statistical design was as much as possible respected. The single tree progenies were kept separate. Identification was done by using cedar poles (photo 3).

The seedlings grew there during the summer of 1972 without any fertilization to reduce their growth as much as possible because the seedlings were to be transplanted in the field.

In the spring of 1973, the seedlings were root-pruned to prepare them for the lifting which took place in October, 1973.

Weeding was done by hand as many times as necessary.





Photo 3. Each progeny was kept separate and identified by using cedar poles.  
Photo by the author.



## 2.3 Measurements techniques

### Germination rate

The number of cavities with at least one germinant was counted for each progeny and each replication using toothpicks of different colour to differentiate the dates of estimation, on the following dates: 3rd, 9th, 15th, 21st, 28th of June, 1971, the 5th, 12th of July, 1971. This is equivalent, for a given progeny, to a random sampling of 4 x 24 cells quoted 0 for absence of germinants and one for presence of at least one germinant. Consequently, the frequency distribution of the total number of cavities with at least one germinant should be distributed according to the Binomial distribution.

As block A had been partly submitted to some mild cold humid treatment, the germination rates observed in that block will be contrasted to those of the other blocks B, C and D.

### Bud set

Bud setting was determined for each provenance and each block separately at two dates in 1971 and at one date in 1972. The following procedure was used: bud set was observed at different dates during late August and in September until the provenances displayed a variety of stages of bud set. Then an empirical scale of six bud stages was determined by careful examination of the provenance shoot apices. The following classes were distinguished.

### Stage

0

No terminal bud visible. Apex obviously still growing.

Stage

- Generally a cluster of twisted, spiraling, pale, green needles.
- 1 Apex flat, not growing any more, greenish or whitish, covered by twisted needles.
  - 2 Small terminal bud greenish, covered by twisted and spiraling needles.
  - 3 Small bud, brownish, smaller than in stage 4, always more or less covered by the needles.
  - 4 Terminal bud well visible, but smaller than in stage 5, brownish, some resinous scales are visible. The needles tend to form a crown around the bud, but cover more of the bud than in stage 5.
  - 5 Terminal bud well visible, brown with some visible scales, the needles generally forming, more or less, a crown. The terminal bud will overwinter under this aspect.

For each replication and each provenance, at least 30 seedlings were randomly chosen and classed according to this scale in order to establish a frequency distribution of the six bud stages and to calculate a weighted mean for each provenance and each block, A, B, C, or D. No attempt was made to estimate the average bud stage on a progeny basis because of the length of time involved and the irregular and often small number (less than 24) seedlings representing each progeny. Only the apex of the main shoot was considered because lateral branches may be at a stage different from the one of the leading shoot.

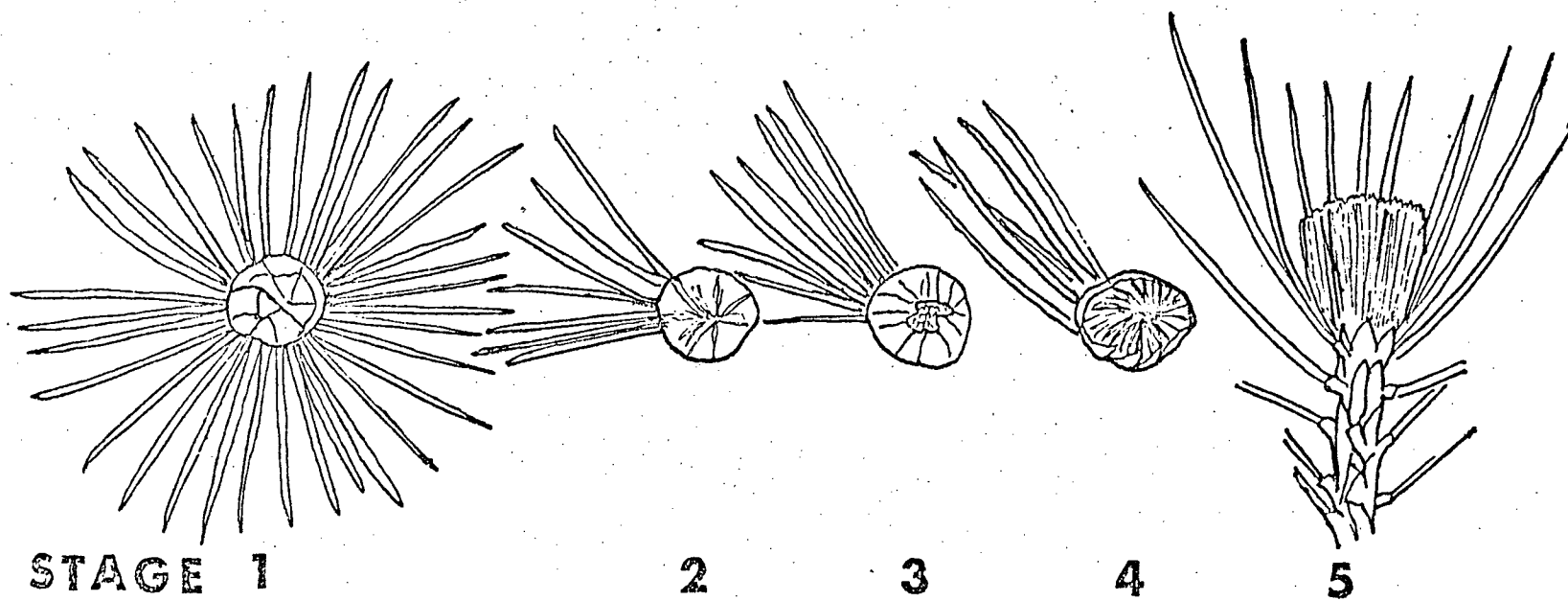


Fig. 21. Schematic representation of the bud burst stages as observed in April 1972.

Bud set was estimated on the 26th of September, 1971 and on the 2nd of October, 1971. All the blocks were measured in one day to avoid any effect due to a sudden weather change.

Bud setting was also estimated on the 8th of September, 1972 using the same procedure.

### Bud burst

Flushing rate was assessed using the same procedure as for bud set on the 3rd of April, 1972.

Five stages of bud bursting of the dominant (leader) bud were distinguished (Fig. 21):

#### Stages

- |   |  |
|---|--|
| 1 | Bud swollen, still brownish.   |
| 2 | Bud swollen, green pale spots appearing.   |
| 3 | Bud swollen, tips of leaves visible, at the center, the bud is more or less fractured. |
| 4 | The bud is open, the leaves are well visible, but some scales are still visible.       |
| 5 | The bud is wide open, the young needles are elongating.                                |

### Colour of the needles

Needle colour was estimated on the 13th of September, 1972. A similar procedure as for bud set was used for studying this characteristic. Three classes were distinguished: 0 = needles green pale (carrot-like); 1 = glaucous, intermediate; 2 = bluish.

### Length of the epicotyl

The length of the epicotyl of the three greatest seedlings, for each progeny and for each replication, was measured to the nearest mm during the winter 1971/72. We tried only to measure a constant upper percentage ( $\pm 20\%$ ) of the seedlings present in a progeny. Therefore, we eliminated the replication which were represented by only a few seedlings. This length is supposed to represent the total height growth after the first growing season, irrespective of the size of the embryo hypocotyl. About 3,000 measurements were made.

### Total height

In February, 1973, a random sample of 5 (1 - 1) seedlings was measured for each replication and each progeny so as to estimate the natural variation occurring in a progeny and a provenance. About 10,000 measurements of total height were made to the nearest 1/2 cm.

### Survival after the first growing season

The number of living seedlings was determined for each progeny and each replication in order to have some idea of the material present, in October, 1971. Little information can be gained from these figures and they will not be analyzed.

### Survival at the end of the second growing season

The seedlings (1 - 0) were transplanted with the soil attached to their roots, under optimum conditions and, there was no noticeable mortality at the end of 1972: all the transplants survived the winter 72/73 as well as the summer, 1973.

## 2.4 Methods used in the analyses

### 2.4.1 Multiple correlation and regression analyses

As it has already been pointed out in Part III, Chapter 2.1, the correlation and regression analyses do not preclude the use of divisive methods like the ANOVAS in provenance tests. Therefore, the ANOVAS and the multiple regression techniques will be systematically used.

A simple correlation matrix was calculated between the provenance means of the traits assessed and between the characters and latitude, longitude and altitude of the place of origin of the provenances, over all the provenances, ignoring regions. The geographical coordinates were chosen because the climatic data were not sufficient or warranted for each place of origin as it has been mentioned in Part I. The traits were plotted against these geographical coordinates in order to check visually the relationships between the provenance means and these variates. A multiple regression equation was calculated between each trait and all the variables or between the trait and a few selected variates. The backward step-wise programme described in Part II was used.

### 2.4.2 Analysis of variance models

For all the traits studied, on a provenance basis, i.e., all the traits except the two growth measurements, univariate analyses of variance were done, using a randomized complete block design model - completely random - (Model II) with four replications. The provenances not represented by four blocks were not included.

For the growth characteristics, two elaborate models were used to take into account the family (single tree progeny) level. As the number of families varied from one provenance to the other, the data were unbalanced for this level and special computing techniques for unbalanced models were used.

#### 2.4.2.1 Model for maximum epicotyl length

A nested model crossed with the blocks was used so as to get the different interactions corresponding to the different levels of variation. For such a complicated model, no formulas to obtain the components of variance was found for unbalanced data, in the specialized literature and the general formulas were developed. It is to be hoped that these original formulas will be useful to other geneticists.

The actual calculation of the unbalanced model was realized by combining two techniques: the linear hypothesis model technique suited to the analyses of unbalanced design and the usual ANOVA technique, because the programme available at the U.B.C. computing centre for applying the linear hypothesis model could not handle the large number of data involved in the analyses. The linear hypothesis model was used to estimate the sums of squares corresponding to the provenances, blocks and block by provenance interaction. To estimate the other sums of squares, the data were re-arranged and the usual ANOVA programme was used. The final sums of squares and degrees of freedom were got by appropriate subtractions.

The statistical model, completely random (or Model II), is as follows:

$$(1) \dots y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{ik} + (\alpha\beta)_{ij} + (\beta\gamma)_{ik/j} + \epsilon_{ijkl}$$

$\mu$  : general mean

$\alpha_i$  : provenance effect;  $\alpha_i$  is N.D.  $(0, \sigma_\alpha^2)$

$\beta_j$  : block effect;  $\beta_j$  is N.D.  $(0, \sigma_\beta^2)$

$\gamma_{ik}$  : progeny within provenance effect;  $\gamma_{ik}$  is N.D.  
 $(0, \sigma_\gamma^2)$

$(\alpha\beta)_{ij}$  : provenance by block interaction;  $\alpha\beta$  is N.D.  
 $(0, \sigma_{\alpha\beta}^2)$

$(\beta\gamma)_{ik/j}$  : progeny within provenance by block interaction;  
 $(\beta\gamma)_{ik/j}$  is N.D.  $(0, \sigma_{\beta\gamma}^2)$

$\epsilon_{ijkl}$  : error term;  $\epsilon_{ijk}$  is N.D.  $(0, \sigma^2)$

SEARLE (1971) has extensively reviewed the actual methods for estimating the variance components from unbalanced data. The estimation of variance components from balanced data rests almost entirely on one method, the analysis of variance method described as: for any model, calculate the ANOVA as if the model was a fixed effect model and then derive the expected values of the mean squares. The expected values will be linear functions of the variance components. Equating these expected mean squares to their calculated values leads to linear equations in the variance components, the solutions of which are taken as estimators of these components. HARTLEY (1967) has given a



general procedure applicable to any unbalanced model and yielding directly the numerical values of the coefficients in the formulas for expected mean squares.

In contrast, there are several methods available for use with unbalanced data, which reduce to the ANOVA method when the data are balanced. These methods have advantages and disadvantages and their properties are not well known, nor are there objective criteria to judge them. The ANOVA method is the simplest one and most used. However, this method, for mixed models, leads to biased estimators of variance components and cannot be used as such for these models (SEARLE, 1971). Ours is completely random and it was decided to use the ANOVA method. However, it should be noted that the analogous sums of squares used in this method for unbalanced data do not, under normality assumptions, have chi-square distributions nor are they distributed independently of one another. Despite this, variances of these estimators, under normality assumptions, can be derived (SEARLE, 1971, p. 433). It must also be noted that our design is totally balanced, but for one nested factor and hopefully the estimators should be only very slightly biased.

For the totally unbalanced case of a completely nested design, the mean squares are not independent because the observations themselves have a covariance structure (SCHEFFE, 1959).

The application of the analysis of variance method is thus based on the calculation of the expected values of the analogous sums of

squares corresponding to the unbalanced model. The mathematical model

(1) can be translated by the following equations:

$l$  goes from 1 to  $n_{ijk}$

then  $N_{ij.} = \sum_k n_{ijk}$  and  $y_{ij.} = \sum_k y_{ijk}$ , etc

$K_1, K_2, \dots, K_5$  are constants to be determined

$$\begin{aligned} \sum_i \sum_j \sum_k \sum_l (y_{ijkl} - \bar{y}_{i...})^2 &= K_1 \sum_i (\bar{y}_{i...} - \bar{y}_{....})^2 \\ &+ K_2 \sum_j (\bar{y}_{.j..} - \bar{y}_{....})^2 + K_3 \sum_i \sum_k (\bar{y}_{i.k.} - \bar{y}_{i...})^2 \\ &+ K_4 \sum_i \sum_j (\bar{y}_{ij..} - \bar{y}_{i...} - \bar{y}_{.j..} + \bar{y}_{....})^2 + K_5 \sum_i \sum_j \sum_k (\bar{y}_{ijk.} - \bar{y}_{ij..} - \bar{y}_{i.k.} + \bar{y}_{i...})^2 \\ &+ \sum_i \sum_j \sum_k \sum_l (y_{ijkl} - \bar{y}_{ijk.})^2 \end{aligned}$$

Sums of Squares (SS)

Analogous Sums of Squares

$\alpha$	$\sum_i \frac{1}{N_{i...}} y_{i...}^2 - \frac{1}{N_{....}} y_{....}^2$
$\beta$	$\sum_j \frac{1}{N_{.j..}} y_{.j..}^2 - \frac{1}{N_{....}} y_{....}^2$
$\gamma$	$\sum_i \sum_k \frac{1}{N_{i.k.}} y_{i.k.}^2 - \frac{\sum_i y_{i...}^2}{N_{i...}}$

$\alpha\beta$	$\sum_i \sum_j \frac{y_{ij..}^2}{N_{ij..}} - \frac{1}{N...} Y_{....}^2 - SS_\alpha - SS_\beta$
$\gamma\beta$	$\sum_i \sum_j \sum_k \frac{y_{ijk.}^2}{n_{ijk.}} - \sum_i \frac{1}{N_{i..}} Y_{i...}^2$ $- SS_{\alpha\beta} - SS_\gamma - SS_\beta$
$\epsilon$	$\sum_i \sum_j \sum_k \sum_l \frac{y_{ijkl}^2}{n_{ijkl}} - \frac{1}{n_{ijkl}} \sum_i \sum_j \sum_k Y_{ijk.}^2$

TABLE OF DEGREES OF FREEDOM

<u>Sums of Squares</u>	<u>Degrees of Freedom</u>
$\alpha$	$\sum_i (1) - 1$
$\beta$	$\sum_j (1) - 1$
$\gamma$	$\sum_i \sum_k (1) - \sum_i (1)$
$\alpha\beta$	$\sum_i \sum_j (1) - \sum_i (1) - \sum_j (1) + 1$
$\gamma\beta$	$\sum_i \sum_j \sum_k (1) - \sum_i \sum_k (1) - \sum_i \sum_j (1)$ $+ \sum_i (1)$
$\epsilon$	$N... - \sum_i \sum_j \sum_k (1)$
Total	$N... - 1$

$\sum_i (1)$  means sum of unity over  $i$ , etc.

