




Article

Qualitative Wood Anatomy Study of Ottobratica and Sinopolese Cultivars of *Olea europaea* L.

Tiziana Urso ¹, Michela Zanetti ^{1,*}, Annalisa Magnabosco ¹, Angelo Mammoliti ², Marco Paccagnella ¹ and Andrea Rosario Proto ²

¹ Department of Land, Environment, Agriculture and Forestry, University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy; tiziana.urso@unipd.it (T.U.); annalisa.magnabosco@unipd.it (A.M.); marco.paccagnella.6@studenti.unipd.it (M.P.)

² Department of Agriculture, University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy; angelo.mammoliti@unirc.it (A.M.); andrea.proto@unirc.it (A.R.P.)

* Correspondence: michela.zanetti@unipd.it

Abstract: Olive wood is used in a niche economic context but is attracting growing interest. In this study, the wood anatomy of *Olea europaea* L. belonging to two cultivars cultivated in the Plain of Gioia Tauro in Calabria (RC) is qualitatively described. Wood samples were obtained along the diameter of wood slices to investigate any anatomical differences between the inner and outer zones of the stem. The microscopic slides were investigated using an optical microscope. The anatomical characteristics observed were compared with existing literature data. The two cultivars show parenchyma rays arranged not only in one to two rows (typical of this species), but also in three rows. Furthermore, in both cultivars, the presence of starch deposits in procumbent parenchyma cells was observed. The Ottobratica cultivar seems to have more starch than the Sinopolese one, but given the high variability of olive wood, further quantitative analysis is needed to determine whether these differences are statistically valid and due to the different cultivars. This work can contribute to a better understanding of the *Olea europaea* L. species and to a better technical valorisation of its wood.

Keywords: *Olea europaea* L.; olive wood; wood vessels; starch; sapwood; heartwood



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1. Introduction

The cultivation of olive trees for oil production is widespread in the Calabria Region (Southern Italy), where it occupies 70% of the territory. Olive trees in these areas grow vigorously, especially local varieties, and have a significant impact on the economy of the entire region [1,2], with numerous companies involved in both cultivation and product processing. The olive tree (*Olea europaea* L.) is an evergreen tree belonging to the genus “*Olea*”, family Oleaceae, and is primarily cultivated for food purposes. It is usually a tree of modest size, and rather long-lived. In certain situations, the tree’s growth habits can become shrub-like; for example, when it is found in Mediterranean scrub formations. The species thrives in generally temperate and warm climates, on limestone and rocky soil [3]. To date, the greatest economic relevance of the olive trees comes from the harvesting of olives for oil production, used for food, medicinal, and cosmetic purposes.

In the “Plain of Gioia Tauro”, located in the province of Reggio Calabria (Italy), the cultivation of olive trees spans over 20,000 hectares. Different to other olive-growing areas, two olive cultivars (Sinopolese and Ottobratica) are characterised by a remarkable growth, perhaps unique in the world, with trees reaching and often exceeding 25 m in height: a real “forest of olive trees” [4]. The stem is robust, and its circumference can vary considerably depending on the age and variety of the olive trees. Wood is not the primary product of olive tree cultivation, but it finds a good place in the market. The wood is mainly used in handicraft products, for cabinetmaking, parquet flooring, carving, and turning. Olive wood is hard, with differentiated sapwood and heartwood; the sapwood is lighter than the

