productivity. Although specific gravity of Japanese larch is slighly lower than tamarack (Loo *et al.* 1982), the difference is negligible considering the superiority of Japanese larch in volume production. Based on volume production per unit area which is based in turn on height, diameter, and survival, provenances, MS 400 (Mt. Shirane), 401 (Mt. Asama), 407 (Mt. Komaga), 406 (Hida Mts.), and 388 (Mt. Azusa) performed best in central New Brunswick. These are the five most productive provenances with an average volume of 156 m^3/ha at age 19, and 29.6% higher than the average of all the Japanese larch provenances, and more than double that of tamarack.

Acknowledgements

The authors wish to acknowledge the major contribution to this study by Mr. H. G. MacGillivray, Maritimes Forest Research Centre (retired). Without his foresight in establishing these trials and in monitoring detailed record for almost 20 years, this genetic evaluation of Japanese larch would not have been possible.

Literature Cited

Cook, D. B.: Genetic improvement as applied to larch. Proc. 18th Northeast. For. Tree Improv. Conf. 69—72 (1971). — Eliason, E. J., and Carlson, D. E.: Comparative volume production of some species of larch. N. Y. Cons. Dep. Notes on Forest Investigations 46. 2 p (1963). — Farnsworth, D. H., Gatherum, G. E., Jokela, J. J., Kriebel, H. B., Lester, D. T., Merritt, C., Pauley, S. S., Read, R. A., Sajdak, R. L., and Wright, J. W.: Geographic variation in Japanese larch in north central United States plantations. Silvae Genet. 21: 139—147 (1972). — Gates, C. E. and Bilbro, J. D.: Illustration of a cluster analysis method for mean separations. Agronomy. J. 70: 462—465 (1978). — Genys, J. B.: Diversity in Japanese larch from different provenances studies in Maryland. Proc. 19th Northeast. For. Tree Improv. Conf. 2—11 (1971). — Hattemer, H. H.: Versuche zur geographischen variation bei der Japanischen Lärche. Silvae Genet. 18: 1—23 (1969). — Honer, T. G.: Standard volume tables and merchantable conversion factors for the commercial tree species of central and eastern Canada. Can. Dep. For. Rural Dev., For. Br., Inf. Rep. FMR-X-5 (1967). — Krusche, D. and S. Reck: Ergebnisse 15jähriger Herkunftsversuche mit Japanlärche (*Larix leptolepis* (Gord.)). I. Wuchsleistung von 25 Herkünften auf 11 Versuchsstandorten in der Bundesrepublik Deutschland. Allg. Forst- u. J.-Ztg. 151: 127—135 (1980). — Lagner, W.: An international provenance trial with *Larix leptolepis* Proc. 8th. Northeast. For. Tree Improv. Conf. 6—8 (1961). — Lee, C. H.: Geographic variation of growth and wood properties in Japanese larch in southwestern low Michigan. Proc. 12th Lake States For. Tree Improv. Conf. 35—46 (1975). — Lester, D. T.: A provenance test of Japanese larch. Univ. Wisconsin For. Res. Notes 112. 6 pp. (1964). — Littlefield, E. W., and Eliason, E. J.: Report on an experimental plantation of several species of larch in New York. Z. Forstgenet. 5: 166—169 (1956). — Loo, J., Fowler, D. P., and Schneider, M. H.: Geographic variation in specific gravity among Japanese larch from different provenances. Wood and Fiber 14: 281—286 (1982). — MacGillivray, H. G.: Larches for reforestation and tree improvement in eastern Canada. For. Chron. 45: 440—444 (1969). — Pauley, S. S., Mohn, C. A., and Crownwell, W. H.: NC-51 Japanese larch seed source tests in Minnesota. Univ. Minnesota For. Note 157. 2 pp. (1965). — Schober, R.: Ergebnisse von Larch- Art- und provenienzversuchen. Silvae Genet. 7: 137—154 (1958). — Scott, A. J., and Knott, M.: A cluster analysis method for grouping means in the analysis of variance. Biometrics 30: 507—512 (1974). — Stairs, R. S.: Geographic variation in Japanes larch. State Univ. N. Y. Coll. For. Syracuse Univ. Mimeo. 7 pp. (1965). — Toda, R., and Mikami, S.: The provenance trials of Japanese larch established in Japan and the tentative achievements. Silvae Genet. 25: 209—216 (1976). — Wright, J. W.: Genetics of Forest Tree Improvement. FAO Forestry and Forest Products Studies No. 16 Rome, Italy. 399 pp. (1962).

Studies of variation in Central American Pines V: a numerical study of variation in the Pseudostrobus group

By J. W. Stead[1])

Commonwealth Forestry Institute, Oxford, England

(Received 25th October 1982)

Summary

A study of the variation and taxonomy of the *Pseudostrobus* group *(sensu* Martínez) has been carried out at the Commonwealth Forestry Institute, Oxford.

The approach adopted in the investigation was to make site collections of approximately 25 trees each throughout the ranges of the taxa involved, to assess morphological and micromorphological characters of needles and cones on the material collected, and to submit the resulting data to numerical analysis . Principal components analysis was used to reveal patterns of variation in the data and to suggest possible taxonomic groupings. Canonical Discriminant Analysis was used to examine the relationships between the resulting groups, to suggest the best ways of discriminating between them and to measure the success with which groups had been formed. Individual collections made throughout the range, and taken on loan from various herbaria, were used to augment the coverage of the site collections.

It was confirmed that the *Pseudostrobus* group is composed of three species only. *Pinus pseudostrobus* Lindl. was found to be a very variable species with two identifiable infraspecific taxa. *P. tenuifolia* Benth. and *P. douglasiana* Martínez are relatively homogeneous species, more closely related to each other than to *P. pseudostrobus*. The character of hypodermal intrusions, found in the internal needle anatomy, was confirmed to be the most reliable for distinguishing *P. tenuifolia* and *P. douglasiana* from *P. pseudostrobus*.

On the basis of this morphological study a revised classification is proposed. The *Pseudostrobus* group now contains three species *P. pseudostrobus, P. maximinoi* H. E. Moore (the correct name for *P. tenuifolia)* and *P. douglasiana. P. pseudostrobus* has two infraspecific taxa *P. pseudostrobus* subsp. *apulcensis* (Lindl.) Stead and *P. pseudostrobus* var. *oaxacana* (Mirov) Harrison. Details of the taxonomic background and conclusions will be given in a subsequent paper.

Key words: Pinus pseudostrobus, Pinus tenuifolia, Pinus maximinoi, Pinus douglasiana, numerical taxonomy.

Zusammenfassung

Am Commonwealth Forestry Institute in Oxford wurde eine Untersuchung zur Variation und zur Taxonomie der *Pseudostrobus*-Gruppe bei *Pinus* (sensu Martínez durchgeführt.

[1]) NTB2, Shell Centre, London, England.

Dabei wurde angenommen, daß jeweils 25 Bäume jeder Standorteinheit der Taxa ausreichend sein würden, um auf Grund morphologischer und mikromorphologischer Eigenschaften eingesammelter Nadeln und Zapfen zu einer zahlenmäßigen Analyse zu gelangen. Die Hauptkomponenten der Analyse wurden benutzt, um ein Variationsmuster zu erarbeiten, aus dem taxonomische Gruppierungen abzuleiten waren. Daraus resultierende Gruppen wurden mit Hilfe der kanonischen Diskriminanzanalyse auf optimale Zugehörigkeit hin geprüft. Die in den Verbreitungsgebieten gesammelten Nadel- und Zapfenproben wurden dazu noch mit Herbarmaterial verglichen, um die Standortszugehörigkeit abzusichern. Es stellte sich heraus, daß die *Pseudostrobus*-Gruppe von *Pinus* nur drei Arten enthält, nämlich:

1.) *Pinus pseudostrobus* Lindl. mit zwei feststellbaren intraspezifischen Taxa,
2.) *Pinus tenuifolia* Benth. und
3.) *Pinus douglasiana* Martínez als relativ homogene Arten, die mehr zueinander tendieren als zu *Pinus pseudostrobus*.

Der Charakter der hypodermatischen Intrusionen, der in der inneren Nadelanatomie gefunden wurde, bestätigte sich als zuverlässig zur Unterscheidung von *P. tenuifolia* und *P. douglasiana* von *P. pseudostrobus*.

Auf Grund dieser morphologischen Studie wird eine revidierte Klassifizierung vorgeschlagen. Danach enthält die Pseudostrobus-Gruppe jetzt drei Arten, *Pinus pseudostrobus, P. maximinoi* H. E. Moore (Der korrekte Name für *P. tenuifolia)* und *P. douglasiana. P. pseudostrobus* hat zwei intraspezifische Taxa: *P. pseudostrobus* ssp. *apulcensis* (Lindl.) Stead und *P. pseudostrobus* var. *oaxacana* (Mirov) Harrison. Einzelheiten des taxonomischen Hintergrundes sowie eine Zusammenfassung folgen in einer weiteren Veröffentlichung.

1. Introduction

The Commonwealth Forestry Institute has been involved in the exploration, collection and evaluation of Central American Pines for more than 15 years. Efforts were initially concentrated on *Pinus caribaea* Mor. and *P. oocarpa* Schiede and later a group designated *P. pseudostrobus* Lindl. (including *P. tenuifolia* Benth.) was incorporated. The field exploration phase presented an ideal opportunity to study the botany of these species and certain taxonomic problems have been considered *(e.g.* Styles (1976); Styles *et al.* (1982)).

The use of the name *P. pseudostrobus* (including *P. tenuifolia)* reflects the uncertain taxonomy of the complex, and over the last three years a study has been carried out to examine the patterns of variation within it, and to clarify the relationship between *P. pseudostrobus* and *P. tenuifolia.* The intention was to examine and resolve all the problems within the Pseudostrobus group *sensu* Martínez (1945, 1948). The results are to be published in two parts. This article will deal with materials, methods and analyses and give the taxonomic conclusions in outline. A second article will give the taxonomic background and conclusions in detail.

