STUDIES OF VARIATION IN CENTRAL AMERICAN PINES PUTATIVE HYBRIDIZATION BETWEEN *Pinus caribaea* var. *hondurensis* and *P. oocarpa* II[1] */

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Resumen

Con frecuencia se ha sugerido que Pinus caribaea *var.* hondurensis *(Sénécl.) Barr. y Golf. y* P. oocarpa *Schiede, dos especies muy importantes en los bosques tropicales, podrían hibridizar en algunos lugares de Centro América en los que su área de crecimiento natural coincide. En este artículo se presentan los resultados de un estudio detallado, diseñado para investigar este tema en profundidad. Se escogió un lugar en Honduras, en donde las dos especies crecen juntas y se recogió material de 80 árboles a lo largo de una sección transversal en una zona de mezcla. El estudio del material y el análisis de gran número de mediciones por diversos métodos, han revelado la presencia de algunas formas intermedias. Estas se interpretan provisionalmente como híbridos pero la confirmación queda pendiente de los resultados de experimentos de polinización controlada.*

1 Received for publication in July 1980.

* Paper I in this series was published in Silvae Genetica 25(3-4):109-117 (1976).
Post-Scriptum
During the protracted period in which this paper was being revised for publication, considerable work has been carried out on the genetical relationships between these two species. Successful artificial hybridasation between them has now apparently been achieved in both Queensland (Australia) and Honduras (*C. America*) hopefully vindicanting the results of this field study. F_1 hybrids already show faster growth than either of the two parents. Isozyme analysis of putative hybrid seed is being undertaken to confirm hybridity.

We would like to express our thanks to the many people who have assisted us in this study. Our colleagues Mr. G. Chaplin and Mr. A. Greaves, and Mr. W. L. Mittak, INAFOR/FAO, Guatemala, Prof. A. Molina R., EAP, El Zamorano, Honduras and Mr. M. Robbins ESNACIFOR, Siguatepeque, Honduras provided information on the species in the field and material used in the study. Mr. M. Taylor helped with the often tedious job of measuring the material and Dr. C. Green and Mr. B. Keeble analysed the resin samples. Considerable advice on the analysis of the results was freely given by Mr. H. L. Wright, Mrs. J. Stone, Prof. G. Namkoong and Dr. J. Burley. Mrs R. Wise did the drawings, Mr. A. Allen took the photographs, Mrs. Coral Taylor and Mrs. Cynthia Styles typed the manuscript.

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Introduction

The importance of *Pinus caribaea* Mor. including variety *hondurensis* (Sénécl.) Barr. & Golf. is indicated by the very large scale on which it is grown commercially outside its natural area of distribution. It is among the four five most important timber trees at present in cultivation in softwood plantations in the tropics. Though not grown so widely as yet, *P. oocarpa* Schiede is being increasingly planted, and both species will probably assume even greater importance after selection and breeding have produced better adapted genotypes with improved commercial characteristics. Both taxa are included in the Central American pine provenance research programme being conducted by the Unit of Tropical Silviculture at the Commonwealth Forestry Institute, Oxford*, as part of the FAO global programme for the improved use of genetic resources (Kemp 18, 19; Styles, 28; Greaves, 14; Greaves and Kemp, 16; and Greaves 15).

Although both are currently accepted as good Linnean species with different ecological requirements and are classified in quite different subsections of the genus *Pinus, P. caribaea* and *P. oocarpa* are nevertheless sometines difficult to distinguish botanically. Where the two grow together their similarities, and the overlap in their variation patterns, are such that a number of observers in Central America have suggested the possibility of hybridization. Botanical specimens showing intermediate morphological characters have been collected on numerous occasions.

Bearing in mind the commercial potential of the two species, and the experimental work now in progress, uncertainty about variation patterns and the possibility of hybridization is a major problem. In order to clarify this a detailed investigation of certain trees growing in a mixed population of the two species in the Republic of Honduras, Central America was carried out. A number of characters, mainly those of needle and cone morphology which have long been found by taxonomists to be useful in distinguishing between taxa in the genus were examined on herbarium specimens, together with a limited amount of chemotaxonomic information obtained from resin. The data obtained were analysed using a variety of established computer and pictorial techniques. The results have revealed the presence of genuine intermediates. These intermediates may represent natural interspecific hybrids, introgressants or perhaps extreme morphological deviants of either of the two parents. The artificial cross between the two species is now being attempted in several tropical countries (Nikles, Queensland, Australia and Houkal, Honduras, Central America, personal communications).

* Instituto Nacional de Investigaciones Forestales, Mexico, is cooperating with this programme and is responsible for seed collections of *P. oocarpa* in that country. Seed of 36 provenances has so far been obtained for distribution in the near future, and more collections are being organised (12)

Geographical range and Ecology

P. oocarpa is a very widespread and common species and has the largest area of distribution of any Mexican or Central American pine, ocurring almost continuously from lat. 28° 20'N in the States of Sonora and Chihuahua, Mexico (Martínez 25, Loock, 23), and throughout Central America as far as lat. 12° 40'N in the Departament of Leon, Nicaragua. It is essentially sub-montane, growing between 700 and 1500 m, on steep mountain slopes with good drainage and within a rainfall range of 700-1500 mm. (Kemp, 18). *P. caribaea* var. *hondurensis* has a more disjunctive distribution in Central America, occurring very locally in Mexico (Quintana Roo, approximate lat. 18° 05'N, long. 88° 35'W, Stead and Styles, unpublished information), Belize, Guatemala and El Salvador. Further south in the Honduran Republic and in Nicaragua its range is more extensive and here there is considerable overlap with *P. oocarpa* at intermediate altitudes (Figure 1). In contrast to *P. oocarpa* it is a tropical or subtropical lowland species, found from sea level to 800 m with a few populations extending up to 1000 m. Further detailed accounts of its taxonomy, ecology and distribution are given by Barret and Golfari (3), Loock (23) Luckhoff (24), Nikles (27) and Lamb (21).