heartwood, which has a reddish colour. In general, the colour of olive wood ranges from brown to yellowish-pink, with evident and varied tonal variations, alternating with streaks, which make it particularly appreciated aesthetically. Olive wood is a diffuse-porous wood with numerous vessels, isolated or in groups of 2–5, arranged in radial lines. The rays are uni- to biseriate and the parenchyma is paratracheal. The growth ring boundaries are often indistinct [5,6]. Concerning olive wood anatomy, the literature presents mainly archaeobotanical study related to climate factors and adaptation [3,5,7]. In popular culture, olive wood is typically considered “oily” due to the high presence of oils and extracts [8], and this makes it burn even when still green [9]. Due to these characteristics, and the low cost of pruning residues, olive wood has been the object of several studies concerning its valorisation in biorefinery [10–13]. Given the widespread presence of plantations throughout the Italian peninsula, the use of olive-tree-pruning residues has also been evaluated for biomass energy production [3,14]. In the Gioia Tauro Plain area, olive trees significantly shape the landscape and characterise the region’s agroecosystems. Despite the extensive cultivation of olive trees, especially in southern Italy and in the Mediterranean basin, to the author’s knowledge, olive wood is still little studied.

In addition to the little-studied anatomy of olive wood, its physical and mechanical characteristics have also been under-investigated [15,16], or the information is difficult to access because the studies are not presented in English [17]. A recent study by Mammoliti et al. [18] compared the physical and mechanical properties of branch and stem wood from the two olive cultivars, “Sinopolese” and “Ottobratica”. The authors found some differences between the two cultivars, primarily in mechanical properties such as modulus of elasticity, modulus of rupture, compression strength, footprint, and screw withdrawal resistance. Overall, the “Sinopolese” cultivar exhibited higher values in these properties compared to the “Ottobratica” cultivar. A solid understanding of the theoretical background of the wood structure and its mechanical and physical properties is essential, as these factors are closely linked to wood’s performance and potential applications [19]. For example, fibre length and fibre-wall thickness are also determinants to predict the density and mechanical properties [20]. On the other hand, vessel size is related to the treatment ability, where a large vessel indicates easy treatment compared to small vessels [21,22]. Additionally, the availability and cost of wood as a raw material are key determinants of its overall suitability for use [23,24].

For these reasons, studies on the anatomical property need to be performed on the little-known species to increase scientific knowledge and to explore the suitability of this wood for different uses. In fact, investigations about cell structure and fibre morphology are very important to fill the gap in knowledge and to determine the different areas of application [19]. Based on the differences highlighted by Mammoliti et al. [18] between “Sinopolese” and “Ottobratica” olive wood cultivars, this study aims to present a comparison of wood anatomical properties of the two olive cultivars cultivated in the Gioia Tauro Plain (Calabria Region), in order (i) to see if there is an anatomical difference between the two cultivars and, if there is, (ii) to understand if this plays a role in the differences highlighted in the study by Mammoliti et al. [18].

2. Materials and Methods

2.1. Study Area

For this study, two olive trees (*Olea europea* L.) of two different cultivars (Sinopolese and Ottobratica) were selected from plants grown in a family-run orchard located in the municipality of Oppido Mamertina, a hamlet of Reggio Calabria (38°16′48″ N, 15°58′55″ E). This orchard is located at 347 m a.s.l. and extends over an area of 0.8 ha, of which 47% is sloped and 53% is flat. The plantation has an 8 × 10 m planting space.

In this plantation, some pathologies such as Peacock eye, leprosy, and olive fly have been found, but Xylella is not present. During the year, the temperature generally ranges from 7 °C to 28 °C and is rarely below 4 °C or above 30 °C. There have been no fires in this orchard, and every two years ammonium sulphate-based fertilisers are applied at 8–10 kg

per tree. Normally, plants are pruned every three/four years, but in 2023, notably extensive pruning and felling work was carried out which gave rise to large-sized wood residues. The two cultivars studied showed different dendrometry measurements (Figure 1):

- Sinopolese cultivar: age: 70 y; 17.0 m height; DBH (diameter breast height): 54 cm.
- Ottobratica cultivar: age: 70 y; 19.0 m height; DBH: 61 cm.



Figure 1. Olive trees (*Olea europaea* L.) examined in this study.

