Parent tree variation in Sitka spruce provenances, an example of fine geographic variation[1]

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Summary

On the basis of five seed and cone traits, 32 Sitka spruce provenances are shown to present a fine pattern of geographic variation. This variation pattern was best detected when canonical analysis was performed on an ecological region basis. Discussion of the present literature leads to the conclusion that complex stepped eco-clines may exist in the species.

Key words: Sitka spruce provenances — seed and cone traits — parent trees — canonical analyses — intra-regional variation.

Zusammenfassung

An Hand der Untersuchung der Zapfen und Samen (Messung der Zapfenlänge, der Samenlänge und -breite sowie der Samenflügellänge und -breite) von 32 ***Picea sitchensis***-Herkünften aus dem natürlichen Verbreitungsgebiet der Art konnte eine geographische Variation bzw. geographische Merkmalsprogression festgestellt werden.

Introduction

In 1971, an extensive provenance study of Sitka spruce ***(Picea sitchensis*** (Bong.)Carr.) was started which included also the study of variation between trees, within provenances. In a recent paper (Falkenhagen and Nash, in press), it was shown that the 5 seed and cone traits measured on 557 Sitka spruce parent trees grouped into 39 provenances varied geographically to such an extent that the place of origin of the populations studied could be used to predict fairly well their cone and seed morphology. Furthermore, it was also shown statistically that this pattern of variation was best detected when the provenances were grouped and analyced according to subsets called ecological regions. In that paper, the biological aspects were not treated extensively.

It is proposed to present this geographic variation in detail here and to discuss the possible reasons and implications of this variability for tree breeding and forestry in general.

Burley (1965) has studied seed weight variability in 30 provenances of Sitka spruce but did not find significant relationship with latitude. There was however a trend for northern provenances to have heavier seeds. Daubenmire (1968) studied the morphological variability of adult trees in ten natural populations of Sitka spruce. He claims that there is a clinal variation in cone size and sterigma angle which decreased from south to north. Possible introgression with ***Picea glauca*** of some populations of the Skeena river watershed was discussed by the author who proposed that length : width ratio of the cone scales be used to differentiate insular from mainland populations (Daubenmire, 1968).

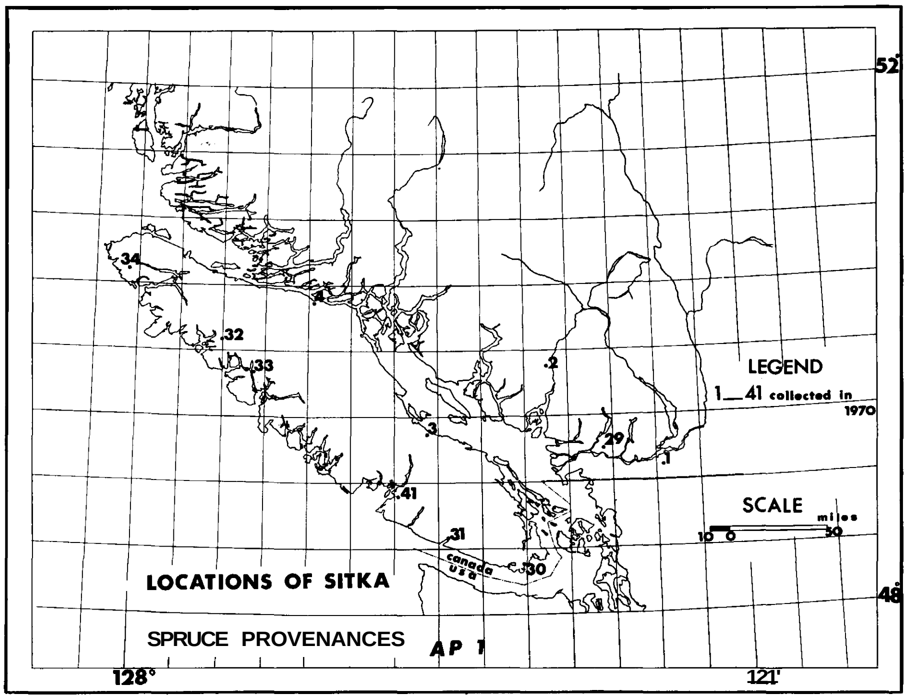

Figure 1. — Geographical locations of the Sitka spruce provenances of the 1970 I.U.F.R.O. collection: The numbers are the provenance numbers. First map.

