COMPARATIVE ANATOMY OF THE WOOD OF ABIES PINSAPO AND ITS TWO MOROCCAN VARIETIES

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SUMMARY

This study describes the structure of the wood of *Abies pinsapo* from samples taken from its three natural distribution areas in the Iberian Peninsula (Sierra de Grazalema, Sierra de las Nieves and Sierra Bermeja) and compares them with the varieties from the north of Africa, *Abies pinsapo* var. *marocana* from the Talassemiane mountains and *A. pinsapo* var. *tazaotana* from the Tazaout mountains. All the samples were collected in their regions of provenance. To put the results into perspective, a comparison was also made with the wood of *Abies alba* and *A. numidica*. The wood of the Iberian *A. pinsapo* and of its two varieties from the Rif mountains in Morocco is anatomically similar, and there are no qualitative differences that enable the wood to be differentiated except for the presence of resin deposits in the tracheids adjacent to the rays in the samples from Grazalema. Quantitatively, for tracheid diameter and tracheid length there are statistically significantly differences (p<0.05) between those of Spanish provenance and the Moroccan varieties, but for tracheid pit diameter, largest diameter of cross-field pits and tall ray frequency the samples from Sierra Bermeja have more in common with the African samples.

**Key words:** *Abies pinsapo*, *A. pinsapo* var. *marocana*, *A. pinsapo* var. *tazaotana*, *A. alba*, *A. numidica*.

INTRODUCTION


Although some authors cite the presence of the pinsapo fir in the province of Granada (Liu 1971; Farjon 1990), it is really only found in the provinces of Cádiz and Málaga, in the western part of the Bética range, specifically in the area known as Serranía de
Ronda. In the province of Málaga its geographical distribution is in two differentiated areas: on the one hand, the small area of some 40 ha in Los Reales de Sierra Bermeja, a single enclave on peridotites from 1200 to 1400 m, very near the Strait of Gibraltar; and on the other hand, the forests of Sierra de las Nieves, with some 3000 ha distributed over the municipalities of Ronda, Tolox and Yunquera, at altitudes ranging from 1000 to 1800 m, in this case on limestone substrates. There are also minor spots in the Alcor, Caparain, Real, Istán, Río Verde and Gialda ranges. In the province of Cádiz the species occurs in the Sierra del Pinar, in the municipality of Grazalema, in an area of approximately 500 ha on limestone substrates, at altitudes ranging from 1000 to 1650 m. There are also specimens and isolated groves in the western part of Monte Prieto, on the slopes of El Montón and the northern slope of Zafalgar and of Los Pinos (Fig. 1) (Ceballos & Vicioso 1933; Ceballos & Ruiz de la Torre 1979).

The so-called Rifian firs (from the Rif mountains) are located in the north of Morocco. *Abies pinsapo* var. *marocana* is distributed from 1450 to 1900 m following the southeast-northwest mountain chain from the western part of the Beni Selman region along the massifs of Tunian, Bab-Tizimando, Isilan and Tisuka as far as mount Magot (Chefchaouen). It is found in northern and eastern exposures, over an area of 2600 ha. *Abies pinsapo* var. *tazaotana* is located on mount Tazaout in the Beni Sey-yel region, northwest of Talambot, over an area of 1500 ha at altitudes ranging from 1280 to 1800 m (Fig. 1 & 2) (Ceballos & Martín Bolaños 1928; Sánchez Cózar 1946; Charco 1999).
The wood anatomy of *Abies pinsapo* and its two varieties has previously been described by several researchers. However, the studies were based on a limited number of samples, mostly of unknown provenance or in some cases belonging to branches, as their biometry shows. Krauss (1864) in Viguié et Gaussen (1928), included *A. pinsapo* in an identification key, distinguishing between *A. cephalonica, A. sibirica, A. fraseri, A. alba, A. pinsapo, A. nordmanniana, A. cilicica* and *A. balsamea*. Castellarnau (1880) made a very thorough description of *A. pinsapo*, although it was based on a single sample from the Serranía de Ronda. Saint-Laurent (1932) made another description of *A. pinsapo* var. *marocana*, without stating its provenance. Greguss (1955) described *A. pinsapo*, similarly failing to mention its provenance. Peraza (1964) described *A. pinsapo* var. *tazaotana* from samples collected by the Spanish Forest Service in the Rif and proposed the separation of *Abies alba* from the group *Abies pinsapo* and *A. pinsapo* var. *tazaotana*, on the basis of the higher frequency of rays over 30 cells high in *Abies alba*. The present authors, García Esteban et al. (2002), previously described two samples of *A. pinsapo* from specimens provided by the Málaga Forest Service.