2.2. Olive Wood Samples

A wood slice was taken from both trees of the two cultivars (Figure 2). The wood slice obtained from the Sinopolese cultivar had a circumference of 135 cm; the maximum diameter was 46 cm, the minimum was 37 cm (the average diameter is 40.5 cm), and the thickness was 4.5 cm. The slice had an irregular shape with an obvious eccentricity with

the pith displaced laterally. The growth rings were irregular, like the shape of the wood slice, and tension wood was present on the larger portion of the piece. There were some radial cracks caused by wood shrinkage and two ring shakes (Figure 2A). Both slices were obtained from each stem at 5.50 m height during extraordinary reform pruning activity. The presence of tension wood in the olive tree is very common because the tree has a very irregular trunk (Figure 1). In the case of the extraordinary pruning activity, the choice of two specimens with similar morphological characteristics was not possible because the selection of the trees to be felled is made upstream and follows a specific authorised felling plan. The “Ottobratica” cultivar wood slice shape was more regular than Sinopolese and it had a circumference of 94 cm, a maximum diameter of 32.5 cm, and a minimum diameter of 26 cm (the average diameter was 29.5 cm). The thickness was 4 cm. This slice had a more regular shape, and the pith was located in the middle of the piece. There were ring shakes and some radial cracks (Figure 2B).



Figure 2. Olive wood (*Olea europaea* L.) slices: (A) Sinopolese cultivar wood and (B) Ottobratica cultivar wood.

2.3. Microscopic Analysis

The slides for the anatomical analysis were obtained from wood samples taken from two strips approximately 1 cm wide cut from the Sinopolese and Ottobratica slices (Figure 3). The samples were cut from the pith of the wood outwards, at a distance of 2 cm from each other, along the diameter shown in pencil in Figure 2. Then, the samples were cut to obtain a cube of 1 cm each side. For the cutting, an automatic fretsaw with a thin blade was used for a precise cut. Due to the different sizes of the two wooden slices, 20 and 13 cubes were obtained from Sinopolese and Ottobratica, respectively.

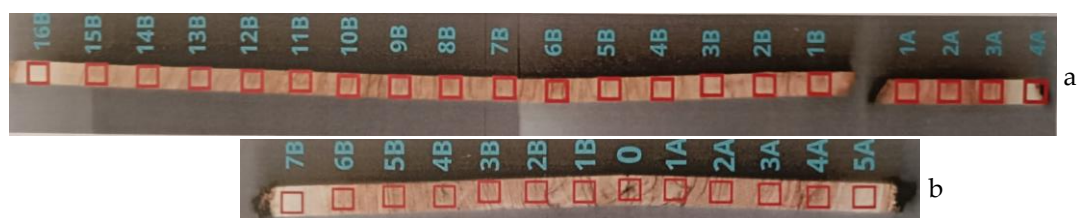


Figure 3. Olive wood cultivar samples: Sinopolese (a) and Ottobratica (b). Small red squares indicate where the samples were cut with the respective codes (sample 0 is the pith).

After boiling to soften the wood [25], the cubes were cut with a Leica RM2245 semi-automatic rotary microtome (Leica Biosystems, Milan, Italy) to obtain 12–14 μm thick slices.

For the anatomical analysis, three sections were cut for each sample: radial, tangential, and cross-section. In the end, 20 slides for Sinopolese and 13 slides for Ottobratica cultivars were prepared and 99 sections were analysed to identify the olive wood's anatomical characteristics. From the analysis of the slides, some characteristics not described in the literature were identified. Therefore, to further the analysis, additional analyses were conducted; 4 cubes were selected for each cultivar, with 2 of sapwood and 2 of heartwood. Radial and tangential sections were cut to obtain 16 sections, 8 sections for each cultivar. To prepare permanent slides, a photo-hardening gel, Eukit UV R Low Viscosity gel (ORSAtec, Viterbo, Italy), was used and the slides were put in an oven with a UV lamp for 15–20 min.

