Geographic Variation in Needles, Cones and Seeds of Pinus tecunumanii in Guatemala[1])

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Abstract

A geographic variation study of *Pinus tecunumanii* Eguiluz et Perry was based on 108 trees from 5 geographic regions of Guatemala. Twenty four of the 29 traits measured on needle anatomy and morphology and on cones and seed were analyzed. Statistical differences were significant among regions and among trees within regions. The variance component analysis showed that almost 2/3 of the variation was attributable to tree to tree differences, and the remaining 1/3 was shared among regions and sampling error. A cluster analysis showed regions 2 and 4 closely linked while region 5 was quite different from the other regions. Regional provenances can be recognized based on morphological information when proper key characteristics are used. *Pinus tecunumanii* is a variable, but distinctive species. It showed an ecotypic variation pattern, since no clear patterns were observed in the characters studied.

Key words: Pinus tecunumanii, Tecun Umán pine, geographic variation, morphologic variation, ecotypic variation.

Zusammenfassung

Bei *Pinus tecunumanii* Eguiluz et Perry wurde auf der Basis von 108 Einzelbäumen aus 5 geographischen Regionen Guatemalas eine geographische Variationsstudie durchgeführt. Dabei wurden 24 von 29 der untersuchten Merkmale zur Nadelanatomie und Morphologie, sowie Zapfen- und Samen-Merkmale analysiert.

Signifikante Unterschiede konnten zwischen den Regionen und zwischen den Einzelbäumen innerhalb der Regionen festgestellt werden. Die Varianzkomponentenanalyse zeigte, daß nahezu zwei Drittel der Varianz auf Baum-zu-Baum-Unterschiede zurückzuführen waren. Das restliche Drittel verteilte sich auf die Regionen und Versuchsfehler. Eine Cluster-Analyse zeigte, daß die Regionen 2 und 4 eng verknüpft sind, während sich die Region 5 von den anderen stark unterscheidet. Wenn saubere Schlüsselmerkmale benutzt werden, können regionale Provenienzen aufgrund morphologischer Informationen erkannt werden. *Pinus tecunumanii* ist eine variable, aber charakteristische Art. Die Baumart zeigt ein ökotypisches Variationsmuster, da in den untersuchten Merkmalen kein klares Muster zu erkennen war.

Introduction

Pines of Central America are very important when used as exotics in many parts of the world. Many subtropical and tropical pines of the Subgenus *Pinus* have shown good adaptability in South and Central African and South American countries. Among the subtropical pines, the *P. patula* Schl. et Cham., *P. oocarpa* Schiede and *P. caribaea* Morelet complexes appear to be the most promising taxa for operational use because they have shown wide adaptability to a number of different environments. Because of the great variation within these pine complexes, a tree breeder, through carefully conducted provenance trials followed by development of land races, can create adaptive strains suited for planting on marginal sites.

Along with *P. patula, P. oocarpa* and *P. caribaea,* the little known and relatively untested *P. tecunumanii* Schw. ex Eguiluz et Perry appears to be a promising species for tree breeders to test as an exotic in tropical countries. This species was first described by Schwerdtfeger (1953) as *P. tecumumani.* He emphasized that it resembled *P. oocarpa* and *P. pseudostrobus* Lindl., but decided to consider it a new species, based on morphological differences of the cones. However, its designation was invalid because neither nomenclatural type nor latin diagnosis was provided (Eguiluz and Perry, 1983).

Pinus tecunumanii develops into large trees with straight stems and relatively small crowns. Few papers have been published about its natural distribution in Central America (Schwerdtfeger, 1953; Aguilar, 1958 and Eguiluz and Perry, 1983). To better understand the distribution and stand composition of *P. tecunumanii,* two collection trips were made to Guatemala to identify good stands and trees of the species and to collect seeds, foliage samples, stem oleoresin and wood cores. Site characteristics, stand composition and climatic conditions of the stands were also recorded.

The foliage, cone and seed forms the basis of the study reported here, while the terpene analysis of the oleoresin and wood properties will be published as separate papers. The two objectives of this study are: i) to determine the variation patterns in 29 morphological characters of *P. tecunumanii* from 5 regions of Guatemala, ii) to determine the relationships among the characteristics measured.

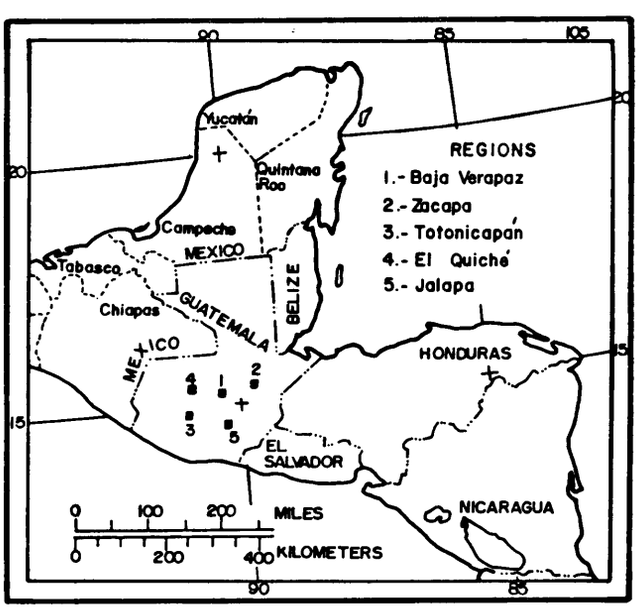

Figure 1. — Location of the 5 regions in Guatemala where *P. tecunumanii* was studied.

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Table 1. — Location of the 5 regions studied.

Region No.	Municipality	Department	Latitude N	Longitude W	Elevation range (m)
1. Finca INAFOR	San Jerónimo	Baja Verapaz	15° 03'	90° 18'	1500-1860
2. Sierra de las Minas	Río Hondo	Zacapa	15° 14'	89° 35'	1750-2080
3. Sierra Sta. Maria Tecun	Pachóc	Totonicapán	14° 57'	91° 17'	2500-2550
4. Road Zacapulas-Quiché	Patsajón	El Quiché	15° 10'	91° 10'	2150-2295
5. La Soledad	Mataques-cuintla	Jalapa	14° 31'	90° 15'	2390-2645

Materials and Methods

1. Sampling scheme. The Tecun Umán pine occurs in other locations but only the 5 regions of Guatemala *(Fig. 1.* and *Table 1)* were sampled:

About 120 trees were collected over all regions and each selected tree was identified in the field on a morphological basis. Trees with intermediate characteristics, pest damage or abnormal looking phenotypes were excluded, leaving only 108 trees for statistical analysis.