TABLE XX

EXPECTATIONS OF SUMS OF SQUARES FOR THE TOTALLY  
UNBALANCED CROSSED MODEL

Sums of squares

Expectations

 $\alpha$ 

$$\begin{aligned}
 & \left[ N_{...} - \frac{1}{N_{...}} \sum_i N_{i..}^2 \right] \sigma_\alpha^2 + \left[ \sum_i \frac{1}{N_{i..}} \sum_j N_{ij.}^2 - \frac{1}{N_{...}} \sum_j N_{.j.}^2 \right] \sigma_\beta^2 \\
 & + \left[ \sum_i \frac{1}{N_{i..}} \sum_k N_{i.k}^2 - \frac{1}{N_{...}} \sum_i \sum_k N_{i.k}^2 \right] \sigma_\gamma^2 + \left[ \sum_i \frac{1}{N_{i..}} \sum_j N_{ij.}^2 \right. \\
 & \left. - \frac{1}{N_{...}} \sum_i \sum_j N_{ij.}^2 \right] \sigma_{\alpha\beta}^2 + \left[ \sum_i \frac{1}{N_{i..}} \sum_j \sum_k n_{ijk}^2 - \sum_i \sum_j \sum_k \frac{1}{N_{i..}} n_{ijk}^2 \right] \times \\
 & \sigma_{\gamma\beta}^2 + \left[ \sum_i (1) - 1 \right] \sigma_e^2
 \end{aligned}$$

 $\beta$ 

$$\begin{aligned}
 & \left[ \sum_j \frac{1}{N_{.j.}} \sum_i N_{ij.}^2 - \frac{1}{N_{...}} \sum_i N_{i..}^2 \right] \sigma_\alpha^2 + \left[ N_{...} - \frac{1}{N_{...}} \sum_j N_{.j.}^2 \right] \sigma_\beta^2 \\
 & + \left[ \sum_j \frac{1}{N_{.j.}} \sum_i \sum_k n_{ijk}^2 - \frac{1}{N_{...}} \sum_i \sum_k N_{i.k}^2 \right] \sigma_\gamma^2 +
 \end{aligned}$$

TABLE XX - Continued

Sums of squares

Expectations

$$\left[ \sum_j \frac{1}{N_{j.}} \sum_i N_{ij.}^2 - \frac{1}{N_{...}} \sum_i \sum_j N_{ij.}^2 \right] \sigma_{\alpha\beta}^2 + \left[ \sum_j \frac{1}{N_{j.}} \sum_i \sum_k n_{ijk}^2 \right. \\ \left. - \sum_i \sum_j \sum_k \frac{1}{N_{...}} n_{ijk}^2 \right] \sigma_{\beta\gamma}^2 + [\sum_j (1) - 1] \sigma_e^2$$

$$\gamma \quad \left[ \sum_i \sum_k \frac{1}{N_{i.k}} \sum_j n_{ijk}^2 - \sum_i \frac{1}{N_{i..}} \sum_j N_{ij.}^2 \right] \sigma_{\beta}^2 + \left[ N_{...} - \sum_i \frac{1}{N_{i..}} \right. \\ \left. \sum_k N_{i.k}^2 \right] \sigma_{\gamma}^2 + \left[ \sum_i \sum_k \frac{1}{N_{i.k}} \sum_j n_{ijk}^2 - \sum_i \frac{1}{N_{i..}} \sum_j N_{ij.}^2 \right] \sigma_{\alpha\beta}^2 \\ + \left[ \sum_i \sum_k \frac{1}{N_{i.k}} \sum_j n_{ijk}^2 - \sum_i \frac{1}{N_{i..}} \sum_j \sum_k n_{ijk}^2 \right] \sigma_{\beta\gamma}^2 \\ + [\sum_i \sum_k (1) - \sum_i (1)] \sigma_e^2$$

$$\alpha\beta \quad \left[ \frac{1}{N_{...}} \sum_i N_{i.}^2 - \sum_j \frac{1}{N_{j.}} \sum_i N_{ij.}^2 \right] \sigma_{\alpha}^2 + \left[ \frac{1}{N_{...}} \sum_j N_{j.}^2 - \right.$$

TABLE XX - Continued

Sums of squares

Expectations

$$\begin{aligned}
& - \sum_i \frac{1}{N_{i..}} \sum_j \sum_k N_{ij.}^2 \Big] \sigma_B^2 + \left[ \sum_i \sum_j \sum_k \frac{n_{ijk}^2}{N_{ij.}} - \sum_i \frac{1}{N_{i..}} \sum_k N_{i.k}^2 \right. \\
& + \frac{1}{N_{...}} \sum_i \sum_k N_{i.k}^2 - \sum_j \frac{1}{N_{.j.}} \sum_i \sum_k n_{ijk}^2 \Big] \sigma_j^2 + \left[ N_{...} - \right. \\
& \left. \sum_i \frac{1}{N_{i..}} \sum_j \sum_k N_{ij.}^2 + \frac{1}{N_{...}} \sum_i \sum_j \sum_k N_{ij.}^2 - \sum_j \frac{1}{N_{.j.}} \sum_i \sum_k N_{ij.}^2 \right] \sigma_{AB}^2 \\
& + \left[ \sum_i \sum_j \sum_k \frac{n_{ijk}^2}{N_{ij.}} - \sum_i \frac{1}{N_{i..}} \sum_j \sum_k n_{ijk}^2 - \sum_j \frac{1}{N_{.j.}} \sum_i \sum_k n_{ijk}^2 \right. \\
& \left. + \frac{1}{N_{...}} \sum_i \sum_j \sum_k n_{ijk}^2 \right] \sigma_{jB}^2 + \left[ \sum_i \sum_j (1) \right. \\
& \left. - \sum_i (1) - \sum_j (1) + 1 \right] \sigma_e^2
\end{aligned}$$

YB

$$\left[ \sum_i \frac{1}{N_{i..}} \sum_j \sum_k N_{ij.}^2 - \sum_i \sum_k \frac{1}{N_{i.k}} \sum_j n_{ijk}^2 \right] \sigma_B^2 +$$

TABLE XX - Continued

Sums of squares	Expectations
	$ \begin{aligned} & + \left[ \sum_i \frac{1}{N_{i..}} \sum_k N_{i.k}^2 - \sum_i \sum_j \sum_k \frac{n_{ijk}^2}{N_{ij.}} \right] \sigma_\gamma^2 + \left[ \sum_i \frac{1}{N_{i..}} \sum_j N_{ij.}^2 \right. \\ & \quad \left. - \sum_i \sum_k \frac{1}{N_{i.k}} n_{ijk}^2 \right] \sigma_{\alpha\beta}^2 + \left[ N_{...} - \sum_i \sum_j \sum_k \frac{n_{ijk}^2}{N_{ij.}} \right. \\ & \quad \left. - \sum_i \sum_k \frac{1}{N_{i.k}} \sum_j n_{ijk}^2 + \sum_i \frac{1}{N_{i..}} \sum_j \sum_k n_{ijk}^2 \right] \sigma_{\gamma\beta}^2 \\ & \quad + \left[ \sum_i \sum_j \sum_k (1) - \sum_i \sum_j (1) - \sum_i \sum_k (1) + \sum_i (1) \right] \sigma_e^2 \end{aligned} $
$\varepsilon$	$[N_{...} - \sum_i \sum_j \sum_k (1)] \sigma_e^2$

TABLE XXI

EXPECTATIONS OF MEAN SQUARES FOR THE  
PARTIALLY UNBALANCED MODEL

Sums of squares	Expectations
$\alpha$	$\frac{\left[ N_{...} - \frac{1}{N_{...}} \sum_i N_{i..}^2 \right] \sigma_\alpha^2}{\left[ \sum_i (1) - 1 \right]}$ $+ \frac{\left[ N_{.j.} - \frac{1}{N_{.j.}} \sum_i N_{ij.}^2 \right] \sigma_{\alpha\beta}^2}{\left[ \sum_i (1) - 1 \right]}$ $+ 12 \sigma_\gamma^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2$
$\beta$	$\left[ N_{.j.} \right] \sigma_\beta^2 + \left[ \frac{1}{N_{.j.}} \sum_i N_{ij.}^2 \right] \sigma_{\beta\gamma}^2$ $+ 3 \sigma_{\gamma\beta}^2 + \sigma_e^2$
$\gamma$	$12 \sigma_\gamma^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2$
$\alpha\beta$	$\frac{\left[ 3 N_{.j.} - 3 \frac{1}{N_{.j.}} \sum_i N_{ij.}^2 \right] \sigma_{\alpha\beta}^2}{3 \left[ \sum_i (1) - 1 \right]}$ $+ 3 \sigma_{\gamma\beta}^2 + \sigma_e^2$
$\gamma\beta$	$3 \sigma_{\gamma\beta}^2 + \sigma_e^2$
$\epsilon$	$\sigma_e^2$



The expectations of the sums of squares are given in Table XX.

By dividing these expectations by the corresponding degrees of freedom and by taking into account the fact that the data were chosen so that  $n_{ijk} = 3$  and  $j = 1, \dots, 4$ , the expectations of the mean squares for the partially unbalanced model used to analyze the epicotyl length measurements are obtained (Table XXI). The equations of the Table XXI have been double-checked by using HARTLEY's method "by synthesis" (HARTLEY, 1967), on a sample of 252 data.

The tests of the different factors are self evident if the expectations of the mean squares are considered. The tests of the different factors are exact, in our partially balanced case, providing there is no interactions between the provenance and block effects. If the latter interaction contributes significantly to the different mean squares, it is necessary to use complicated linear combinations of the mean squares in order to isolate the main effects from their interaction and equalize the coefficients of the components. The tests are then only approximate, (SCHEFFE, 1959). Anyhow, the F tests lose their interest because of the presence of interaction.

The errors of the variance components have been estimated according to ANDERSON and BANCROFT (1952, p. 321). However, the formula used is strictly valid for balanced data. The error of the variance component is given as:

$$\sigma^2 = \frac{2}{c^2} \frac{\sum_i V_i^2}{f_i + 2}$$

- $c$  : coefficient of the variance component  
 $V_i$  : mean square used to get the component  
 $f_i$  : degrees of freedom corresponding to  $V_i$

Total height after the second growing season was analyzed by using a completely nested model (Model II) with the blocks considered as simple replications:

$$y_{ijkm} = \mu + \alpha_i + \beta_{ij} + \gamma_{ijk} + \epsilon_{ijkm}$$

$$i = 1, 2, \dots, a$$

$$j = 1, 2, \dots, b_i$$

$$k = 1, 2, \dots, c_{ij}$$

$$m = 1, 2, \dots, n_{ijk}$$

All the elements are normally distributed with mean 0 and some appropriate variance. Again, the design is completely balanced except for the family level. The data were so selected as to ensure that always  $k = 1, \dots, 3$ :  $c_{ij} = 3$  and  $n_{ijk} = 5$ .

The components of variance and their error were obtained by using the formulas given by MAHAMUNULU (1963). The formulas are extremely complicated and a special programme was written in order to compute the components of variance and their errors once a certain number of parameters have been calculated. The programme is available to the interested reader. The errors of the components are slightly biased because the covariance of the components have not been

calculated as the formulas used originally were based on SEARLE's book (1971) which does not mention the covariance formulas.

The pooled sums of squares used in MAHAMUNULU's formula are not chi-square distributed, hence the error variance is not correctly derived either. In spite of the fact that for a totally unbalanced case of a completely nested design, the mean squares are not independent, independent sums of squares - not chi-square distributed - can be derived (personal communication of Dr. G. Namkoorg, North Carolina State University).

## CHAPTER 3

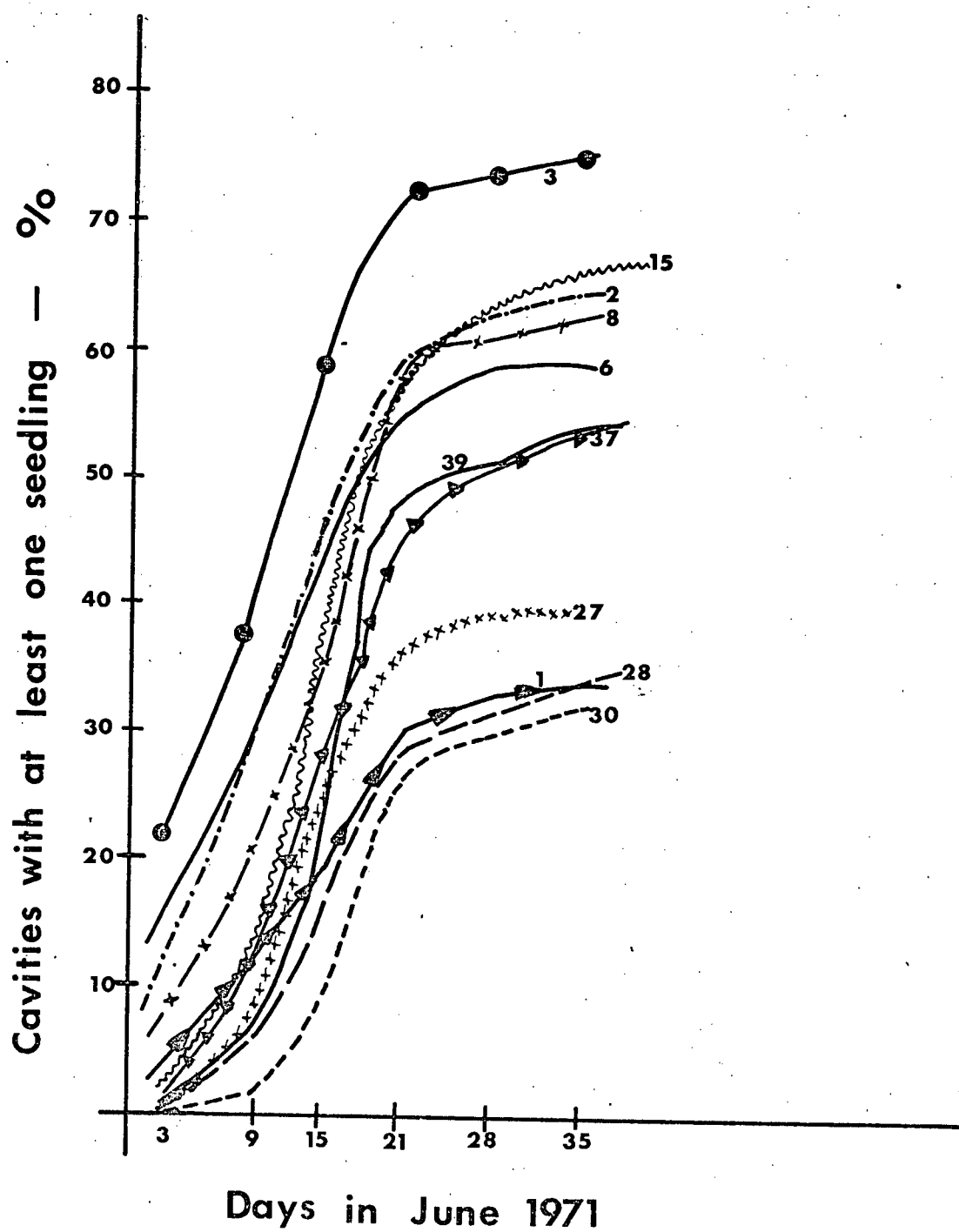
## RESULTS AND CONCLUSIONS

3.1 Germination rate

For each provenance, the total number of cavities with at least one germinant was calculated for each date. As the number of families sown varied from one provenance to another, a correction was made by dividing this total number by the total number of cavities sown. As the exact number of seeds sown in each cavity was not known, the results have only a comparative value. The rates have been plotted against time in number of days, for some selected provenances. The germination rate curves affect the form of a sigmoid (Fig. 22). These curves are different, but the differences cannot be explained. There is no clear geographical pattern of variance; for instance, the curve for provenance 1 (Lower Mainland, B.C.) has a curve very similar to the one of provenance 23 (Southeast Alaska). The provenances slow to germinate may or may not evolve slowly and the total germination may or may not remain small (provenances 1, 23, 27, 30).

