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NOTE

Variation In Seedlings of *Cupressus lusitanica*

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Abstract

Seedlings from 28 lots of "*Cupressus lusitanica"* seed, of indigenous provenances in Mexico and Guatemala, and of exotic provenances in Portugal, Kenya and New Zealand, were arranged to a replicated design. At one year of age a visual appraisal led to a tentative classification, the indigenous material being divided into four groups:

1. *C. lusitanica* var. *lusitanica,* from a central region in Mexico;
2. *C. lusitanica* var. *benthamii,* from Hidalgo province in Mexico;
3. *C. lusitanica* of uncertain status, from Guatemala; and
4. two seedlots of doubtful status, believed to be *C. arizonica* from Durango, Mexico.

Statistical analysis suggests that Number of Cotyledons, from 2 to 6 per seedling, varied significantly ($p < 0.05$) from one seedlot to another and possibly varied on a regional basis in the wild. Measurements of Height, Leaning of the Stem, Length of Longest Lateral and Number of Laterals, taken as single variates, all showed significant seedlot variation within and between geographical groups. Means of the 26 seedlots (excluding the two from Durango) taken in pairs, showed significant positive correlation from 0.37 to 0.88. Many of the differences between seedlots of indigenous origin may be ascribed to differences in the intensity of inbreeding. This speculation also applies to the apparently wider variation of the seedlots from cultivated trees. Another possible source of genetic variation in cultivated material is hybridisation between *C. lusitanica* and *C. macrocarpa,* which is known to occur.

Keywords: *Cupressus lusitanica*; taxonomy; varieties; provenance

Introduction

The name "*Cupressus lusitanica"* was established by Miller (1768), whose type specimen came from a tree beside the Chapel of San José at Bussaco, Portugal (Elwes & Henry, 1910). Since then, cypresses with affinities to the type specimen have been grown in many parts of the world. I have seen examples not only in New Zealand but also in Tasmania, Queensland and Chile, and there are countless references to *C. lusitanica* cultivated in other countries (e.g. Great Britain, USA (California), El Salvador, Costa Rica and Colombia); and particularly in East Africa (Parry, 1956; Pudden, 1957; Griffith, 1958).

It is generally accepted, now, that in cultivation *C. lusitanica* has given rise to many different forms. Some botanists have classified these various forms under different names, either as more than one species or as sub-species and varieties (e.g. Franco, 1945), but the variation is still not well understood. For many years it was thought that *C. lusitanica*, recognised as an exotic in Portugal, must have originated in Goa, a small Portuguese colony on the west coast of India. However, it is now generally accepted that the tree at Bussaco originated not in Goa, but in Mexico. There is still very little information on the origins and genetic history of

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the cypresses now in cultivation as "*C. lusitanica*".

In 1959, two foresters, H. V. Hinds and E. Larsen, were sent from New Zealand to Mexico to collect seed from conifers in their natural habitats. Among their collections were seedlots of cypresses from eight different localities, and these were incorporated in a trial designed to compare trees of natural origin with others of exotic origin. Three more seedlots, from Guatemala, and one from the type locality in Portugal, were included in the trial. Another objective was to evaluate the merits of the material for plantation forestry. Among the exotic origins were seedlots from special trees, selected by foresters in Kenya. This paper records observations made during the first year of the New Zealand trial. The one-year-old seedlings were planted in 1962 in field trials, where observations have continued.

Materials and Methods

Details of the seedlots of indigenous origin are shown in Table 1, and those of exotic origins in Table 2. The 28 seedlots were stratified for four weeks and then sown, without randomisation, in boxes of soil on a glass-house bench. Germination was prompt and even. The seedlings were then pricked out into jiffy-pots, each seedling being labelled with its seedlot number. While still in the cotyledon stage the number of cotyledons was counted on samples of 36 seedlings per seedlot. This done, they were arranged on a glass-house bench, in a randomised, balanced, incomplete-block design (Cochran & Cox, 1950; Plan No.1138). When they were judged to be strong enough they were moved from the glasshouse to a suitable place outdoors in the nursery of the Forest Research Institute at Whakarewarewa.