A nested design was used, with 3 sources of variation (regions, trees within regions and measurements within trees) for 19 variables *(Table 3).* One branchlet was collected at random, from the lower-half of the crown of each tree, and about 50 needle fascicles were randomly removed from the branchlet (10—15 cm from the bud).

2. Characteristics measured.)* With the exception of needle anatomy, a preliminary sample size determination was calculated for each trait recorded, using the formula of Snedecor and Cochran (1976; p. 516):

$$n = \frac{4\,S^2}{L^2}$$ where;

n = sample size required, with a 5% risk that the error will exceed (L).

S^2 = variance estimated on the preliminary sampling.

L^2 = desired confidence limits (in units).

*) A listing of characteristics and their abbreviations are provided in *Fig. 2* and *Table 2.*

The number of observations needed per trait was rounded to a convenient level.

Needle morphology. Traits recorded on the needle fascicles concerned both external morphology (9 traits) and needle anatomy (5 traits) and were sampled as follow: i) Number of needles per fascicle (NNPF). The number of needles of 40 fascicles per tree were recorded, *(Fig. 2),* but only 15 were used in the analysis of variance and in subsequent measurements. ii) Needle length (NL). One needle was removed at random from each fascicle, and 15 needles per tree were measured to the nearest mm *(Fig. 2).* iii) Fascicle sheath length (FSL). Fifteen entire fascicle sheaths were measured to the nearest mm *(Fig. 2).* iv) Number of stomata rows on face one (NSRF1) of 10 needles per tree. v) Number of stomata rows on face two (NSRF2) of 10 needles per tree. vi) Number of stomata rows on face three (NSRF3) of 10 needles per tree. The stomata rows were counted at needle mid-section *(Fig. 2).* vii) Number of stomata on external face 3 (NSEF). This characteristic was counted on a 5-mm long section of the needle, located at its mid point. The number of stomata within that distance were counted on one of the central rows *(Fig. 2).* viii) Number of serrations on the left edge (NSLE). ix) Number of serrations on the right edge (NSRE). Despite the presence of serrations on the 3 edges of the needle, only the right and the left edges were counted. The number of defined serrations was counted on the same 5-mm section used for stomata counts *(Fig. 2).*

Table 2. — Tree means and other statistics of 29 characteristics of *Pinus tecunumanii* from Guatemala, C. A. n = number of observations per tree; N = total number of trees measured; S. E. = standard error; C. V. = coefficient of variation; M = medial; I = internal; E = external and S = septal. * n = 5 but, it varied from 1 to 10.

Variables:	n	N	MEAN	S.E.	MODE	C.V.	MINIMUM	MAXIMUM
1. Number of needles per fascicle (NNPF)	15	108	4.4	.33	4.0	8.1	3.5	5.1
2. Needle length (NL), cm.	15	108	17.5	.20	17.7	12.0	13.3	25.0
3. Fascicle sheath length (FSL), mm	15	108	18.4	.36	20.0	20.4	11.7	26.2
4. Number of stomata rows on face 1 (NSRF1)	10	108	3.6	.07	3.0	19.8	2.2	5.8
5. Number of stomata rows on face 2 (NSRF2)	10	108	3.6	.06	4.0	18.5	2.1	5.3
6. Number of stomata rows on face 3 (NSRF3)	10	108	5.1	.10	5.0	21.5	2.7	8.4
7. Number of stomata on a 5 mm section of the external face (NSEF)	10	108	60.9	.53	60.0	9.0	46.9	83.9
8. Number of serrations on the left edge (NSLE)	15	108	25.0	.31	25.0	12.9	18.4	32.9
9. Number of serrations on the right edge (NSRE)	15	108	24.7	.31	34.0	13.0	18.7	34.0
10. Number of resin canals (NRC)	1	108	3.4	.10	3.0	29.6	1.0	7.0
11. Number of schlerenchyma cells surrounding the resin canal (NSCSRC)	1	94	8.3	.08	8.5	9.8	7.0	11.0
12. Number of 'Medial' resin canals (NMRC)	1	108	2.9	.08	3.0	29.1	0	5.0
13. Number of endodermal cells (NEC)	1	108	26.8	.24	25.0	9.4	22.0	39.0
14. Number of cell rows forming the hypodermis (NCRFH)	1	98	2.1	.04	2.0	17.6	2.0	4.0
15. Peduncle length (PL), cm ca.	5*	107	1.3	.04	1.0	28.6	0.6	3.2
16. Cone length (CL), cm. ca.	5	107	5.2	.08	5.3	15.3	3.4	7.3
17. Closed cone width (CCW), cm. ca.	5	107	3.0	.04	3.0	14.0	2.0	4.0
18. Ratio CL/CCW ca.	5	107	1.8	.02	2.0	14.1	1.2	2.6
19. Cone axis diameter (CAD), mm.	2	104	6.4	.15	6.0	24.5	3.5	12.3
20. Cone scale length (CSL), mm.	10	104	20.4	.23	20.0	11.8	15.1	26.7
21. Apophysis length (AL), mm.	10	104	9.7	.12	10.0	12.4	6.3	12.8
22. Apophysis width (AW), mm	10	104	6.6	.09	6.0	13.3	4.6	8.9
23. Ratio AL/AW	10	104	1.5	.02	1.4	11.0	1.0	1.9
24. Apophysis height (AH), mm	10	104	3.6	.05	3.5	14.4	2.4	5.0
25. Wing length (WL), mm.	10	104	9.6	.16	10.0	17.4	6.2	13.6
26. Wing width (WW), mm.	10	104	5.2	.06	5.1	11.2	4.0	6.4
27. Seed length (SL), mm.	10	114	5.6	.04	5.1	8.6	4.4	6.7
28. Seed width (SW), mm.	10	114	2.8	.02	3.1	8.7	2.2	3.5
29. Ratio SL/SW	10	114	2.0	.02	2.0	9.9	1.6	3.1
Frequency of the NNPF (in percentage)	50	108	3's (4.2); 4's (50.0); 5's(45.2); 6's (.6).					
Frequency of the position of the resin canals (in percentage)	1	108	M= (80.5); I (13.0); E (3.5); S (3.1).					

Table 3. — Analysis of variance and variance components for the means of 24 characteristics of *P. tecunumanii*.