Botanical variation and classification

The number of differentiating characters which can be used to distinguish between *P. oocarpa* and *P. caribaea* is small and the fact that these characters are themselves extremely variable makes their identification in the forest and in the herbarium difficult at times. The most important botanical differences between the two species are summarized in Table 1.

P. caribaea and *P. oocarpa* are presently classified in different subsections of the genus *Pinus* (Little and Critchfield, 22). Caribbean pine is placed in the subsection Australes Loud., 'southern yellow pines," and is the only one of the group to extend into Central America. It has 2-3 needles per fascicle with internal or medial resin canals; the female cone is

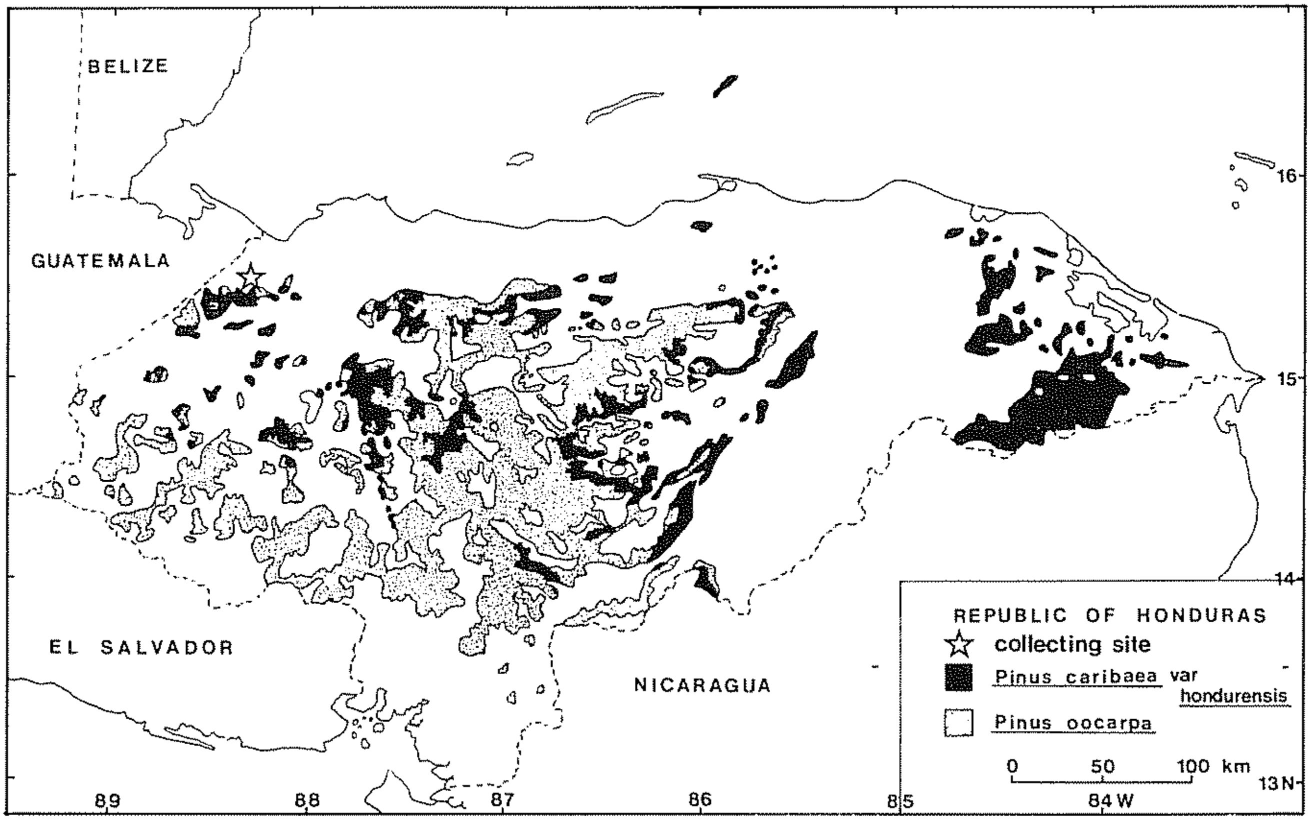

Fig. 1. Map of Honduras Republic showing the distributions of *Pinus oocarpa* and *P. caribaea* var. *hondurensis* and location of collecting site

Table 1. Summary of the chief botanical differences between *Pinus caribaea* var. *hondurensis* and *P. oocarpa*

Character	*Pinus caribaea* var. *hondurensis*	*Pinus oocarpa*
Needles	Fascicles with groups of 3 (sometimes 4, very rarely 2 or 5); needles stiff, thick, erect; drying light green.	Fascicles with groups of 5 (sometimes 3 or 4, rarely 6); needles stiff, sometimes slender, erect or spreading; drying dark green.
Number and positions of resin canals	Generally 2-3 (4); usually all internal, sometimes with 1 or 2 medial. (Higher numbers recorded by Lückhoff (24) for other varieties).	Generally (3)4-5(6); mostly all septal, or with 1-2 external, or less frequently 1-2 medial or internal.
Female cone (open)	Broadly elongate, cylindrical to elongate, ovoid (barrel shaped); symmetrical or slightly asymmetrical; markedly deciduous, falling without peduncle; reddish brown, turning dark dull brown to black.	Broadly ovoid, sometimes conoidal (egg-shaped); opening in the form of a rosette; slightly asymmetrical; with a stout peduncle; long persistent on tree after maturity; tawny-brown, shining.
Cone scales	Softly woody, thin and flexible; widely spaced; apophysis raised, sometimes recurved, keeled, rugose; umbo raised with a short persistent prickle.	Very woody and thick; compact; apophysis flat or convex, keeled, smooth; umbo often prominent and raised, sometimes prolonged; prickle deciduous.

asymmetrical (oblique) and persists on the tree for a long time after maturity; the cone scales remain closed for a considerable time and open irregularly and slowly over an extend period to release the seeds (serotinous); they generally possess a prickle (often deciduous), or sometimes a protruding umbo.