While there are very few descriptions of *A. pinsapo* var. *marocana* and var. *tazaotana*, Saint-Laurent (1932) and Peraza (1964), there are many more in the case of *A. pinsapo*, although a divergence in data can be observed, particularly in the average ray height in number of cells, one of the characteristic features of the genus *Abies*: 3–30 (Kraus 1864 in Viguié et Gaussen 1928), 1–30 (Castellarnau 1880), 1–8 (Greguss 1955), 2–30 (Peraza 1964), 4–15 (Richter & Dallwitz 2000), 1–10 (García Esteban et al. 2002). Descriptions with such a low number of cells most likely correspond to samples taken from juvenile wood.

The objectives of this study are to describe, for the first time, the anatomy of the wood of *A. pinsapo* using samples from the three natural areas in the Iberian Peninsula; to describe the two Moroccan varieties using samples from their two places of origin; to compare the anatomy of the wood of *A. pinsapo* and its two varieties; and to analyse whether the variation found constitutes a distinct pattern.

**MATERIALS AND METHODS**

The material used for this study was collected in natural forests of *Abies pinsapo* in both the Iberian Peninsula and the north of Africa, in Morocco. In Spain samples were taken from the three natural areas: Sierra del Pinar de Grazalema (AP1-5) in the province of Cádiz, Los Reales de Sierra Bermeja (AP6-10) and La Nava de San Luis in Parahuta de la Sierra de las Nieves (AP11-15), in the province of Málaga. In Morocco the samples of *A. pinsapo* var. *marocana* were taken from the Talassentnane National Park (TL1-5), and of *A. pinsapo* var. *tazaotana* from Mount Tazaout (TZ1-5).

Five trees were cut in each zone, all adult specimens over 70 years old and representative of the forest. The study included a total of 25 trees, 15 from Spain and 10 from Morocco. In order to locate the natural forests, the publications of Ceballos and Martín Bolaños (1930) were used for the province of Cádiz and of Ceballos and Vicioso
(1933) for the province of Málaga. For the forests in Morocco the studies of Ceballos and Martín Bolaños (1928) and Sánchez Cózar (1946) were used.

*Abies alba* samples were also taken in Mount Martinier-La Mascarina in the municipality of Forcayo El Cornato (AA1-5), in the province of Huesca, and samples of *A. numidica* from the wood collection of the Royal Botanic Garden, Kew (K-Jw 18393) and the Nationaal Herbarium Nederland (UN192) were also used for comparison with the pinsapo fir wood, as the Mediterranean fir group is a closed group and is very differentiated from the groups in Asia and North America (Kormutak *et al.* 2004). In addition, this group supports the hypothesis of hybridisation in the fir species (Conte & Cristofolini 2003).

Microtome sections were made using standard methods, and the descriptions were made in accordance with the IAWA Committee (2004).

The samples were observed by using light microscopy and scanning electron microscopy SEM mod. JEOL JSM-6380, both without staining and after staining with safranine and Sudan 4 in order to stain the resin (Jane 1970). The test pieces for SEM were prepared following the method described by Heady and Evans (2000). The tracheid length was measured following Ladell’s indirect method (Ladell 1959).

The measurements of tangential tracheid diameter, tracheid length, diameter of tracheid pits, height and frequency of rays and size of cross-field pits were made on the microtome sections using the WinCell image analysis programme. The biometry was done on 5 preparations from each tree, in all cases on mature wood from a basal disc.

Figure 2. Pinsapo fir forest in its ecological optimum in Tazaout. — Figure 3. Sapwood and heartwood similar (Grazalema).
between rings 70 and 100. On each preparation 35 random measurements were made (5 trees × 5 preparations × 35 measurements = 875 measurements/provenance). The number of pits per cross-field was measured on the five preparations from each tree, in ten different rays. The measuring of the height and frequency of the rays was done on all the rays contained in one square millimetre on the tangential section.

An analysis of variance was made in order to determine the significance level in the variables of tangential tracheid diameter, tracheid length, ray height and frequency, number of rays per square millimetre, diameter of tracheid pits, largest diameter of the pits in the cross-fields and the number of pits in the cross-fields.

The Lilliefors normality test and Bartlett’s homogeneity of variance test were done, both for a significance level of 95 % (p < 0.05). As in all cases at least one of the conditions was not met, the ANOVA Kruskal-Wallis test was used to analyse the samples. To determine statistically significant differences between provenances LSD tests were done using the data from the ANOVA Kruskal-Wallis test. The statistical calculations were done with the MATLAB V.6.5 Release 13 programme, for a significance level of 95 %.