VARIABLES	SOURCES OF VARIATION: REGIONS			TREES (REGIONS)			ERROR			d. f.		Expected means squares
	S S'	V C	SE VC	S S	V C	SE VC	S S	V C	SE VC	R[6]	4	$\sigma_e^2 + 15\,\sigma_{t(r)}^2 + 317.638\,\sigma_r^2$
										T(R)	103	$\sigma_e^2 + 15\,\sigma_{t(r)}^2$
										E	1512	σ_e^2
NNPF	9.411*	0.001	0.004	196.477*	0.113	0.018	323.466	0.214	0.008			Coefficients"
NL	729.503*	0.389	0.332	6321.578*	3.958	0.565	3024.577	2.000	0.073			
FSL	11183.533*	8.452	5.079	11449.778*	7.184	1.023	5150.666	3.406	0.124			
NSLE	2753.974*	1.739	1.252	14026.788*	8.498	1.253	13175.466	8.714	0.317			
NSRE	3135.028*	2.051	1.425	13607.673*	8.299	1.216	28283.101	7.632	0.277			
NSRF1	147.229*	0.155	0.100	408.247*	0.358	0.054	375.700	0.386	0.017	R	4	1 + 10 + 211.759
NSRF2	122.641*	0.128	0.184	365.799*	0.317	0.049	374.800	0.386	0.017	T(R)	103	1 + 10
NSRF3	397.707*	0.429	0.271	882.659*	0.765	0.118	892.000	0.918	0.042	E	972	1
NSEF	9248.214*	9.851	6.305	23279.171*	19.486	3.122	30283.000	31.155	1.412	R	4	1 + 4.416 + 84.872
PL	6.539*	0.017	0.013	39.048*	0.071	0.013	27.823	0.080	0.006	T(R)	102	1 + 4.246
CL	25.552*	0.057	0.050	238.657*	0.464	0.077	129.631	0.372	0.028	E	349	1
CCW	9.266*	0.030	0.021	58.201*	0.111	0.019	34.961	0.100	0.008	R	4	1 + 4.385 + 84.633
CL/CCW	2.187*	0.004	0.004	21.275*	0.042	0.007	10.245	0.029	0.002	T(R)	102	1 + 4.237
CAD	104.956*	0.667	0.442	369.585*	1.599	0.286	67.265	0.739	0.108	E	348	1
WL	618.356*	0.691	0.467	2157.394*	2.190	0.319	640.567	0.715	0.034	R	4	1 + 1.925 + 38.156
WW	30.872*	0.028	0.026	301.147*	0.296	0.044	176.098	0.196	0.009	T(R)	99	1 + 1.872
SL	46.245*	0.045	0.031	203.246*	0.175	0.025	152.632	0.152	0.007	E	91	1
SW	9.618*	0.010	0.007	57.526*	0.046	0.007	81.132	0.081	0.004	R	4	1 + 9.339 + 178.422
SL/SW	2.553*	0.002	0.002	40.520*	0.022	0.005	155.883	0.155	0.007	T(R)	99	1 + 9.625
										E	896	1
										R	4	1 + 9.831 + 208.860
										T(R)	109	1 + 9.787
										E	1002	1

VARIABLES	REGIONS			TREES (REGIONS)			CONES (TREES)			ERROR			d. f.		Expected Means squares
CSL	536.728*	0.433	0.408	4991.261*	4.644	0.765	631.040*	1.277	0.203	427.608	0.548	0.028			$\sigma_e^2 + 5\,\sigma_c^2 + 9.626\,\sigma_t^2 + 190.78\,\sigma_r^2$
AL	247.065*	0.262	0.187	1127.838*	1.041	0.173	149.816*	0.271	0.048	228.172	0.292	0.015	R	4	$\sigma_e^2 + 5\,\sigma_c^2 + 9.361\,\sigma_{t(r)}^2$
AW	96.119*	0.092	0.073	619.526*	0.540	0.096	109.319*	0.173	0.072	261.008	0.335	0.017	T(R)	99	
AL/AW	6.162*	0.007	0.005	19.694*	0.016	0.003	4.159*	0.006	0.001	11.508	0.015	0.001	C(T)	91	$\sigma_e^2 + 5\,\sigma_{c(t)}^2$
AH	4.157*	0	0.004	254.152*	0.232	0.039	35.896*	0.055	0.012	93.520	0.120	0.006	E	780	σ_e^2

'In this table, the S S are type IV, as generated by SAS; but the SAS package uses S S type I to calculate the V C. - & Symbology: R = regions, T = trees, E = error, S S = sum of squares, V C =variance component, S E = standard error, and d. f. = degrees of freedom. - * = Significant at the 0,01% probability level. - " for the above E. M. S.

Needle anatomy. One needle per tree was removed and rehydrated in distilled water for 1—3 hours. The needle was inserted in a fresh carrot for support and sectioned with a double-edged razor blade. One slide per needle was prepared with several sections placed on glycerine. The slide was studied under the light microscope recording the following characteristics: i) Number of resin canals (NRC). ii) Position of the resin canals (PRC). The 4 positions of resin canals were recorded as internal, medial, external and septal *(Fig. 2).* iii) Number of schlerenchyma cells surrounding the resin canal (NSCSRC). iv) Number of endodermal cells (NEC). v) Thickness of the outer wall of the endoderm cells (OWEC). The thick outer walls of the endoderm cells was reason for excluding the trait from statistical analysis. vi) Number of cells rows forming the hypoderm (NCRFH).

Due to the limited number of observations per tree, the anatomical data were excluded from the analysis of variance. Several other features were occasionally recorded on the slides, such as: the closeness of the vascular bundles, the shape of the endoderm, the shape of the needle at cross section, the location and size of the resin canals, the type of connection between the external canals and the hypoderm *(Fig. 2),* and the internal canals with the endoderm (Doi and Morikawa, 1929).

Cone morphology. The number of mature cones on each branchlet varied from 1 to 10 per tree, but 4 to 5 were the most common. The 5 traits measured on each cone were *(Fig. 2):* i) Peduncle length (PL), in cm. ii) cone length (CL), in cm. iii) Closed cone width (CCW), in cm. iv) Cone length to width ratio (CL/CCW). v) Cone axis diameter (CAD). Two cones per tree were measured at the widest closed cone diameter, and cross sectioned, usually 2 to 3 cm from the base. From the upper half of the cone, 10 scales were removed and the following 5 characteristics were measured to the nearest mm *(Fig. 2):* vi) Cone scale length (CSL). vii) Apophysis length (AL). viii) Apophysis width (AW). ix) Apophysis length to width ratio (AL/AW). x) Apophysis height (AH). In this case, the height of three characteristics were effectively measured: apophysis, umbo and prickle *(Fig. 2).*

Seed morphology. Most of the collected cones bore seed. However, the 10 winged seeds measured were randomly chosen from a composite sample of several cones from the same tree. The wing was removed and both seed and wing were measured to the nearest mm *(Fig. 2).* Five seed traits were quantified: i) Wing length (WL), in mm. ii) Wing width (WW), in mm. iii) Seed length (SL), in mm. iv) Seed width (SW), in mm. v) Seed length to width ratio (SL/SW).