2.4. Staining and Mounting of Slides

The characterisation of the not-yet-classified substances observed in the parenchyma cells was possible thanks to a specific stain applied to the newly prepared slides. Assuming the presence of starch, it was decided to use Lugol's solution based on iodine (Marco Viti, 7% iodide solution and 5% potassium iodide solution in deionised water and ethanol). If starch is present, the colouring solution reacts and the starch takes on a shade tending towards purple or dark blue. Some drops of staining solution were applied to each of the 16 sections, and after 1 min the sections were washed, first with deionised water and then with a 50% solution of deionised water and ethyl alcohol. Finally, the sections were dehydrated with ethyl alcohol [15]. At the end of this procedure, the permanent slide was mounted. The slides made for the anatomical characteristics study were observed with a Leica DMC 4500 microscope (Leica Biosystems, Milano, Italy), and pictures of the main anatomical characteristics were taken. Stained slides were digitalised with the Axioscan 7 scanner (Zeiss, Milano, Italy), and images were obtained with a specific application, ZEN ZEISS 3.9 (Zeiss, Milano, Italy).

3. Results and Discussion

3.1. Microscopic Features

The anatomical characteristics were coded using the “IAWA list of microscopic features for hardwood identification” [26] and are reported in Table 1.

Table 1. IAWA features of Ottobratica and Sinopolese cultivars of olive wood (*Olea europaea* L.) (source: [26]).

IAWA Feature	General Description
5. Wood diffuse-porous	Wood in which the vessels have more or less the same diameter throughout the growth ring
8. Vessels in dendritic patterns	Vessels arranged in a branching pattern, forming distinct tracts, separated by areas devoid of vessels
10. Vessels in radial multiples of 4 or more common	Radial files of 4 or more adjacent vessels of common occurrence
11. Vessel clusters common	Groups of 3 or more vessels having both radial and tangential contacts, and of common occurrence
13. Simple perforation plates	A perforation plate with a single circular or elliptical opening
22. Inter-vessel pits alternate	Pits between vessel elements (inter-vessel pits) arranged in diagonal rows (alternate)
36. Helical thickenings in vessel elements present	Ridges on the inner face of the vessel element wall in a roughly helical pattern
38. Helical thickenings only in vessel element tails	Ridges on the inner face of the vessel element wall in a roughly helical pattern only in vessel element tails

Table 1. Cont.

IAWA Feature	General Description
56. Tyloses common	Outgrowths from an adjacent ray or axial parenchyma cell through a pit in a vessel wall, partially or completely blocking the vessel lumen, and of common occurrence (except in outer sapwood)
58. Gums and other deposits in heartwood vessels	Includes a wide variety of chemical compounds, which are variously coloured (white, yellow, red, brown, black)
66. Non-septate fibres present	Fibres without septa
70. Fibres very thick-walled	Fibre lumina almost completely closed
78. Axial parenchyma scanty paratracheal	Axial parenchyma associated with the vessels or vascular tracheids, and types of paratracheal parenchyma are scanty paratracheal, vasentric, aliform, confluent, and unilateral paratracheal
79. Axial parenchyma vasentric	Parenchyma cells forming a complete circular to oval sheath around a solitary vessel or vessel multiple
97. Ray width 1 to 3 cells	Ray width in cell numbers as per feature descriptor
107. Body ray cells procumbent with mostly 2–4 rows of upright and/or square marginal cells	Procumbent ray cell = a ray parenchyma cell with its longest dimension radial as seen in radial section; square ray cell = a ray parenchyma cell approximately square as seen in radial section
109. Rays with procumbent, square, and upright cells mixed throughout the ray	Procumbent ray cell (see above description); square ray cell (see above description); upright ray cell = a ray parenchyma cell with its longest dimension axial as seen in radial section

In the analysed microscopic images of both Sinopolese and Ottobratica, the growth rings were indistinct or absent [27]. Furthermore, the microscopic images confirm that there were no variations in the diameter of the vessels between earlywood and latewood, which is a typical anatomical trait of diffuse-porous wood (IAWA feature 5 [26]) such as olive wood (Figure 4). In this type of wood, growth rings should be marked by a radial dimension of fibres; in the latewood, the fibres could be radially flattened with or without thick walls, while in the earlywood, the lumen is larger [6].