**Anatomical description and biometry**

**Cross section** (Fig. 3–8). Sapwood and heartwood colour similar, although the heartwood colour is quite marked in the fresh wood, particularly in the Sierra Bermeja sample, which has shades of red (Fig. 3). Fresh wood has a fetid odour. Growth ring boundaries distinct (Fig. 3). Abrupt transition from earlywood to latewood (Fig. 4), with the transition being more pronounced in the wood from Sierra Bermeja (Fig. 5) where the rings are narrower. The Moroccan varieties show a transition closer to that of Sierra Bermeja, although the number of cells in the latewood is greater: 8–15 in var. marocana and 6–10 in var. tazaotana. Tracheids rectangular in transverse section. Tangential wall pits generally present in the tracheids near the growth ring boundary (Fig. 6a). Axial parenchyma present but sparse, generally in marginal position, in isolated cells with high cellular content (Fig. 6b). Resin canals absent. Occasional presence of traumatic resin canals, surrounded by subsidiary cells with high cellular content and crystals (Fig. 7). Resin deposits in the tracheids adjacent to the rays only observed in the samples from Grazalema (Fig. 8).

**Tangential section** (Fig. 9–12). Rays of over 30 cells tall. Rays almost exclusively uniseriate. Rays 2-seriate in part, constituting less than 10 % of the total (Fig. 11). Transverse end walls of axial parenchyma cells nodular (Fig. 12).

**Radial section** (Fig. 13–18). Cross-field pits taxodioid (Fig. 13). Trabeclula common (Fig. 14). Tracheid pits in radial walls of earlywood predominantly biseriate, in opposite arrangement (Fig. 15). Horizontal walls of ray parenchyma cells distinctly pitted. Indentures present (Fig. 16). Calcium oxalate crystals in ray parenchyma cells prismatic, located in both the marginal and interior rows of the rays, more abundantly in the former (Fig. 17:a). Irregularly shaped cells present in the marginal rows of the rays (Fig. 17:b & 18). Tori well defined, not scalloped, but extensions present (Fig. 19). Warty layer present (Fig. 20).

All biometric data are summarised in Table 1.

(Text continued on page 294)
Table 1. Biometry of species of the genus *Abies* from the western Mediterranean, including *A. alba* from the Pyrenees and *A. numidica*.