3. Statistical analyses. The *P. tecunumanii* data set was analysed utilizing the SAS (Statistical Analysis System) package. A univariate procedure was used to test for deviations from normality on each variable before the statistical analysis was performed. Due to the large sample sizes, the test was extremely powerful and showed deviations from normality; however, graphical examination of the data showed that these deviations were not extreme and transformations were deemed unnecessary, due to the robustness of the analysis of variance procedure.

Analysis of variance (ANOVA). The ANOVA was performed on tree means, for 24 variables. The General Linear Models procedure of SAS was used to account for the different number of observations and trees per region, and an F-ratio test was performed to detect any differences among regions, trees and cones.

Variance components. Using the Variance Components procedure of SAS, the expected mean squares (all effects considered random) and the variance component estimates were obtained for each source. A percentage of each component was calculated by adding all the variance components and dividing the component of each source by the total. The standard error of the variance component estimates was calculated using the formulae reported by Becker, (1975; p. 44):

$$\widehat{\mathrm{Var}}(\sigma^2_g) \cong \frac{2}{k^2} \sum g \frac{MS^2_g}{f_g + 2}$$

where:

k = coefficient of the variance component being estimated

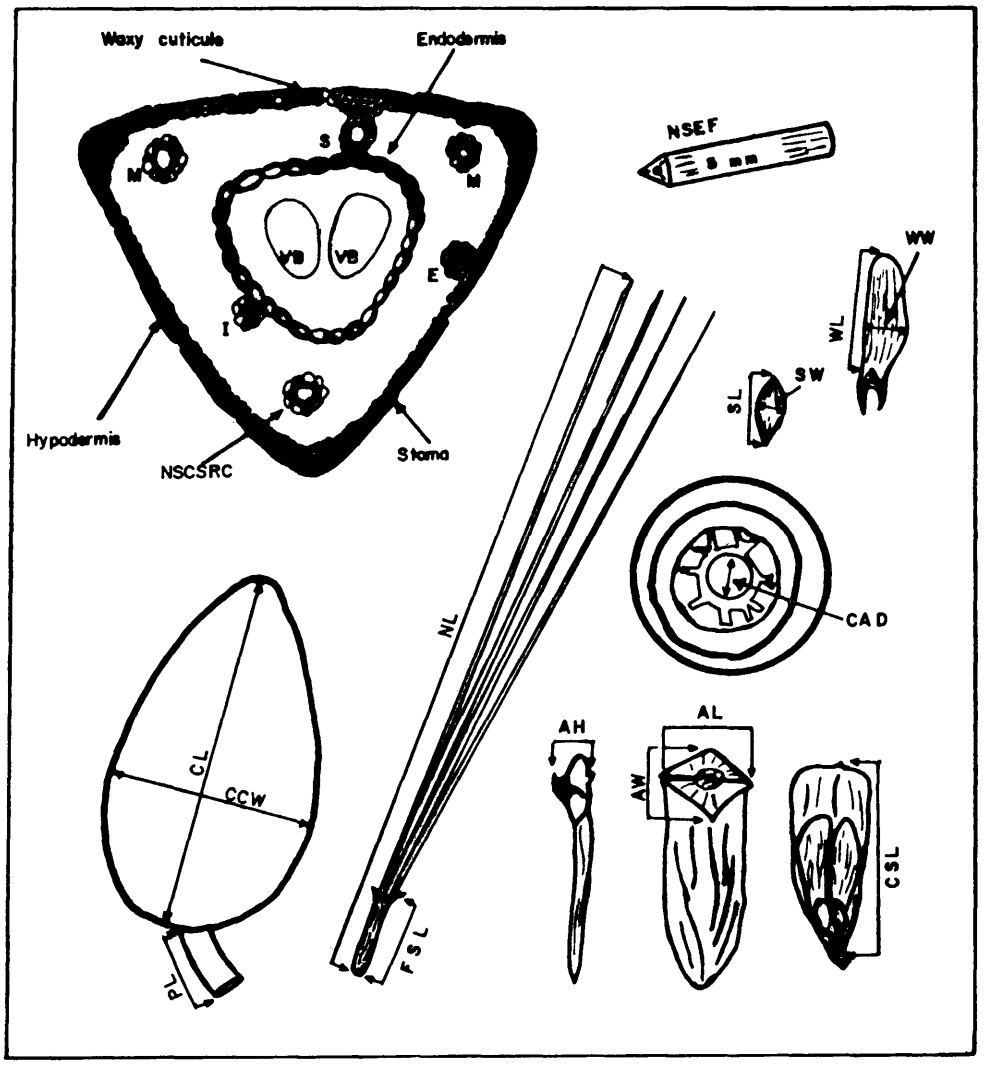

Figure 2. — Schematic drawing of cone, scales, seed and needles, indicating the site of measurement for each characteristic. (The cross section of the needle is oriented horizontally with the tip pointed into the page).
NL = needle length, FSL = fascicle sheath length, NSEF = No. of stomata on a 5 mm section of the external face, NSCSRC = No. of schlerenchyma cells surrounding the resin canal, VB = vascular bundel, I = internal, M = medial, E = external, S = septal, PL = penduncle length, CL = cone length, CCW = closed cone width, CSL = cone scale length, AL = apophysis length, AW = apophysis width, AH = apophysis height, CAD = cone axis diameter, SL = seed length, SW = seed width, WL = wing length, WW = wing width.

$$\text{S.E.}(\sigma^2_g) \cong \sqrt{\text{Var}\,(\sigma^2_g)}$$

MS_g = the g^{th} mean square used to estimate the variance component

g = the g^{th} mean square

f_g = the degrees of freedom of the g^{th} mean square

The coefficient of variation of the variance components was estimated using the traditional formula,

$$\text{C.V.} = \frac{\text{S.E. of the variance component}}{\text{Variance component estimate}}\,(100)$$

Correlation coefficients. Using the correlation procedure of SAS, a correlation analysis was performed on the means of 25 variables (including elevation), in order to remove any trait highly correlated with other(s) in future studies of this species.