Materials and Methods

The geographical coordinates of the Sitka spruce provenances studied have been given elsewhere (Falkenhagen, 1971). The five reproductive characters measured were: cone length, seed and wing length and seed and wing width.

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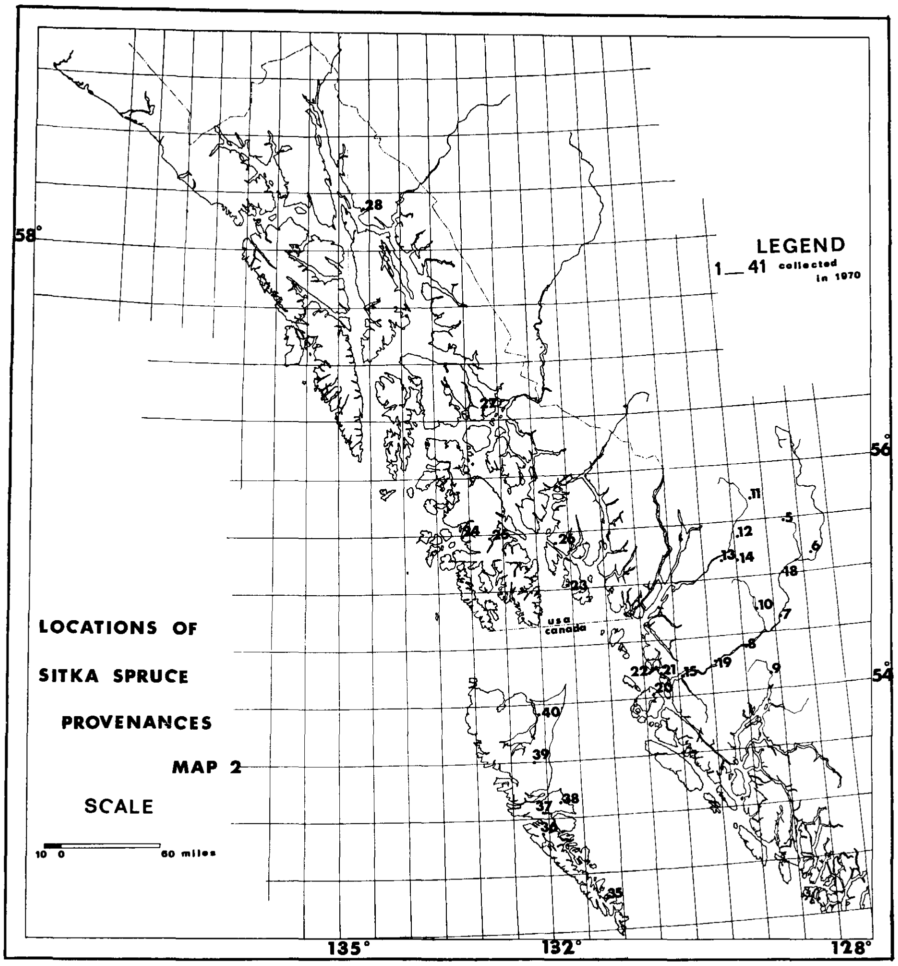

Figure 2. — Same as for *Figure 1* but Second map.

Five seeds and 10 cones per tree and up to fifteen trees per provenances were measured, with the following accuracy: seed traits to the nearest 0,01 mm, cone length to the nearest mm. Only the provenances with 15 trees are dealt with in this study (Falkenhagen and Nash, in press). *Fig. 1* and *2* show the location of the provenances studied and are presented here because they are important to the following discussion.