Hybridization

Hybridization between species in different subsections is rare among the pines, but not unknown, (Critchfield, 8, 9; Barnes, 2), which makes the supposed cross between *P. oocarpa* and *P. caribaea* all the more interesting. Nevertheless, many botanists and foresters with field experience in the Central American pine forests believe that these two do intergrade or hybridize in certain areas [Williams, 29; Denevan, 11; Barrett and Golfari, 3; and Luckhoff, 24)]. Such hybrids occur in areas of "intermediate' altitude between 500-700 m where trees of the two grow in intimate mixture. The characters of the intermediate forms cannot be due to environmental conditions. *P. oocarpa* specimens have been studied (by BTS) from trees growing as low as 200 m in Mexico and *P. caribaea* var. *hondurensis* has been investigated at 900 m in Guatemala. Specimens from 'extreme' altitudes were typical in all respects of cone morphology and needle anatomy. Over most of their range the two taxa apparently remain distinct (Coyne & Critchfield, 6; Styles, unpublished).

Natural hybridization in some zones of overlap appears possible on biological grounds since flowering times sometimes coincide. Although the main peak periods of pollen shedding and female conelet receptivity are different in the two species, in some areas in Central America there is considerable overlap, and occasional trees of both species can be found at the same time with receptive female cones and/or mature flowers with well formed pollen. Phenological observations in so far as they have been made suggest that *P. caribaea* var. *hondurensis* sheds the main bulk of its pollen earlier than *P. oocarpa*, and Critchfield (7) noted in mid-January 1965 that trees in populations of both species in Honduras had mostly or completely shed their pollen by this time. Robbins* visited the experimental area at Pinalejo, from where the study material was obtained (see below) in mid-November 1977, and noted then that some trees of *P. caribaea* var. *hondurensis* there had mature male and receptive female flowers (strobili) with many more still developing. On some other trees the male flowers had finished shedding their pollen and newly fertilized female cones were already developing. *P. oocarpa* was later in development with the male flowers present as 3-4 mm long buds, but some more or less symmetrical, quickly deciduous, with scales which all open more or less simultaneously at maturity. The scales generally possess a persistent prickle or apiculus.

P. oocarpa is placed with the closed-cone pines, in subsection Oocarpae, which consists of seven species confined to the southern United States, Mexico and Central America. The group is characterised by having the leaves in fascicles of 2-5, with medial, internal or septal resin canals; the female cone is mostly female conelets were however at the receptive stage. Local environmental conditions are thought to be responsible for the wide variations noted by field workers regarding the flowering times of the two.

Materials and methods

In order to investigate the possibility of naturally occurring hybrids between these two pines are fully, one of the intermediate zones was chosen for special study. Following advice from the staff of the Banco de Semillas, ESNACIFOR, Siguatepeque an area in Honduras was selected. This lies in the Department of Santa Barbara near the village of Pinalejo (lat. 15° 23'N, long. 88° 23'W), between 300 and 900 m altitude, on the southern slopes of the Sierra de Espiritu Santo (Figure 1). The terrain is one of open pine forest which has been logged in the past, but which has suffered relatively little human interference since. The stands contain trees of all ages and a number of large malformed specimens of both species still remain. At lower altitudes, forest with only *P. caribaea* var. *hondurensis* occurs passing through a transition zone where it grows in mixture with *P. oocarpa*. At higher elevations trees of the latter occur as the only species, but these in turn give way to forest of pure *P. tenuifolia* Benth. (= *P. maximinoi* H. E. Moore) at and above 1500 m. Within the transition zone (altitudes between 500 and 700 m) trees are found which cannot be satisfactorily identified as either *P. oocarpa* or *P. caribaea* var. *hondurensis*, a number showing characteristics intermediate between the two. Furthermore, some specimens appear to possess foliage typical of *P. caribaea* var. *hondurensis* and mature female cones resembling more those of *P. oocarpa*. The reverse situation is also true.

A transect was made through the forest from a low elevation of approximately 400 m with trees of pure *P. caribaea* only, passing through a zone of mixture

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and reaching forest of pure *P. oocarpa* at approximately 900 m. Along the transect eight sites were selected at approximately 75-100 m intervals, and within each site ten mature trees were chosen at random (Table 2).

For each tree two foliage samples were collected from the crown, together with four mature, open female cones. These samples were selected at random within the crown, to represent each tree. No effort was made, in this study, to examine the pattern of within tree variation.

A small (7 cm^3) bottle was screwed into the base of the bole and a sample of resin collected overnight. Thus from each of ten trees at eight sites fully correlated botanical material consisting of two herbarium specimens, four cones and a sample of resin was available. The morphological characters assessed are listed in Table 3 (see also Figures 2a, b, c and d).

Analyses and results

Various methods for the analysis of this type of data have been used and have been critically appraised in papers by Namkoong (26) and Goodman (13). Dancik and Barnes (10) recommend, for the analysis of hybrid populations, sequential use of various methods *e.g.* Principal Components Analysis Discriminant Analysis and Canonical Analysis, in order to produce a detailed picture of the relationships between taxa and their relative variability.

Numerous analyses were performed in an effort to reveal the pattern of relationships between the individual trees included in this study. Each analysis contributed something to our understanding of the data but it would be cumbersome and confusing to present all of them here. The results of analyses using two different techniques are shown below. It was felt that, of all the multivariate techniques used, canonical correlation analysis (CCA) most clearly demonstrated the taxonomic relationships within the material, and the results of this analysis are shown below. Also included are the results of analyses using the Pictorialised Scatter Diagram technique developed by Anderson (1). This is a much simpler method and it is interesting to compare the results of the two different analyses.

Canonical Correlation Analysis (CCA): Canonical correlation analysis was developed to investigate the relationship between two or more sets of variables. The method used is to calculate the principal components of each set and then to correlate the resulting components. For a summary of the origins and theory of the analysis and the terminology used, see Jeffers (17).

In this case the two sets of variables are cone characters and needle characters. A CCA will therefore reveal the relationship between the individuals in the analysis in terms of the variation in their cone and needle characters.