The *Pseudostrobus* group contains three species, *P. pseudostrobus, P. tenuifolia* and *P. douglasiana* Martínez. The following infraspecific taxa, within *P. pseudostrobus,* are included in the group by Martínez: —

P. pseudostrobus var. *oaxacana* Martínez
P. pseudostrobus var. *apulcensis* (Lindl.) Martínez
P. pseudostrobus var. *coatepecensis* Martínez
P. pseudostrobus var. *estevezii* Martínez
P. pseudostrobus f. *protuberans* (Roezl) Martínez

The Pseudostrobus group extends from northern Mexico, south through Guatemala, El Salvador and Honduras, reaching its southern limit in Nicaragua, close to the southern limit of *Pinus* in the new world (see *Fig. 1).* Taxonomic confusion makes it difficult to map *P. pseudostrobus* and *P. tenuifolia* separately (see below). *P. douglasiana* is said to overlap with *P. pseudostrobus* in the north-western part of its range in Sonora, Sinaloa, Nayarit, Jalisco, Michoaćan and Oaxaca. The geographical distributions of the infraspecific taxa of *P. pseudostrobus* are poorly defined but the type localities, all in Mexico, are well known. *P. pseudostrobus* var. *oaxacana,* as its name suggests, is found

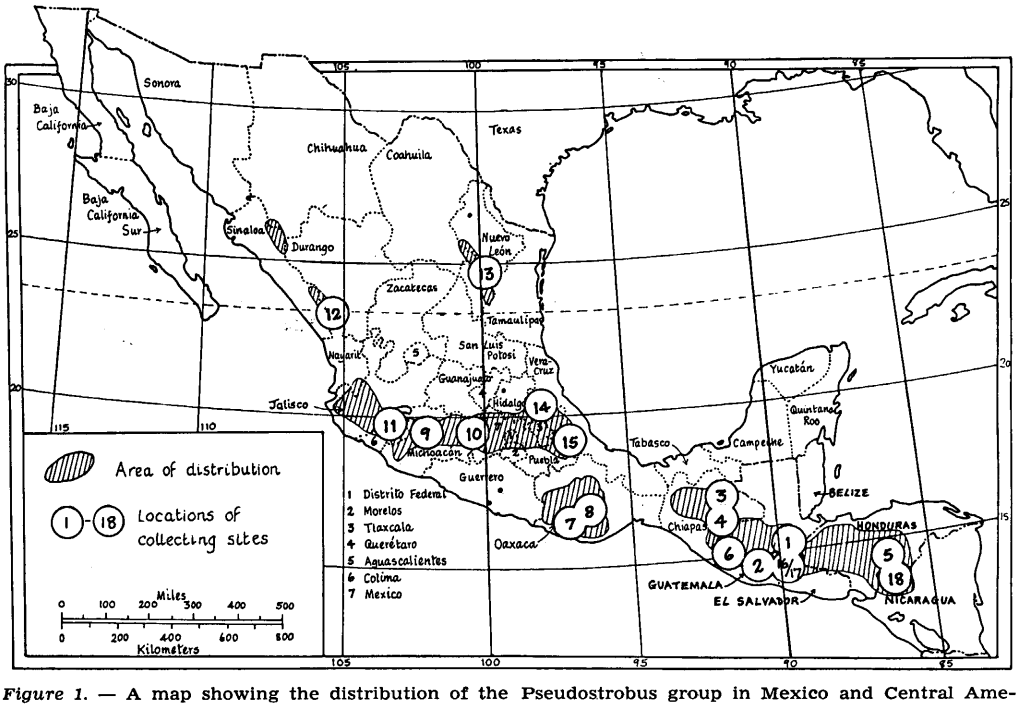

Figure 1. — A map showing the distribution of the Pseudostrobus group in Mexico and Central America (adapted from Critchfield and Little (1966)).

Table 1. — Details of Collecting Sites

Site Number	Identification at collection	Site	State/Dept. Country	Latitude Longitude	Altitude m	Number of trees in collection
1	P. tenuifolia	Finca Bucaral Morazán	El Progreso Guatemala	15° 00'N 90° 00'W	1400	25
2	P. pseudostrobus	Tecpán	Chimaltenango Guatemala	14° 45'N 91° 00'W	2325	25
3	P. tenuifolia	Ocosingo	Chiapas Mexico	17° 04'N 92° 15'W	1280	25
4	P. pseudostrobus	Comitán	Chiapas Mexico	16° 15'N 92° 08'W	1800	25
5	P. tenuifolia	La Fortuna	El Paraiso Honduras	14° 10'N 86° 35'W	1250	25
6	P. pseudostrobus	Palestina	San Marcos Guatemala	14° 55'N 91° 40'W	2500-2950	18
7	P. tenuifolia	San Gabriel Mixtepec	Oaxaca Mexico	16° 05'N 97° 06'W	700-1300	25
8	P. pseudostrobus var. oaxacana	Miahuatlán	Oaxaca Mexico	16° 20'N 96° 36'W	2000-2320	25
9	Pseudostrobus group	Uruapan	Michoacán Mexico	19° 25'N 101° 58'W	2050-2200	24
10	P. pseudostrobus	Angangueo	Michoacán Mexico	19° 37'N 100° 18'W	2300	25
11	Pseudostrobus group	Gomez Farías	Jalisco Mexico	19° 47'N 103° 29'W	2000	25
12	Pseudostrobus group	El Batel Concordia	Sinaloa Mexico	23° 17'N 106° 04'W	1310-1750	25
13	P. pseudostrobus var. estevezii	Monterrey & Iturbide	Nuevo León Mexico	25° 40'N 100° 19'W 24 °44'N 99 °54'W	950-1150 2000	25
14	P. pseudostrobus var. apulcensis	Apulco	Hidalgo Mexico	20° 19'N 98° 20'W	2100	25
15	P. pseudostrobus var. coatepecensis	Perote Coatepeque	Veracruz Mexico	19° 34'N 97° 14'W	2300-2750	23
16	P. tenuifolia	Finca Agua Tibia San José Pinula	Guatemala Guatemala	14° 30'N 19° 25'W	1840-2000	15
17	P. pseudostrobus	"	"	"	850-2100	10
18	P. tenuifolia	Jinotega	Jinotega Nicaragua	13° 06'N 86° 00'W	1380	10

typically in the state of Oaxaca. *P. pseudostrobus* var. *apulcensis* is from Apulco, Hidalgo, *P. pseudostrobus* var. *coatepecensis* from Veracruz, *P. pseudostrobus* var. *estevezii* from Nuevo León and *P. pseudostrobus* f. *protuberans* from Michoacán.

Taxonomic problems in the *Pseudostrobus* group were best summarised for the author by Critchfield (pers. comm.). In a publication on the geographic distribution of pines (Critchfield and Little (1966)) *P. pseudostrobus* and *P. tenuifolia* are treated as synonymous and referred to as "*P. pseudostrobus*, including the doubtfully distinct *P. tenuifolia* Benth.". In the same publication *P. douglasiana* is mapped together with *P. pseudostrobus*.

Critchfield (pers. comm.) suspected the existence of two taxa (species) within the very variable taxon called *P. pseudostrobus* but felt that misidentifications and misuse of names in the literature made it impossible to map them separately. His experience in the field, however, confirmed the obvious problems which other workers had encountered. In Michoacán, Mexico, where *P. pseudostrobus* (including *P. tenuifolia)* and *P. douglasiana* occur together, he was unable to make definite identifications and, in some areas of forest, was unsure whether he was dealing with three separate taxa or one very variable taxon. This view is summarized in Coyne and Critchfield (1974).

It seems that complicated patterns of variation involving three separate taxa caused the confusion found in earlier treatments of the *P. pseudostrobus* complex, based mainly on Mexican material. This confusion has pervaded treatments of this complex in Central America. Initial observations in Central America by the CFI suggested that two fairly distinct taxa, *P. pseudostrobus* and *P. tenuifolia*, are to be found in Guatemala and that the latter extends south into Hondoras, Nicaragua and probably El Salvador. Studies of the pines of Guatemala by Schwerdtfeger (1953) and Aguilar (1961) treated these two as separate species. Standley and Steyermark (1958) however, considered them to be identical, and referred to them as *P. pseudostrobus* (the oldest name). This view was accepted by Molina (1964).

Thus a solution to the taxonomic problems within the *P. pseudostrobus* complex requires a study of material from throughout the ranges of the taxa in Central America and Mexico. Patterns of variation of *P. pseudostrobus* and *P. tenuifolia* are probably confused by the existence of the closely related species *P. douglasiana* in the northwestern part of the range. Study of information available in the literature suggests that *P. pseudostrobus* is a very variable taxon, and that divisions within it require further study.

2. Materials

2.1 *Collection of specimens*

Most of the material obtained for this study comprised "population " collections at specified sites throughout the

Table 2. — Characters of needles and cones assessed on all specimens.

Character	Code*
Length of needle (cm)	NLTH
Width of needle (mm x 40)	NWTH
Number of needles per fascicle	LVES
Length of sheath (mm)	SHTH
Number of stomatal lines on dorsal surface	SLDO
Number of stomatal lines on ventral surface	SLVE
Number of stomata per 5 mm line on dorsal surface	STOM
Number of marginal serrations per 5 mm	SERR
Presence/absence of pruinose bloom on branches	PRUI
Number of resin canals	RCAN
Number of intrusions of hypoderm to endoderm	INTR
Number of hypoderm cells touching endoderm	HTOE
Number of endoderm cells on dorsal surface of vascular bundle	ENDO
Lenth of cone (cm)	CLTH
Width of cone at widest point (cm)	WTH1
Width of cone at right angles to WTH1 (cm)	WTH2
Width of apophysis (mm)	WAPO
Depth of apophysis (mm)	DAPO
Height of apophysis (mm)	HAPO
Presence/absence of peduncle	PEDU

* This four letter code was used to refer to the characters in computer analyses and will sometimes be used in tables and text.

range of the *Pseudostrobus* group. At these sites up to 25 specimens from well spaced trees were collected. The aim of these site collections was to indicate the range of variants of a species within one site and that together they would give a good indication of the complete variation pattern of that species. Individual collections were made, or taken on loan from various herbaria, and used to supplement the site collections.

The location of site collections are shown on *Fig. 1* and listed in *Table 1.*

2.2 *Collection of data*

2.2.1 *Selection of characters*

Based on previous experience, a search of the literature and an investigation of a limited number of specimens available in Oxford before the project began, a set of 20 characters thought to be of value for discriminating between taxa of the *Pseudostrobus* group was selected for use in this study. The characters are listed in *Table 2.*

Several characters refer to the morphology of the needles the needle sheath and cones. The intention was to define their size and shape in terms of a set of measurements in such a way as to describe differences between taxa in the group. Several micromorphological characters of the stomata and needle serrations are included. These have been used in previous investigations (see MERGEN *et al. (1965)* and STYLES *et al.* (1982)) but have not been examined in the Pseudostrobus group before.

Details of the internal needle anatomy of species of the Pseudostrobus group were considered by SHAW (1909). He discusses the intrusions of the hypoderm which extend to the endosperm partitioning the chlorenchyma, which characterize his *P. pseudostrobus* var. *tenuifolia.* MARTÍNEZ (1948) cites this character when reinstating *P. pseudostrobus* var. *tenuifolia* to specific rank, and in his description of *P. douglasiana.* Intrusions were measured in this study by two characters *i.e.* the number of intrusions, and the number of hypoderm cells touching the endoderm. From the initial survey of specimens it was thought that the number of endoderm cells across the dorsal side of the vascular bundle might be a useful character and this was also included. In the assessment of the specimens the number and position of the resin canals was noted. It was found that the vast majority of resin canals were medial in position so only the average number per needle was used as a character in the analyses.

Considering the branchlets, SHAW (1909) described *P. pseudostrobus as* "usually conspicuously pruinose" *(i.e.* having a waxy bloom). The presence or absence of this character was recorded. Similarly the presence or absence of a cone peduncle was recorded. *P. tenuifolia* cones are said to retain the peduncle when they fall. *P. pseudostrobus* cones leave the peduncle, and sometimes a few basal scales, behind when they fall.

2.2.2 *Assessment of characters*

The characters assessed are shown diagrammatically in *Fig. 2.*

Needle characteristics were assessed on 5 separate fascicles from each branchlet. Length of the complete fascicle and the length of the sheath around the bottom were taken from a metal rule. The number of needles per fascicle was simply recorded.

The remaining characters of the needle were assessed on one needle from each fascicle using a 5 mm length taken from around the mid-point, observed using a low-power binocular microscope (Magnification x80). It was found to be most convenient to deal with all five of the 5 mm lengths from one branchlet specimen at one time, mounted on a glass slide. These micromorphological characters are demonstrated in *Fig. 2(a).* The number of stomata per 5 mm line was counted for one line chosen at random on the dorsal surface and the number of marginal serrations was counted for one side of the needle. The width of the needle was assessed using a calibrated graticule mounted in the microscope eye-piece.

