

Microscopic Distinction of Wood Between Two *Pinus* Species; *P. Halepensis* and *P. Brutia*

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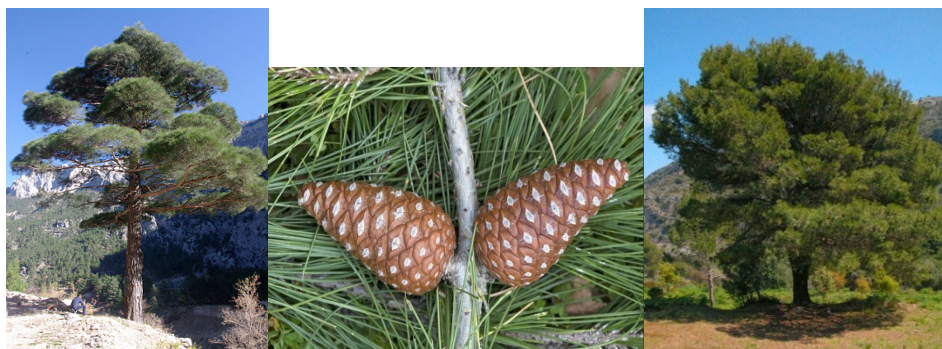
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ABSTRACT

This research aims to develop a pine-wood identification methodology concerning *Pinus halepensis* (Aleppo pine) and *Pinus brutia* (Turkish pine), based on microscopic observations and anatomical features that could be used to identify and distinguish the above two species (in particular the dimensions of the rays). We concluded that the maximum ray height of the *Pinus halepensis* wood is lower than that of the *Pinus brutia* wood (18 vs. 26 cells respectively). The average ray height of *Pinus halepensis* wood is lower than that of the *Pinus brutia* wood. It's the first attempt to identify these two different *Pinus* species.

Keywords: *Pinus halepensis*, Aleppo pine, *Pinus brutia*, microscopic separation





The average ray's height of *Pinus halepensis* wood is lower than that of *Pinus brutia* wood.

INTRODUCTION

Identification of wooden material is a major aspect for many scientists and professionals interested in identifying wood origination (tree botanical identity) on the basis of the variability in the macroscopic and microscopic characteristics of wood among different genera and species [1]. In addition to the scientific interest, wood identification is also characterized of great practical importance. Wood can be identified at three levels: macroscopic, microscopic and by biochemical methods (this category includes the methods of DNA determination, isoenzyme analysis and resin contained in pine plant tissues). The pine tree genus (*Pinus*) comprises about 90 species found in Northern Hemisphere and is of great importance for the global wood trade and manufacture. In the Greek area, seven pine species are mainly found: *Pinus halepensis*, *Pinus brutia*, *Pinus pinea*, *Pinus nigra*, *Pinus silvestris*, *Pinus leucodermis* (*Pinus heldreichii*) and *Pinus peuce*. Especially for pine species, macroscopic and microscopic identification keys had shown in the past [2,3] that there is only a possibility of classifying the pinewoods in common like groups such as *Pinus halepensis* with *Pinus brutia*, *Pinus nigra* with *Pinus silvestris*, *Pinus leucodermis* with *Pinus pinea* etc. The distinction between two pinewood species forming a common group is sometimes very difficult, as in the case of this research, which deals with the group of two important Mediterranean species (Table 1), *Pinus halepensis* (Aleppo pine) and *Pinus brutia* (Turkish pine) [4,5,6].

Table 1. Geographical distribution of *Pinus halepensis* and *Pinus brutia*

Species	Greece		Other countries	Literature
	Mainland	Islands		
<i>Pinus halepensis</i> (Aleppo pine)	Epirus, Halkidiki, Central Greece, Peloponnese, Pelion	Euboea, Skyros and other islands of the Aegean and Ionian seas.	North Africa, Spain, N. France, Italy, Serbia, Croatia, Turkey (Adana), Albania, Lebanon, Palestine From Morocco to Tunisia and Libya. On the Adriatic coast and in Israel and Jordan.	Athanasiadis 1986 Vidakovic 1991
<i>Pinus brutia</i> (Turkish pine)	Thrace (DadiaSoufliou), Halkidiki (Stratoniki, Olympiada)	Thassos, Crete, Samothrace, Lesvos, Chios, Samos, Ikaria, Dodecanese	Cyprus, NE Caucasus and NE Iraq, North Iran, Afghanistan, Syria, North Lebanon, South Italy, Anatolia, Crimea, coasts of Turkey and Black Sea	Athanasiadis 1986 Arabatzis 1998