The statistical methods used to analyse the 5 traits were: analyses of variance, multiple correlation and regression analyses, canonical correlation analyses and discriminant function analyses (also called canonical analysis by Seal (1964)). Only the results of the canonical analyses will be presented here. Canonical analysis has been developed in order to discriminate between several multivariate groups. It is another interpretation of discriminant function analysis. Suppose that we have h p-variate normal universes represented by samples, then 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 of the samples, 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. The centroids of the different universes can be expressed in canonical form and their mutual positions visualized in a diagram. Confidence circles can be drawn around the points representing the universes in the graphs. (Seal, 1964; Falkenhagen and Nash, 1976).

Results and Discussion

The results are best shown in a series of graphs *(Fig. 3, 4, 5* and *6)*. Each figure corresponds to some "a priori" delimited ecological region. The ecological regions were defined as follows:

Region 1: Eastern coast of Vancouver island and the Lower Fraser Valley.
Region 2: Western coast of Vancouver island.
Region 3: Queen Charlotte islands.
Region 4: Alaska panhandle.
Region 5: Skeena river basin and adjacent areas.

The following comments can be made if we carefully compare the plotting of the provenances in the different canonical spaces and their physiographic position in *figures 1* and *2*. The first two axes are used here as they have proved the most useful in each case.

The two first canonical axes account for 66% of the total variation for Regions 1 and 2 pooled, 63% for Region 3, 62% for Region 4 and 62% for Region 5. The other canonical axes did not lead to any meaningful interpretation.

Regions 1 and 2 pooled (= Vancouver island) (Fig. 3.)

The first canonical axis represents a gradient parallel to the longitudinal axis of the island while the second axis is parallel to the small east-west axis of the island mass and seems to contrast the west coast from the east coast which belong to different climatic subregions. Only the provenance 31 seems oddly placed between the provenances 3 and 4 of the east coast of the island. There is no ready made explanation for this anomaly except for random drift, perhaps. Thus 1 out of 7 provenances (or 14%) is not plotted according to its geographic origin.

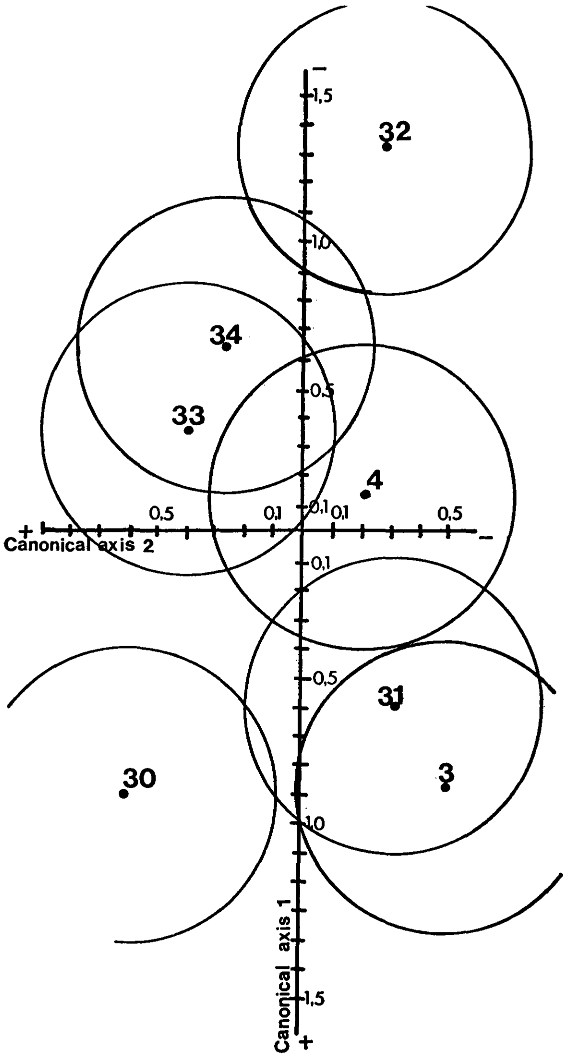

Figure 3. — Canonical analysis for Region 1 and 2 pooled. Plotting made using the first two canonical axes. The numbers are the provenance numbers. The circles shown are 95% confidence circles (Seal, 1964). The more the circles overlap, the less different are the provenances.