The pruinose bloom on the branchlet specimens was recorded as 0 for presence and 1 for absence.

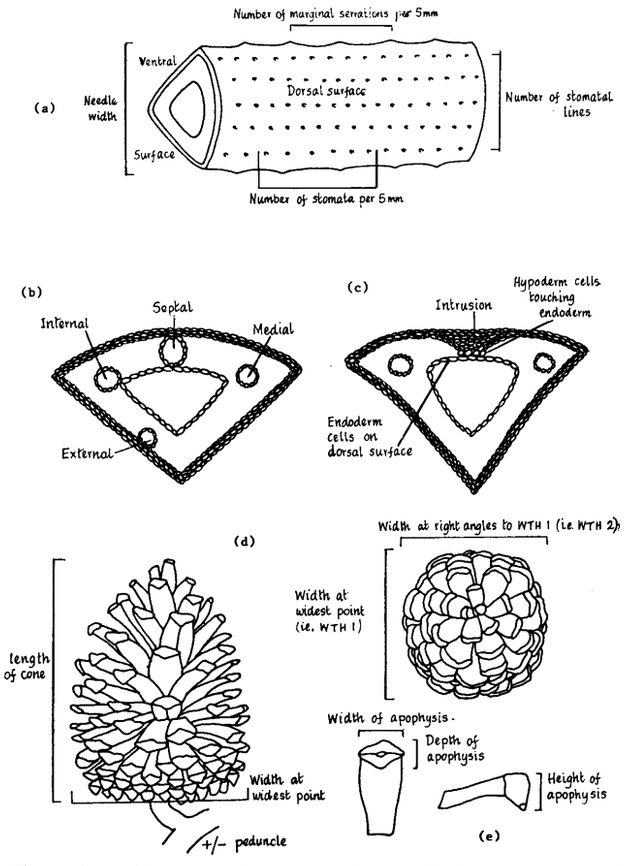

Figure 2. — Diagrammatic representation of characters assessed.

Details of the internal needle anatomy were observed by taking sections from the mid-point of each of the five needles already investigated above. The needles were boiled for approximately 30 minutes before sectioning by hand using a one-sided razor blade. The resulting sections were fumed in latic acid to clear them for easier observation of the internal structure.

The possible positions for resin canals are shown in *Fig. 2(b)*. The characters of the intrusions were also recorded from the sections. A section showing one intrusion and four hypoderm cells touching the endoderm is shown in *Fig. 2(c)*. The number of endoderm cells on the dorsal surface of the vascular bundle was recorded for each section. The section in *Fig. 2(c)* has ten cells.

Characters were assessed on the two cones collected from each tree sampled. These are demonstrated in *Fig. 2(d)*. Length and width were measured to two decimal places using a pair of calipers. The value of Width 1 was taken at the widest point of the cone and the value of Width 2 was measured in the same plane, at right angles to Width 1.

Three measurements of the apophysis of the cone scale were recorded from five scales evenly spaced around the widest point of the cone. These are shown in *Fig. 2(e)*. They were assessed using a pair of dividers and a metal rule calibrated to 0.5 mm. In an effort to make these small measurements as accurately as possible they were recorded to the nearest 0.25 mm depending on whether the point of the divider was placed on a line or between two lines.

Presence of a peduncle was indicated with 1, absence with a 0.

3. Methods of Analysis

The genus *Pinus* does not exhibit the same obvious patterns of variation found in angiosperms. Most characters, of leaf and cone, show continuous variation and the ranges of variation for different species often overlap. Therefore herbarium specimens are superficially very similar, and it is necessary to study many of them to resolve the complex patterns of variation found.

Bearing these problems in mind it was felt that a numerical approach would be helpful in this study. All of the data available for each specimen could be recorded onto data sheets and undergo initial sorting using mathematical methods. This would allow full consideration of data from all sources and by-pass the process of matching all the herbarium specimens and data sheets. This approach has been pursued at Oxford in previous studies (Styles *et al.* (1982)).

Numerical techniques have been used in studies on several plant groups including *Pinus*. Jeffers and Black (1963) applied three different techniques (Q-techniques, Discriminant Analysis, Component Analysis) to study variation within *P. contorta* Dougl.. The analysis integrated data from needles, cones, seeds and information on wood anatomy. A multivariate study of the *P. chiapensis-monticola-strobus* group suggested that the former species, originally described as a variety of *P. strobus* L., should be elevated to specific rank (Andresen (1966)). Smouse and Saylor (1973) used Canonical Analysis to reveal relationships between three species of pine (*P. taeda* L., *P. echinata* Mill. and the *P. rigida-serotina* complex).

Typically, methods of analysis have been used in combination to reveal patterns of variation contained in data. Dancik and Barnes (1975) applied five different methods of analysis (Discriminant Analysis, Principal Components Analysis, Canonical Variates Analysis, Prim network Analysis and Cluster Analysis) to a set of morphological measurements from two species of birch and hybrids between the two. They too suggested the use of combinations of different analyses to reveal details of a pattern of variation. They recommended particularly the use of Principal Components Analysis and Canonical Variates Analysis.

Principal Components Analysis (PCA) can be used to reveal the broad patterns of variation within data and to suggest possible groupings of individuals into taxa. The relationship between the groupings can then be examined using a form of Canonical Analysis (Canonical Discriminant Analysis (CDA) was chosen). CDA can show how similar groups are to each other, suggest the best characters for discriminating between groups, and demonstrate the sucess with which individuals have been classified into different groups.

It was decided to apply these analyses to the data available for this study.

4. Analyses

The first objective was to investigate the patterns of variation and possible groupings within the *Pseudostrobus* group. Data from sites 1—12, 16, 17 and 18 (see *Table 1)* were submitted to a combination of PCA's and then CDA's. Sites 1—12 form a sequence moving approximately south to north covering the area where the ranges of the three species of the group overlap. Later assessments, of sites 16 and 17 which represent *P. tenuifolia* and *P. pseudostrobus* growing together at one site, and from site 18 which is the most southerly site collection of *P. tenuifolia,* were included in these analyses.

The second objective was an investigation of the variation pattern and groupings within *P. pseudostrobus* and the status of the various infraspecific taxa. Site collections 8, 13, 14 and 15 represent the four varieties of *P. pseudostrobus* which have been described.

4.1 *Patterns of variation within the Pseudostrobus group*

4.1.1 *Principal Components Analysis (PCA)*

The site collections were assessed and analysed approximately in the order in which they are numbered. The resulting data were analysed, using PCA, as it accumulated. The first analyses revealed a clear division of the material into two groups representing *P. tenuifolia* and *P. pseudostrobus*. Typically, PC1 (Principal Component 1) explaind a large proportion of the variation, and most of the characters were highly correlated with that PC. A graph plot of PC1 vs. PC2 produced a clear separation of the individuals in the analysis into two groups, representing the two species.

As more site collections were assessed, data were added in to these analyses, and for the southern half of the range of the *Pseudostrobus* group the resulting picture remained more or less the same. All of the individuals from a site were found to be of the same species. Inevitably as the area covered by the collections was expanded, the range of variation within the data increased. The spread of points for each species group, on the graph of PC1 vs. PC2, increased and the gap between them decreased. The following analysis summarizes the picture for the southern half of the range.

Table 3. — PCA sites 1, 2, 3, 4, 5, 6, 7, 8, 16 and 17 — roots of the correlation matrix.

Root		% of variation
1	10.587	52.93
2	2.138	10.69
3	1.109	5.55
4	0.976	4.88
5	0.925	4.62
6	0.848	4.24
7	0.620	3.10
8	0.559	2.80
9	0.432	2.16
10	0.345	1.73
11-20	1.461	7.30

Table 4. — PCA sites 1, 2, 3, 4, 5, 6, 7, 8, 16 and 17 — scaled eigenvectors (largest scaled to unity).

NLTH	NWTH	LVES	SHTH	SLDO	SLVE	STOM	SERR	PRUI	RCAN
INTR	HTOE	ENDO	CLTH	WTH1	WTH2	WAPO	DAPO	HAPO	PEDU
Vector 1									
0.669	0.934	0.255	0.871	0.929	0.885	0.062	-0.235	-0.669	0.738
-0.781	-0.714	0.804	0.909	1.000	0.993	0.954	0.894	0.869	-0.391
Vector 2									
0.692	0.129	-0.017	0.399	0.187	0.148	0.963	1.000	0.428	-0.462
0.718	0.756	0.374	0.027	0.027	0.008	0.145	0.188	0.130	0.283
Vector 3									
-0.193	-0.367	1.000	-0.060	-0.261	-0.220	-0.435	0.210	-0.313	-0.486
0.324	0.406	-0.219	0.291	0.183	0.185	0.402	0.291	0.142	-0.645

Characters (see *Table 2*)

4.1.1.1 *PCA Sites 1, 2, 3, 4, 5, 6, 7, 8, 16 and 17*

The data from these site collections were submitted to a PCA. The roots of the correlation matrix and the percentage of the total variation they represent are shown in *Table 3* for the first 10 roots. It can be seen that PC1 explains a fairly large proportion of the total variation in the data (52,93%) and that subsequent PC's explain small and decreasing amounts.

The relative contribution of each variable to each PC is obtained from the table of eigenvectors (see *Table 4* scaled eigenvectors (largest scaled to unity)). In fact the majority of characters make an important contribution to PC1 (± 0.700 will be taken as an arbitrary level of importance throughout this study). PC1 represents cone size (CLTH, WTH1, WTH2, WAPO, DAPO, HAPO), needle size (NWTH, SHTH), number of stomatal lines (SLDO, SLVE), number of resin canals (RCAN) and number of endodermal cells (ENDO), negatively correlated with intrusions (INTR, HTOE).

PC's 2 and 3 which account for 10.69% and 5.55% of the variation represent serrations (SERR), stomata (STOM) and intrusions (INTR, HTOE) and needles/fascicle (LVES) respectively.

The results of the analysis are well represented by a graph plot of the PC scores for each individual from the two sites for PC1 vs. PC2 (see *Fig. 3*). The individuals are marked on the graph with a 1(= site 1) or a 2 (= site 2) *etc.* and they are divided into two groups. The dashed line indicates the division between sites 1, 3, 5, 7 and 16 *(P. tenuifolia* and sites 2, 4, 6, 8 and 17 *(P. pseudostrobus).*

The separation is along the PC1 axis only and it can be concluded that, for this sample of the range, *P. pseudostrobus* and *P. tenuifolia* are distinct, and differ in all the characters which contribute to PC1. *P. pseudostrobus* has larger, wider cones with better developed apophyses and wider needles with larger sheaths. As for micromorphological characters and internal needle anatomy, *P. pseudostrobus* has more stomatal lines, resin canals and endoderm cells and fewer hypodermal intrusions. The second axis (PC2) accounts for a small proportion of the variation and reveals no pattern in the data.