That the type of measurements made could be used to separate individuals from two taxa into their respective groups by this type of analysis was demonstrated using data from two isolated and presumably pure parent populations. Material from 25 trees (numbered 1-25) from a pure stand of *P. oocarpa* at La Lagunilla, Guatemala (lat. 14° 42'N, long. 89° 57'W) and from 25 trees (numbered 26-50) from a pure stand of *P. caribaea* var. *hondurensis* at Alamicamba, Nicaragua (lat. 13° 34'N, long. 84° 17'W) were measured for the characters listed in

Table 2. Location of trees within sites.

Site	Species	Altitude (m)	Tree Number
1	*P. caribaea* var. *hondurensis*	395 – 410	1 – 10
2	P. *caribaea* var. *hondurensis*	445 – 475	11 – 20
3	*P. caribaea* var. *hondurensis, P. oocarpa*, and *intermediates*	500 – 535	21 – 30
4	*P. caribaea* var. *hondurensis, P. oocarpa*, and *intermediates*	585 – 600	31 – 40
5	*P. caribaea* var. *hondurensis, P. oocarpa*, and *intermediates*	660 – 680	41 – 50
6	*P. oocarpa*	700 – 710	51 – 60
7	*P. oocarpa*	795 – 810	61 – 70
8	*P. oocarpa*	890 – 910	71 – 80

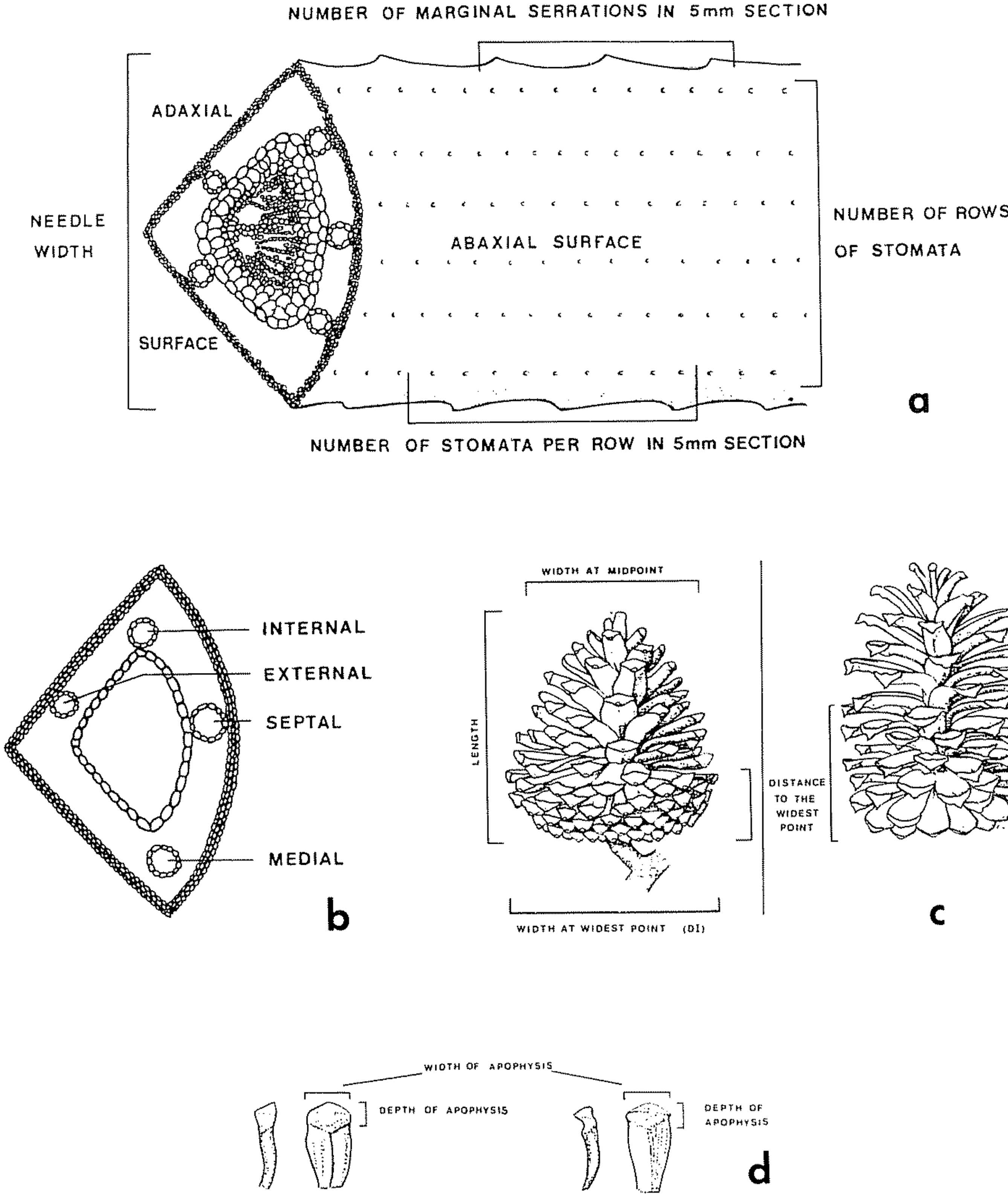

Fig. 2 Diagrams to show the characters measured: a. Part of a needle; b. Cross section of a needle; c. Cones; d. Cone scales

Table 3. Only one herbarium specimen and two cones per tree were available in this study.

In this application we were not interested in within tree variation so that for each character, tree means were calculated for use in the analysis. We have assumed for the purposes of this technique, that the data are approximately multivariate normal. This would seem to be reasonable particularly in the light of previous work (Cotton *et al.* 5; and Dancik and Barnes, 10). Problems arising from the use of different scales in the assessment of characters were circumvented by using the correlation matrix.

A CCA, was carried out and five roots were estimated. The first, which accounted for 41.4% of the variation, had a canonical correlation coefficient of 0.9755 (significant at 0.1% by Bartlett's test). None of the remaining four was significant and they will not be considered further. Table 4a gives the canonical vectors for the first pair of canonical variables. Note that the vectors have been scaled (the largest set to 1.0 with the others in proportion) to make them easier to interpret.