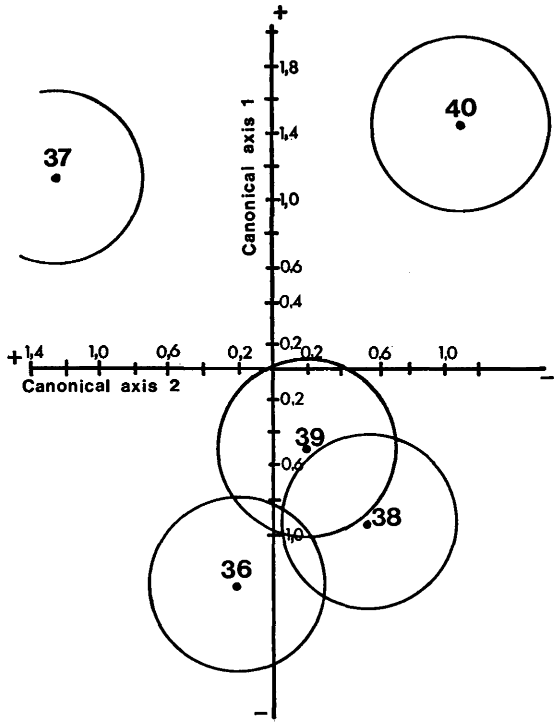

Figure 4. — Canonical analysis for Region 3. Same comments as for *Fig. 3.*

Region 3. (Fig. 4).

The first canonical axis seems to represent a latitudinal gradient, while the second axis positions the provenances according to their longitudinal position. The only seemingly aberrant provenance is the provenance 37, well isolated (as is the provenance 40) in the canonical space. 4 out of 5 provenances (or 80%) are plotted according to their geographic origin.

Region 4. (Fig. 5).

Again the two first canonical axes seem to represent geographical gradients if we discount the provenance 26 and the provenance 23 which is completely isolated from the other provenances. Again 4 out of 6 provenances (or 67%) are plotted according to their geographic origin.