4.1.1.2 *PCA Sites 3, 4, 7, 9, 10, 11 and 12*

The next phase of the study involved the assessment and analysis of data from collections in the north-western part of the range in Mexico *i.e.* sites 9, 10, 11 and 12. Site 10 is Angangueo, Michoacán, the type locality of *P. pseudostrobus*. Sites 9, 11 and 12 were noted at collection to be a mixture of variants of the Pseudostrobus group. Data for sites 3 and 7 were included to represent known collec-

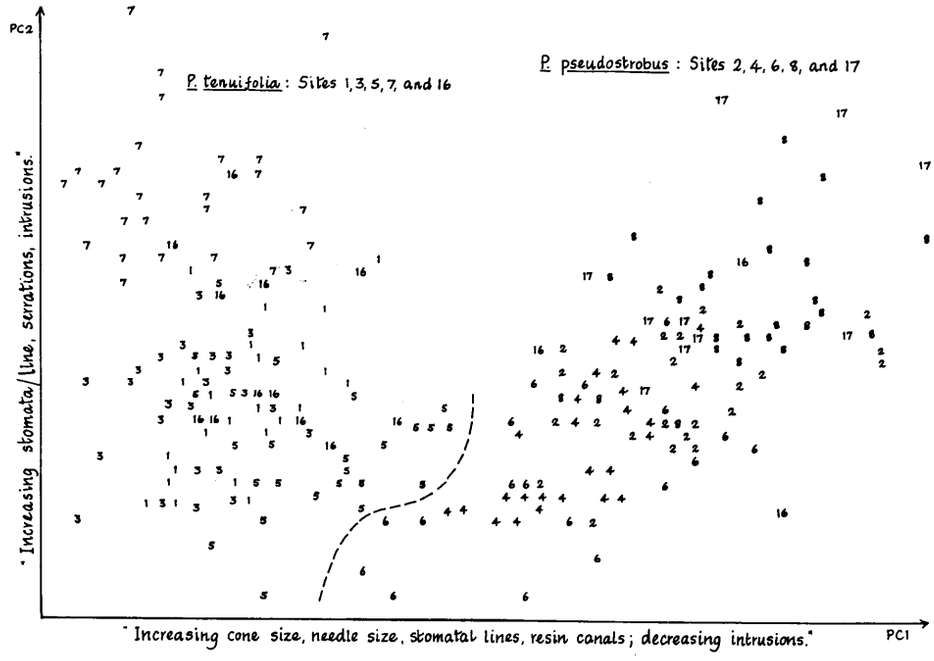

Figure 3. — PCA Sites 1, 2, 3, 4, 5, 6, 7, 8, 16 and 17 (PC1 vs. PC2). 1 = Site 1, 2 = Site 2 *etc.*

Table 5. — PCA sites 3, 4, 7, 9, 10, 11 and 12 — roots of the correlation matrix.

Root		% of variation
1	7.667	38.33
2	3.617	18.08
3	1.528	7.64
4	1.332	6.66
5	1.011	5.06
6	0.798	3.99
7	0.771	3.86
8	0.564	2.82
9	0.465	2.32
10	0.462	2.31
11-20	1.785	8.93

Table 6. — PCA sites, 3, 4, 7, 9, 10, 11 and 12 — scaled eigenvectors (largest scaled to unity)

NLTH	NWTH	LVES	SHTH	SLDO	SLVE	STOM	SERR	PRUI	RCAN
INTR	HTOE	ENDO	CLTH	WTH1	WTH2	WAPO	DAPO	HAPO	PEDU
Vector 1									
0.308	0.660	0.938	0.536	0.781	0.841	0.206	0.032	-0.432	0.733
-0.577	-0.548	0.652	0.845	1.000	0.995	0.908	0.843	0.761	-0.681
Vector 2									
1.000	0.675	0.093	0.885	-0.247	0.282	0.402	0.625	0.463	-0.311
0.873	0.862	0.652	-0.212	-0.011	-0.039	0.085	0.245	-0.104	0.559
Vector 3									
-0.127	-0.562	0.479	-0.041	0.192	0.084	1.000	0.935	-0.018	-0.303
-0.228	-0.171	-0.154	-0.005	-0.058	-0.056	0.190	0.098	-0.429	-0.347

tions of *P. tenuifolia,* and for site 4 to represent known collections of *P. pseudostrobus.* Details of the roots and the eigenvectors are shown in *Tables 5* and *6.*

Consideration of *Tables 5* and *6* alone suggests that the pattern of variation in this set of data is more complex than any so far examined. PC1 accounts for a relatively small proportion of the total variation (38.33%) and PC2 a much higher proportion than previously found (18.08%). The remaining PC's are relatively insignificant. Similarly fewer characters contribute significantly to PC1 and more characters contribute significantly to PC2. PC1 represents mainly cone size (CLTH, WTH1, WTH2, WAPO, DAPO, HAPO), stomatal lines (SLDO, SLVE), and resin canals (RCAN). PC2 represents needle length (NLTH), sheath length (SHTH) and intrusions (INTR, HTOE).

The greater complexity of the variation pattern in these data is shown by the graph plot of all the individuals in the analysis for PC1 vs. PC2. (see *Fig. 4).* Three separate groups can be identified on the graph. It can be seen that *P. tenuifolia* and *P. pseudostrobus* are spread along PC1 to form two groups less well separated than has been seen previously. The two more or less vertical dotted lines in *Fig. 4* indicate an area of uncertainty between the two species groups. Of great interest is the fact that a third group has been separated out along PC2, indicated on *Fig. 4* by the horizontal dotted line. The individuals in this group were found to conform to the description of *P. douglasiana.* The *P. tenuifolia* group is comprised of individuals from sites 3 and 7 and the *P. douglasiana* group of selected individuals from sites 9 and 12. The *P. pseudostrobus* group is comprised of individuals from sites 4, 10, 11 and the remaining members of sites 9 and 12. These collections cover the known area of confusion within the Pseudostrobus group and include representatives from further south in the range where the pattern of variation is more clear. This PCA, and particularly the graph in *Fig. 4* suggests an explanation for the confusion found.

It would seem that *P. tenuifolia* is fairly well defined as a species. Apart from a few individuals, sites 3 and 7 are grouped quite closely together in one part of the graph and most importantly, no specimens of *P. tenuifolia* were found in the site collections 9, 11 and 12 which were originally labelled "Pseudostrobus group" only (with the exception of one deviant from site 12). The majority of the specimens from sites 9, 10, 11 and 12 were *P. pseudostrobus* and they form a fairly well defined group in *Fig. 4.* The gap between *P. tenuifolia* and *P. pseudostrobus* is not very clearly defined and the specimens which fall between the two (bounded by the vertical dotted lines) were examined in detail.

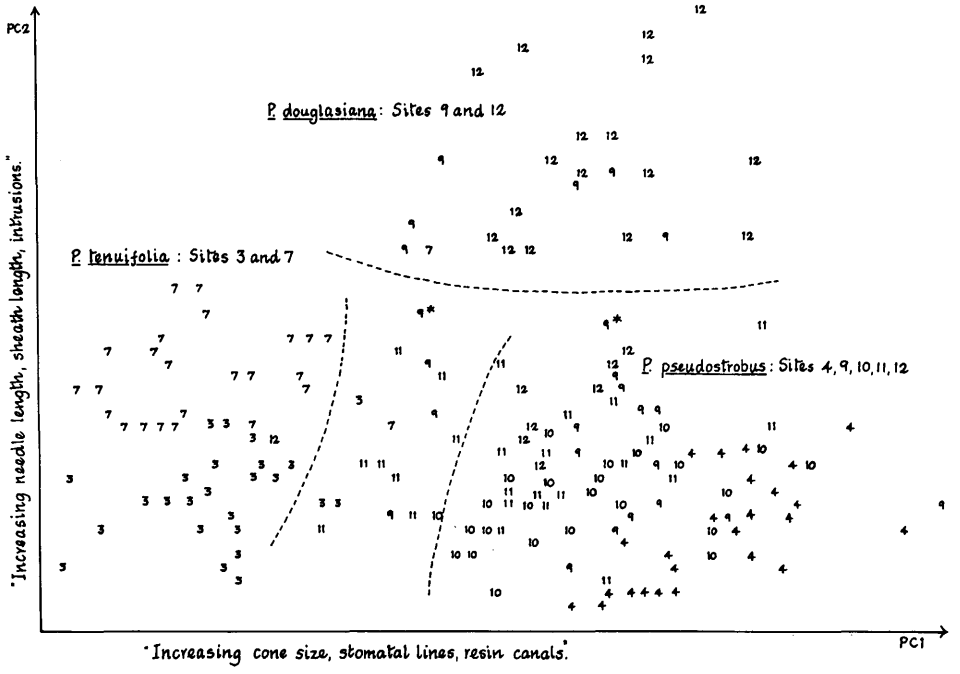

Figure 4. — PCA Sites 3, 4, 7, 9, 10, 11 and 12 (PC1 vs. PC2). 3 = Site 3, 4 = Site 4 *etc.*

Table 7. — PCA sites 1, 3, 5, 7 and 18 + *P. douglasiana* collections from sites 9 and 12 + 41 individual collections of *P. tenuifolia* and *P. douglasiana*-roots of the correlation matrix.

Root		% of variation
1	7.923	39.61
2	2.900	14.50
3	1.402	7.01
4	1.199	6.00
5	1.073	5.36
6	0.951	4.76
7	0.808	4.04
8	0.747	3.73
9	0.585	2.93
10	0.470	2.35
11-20	1.942	9.71

It can be seen that most of the specimens in this intermediate zone are from sites 9 (Uruapan, Michoacán) and 11 (Gomez Farías, Jalisco). Close inspection of these individuals revealed that they were most like *P. pseudostrobus*. The overall morphology resembled *P. tenuifolia* and in particular the cones were smaller with more delicate scales than typical *P. pseudostrobus*. Most importantly however, the needles were not found to have any intrusions and it was on the basis of this character, the value of which will be discussed in detail below, that these intermediates were provisionally included within a broader concept of *P. pseudostrobus*.

The identity of *P. douglasiana* has also been the source of confusion in the field. As can be seen from *Fig. 4* all but one of the specimens which are grouped together as *P. douglasiana* are from sites 9 and 12 which means that this species grows in close association with *P. pseudostrobus*. Also, from the relative positions of the *P. pseudostrobus* group and the *P. douglasiana* group, it can be seen that, in terms of PC1 which represents mainly cone size and shape, *P. pseudostrobus* and *P. douglasiana* are very similar. They are separated along the PC2 axis on the basis of needle length, sheath length and the intrusions character. This might explain why the two species could be confused in the field when heavy reliance is placed on characters of gross morphology, particularly of cone characters.

Thus *Fig. 4* suggests a possible explanation of the problems within the *Pseudostrobus* group. *P. pseudostrobus* is shown to be a very variable species which in the northwestern part of its range can resemble *P. tenuifolia* enough to blurr the differences between these two species in the field. As will be discussed later, detailed study of internal anatomy helps to clarify the differences. *P. douglasiana* resembles *P. pseudostrobus* in the field, and these two species are found growing in close association. They can in fact be separated on needle characters, particularly the presence or absence of intrusions. This proposed explanation of the relationships between *P. pseudostrobus, P. tenuifolia* and *P, douglasiana* will be examined further in subsequent analyses.

4.1.1.3 *PCA P. tenuifolia and P. douglasiana*

Of particular interest is the relationship between *P. tenuifolia* and *P. douglasiana*, both said to be characterized by the presence of hypodermal intrusions, and obviously closely related. The results of the previous analysis would suggest that they are well defined and distinct species. It was decided to submit data from *P. tenuifolia* and *P. douglasiana* site collections to a PCA to examine the relationship further, and to include data from other individual

Table 8. — PCA sites 1, 3, 5, 7 and 18 + *P. douglasiana* collections from sites 9 and 12 + 41 individual collections of *P. tenuifolia* and *P. douglasiana* — scaled eigenvectors (largest scaled to unity).