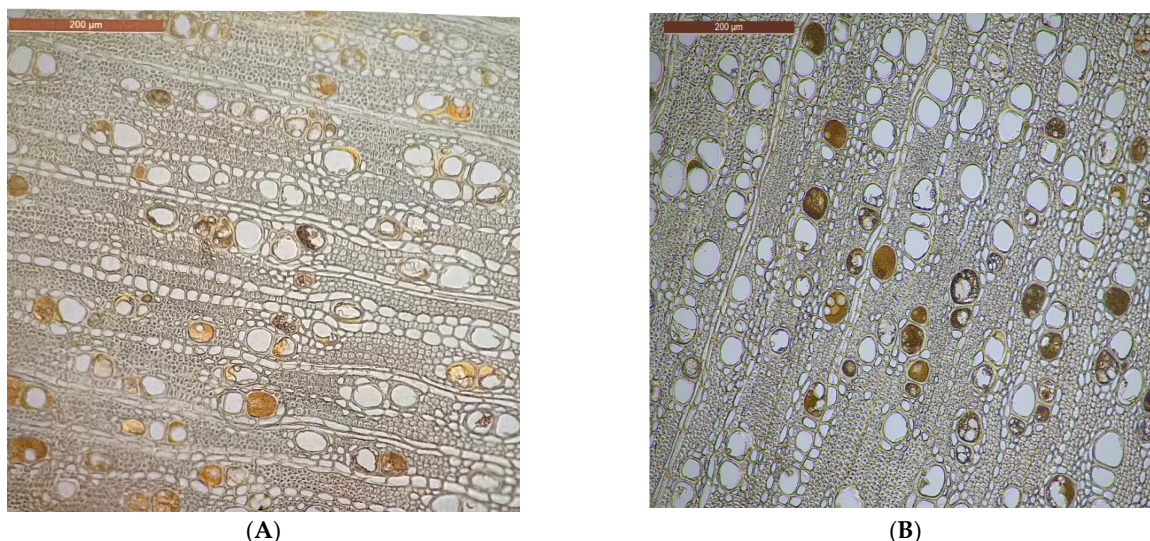


Figure 4. Olive heartwood cross-section: (A) Sinopolese cultivar: sample 1B; (B) Ottobratica cultivar: sample 2A. Diffuse-porous wood (IAWA feature 5). Vessels in radial/diagonal pattern (IAWA feature 7). Vessels partly solitary, partly in radial multiples of 2–4, or in very small clusters (IAWA features 10, 11). Parenchyma scanty paratracheal/vasentric (IAWA features 78,79). Presence of common tylosis (IAWA features 56), gums, and other deposits (IAWA feature 58) [26].

The vessels are distributed in a radial pattern, and they are partly solitary and partly in radial multiples of 2–4, or in very small clusters (IAWA features 8, 10, 11 [26]; Figure 4). In cross-section, it is also possible to note axial scanty paratracheal vasicentric parenchyma (IAWA feature 78, 79 [26]), and parenchyma cells are associated with the vessels forming complete circular or oval sheath around solitary or multiple vessels (Figure 4).

In the radial section, the vessels show simple perforation plates (IAWA feature 13 [26]), while in the tangential section, it is possible to see the alternate inter-vessel pits arranged in diagonal rows (IAWA feature 22 [26]; Figure 5). Together with vessel perforations, there are few and dense helical thickenings only in the vessel element tails (features 36 and 38 [26], Figure 6).