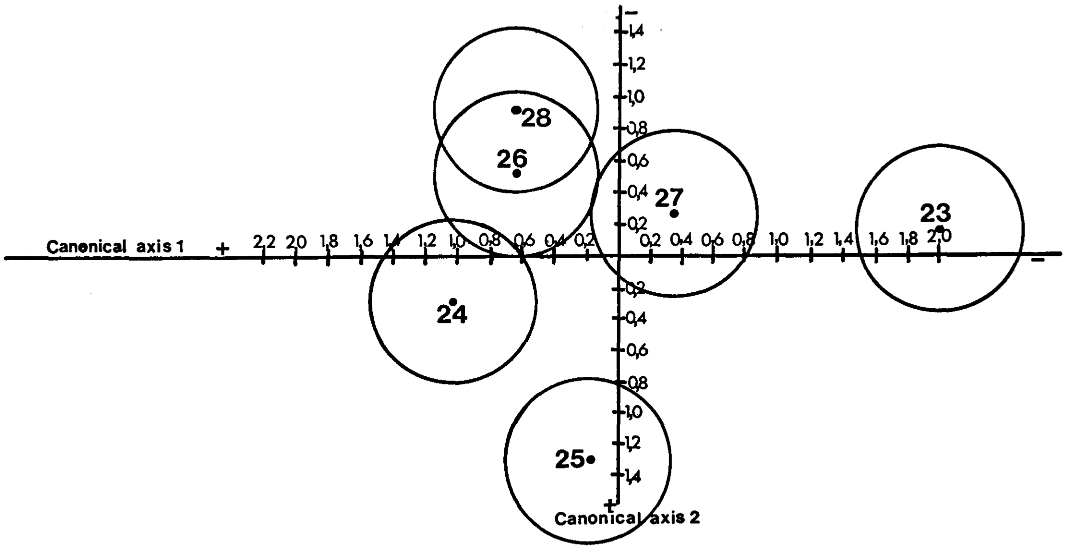

Figure 5. — Canonical analysis for Region 4. Same comments as for *Fig. 3.*

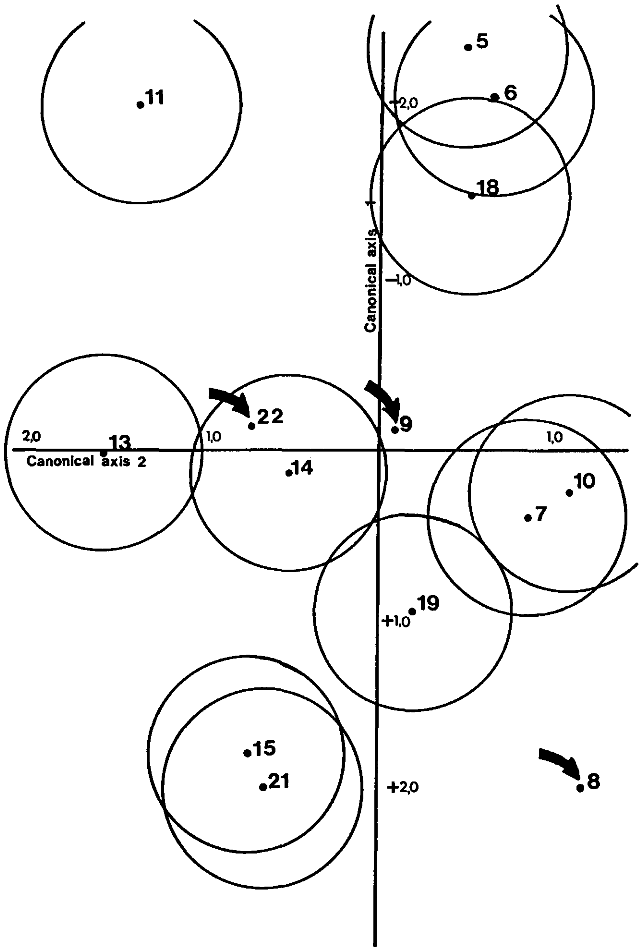

Figure 6. — Canonical analysis for Region 5. Same comments as for *Fig. 3.*

Region 5 (Fig. 6) represents the most striking example of geographic variation. The two first canonical axes remarkably position the provenances according to the latitude, longitude and elevation of their place of origin. Provenance 9 appears to be oddly placed but in fact, its plotting reflects the high altitude of its place of origin. The same is true for the provenance 22 which is a high elevation coastal provenance. The position of the provenance 8 is perhaps the least explainable. The provenances 8, 9 and 22 are indicated by arrows and for clarity reasons do not have 95% confidence circles. More than 90% of the provenances are plotted according to geographic origin.

Thus, for each region, there is a striking similarity between the geographic location of the provenances on the maps and their plotting in the canonical spaces resulting from the transformation of the original hyper-space of the morphological traits. This clearly indicates that the reproductive characters of the parent trees are correlated with their local environments.

When the provenance means are used, a correlation matrix *(Table 1)* between the traits studied and latitude, longitude and elevation of the place of origin of the provenances shows that wing width, seed width and cone length are significantly correlated with the geographic coordinates, thus indicating some continuous variation. However the coefficients of determination vary between 12% and 32%. Multiple correlation and regression analyses furthermore show that wing length can be predicted by longitude and latitude together, wing width by longitude alone, seed width by longitude and altitude together and cone length by altitude alone. For more details on the method used see FALKENHAGEN (1978). However the coefficient of determination of these regression equations oscillates between 19 and 38% leaving much of the individual trait variation unexplained.

Nested analyses of variance were performed on each trait, neglecting the five ecological regions. The results are shown in *Table 2*.