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>A. pinsapo</em></th>
<th><em>A. pinsapo</em></th>
<th><em>A. pinsapo</em></th>
<th><em>A. alba</em></th>
<th><em>A. numidica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grazalema</td>
<td>Sierra</td>
<td>Sierra</td>
<td>Tazaout</td>
<td>Talassentane</td>
</tr>
<tr>
<td>Tangential tracheid diameter (µm)</td>
<td>46.9 ± 9.4</td>
<td>44.4 ± 9.8</td>
<td>44.9 ± 10.8</td>
<td>51.4 ± 7.3</td>
<td>62.0 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>(23.4–76.0)</td>
<td>(22.7–71.3)</td>
<td>(25.3–73.4)</td>
<td>(30.1–67.2)</td>
<td>(42.5–76.7)</td>
</tr>
<tr>
<td>Diameter tracheid pits (µm)</td>
<td>18.5 ± 1.9</td>
<td>20.2 ± 1.7</td>
<td>18.6 ± 2.7</td>
<td>20.7 ± 2.6</td>
<td>21.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>(12.7–24.6)</td>
<td>(16.0–25.3)</td>
<td>(7.5–28.6)</td>
<td>(9.8–29.6)</td>
<td>(10.5–26.0)</td>
</tr>
<tr>
<td>Tracheid length (µm)</td>
<td>2591 ± 793</td>
<td>3154 ± 861</td>
<td>2946 ± 934</td>
<td>3518 ± 976</td>
<td>3405 ± 1255</td>
</tr>
<tr>
<td>Most frequent average height in</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Most frequent average height of</td>
<td>169.7 ± 24.6</td>
<td>95.3 ± 8.9</td>
<td>114.0 ± 12.2</td>
<td>133.7 ± 12.4</td>
<td>134.0 ± 15.9</td>
</tr>
<tr>
<td>rays (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rays per mm²</td>
<td>24.4 ± 4.0</td>
<td>23.6 ± 2.9</td>
<td>23.8 ± 4.6</td>
<td>22.9 ± 3.8</td>
<td>23.5 ± 3.2</td>
</tr>
<tr>
<td>Largest diameter cross-field pits (µm)</td>
<td>7.5 ± 1.2</td>
<td>7.1 ± 1.2</td>
<td>6.6 ± 1.1</td>
<td>7.2 ± 1.1</td>
<td>7.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>(4.7–10.9)</td>
<td>(3.6–11.3)</td>
<td>(3.5–9.8)</td>
<td>(3.4–10.7)</td>
<td>(3.4–11.1)</td>
</tr>
<tr>
<td>Smallest diameter cross-field pits (µm)</td>
<td>4.4 ± 1.2</td>
<td>4.2 ± 1.1</td>
<td>3.8 ± 0.9</td>
<td>3.0 ± 0.9</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>(1.9–7.3)</td>
<td>(1.3–7.5)</td>
<td>(1.7–6.8)</td>
<td>(0.9–6.0)</td>
<td>(1.1–5.2)</td>
</tr>
<tr>
<td>Number of pits per cross-field</td>
<td>1.6 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.7</td>
<td>1.9 ± 0.6</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>(1–4)</td>
<td>(1–4)</td>
<td>(1–4)</td>
<td>(1–4)</td>
<td>(1–3)</td>
</tr>
<tr>
<td>Number of rays / mm² &gt; 30 cells</td>
<td>0.025 ± 0.041</td>
<td>0.002 ± 0.009</td>
<td>0.021 ± 0.047</td>
<td>0.004 ± 0.017</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>
The genus *Abies* shows very pronounced wood anatomical homogeneity (Jacquiot 1955; Peraza 1964), and the wood of its species cannot be differentiated anatomically (Schweingruber 1990). However, many authors have proposed keys based on very different criteria to differentiate the species of *Abies* (Krauss 1864 in Viguie et Gaussen 1928; Castellarnau 1880; Penhallow 1896; Saint-Laurent 1932; Wiesehuegel 1932; Record 1934; Phillips 1948; Brown et al. 1949; Greguss 1955; Kukachka 1960; Peraza 1964; Core et al. 1979).

The *Abies pinsapo* wood from the three Spanish provenances and its two varieties from the Rif mountains are anatomically similar, and there are no qualitative differences to allow them to be differentiated from each other except for the presence of resin deposits in the tracheids adjacent to the rays in the samples from Grazalema, similar to those described in Araucariaceae (Jones 1912; Patton 1927; Heady et al. 2002). The presence of resin deposits in this provenance may be related to the high resin content in the ray parenchyma, which is much higher than in any of the other provenances, and the subsequent overflow of the resin into the tracheid lumina through the cross-field pits (Heady et al. 2002; García Esteban et al. 2005). While resin deposits are common in Araucariaceae and some genera of Podocarpaceae and Cupressaceae, they are very rare in Pinaceae, *e.g.* *Picea sitchensis* (Kukachka 1960).

Although Sargent (1902) used wood colour to differentiate *A. nobilis* and *A. magnifica* from the rest of the American firs, the more reddish colour of the Sierra Bermeja provenance is probably influenced by the edaphic substrate made up of peridotites.

Wiesehuegel (1932) used the early-latewood transition of the rings in the American species as a diagnostic feature, although it should not be considered as such, as these variations are a function of climatic conditions (Core et al. 1979). In fact, Sierra Bermeja has the least favourable ecological conditions of all the sites where *A. pinsapo* grows. On the other hand, the ecological optimum in the Moroccan forests (Sánchez Cózar 1946) explains the significantly wider diameter of the tracheids than those of the Spanish provenances (Fig. 21:a). This behaviour is also seen when the mean tracheid length values are analysed (Fig. 22).
As for the tracheid pit diameter and the largest diameter of the cross-field pits, the results show an affinity between the Sierra Bermeja provenance and the two Moroccan ones. The number of pits per cross-field is a homogeneous feature for all the provenances except in the case of the Grazalema provenance (Fig. 21:b, c, d).