NLTH	NWTH	LVES	SHTH	SLDO	SLVE	STOM	SERR	PRUI	RCAN
INTR	HTOE	ENDO	CLTH	WTH1	WTH2	WAPO	DAPO	HAPO	PEDU
Vector 1									
0.908	0.957	0.063	1.000	0.448	0.905	0.563	0.584	0.250	0.337
0.616	0.565	0.927	0.483	0.827	0.759	0.906	0.966	0.658	-0.003
Vector 2									
-0.134	0.238	0.160	-0.073	0.415	0.110	-0.495	-0.579	-0.669	0.816
-0.962	-1.000	-0.332	0.895	0.734	0.744	0.157	-0.086	-0.026	0.101
Vector 3									
-0.191	-0.552	0.885	-0.311	-1.000	-0.694	-0.238	-0.304	-0.112	-0.235
0.447	0.625	-0.259	0.600	0.468	0.395	0.371	0.537	0.350	0.435

Characters (see *Table 2*)

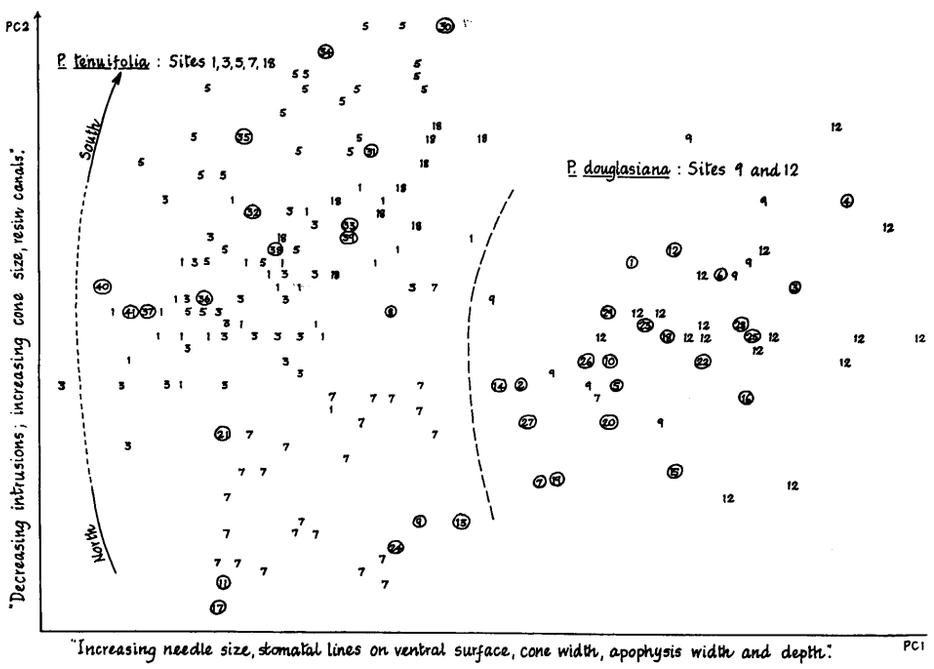

Figure 5. — PCA *P. tenuifolia* and *P. douglasiana* (PC1 vs. PC2) 1 = Site 1, 3 = Site 3. etc.

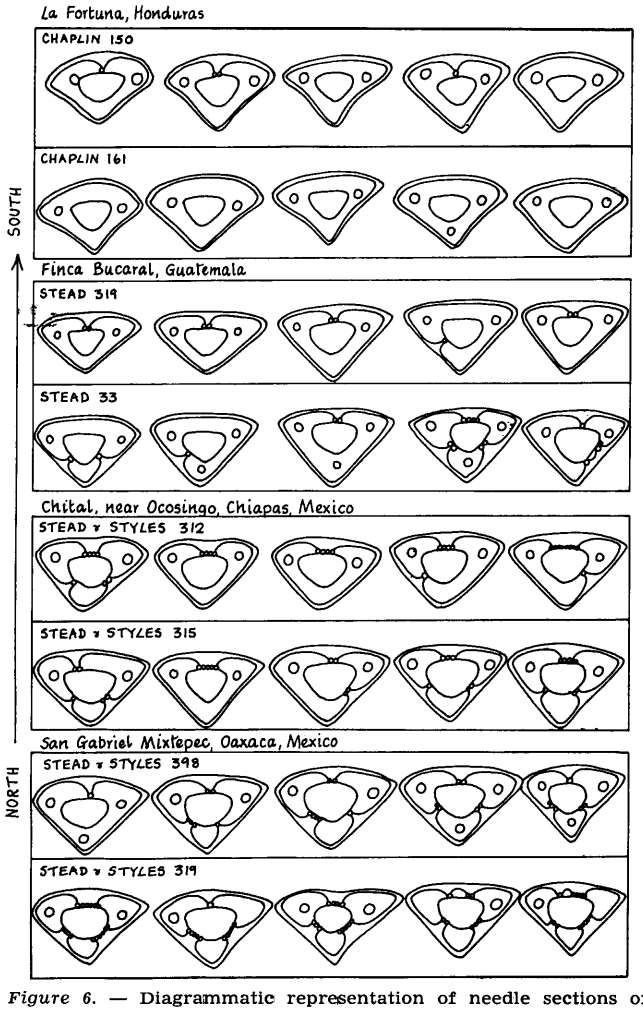

Figure 6. — Diagrammatic representation of needle sections of *P. tenuifolia* from throughout its range showing the clinal pattern of variation in the intrusions characters (INTR, HTOE).

collections which had been found to have the intrusions character.

Data for sites 1, 3, 5, 7 and 18 were included in the analysis representing *P. tenuifolia.* Individuals of *P. douglasiana* were extracted from site collections 9 and 12 based on the results of the previous analysis. Two individuals from site 9 (marked with an asterisk (*) on *Fig. 4)* were also included because the presence of intrusions in the needles separated them from *P. pseudostrobus,* with which they might have otherwise been grouped. A total of 41 individual tree collections thought to be *P. tenuifolia* or *P. douglasiana* were included with this site material in a PCA. Details of the roots and eigenvectors from this analysis are shown in *Tables 7* and *8.* The graph plot of PC1 vs. PC2 is shown in *Fig. 5.* It can be seen that the individuals in the analysis have been divided into two groups along the horizontal axis (PC1) The groups represent sites 1, 3, 5, 7 and 18 *(P. tenuifolia)* and sites 9 and 12 *(P. douglasiana)* and the 41 individual collections (indicated by ringed numbers) are divided between the two.

It is interesting to note that despite the addition of extra site collections and 41 individual collections, the separation into two groups is as clear as was found in the previous analysis (see *Fig. 4)* suggesting that *P. tenuifolia* and *P. douglasiana* are indeed two separate species. According to this analysis *P. douglasiana* has longer and wider needles and longer sheaths, more stomatal lines on the ventral surface of the needles and wider cones with bigger apophyses than *P. tenuifolia.*

Comparing the two species groups it is apparent that there is greater variation within *P. tenuifolia* than within *P. douglasiana,* along the vertical axis (PC2) which primarily represents the intrusions character (INTR, HTOE). This means that the intrusions character is very variable within *P. tenuifolia* (as was demonstrated by SHAW (1909)) but this analysis exhibits very clearly that the variation presents a regular, clinal pattern. There is a decreasing frequency of intrusions going from north to south starting with site 7 (Oaxaca, Mexico) grouped at the bottom of the graph, moving through site 3 (Chiapas, Mexico) and site 1 (Guatemala) to sites 5 and 18 (Honduras and Nicaragua respectively). The nature of the variation and its pattern is demonstrated in *Fig. 6.*

Typically specimens from the northern part of the range of *P. tenuifolia* have an average of three intrusions per needle. Specimens from the mid-part of the range in Chiapas, Mexico, and Guatemala have an average of one or two. In specimens from the very south of the range intrusions are infrequent, averaging less than one per needle and from some trees five fascicles have been sampled without finding a single intrusion.

These few site collections represent a rather sparse sample of the cline but the individual collections from throughout the range tended to confirm the pattern. In the northern part of the range in Mexico, specimen 17 is from Jalisco, 13 and 24 from Michoacán, and 8, 9, 11 and 21 from Guerrero. In the middle part of the range 40 and 41 are from Chiapas, Mexico, 37, 38 and 39 from Guatemala and 36 from El Salvador. In the south, 34 and 35 are from Honduras and 30, 31, 32 and 33 from Nicaragua. All of these specimens follow approximately the clinal pattern of the site collections.

The fact that the intrusions character has been shown to vary in a consistent manner establishes it as a useful character for distinguishing between *P. pseudostrobus* and *P. tenuifolia.*

The specimens from the intermediate zone on *Fig. 4* are largely from Jalisco and Michoacán where *P. tenuifolia* typically has 2 or 3 intrusions per needle. The fact that these specimens were found to have no intrusions strongly suggests that they are indeed *P. pseudostrobus* despite their similarities with *P. tenuifolia* in overall morphology.

4.1.2 *Canonical Discriminant Analysis (CDA)*

4.1.2.1 *CDA Species Groups, all variables (characters)*

Analysis of the data using PCA has revealed the existence of three species groups and has helped to provide an explanation for the confusion between them.

It was decided to divide the material into species groups and examine the relationships between them using CDA. This analysis can also be useful in identifying the best characters for distinguishing groups and can measure the success with which individuals have been classified into groups.

Data from sites 1—12 were used in this analysis. Sites 2, 4, 6, 8, 9, 10, 11 and 12 (167 individuals) were grouped as *P. pseudostrobus.* Sites 1, 3, 5 and 7 (100 individuals) were grouped as *P. tenuifolia* and individuals from sites 9 and 12 identified by previous analyses were grouped together as *P. douglasiana* (25 individuals). It was assumed that site collections were of one species only (except in the

Table 9. — CDA species groups all variables (characters) — eigenvalues.

	Eigen value	% of variance
1	6.22079	70.52
2	2.60016	29.48

Table 10. — CDA species groups all variables (characters) — standardized canonical discriminant function coefficients.

Variable (character)	Function 1	Function 2
NLTH	-0.02190	-0.02816
NWTH	-0.54860	0.29359
LVES	-0.02476	0.00092
SHTH	0.05955	0.52977
SLDO	0.32795	-0.58878
SLVE	0.19629	0.37021
STOM	-0.10802	0.08003
SERR	0.08913	0.05074
PRUI	-0.00699	0.21638
RCAN	0.19838	0.35538
INTR	-1.10963	0.18765
HTOE	0.45086	0.27433
ENDO	0.38115	0.03799
CLTH	-0.31333	-0.47837
WTH1	-0.73811	0.74506
WTH2	0.45918	-0.15355
WAPO	0.42834	0.01455
DAPO	0.45217	0.28789
HAPO	0.17936	-0.21001
PEDU	-0.34281	0.10616

case of mixtures of *P. pseudostrobus* and *P. douglasiana).* Previous analysis had suggested that a few specimens might be different from the majority species at a site but they were considered to be extreme forms of that species and were included with it in this analysis.