Table 1. — Correlation matrix between the seed characteristics and cone length and the geographical coordinates of the place of origin of the 39 provenances studied.

	a	b	c	d	Cone	Long.	Lat.	Alt.
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

a = wing length.
b = wing width.
c = seed length.
d = seed width.
cone = cone length.
Long = longitude in degrees; hundredth.
Lat = latitude in degrees; hundredth.
Alt = in feet.

NS Not significant at 5% level.
* significant at 5%.
** significant at 1%.
*** significant at 0.1%.

Table 2. — F Values of the nested anova performed on the different traits measured.

Sources of Variation	D.F.	Seed Traits a	b	c	d	D.F.	Cone Length
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, because of heterogeneity of variance problems.

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.

Nested analyses of variance were also performed on each trait and for each region separately with two levels of variation: trees and provenances. The components of variance calculated show that the variability between provenances is fairly large for seed length and width and cone length. However, the components of variance for the between trees variability remain always above 50%. Taken individually, the 5 traits do not tell much about the geographic differentiation of the populations but a multivariable approach clearly indicates that there are complex topoclines combined with large topotypes in Sitka spruce. Within each ecological region, it is possible that ecoclinal variation exists as it is examplified by the ordering of the provenances of Region 5 along altitudinal gradients corresponding to river valleys.

The hypothesis which comes to mind first is that there exist stepped ecoclines, with discontinuities at the ecological region levels. These regions themselves change according to ecogeographic gradients and, within each region, regional ecoclines exist. The reproductive traits measured are phenotypic and as such their ontogeny is under the influence of the local environment. It has been suggested (Rowe, 1964) that direct preconditioning by the environment of the seed (and of the resulting seedling) is possible. A survey of the forestry literature of the past decade has failed to show us further research on this very important question. Mention of the problem has been made but no experimental research started (McGee, 1973). Stern uses the word preconditioning in a very different way from Rowe's one (Stern and Roche, 1974). Langlet (1967 and 1971) is convinced that habitat correlated variation shown in a comparative test of seeds of different origins is heritable. Langlet (1967 p. 440) says: "what is remarkable is that Engler (1913), also proved that offspring from lowland spruce that had been cultivated for 30—40 years at high altitudes, preserved most of their lowland — spruce characters when tested in the vicinity of Zürich. He was thus able to demonstrate that the actual adaptive characteristics were in fact hereditary" Heslop — Harrison (1964) contents that there is no direct evidence of habitat correlated variation which is not adaptive and not hereditary. Some genecologists do not even mention preconditioning as factors of differentiation (Timofeeff — Ressovsky (1940), Wilkins (1968) and Ehrendorfer (1968)).

Simak (1967) in a careful study of the variation of seed weight of different larch (*Larix decidua* Mill) provenances has shown that those larch provenances cultivated as exotics in different European countries produce seed whose thousand seed weight (and seed length which is strongly correlated with this trait) falls within the limits for this character for the original provenances. In other words, thousand seed weight seems to be highly heritable in European larch. However, thousand seed weight of Scots pine is not so strongly inherited (Simak, 1967).

On the basis of the present scientific literature, one can thus accept that the seed and cone characters of the Sitka spruce parent trees are probably adaptive because of their fine geographic variation. This fine adaptive variation is genetically based and perhaps highly heritable. Naturally more research is eneded. Only the comparison of cone and seed traits of second generation stands of known origin or of crosses in seed orchards of Sitka spruce with the same traits of autochthonous stands can solve the problem of the heritability of these traits.