It is apparent from the table that of the needle characters, the number of needles per fascicle, with

Table 3. Characters used in the analyses.

Needle characters

Characters 1 – 3 were assessed on 5 mature fascicles from each of the two herbarium specimens per tree (i.e. 10 fascicles).

1. Number of needles per fascicle.
2. Fascicle sheath length (to the nearest 0.5 mm).
3. Needle length, of the longest needle in each fascicle (to the nearest 0.1 cm).

Characters 4 – 8 were assessed on a 5 mm section from the central portion of the longest needle, from each of the 10 fascicles used for 1 – 3. (Figure 2a).

4. Needle width (x40), measured with a stage micrometer (to the nearest 0.1 mm).
5. Number of rows of stomata on the adaxial surface.
6. Number of rows of stomata on the abaxial surface.
7. Number of stomata per row counted on the abaxial surface (the most easily defined row of stomata was chosen).
8. Number of marginal serrations counted along one side

Characters 9 – 12 were assessed on hand sections, cleared in fuming lactic acid, cut from the central portion of the needles used for characters 4 – 8 (Figure 2b).

9. Number of external resin canals
10. Number of internal resin canals.
11. Number of medial resin canals.
12. Number of septal resin canals

Cone characters

Characters 13 – 17 were assessed on the four cones per tree to the nearest 0.1 cm using a metal gauge (Figure 2c).

13. Cone length.
14. Cone width at the widest point, DI.
15. Cone width at 90° to DI, i.e. DII.
16. Distance to the widest point i.e. from the base of the cone to the point at which DI was taken.
17. Cone width at the mid point.

Cone scale characters

Characters 18 – 19 were assessed on 5 scales from each of the 4 cones per tree, to the nearest 0.25 mm using calipers (Figure 2d).

18. Width of apophysis.
19. Depth of apophysis.

Note: The data are not reproduced here but are filed on specially prepared proformas and are available at Oxford.

a vector score 1.0000, is the most important. None of the others makes any sizeable contribution, and consequently we consider that the first canonical vector represents essentially "needles per fascicle".

For the cone measurements, the vector expresses mainly the width at the widest point (DI) (score 1.0000), contrasted with the width taken at right angles to DI (i.e. D II) (score –0.4447). This would seem to be an expression of both "basal shape" and the "degree of symmetry" of the cone. It is interesting that the degree of symmetry could be an important taxonomic character and, whilst it is difficult to observe and quantity, it has been highlighted by this analysis.

The results for the first pair of canonical variables are presented in Figure 3. This graph shows a well-defined partition of the 50 individuals into two species groups on the basis of this first pair of canonical variables. Note that *P. oocarpa* is characterised by high values for 'Needles per fascicle' and 'Basal shape and symmetry of cone' whilst *P. caribaea* var. *hondurensis* is characterised by low values for the same 'character.'

The same analysis was then carried out using data from the 80 trees collected in the transect study. Of the five roots estimated, the first two accounted for 39.3% and 22.9% of the variation respectively and had canonical correlation coefficients, of 0.8215 and 0.6277 (significant at 0.1% by Bartlett's test). The canonical vectors for the first two pairs of canonical variables are given in Table 4b. Looking at the needle characters for the first pair, it appears that the number of stomatal lines on the abaxial surface is most important (score 1.0000), contrasted with the number of septal resin canals (score –0.4528). In the case of the cone characters it is again a measure of cone basal shape but this is contrasted with depth of the apophysis (score –0.7679).

Although the second canonical correlation was significant, a plot of individual tree scores did not show any separation even between species. This shows that there is some pattern of variation within the data independent of the accepted division into two taxa. As we have accepted the division into two species, and are looking for possible intermediates between them this part of the analysis is of no value and will not be considered further.

A graph plot of the first pair of canonical variables is shown in Figure 4. It can be seen here that the individuals have been separated into two groups representing *P. oocarpa* and *P. caribaea* var. *hondurensis* very much as was observed in Figure 3. The interesting feature is that ten trees are found, with varying degrees of intermediacy between the two main groups. These ten trees, numbers 19, 23, 27, 30, 36, 37, 38, 48, 50, 61 are almost all from the "intermediate" altitudinal zones and the herbarium specimens collected were labelled as "possibly hybrid" by one of the authors (Styles).

Comparing Figures 3 and 4 it will be noted that different sub-sets of the characters are involved in the separation of the individuals in the two analyses. In explaining this it must be remembered that CCA was performed 'independently,' on the data from the pure parent populations and the data from the transect, to reveal the relationship between individuals in terms of variation patterns in cone and needle characters. The fact that a more extensive and different sub-set of characters is significant in the transect analysis, is due to the greater variation in these data introduced by the presence of intermediates. Thus, for example, difference in needle number is no longer sufficient to discriminate between the two species and two other characters ("Rows of stomata on abaxial surface/Number of septal resin canals") largely account for the separation.

Pictorialized Scatter Diagram: In this analysis a reduced number of characters was used, to avoid undue complexity. Three were combinations of characters used individually in the canonical correlation analysis. Table 5 shows the characters used and

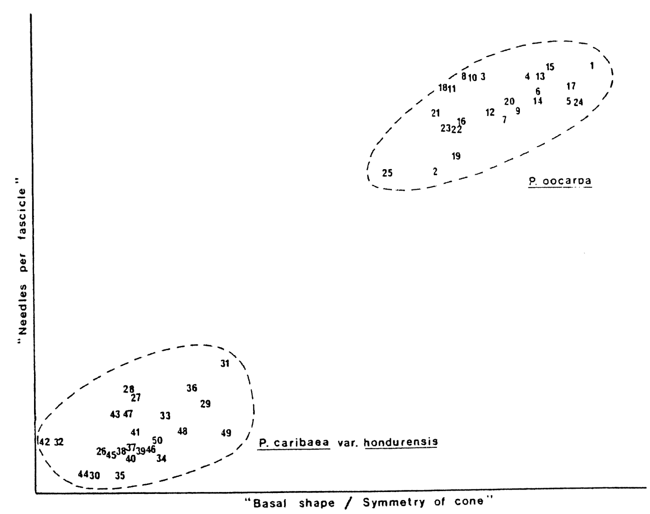

Fig. 3. Canonical correlation Analysis of two pure parent populations (*P. oocarpa* (Guatemala) & *P. caribaea* (Nicaragua)): graph of canonical vector scores for root 1.