There were striking differences between block A and the other blocks. Block A was faster to be covered by seedlings and the block B, C and D presented late germinants throughout the growing season. The mean number of cavities occupied by at least one germinant, its standard error and coefficient of variation have been calculated for each date and each provenance, for block A and for the blocks B, C

Fig.22. Germination rates plotted against time for some selected provenances. The numbers are the provenance numbers.



and D pooled. The differences are striking between block A and the other and need not be studied statistically. The differences are most important at the beginning of the germination process: there are often two times as many seedlings in block A as in the other blocks. The coefficient of variation is much lower in block A attesting that the few days of cold and humid treatment might have equalized the germination rates of the Sitka spruce families for a given provenance, while increasing the rates themselves. The differences, however, tend to disappear with time and by the end of the germination process, the number of germinants and their coefficient of variation are much closer in the two series of blocks. With time, the coefficient of variation decreases, pointing out a levelling of the rates of the different families within the same provenance. Table XXII shows a few germination statistics for some provenances.

According to our data, the rate of growth does not seem to be affected by the germination rate: the differences between the provenances in germination rates tend to disappear. Furthermore, there is no relationship between height growth and the germination rates: there is no apparent geographic trend in the differences between the provenances while there is a strong one for height growth.

### 3.2 Phenological observations

Bud burst and bud set rates are important adaptative traits as they determine the co-adaptation of the vegetation period of the tree to the periodic variation of the climatic components of the environment

TABLE XXII

MEAN NUMBER OF CAVITIES WITH AT LEAST ONE  
GERMINANT AND COEFFICIENT OF VARIATION (C. V.)  
FOR BLOCK A AND THE BLOCKS B, C, AND D POOLED

Provenance	Date	Block A		Block B, C and D	
		Mean	C. V.	Mean	C. V.
1	first	2.8	96.0	0.07	460.7
	last	8.5	87.3	5.5	99.5
5	first	6.1	55.5	1.5	144.9
	last	18.9	20.6	16.0	30.7
36	first	0.7	159.4	0.02	648.3
	last	16.2	21.8	6.6	60.8
39	first	0.5	279.0	0.09	323.7
	last	15.1	26.5	12.4	48.41

where the tree thrives. This co-adaptation is particularly important for the species growing in the temperate regions where important cyclic variation in day length, temperature regime, rainfall, etc. may exist.

Bud set is linked with the cessation of the elongation of the stem, but it is also related to the adaptation to the cold season and the entrance in dormancy which generally accompanies it, in the temperate tree species.

Bud set and bud flushing have been particularly studied in EUROPE, for different tree species, in connection with height growth, late and early frost damage (see SCHOBER, 1962 or NANSON, 1964). In a maritime, irregular climate as the one of the distribution area of Sitka spruce, these traits could be of paramount importance, especially

if this maritime climate is superimposed to a wild, mountainous topography with abrupt changes in aspect, altitude, rainy windward and drier lee-slopes or frost pockets due to temperature inversion.

The methodology of the study of bud set or bud burst is particularly important if precise comparisons are needed.

SCHOBER (1962) has studied the late frost and winter frost damage variability of ten Sitka spruce provenances. Flushing rate was assessed every 3-4 days using an empirical scale of four bud stages. The development of the terminal bud takes the shape of a sigmoid (Fig. 23). However, tree to tree variation was noticed. Bud set also takes a sigmoidal form.

Several methods can be used to assess bud set and bud burst. One can estimate the percentage of bud at a given stage of development every two or three days and draw a curve expressing the evolution of the percentage with time, or count the number of days until some type of bud appears. This latter method was used by BURLEY (1966<sub>a</sub>). These procedures may be long and tedious, depending on the size of the material and also imprecise because of the lack of clear definition of the type of bud considered or the small number of trees assessed: BURLEY (1966<sub>a</sub>) assessed two seedlings per treatment. Another method used in this study, consists of taking into account the continuity of the bud formation process, in distinguishing different stages and in establishing, for each population, the frequency distribution of the stages and in calculating the average bud stages. The



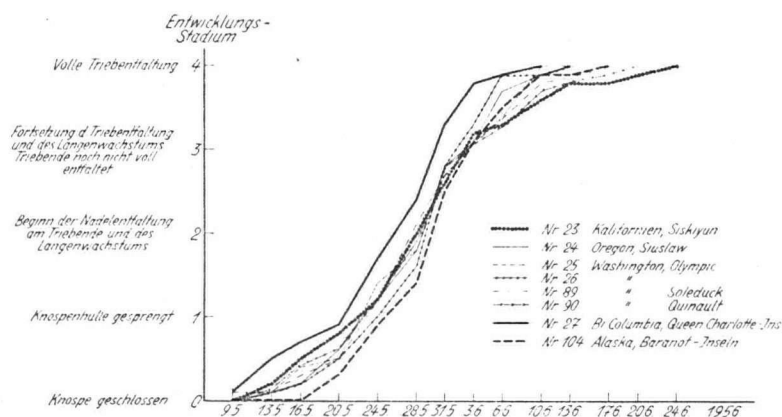


Abb. 33: Austreiben der Sitka im Frühjahr 1956 im Mittel von je 9 Stämmen  
Versuch Forstamt Gahrenberg Abt. 174

Flushing of Sitka spruce in spring 1956, mean of 9 trees in each sample.  
Experiment Forstamt Gahrenberg, compt. 174.

Fig. 23. Bud burst evolution of different Sitka spruce provenances growing in Germany in the spring of 1956. ( SCHÖBER, 1962 ).

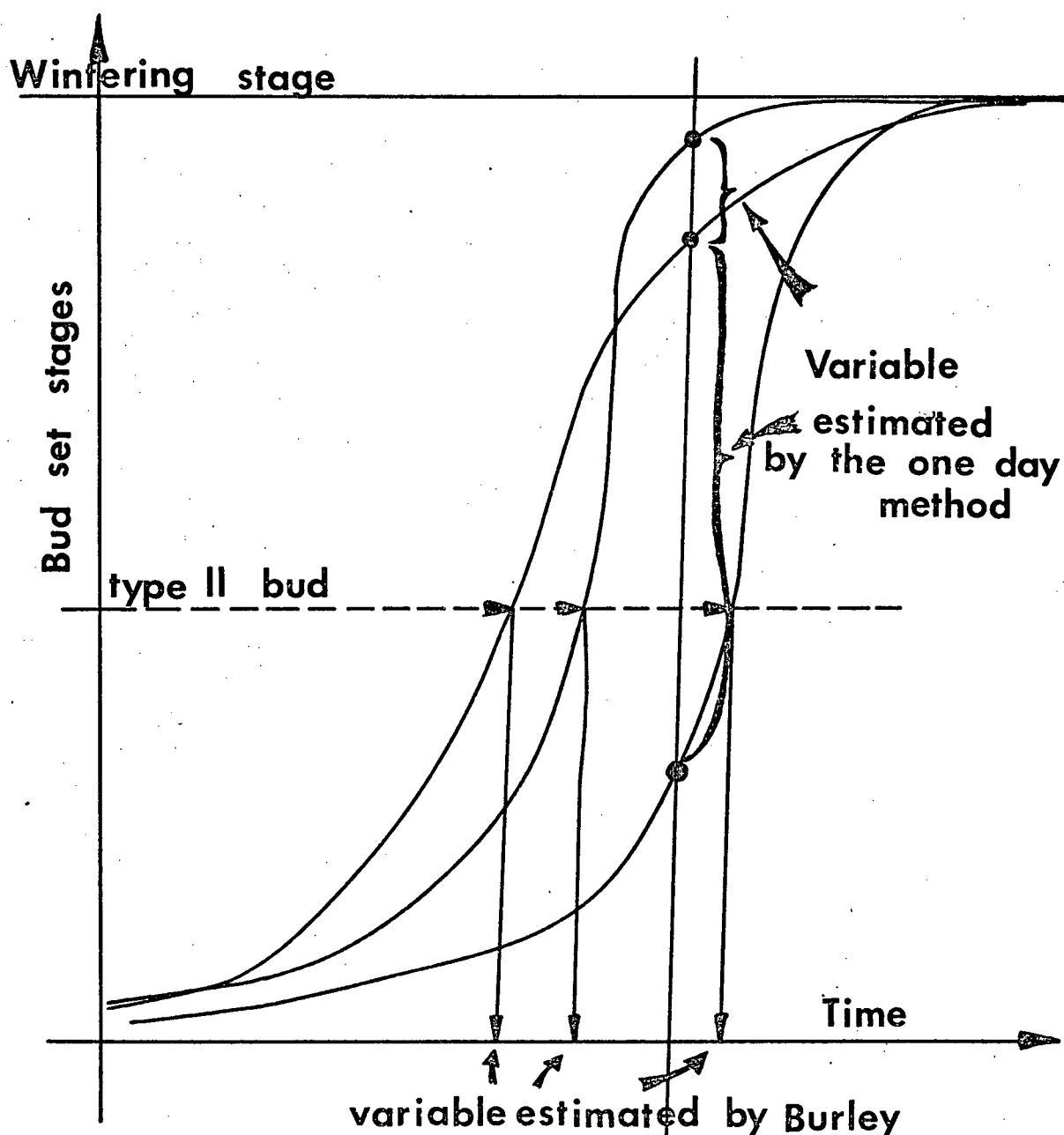


Fig. 24. Relationships between the one-day method to assess bud set and BURLEY's method.

relationship between the two methods are shown in Fig. 24, for an idealized example of three provenances. The method used in this study estimates the average bud set of a given provenance at a given date. It is a one-day estimation; therefore, the differences expressed by the provenances vary with the date chosen for the estimation. The differences estimated are indicated along the vertical line in Fig. 24. The differences in days given by BURLEY's method are indicated by the arrows corresponding to the horizontal line. Before bud set starts, all the provenances are growing and the differences in bud setting, between them, are equal to zero; when all the provenances are resting, again, the differences in bud set are equal to zero; consequently, there must be some date where, on the average, the differences are maximum and consequently, the genetic variance maximum (FALKENHAGEN, 1968).

The nature of the frequency distribution of bud flushing stages within different provenances of European beech (Fagus sylvatica L.) has been intensively studied by GALOUX and FALKENHAGEN (GALOUX, 1966). Two theoretical distributions, normal and lognormal, have been adjusted to the data accumulated for different beech progenies. Different numbers of bud stages: 5, 6 or 7 have been considered. The number of stages or classes to be distinguished is a problem in itself. Generally, the distribution was found to be skewed and not normal, rarely log-normal.

A correlation matrix (Table XXIV) between all the traits which presented some systematic variation has been calculated and between these traits and the geographical coordinates of the place of origin

of the provenances. The means of the provenances have been used to calculate this correlation matrix, disregarding the regions.

The legend of the matrix is as follows:

- LAT = latitude ( $^{\circ}$ , 1/10).
- LON = longitude ( $^{\circ}$ , 1/10).
- ALT = altitude (feet).
- BST 1 = bud set estimated on the 26th of September, 1971
- BST 2 = bud set estimated on the 2nd of October, 1971.
- BST72 = bud set estimated on the 8th of September, 1972.
- BBT = bud burst estimated on the 3rd of April, 1972.
- EPL = maximum epicotyl length.
- HET = mean total height after the second growing season.
- HMN = mean minimum total height after the second growing season.
- HMX = mean maximum total height after the second growing season.
- COL = needle colour estimated in 1972.

Table XXIII, with similar legends, shows the average values for the same variates studied.

A look at this matrix shows that latitude and longitude of the place of origin of the provenances are positively correlated ( $r = 0.78$ ). This fact results from the particular physiography of the northern part of the west coast of North America. Therefore, multiple regression techniques or partial correlation coefficients are necessary to dissociate the effects of these two variates.

TABLE XXIII

GEOGRAPHICAL COORDINATES OF THE PROVENANCES AND  
AVERAGE VALUES OF THE NURSERY TRAITS STUDIED

PROV.	LAT.	LONG.	ALT.	BUD- SET 1	BUD- SET 2	BUD BURST	COL.	BUD- SET 72	EPL (mm)	HET (mm)	HMN	HMX
1	49.12	121.93	100.	0.84	1.10	2.67	0.81	0.53	80.24	196.92	150.37	238.70
2	49.92	123.25	100.	1.10	1.41	3.21	1.25	1.61	79.77	206.58	148.53	263.47
3	49.38	124.62	0.	1.58	1.64	3.10	1.09	1.53	87.37	230.50	171.41	283.39
4	50.38	125.95	0.	1.14	1.99	2.85	1.18	1.84	85.01	202.59	143.08	260.53
5	55.47	128.23	1700.	2.52	2.96	3.05	1.36	3.28	60.29	143.70	99.45	194.88
6	55.17	127.87	2200.	2.55	2.85	3.35	1.34	3.20	59.95	125.61	83.52	175.75
7	54.63	128.40	450.	2.57	2.79	2.89	1.39	2.47	72.47	181.23	128.15	234.43
8	54.40	128.95	100.	2.80	2.98	2.86	1.37	2.79	72.63	167.60	119.36	217.59
9	54.13	128.62	550.	2.48	2.75	2.90	1.55	2.89	66.96	167.15	123.96	214.92
10	54.72	128.77	450.	2.37	2.79	2.92	1.27	2.32	71.48	184.91	130.33	244.79
11	55.68	128.68	800.	2.44	2.95	3.13	1.20	3.07	57.66	135.63	90.07	184.56
12	55.35	128.95	850.	2.38	2.74	2.94	1.19	2.79	64.31	153.38	106.04	205.26
13	55.15	129.22	50.	2.24	2.68	3.01	1.28	2.80	67.03	155.09	99.64	209.70
14	55.15	128.97	1300.	2.50	2.87	2.84	1.29	3.00	66.11	151.96	105.87	205.97
15	54.20	129.92	0.	1.94	2.39	2.56	1.42	2.05	81.71	184.39	135.34	238.34
18	55.02	128.32	800.	2.30	2.81	3.44	1.25	3.13	59.18	141.28	95.09	193.55
19	54.28	129.42	100.	2.35	2.79	2.91	1.33	2.29	65.25	174.05	121.53	228.10
20	54.03	130.37	1000.	2.01	2.49	2.06	1.49	2.49	65.90	142.91	92.47	192.94
21	54.20	130.25	50.	1.91	2.00	2.24	1.35	2.20	75.02	172.38	125.47	222.09
23	55.03	131.55	0.	1.71	2.22	2.09	1.40	1.85	67.85	153.44	106.73	204.27
24	55.50	133.13	0.	2.05	2.86	2.35	1.54	2.79	73.13	170.18	116.91	222.89

TABLE XXIII - Continued

PROV.	LAT.	LONG.	ALT.	BUD- SET 1	BUD- SET 2	BUD BURST	COL.	BUD- SET 72	EPL (mm)	HET (mm)	HMN	HMX
25	55.47	132.67	0.	2.56	3.03	2.70	1.64	2.84	73.66	173.30	120.48	324.35
26	55.42	131.70	50.	2.10	2.69	2.34	1.60	2.30	67.56	156.20	103.37	211.09
27	56.58	132.73	25.	2.48	2.95	2.54	1.68	2.97	66.29	154.94	113.32	205.00
28	58.37	134.58	100.	2.71	3.08	2.18	1.54	3.37	68.61	144.76	95.72	200.87
29	49.25	122.60	650.	0.89	0.96	2.75	0.74	1.59	82.89	205.65	156.38	251.04
30	48.38	123.87	0.	0.51	0.55	3.13	0.61	0.51	91.37	217.58	162.32	279.87
31	48.58	124.40	25.	0.56	0.34	2.43	1.33	0.60	76.87	202.95	148.84	259.68
32	50.08	127.50	100.	1.28	1.51	2.95	1.35	1.68	79.32	193.57	145.76	239.69
33	49.83	126.67	10.	0.55	0.74	2.54	1.32	1.69	78.87	191.25	132.14	249.29
34	50.62	128.12	100.	1.35	1.72	2.60	1.42	1.78	74.89	169.88	126.07	213.73
35	52.28	131.22	50.	1.17	1.49	2.34	1.21	1.66	77.21	208.62	151.33	266.87
36	52.87	132.08	50.	1.35	1.91	2.47	1.33	2.03	71.53	158.17	106.05	211.04
37	53.05	132.08	200.	1.54	2.43	2.55	1.49	2.16	87.96	199.86	137.24	258.03
38	53.13	131.80	250.	1.61	2.08	2.19	1.29	2.04	77.03	170.57	114.08	233.84
39	53.50	132.17	300.	1.52	1.99	2.77	1.20	1.96	72.62	186.95	133.07	234.97
40	53.92	132.08	0.	1.22	1.60	2.60	1.24	1.86	89.55	207.83	148.13	263.69
41	48.90	124.95	700.	0.95	0.80	2.90	1.12	1.34	81.77	211.13	154.39	263.95