Figure 21. LSD statistical analysis. Provenance of species: 1: *Abies pinsapo* (Sierra de Grazalema); 2: *A. pinsapo* (Sierra Bermeja); 3: *A. pinsapo* (Sierra de las Nieves); 4: *A. pinsapo* var. *tazaotana* (Tazaout); 5: *A. pinsapo* var. *marocana* (Talassemotane); 6: *A. alba* (Pyrenees); 7: *A. numidica* (collections). – a: Tangential diameter of tracheids. – b: Diameter of tracheid pits. – c: Largest diameter of cross-field pits. – d: Number of pits per cross-field. – e: Number of rays per mm². – f: Number of rays per mm² > 30 cells.
Ray size parameters are usually considered to be good indicators for *Abies* (Wiesehuegel 1932; Greguss 1955) and have been used by some authors for characterising its species (Castellarnau 1880; Wiesehuegel 1932; Phillips 1948; Greguss 1955; Peraza 1964). In *A. pinsapo* the number of rays per square millimetre does not show significant differences (Fig. 21:e), but the frequency of rays over 30 cells high does. This value is used by some authors as a threshold for differentiating *A. alba* from the other *Abies* species (Jacquiot 1955; Peraza 1964). Analysis of the maximum ray height allows two clusters to be distinguished: on the one hand the pinsapo firs from Grazalema and Sierra de las Nieves, and on the other hand the pinsapo firs from Sierra Bermeja and the two Moroccan varieties. The values place the woods from Grazalema and Sierra de las Nieves closer to *A. alba* than to the Rifian subspecies (Table 1). The rays in the wood from Sierra Bermeja are intermediate in height between those from Grazalema-Sierra de las Nieves and the Tazaout-Talassentane. The Iberian pinsapo firs attain higher values of ray height than the Rifian firs.

Analysis of the number of rays with over 30 cells shows that the Grazalema-Sierra de las Nieves provenance is closer to *A. alba*, but yet statistically different from each other, while the Sierra Bermeja provenance once again forms a cluster with the Moroccan firs and even with *A. numidica* (Fig. 21:f). The Moroccan provenances do not have very high rays in spite of the fact that they are located in the ecological optimums.
of the pinsapo fir forests, and therefore a relation between maximum ray height and ecological conditions seems to be ruled out.

The wood structure of the Rifian firs is statistically different from the structure of *A. numidica* in their maximum ray height, although there are no significant differences in terms of the number of rays of over 30 cells between the Rifian and the Algerian firs.

Resin canals were only observed in the wood from Grazalema, but their presence should not be considered to have diagnostic value either, as the canals in *Abies* appear in response to injuries (Anderson 1897; Jeffrey 1905; Rhoades 1923; Chamberlain 1935; Jane 1970).

Calcium oxalate crystals were present in the ray parenchyma in all the provenances, both in the marginal and submarginal rows. Their presence cannot, in this case, be used with diagnostic value to differentiate provenances, although it can be used to characterise the pinsapo fir woods, as in the case of most of the *Abies* species (Greguss 1955; Jane 1970; Core *et al.* 1979; IAWA Committee 2004).

Ray tracheids were not observed in any of the provenances, and although some authors maintain that some species of *Abies* can contain ray tracheids (Penhallow 1907 in *A. balsamea*; Thompson 1912 in *A. homolepis* and *A. veitchii*; Jane 1970 in *Abies* spp.), their presence is related to injuries (Jeffrey 1917; Chamberlain 1935; Phillips 1948). The presence of irregularly shaped cells, generally in the marginal position, is usual in pinsapo fir wood, particularly in the wood from Grazalema and Las Nieves. Thompson (1912) referred to these cells as degenerate cells and considered them a characteristic element of the genus *Abies*. Later on, Chrysler (1915) also cited them in *Cedrus*.

The warty layer on the tracheid walls is present in all the provenances. No quantitative or qualitative differences are observed between the provenances.

With the exception of two biometric variables (tracheid diameter and tracheid length), it can be stated that, in general, the wood structure of the two Moroccan varieties is similar to the firs of the Iberian Peninsula, particularly the Sierra Bermeja provenance. This provenance could perhaps present an intermediate phyletic stage between the provenances from Grazalema and Sierra de las Nieves on the one hand, and the Moroccan firs on the other, in the same way as *A. nebrodensis* does between *A. alba* and *A. numidica* (Ducci *et al.* 1999; Parducci *et al.* 2001; Conte & Cristofolini 2003).

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IAWA List of Microscopic Features for Hardwood Identification
by an IAWA Committee; edited by E.A. Wheeler, P. Baas and P.E. Gasson

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This IAWA List offers guidance to everyone who wants to interpret descriptive information from the vast wood anatomical literature or who needs to include descriptive wood anatomical information in original publications. Thanks to the concise definitions and high-quality illustrations, the IAWA Hardwood List is also very suitable for teaching purposes.

IAWA List of Microscopic Features for Softwood Identification
by an IAWA Committee; edited by H.G. Richter, D. Grosser, I. Heinz and P.E. Gasson

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