Are the cone and seed traits studied related to the characteristics of the corresponding progenies? A detailed multiple correlation and regression analysis of the correlation matrix between the parental traits and some characteristics of the progenies raised in a nursery (see Falkenhagen, 1976) has given the following results (only the most important results are shown here): wing width was positively correlated with bud set measured in 1971 ($r = 0.35$) and needle colour ($r = 0.39$); seed length was positively correlated with epicotyl length and total height after the second growing season ($r = 0.36$); seed width was negatively correlated with bud set ($r = —0.38$) and positively correlated with epicotyl length and total height ($r = 0.68$) ($r_{0.05} = 0.32$). Of the variation in bud set 52.2% was explained by the five seed and cone traits, 37.7% by wing width and cone length together. For bud burst, 35.0% of its variation is explained by the 5 traits studied, 31.5% by wing length and seed width together. For epicotyl length, 68.4% of the variation is explained by the 5 traits studied, 47.7% by cone length alone. For total height, these figures become 71.2% and 44.5%. These figures point to the fact that parental phenotypic traits are correlated with the corresponding progeny traits as well as with the geographical coordinates of the place of origin of the Sitka spruce provenances.

Thus, five seeds per tree and fifteen trees per provenance can be used not only to identify the origin of a provenance but also to predict the characteristics of the progenies growing in a nursery. If multivariate statistical techniques are used, then accurate predictions are possible.

Among the factors of genecological differentiation which are probably at play for the type of variation described, natural selection is likely to be predominant with random drift negligible in overcoming possible migration from neighbouring populations of spruces.

If the phenotypic and genetic plasticity of Sitka spruce were small, then the pattern of variation would point to a narrow range for seed transfer for reforestation or afforestation purposes. However, widespread success of Sitka spruce as exotics in Europe and elsewhere does seem to indicate a great plasticity provided that its minimum ecological requirements are met.

Literature

Burley, J.: Variation in seed characteristics of Sitka spruce. Advancing frontiers of plant sciences **10**: 11—24 (1965). — Daubenmire, R.: Some geographic variation in *Picea sitchensis* and their ecologic interpretation. Can. Jl. of Botany **46**: 787—798 (1968). — Ehrendorfer, F.: Geographical and ecological aspects of intraspecific differentiation. In: Modern Methods in Plant Taxonomy (V. H. Heywood, p. 261—296). — Academic press. 1 vol. 312 pp. (1968). — Falkenhagen, E. R. and Nash, S. W.: Multivariate classification in provenance research: a comparison of two statistical techniques. Silvae Genetica **27**: 14—23 (1978). — Falkenhagen, E. R.: Genetic variation in 38 provenances of the I.U.F.R.O 1970 Sitka spruce samples (in press). — Langlet, O.: Regional Intra-specific variousness. München XIV. I.U.F.R.O.-Congress proceedings. Section 22 p. 435—458 (1967). — Langlet, O.: Two hundred years genecology. Taxon **20** (5/6): 653—722 (1971). — McGee, C. E.: Is variation in budbreak of Red oak the result of heredity or environment? Proceedings, 12th Southern Forest Tree Improvement Conference: p. 185—189 (1973). — Rowe, J. S.: Environmental preconditioning, with special reference to forestry. Ecology 45 (2): 399—402 (1964). — Seal, H. L.: Multivariate statistical analysis for biologists. Methuen & Co. (Ltd.). 1 vol. 207 pp. (1964). — Simak, M.: Seed weight of larch from different provenances (*Larix decidua* Mill.). Studia forestalia suecica (57). 31 pp. (1967). — Stern, K. and Roche, L.: Genetics of forest ecosystems. Springer Verlag. 1 vol. 330 pp. (1974). — Timofeeff-Ressovsky, N. W.: Mutation and geographical variation. In: The New Systematics (J. Huxley, ed. p. 73—136). Oxford University Press. 1 vol. 583 pp. (1940). — Wilkins, D. H.: The scale of genecological differentiation. In: Modern Methods in Plant Taxonomy (op. cit) p. 227—239 (1968).

Pollen and Stigma Viability in Teak (Tectona grandis L. f.)