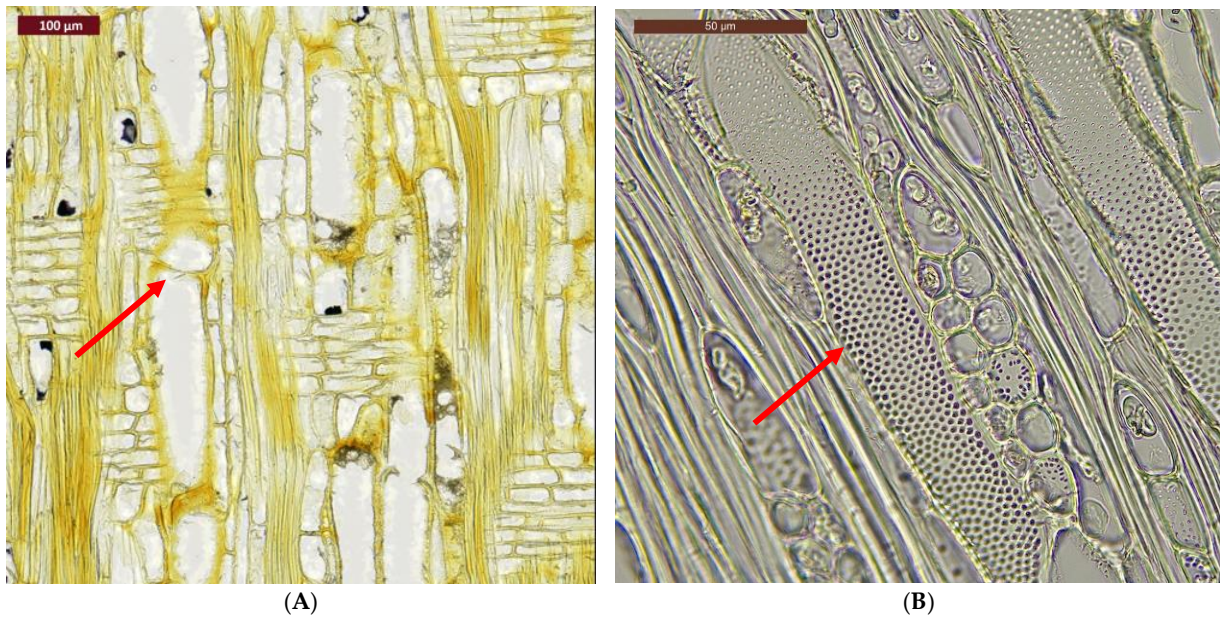


Figure 5. Sinopolese olive wood cultivar: (A) 15B sample, sapwood. Radial section, red arrow: simple perforations plate (IAWA feature 13); (B) 12B sample, heartwood. Tangential section, red arrow: alternate inter-vessel pits (IAWA feature 22) [26].



Figure 6. Ottobratica olive wood cultivar: 5A sample, sapwood, tangential section. Red circles: helical thickenings only in vessel element tails (IAWA features 36 and 38 [26]).

Olive heartwood is also characterised by the presence of common tylosis (IAWA features 56 [26]) and other coloured deposits (IAWA feature 58 [26]). Tylosis and deposits completely filled some vessels, as is evident in the cross-sections and tangential sections (Figures 4 and 7). It is probable that the deposits in the heartwood are oily substances, and they take on a colour tending towards orange.