Details of the eigenvalues *(i.e. roots)* and the canonical discriminant functions *(i.e.* eigenvectors) are shown in *Tables 9* and *10.*

Table 9 suggests that between the three groups, function 1 makes one very clear separation and that function 2 makes a somewhat less clear but still important separation. It is evident from *Table 10* that the important influence in function 1 is the intrusions character (INTR). All the other characters are relatively unimportant. With respect to function 2 cone width (WTH1) and sheath length (SHTH) are the most important, inversely correlated with stomatal lines on the dorsal surface (SLDO).

The results of the analysis are represented graphically (see *Fig. 7).* The two axes of the graph represent canonical discriminent functions 1 and 2 and the positions of the group means relative to these axes *(i.e.* the centroids) are marked with an asterisk (*). The individual members of each group are plotted relative to the same axes and are marked with a 1 (= *P. pseudostrobus),* 2 (= *P. tenuifolia)* and 3 (= *P. douglasiana).*

It can be seen from *Fig. 7* that the main separation between the groups is between *P. pseudostrobus* and *P. tenuifolia/P. douglasiana.* This separation is along function 1 and is based mainly on the intrusions character (INTR).

This confirms the importance of the intrusions character in identifying *P. tenuifolia* and *P. douglasiana.* Function 2 serves to separate *P. douglasiana* from *P. tenuifolia* on the basis of cone width (WTH1), sheath length (SHTH) and decreasing stomatal lines on the dorsal surface (SLDO).

It is possible to predict the group an individual should belong to from its position relative to the group centroids. The analysis used here presents these results in the form of a table from which can be determined the success with which individuals have been classified into groups. These results are shown in *Table 11.*

From *Table 11* it can be seen that the species groups suggested by PCA are remarkably correct. The CDA has separated the species and revealed that the individuals are almost 100% correctly classified. The one individual of *P. pseudostrobus* which was predicted to belong to *P. tenuifolia* was a specimen from site 11 (Gomez Farias, Jalisco). This might be expected as *P. pseudostrobus* in that part of the range does resemble *P. tenuifolia.*

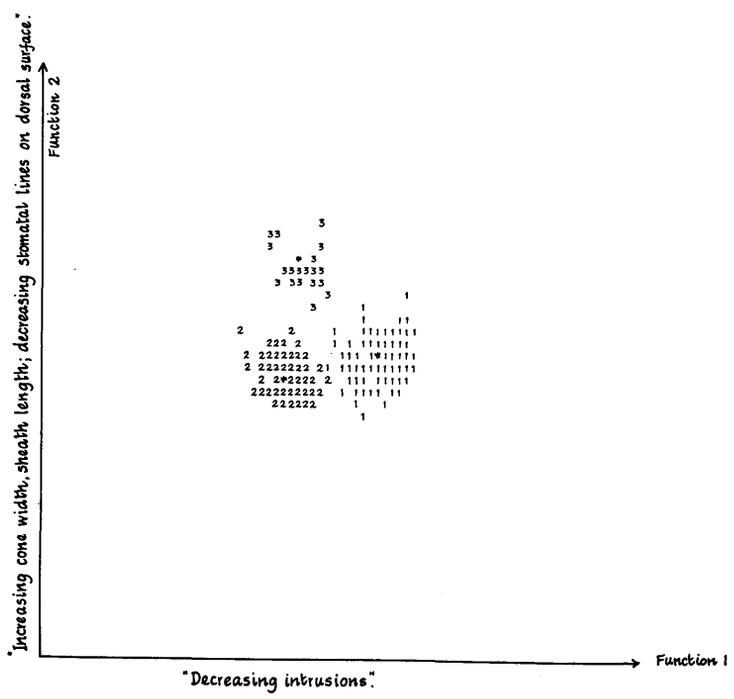

Figure 7. — CDA species groups all variables (Function 1 vs. Function 2). 1 = *P. pseudostrobus,* 2 = *P. tenuifolia,* 3 = *P. douglasiana,* * = group centroid.

Table 11. — CDA species groups all variables — classification results.

Actual group	Individuals in group	Predicted group membership 1	2	3
P. pseudostrobus	167	166 99.4%	1 0.6%	0 0.0%
P. tenuifolia	100	0 0.0%	100 100.0%	0 0.0%
P. douglasiana	25	0 0.0%	0 0.0%	25 100.0%

Thus CDA highlighted the intrusion character as being the most important for distinguishing *P. tenuifolia* and *P. douglasiana* from *P. pseudostrobus*. The same analysis was re-run excluding the intrusions character and the rest of the laboratory characters in order to examine the problems of field identification.

The equivalent graph plot is shown in *Fig. 8*. In this case Function 1 serves to separate *P. pseudostrobus* and *P. douglasiana*, from *P. tenuifolia* and Function 2 then separates *P. douglasiana* from *P. pseudostrobus* and *P. tenuifolia*. The degree of separation is noticeably less clear than in the CDA using all variables. The confusion resulting from the use of field characters only can be seen in the classification table *(Table 12)*.

The degree of successful classification is reduced using field variables and the proportions in which it has been reduced are very interesting. It seems that using only field variables, *P. tenuifolia* and *P. douglasiana* can still be quite clearly identified. The problems arise when *P. pseudostrobus* is identified on field variables, when in 9% of the cases it is misidentified as *P. tenuifolia* and in 3.6% of cases it is misidentified as *P. douglasiana*. This confirms the suggestion made on the basis of PCA *(Fig. 4)* that problems in the field arise from the misidentification of *P. pseudostrobus* as *P. tenuifolia*, and sometimes *P. douglasiana*.

The use of CDA has confirmed the tentative conclusions based on PCA of sites 3, 4, 7, 9, 10, 11 and 12 that in the north-western part of the range *P. pseudostrobus* strongly resembles *P. tenuifolia*, (hence the area of uncertainty between the two species groups in *Fig. 4)*. If however all characters are considered in a detailed investigation, the two can be separated, largely on the character of the intrusions. When field characters alone are considered *P. pseudostrobus* is regularly misidentified as *P. tenuifolia* and sometimes *P. douglasiana*.

4.2 *Patterns of variation within P. pseudostrobus*

4.2.1 *Principal Components Analysis (PCA)*

4.2.1.1. *PCA sites 2, 4, 6, 8, 10, 11, 13, 14 and 15*

The analyses already discussed have demonstrated that *P. tenuifolia* and *P. douglasiana* are quite homogeneous and well defined species. *P. pseudostrobus* on the other hand is very variable and several authors have attempted to describe the variation they found. It was considered necessary therefore to examine the patterns of variation within *P. pseudostrobus* and to account for the infraspecific taxa which have been described.

Table 12. — CDA species group field variables — classification results.

Actual group	Individuals in groups	Predicted group membership 1	2	3
P. pseudostrobus	167	146 87.4%	15 9.0%	6 3.6%
P. tenuifolia	100	2 2.0%	98 98.0%	0 0.0%
P. douglasiana	25	0 0.0%	0 0.0%	25 100.0%

As a first step, data from site collections determined to be *P. pseudostrobus* from the above analyses, were examined using PCA. Also included were data from site collections 13, 14 and 15 which were collected as *P. pseudostrobus* var. *estevezii*, *P. pseudostrobus* var. *apulcensis* and *P. pseudostrobus* var. *coatepecensis respectively*. (site 8 which has been included with various analyses already, was collected at a locality mentioned by Martínez in his discussion of *P. pseudostrobus* var. *oaxacana)*.

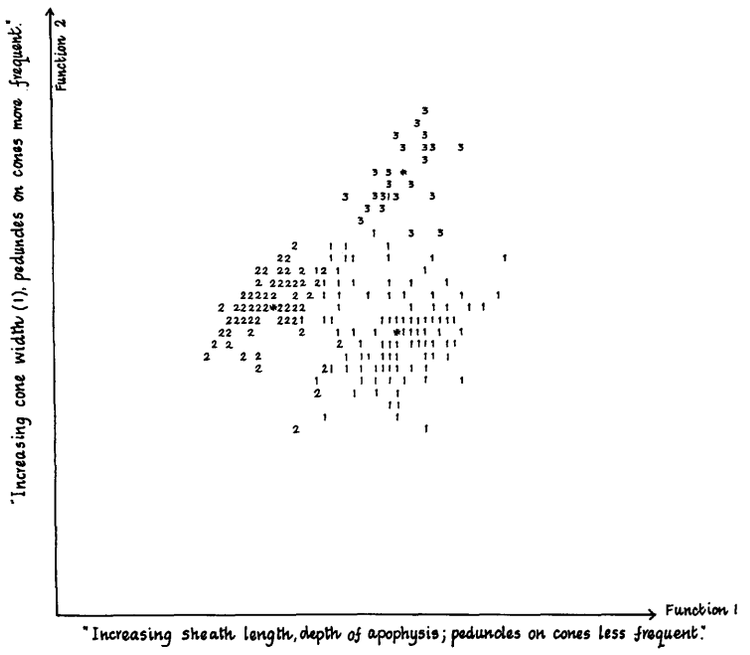

Figure 8. — CDA species groups field variables (Function 1 vs. Function 2). 1 = *P. pseudostrobus*, 2 = *P. tenuifolia*. 3 = *P. douglasiana*, * = group centroid.

Details of the roots and the scaled eigenvectors are shown in *Tables 13* and *14*. Considering *Table 13*, PC1 accounts for only 25.94% of the variation and PC2 accounts for almost the same amount (21.16%). These two together however account for less than half of the total variation (47.10%), indicating that there is no clear pattern which can be represented in one or two dimensions as was found in previous PCA's.

From *Table 14* it can be seen that PC1 represents mainly cone size (CLTH, WTH1, WTH2, WAPO and HAPO) and needle width (NWTH) and that PC2 represents needle width (NWTH, ENDO), stomatal lines (SLDO, SLVE) pruinose bloom on the branchlets (PRUI) *(i.e.* its absence) and resin canals (RCAN). The pattern revealed by PC1 and 2 is demonstrated in the graph of PC1 vs. PC2 shown in *Fig. 9*, which immediately demonstrates the fact that there are no very clear patterns of variation within *P. pseudostrobus.* The individuals comprising the site collections in the analysis have been spread out with only one possible grouping which can be identified. The majority of specimens from sites 13, 14 and 15 are grouped together in the top right hand corner of the graph and are isolated by a gap in the distribution (marked on *Fig. 9* with a feint dotted line.

Inevitably the taxonomic groupings within *P. pseudostrobus*, based on this analysis, will be somewhat ill-defind but some definite conclusions can be made based on the results shown in *Fig. 9*. It seems that there are no groupings within *P. pseudostrobus* which are worthy of specific rank. Similarly, the collections from sites 13, 14 and 15 may be worthy of some infraspecific status as a group but the three taxa they represent *(i.e. P. pseudostrobus* var. *estevezii, P. pseudostrobus* var. *apulcensis* and *P. pseudostrobus* var. *coatepecensis)* cannot be retained as separate entities. Finally, the taxon *P. pseudostrobus* var. *oaxacana,* characterized by the highly developed apophyses and represented here particularly by site 8, seems to be doubtfully distinct from the very variable *P. pseudostrobus.*

Table 13. — PCA sites 2, 4, 6, 8, 10, 11, 13, 14 and 15 — roots of the correlation matrix.

Root		% of variation
1	5.188	25.94
2	4.231	21.16
3	2.030	10.15
4	1.475	7.38
5	1.347	6.73
6	1.021	5.10
7	0.850	4.25
8	0.718	3.59
9	0.650	3.25
10	0.451	2.25
11-20	2.039	10.20

Table 14. — PCA sites 2, 4, 6, 8, 10, 11, 13, 14 and 15 — scaled eigenvectors) largest scaled to unity).