Some additional features proposed for wood identification of pine species cannot be observed macroscopically but only with the use of microscopic observation on exceptionally fine / clean cuts at sufficiently large magnifications. Such features are: type and size of wood-forming cells, type and characteristics of rays (radius width and height, ray structure, presence of radial resin canals), pits (appearance, types of pits, cross-field pits), radial tracheids inner walls, parenchyma etc. [7,8]. This research deals particularly with the dimensions of the rays.

MATERIALS AND METHODS

Material

The material was derived from four individual trees of *Pinus halepensis* and four individual trees of *Pinus brutia* placed in natural forest stands in the region of Halkidiki. Core samples were taken from the stem-wood of each tree, with an increment borer placed at breast-height. The samples promoted for the creation of microtomes were selected from the mature wood area of the initial core samples (Figure 1, Figure 2).

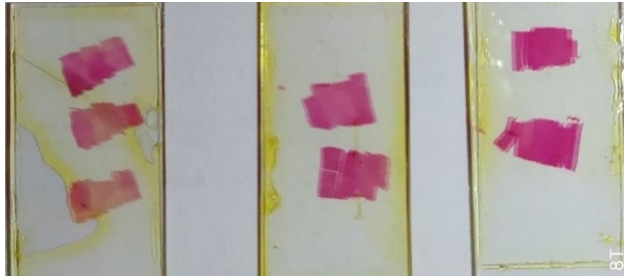


Figure 1. Samples from *P. brutia*



Figure 2. Samples from *P. halepensis*

Method

Wood samples must be softened with appropriate treatment before getting cut into the microtome, especially when the wood is hard or air-dry. In this research, the samples were initially softened by boiling in water. The boiling continued until the samples were submerged under the influence of their weight (2.5-3 hours). After boiling, the samples were stored in a 3: 2: 1 ratio of glycerol, ethyl alcohol, and water, respectively, in order to soften sufficiently enough to be sectioned by the microtome. Afterwards, transverse, radial and tangential sections were obtained from all 8 samples of typical mature wood (4 from *Pinus halepensis* and 4 from *Pinus brutia*). The obtained sections were initially washed to remove foreign particles and wood residues. Safranin was used for the sections' coloring (2-3 drops in water for less than one minute) before washing in 50% ethyl alcohol solution and twice in 100% ethyl alcohol solution for dehydration. Finally, xylene wash was applied to remove the ethyl alcohol, while Canadian Balsam was used as a glue to secure the sections between covers and slides [9]. Sections' observation was performed with a simple 10x magnification microscope with an eyepiece 8. The observation field allowed the measurement of 40-45 rays.

Table 2. Number and height of *Pinus halepensis* uniseriate wood rays

Ray height (cells)	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sample	Number of rays															
X1α	3	7	3	6	6	2		3	4	1	1	3		1		
X1β	2	7	4	6	6	9	4	4		3	1		1	1		
X1γ	6	6	8				1	2	3		1	1		1		
X1δ	3	5	3	4	4	4	5	3		2	1	1				
X2α	5	5	8	3	8	3	3	2		1	1					
X2β	3	4	6	6	8	4	3	1	2		1		1	1		
X2γ	4	4	5	5	7	3	4	4	5		1	1				
X3α	5	5	7	1	6	8	1	1	2	1	2	2	1			1
X3β	4	5	7	4	2	5	1	2	3		1	1		1		
X3γ	5	4	5	4	6	3	4	2		2		2	1			
X4α	5	4	5	8	4	3	1	1	4	3	1					
X4β	4	5	5	7	5	3	2	2	4		2	1		1		
X4γ	4	3	5	6	4	3	2	2		3	1	1				