TABLE XXIV

CORRELATION MATRIX BETWEEN THE TRAITS STUDIED AND THE GEOGRAPHICAL  
COORDINATES OF THE PLACE OF ORIGIN

	LAT	LON	ALT	BST1	BST2	EPL	BBT	BST72	HET	HMN	HMX	COL
LAT	1.0											
LON	.78	1.0										
ALT	.26	-.12	1.0									
BST1	.89	.53	.39	1.0								
BST2	.92	.63	.32	.96	1.0							
EPL	-.71	-.34	-.56	-.74	-.70	1.0						
BBT	-.14	-.51	.42	.13	.066	-.13	1.0					
BST72	.88	.59	.47	.91	.92	-.75	.12	1.0				
HET	-.78	-.49	-.52	-.74	-.74	.91	.037	-.80	1.0			
HMN	-.80	-.54	-.49	-.72	-.76	.88	.069	-.81	.98	1.0		
HMX	-.74	-.43	-.53	-.72	-.72	.90	.019	-.77	.98	.95	1.0	
COL	.64	.72	-.039	.58	.62	-.44	-.37	.61	-.50	-.53	-.48	1.0

36 D.F.

 $r .05 = .32$  $r .01 = .41$  $r .001 = .51$

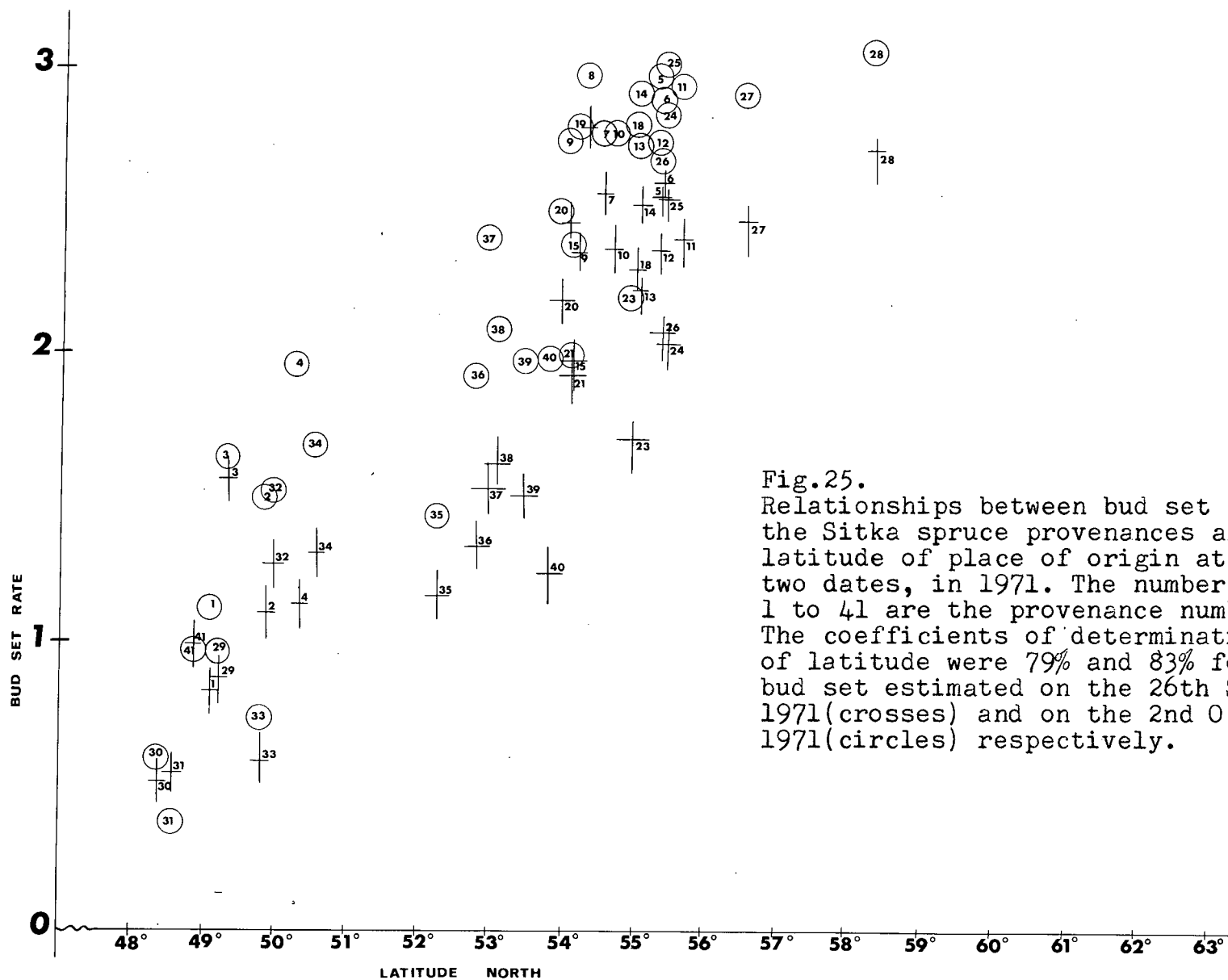


Fig.25.  
Relationships between bud set of the Sitka spruce provenances and latitude of place of origin at two dates, in 1971. The numbers 1 to 41 are the provenance numbers. The coefficients of determination of latitude were 79% and 83% for bud set estimated on the 26th Sept. 1971(crosses) and on the 2nd Oct. 1971(circles) respectively.



Bud set 1 and 2 and bud set 72 are strongly correlated with latitude ( $r = 0.89$  or  $0.92$  and  $0.88$ ). There are also at least significantly correlated with longitude and altitude; however, the relationships are somewhat weaker. What is most important is the constancy of all correlations over the years '71 and '72. This would indicate a strict genetic control of bud set, at least in comparison with the differences in the growing conditions of the two years and the two nursery locations.

Fig. 25 shows the relationship between bud set 1 and 2 and latitude. The general relationship is linear.

Multiple regression and correlations analyses show that altitude does not contribute significantly to the variation of bud set 1 and 2. 86.2 percent of the variation in bud set is explained by longitude and latitude, 79.0 percent by latitude alone. 84.8 percent of the variation in bud set two is explained by longitude and latitude, 83.0 percent by latitude alone. The regression equations are:

$$\text{budset 1} = -3.28 - 0.0936\text{Long} + 0.322\text{Lat}$$

$$\text{or} = -0.0105 + 0.232\text{Lat.}$$

$$\text{budset 2} = -8.16 - 0.052\text{Long} + 0.32\text{Lat}$$

$$\text{or} = -12.20 + 0.269\text{Lat}$$

Fourty-four percent of bud burst is explained by latitude and longitude and 26 percent by longitude alone.

The multiple regression equations are:

$$\text{bud burst} = 12.8 + 8.94\text{Lat} - 0.0015\text{Long}$$

$$\text{or} = 10.0 - 0.0566 \text{ ong}$$

Partial correlation coefficients are very useful in representing multiple relationships.

If bud burst is symbolized by  $y$

If altitude is symbolized by  $X_1$

If longitude is symbolized by  $X_2$

If latitude is symbolized by  $X_3$

then:

$$r_{yX_1/X_2X_3} = 0.20 \text{ NS}$$

$$r_{yX_2/X_1X_3} = -0.51^{***}$$

$$r_{yX_3/X_1X_2} = 0.27 \text{ NS}$$

Thus, there is a negative correlation of 0.51 between bud flushing and longitude for fixed latitude and altitude, but not with the other parameters: the more maritime the local climate, the later the provenances flush. Thus, there is some evidence that the differences in bud flushing are related to the thermic conditions of the place of origin, probably with the local late frost distribution as already observed by SCHOBBER (1962).

Ninety-one percent of the total variation in bud set 72 can be attributed to the interaction of latitude, altitude, bud set 2, bud burst and colour; all these variables contributing significantly to the variation in bud set 72. Eighty-seven percent is explained by latitude, altitude and bud burst; eighty-four percent by latitude and bud burst; seventy-eight percent by latitude alone.

Thus, there is a covariance structure between bud set 72 and bud burst, bud set 2 and needle colour. As these traits are strictly heritable, it is possible that genetic correlations exist between these traits, positive or negative. Therefore, in a selection experiment, care should be taken of the correlated responses when selecting for a given trait such as late flushing or early bud setting.

Bud set 1 and 2 may be considered as one trait because of the strong correlation coefficient between them and the other traits. Bud set 72 is strongly correlated with epicotyl length and total height ( $r = -0.75$  or  $-0.81$ ). Bud set, in general, is positively correlated with needle colour.

The higher the latitude of the place of origin of the provenances, the earlier these provenances set their bud and the shorter the height of the provenances. Thus, the earlier they set their bud, the shorter the provenances.

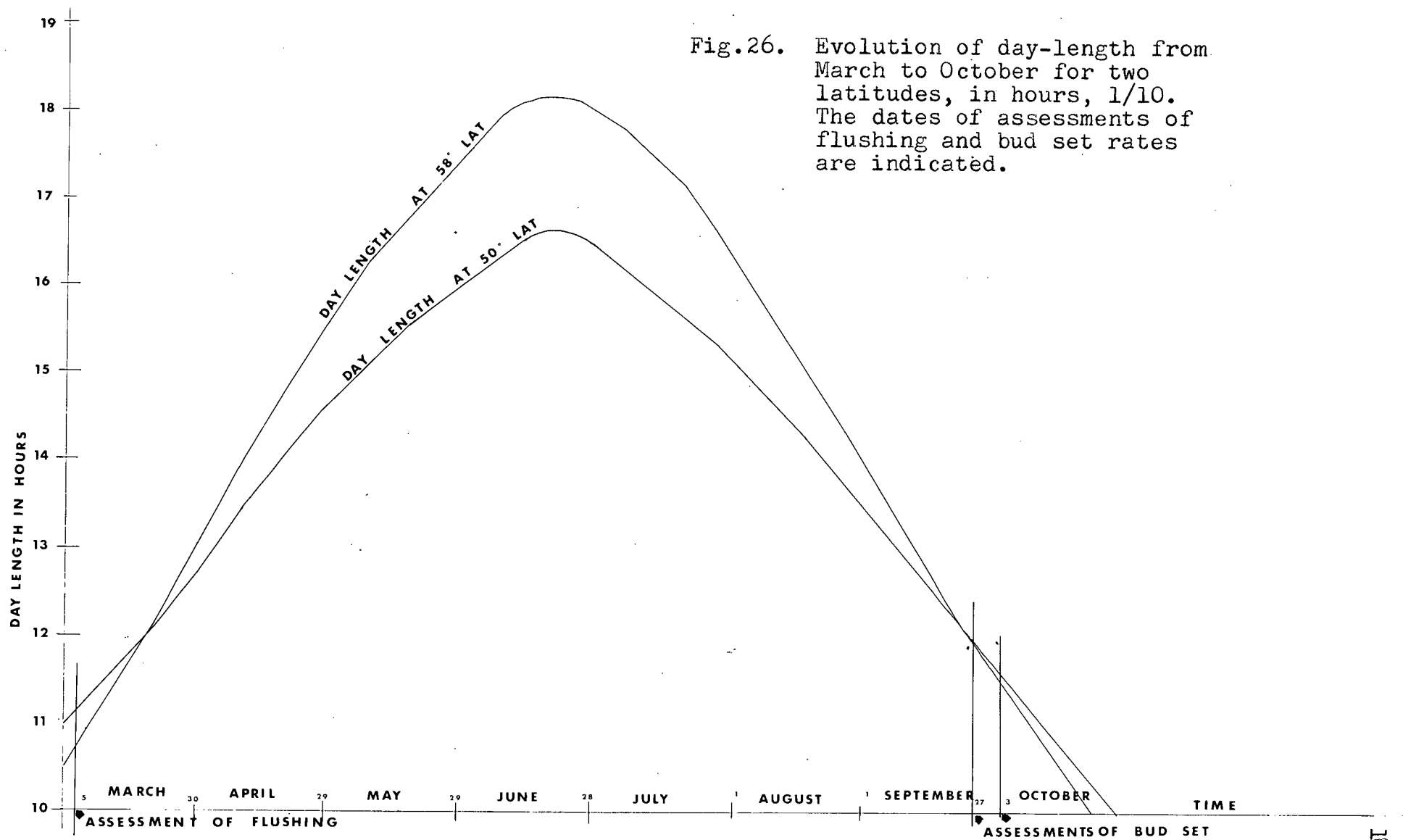
Bud burst is not significantly correlated with bud set or epicotyl length.

Our data, therefore, confirm BURLEY's (1966<sub>a</sub>) findings that seedlings from northern sources form their buds before the southern provenances, findings based on the study of the formation of a certain type of bud (Type II, according to BURLEY). However, some of his observations on the Type III bud could contradict his relationships based on his Type II bud.

However, our research does not confirm BURLEY's con-

tention that flushing rate is not related to the place of origin of the Sitka spruce provenances. It is also important to note that BURLEY has not studied the relationship between the number of leaf primordia and internodes and the next year's shoot growth. According to ROMBERGER (1963), for some species of Pinus or Picea, the number of internodes telescoped in the terminal bud determines the total shoot growth of the next growing season. It is not known if Sitka spruce has terminal buds with predetermined shoots and fully preformed needles and internodes. At the Surrey nursery, the Sitka spruce provenances grew continuously until fall. No repeated flushes have been noticed by us, which would indicate (KOZLOWSKI, 1971) that Sitka spruce has no shoots preformed in the dormant buds.

Bud set in trees is considered by many authors as often set in motion by declining photoperiod during late summer through some "phytochrome" mechanism (ROMBERGER, 1963; KOZLOWSKI, 1971). The data presented here do not support this theory, as Fig. 26 seems to show, because at the time of measurements, the differences between the photoperiods at latitudes  $50^{\circ}$  and  $58^{\circ}$  are too small to explain the differences in bud setting expressed by the provenances growing at Surrey. Perhaps the mechanism triggering the onset of dormancy is set in motion earlier, during the summer. The same holds true for bud flushing as observed in the nursery. Thus, it is difficult to attribute the differences in bud flushing and bud set observed in the nursery as due to different adaptations to different photoperiods at the place of origin of the provenances.



However, the thermal energy accumulated over the growing season varies much in quantity and rate of accumulation with latitude, as a look at Fig. 5 may suggest. The effect of altitude could be the same. It has been suggested by GALOUX (1966) that the rate of bud flushing in Fagus sylvatica L. is a physiological process depending on an adequate accumulation of thermal energy in the environment - most particularly the soil, once dormancy has been broken. This accumulation of calories is linked with the number of degree-days accumulated in the spring. There would be a need for reaching some accumulated threshold before flushing can take place, once the buds are quiescent and ready to flush. Fig. 6 might indicate that the differences in bud burst might be linked with adaptation to different temperature thresholds: the higher the altitude of the place of origin, the quicker to flush when growing in a milder environment. Note that the correlation of bud burst with altitude is  $+ 0.42^{**}$ .

Genetic control of time of bud break is well known and the beginning and cessation of shoot growth often are genetically fixed in relation to a given photoperiod (KOZLOWSKI, 1971). Bud burst in Fagus sylvatica L. has been shown to be impossible without an adequate photoperiod for some time (LAVARENNE-ALLARY, 1965 in GALOUX, 1966). Complex temperature-photoperiod interactions may play some role in triggering both bud flushing and bud set in forest trees (VEGIS, 1965).

Naturally, the study of the growth responses of Sitka spruce provenances to shortened and extended photoperiods and under different temperature regimes should provide some clues as to the mechanisms

of bud burst and bud set prevalent in this tree species.