Factor analysis. A factor analysis was carried out on the correlation matrix. The factor analysis assumes that each factor explains different portions of the total variance. The tendency of this analysis is to produce a general factor (Factor 1) on which almost all variables are highly correlated (Green, 1978). The intention of this analysis is to rotate the factor axes, in such a way that each dimension (including Factor 1 which is a linear composite of the original variable) has only a few correlated variables defining each factor. The first 7 principal components (dimensions), in the principal component analysis (SAS, 1979) were retained for the factor analysis. The principal component axes were rotated using both an orthogonal (Varimax) and oblique or nonorthogonal (Promax) rotation to better differentiate the groups of variables. The Promax rotation results in new factor *loadings* or weightings in which the factors are correlated rather than being mutually orthogonal (Green, 1978). This is useful because some groups of variables may not exist in orthogonal directions. By

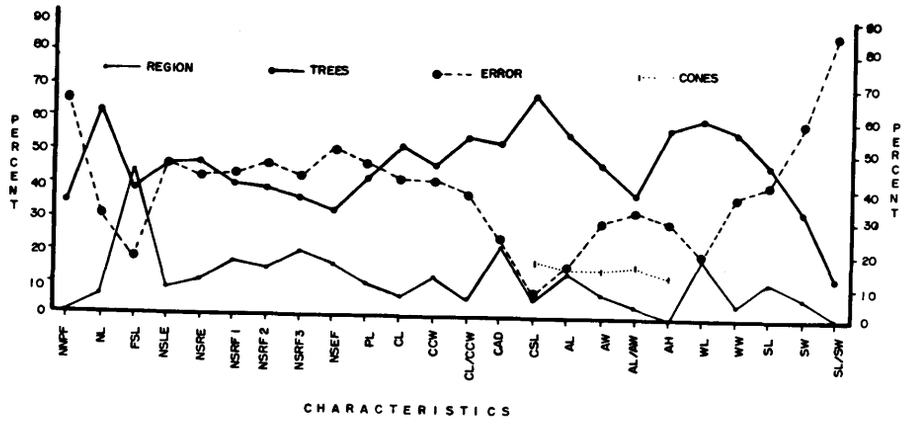

Figure 3. — Percentage of the variance component for each source of variation calculated on 24 characteristics (abbreviations are listed in *Table 1)* of *Pinus tecunumanii* from Guatemala.

loading is meant the correlation of some original variables (trait) with a principal component (factor). More generally, GREEN (1978) defines the *loading* of a variable on a factor as a weight that measures the contribution to variance in a variable (factor) that is made by a factor (variable). The interpretation given to the factor pattern is: i) The degree of similarity between a set of characteristics which a factor represents is called its factor *loading.* ii) Each variable shows a high correlation (= weighting) only with the factors which explained most of its variation, and vice versa. iii) If the same factor has different variables with high weightings, this implies high correlations among such variables.

Cluster analysis. A cluster analysis was carried out on the means of 24 characteristics of each region using the Maximum method (JOHNSON, 1967). The purpose of this analysis was to group the regions according to their affinities and differences, based on their high similarity coefficients. This anlysis is analogous to the joining of mountain peaks on a topographic map by successively lowering contour lines, until two clusters overlap in the valley. A phenogram or cluster map was constructed based on the maximum distance within a cluster.

Results and Discussion

The F-test performed on the ANOVA results showed highly statistically significant differences for all sources of variation of the 24 traits *(Table 3).* Results of the variance component analysis indicated that 2/3 of the total variation in the most stable traits is attributable to the differences among trees within regions *(Fig. 3).* On the other hand, the 9 most variable traits, had the maximum variation as sampling error, indicating that the variation of these traits was related to sources not included in the analysis. All traits except fascicle sheath length (FSL), showed little variation among regions. Possibly, the FSL regional variation is inflated because the fascicle sheaths disintegrate quickly as they dry out, and regions were sampled at different dates.

The coefficients of variation (C. V.) of the variance components of all the variables but one were below 19% for the source of variation trees withing regions. But, the C. V. for observations within tree (error) were always below 8% (except for the trait cone axis diameter) which suggests that the sample size used was appropiate. Cones within trees showed C. V. from 15 to 21% but regions showed very large C. V., largely because of the reduced degrees of freedom. This indicates that additional regions should be included in future studies.

The results of the correlation analysis produced high coefficients among variables within a needle *(Table 4).* For example, the correlations between stomata rows were .92, .78 and .76 for the face 1 with face 2, face 1 with face 3, and face 2 with face 3. Another expected high correlation

Table 4. — Correlation coefficients calculated on the means of 25 variables of *Pinus tecunumanii.*

Variables	NNPF	NL	FSL	NSRF1	NSRF2	NSRF3	NSEF	NSLE	NSRE	PL	CL	CCW	CL/CCW	CAD	CSL	AL	AW	AL/AW	AH	WL	WW	SL	SW	SL/SW
NL	.21*																							
FSL	.28**	.21*																						
NSRF1	.13	.28**	-.15																					
NSRF2	.14	.25**	-.12	.92**																				
NSRF3	-.25**	.05	-.30**	.78**	.76**																			
NSEF	-.08	-.13	-.37**	.09	.03	.16																		
NSLE	.05	.02	-.16	.44**	.41**	.36**	.39**																	
NSRE	.08	-.01	-.21*	.44**	.40**	.36**	.38**	.97**																
PL	.27**	.27**	-.06	.42**	.39**	.26**	.04	-.03	0															
CL	-.08	.05	.01	.22*	.23*	.22*	.02	.05	.02	.18														
CCW	-.07	.04	.13	.12	.14	.13	-.18	-.05	-.10	-.09	.55**													
CL/CCW	0	.03	-.11	.14	.11	.08	.21*	.15	.17	.27**	.53**	.40**												
CAD	-.03	.11	-.01	.29**	.25*	.27**	0	.05	0	-.04	.46**	.69**	-.19											
CSL	.17	.11	.20*	.19*	.20*	.05	.02	0	-.04	.13	.57**	.59**	.06	.49**										
AL	-.09	-.02	.24*	-.01	-.02	-.08	-.09	-.04	-.08	-.05	.43**	.63**	-.14	.46**	.55**									
AW	.13	.08	.22*	-.02	-.09	-.18	0	-.06	-.07	-.01	.39**	.40**	.07	.22*	.55**	.60**								
AL/AW	-.21*	-.12	-.02	-.01	.04	.10	-.08	0	-.03	-.06	0	.22*	-.24*	.24*	-.03	.39**	-.48**							
AH	-.13	-.07	-.23*	.09	.06	.11	.07	.01	.04	-.14	.30*	.40**	-.03	.36**	.28**	.35**	.39**	-.04						
WL	.10	.08	.08	.38**	.35**	.30**	.01	.09	.08	.15	.51**	.61**	-.05	.45**	.77**	.53**	.38**	.11	.20					
WW	-.16	-.01	.07	.13	.12	.19	-.01	.07	.02	-.01	.40**	.64**	-.20*	.54**	.42**	.66**	.36**	.25*	.21*	.62**				
SL	-.04	.08	-.24*	.25*	.26**	.27**	.07	.12	.10	.22*	.34**	.17	.19*	.19	.22*	.08	-.13	.24*	.12	.24*	.31			
SW	-.16	.15	-.19	.26**	.25*	.34**	.05	.25*	.20*	.13	.30**	.32**	-.01	.28**	.19	.23*	.09	.13	.25*	.26**	.56**	.52**		
SL/SW	.10	-.14	.03	-.09	-.06	-.14	-.01	-.11	-.09	.09	0	-.19	.21*	-.12	-.04	-.14	-.23*	.13	-.19	-.10	-.29**	.36**	-.50**	
Altitude	.15	-.28**	-.08	-.22*	-.24*	-.29**	.22*	-.04	.01	-.01	-.17	-.40**	.23*	-.47**	-.21*	-.22*	.11	-.36**	-.06	-.40**	-.29**	-.09	-.21*	.16