Table 3. Number and height of *Pinus brutia* uniseriate and biseriate wood rays

Sample	Number of uniseriate rays by ray height																											
Ray height	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	28				
B1a	3	3	2	3	4	4	3	2	4	2	1	1	1	1	2	2	1	1					1					
B1b	2	1	1		4	1	1	6	4	1		2	1	1	2	2		1										
B1c	3	3	3	4	4	5	3	3		2		2	1	1	2	1		1		1								
B2a	1	2	1	3	5	2	2	5	3		1	1	2	1	1	1		1		1								
B2b	2	3	2	4	6		3	1	4	4	2		3	2	1			1										
B2c	1	2	3	3	3	3	1	4	4	3	2	1	1	1		1			2									
B3a	2	4	8	7	5	6	5	4	4		1					1												
B3b	1	2	5	6	1	2	3	3	2	1			1		1	1												
B3c	1	2	3	3	3	3	2	3			2	3	2	2	2		1											
B4a			3	1	1	2		3	3	1	2	3	3	1	1	1	1	1	1					1				
B4b		3	2	3	2	4	3	2		3	2	2	1	2	1	1				1		1						
B4c		3	3	4	2	2	3	4	3		2	1	1		1	2		1	1		1							
Sample	Number of biseriate rays by ray height																											
Ray height	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	28				
B1a								1																				
B1b									1																			
B1c							1																					
B2a								1																				
B2b							1																					
B2c									1																			
B3a								1																				
B3b							1																					
B3c																												
B4a								1																				
B4b																												
B4c								1																				

RESULTS AND DISCUSSION

During tangential section observation, the height and the width of the rays were measured. The results obtained are presented in tables 2 & 3 and figures 1 for all *Pinus halepensis* samples and 2 for all *Pinus brutia* samples, frequency distribution charts. Mean values of rays' height were also determined (table 4).

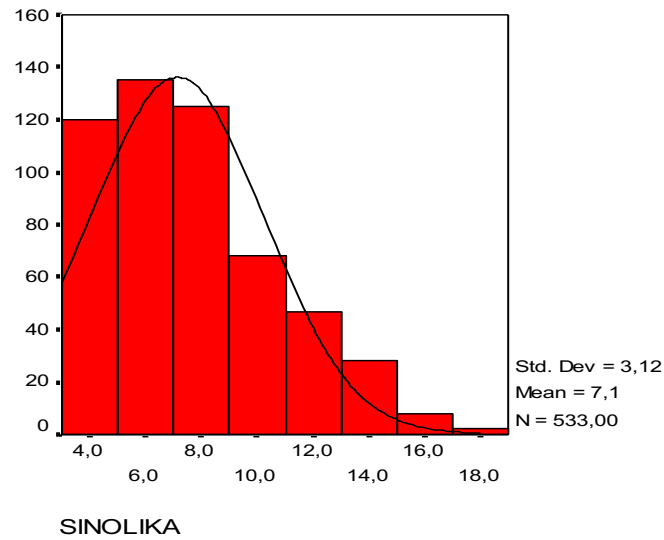


Figure 3. Normal distribution figures of all *Pinus halepensis* samples

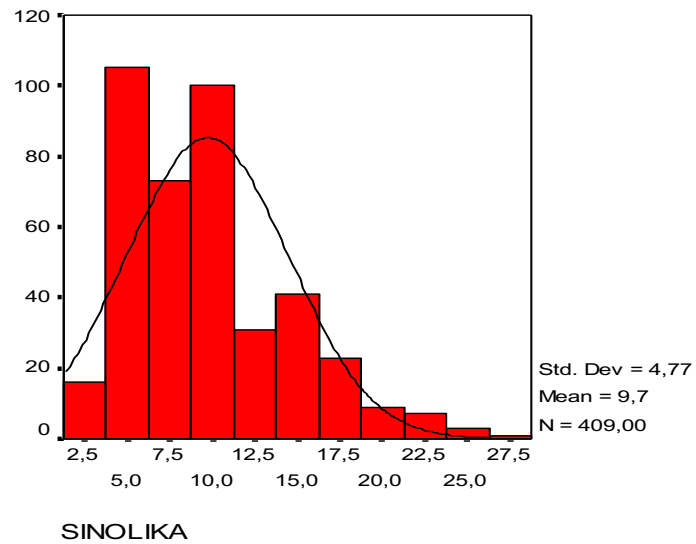


Figure 4. Normal distribution figures of all *Pinus brutia* samples

Table 4. Number of specimens (n), mean ($\bar{x}$) and standard deviation ($S \pm$) of *Pinus halepensis* and *Pinus brutia* samples