In October 1962 the original randomised block design was dismantled by grouping all the members of each seedlot together. At this stage morphological observations clearly indicated that there were separate groups of seedlots. These were evidently related to provenance, so that those from natural sources fell into three classes - Central Mexico, Hidalgo and Guatemala, while the seedlings from cultivated sources were indistinguishable from those from Central Mexico. After photographing examples, the seedlings were re-arranged to the same design as before, but with a completely new randomisation, and planted in Rotoehu State Forest.

To examine the variation more closely, in addition to Number of Cotyledons per Seedling, four other characters were recorded:

1. Leaning of Stem (departure from vertical on a subjective scale from 1 (extreme lean) to 5 (vertical));

TABLE 1: Indigenous Origins of Seedlots

Country	Place or District	Latitude (N)	Longitude (W)	Altitude (m.a.s.l)	Seedlot Number	Female Parentage
Mexico	Chilpancingo	17° 38'	99° 47'	2640	1	One well-formed tree.
		17° 38'	99° 47'	2640	26	Uncertain: Two trees, one predominant.
Mexico	Vera Cruz	19° 30'	98° 30'	2360	5	One tree.
Mexico	Puebla	19° 39'	97° 02'	2990	13	One tree.
Mexico	Michoacan/Distrito Federal	19° 26'	100° 14'	2500	9	One tree on district boundary.
Mexico	San Rafael Distrito Federal	19° 13'	98° 46'	2750	23	One tree.
Mexico	La Venta Distrito Federal	19° 17'	99° 19'	2990	27	One tree.
Mexico	La Cumbre, Hidalgo	20° 22'	98° 19'	2370	7, 12, 18, 20, 28	Five trees, one seedlot each.
Mexico	El Salto, Durango	23° 40'	105° 20'	2860	2, 8	Progeny of two trees of *C. arizonica.*
Guatemala	Chimaltenango	14° 40'	90° 49'	not known	22	Supplied as *C. lusitanica.*
Guatemala	Near Guatemala City	Approx. 14° 37′	Approx. 90° 31'	1610	24	Mixed progenies of several trees.
Guatemala	Quezaltenango	14° 48'	91° 27'	not known	25	Supplied as "*Cupressus pyramidal*".

2. Height[1] in centimetres from ground level to the apex of the terminal leaves;

3. Length of longest lateral in millimetres; and

4. Number of Laterals.

Before analysing the resulting data it was decided to exclude two of the 28 seedlots. These came from Durango, a province in the north of Mexico. In their field notes the collectors had recorded doubts about the taxonomic status of these two seedlots, describing them as "*C. lindleyi?"* ("*C. lindleyi"* and "*C. lusitanica"* are synonyms). It has also been recorded that both *C. lusitanica* and *C. arizonica* occur there and that intermediates are common (Martinez, 1947).

Furthermore, in the nursery the seedlings of these two seedlots appeared to grow more slowly than the others; and subsequent observations of their development, during 34 years, have reinforced my belief that they are *C. arizonica,* and should be described in a separate paper. This ruled out the use of the incomplete-blocks analysis, but the results for the remaining 26 seedlots were suitable for analysis as randomised, complete blocks - i.e. 26 Seedlots x 18 Complete Blocks x 2 Trees per Plot: a total of 936 Trees. For Leaning of Stem, the assessment was subjective and was limited to 9 of the 18 Complete Blocks.

The resulting data were examined by analyses of variance. For Number of Cotyledons this was done in two parts: first, to assess the variation between seedlot means; and second, to assess the apparent differences between groups. Block effects did not appear in the Cotyledon analyses because the numbers had been determined during embryogenesis; long before the Seedling x Block layout was imposed. The analyses were done as if all the effects were random.

Results

General observations

The material from Central Mexico, Portugal, Kenya and New Zealand developed into 1-year seedlings, all with much the same habit. The stems generally leaned, and the apical tufts of primary leaves curled over in the same direction as the lean. The laterals were rather

TABLE 2: Exotic Origins of Seedlots[a]

Country	Locality	Seedlot Number	Mother trees, Number at Origin	Remarks
Kenya	Sokoro 2D	16	7	Outstandingly productive in progeny trials from Elburgon[b].
Kenya	Daraja 2G	10 15	33 70	Outstanding for diameter growth.
Kenya	Uplands 7K	4 11	3 8	From Guatemala seed; planted 1910.
Kenya	Kabage 10H	6	-	"Benthami type".
New Zealand	Waipoua	14 19	WP2 WP1	Dominant in stand registered for seed.
New Zealand	Whakarewarewa	3 21	30 10	Excellent stem. Solitary tree
Portugal	Bussaco	17	-	From the type locality.