ANOVAs and DUNCAN's multiple range tests ( $\alpha = 0.05$ ) have been calculated on bud set 1 and 2 and bud burst, for each region separately.

The following tables summarize the analyses done for some regions as examples. The complete analyses are available on request. The model used is a completely randomized block design (Model II) with four blocks.

Region 1 = provenances 1, 2, 3, 4, 29

<u>Sources of Variation</u>	<u>D.F.</u>	<u>F Values and Significance</u>		
		<u>Bud set 1</u>	<u>Bud set 2</u>	<u>Bud burst</u>
Provenances	4	13.67***	7.29**	4.20*
Blocks	3	0.24 NS	0.23 NS	4.13*
Error	12			
TOTAL	19			

Duncan's tests: ( $\alpha = 0.05$ ). The greatest value is always on the left.

Bud set 1

Prov. No:	3	4	2	29	1
Value	1.58	1.14	1.10	0.89	0.84

Bud set 2

Prov. No:	4	3	2	1	29
Value:	1.99	1.64	1.41	1.10	0.96

Bud burst

Prov. No:	2	3	4	29	1
Value:	3.21	3.10	2.85	2.75	2.67

Region 2 = provenances 30, 31, 32, 33, 41

<u>Sources of Variation</u>	<u>D.F.</u>	<u>F Values and Significance</u>		
		<u>Bud set 1</u>	<u>Bud set 2</u>	<u>Bud burst</u>
Provenances	4	15.65***	11.46***	5.02*
Blocks	3	3.01 NS	3.62*	1.82NS
Error	12			
TOTAL	19			

Duncan's tests: ( $\alpha = 0.05$ ).

Bud set 1

Prov. No:	32	41	33	31	30
Value:	1.28	0.95	0.55	0.55	0.51

Bud set 2

Prov. No:	32	41	33	30	31
Value:	1.51	0.80	0.74	0.55	0.34

Bud burst

Prov. No:	30	32	41	33	31
Value:	3.13	2.95	2.90	2.54	2.43

Region 5 = provenances 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20, 21

<u>Sources of Variation</u>	<u>D.F.</u>	<u>F Values and Significance</u>		
		<u>Bud set 1</u>	<u>Bud set 2</u>	<u>Bud burst</u>
Provenances	13	4.68***	9.86***	9.49***
Blocks	3	1.77 NS	0.87 NS	11.49***
Error	39			
TOTAL	55			

Duncan's tests: ( $\alpha = 0.05$ ).

Bud set 1

Prov. No:	7	6	14	5	9	11	10	12	19	18	13	20	15	21
Value:	2.57	2.55	2.50	2.50	2.48	2.44	2.38	2.38	2.35	2.30	2.24	2.01	1.94	1.91

Bud set 2

Prov. No:	5	11	14	6	18	7	10	19	9	12	13	20	15	21
Value:	2.96	2.95	2.87	2.85	2.81	2.79	2.79	2.79	2.75	2.74	2.68	2.40	2.39	2.00

Bud burst

Prov. No:	18	6	11	5	13	12	10	9	7	14	15	21	20
Value:	3.44	3.35	3.13	3.05	3.01	2.94	2.92	2.90	2.89	2.84	2.56	2.24	2.06

Generally, it can be seen that the DUNCAN's tests for the two bud sets 1 and 2 can be different despite the similar correlation coefficients with latitude or longitude. The combined effects of sampling error and specific rates of evolution of the bud setting could be one explanation.





Photo 4. Variation in needle colour was striking. The higher the latitude or the greater the longitude of the place of origin, the more bluish the provenance. Photo by the author.

### 3.3 Needle colour

BURLEY (1966<sub>b</sub>) has observed genetic variation in needle and hypocotyl colour of different Sitka spruce provenances. The bluish bloom occurring on plum fruits, eucalypt leaves and needles of blue spruce, is well known and has been linked with cuticular wax structures. It was decided to study the needle glaucousness on a provenance basis, using an empirical scale of bluish bloom intensity consisting of three classes, in order to accurately check the qualitative observations of BURLEY. Indeed, the differences were striking (photo 4). Needle colour is positively correlated with latitude, longitude, ( $r = 0.64$  and  $0.72$ ), with bud set 1, bud set 2, negatively correlated with epicotyl length or total height, (Table XXIV). Thus, the higher the latitude or longitude, the more bluish the provenances are and the shorter they are.

When the influences of latitude, longitude and altitude of the place of origin are considered simultaneously, only longitude contributes to colour variation: 51.8 percent of the variation in needle colour is explained by longitude.

### 3.4 Growth characteristics

Maximum epicotyl length and total height after the second growing season of the transplants (1-1 seedlings) will be studied using the well known techniques: multiple regression analysis and anovas.

#### 3.4.1 Maximum epicotyl length (EPL)

EPL has been related to all the other variables and with an additional one not included in Table XXIV: a measure of average density

obtained by dividing the number of seedlings at the end of the first growing season by the number of cavities sown, for each progeny and each replication; then the average was calculated for each provenance. The correlations are calculated over all provenances, neglecting the regions.

The average density so calculated only contributed 1.08% of the total coefficient of determination of all the independent variables considered, thus the average number of seedlings per cavity did not influence the growth of the families during the first year; the availability of water and mineral nutrients being sufficient.

Eighty percent of EPL is explained by the linear combination of latitude, longitude, altitude, colour, bud burst and density.

Sixty-five percent by latitude and altitude only.

Fifty percent by latitude alone.

Fig. 27 indicates the relationship between EPL and latitude.

The multiple regression equations are:

$$\text{EPL} = 182.58 - 2.00 \text{ Lat.} - 0.00682 \text{ Alt.}$$

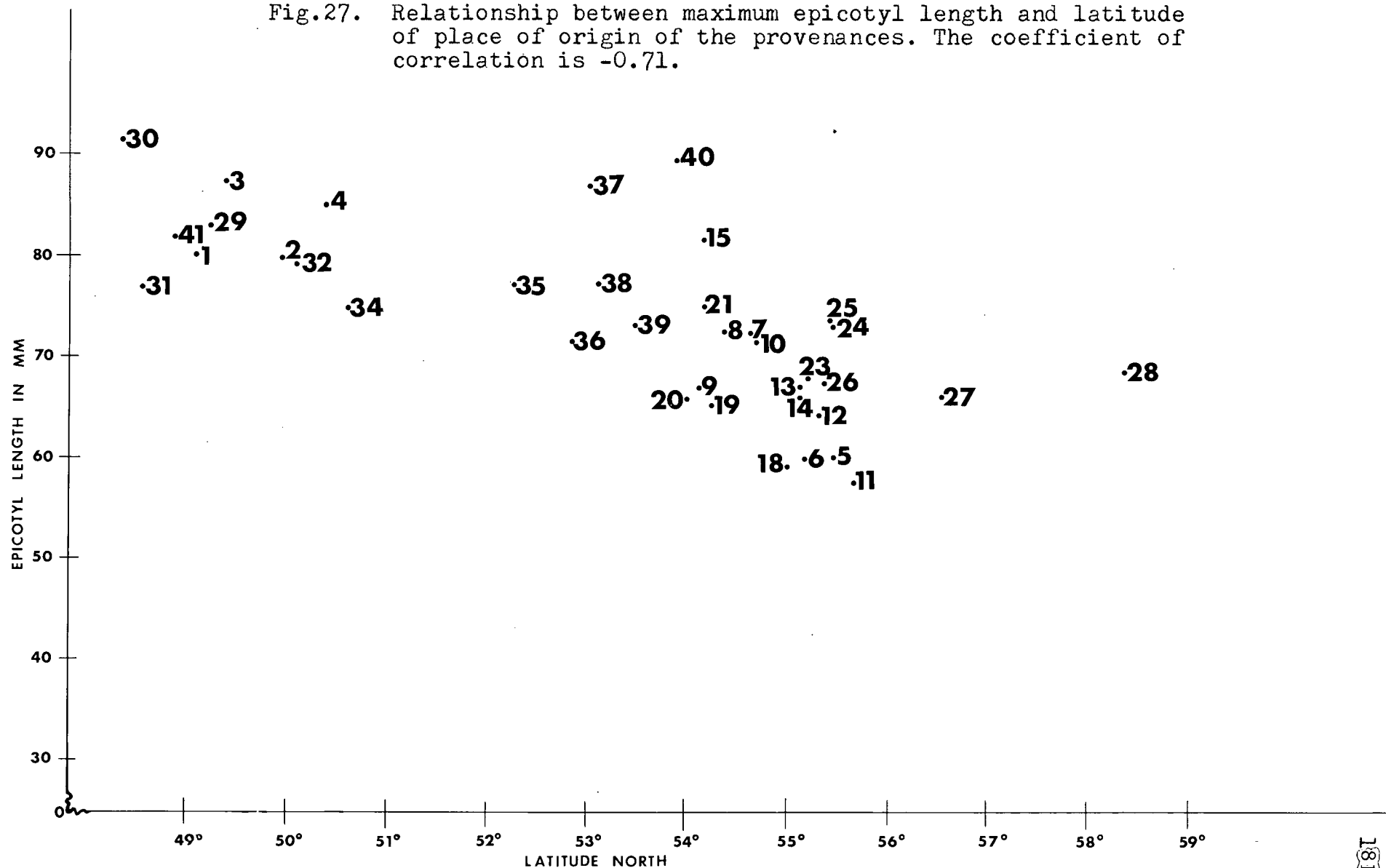
or 
$$\text{EPL} = 198.44 - 2.35 \text{ Lat.}$$

Another multiple regression analysis shows that 74% of EPL is explained by altitude and bud set 1. Sixty-six percent by bud set 1 alone. This might indicate that the rate of bud setting determines somehow height growth.

The calculation of partial correlation coefficients shows that if:

$$\begin{aligned} y &= \text{EPL} \\ X_1 &= \text{altitude} \end{aligned}$$

Fig.27. Relationship between maximum epicotyl length and latitude of place of origin of the provenances. The coefficient of correlation is  $-0.71$ .



$X_2$  = longitude

$X_3$  = latitude

then  $r_{yX_1}$  for fixed  $X_2$  and  $X_3$  = -0.365 NS  
 $r_{yX_2}$  for fixed  $X_1$  and  $X_3$  = + 0.275 NS  
 $r_{yX_3}$  for fixed  $X_2$  and  $X_1$  = -0.535\*

The partial correlation analysis shows - as it should be - the primordial influence of latitude in explaining EPL variation. For a given longitude and altitude, EPL significantly decreases with latitude: the partial correlation coefficient is - 0.535, which is remarkable because the contributions of longitude and altitude have been eliminated.

If only longitude is kept constant, the correlation with latitude is increased  $r_{yX_3}$  for fixed  $X_2$  = -0.75\*\*. Fixing longitude also increases the correlation with altitude  $r_{yX_1}$  for fixed  $X_2$  = -0.627\*\*.

The analyses of variance, the components of variance and the DUNCAN's tests (\*) have been calculated for each region separately, by using the model and methods described in Part III, Section 2.4.2.

The details of the calculations are given for one region only: region 1. The other regions will be presented under the form of short tables.

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(\*) In order to estimate the variance components, a completely random model was assumed. However, the practical tree breeder will be interested in knowing how a particular provenance outperformed, in the nursery, another provenance; therefore, DUNCAN's tests for the provenance means were calculated which would, theoretically, imply that the provenance effect is fixed.

Region 1 = provenances 2, 3, 4, 29

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Blocks	3	454.53895	151.51298	NS
Provenances	3	3,387.58675	1,129.19558	240 NS
Trees wn prov.	41	19,248.41325	469.47349	4.29***
Prov. X block	9	2,163.65625	240.40625	2.20 NS
Tree wn prov X block	123	13,455.57393	109.39491	1.26 NS
Error	360	31,283	86.89722	
TOTAL	539			

No significant differences between provenances, thus no DUNCAN's test.

Components of variance

$$N_{...} = 540$$

$$1/N_{...} \sum_i N_i^2 = 146.4$$

$$N_{.j.} = 135$$

$$1/N_{.j.} \sum_i N_{ij}^2 = 36.6$$

The equations become:

$$131.2 \sigma_{\alpha}^2 + 32.68 \sigma_{\alpha\beta}^2 + 12 \sigma_{\gamma}^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2 = 1,129.19558$$

$$135 \sigma_{\beta}^2 + 36.6 \sigma_{\alpha\beta}^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2 = 151.51298$$

$$12 \sigma_{\gamma}^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2 = 469.47349$$

$$32.8 \sigma_{\alpha\beta}^2 + 3 \sigma_{\gamma\beta}^2 + \sigma_e^2 = 240.40625$$

$$3 \sigma_{\gamma\beta}^2 + \sigma_e^2 = 109.39491$$

$$\sigma_e^2 = 86.89722$$

Solving these equations give:

$$\sigma_e^2 = 86.89722$$

$$\sigma_{\gamma\beta}^2 = 7.49923$$

$$\sigma_{\gamma}^2 = 30.00654$$

$$\sigma_{\alpha}^2 = 4.029807, \text{ etc.}$$

See Table XXV for the error of the components of variance.

Region 2 = provenances 30, 32, 34, 41

#### Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Blocks	3	1,343.51915	447.83971	1.64
Provenances	3	6,062.39993	2,020.79997	3.98*
Tree wn prov.	17	8,631.60007	507.74118	3.77***
Prov. X block	9	2,458.30680	273.14520	2.03 NS
Tree wn prov X block	51	6,862.26898	134.55429	1.30 NS
Error	168	17,277	102.83928	
TOTAL	251			

DUNCAN's test: ( $\alpha = 0.05$ ).

EPL = 90.5      78.8      78.5      78.5 mm

Prov.nr= 30      41      34      32

Components of variance: see Table XXV.

Region 3 = provenances 35, 36, 37, 39, 40

Analysis of variance

Sources of Variance	D.F.	S.S.	M.S.	F
Blocks	3	2,165.02593	721.67531	2.84 NS
Provenances	4	27,397.26486	6,849.31621	16.16 ***
Tree wn prov.	24	10,173.73514	423.90563	3.08 ***
Prov. X block	12	3,051.92880	254.32740	1.85 NS
Tree wn prov. X block	72	9,907.62696	137.60593	1.38 NS
Error	232	23,201	100.00431	
TOTAL	348			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov.No.	37	40	35	39	36
EPL =	92.0	86.8	83.6	70.8	70.5

Components of variance: see Table XXV

Region 4 = provenances 24, 25, 26, 27

Analysis of variance

Sources of variation	D.F.	S.S.	M.S.	F
Blocks	3	1,042.15836	347.38612	2.62 NS
Provenances	3	3,844.71402	1,281.57134	1.81 NS
Tree wn prov.	18	12,770.28598	709.46033	6.44 ***
Prov. X block	9	1,193.48817	132.60979	1.20 NS
Tree wn prov. X block	54	5,947.41183	110.13725	1.57 NS
Error	176	12,313	69.96022	
TOTAL	263			

No significant differences; thus no DUNCAN's test, for the provenance effect.



Components of variance: see Table XXV.

Région 5 = provenances 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, 19, 21

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Blocks	3	3,958.18031	1,319.39343	12.04***
Provenances	13	74,524.47710	5,732.65185	10.68***
Tree wn prov.	83	44,535.52290	536.57256	6.90***
Prov. X block	39	4,274.27871	109.59689	1.41 NS
Tree wn prov. Xblock	249	19,369.45602	77.78898	1.19 NS
Error	776	51,243	66.03479	
TOTAL	1,163			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov.No.	15	21	10	8	7	13	14	9	12	19	5	6	11	18
EPL =	81.0	78.8	75.0	72.6	72.0	66.4	65.6	65.5	62.7	61.9	60.4	59.4	58.6	57.0

Components of variance: see Table XXV.

In the following and preceding analyses, only the provenances represented by at least three families and the families represented by four complete block measurements were considered.

To see if some transformation of the original epicotyl measurements was necessary, in order to stabilize the variances of the means, the means of the EPL of the provenances have been plotted against their variance on a bi-logarithmic paper. The relationship (Fig. 28) is too weak to ensure a useful transformation which would be close to a reciprocal transformation if we use the technique described by JEFFERS (1959, p. 75).