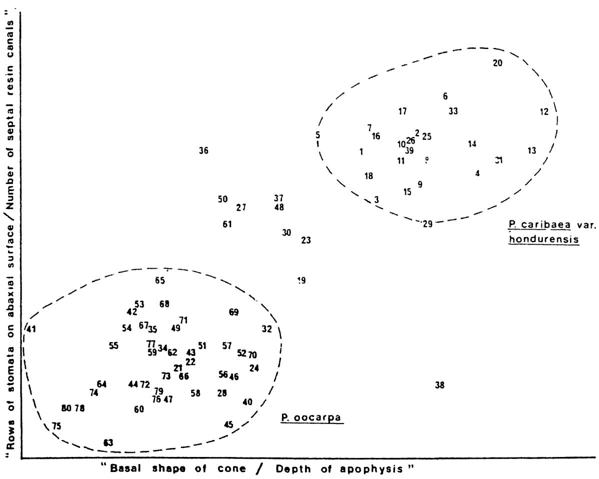

Fig. 4. Canonical Correlation Analysis of 80 trees from the transect study: graph of canonical vector scores for root 1.

how they are represented. Two characters, number of needles/fascicle and cone shape, were chosen as the axes of the graph as multivariate analyses had previously indicated that they were the most valuable for distinguishing the species. Their range of variation was divided into ten classes, and each individual tree was plotted on the graph according to its score. The other characters were incorporated on the graph using the designated symbols (Table 5).

The result in shown in Figure 5 where it can be seen that the scatter produced is very similar to that of Figure 4. On the diagram, 80 individuals from the transect study have been divided into two groups representing the two species, plus several individuals which occupy intermediate positions. More about the characteristics of the two main groups and the intermediates can be determined from the axes and the symbol combinations.

Some of the individuals on the diagram have been marked with numbers, equivalent to those used in the CCA and Figure 4. Those occupying an intermediate position are numbers 3, 5, 19, 23, 27, 29, 30, 31, 36, 38, 48, 61. This list compares favourably with that derived from the CCA. Four trees, 3, 5, 29 and 31, are included which were considered to be "parent" types in the CCA. Two trees, 37 and 50, which were intermediate on the CCA analyses are here placed in the parent groups.

Discussion and conclusions

This project was designed as a detailed study of a number of pine specimens from a restricted area of Honduran forest. The specimens studied had been collected from along a transect to include, as far as possible, all the types present, and in the study of

Table 4. Canonical Correlation Analysis of (a) two pure parent populations: the canonical vectors of the first pair of canonical variables, and (b) 80 trees from a transect: the canonical vectors of the first two pairs of canonical variables.

	NEEDLE CHARACTERS[1]											
	Needles/ fascicle	Length of sheath	Needle length	Needle width	Rows of stomata abaxial surface	Rows of stomata abaxial surface	Stomata/ row	Serrations	External resin canals	Internal resin canals	Medial resin canals	Septal resin canals
(a)	1.0000	0.1891	−0.0971	−0.0809	−0.0406	0.0396	0.0523	0.0978	−0.0842	−0.0292	0.1177	−0.0488
(b)	−0.2012	−0.1107	0.3775	−0.0830	1.0000	−0.2653	−0.1236	0.2957	−0.1351	−0.0996	−0.2355	−0.4528
	0.8677	−0.7933	−0.0831	1.0000	0.0308	0.5023	0.4463	−0.3064	0.1222	0.1854	0.1058	0.5017

	CONE CHARACTERS[1]						
	Length	Width DI	Width DII	Distance to widest point	Width at the mid point	Width of apophysis	Depth of apophysis
(a)	−0.1634	1.0000	−0.4608	−0.4447	−0.2030	0.2771	0.2812
(b)	0.3836	−0.4956	−0.0162	1.0000	0.3306	0.2984	−0.7679
	−0.0608	−0.6214	1.0000	0.2431	0.0256	−0.0337	−0.1898

1 The parameters were quantified as described in Table 3.

Table 5. Characters, and their score values/symbols, used in the Pictorialised Scatter Diagram (Included in Figure 5).

CHARACTER	SCORE									
	1	2	3	4	5	6	7	8	9	10
Number of needles per fascicle (y axis)	≥4.9	4.8–4.7	4.6–4.5	4.4–4.3	4.2–4.1	4.0–3.9	3.8–3.7	3.6–3.5	3.4–3.3	≤3.2
Needle length x Needle width	≤19.9		20.0–23.9		24.0–27.9		28.0–31.9		≥32.0	
Number of rows of stomata on the abaxial surface	≤4.9		5.0–6.9		7.0–8.9		9.0–10.9		≥11.0	
Percentage septal + external resin canals	≥71.0				70.9–31.0				≤30.9	
	1	2	3	4	5	6	7	8	9	10
Cone Shape (x axis): Length x distance to widest point / DI + DII/2	≤1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	≥5.6

these individuals numerous measurements were made and several different analyses performed. Both types of analyses presented here reveal individual trees which are intermediate in morphology, particularly in cone shape, needle number and in their internal needle anatomy, between the two parents.