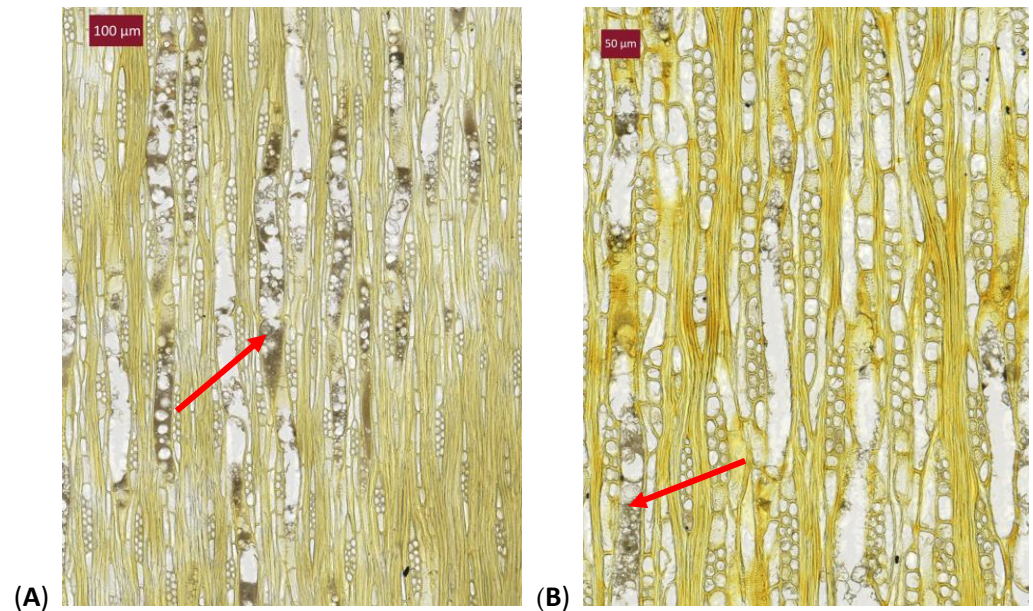


Figure 7. Olive wood. (A) Ottobratica cultivar, 4B sample. (B) Sinopolese cultivar, 1B sample. Heartwood, tangential section. Presence of common tylosis (red arrows, IAWA features 56), gums and other deposits (IAWA feature 58) [26].

The analysed olive wood fibres are very thick-walled (IAWA feature 70 [26]), and there are no septate fibres (IAWA feature 66 [26]). Tension wood fibres have a thick wall with an unligified gelatinous (G) layer [26], but since this character is present in both cultivars, and the G layer was not observed, the authors cannot conclude that the presence of very thick-walled fibres means that tension wood fibres are detected. This anatomical characteristic certainly influences the high density of the wood of these two cultivars, with 0.89 g/cm^3 (moisture content: 7.2%) found for the Sinopolese and 0.92 g/cm^3 (moisture content 5.9%) for the Ottobratica.

Rays are multiseriate with 1 to 3 cells (IAWA feature 97 [26]), and in radial sections, is possible to distinguish procumbent cells with mostly 2 to 4 rows of square marginal cells (IAWA feature 107 [26]). Some rays had procumbent, square, and upright cells mixed throughout the rays (IAWA feature 109 [26]) (Figures 8 and 9).

In the sapwood of each cultivar, the presence of granular material similar to starch was observed.



Figure 8. Sinopolese olive wood cultivar: (A) 1B sample, heartwood; (B) 15B sample, sapwood. Tangential section. Yellow boxes: ray width with one to three cells (IAWA feature 97 [26]).