* Correlation significant at the .05% probability level, and ** correlation significant at the .01% probability level. Abbreviation of variables are listed in *Table 2.*

Table 5. — Results of the factor analysis performed on the correlation matrix of 25 variables of *P. tecunumanii* from Guatemala (abbreviations of variables are listed in *Table 2).*

ROTATED FACTOR PATTERN: "Varimax" and "Promax" rotations respectively*								
		F	A	C	T	O	R	S
	1	2	3		4	5	6	7
Variance explained by each factor	5.06(5.28)	3.26(3.75)	2.05(2.25)		2.38(2.63)	2.06(2.30)	1.93(2.03)	1.76(2.67)
% of total variance	27.4(25.7)	17.6(17.9)	11.1(10.8)		12.9(12.6)	11.1(11.0)	10.4(9.7)	9.5(12.8)
Variables								
NEEDLES NNPF							0.71(0.71)'	
NEEDLES NL							0.49(0.49)	
NEEDLES FSL							0.69' 0.70)	
NEEDLES NSRF1		0.89(0.89)						
NEEDLES NSRF2		0.89(0.89)						
NEEDLES NSRF3		0.82(0.83)						
NEEDLES NSEF					0.58(0.55)			
NEEDLES NSLE					0.91(0.92)			
NEEDLES NSRE					0.91(0.92)			
CONE PL		0.45(0.40)				0.51(0.51)		
CONE CL	0.66(0.66)					0.51(0.51)		
CONE CCW	0.84(0.82)							
CONE CL/CCW						0.75(0.74)		
CONE CAD	0.70(0.69)							
CONE CSL	0.81(0.85)							
CONE AL	0.82(0.83)							
CONE AW	0.62(0.62)		-0.63(-0.66)					
CONE AL/AW			0.86(0.87)					
CONE AH	0.46(0.46)						-0.48(-0.51)	
SEED WL	0.78(0.78)							
SEED WW	0.71(0.63)							0.44(0.40)
SEED SL			0.46(0.47)			0.67(0.69)		
SEED SW								0.83(0.86)
SEED SL/SW			0.32(0.41)			0.40(0.41)		-0.74(-0.78)
Elevation		-0.40(-0.39)	-0.40(-0.36)					

* The Promax values are in parentheses. 'These values are correlations of each variables to each factor or principal component (correlations above or below ca. ± .35 were not included here).

(r = .96) was obtained on needle serration from the right edge to the left edge. However, cone and seed characteristics showed lower r values, especially when correlated with needle variables. Similar results occurred when needles were correlated to cone and seed morphology. The low relationship between needle length and elevation, was not surprising since few and some times repeated elevations were sampled.

The relationship among the correlated variables was supported by the factor analysis. Each of the 7 factors retained characterized a particular set of traits *(Table 5).* For instance, Factors 1, 3, 5, and 7 showed high weights for cone and seed variables; while Factors 2, 4, and 6 showed high weights for specific characteristics on the needles. Thus, Factor 2 was concerned with the number of stomata rows (NSRF1, NSRF2 and NSRF3), accounting for most of the variance explained by this factor *(Table 5).* On the other hand, Factor 4 was mainly concerned with the needle serrations (NSLE und NSRE) and to a lesser degree with the number of stomata (NSEF). Factor 6 was highly weighted with needle fascicle variables (NNPF, NL and FSL), in addition to AH *(Table 5).*

The results of the factor analysis were interpreted as follows:

i) When several variables assigned to the same factor are highly weighted, it is possible that either genetic effects, such as pleiotropy, linkage or environmental correlations, or a combination of the two, are the cause of their relationship. Factors 2 and 4 are used to illustrate this point. Three traits of Factor 2 (NSRF1, NSRF2 and NSRF3) showed high weightings *(Table 5)* and high correlation coefficients *(Table 4)* among themselves. For Factor 4, the 2 traits (NSLE and NSRE) also had high r values, indicating that each group of traits is either affected by the environment in the same way or controlled by the same gene(s) or different genes with pleiotropic effects; furthermore, the lower weight of stomata number (NSEF) on Factor 4, indicates a weaker relationship of this trait with needle serrations, which may be caused by loosely linked genes or independent environmental effects.

ii) Characteristics showing high weights on different factors imply that they are controlled by different gene complexes, and/or the effects of the environment are independent. This occurs for PL, CL, AW and AH.

The phenogram resulting from the cluster analysis showed 2 defined clusters *(Fig. 4).* Within the first cluster, the close affinity of the trees from regions 2 and 4 was not disclosed from field observations. The population of region 3 which is nearly extinct from damage by the bark beetle, *Dendroctonus* sp. represents one polarity of this cluster. It has more variable traits than the population from region 1 *(Table 6)* and definitely has more internal variability than populations from regions 2 and 4. The minimum values of 13 traits of the population from regions 3 were probably the cause for its difference, while the population from region 2 with 19 traits showing the maximum values, also differed from the populations in regions 2 and 4. The populations of these 2 regions showed less variability in most of the traits than those of the other regions and their means are closer to those of the population from region 1.

Based on geographic isolation and closer association of the population of region 5 with the *P. oocarpa* and the *P. pseudostrobus* complexes, it was not surprising to find that the population of region 5 exemplified one cluster. Lower latitude and different climatic conditions, as well as interspecific hybridization, is a logical explanation for the great variability among the trees of this region, while those of regions 1, 2, and 4 which are distributed along the same mountain chain, reflect the common environmental similarities among these locations.

There were no definite regional gradations in the 30 traits reported in Table 6. The gradient was discontinuous when followed from East to West or North to South. So,

Table 6. — Number of trees, means and standard errors respectively, of 29 characteristics of *P. tecunumanii* from 5 regions of Guatemala, C. A. (variable abbreviations are listed in *Table 2*).