Fig.28. Mean epicotyl length of the provenances plotted against their variance, on a double logarithmic paper.

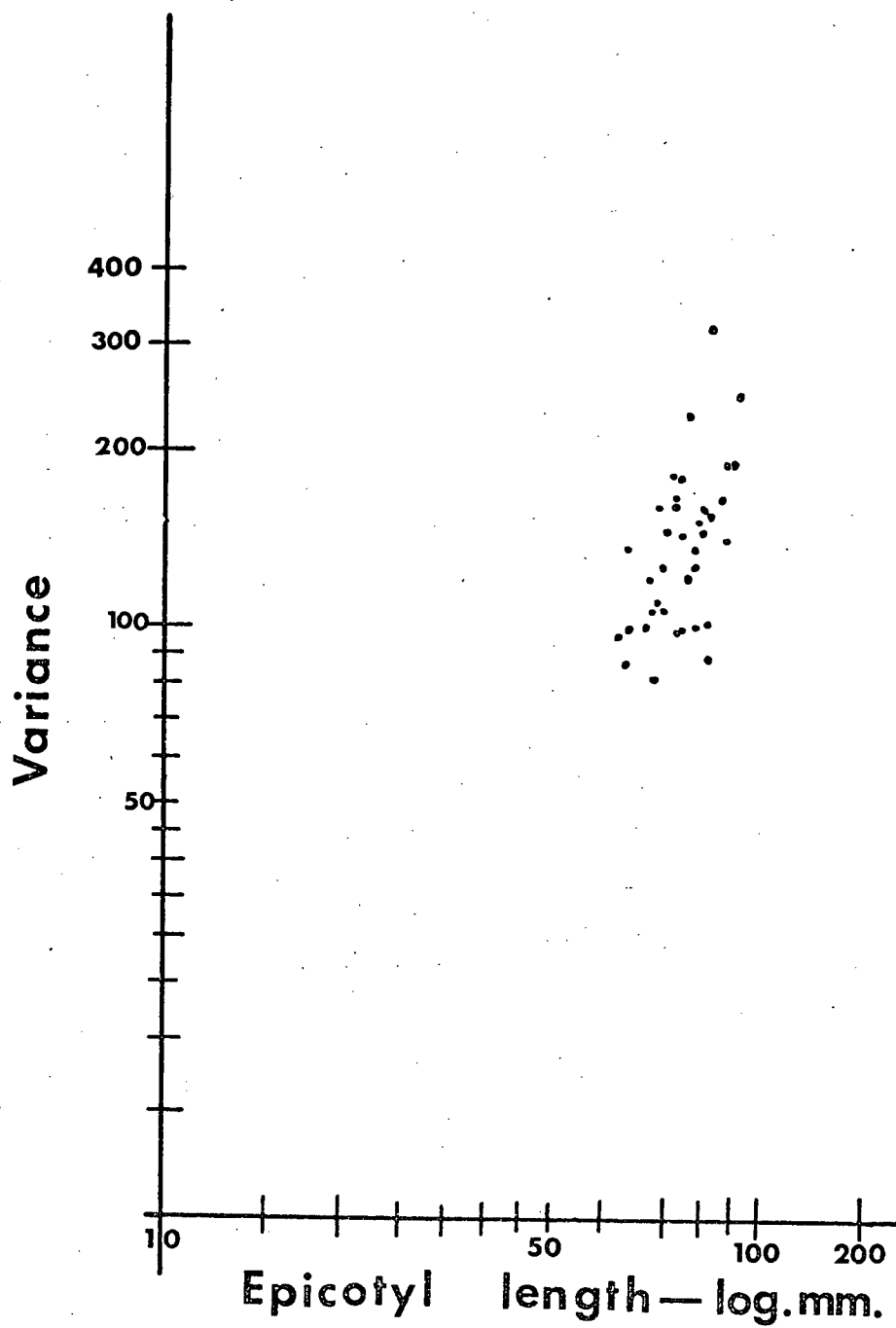


TABLE XXV

VARIANCE COMPONENTS OF SOME IMPORTANT SOURCES  
OF VARIATION AND THEIR STANDARD ERROR FOR  
MAXIMUM EPICOTYL LENGTH

Number of the Region	Sources of the Effect					
	Provenances		Trees		Error	
1	4.0 ±	5.5	30.0 ±	8.5	86.9 ±	6.4
2	23.2 ±	21.8	31.1 ±	13.9	102.8 ±	11.2
3	95.0 ±	59.6	23.8 ±	10.0	100.0 ±	9.2
4	8.7 ±	13.4	49.9 ±	18.8	70.0 ±	7.4
5	63.0 ±	25.5	38.2 ±	6.9	66.0 ±	3.3

#### 3.4.2 Total height after the second growing season (HET)

HET was measured in 1972 on the basis of a random sample of five seedlings per replication and per progeny. In order to study the relationships between HET and EPL, the average maximum height (HMX) and the average minimum height (HMN), for each provenance, have been calculated by taking the greatest and the smallest seedling of each progeny and summing up over all the replications and progenies for each provenance.

The standard deviation (SD), the standard error (SE) and the coefficient of variation (CV) of HET have also been calculated for each provenance.

The correlation matrix (Table XXIV) shows that the correlations between HMX, HMN, EPL and HET are very high as well as

TABLE XXVI

CORRELATION MATRIX BETWEEN THE GEOGRAPHICAL  
COORDINATES OF THE PROVENANCES AND

	<u>HET, SD, SE AND C. V.</u>						
	Lat	Long	Alt	HET	C. V.	SD	SE
Lat	1.00						
Long	0.78	1.00					
Alt	0.26	-0.12	1.00				
HET	-0.78	-0.49	-0.52	1.00			
C. V.	0.59	0.39	0.30	-0.74	1.00		
SD	-0.16	-0.009	-0.33	0.25	0.45	1.00	
SE	-0.41	-0.24	-0.26	0.34	0.10	0.62	1.00

$r_{.05} = 0.32$

highly significant ( $r = 0.88; 0.90; 0.91$ , etc.). Consequently, the conclusions concerning EPL should be equally valuable for HET, at least as far as the correlation analyses are concerned.

A correlation matrix has been calculated between the geographical coordinates and HET, C. V., SD and SE (Table XXVI). This matrix shows that C. V. increases with latitude ( $r = 0.59$ ), but that SE decreases with latitude. This would indicate that the populations become more homogeneous with latitude. HET is negatively correlated with C. V. ( $r = -0.74$ ).

Seventy-three percent of the total variation in HET is explained by latitude and altitude. Longitude does not contribute significantly to the total variation in HET.

Sixty-one percent is explained by latitude alone.

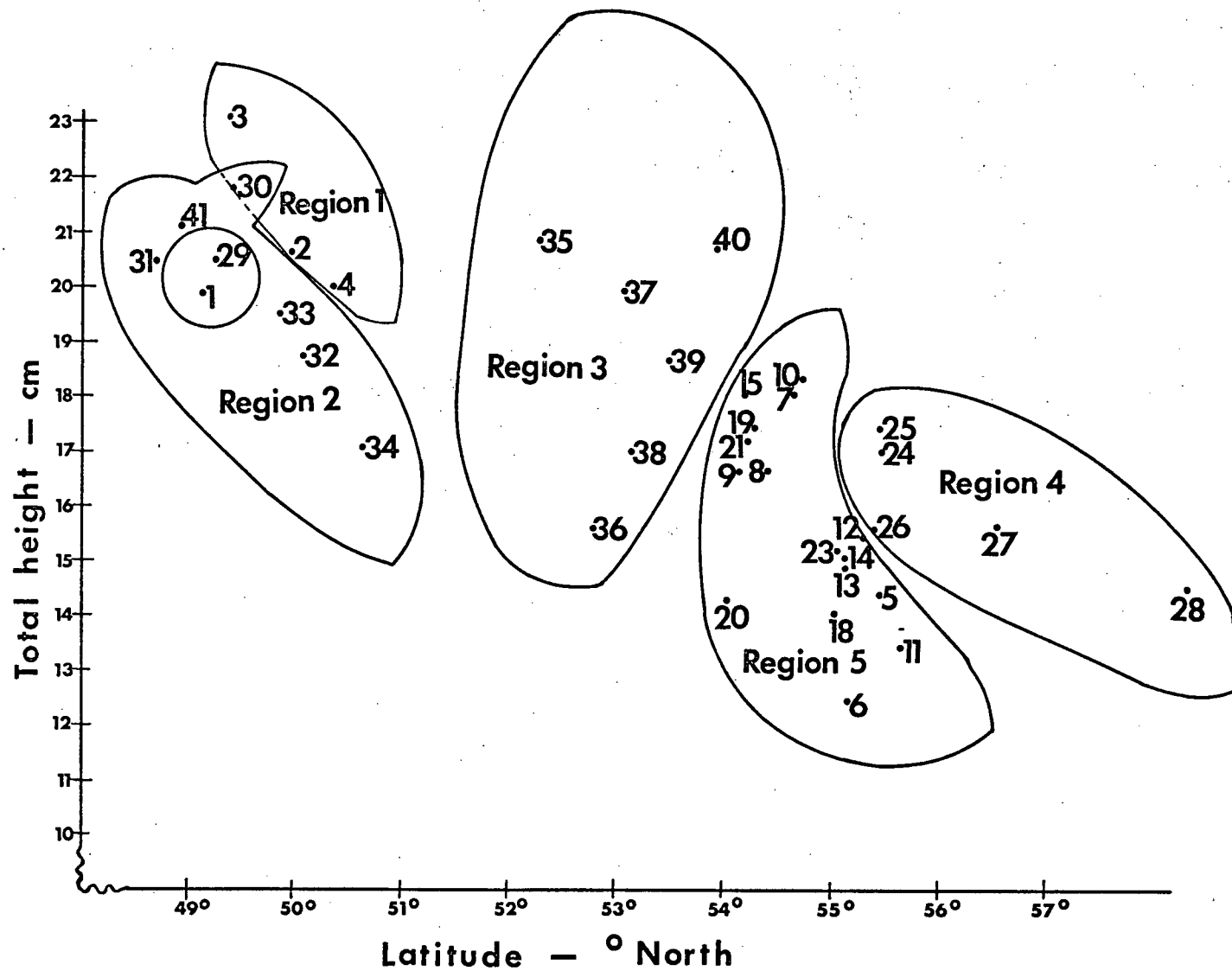


Fig.29. Relationship between total height after the second growing season and latitude of place of origin of the provenances.

Eighty-nine percent of the variation is explained by bud set 72, EPL and bud burst.

Eighty-two, point four percent by EPL alone.

The plotting of HET on latitude shows that if the relationship is linear, it is also very complex if the place of origin of the provenances is taken into account (see Fig. 29). The provenances of Vancouver Island can be splitted into two sub-groups: the west coast and the east coast groups of the Island. On this Island, HET sharply decreases with latitude. Altitude cannot be responsible for this behaviour, because the altitude of the provenances remains constant.

The provenances of the Queen Charlotte Islands seem to behave differently from the other groups of provenances: HET increases with latitude. Altitude cannot be the factor responsible for this odd behaviour.

The Alaska and the Skeena River watershed provenances do not form two distinct groups, but some divergences are noticeable. HET again decreases sharply with latitude.

The analyses of variance, the components of variance and their standard error, and the DUNCAN's tests of the means of the provenances have been calculated for each region separately.

In this case, only the provenances represented by at least five families and the families with three replications were considered.

It is important to remind the reader that the model and the formulas for the variance components and their error are quite different

from those used to analyze EPL because it was believed that there was no need to use a complicated model to analyze data as no interaction - not important at least - was noted and no exact formula for the error of the components existed for this crossed model. Thus, we used a three level nested model which has been well worked out by SEARLE and his students (SEARLE, 1971).

Again, to see if some transformation of the original height measurements was necessary, the mean HET of the provenances have been plotted against the corresponding variances, in a double logarithmic graph. The square root transformation would be the closest possible transformation - using JEFFERS' method (JEFFERS, 1959), but again, the relationship is too loose to warrant any useful results (Fig. 30).

The following tables summarize the analyses done:

Region 1 = provenances 2, 3, 4 and 29

Analysis of variance

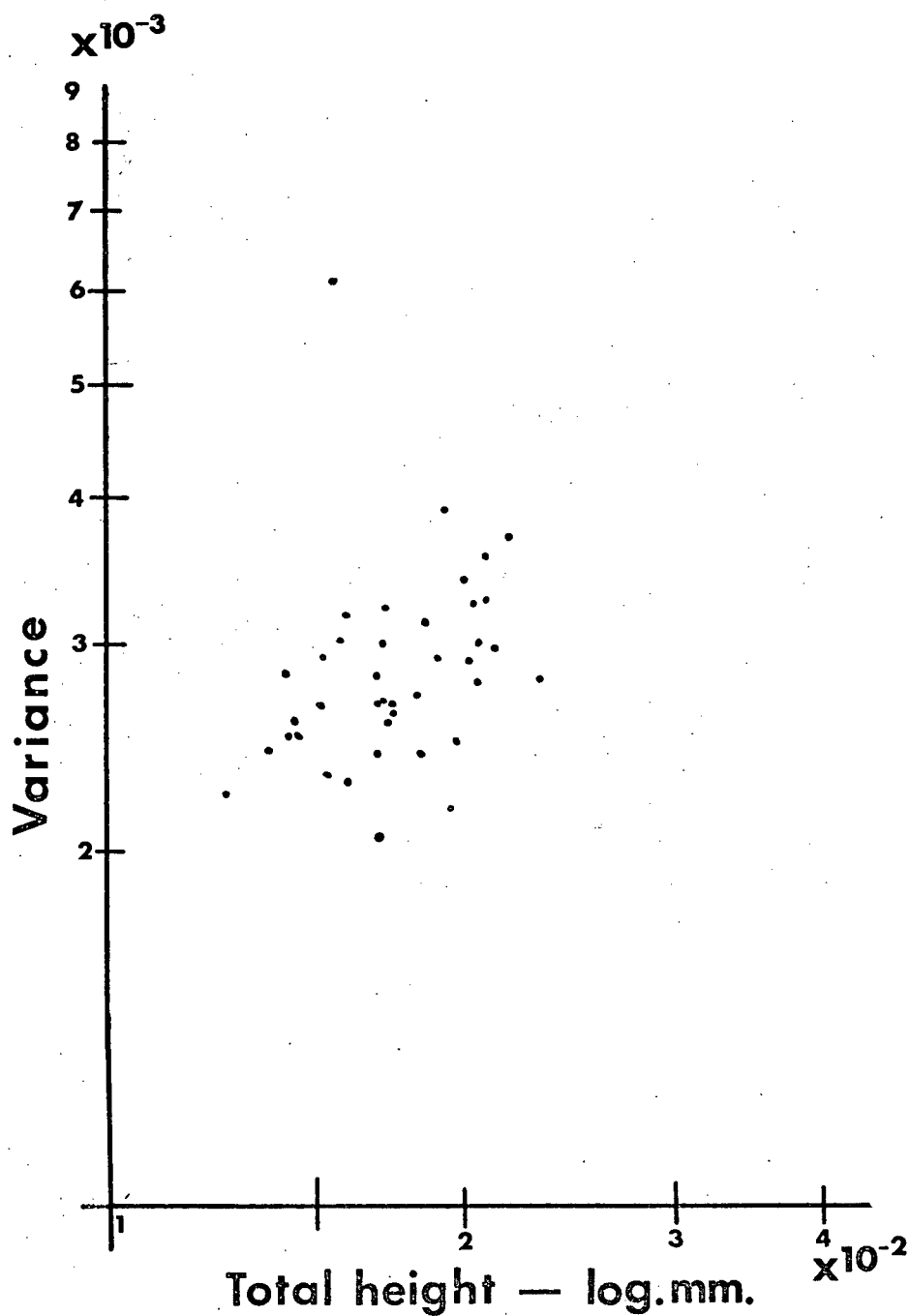
Sources of Variation	D.F.	S.S.	M.S.	F
Provenances	3	129,681.4	4,322.713	8.25 ***
Tree wn prov.	43	225,185.8	5,236.879	1.19 NS
Rep wn tree wn prov.	94	410,880.1	4,371.063	1.75 ***
Error	564	1,406,640	2,494.043	
TOTAL	704			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov. No.	4	2	29	3	
HET	=	203.3	206.3	212.5	234.4 mm

Components of variance: see Table XXVII.