[a]Each seedlot was from a single mother tree.

[b]The Elburgon strain is described by Kenyan foresters as small-branched trees of desirable habit, preferred to other strains for plantation forestry.

[1] *i.e. the distance between ground level and the apex of the terminal leaves. Where there was curvature, most of it was removed by straightening the stem. With any residual curves, the true length of the stem would have been somewhat greater than the measured "height".*

wide-spreading and lax, and they too tended to curl downwards at the tip. The colour was generally a soft blue-grey, commonly tinged with mauve or purple where the tissues were most exposed to winter temperatures.

The seedlings from Hidalgo were more compact, with more upright stems and candelabra-like branching; and in colour they were closer to the green part of the spectrum than to the blue or blue-green.

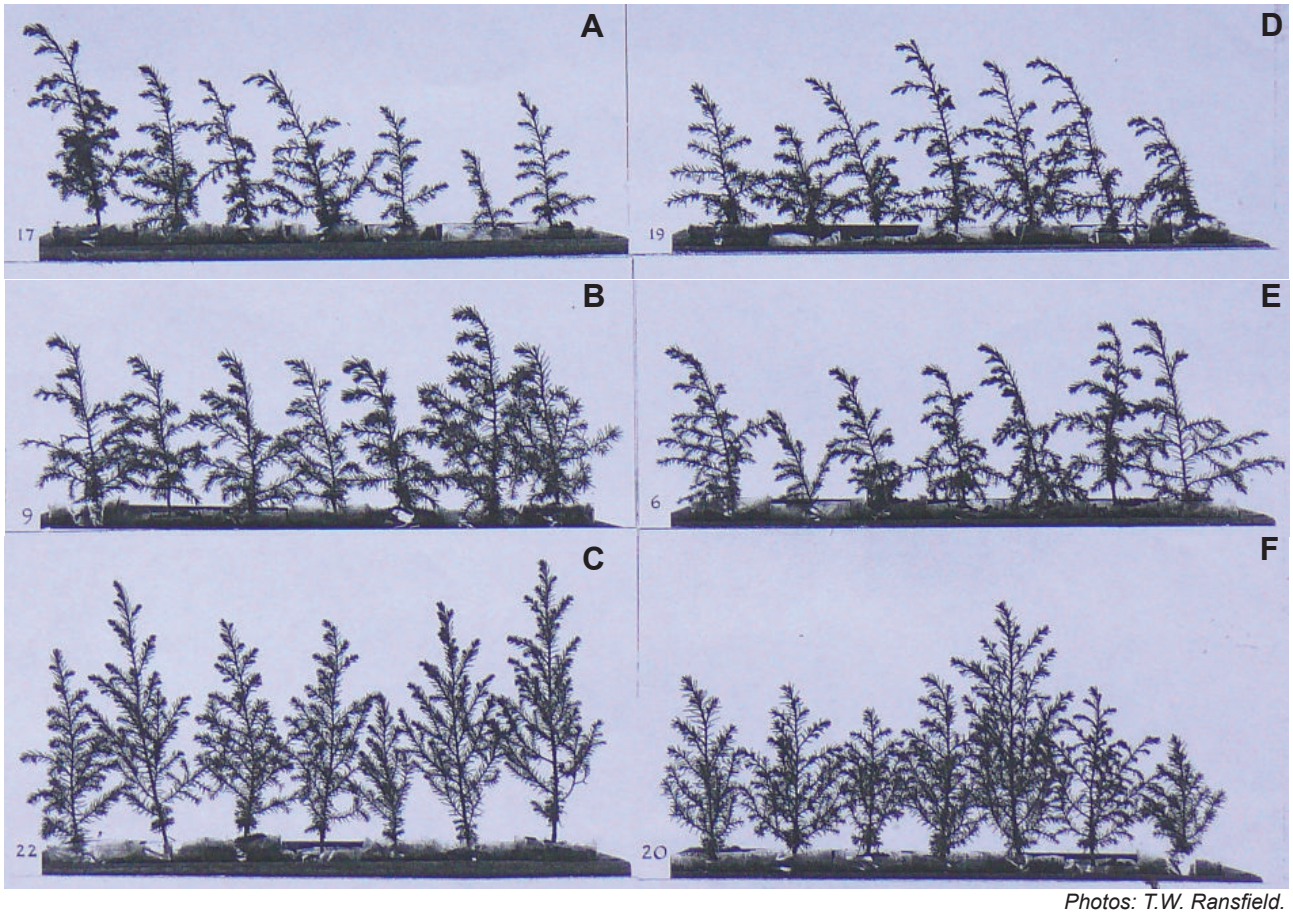
Those from Guatemala differed from the others in having more erect, and generally taller, stems; they were yellowish-green rather than blue-grey; and their leaves seemed to lie closer to the supporting shoots than in the other seedlots. Samples from six seedlots are shown in Figure 1. (For photography they were oriented so that they all leaned in the same direction. In nature they seemed to lean in random directions).