	CHARACTERISTIC	1. BAJA VERAPAZ			2. ZACAPA			3. TOTONICAPAN			4. EL QUICHE			5. JALAPA		
NEEDLES	NNPF	28	4.3*	.08	29	4.4	.07	15	4.6	.07	15	4.4	.09	21	4.5	.07
	NL (cm)	28	17.7	.30	29	18.2'	.50	15	16.1*	.50	15	17.3	.41	21	17.4	.38
	FSL	28	18.7	.44	29	18.5	.68	15	22.1'	.62	15	20.4	.76	21	13.7*	.31
	NSRF1	28	4.0'	.13	29	3.5	.12	15	3.0*	.17	15	3.4	.10	21	4.0'	.14
	NSRF2	28	4.0'	.12	29	3.5	.11	15	3.1*	.15	15	3.4	.12	21	3.9	.13
	NSRF3	28	5.8'	.21	29	4.8	.17	15	4.0*	.22	15	4.8	.18	21	5.5	.18
	NSEF	28	60.3	.62	29	59.3	.92	15	59.5	.92	15	58.2*	1.50	21	66.7'	1.40
	NSLE	28	25.7	.56	29	23.9	.59	15	23.3*	.60	15	24.9	.82	21	27.0'	.69
	NSRE	28	25.3	.54	29	24.4	.55	15	23.2*	.62	15	24.4	.80	21	27.0'	.73
NEEDLE ANATOMY	NRC	28	3.3	.11	29	3.0*	.10	15	3.5	.34	15	3.3	.32	21	4.3'	.23
	NSCSRC	19	8.6'	.18	26	8.0*	.12	13	8.1	.19	15	8.6'	.19	21	8.6'	.20
	NMRC	28	3.1	.09	29	2.7	.13	15	3.1	.29	15	2.2*	.27	21	3.3'	.21
	NEC	28	26.1	.41	29	26.4	.37	15	25.9*	.43	15	27.6	.88	21	28.3'	.54
	NCRFH	28	2.4'	.10	20	2.0*	.05	15	2.0*	0	15	2.1	.01	21	2.0*	0
CONE	PL (cm)	28	1.3	.05	29	1.3	.10	15	1.2	.05	15	1.0*	.04	20	1.5'	.07
	CL (cm)	28	5.6'	.14	29	5.1	.12	15	5.1	.19	15	4.9*	.20	20	5.1	.22
	CCW (cm)	28	3.2'	.06	29	3.0	.06	15	2.9	.12	15	3.0	.11	20	2.6*	.08
	CL/CCW	28	1.7	.04	29	1.7	.03	15	1.8	.05	15	1.6*	.07	20	1.9'	.07
	CAD (mm)	27	7.1'	.35	28	7.1'	.24	14	5.4*	.34	15	5.9	.28	20	5.6	.27
	CSL (mm)	27	21.4'	.33	28	20.3	.43	14	20.4	.69	15	19.5*	.73	20	19.6	.56
	AL (mm)	27	10.2'	.23	28	9.6	.20	14	10.2'	.26	15	9.7	.32	20	8.8*	.23
	AW (mm)	27	6.4	.12	28	6.4	.14	14	7.0	.20	15	7.1'	.25	20	6.3*	.24
	AL/AW	27	1.6'	.02	28	1.5	.03	14	1.5	.03	15	1.4*	.03	20	1.4*	.03
	AH (mm)	27	3.7'	.10	28	3.5*	.10	14	3.5*	.10	15	3.5*	.14	20	3.6	.12
SEED	WL (mm)	42	10.6'	.22	28	9.0	.27	15	9.0	.48	11	9.2	.50	8	8.4*	.42
	WW (mm)	42	5.4'	.08	28	5.2	.11	15	5.1	.16	11	5.1	.16	8	4.7*	.20
	SL (mm)	42	5.6	.07	28	5.5	.08	15	5.2*	.10	11	5.3	.19	18	5.9'	.08
	SW (mm)	42	2.9'	.04	28	2.8	.05	15	2.6*	.06	11	2.8	.06	18	2.9'	.04
	SL/SW	42	2.0'	.03	28	2.0'	.03	15	2.1	.09	11	1.9*	.05	18	2.0'	.02
	Frecuency of NNPF (%) n=50 fascicles/tree	3,s: 4,s: 5,s: 6,s:	5.8 54.6' 38.6* 1.0'			5.1 48.8 46.0 0.1*			2.2 44.5* 53.2' 0.2			6.8' 50.3 42.0 0.9			0.5* 49.5 49.4 0.6	

Extreme values among the 5 regions: * = minimum and ' = maximum.

based on morphology *P. tecunumanii* appears to have ecotypic rather than clinal variation. There are certain traits that characterize particular regions *(Table 6)*. Thus, Baja Verapaz followed by Jalapa, showed 18 and 13 traits respectively, with the maximum values. However, while these maximum values concerned basically cone and seed traits on Baja Verapaz, they mainly regarded needle morphology and anatomy on Jalapa. On the other hand, the population of Totonicapán followed by that of El Quiché, showed 13 and 9 traits respectively, with the minimum values, involving needle, cone and seed morphology in the former, and mainly cone morphology in the latter. The population of Zacapa had the least number of extreme values; here, *P. tecunumanii* forms many extensive monospecific stands of high quality. Furthermore, the sampling included trees from the lowest to the highest elevations in the region of Zacapa, but not in the other regions.

Conclusions and Recommendations

Like most species of the Subsection *Oocarpae* (Little and Critchfield, 1969), *P. tecunumanii* showed great variation in cone, seed and needle morphology, as well as needle anatomy. A discontinuous pattern of variation was evident, without signs of any latitudinal or longitudinal trends. Most of the variation was attributed to tree to tree differences, rather than to regional differences. However, the within tree component of variation (error) of the ANOVA contained considerable portions of the variance components, definitely, including sources of variation not considered in the analysis. The F-test indicated high sta-