Fig.30. Mean total height of the provenances, plotted against their variances, on a double logarithmic paper.





Region 2 = provenances 30, 32, 34, 41

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Provenances	3	249,628.2	82,209.328	14.91***
Tree wn prov.	30	167,355.2	5,578.504	1.17 NS
Rep wn tree wn prov.	68	321,681.2	4,730.605	2.47***
Error	408	778,323.2	1,907.655	
TOTAL	509			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov. No.	30	41	32	34	
HET	=	220.7	215.5	198.9	164.6 mm

Components of variance: see Table XXVII.

Region 3 = provenances 36, 37, 39, 40

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Provenances	3	236,654.9	78,884.94	11.29***
Tree wn prov.	48	335,373.5	6,986.945	0.94 NS
Repwn tree wn prov.	104	772,471.5	7,427.609	3.19***
Error	624	1,450,453	2,324.444	
TOTAL	779			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov. No.	40	37	39	36	
HET	=	205.7	200.1	187.2	158.5 mm

Components of variance: see Table XXVII.

Region 4 = provenances 23, 24, 25, 26, 28

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Provenances	4	78,252.9	19,563.22	3.21*
Tree wn prov.	45	273,915.2	6,087.004	1.23 NS
Rep wn tree wn prov.	100	493230.7	4,932.307	2.26***
Error	600	1,306,665	2,177.775	
TOTAL	749			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov. No.	25	24	23	28	26
HET	= 168.7	168.6	152.0	147.4	144.5

Components of variance: see Table XXVII.

Region 5 = provenances 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 18, 19

Analysis of variance

Sources of Variation	D.F.	S.S.	M.S.	F
Provenances	11	835,438.9	75,948.94	11.83***
Tree wn prov.	118	757,167.6	6,416.672	1.20 NS
Rep wn tree wn prov.	260	1,386,704	5333.476	2.71***
Error	1560	3,068,979	1,967.294	
TOTAL	1949			

DUNCAN's test: ( $\alpha = 0.05$ ).

Prov. No.	15	10	7	19	8	9	5	14	13	11	18	6
HET	= 188.5	183.1	181.9	180.0	171.5	170.2	150.7	150.7	142.0	133.8	131.9	128.2

mm

Components of variance: see Table XXVII.

TABLE XXVII  
SECOND YEAR HEIGHT VARIANCE COMPONENTS  
AND STANDARD ERROR

Region	Sources of the Effect			
	Provenances	Trees	Repetitions	Errors
1	224.6 $\pm$ 218.0	57.7 $\pm$ 86.5	375.4 $\pm$ 130.9	2,494.0 $\pm$ 148.5
2	639.1 $\pm$ 587.9	56.5 $\pm$ 110.2	564.6 $\pm$ 164.6	1,907.7 $\pm$ 133.6
3	370.2 $\pm$ 105.2	-29.4 $\pm$ 117.3	1,020.6 $\pm$ 207.7	2,324.4 $\pm$ 131.6
4	91.6 $\pm$ 96.0	77.0 $\pm$ 97.4	550.9 $\pm$ 141.8	2,177.8 $\pm$ 125.7
5	430.0 $\pm$ 204.6	72.2 $\pm$ 63.8	673.2 $\pm$ 94.6	1,967.3 $\pm$ 70.4

For both EPL and HET, the components of variance differ according to the region. EPL is characterized by smaller components of variance, in absolute value, as it should be expected, than those for HET.

The components of variance estimated for HET indicate that, generally, the genetic variance between provenances is larger than the tree to tree within provenance genetic variation, while in the case of EPL, the within provenance variation can be larger than the between variation.

The so-called error variance is the largest component. This large component might suggest that the within progeny group genetic variation is quite large, attesting a genetic plasticity in harmony with the idea that Sitka spruce might be an "opportunistic" species (see Part I, Chapter 3). However, in the absence of data on the frequency of self-pollination and the inbreeding processes taking place in Sitka spruce, no

definite interpretation is possible (personal communication with Dr. R. PETERSON).



Photo 5. Abnormal seedling with stunted growth and short and thick needles. Probably a trisomic individual. Photo by the author.

## CHAPTER 4

## THE HERITABILITY PROBLEM:

## ITS APPLICATION TO ONE CHARACTERISTIC:

## TOTAL GROWTH AFTER TWO GROWING SEASONS

Classically, the nested structure of the height data: replications within progeny/progenies within provenances/provenances is interpreted as follows: the single tree progenies come from open pollinated mother trees and can be assimilated to families of half sibs. Therefore, the variance between tree progenies within provenances is equivalent to the covariance among half sibs (after FALCONER, 1964).

But:

$$(1) \quad \sigma_{\text{Tree}}^2 = \text{cov}_{\text{half sibs}} = 1/4 V_A + 1/16 V_{AA} + 1/64 V_{AAA}$$

with  $V_A$  = additive genetic variance  
 $V_{AA}$  = additive by additive interaction variance  
 $V_{AAA}$  = additive by additive by additive interaction variance

(after BECKER, 1967).

Generally,  $\sigma_{\text{Tree}}^2$  is taken to be equal to  $1/4$  of  $V_A$ . But assuming that this last formula is true, by neglecting the non-additive gene effects, gives an overestimation of the additive genetic variance. NAMKOONG (1966) has discussed different sources of bias in using open pollinated seed for estimating the additive genetic variance: dominance effects, relatedness of the parent trees (inbreeding), restricted number

of pollen parents, all are factors which might lead to an overestimation of the true additive genetic variance. Therefore, the estimation:

$$(2) \quad V_A = 4 \cdot \sigma_{\text{tree}}^2$$

can always be accepted as an upper limit of the true variance.

An estimation of the heritability is given by:

$$(3) \quad h^2 = \frac{4 \sigma_{\text{tree}}^2}{\sigma_{\text{total}}^2}$$

with  $\sigma_{\text{total}}^2$  = sum of the components of variance estimated; it is the total phenotypic variance (after FALCONER, 1964 and BECKER, 1967).

The heritability defined by the formula (3) is the so-called narrow sense heritability, on an individual basis; it is the parameter used in the formulas to estimate possible genetic gains under different selection schemes. This heritability should not be confused with the heritability of the family means as defined by WRIGHT (1963, formula 61), (WRIGHT, 1963).

A useful approximation of the standard error of the heritability is given by:

$$(4) \quad \text{S.E. } (h_s^2) = \frac{4 \cdot \sqrt{\text{var } \sigma_{\text{tree}}^2}}{\sigma_{\text{total}}^2}$$

(According to BECKER, 1967)

The narrow sense heritability has been estimated only for the second year height growth which takes into account the natural varia-

TABLE XXVIII  
NARROW SENSE HERITABILITY FOR TOTAL HEIGHT  
AFTER THE SECOND GROWING SEASON:  
WITH THEIR ERROR - BY REGION

Region	Heritability	Standard Error
1	0.07	0.11
2	0.07	0.14
3	negligible	-
4	0.11	0.13
5	0.09	0.08

bility encountered in nursery practices with Sitka spruce.

The heritability - narrow sense - has been estimated for each region separately. Table XXVIII indicates their values and their error. The formulas (2), (3) and (4) were applied.

The study of HET was based on a random sample of five (1 - 1) seedlings per replication and per family. The narrow sense heritability calculated is generally less than 10% and is affected by a large error. However, four regions give consistent estimations for  $h^2$ .

That the narrow sense heritability, i.e., the additive genetic variance could be small for growth traits is confirmed by the results of the diallel cross performed on Sitka spruce already mentioned (SAMUEL, et al. 1972).

Therefore, family selection would be appropriate (FALCONER, 1964). The general combining ability seems to be low, therefore specific combining ability should be used through crossing between outstanding individuals and populations.



## CHAPTER 5

### RELATIONSHIPS BETWEEN SEED, CONE AND SEEDLING TRAITS STUDIED

It is interesting to relate the four seed traits studied and cone length to the seedling characters measured in the nursery in order to know if the former parental characteristics could be used to accurately predict some juvenile characters of the progenies growing in the nursery. If this was the case, then some of these parent characters could be used in an "early test" to predict the behaviour of these progenies.

A simple correlation matrix has been calculated between all the seed, cone and seedling characters, as well as with latitude, longitude and altitude of the place of origin of the provenances, (see TABLE XXIX). The legend is the same as the legends of Tables XI and XV.

A look at Table XXIX shows that the seed and cone traits are generally not significantly related with the seedling characters studied. However, wing width (b) is positively correlated with bud set measured in 1971 ( $r = 0.32$  and  $0.37$ ), needle colour ( $r = 0.39$ ); seed length (c) is positively correlated with epicotyl length and total height ( $r = 0.36$ ); seed width (d) is negatively correlated ( $r = -0.40$ ) with bud burst, positively with needle colour ( $r = 0.48$ ); cone length is negatively correlated with bud set 72 ( $r = -0.38$ ) and positively correlated with epicotyl length and total height ( $r = 0.69$  and  $0.67$ ).

TABLE XXIX

CORRELATION MATRIX BETWEEN SEED, CONE AND SEEDLING TRAITS STUDIED

	LAT	LONG	ALT	BST1	BST2	BBT	COL	BST72	EPL	HET	a	b	c	d	cone
LAT	1.00														
LONG	0.78	1.00													
ALT	0.26	-0.12	1.00												
BST1	0.89	0.53	0.39	1.00											
BST2	0.92	0.63	0.32	0.96	1.00										
BBT	-0.14	-0.51	0.42	0.13	0.07	1.00									
COL	0.64	0.72	-0.039	0.58	0.62	-0.37	1.00								
BST72	0.88	0.59	0.47	0.91	0.92	0.12	0.61	1.00							
EPL	-0.71	-0.34	-0.56	-0.74	-0.70	-0.13	-0.44	-0.75	1.00						
HET	-0.78	-0.49	-0.52	-0.74	-0.74	0.037	-0.50	-0.80	0.91	1.00					
a	-0.17	-0.099	0.0054	0.30	0.27	0.24	0.053	0.22	-0.093	-0.017	1.00				
b	0.39	0.45	-0.15	0.32	0.37	-0.21	0.39	0.28	0.0041	-0.010	0.57	1.00			
c	-0.19	-0.10	-0.28	-0.13	-0.12	-0.11	-0.052	-0.21	0.36	0.36	0.62	0.62	1.00		
d	0.34	0.49	-0.40	0.25	0.30	-0.40	0.48	0.22	0.066	-0.075	0.30	0.75	0.451	1.00	
cone	-0.30	-0.033	-0.57	-0.28	-0.28	-0.24	0.10	-0.38	0.69	0.67	0.32	0.48	0.66	0.49	1.00

 $r_{0.05} = 0.32$

The multiple regression technique used to study the other correlation matrices has been applied to test how well the seed and cone traits predict the seedling traits measured.

Fifty, point two percent of the variation in bud set 1 is explained by the five seed and cone traits, 37.7% by wing width and cone length alone. For bud set 2, these figures become 53.3% and 43.0%. Fifty-four, point nine percent of bud set 72 is explained by the five traits studied, 44.3% by wing width and cone length alone.

Thirty-five, point zero percent of the variation in bud burst is explained by the five traits studied, 31.5% by wing length and weed width alone.

Thirty-seven, point nine percent of needle colour is explained by the five traits studied; 33.3% by seed length and seed width alone.

Sixty-eight, point four percent of epicotyl length is explained by the five traits studied; 47.7% by cone length alone. For total height, these figures become 71.2% and 44.5%.

However, the relationships established between latitude, longitude and altitude of the place of origin of the provenances and the seedling traits studied (see Chapter 3, Part II) show that a higher percentage of the variation in these traits is explained by the place of origin of the provenances. For example, up to 86.2% of the variation in bud set is explained by longitude and latitude of the place of origin. Sixty-five percent of epicotyl length variation is explained by latitude and altitude

alone. Therefore, the seed and cone traits studied are not as efficient as the geographical coordinates of the provenances as predictor of the progeny characteristics. They are also more time consuming to obtain and should be used only when the origin of the provenance is unknown and as gross predictors.

It would be interesting to use the canonical correlations to study the relationship between the two sets of variates.

CHAPTER 6  
GENETIC VARIATION IN FOLIAR MACRO AND MICRO  
NUTRIENTS OF TEN SELECTED ONE YEAR OLD  
SITKA SPRUCE PROVENANCES

6.1 Introduction

So many traits present genetic differences between provenances, that it is tempting to try finding differences in mineral content that could be associated with and perhaps explain these differences.

However delicate is the interpretation of the results of plant analysis (GOUNY, 1956), more particularly foliar analysis, the recent years have seen studies of plant tissue mineral composition in relation with seed sources (MERGEN and WORRALL, 1965; STEINBECK, 1966; VAN DEN DRIESSCHE, 1969, 1973). The work of ADDISON (1966) has already been mentioned in Chapter 3, Part I.

There is also some need to know the average mineral composition of the most important tree species if fertilizer applications must be done in the nursery.

Therefore, foliar analysis of ten selected provenances of Sitka spruce were performed in order to detect any genetically based variation in mineral content which could be related to their geographic origin or which could find some explanations and also to provide some data on the mineral composition of the foliage of Sitka spruce seedlings under optimum nursery conditions.

## 6.2 Material and methods

The material used comes from 1-0 seedlings which were growing in the styro-foam containers in 1971. During the first growing season, the seedlings were fertilized regularly.

The fertilizers used were different highly soluble commercial preparations (Plant-Prod: Plant- Products Co. Ltd. Port Credit, Ont.), formulated as follows:

28 - 14 - 14

15 - 15 - 30

21 - 0 - 0

For instance, the guaranteed minimum composition advertised was, for the fertilizer 28 - 14 - 14.

Total nitrogen: 28%

Available phosphoric acid: 14%

Soluble potash: 14%

Chelated trace elements:

Manganese:	0.045%
Iron:	0.03 %
Copper:	0.0025%
Zinc:	0.014 %
Molybdenum:	0.001 %
Boron:	0.04 %

The fertilizers were fed to the seedlings through the irrigation system. The guiding principles were to supply the seedlings with high levels of nitrogen through mid-summer and to reduce nitrogen supply and increase potassium towards the end of the growing season. To reduce high salt accumulation, the fertilizers 28 - 14 - 14 and 15 - 15 - 30 were alternated with ammonium sulfate (21 - 0 - 0). Leaching with

water avoided build-up of fertilizer to any toxic level in the plug soil (after ARNOTT, 1971). A complete record of the fertilizer applications which began in June, 1971 shows that the applications were made weekly or bi-weekly and varied from 1.03 gr to 9.44 gr of a given fertilizer per styro-foam container. The last application was made on the 30th of November, 1971 (15 - 15 - 30). Most applications were of the fertilizer 28 - 14 - 14.

To avoid any growth artifact effects as evidenced by the studies of MERGEN and WORRALL (1965) and other authors (GOUNY, op. cit.), the ten selected provenances were sampled on the 23rd of February, 1972, still quiescent. The provenances were chosen according to their place of origin along a latitudinal gradient, to study a major source of variation.

Three blocks A, B and D were sampled.

Healthy seedlings were clipped, immediately put in plastic bags and freeze dried the same day in a "Thermovac Industries Corporation Freeze Drier". The condenser temperature was at  $-64^{\circ}\text{F}$ , the shelves at  $+70^{\circ}\text{F}$  and the vacuum reached 80-90 microns of Mercury. The freeze drying process lasted five days and the freeze dried seedlings were put in sealed plastic bags and kept in deep freeze until the analyses were done.

The dry needles were separated from the stems and milled in a WILEY mill until the powder obtained passed through a 60 mesh sieve.

Then digestion in perchloric acid mixture was performed.

Iron contamination by the mill was considered negligible and in any case, random.

Phosphorus was determined by the molybdenum blue method of DICKMAN and BRAY.