Two types of analysis were chosen to demonstrate the conclusions, but it must be remembered that all

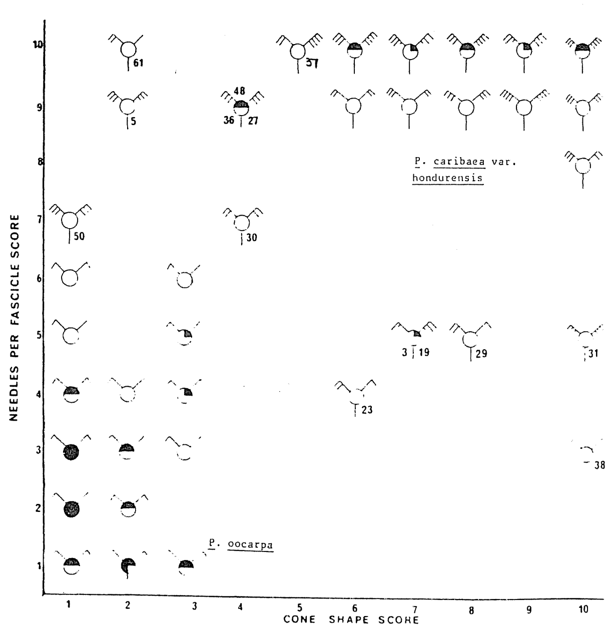

Fig. 5. Pictorialised Scatter Diagram to demonstrate relationship between the 80 individuals in the transect study (see Table 5).

the analyses performed helped to reveal the patterns within the data. It is our opinion that, of these two analyses, the CCA most accurately and clearly separated the individuals into their groups. The simpler Pictorialized Scatter Diagram placed some individuals in the intermediate zone which, according to the CCA and on closer inspection, were considered to be good *P. oocarpa* or *P. caribaea* var. *hondurensis*, and *viceversa*. This simpler method will still be very useful however where more sophisticated computer analyses are not available.

Considering further the characteristics of the intermediates revealed in this study, reference to Figures 4 and 5 demonstrates that cones from these trees are intermediate in form, between the 'egg-shape' of *P. oocarpa* and the 'barrel-shape' of *P. caribaea* var. *hondurensis*. Compare the photographs and diagrams of cones from this study shown in Figure 6. The cones of most of the intermediates resemble those of *P. oocarpa* but are more elongate with rounded (not more or less flat) bases. The cone scales are narrower, more widely spaced and often with a persistent prickle. Similarly there are cones of a *P. caribaea* var. *hondurensis* type which are more ovoid and with a persistent peduncle (a feature not studied in the analyses) as particularly shown in Figure 6d.

Intermediacy is also found in the needle characters e.g. number of needles/fascicle, number of rows of stomata on the abaxial surface and internal anatomy. The intermediates generally possessed fascicles with 3, 4 and 5 needles, with cross-sections showing a

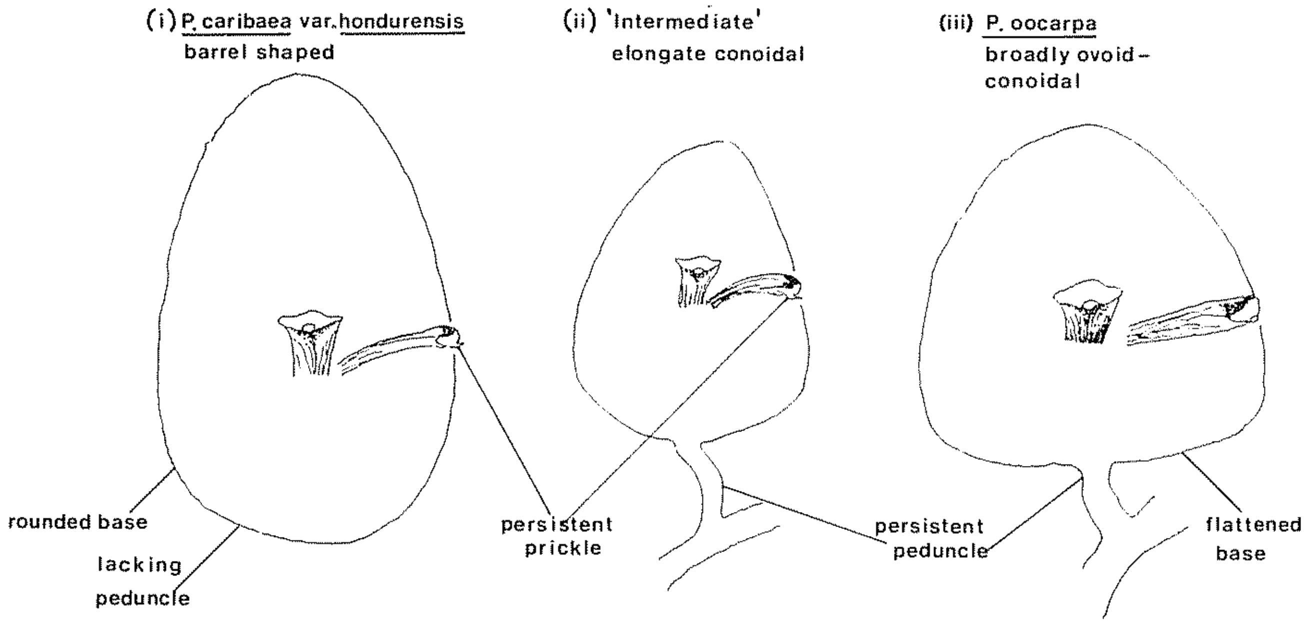

Fig 6. Female cones, (iii): *P. oocarpa*. (ii): 'Intermediate'; (i): *P. caribaea* var *hondurensis*: d: Outlines.

mixture of internal, medial and septal resin canals (Figure 7).

Another point suggested by the analyses is the existence of not only intermediates but also of some individuals with a cone typical of one species and needles typical of the other and *vice versa*. Referring to Figure 4, such types are represented by those individuals tending towards the top left and the bottom right hand corners of the graph. For example tree 38 has needles typical of *P. oocarpa* and cones more typical of *P. caribaea* var. *hondurensis* whilst the reverse in true of tree 36. These trees are probably F_2 transgressants which cannot be considered as mere genetic intergradations.

Further evidence as to the intermediate nature of some of these trees comes from analyses of the correlated resin samples which were collected from each tree.