For Number of Cotyledons the first part of the analysis is shown in Table 3, and the second part in Table 4. Results for the other four characters are shown in Tables 5 - 8. The means of the five characters in each group are shown in Table 9.

Discussion

Number of cotyledons

In the whole of Africa and Eurasia there are about eight or nine native species of cypresses, all of which have two cotyledons per seedling. In North America there are about eight or nine native species, three of which regularly have two cotyledons per seedling; the others all produce seedlings with cotyledons varying in number, usually three or four per seedling, but quite often two or five, and very rarely, six.



Photos: T.W. Ransfield.

FIGURE 1: Samples of one-year-old seedlings from six provenances:

A - seedlot 17 - Bussaco, Portugal
B - seedlot 9 - Michoacan, Mexico
C - seedlot 22 - Chimaltenango, Guatemala
D - seedlot 19 - Waipoua, New Zealand
E - seedlot 6 - Kabage, Kenya
F - seedlot 20 - Hidalgo, Mexico

TABLE 3: Analyses of Variance in Number of Cotyledons in Different Groups.

Group	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Central Mexico	Seedlots	4.808	6	0.801	3.81	~0.002
	Error	51.492	245	0.210		
	Total	56.300	251			
Hidalgo, Mexico	Seedlots	0.286	4	0.071	<1.00	
	Error	41.479	175	0.2		
	Total	41.765	179			
Guatemala	Seedlots	2.532	2	1.266	4.32	~0.02
	Error	30.769	105	0.293		
	Total	33.301	107			
Kenya	Seedlots	6.090	5	1.218	6.88	<0.001
	Error	37.170	210	0.177		
	Total	43.260	215			
New Zealand	Seedlots	0.538	3	0.179	<1.00	
	Error	25.791	140	0.184		
	Total	26.329	143			
Bussaco, Portugal	Seedlots	-	0	-	-	
	Error	8.930	35	2.555		
	Total	8.930	35			
Total of Seedlots ignoring Groups	Seedlots	24.680	25	0.981	4.59	<0.001
	Error	195.631	910	0.215		
	Total	220.311	935			

TABLE 4: Analysis of Variance in Number of Cotyledons

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Groups	10.440	4	2.610	3.85	~0.02
Seedlots within Groups	14.240	21	0.678	3.15	<0.001
Error	195.631	910	0.215		
Total	220.311	935			

TABLE 5: Analysis of Variance in Scores for Leaning

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Groups	122.04	4	30.51	11.01	<0.001
Seedlots within Groups	58.19	21	2.77	5.54	<0.001
Blocks	7.30	8	0.91	1.82	~0.03
Interaction	100.82	200	0.50	<1.00	
Error	120.00	234	0.51		
Total	408.30	467			

Tables 3 and 4 suggest that variation in this character depends partly on provenance, and that a major part of it arises from differences between the seedlots. It appears to be quite independent of the other characters considered here. At the species level it appears to be genetic; there also appears to be some genetic variation within the species, *C. lusitanica* itself.