Potassium, Calcium, Magnesium, Manganese, Iron and Zinc were determined using an atomic absorption flame-emission JARRELL-ASH spectro-photometer.

Nitrogen was determined by the well known semi-micro KJELDAHL method.

The methods used are all outlined by CHAPMAN and PRATT (1961).

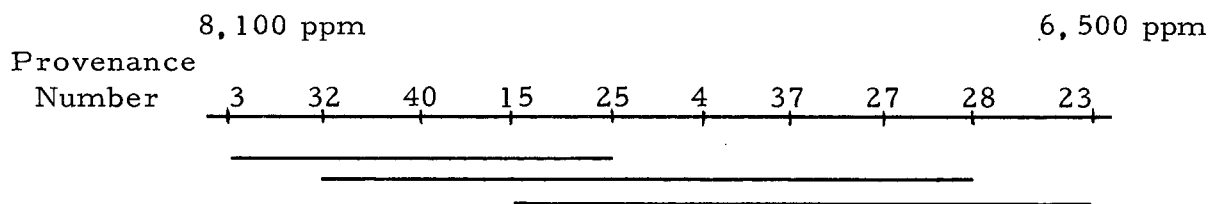
### 6.3 Results

The results of the analyses are shown in Tables XXX and XXXI. They are quite remarkable. There are no differences in macro and micro nutrients contents which could be attributable to the geographic origin of the provenances. However, the epicotyl length of the ten provenances varies between 66.5 mm (provenance 27) and 89.6 mm (provenance 40).

The analyses of variance show that there are no significant differences between the provenances for all the elements analyzed except for Potassium. The anova model was the randomized complete block design with three blocks.

The DUNCAN's test for Potassium is as follows:





The interpretation of these results is difficult, but no more than if we had found genetic variation in mineral contents of the needles of Sitka spruce provenances. Thus, there are no differences in absorption and storage of the nutrients studied in the foliage of one year old Sitka spruce seedlings or there are differences which appear at some time of the year; but these differences disappear because there are counterbalancing differences in rates of leaching or in rates of internal translocation of the same element. Another hypothesis would be that there are different rates of change in carbohydrate content as, according to REUTHER and SMITH (in CHILDERS, ed. 1954), a change in concentration of a mineral nutrient in leaves of citrus species may be caused by an accumulation or depletion of dry matter. This latter hypothesis is difficult to visualize in our case.

Nutrient absorption, translocation and loss in forest trees are not well understood (VOIGT, 1968). Discussions are generally carried out at a general level that gives little or no indication of the reasons for the differences - if any - in the ability of plants to accumulate nutrients.

GOUNY (op. cit.) has reviewed the difficulties in interpreting the results of foliar and other plant tissue analysis. However,

TABLE XXX

FOLIAGE ANALYSIS OF 10 ONE YEAR OLD DORMANT SITKA SPRUCE PROVENANCES

## 100 PPM DRY NEEDLES

<u>Nr PROV</u>	<u>Fe</u>			<u>Mn</u>			<u>K</u>			<u>Mg<sup>(*)</sup></u>			<u>Ca</u>		
	<u>A</u>	<u>B</u>	<u>D</u>	<u>A</u>	<u>B</u>	<u>D</u>	<u>A</u>	<u>B</u>	<u>D</u>	<u>A</u>	<u>B</u>	<u>D</u>	<u>A</u>	<u>B</u>	<u>D</u>
3	1.4	3.5	1.4	1.5	1.5	1.5	78	79	87	.58	.58	.62	16	15	18
4	1.4	1.4	1.4	1.6	1.4	1.3	71	70	74	.62	.58	.54	14	17	17
15	1.4	2.2	1.4	1.5	1.7	1.5	71	78	74	.62	.58	.58	10	14	18
23	1.4	3.3	1.4	1.5	1.4	1.5	62	62	70	.66	.62	.54	17	13	18
25	1.4	5.1	1.4	1.6	1.5	1.5	72	80	66	.62	.71	.50	17	16	16
27	1.4	3.3	1.4	1.5	1.5	1.5	62	75	70	.66	.62	.58	15	17	18
28	3.3	1.4	1.4	1.7	1.5	1.5	76	69	62	.75	.66	.58	14	18	15
32	1.4	2.2	1.4	1.6	1.5	1.6	71	78	81	.62	.56	.54	14	16	15
37	1.4	1.4	1.4	1.5	1.5	1.5	72	76	66	.58	.58	.62	15	17	18
40	1.4	1.4	2.6	1.6	1.5	1.5	74	76	76	.62	.56	.58	15	17	15

ZN: content less than 20 PPM

(\*) The figures for the Mg content must be multiplied by 5,000.

TABLE XXXI

FOLIAGE ANALYSIS OF 10 ONE YEAR OLD DORMANT SITKA SPRUCE PROVENANCES

% NEEDLE DRY WEIGHT

<u>Nr PROV</u>	<u>P</u>			<u>N</u>		
	<u>A</u>	<u>B</u>	<u>D</u>	<u>A</u>	<u>B</u>	<u>D</u>
3	.53	.44	.49	1.24	1.16	1.26
4	.45	.39	.51	1.27	1.12	1.46
15	.35	.46	.57	1.26	1.34	1.38
23	.49	.42	.32	1.08	1.24	1.22
25	.50	.35	.45	1.24	1.34	1.35
27	.58	.53	.50	1.44	1.21	1.40
28	.50	.39	.43	1.40	1.36	1.35
32	.50	.42	.55	1.18	1.16	1.32
37	.43	.46	.49	1.33	1.25	1.24
40	.42	.47	.58	1.14	1.32	1.22

LEYTON (in I.H.R.O., 1956) has found significant linear correlations between tree height and concentrations of N, P, K and ash in needles of Japanese larch.

Authors diverge as to the significance of mineral concentration of plant tissue: time is important as well as the precise physiological stage of the plant (GOUNY, op. cit.); LAVENDER and CARMICHAEL (1966), WARING and YOUNGBERG (1972), LEAF (1968), believe that much biological information is lost by sampling foliage during the dormant season or in the fall. The best time to characterize the differences would be while growth takes place. Foliage might not necessarily reflect the total absorption of the elements by the seedlings (LEAF, op. cit.). However, VAN DEN DRIESSCHE (1969<sub>b</sub>) does not think so on the basis of his studies on mineral nutrition of Douglas-fir and Sitka spruce. LAVENDER (1970) believes that if the mineral content of a given plant population could be shown to be correlated with growth, this would be evidence of a deficiency.

Despite the difficulties in interpreting the results of plant tissue analysis, several authors have researched genetically based differences in mineral composition of plant tissues.

MERGEN and WORRALL (1965) have found differences in mineral content of seed sources of Jack pine: the differences might be due to differences in ability of utilizing the materials from the soil medium and partly to differences in growth responses to different environments. The two authors do not mention the precise development stage at

the time of harvest: 90 days old. Nitrogen content was correlated with weight.

STEINBECK (1966) has found great differences between provenances of Scots pine for N, P, Na, Mg and B. However, interactions were noted and no explanations have been proposed for the differences observed.

VAN DEN DRIESCHE (1969) has studied the relationships between the growth of Douglas-fir seedlings and levels of some soil and tissue nutrients. Only 19% of the two year old shoot dry weight variability was accounted for by the first year concentrations in P, K, Mg. The same author (1969<sub>b</sub>), has extensively studied the tissue nutrient concentrations of Sitka spruce and Douglas-fir under different nutrient solutions. Generally, needle concentrations paralleled stem and root concentrations. Seed mineral composition was irrelevant. Differences in all nutrients except P were found in four seed sources of Douglas-fir. The same author (1973) has further studied the differences in nutrient concentrations of foliage of different provenances of Douglas-fir. Significant differences were found, but different kinds of interactions existed. Deductions about nursery, site nutrient availability made from foliar analyses would only be applicable for a given provenance grown on that site because the differences between provenances varied from one nursery to another, as well as the growth relationships.

It is important to note that all the studies mentioned were based on seedlings still growing or sampled at the beginning of the rest-

ing period (October to December). No precise description of the physiological stage has ever been attempted.

Our results show that time is important and the differences, if they ever existed, could disappear late in the cold season. The seedlings were sampled in February. They were still dormant. If the seedlings were in a state of rest imposed by the environmental conditions or still in a state of irreversible dormancy, is not known. The mechanisms of such uniformization of the mineral composition of the foliage of Sitka spruce are difficult to visualize or to understand as to their possible significance in the physiological processes of the tree.

The results of some authors seem to point out that, even when nutrient supply is abundant, there is higher use of minerals by the trees growing the fastest, as attested by their higher mineral content. Consequently, our data would show that the differences in mineral compositions detected by the authors mentioned, would be due to higher metabolic activities connected with higher growth rates.

It would be interesting to study the mineral composition of provenances of Sitka spruce under different mineral nutrition stresses to further test the hypothesis that Sitka spruce provenances might have different abilities to absorb the mineral elements.

The mineral contents of the needles of ten Sitka spruce provenances are within the range of mineral compositions of Sitka spruce foliage compiled by VAN DEN DRIESSE (1969). However, it seems that the concentrations obtained by us are fairly high, in comparison with those shown by the latter author.

The recent analyses of Sitka spruce seedlings by BENZIAN and SMITH (1973) are not directly comparable as they are based on whole plants.

## PART IV

### SUMMARY AND CONCLUSIONS



## SUMMARY AND CONCLUSIONS

(1) In a first part, seed and cone morphology of 557 Sitka spruce trees representing 39 locations were studied on a single tree basis. Ten cones per progeny were randomly selected and the length of each cone measured to the nearest mm. Five randomly selected seeds from each tree were mounted on a special sheet, and seed length, seed width, wing length and wing width were measured to the nearest 0.01 mm. Nested analysis of variance and DUNCAN's multiple range tests for all the characteristics studied have been performed using five working subregions based on biogeoclimatic data. No definite classification of the provenances was possible by using univariate anova procedures. A simple correlation matrix has been calculated between all the traits studied and longitude, latitude and altitude of the place of origin of the provenances, using the provenance means. Multiple regression analyses have been used for investigating this correlation matrix. The percentage of variation accounted for by the geographical coordinates varies between 10.2% to 43.6%. Components of variance calculated for the traits studied indicate that generally the variability is mostly confined to the between tree, within provenance variability. Therefore, the traits studied are not likely to be good discriminatory characters for distinguishing the provenances. Tentatively, the traits with the best discriminating power were chosen as seed length, seed width and cone length because they show a greater variability between provenances.

To see if the simultaneous consideration of the five reproductive traits studied could result in a better classification of the Sitka spruce provenances, some multivariate statistical techniques were tried. Prior to any definite classification, the basic problem of finding the best classification method was attempted.

(2) Using the seed and cone traits studied, a comparison of several multivariate statistical analyses which can be used for classification purposes has been attempted. The so-called canonical analysis, discriminant function analysis and principal component analysis have been compared and applied for classifying the provenances. The subregions were analyzed separately. Dendrograms were also constructed and analyzed. Advantages and disadvantages of each multivariate method have been discussed. It was found that the discriminant function analysis, its associated generalized distances of MAHALANOBIS and dendrograms provided the most rational classification of the provenances. A comparison of the techniques with the classification possible by a MANOVA followed by a multivariate multiple comparison of the centroids would be useful. Another approach to be tried would be to apply the techniques studied to a "plasmode" for a definition, see CATTELL (1965), i.e., a set object whose properties and clustering are known, in order to compare the classifications made by these techniques.

(3) In a second part, the genetic variability of 545 Sitka spruce

single tree progenies was studied in a nursery test during the 1971 and 1972 growing seasons.

A total of 545 single tree progenies grouped into 38 provenances was sown in April, 1971, using a randomized complete block design with four replications and 24 seedlings per replication or 96 seedlings per progeny. The seeds were placed in the cavities of styro-blocks using the method developed by the Pacific Forest Research Centre in cooperation with the B.C. Forest Service and they have been treated by the most recent nursery methods, in the new B.C.F.S. nursery at Surrey (B.C.). Germination rate, bud set, length of the epicotyl and survival after the first growing season were assessed in 1971. The seedlings were transplanted in plain soil seedbeds in May, 1972, to a distance of 6" to 6", each progeny being kept separate while respecting the same statistical design as in 1971. Bud burst, bud set, colour of the needles and total height after the second growing season were assessed in 1972. There was a clinal variation in bud burst, bud set, colour of the needles and epicotyl length. Bud burst was negatively correlated with longitude ( $r = -0.50$ ) and positively correlated with altitude ( $r = 0.42$ ). Bud set appeared under strict genetic control as indicated by the second estimation of this trait, at the end of the second growing season (with latitude:  $r = 0.88$ ). Latitude and altitude of the seed sources explained 65% of the total variation in epicotyl length. Total height after the second growing season showed the same relationships as epicotyl length.

Therefore, there is a clinal variation, mostly with latitude, for all the traits studied as well as a covariance structure between traits which might result in correlated responses when selection for one character is attempted. Bud set and bud burst are important adaptative characteristics which might be adversely affected when selection for height growth is tried. Bud set determines somehow the cessation of height growth as well as the cold hardiness of the Sitka spruce provenances.

(4) The important question of the physiological mechanism controlling bud set and bud burst in Sitka spruce remains to be solved as our data do not support directly the idea that bud set is controlled by the declining day-length of late summer. BURLEY's growth chamber studies suggest that declining photoperiod might hasten bud setting in Sitka spruce. The present study shows that, in natural conditions, 86.2% of the variation in bud set can be explained by longitude and latitude, 79% by latitude alone. Forty-four percent of bud burst variation is explained by latitude and longitude, 26% by longitude alone. Our research shows that there is some evidence that the differences in bud flushing are related to the local late frost distribution of the place of origin.

The study of the growth responses of Sitka spruce provenances to shortened and extended photoperiods and under different temperature regimes should provide some clues as to the mechanisms of bud burst and bud set prevalent in this tree species.

- (5) General equations for components of variance for unbalanced data were originally calculated for a nested-crossed model. Components of variance and their standard error were calculated for epicotyl length and total height after the second growing season. Depending on the subregions, the genetic variance among provenances is generally larger than the tree to tree genetic variation.
- (6) The narrow sense heritability, on an individual basis, and its standard error, for total height after the second growing season were estimated on a subregion basis. Heritability varied between 0.07 and 0.11. These results, as well as those of SAMUEL, et al. (1972), suggest that family selection would be appropriate and that the specific combining ability should be used through crossing between outstanding individuals and populations.
- (7) The correlations between the seed, cone and seedling traits were studied. Wing width was positively correlated with bud set ( $r = 0.32$  and  $0.37$ ), and needle colour. Seed length was positively correlated with epicotyl length and total height ( $r = 0.36$ ), cone length is negatively correlated with bud set 72 and positively with epicotyl length and total height ( $r = 0.69$  and  $0.67$ ). However, the geographical coordinates of the place of origin of the provenances explain more of the variation of the seedling traits than the cone and seed characters which appear to be less useful as "early test characters". The relationships established might indicate some "maternal" effects.

(8) Variation in foliar macro- and micro- nutrients of ten provenances was studied but no geographical pattern of variation detected in K, Ca, Mg, Fe, Mn, Zn, P and N needle contents. Only K showed some provenance to provenance variations. Possible physiological explanations for this absence of variation are discussed. The data obtained in this study would show that the differences in mineral composition detected by some authors might be due to different metabolic activities connected with different growth rates. It would be interesting to study the mineral composition of different Sitka spruce provenances under different mineral nutrition stresses to further test the hypothesis that Sitka spruce provenances might have different abilities to absorb the mineral elements.

(9) The phenotypic variation of the parent trees of 545 Sitka spruce progenies has been studied on the basis of five seed and cone traits in order to begin classifying the natural populations of this tree species. Important adaptative characteristics of the progenies growing in a nursery near Vancouver (B.C.) have also been studied as early test characters. The progenies have been outplanted in October, 1973 and spring 1974 on the Queen Charlotte Islands and on Vancouver Island by the B.C. Forest Service, to study their genetic variability in different field environments. Genotype by environment interactions and susceptibility to weevil attack will be studied.

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