NLTH INTR	NWTH HTOE	LVES ENDO	SHTH CLTH	SLDO WTH1	SLVE WTH2	STOM WAPO	SERR DAPO	PRUI HAPO	RCAN PEDU
Vector 1									
0.404	0.718	0.136	0.415	0.554	0.505	0.123	-0.168	-0.296	0.369
-0.317	-0.310	0.550	0.907	1.000	0.993	0.895	0.545	0.739	-0.221
Vector 2									
-0.507	0.817	-0.312	-0.357	1.000	0.990	0.361	0.398	0.751	0.806
-0.170	-0.176	0.894	-0.182	-0.344	-0.335	-0.327	-0.524	-0.484	0.571
Vector 3									
0.427	0.105	-0.104	0.340	0.092	0.070	0.235	0.104	-0.081	0.050
1.000	0.994	0.211	0.097	-0.026	-0.036	-0.004	0.093	-0.039	0.224

Characters (see *Table 2*)

The fact that almost all of the individuals from sites 13, 14 and 15 have grouped together on *Fig. 9*, and that no individuals from any other site are grouped with them, strongly suggests that together they may constitute a separate taxon. According to *Fig. 9* and *Table 14* it would seem that this taxon, as compared to typical *P. pseudostrobus*, is characterized by larger than average cones, wider needles, more stomatal lines and resin canals, and a less frequent pruinose bloom on the branchlets. As might be expected this taxon merges into the typical species and several individuals from sites 13, 14 and 15 are found

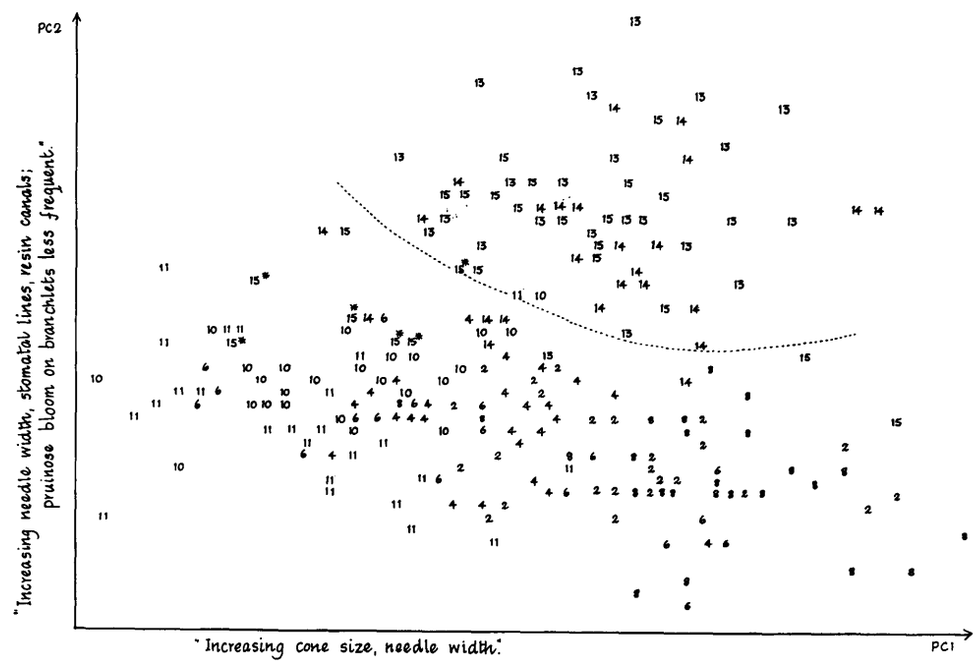

Figure 9. — PCA Sites 2, 4, 6, 8, 10, 11, 13, 14 and 15 (PC1 vs PC2). 2 = Site 2, 4 = Site 4 *etc.*

grouped with the main body of *P. pseudostrobus* specimens. Several specimens from site 15, marked with an asterisk (*) on *Fig. 9* were collected on pedregal (lava flow) and were noted at the time of collection to be depauperate with smaller cones and finer needles. This may explain their position relative to typical *P. pseudostrobus* and the proposed infraspecific taxon. The identity of this taxon will be examined further using CDA.

It is interesting to note from *Fig. 9* and *Table 14* that specimens called at the time of collection *P. pseudostrobus* var. *oaxacana* are not separated out, nor does the character which typifies this taxon contribute greatly to any PC. Most of the specimens collected at site 8 were of this type of which the cones are very distinctive (see *Fig. 10)* and these individuals are somewhat grouped at the bottom right hand side of *Fig. 9.* Closer examination of the original material and data sheets revealed however, that they are grouped there because of larger than average cones rather than the development of the apophyses. Inspection of material from other sites particularly sites 2 and 4 showed that this character is found throughout a large part of the range of *P. pseudostrobus.* Some cones with this development were found at site 15. At any one site it can be found in various degrees of development. In *Fig. 11* a selection of scales from different individuals collected at site 4 (Comitán, Chiapas) are shown. It can be seen that every degree of development of the apophyses is represented.

It was decided that specimens of *P. pseudostrobus* var. *oaxacana* though very distinctive because of the development of the apophyses, are in all other respects exactly like typical *P. pseudostrobus.* In addition, the character of the apophyses is very variable, even within a small population. The indication is therefore that *P. pseudostrobus* var. *oaxacana* on morphological grounds, merits a low taxonomic rank.

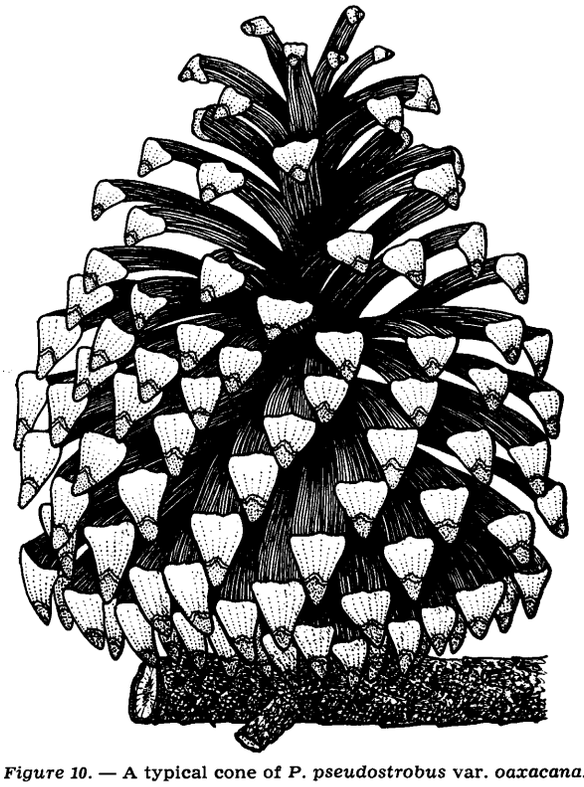

Figure 10. — A typical cone of *P. pseudostrobus* var. *oaxacana.*

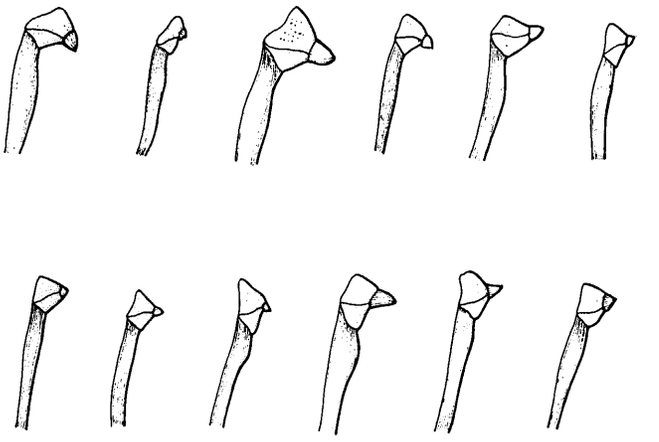

Figure 11. — Cone scales from trees at Comitán, Chiapas, Mexico (Site 4).

4.2.2 *Canonical Discriminant Analysis (CDA)*

4.2.2.1 *CDA P. pseudostrobus and variety*

As CDA had proved so useful in the analysis of the Pseudostrobus group it was decided to use it to investigate subdivisions within *P. pseudostrobus,* and particularly the identity of the subgroup revealed by PCA, comprised of the majority of specimens from sites 13, 14 and 15.

Referring to *Fig. 9* PCA revealed a possible subgroup within *P. pseudostrobus* which is separated by the dotted line. A total of 58 individuals from sites 13, 14 and 15 were placed in a separate group. Only two specimens from other sites (10 and 11) were considered for this group but as they were border line cases they were not included. A CDA was performed on this putative infraspecific group (called here a variety) and a *P. pseudostrobus* group (sites 2, 4, 6, 8, 10 and 11 and the typical *P. pseudostrobus* specimens from sites 13, 14 and 15). Such an analysis generates only one function shown in *Table 15.*

As might be expected when dealing with complex variation at this level of separation, the function is not easy to interpret. The strongest influence would appear to be the character of cone width 1 (WTH1) and, with an opposite sign, cone width 2 (WTH2). As these two values are usually very similar it would seem that they would cancel each other out, however as WTH1 is always greater then WTH2, these characters together represent the degree of asymmetry of the cone. Similarly counterbalanced are the characters of the intrusions (INTR) and the hypoderm cells

Table 15. — CDA *P. pseudostrobus* and variety — standarized canonical discriminant function coefficients.

Character	Function
NLTH	-0.48570
NWTH	0.31983
LVES	0.07968
SHTH	0.04966
SLDO	0.19219
SLVE	0.27367
STOM	0.06612
SERR	-0.02752
PRUI	0.23957
RCAN	0.18497
INTR	0.38537
HTOE	-0.31444
ENDO	0.40912
CLTH	-0.09426
WTH1	1.40150
WTH2	-1.48537
WAPO	0.38126
DAPO	-0.07686
HAPO	-0.21661
PEDU	0.29322

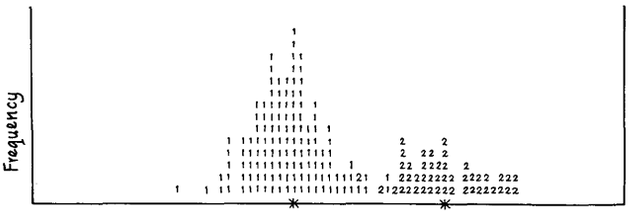

Figure 12. — CDA *P. pseudostrobus* and variety. 1 = *P. pseudostrobus*, 2 = Variety, * = group centroid.

touching the endoderm (HTOE) but as so few individuals in *P. pseudostrobus* possess this character this is a rather spurious result. Of the remaining characters it can be seen that needle width (NWTH, ENDO) is inversely correlated with needle length (NLTH). Width of apophysis (WAPO) is also fairly important.

The results of the analysis are shown graphically in the histogram in *Fig. 12.* It can be seen that the two groups have been quite well separeted. According to the preceding discussion the so called variety is separated from *P. pseudostrobus* on the basis of more asymmetrical cones with wider apophyses and by shorter wider needles. The width of the needles is something that was emphasized as a discrirminating character by PCA.