The terpene composition of each sample was determined by staff of the Tropical Products Institue*. They have studied collections of resin of the two species from all over their range in Central America (Burley and Green, 4). A sample of 20-30 trees is preferred for 'provenance' identification, but results from the transect study were analysed as eight 'provenances' of only ten trees. Each tree was placed in one of a limited number of categories depending on the percentage abundance of six selected terpenes. The percentage of trees in each category was used to define each 'provenance'.

This analysis suggested division of the 'provenances' into the same subjective groups defined at the beginning of the project and listed in Table 2. Most importantly it suggested sites three, four and five were 'intermediate'. Thus on a site basis resin analysis can be used to test for the presence of intermediacy. Unfortunately this is very difficult on an individual tree basis; however a tentative classification of trees in the intermediate zone was made. Of four trees which were found to have unusual resin compositions, three i.e. 19, 30 and 36 are found in the intermediate group in Figure 4. Of the rest of this intermediate group, trees 23, 27, 37 and 50 were labelled as 'uncertain' on the results of resin analysis alone. These results will be published in a more detailed form later.

We therefore interpret these intermediate trees as possible F_1 and F_2 hybrids, but with some reservation since their status can only be confirmed by carefully controlled pollination experiments. There are scarcely any published data on the factors which effect the developmental stages of the leaves and female cones, which still need considerable study. The fertility of such hybrids will need further inves-

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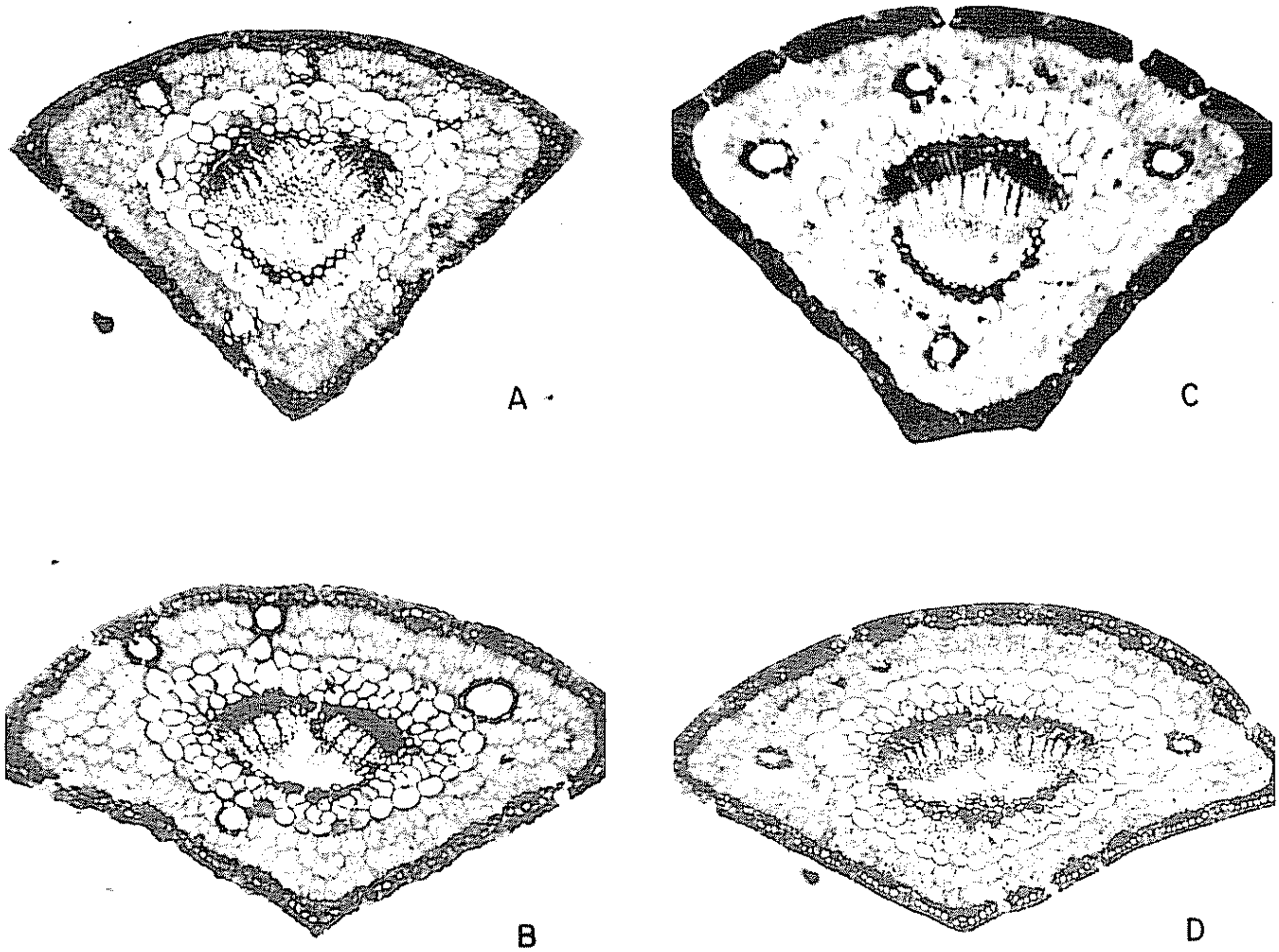

Fig. 7. Cross sections of needles. a(x110): *P. oocarpa*. b (x105), c (x110): 'Intermediate'; d (x90): *P. caribaea* var. *hondurensis*

tigation, since in the present study it is difficult to hypothesise about possible introgression, although some trees in the study certainly suggest this.

Summary

It has been frequently suggested that *Pinus caribaea* var. *hondurensis* (Sénécl.) Barret and Golfari and *P. oocarpa* Schiede, two species very important in tropical forestry, might hybridize in some places where their natural ranges overlap in Central America. In this paper, results are presented of a detailed study designed to examine this further. A site in Honduras was chosen where the two species grow together and material was collected from 80 trees along a transect running through an area of mixture. Study of the material and analysis of numerous measurements by different methods have revealed the presence of some intermediate forms. These are tentatively interpreted as hybrids but confirmation must await the results of controlled pollination experiments.

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