Leaning of the stem

Although it was based on subjective scores by only one observer, the analysis in Table 5 reflects differences that were plainly visible, especially between groups. Leaning may be related to the incidence of "butt sweep", which is known to occur in the early stages of growth in the field, but it seems more likely to be a transient feature, seen only in the nursery.

Height, Longest Lateral and Number of Laterals

Each of these characters indicates variation in growth rate, which is especially significant between seedlots (Tables 6, 7 and 8). The seedlot means show significant positive correlation, (Table 10), which suggests that they are manifestations of an underlying process common to all the groups.

There is also significant variance between groups, which lends some support to the subjective classification based on morphology.

TABLE 6: Analysis of Variance in Height of Seedlings (cm)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Groups	2101.99	4	525.50	5.23	~0.004
Seedlots within Groups	2108.41	21	100.40	7.05	<0.001
Blocks	1401.90	17	82.45	5.79	<0.001
Interaction	6052.80	425	14.24	1.62	<0.001
Error	4108.44	468	8.78		
Total	15773.54	935			

TABLE 7: Analysis of Variance in Length of Longest Lateral (mm)

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Groups	25118.8	4	6279.70	3.81	~0.015
Seedlots within Groups	34598.2	21	1647.53	7.61	<0.0001
Blocks	18491.7	17	1087.75	5.03	<0.0001
Interaction	91978.0	425	216.42	1.13	~0.1
Error	89415.0	468	191.06		
Total	259601.7	935			

TABLE 8: Analysis of Variance in Number of Laterals

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Square	F Ratio	Probability
Groups	2217.46	4	554.37	7.23	<0.001
Seedlots within Groups	1610.04	21	76.67	6.63	<0.001
Blocks	1504.50	17	88.50	7.66	<0.001
Interaction	4913.00	425	11.50	1.15	~0.1
Error	4722.10	468	10.09		
Total	14967.10	935			

TABLE 9: Means for five characters in six groups (provenances)

Provenance	Number of Cotyledons	Height (cm)	Length of Longest Lateral (mm)	Leaning of Stem[a]	Number of Laterals
Mexico (Central)	3.26	14.53	67.07	2.91	17.11
Guatemala	3.46	17.93	72.23	4.07	20.58
Mexico (Hidalgo)	3.32	14.59	67.76	4.11	18.18
Kenya	3.76	14.68	59.20	3.35	18.60
New Zealand	3.17	12.25	52.03	2.79	17.11
Portugal	3.12	13.56	53.80	3.50	17.70

[a] departure from vertical on a subjective scale from 1 (extreme lean) to 5 (vertical)

The seedlot means of Height and Number of Laterals, plotted in a graph, (Figure 2) show, in a central cluster:

1. the native seedlots from Mexico (including Hidalgo);

2. the one exotic from Bussaco, Portugal (the type locality); and

3. most of the exotic seedlots from Kenya and New Zealand combined.

The three from Guatemala appear to form a distinct group (Figure 2). The remainder of the exotic seedlots lie outside the central cluster.

Reasons for this distribution may include:

1. some of the differences between seedlots may have arisen from variation in the intensity of inbreeding. For example, the seedlot represented at the extreme left of Figure 2 came from a tree so isolated that its progeny may have come from self-pollination (Tree No. 21, Table 2);

2. the records from Kenya show that some of the seed-trees may have been abnormal, having been selected artificially by progeny tests (Table 2); and

3. in Portugal, Kenya and New Zealand *C. lusitanica* and *C. macrocarpa* have often been grown close to one another.