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Abstract

Methods and results of tests on the viability of pollen and stigmas in *Tectona grandis* L.f. are described. *In vivo* and *in vitro* techniques revealed the extent of viability of pollen and stigma. Pollen grains from the day of anthesis to three days after were used in controlled pollinations. Pollen grains were also germinated in mixtures of boric acid, gibberellic acid and sucrose. Some pollen grains were stored in a deep freeze, a cooled incubator and a vacuum desiccator and later germinated. Sucrose at 14% gave the best germination; at 1% to 3% sucrose there was rupture and at 35% to 40% there was plasmolysis. Pollen grains appeared viable two days after anthesis although no seeds were obtained when these were used in controlled pollination.

Pollen grains had the highest percentage of viability between 12.00 and 14.00 hours, while viability decreased betwen 07.30 and 10.00 hours.

The use of stored pollen for tree breeding appears possible.

Key words: Pollen viability, in vivo, in vitro, pollen storage, pollen tube measurement, controlled pollination.

Zusammenfassung

Unbehandelter Pollen von *Tectona grandis* L.f. war bis zu 2 Tagen nach der Anthesis keimfähig. Nach Aufbewahrung des Pollens in einem Exsikkator bei Minustemperaturen unterschied sich die Keimung nach 35 Tagen nur dadurch, daß die Pollenschläuche kürzer waren. In einigen Fällen konnte mit aufbewahrtem Pollen Befruchtung erzielt werden.

Introduction

Establishment of forest plantations is currently of great interest in Nigeria because of rapid depletion of the natural forest. However, there is little or no information on the reproductive biology and genetic variation of the species used in plantations. Information on the reproductive biology of such species is essential in designing effective breeding programmes. Bawa and Stettler (1969) stated that although breeding mechanisms of temperate forest-tree species are fairly well known they are poorly understood in tropical tree species.

The teak flower is actinomorphic and hermaphroditic and normally has six petals in a single whorl. The pistil is composed of an ovary with four ovules and a style with a forked stigma. Style and stamens are about 6 mm long and the diameter of the corolla is from 6—8 mm (Bryndum and Hedegart, 1969). The flowers appear in large panicles in the outer, unshaded part of the tree crown. The flowering period for an individual inflorescence may last from 2 to 6 weeks.

The first study of some aspects of the reproductive biology of teak was begun in Thailand and was mainly on flowering and fruiting (Bryndum and Hedegart, 1969). This work showed that the major pollinators of teak are insects; in Nigeria these are *Euphaedra janatta* B., *Belenois calypso* Dr., *Acraea bonasia* F., belonging to the family *Nymphalidae* and the order *Lepidoptera; Nomia tridenta* Sm. of the family *Apidae* and the order *Hymenoptera; Melonospilus sternalis* Dall. of the family *Lygaeidae* and the order *Hemiptera* (Egenti, 1974). Another study by Hedegart (1973) showed that hand-pollination increased the percentage of fruit formation in comparison to pollination by natural agents. This finding led to the present study and the objectives were as follows:

(1) to determine the extent of pollen and stigma viability through *in vivo* and *in vitro* examination;
(2) to investigate the use of stored pollen for pollination.

Materials and Methods

Three studies were made (1) viability of pollen and stigma *in vivo;* (2) the viability of pollen *in vitro* (3) pollen storage in (a) a vacuum desiccator, (b) a cooled incubator and (c) a deep freeze.

Material for these three studies was obtained from 10 to 15 year old teak trees growing in plantations at Onigambari near Ibadan in Nigeria. Thirty trees were randomly selected from the plantations.

(I) For the *in vivo* studies, flowers were taken one day before anthesis (A — 1), on the day of anthesis (A), one day after anthesis (A + 1), two days after anthesis (A + 2) and three days after anthesis (A + 3).

After emasculation, fresh stigmata of the day of anthesis were pollinated with pollen of A — 1, A, A + 1, A + 2, A + 3 and bagged. In addition A and A + 1 stigmata were pollinated with fresh pollen of the day of anthesis (A) and bagged.

In practice it was not possible to use A — 1 and A + 1 stigmata as they could not be freed from the remaining parts of the flower without damage in the case of A — 1 because the flowers had not opened and the stigma had become dry in the case of A + 2. Each bag was numbered and dated together with the time of day when the pollina-