The success of the separation can be judged by the classification table *(Table 16).* The degree of success of the classification is remarkably high though not quite the 99—100% accuracy found when discriminating species.

It would seem from the analyses performed on data for *P. pseudostrobus* that a revision of its infraspecific classification is required. It is proposed that *P. pseudostrobus* should be left with two infraspecific taxa only. The most obvious grouping is that examined in the CDA above which it is felt merits the rank of subspecies. As described, the range of variation of the taxon encompasses three previously described taxa, the oldest of which was first described as *P. apulcensis* by LINDLEY in 1839. According to the rules of botanical nomenclature this newly circumscribed taxon should be called *P. pseudostrobus* subsp. *apulcensis* (LINDL.) STEAD *(stat. nov.).* This taxon though morphologically very similar to *P. pseudostrobus* has a distinct distribution and ecology. *P. pseudostrobus* var. *oaxacana* though very similar to *P. pseudostrobus* is very distinctive in the form of its cones and will be retained as a variety. Its distribution and ecology is much more diverse and it intermingles and intergrades with the species. The remaining varieties and forms of Martínez and Loock are not retained.

Table 16. — CDA *P. pseudostrobus* and variety — classification table.

Actual group	Individuals in groups	Predicted Group 1	2
P. pseudostrobus	158	155 98.1%	3 1.9%
Variety	58	2 3.4%	56 96.6%

5. Conclusions

The analyses have revealed the patterns of variation within the *Pseudostrobus* group. On the basis of these results it is concluded that there are three species in the group: —

P. pseudostrobus
P. tenuifolia (= *maximinoi* H. E. MOORE)
P. douglasiana

Within *P. pseudostrobus* two infraspecific taxa are recognized: —

P. pseudostrobus subsp. *apulcensis* (LINDL.) STEAD
P. pseudostrobus var. *oaxacana* (MIROV) HARRISON

If *P. tenuifolia* is accepted as a separate species it is necessary to follow the recommendations of H. E. MOORE (1966) that the name *P. maximinoi* should be used. This, and other taxonomic points, will be discussed in a subsequent paper.

The approach of using two different analyses in combination proved highly successful. PCA revealed patterns of variation and suggested possible groupings, thus by-passing the traditional process of matching and sorting. CDA suggested relationships between groups, and identified the best characters for discriminating between groups, and provided a test for the success with which individuals had been classified.

Most of the characters used in the study proved to be of some value and will be particularly valuable in distinguishing species of this group from species in other groups. Some characters such as number of stomata per 5 mm, the number of serrations per 5 mm, the number of hypodermal cells touching the endoderm and the endodermal cells on the dorsal side of the vascular bundle cannot be recommended for future studies.

Finally, it is worthy of comment that this detailed revision of the *Pseudostrobus* group has produced a very clear picture of the identities of the species involved and their relationships. Well over 500 specimens were examined in detail and in the vast majority of cases have been exactly classified as one species or another. Some few specimens have proved difficult to identify, as might be expected when dealing with herbarium specimens taken on loan. This is to be expected as such specimens, sometimes only a branchlet, could be a poor sample from a whole tree which might itelf be quite easy to identify.

This outcome is in contrast to previous studies in *Pinus (e.g.* MARTINEZ (1948), ZOBEL and CECH (1957), MIROV (1967)) which have often concluded that the complex patterns of variation encountered are impossible to resolve exactly and are the product of frequent intercrossing between species. Separating two closely related species involves consideration of continuously variable characters with overlapping ranges of variation. These characters are not easily observed and compared on herbarium specimens. The results of this study show however that if material from throughout the complete range of taxa is considered in detail, problems of classification can be resolved.

References

AGUILAR, J. I.: Pinos de Guatemala. Ministerio de Agricultura Dirección General Forestal, Guatemala. 33 p. (1961). — ANDRESEN, J. W.: A multivariate analysis of the *Pinus chiapensis-monticola-strobus* phylad. Rhodora **68**, 1—24 (1966). — COYNE, J. F. and CRITCHFIELD, W. B.: Identity and terpene composition of Honduran pines attacked by the bark beetle *Dendroctonus frontalis (Scolytidae).* Turrialba **24**, 327—31 (1974). — CRITCHFIELD, W. B. and LITTLE, E. L.: Geographic distribution of pines of the world. Misc. Pub. USDA No. 991. 97 p. (1966). — DANCIK, B. P. and BARNES, B. V.: Multivariate analysis of hybrid populations. Naturaliste Can. **102**. 835—43 (1975). — JEFFERS, J. N. R. and BLACK, T. M.: An analysis of variability in *Pinus contorta.* Forestry **36**, 199—218 (1963). — MARTINEZ, M.: Las Pinaceas Mexicanas. Anales del Instituto de Biología, Mexico **16**, 168—201 (1945). — MARTINEZ, M.: Los Pinos Mexicanos,

Mexico, Ediciones Botas. 361 p. (1948). — MIROV, N. T.: The Genus *Pinus*. New York, Ronald Press Company. 602 p. (1967). — MOLINA, A.: Coniferas de Honduras. Ceiba **10**, 5—21 (1964). — MOORE, H. E.: Nomenclatural notes on cultivated conifers. Baileya **14**, 1 (1966). — SCHWERDTFEGER, F.: Informe al gobierno de Guatemala sobre la entomologia forestal de Guatemala. Vol. 1. Los pinos de Guatemala. Informe FAO/ETAP FAO Rome No. 202. 58 p. (1953). — SHAW, G. R.: The pines of Mexico. Publications of the Arnold Arboretum 1. 29 p. (1909). — SMOUSE, P. E. and SAYLOR, LE R. C.: Studies of the *Pinus rigida-serotina* complex. 2. Natural hybridization among the *P. rigida-serotina* complex, *P. taeda* and *P. echinata*. Ann. Mo. bot. Gdn. **60**, 192—203. (1973). — STANDLEY, P. C. and STEYERMARK, J. A.: Flora of Guatemala. Fieldiana, Bot. **24**, 53—5 (1958). — STYLES, B. T.: Studies of variation in Central American pines. I. The identity of *Pinus oocarpa* var. *ochoterenai* MARTINEZ. Silvae Genet. **25**, 109—18 (1976). — STYLES, B. T., STEAD, J. W. and ROLPH, K. J.: Studies of variation in Central American pines. II. Putative hybridization between *Pinus caribaea* var. *hondurensis* and *P. oocarpa*. Turrialba 32, 229—242 (1982). — ZOBEL, B. and CECH, F.: Pines from Nuevo Leon, Mexico. Madrono **14**, 133—44 (1957).

Bud and Shoot Formation in Juvenile Tissue Culture of Pinus nigra

By B. KOLEVSKA-PLETIKAPIĆ, S. JELASKA, J. BERLJAK[1]) and M. VIDAKOVIĆ[2])

(Received 26th October 1982)

Abstract

Adventitious and axillary buds, shoots and needles were generated using tissue cultures from excised embryos of *Pinus nigra* ARNOLD. Formation of these structures in embryo culture was obtained on agar half-strength MS basal medium supplemented with 2 mg. l^{-1} BAP, 250 mg. l^{-1} myo-inositol and NAA and IBA in various concentrations.

Using shoot-tip explants of 20-day seedlings, generation of buds, shoots and needles was also achieved on an agar medium of half-strength MS salts, but supplemented with 1 mg. l^{-1} BAP and 0.001 mg. l^{-1} NAA.

The origins of new buds in these cultures were adventitious, axillary and native from brachyblasts. Long-shoot development and growth of buds and shoots was achieved by transferring them onto a hormone-free medium, sometimes aided by the addition of 0.3% activated charcoal.

The best tissue-media interactions produced bud and shoot formation in 60—70% of cultures.

In-vitro developed shoots were subjected to the same procedures, and typically produced a successive generation of buds, shoots and needles.

Key words: Pinus nigra, tissue culture, auxin, bud induction, shoot formation.

Zusammenfassung

Aus explantierten Embryonen von *Pinus nigra* ARNOLD konnten in Gewebekultur Adventiv- und Achselknospen, Sprosse und Nadeln erzeugt werden. Die Ausbildung dieser Strukturen wurde auf einem halb-konzentrierten MS-Basal-Medium erreicht, dem 2 mg. l^{-1} BAP, 250 mg. l^{-1} Myo-Inositol und NAA und IBA in verschiedenen Konzentrationen zugefügt worden waren. Bei Verwendung von Sproßspitzenexplantaten 20 Tage alter Keimlinge wurde die Ausbildung von Knospen, Sprossen und Nadeln auch erzielt, wenn dem Agar-Medium außer den MS-Salzen 1 mg. l^{-1} BAP und 0,001 mg. l^{-1} NAA zugegeben wurden. Der Ursprung neuer Knospen in diesen Kulturen waren Adventiv- und Achselknospen, die sich aus Kurztrieben entwickelten. Eine Entwicklung von Langtrieben und das Wachstum von Knospen und Trieben wurden erzielt, wenn diese auf ein hormonfreies Medium transferiert wurden, wobei manchmal eine Zugabe von 0,3 prozentiger Aktivkohle hilfreich war. Die beste Gewebe-Medium-Interaktion erbrachte eine Knospen- und Sproßentwicklung von 60—70%. In Vitro entwickelte Sprosse wurden derselben Prozedur unterzogen und produzierten in der Regel so nach und nach Generationen von Knospen, Sprossen und Nadeln.

Introduction

Practical applications of tissue culture techniques for the vegetative propagation of woody plants have recently been emphasized in several reviews (BONGA 1977, WINTON 1978, SOMMER and BROWN 1979, BOULAY 1980).

SOMMER and BROWN (1974) were the first to report plantlet regeneration from mature embryos in *Pinus* sp., and adventitious bud induction has subsequently been reported for several pine species (BROWN and SOMMER 1977, DAVID and DAVID 1977, WEBB and STREET 1977, TRANVAN 1979, BORNMAN and JANSSON 1980, KONAR and SINGH 1980). Shoots and plantlets have been most effectively produced in tissue cultures of pines using juvenile material — embryos and young seedlings. It is encouraging, however, that recent papers indicate the possibility of regeneration from mature material (FRANCLET *et al.* 1980, BONGA 1981).

In the breeding process, which is particularly long with forest trees, one often gets only a small number of plants with particularly desirable combinations of characteristics. To capture their advantages for practical purposes, such plants must be multiplied many times. Tissue culture provides this capability. European black pine *(P. nigra* ARNOLD) is an economically important conifer species in southern Europe, and vegetative propagation of its elite specimens and/or hybrids is worthy of attention.

For the past decade one of us (B. K-P.) has been working on various methods of tissue culture for this important species, but with little success. Based on our detailed observations, we suspected that *P. nigra* might be among those tree species that are more difficult to differentiate under *in vitro* conditions. This viewpoint was supported by the first series of tissue culture studies, since buds, shoots, or other organized structures failed to differentiate in most media combinations attempted. However, one combination worked moderately well, and led us to the second and third set of experiments. These allow us to report successful induction of buds, shoots and needle structures using two kinds of juvenile tissue of European black pine for culture.

Address: [1]) Department of Botany, Faculty of Science, University of Zagreb, Rooseveltov trg 6, P.O. Box 933, 41001 Zagreb, Yugoslavia.

[2]) Department of Forest Genetics and Dendrology, Faculty of Forestry, University of Zagreb, Šimunska cesta 25, P.O. Box 178, 41001 Zagreb, Yugoslavia.