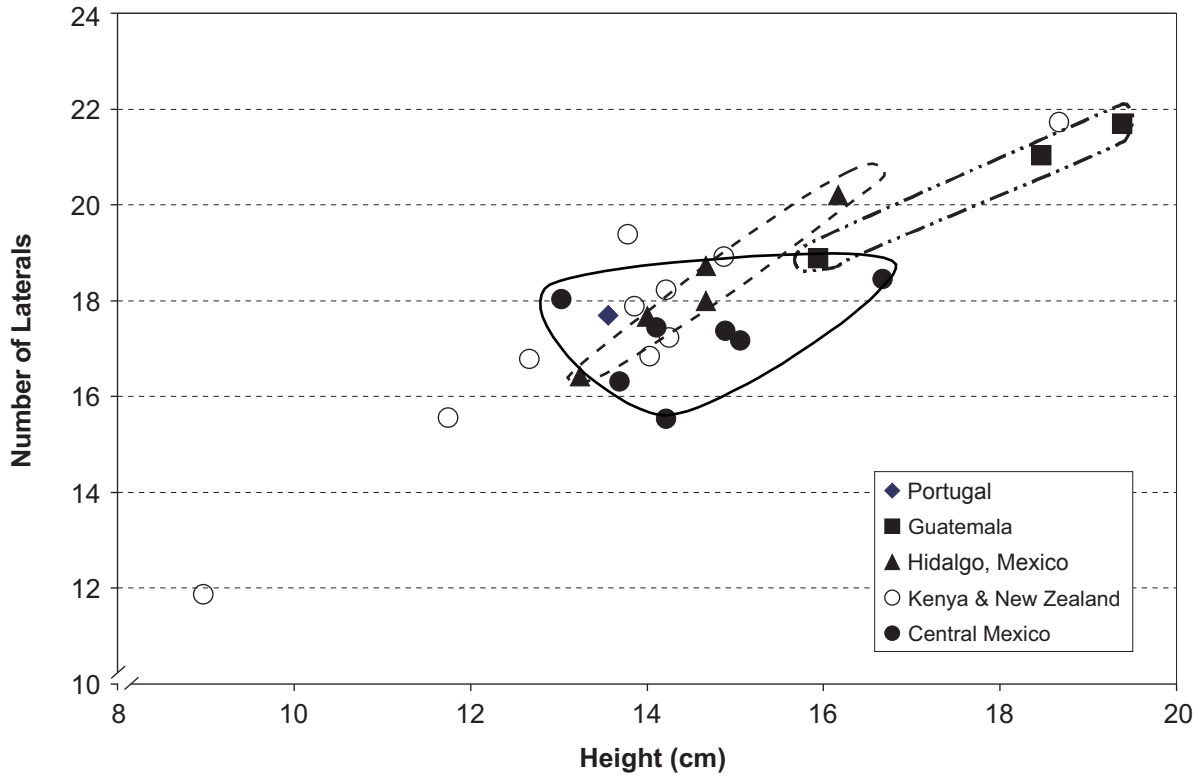


FIGURE 2: Mean number of laterals versus mean height for each seedlot

TABLE 10: Correlation Coefficients for Means of Characters Taken in Pairs (Degrees of Freedom = 24)

	Height	Score for Leaning	Length of Longest Lateral	Number of Laterals
Number of Cotyledons	-0.01	-0.06	-0.06	-0.0016
Height		+0.5**	+0.70***	+0.88***
Scores for Leaning			+0.37*	+0.73***
Length of Longest Lateral				+0.55**

Probabilities: * 0.05; ** 0.005; *** 0.001

The first putative F_1 hybrid between *C. macrocarpa and C. lusitanica* was reported by Gomes & da Costa (1943). There is further evidence from Kenya (*Report of the Forest Department,* 1954). More recent studies have confirmed that the two species are genetically quite compatible, and that introgressive hybridisation from *C. macrocarpa* into *C. lusitanica* has occurred.

In their morphology the Kenyan seedlots closely resembled those from Mexico, Portugal and New Zealand (the central cluster in Figure 2), but not the three from Guatemala in this trial. This is puzzling, because some of them were descended from seed imported from Guatemala (Table 2). The reason may be that as many as five generations could have elapsed between 1910 and 1960, and hybridisation may have taken place, involving provenances other than Guatemala.

Conclusions

All the 26 seedlots, apart from the two from Guatemala, are confidently assigned to *C. lusitanica sensu lato.* At a lower level in the hierarchy, the species comprises at least two varieties:

1. *C. lusitanica - Miller* var. *lusticanica;* and
2. *C. lusitanica* Mill. var. *benthamii* (Endl.) Carrière;

which are based on morphology and geography (Farjon 1993 & 2005). The status of the species in in Guatemala needs further study. So too does the situation in the northern provinces of Mexico where *C. lusitanica* and *C. arizonica* are sympatric (Martinez, 1947).

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