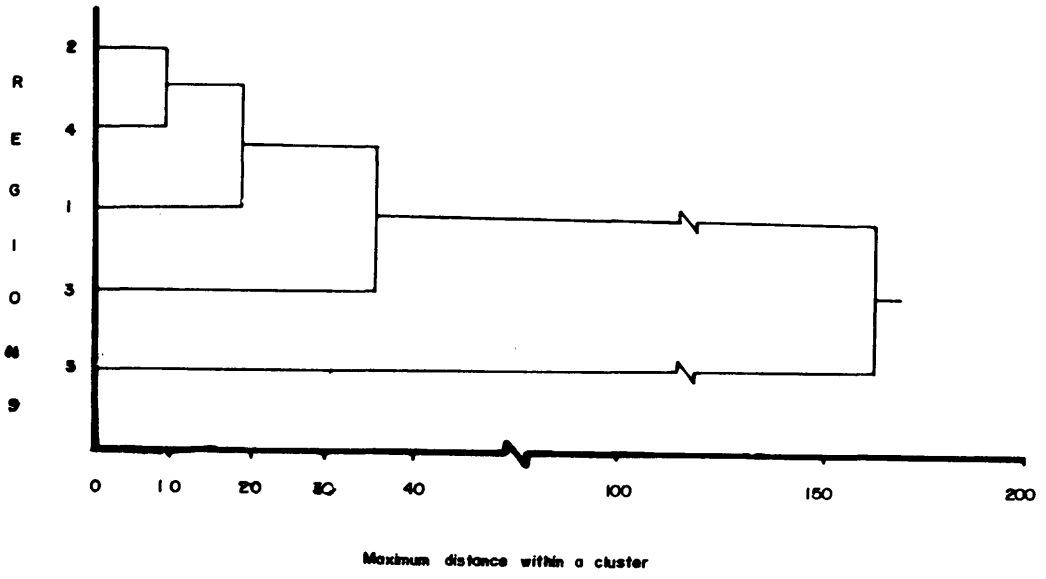

Figure 4. — Phenogram of 5 regions of *P. tecunumanii* based on standardized distance, calculated from the 24 variables listed in *Table 1*. The maximum distance within a cluster represents the similarity values associated with each region in the hierarchical representation.

tistical differences among regions, trees and cones (when applicable). However, future studies must include additional sources such as levels of variation within trees. Also needed would be more trees within stands and stands within regions, rather than more regions. The populations of Baja Verapaz and Jalapa had the maximum values for most seed, cone and needle traits. The cluster analysis, identified the population of Jalapa as being a different cluster, probably due to its geographic isolation, in addition to its association and hybridization with other species, i.e. *P. oocarpa* and *P. maximinoi* Moore, with which it is said to hybridize rather easely. The population of the Sierra de las Minas, Jalapa, followed by the population of the Communal forest of Patsajón, El Quiché, have the most uniform stands of *P. tecunumanii*. A number of traits were correlated, and they should be sorted out in future studies. Only one lateral face of stomata rows need be counted in the future, in addition to the stomata rows of the external face. Since the number of serrations on the right and left edges were highly correlated, recording on one edge is sufficient for future studies. In spite of the presence of high correlations among cone and seed characteristics, all traits should be measured until additional information is obtained which would indicate which might be dropped.

The most highly correlated characteristics were allocated to specific factors. Thus, stomata rows accounted for almost the total variance of Factor 2, while the needle serrations accounted for most of the variance of Factor 4. This meant that the weights to these factors is probably due to similar effects of the environment on each group of traits, although they may be the result of linked genes or genes with pleiotropic effects or a combination of these. High weights of the same trait on different factors, may be the result of either independent gene complexes effects, environmental effects or both combined.

The best phenotypes of the Tecun Umán pine as well as some of the best stands are threatened by woodcutters, uncontrolled forest fires and pest epidemics in most of the regions studied. It is therefore, urgent to encourage seed collections in all locations, since no provenance trial information is available. This would protect from the risk of loosing trees from other regions as has happened in region 3. This is particularly true for phenotypes which will prove to be invaluable in planting programs.

Literature Cited

Anonymus: Statistical Analysis System. SAS User's guide. SAS Institute. 494 pp. Raleigh. 1979. — Aguilar, J. I.: Pinos de Guatemala. Ministerio de Agricultura. 2nd Ed. La Aurora. 32 pp. Guatemala (1958). — Becker, W. A.: Manual of Quantitative Genetics. Students Book Corp. 170 p. p. Pullman, Wa. (1975). — Doi, T. and K. Morikawa: An anatomical study of the leaves of the Genus *Pinus*. Jour. Depart. Agric. Kyushu Imp. Univ. **2** (6): 150—198 (1929). — Eguiluz Piedra, T. and J. P. Perry, Jr.: *Pinus tecunumanii:* una especie nueva de Guatemala. Ciencia Forestal (México) **41** (8): 3—22 (1983). — Green, P. E.: Analyzing Multivariate Data. The Dryden Press. 519 pp. Illinois (1978). — Johnson, S. C.: Hierarchical clustering schemes. Psychometrika **32** (3): 241—254 (1967). — Little, E. L. Jr. and W. B. Critchfield: Subdivision of the Genus *Pinus* (Pines). U. S. D. A. For. Serv. Misc. Pub. No. 1144. 51 pp. Washington (1969). — Schwerdtfeger, F.: Informe al Gobierno de Guatemala sobre la Entomología Forestal de Guatemala. Vol. I. Los Pinos de Guatemala. Informe FAO/ETAP. No. 202. 58 pp. Rome (1953). — Snedecor, G. W. and W. G. and Cochran: Statistical Methods. 6th Ed. Iowa State Univ. Press. 593 pp. Ames (1976). — Standley, P. C.: Notes on some guatemalan trees. Tropical Woods **84**: 1—18 (1945).

Genetic Variation in Resin Canal Frequency and Relationship to Terpene Production in Foliage of Pinus contorta[1])

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1. Abstract

Resin canal frequency was examined in needles from grafts and corresponding open-pollinated families of lodgepole pine (*Pinus contorta* Dougl.). Correlations between tree resin canal frequencies and absolute amounts of monoterpenes and three sesquiterpenes indicate that their synthesis or storage is largely compartmentalized in resin canals. In grafted trees, differences between clones in resin canal frequency were highly significant, indicating genetic control of resin canal frequency. Heritability estimates for resin canal frequency based on degree of resemblance between grafts of the same clone may have been influenced by the stabilization in grafts of position effects from the mother tree. Heritability estimates for resin canal frequency based on resemblance between open-pollinated seedlings of the same families, and correlations between progeny and mother trees, were moderate.

Key words: Pinus contorta, Pinaceae, lodgepole pine, genetics, leaf anatomy, terpenes, resin canals, biosynthesis, compartmentalization, heritability.

Zusammenfassung

Bei *Pinus contorta* Dougl. wurde die Häufigkeit des Auftretens von Harzkanälen in den Nadeln von Pfropflingen und entsprechenden frei bestäubten Familien untersucht. Korrelationen zwischen den Häufigkeiten des Auftretens der Harzkanäle eines Baumes und den absoluten Mengen der Monoterpene und von drei Sesquiterpenen zeigen an, daß ihre Synthese oder Lagerung zum großen Teil auf die Harzkanäle verteilt ist. Bei den Pfropflingen sind die Unterschiede in der Harzkanal-Frequenz zwischen den Klonen höchst bedeutsam, insofern als dadurch auf eine

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