Sample	<i>Pinus halepensis</i>			<i>Pinus brutia</i>		
	n	$\bar{x}$	$S \pm$	n	$\bar{x}$	$S \pm$
1 α	40	7,6	3,57	41	10,2	5,40
1 β	48	7,5	3,06	20	8,2	2,71
1 γ	56	7,4	3,24	39	9,3	5,04
1 δ	37	7,5	2,98	-	-	-
2 α	39	6,3	2,48	33	10,2	4,82
2 β	39	6,3	2,48	38	9,6	4,36
2 γ	43	7,3	2,85	35	10	4,69
3 α	43	7,5	3,71	47	7,4	2,89
3 γ	37	6,9	3,07	32	10	4,59
4 α	33	6,9	2,97	29	12,8	5,42
4 β	41	7,2	3,27	33	10,7	5,28
4 γ	35	7,2	3,29	34	10,4	5,36
Total	533	7,1	3,12	409	9,7	4,77

The experimental results presented in figures 3-4 and in tables 2, 3 & 4, lead to the following determinations:

- Ray Height: The maximum ray height occurs in *Pinus brutia* wood and reaches up to 26 cells, while in *Pinus halepensis* wood it reaches up to 18 cells. The minimum ray height for both species is determined about 3 cells.

- Ray Width: All *Pinus halepensis* wood ray are exclusively uniseriate, while in *Pinus brutia* few biseriate wood rays with a height of about 10 cells occur (1-2 biseriate rays per field),

- Average ray height: Ray height ranged from 3-18 cells for *Pinus halepensis* with an average of 7.1 cells, while for *Pinus brutia* rayheight ranged from 3-26 cells with an average of 9.7 cells. Maximum ray height of *Pinus halepensis* wood is lower than that of *Pinus brutia* wood (18 vs. 26 cells respectively).

The average ray's height of *Pinus halepensis* wood is lower than that of *Pinus brutia* wood.

In *Pinus halepensis* wood only uniseriate rays were observed, while in *Pinus brutia* wood a few biseriate rays were also observed.

A larger and more representative sample of *Pinus halepensis* and *Pinus brutia* wood, derived from pine trees based on their natural distribution in Greece, should be studied in a more extended and detailed research. An extension of such studies to other native pine species in Greece, rather than *Pinus halepensis* and *Pinus brutia*, is also suggested.

ACKNOWLEDGMENTS

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References

- [1]. Timar, M., Gurau L., Porojan, M. & Beldean, E., *Microscopic identification of wood species: An important step in furniture conservation*. European Journal of Science and Theology, 2013. **9**(4): p. 243-252.
- [2]. Tsoumis, G.. *Science and technology of wood: Structure, properties, utilization*, 1991, Van Nostrand Reinhold, New York, USA, pp 494.
- [3]. Wheeler, E. and Baas, P., *Wood Identification - A Review*. IAWA Journal/International Association of Wood Anatomists, 1998. **19**(3): p. 241-264.
- [4]. Athanasiadis, N. (1986). Forest Botany, Volume II, (in Greek) Trees and Shrubs of the Greek Forests, Giahoudis – Giapoulis ed, Thessaloniki, Greece, pp 320.
- [5]. Vidakovic, M. (1991), Conifers Morphology and Variation, Grafičkizavod Hrvatske, pp 754.
- [6]. Arabatzis, T. H. (1998). Shrubs and Trees in Greece, Volume I. (in Greek), T.E.I. Kavalas & Ecologiki Kinisi Dramas ed, Technological Educational Institute of Kavala, Drama, Greece, pp 292.
- [7]. Passialis, C., *Wood utilization (Academic lectures in Greek)*, 1994, Technological Educational Institute of Kavala, Drama, Greece.
- [8]. Payette, S., Garneau, M., Delwaide, A.& Schaffhauser, A., *Forest soil paludification and mid-Holocene retreat of jack pine in easternmost North America: Evidence for a climatic shift from fire-prone to peat-prone conditions*. The Holocene, 2013. **23**(4): p. 494-503.
- [9]. Voulgaridis, E., Passialis, C. and Vasiliou, V., *Wood identification (Academic lectures in Greek)*, 2002, Aristotelian University of Thessaloniki, Greece.