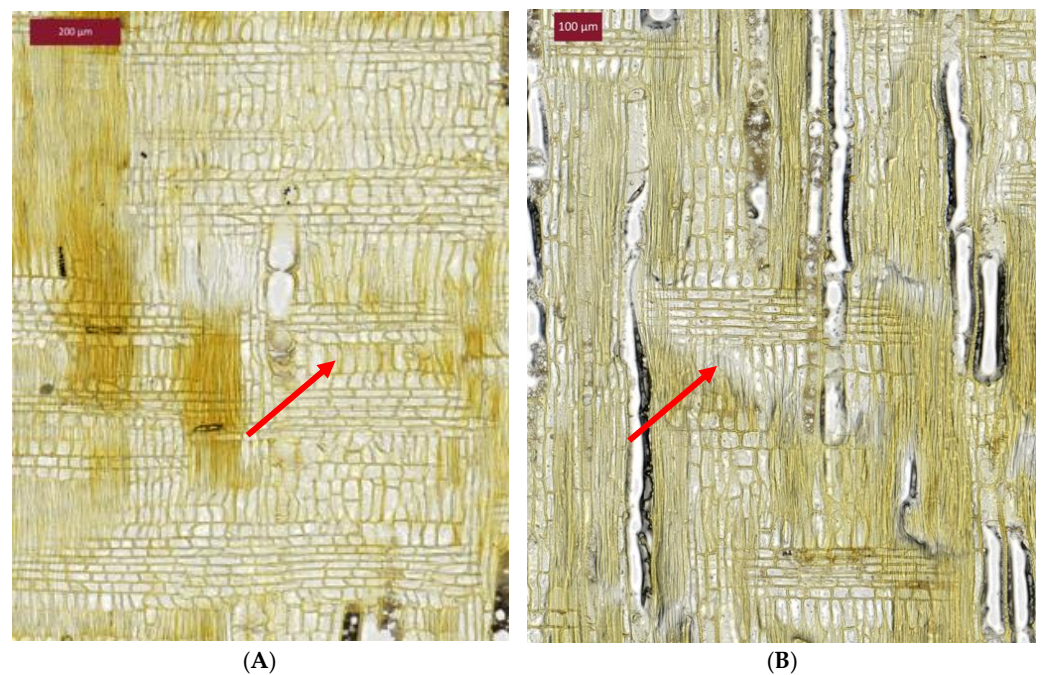


Figure 9. Olive wood, heartwood radial sections. (A) Sinopolese, 1B sample; (B) Ottobratica, 4B sample. Red arrow: body ray cells procumbent with mostly two to four upright rows (IAWA feature 107). Red arrow: rays with procumbent, square, and upright cells mixed throughout the ray (IAWA feature 109) [26].

3.2. Starch

In order to verify the nature of the granules observed in the sapwood of the two cultivars, Lugol's solution, specific for starch, was used to colour the deposits present in the parenchymal cells of the sapwood of both cultivars, confirming the initial hypothesis that they were composed of starch. Microscopic observations of both Sinopolese and Ottobratica stained slides show evident differences between sapwood and heartwood, visible in both the radial and tangential sections. The presence of starch deposits is notice-

able in the sapwood which, following staining, took on a very dark blue–purple colour (Figures 10 and 11). The deposits are localised in the ray parenchyma cells. In particular, they seem to be present in greater quantities in the procumbent cells of the *Ottobratica* cultivar. In the sapwood area of the *Sinopolese* cultivar, a smaller quantity of starch is noted compared to the *Ottobratica* cultivar, and it is mainly deposited in the straight parenchyma cells (Figure 11). As expected, in both *Ottobratica* and *Sinopolese* cultivars, no starch was found in the heartwood (Figure 9A,B). The presence of starch deposits in ray cells in the sapwood, demonstrated by histochemical staining, has not been found in other literature descriptions on olive wood anatomy.



Figure 10. Starch (black spots) in *Ottobratica* olive wood cultivar, 5A sample, sapwood. (A) Radial section, (B) tangential section.



Figure 11. Starch (black spots) in *Sinopolese* olive wood cultivar, 15B sample, sapwood (radial section).

The two cultivars show the same anatomical characteristics; any differences could be evident with measurements and statistical analysis, which were not performed in this

study, as it was only qualitative. In the Sinopolese cultivar, despite what was expected, no tension fibres were detected in anatomical slides. Tension cell wood anatomy varies depending on hardwood species, and different anatomical characteristics could be seen [28]. In this qualitative anatomical study, the magnifications used did not allow, for example, for a verification of the presence or absence of the cell wall's gelatinous layer, typical of tension cell wall wood. Furthermore, the presence of tension wood, unlike the compression wood of gymnosperms, is not easily visible macroscopically, especially in wood with an inhomogeneous appearance, such as olive wood. In fact, in the Ottobratica slice, the only indication of the presence of tension wood is given by the eccentric pith.

It is possible to note that in each cultivar, the main difference is between sapwood and heartwood due to the coloured deposits, tylosis, and granular elements (starch). The first two are mostly present in heartwood, while the last is present in sapwood. The anatomical characteristics observed in both cultivars are consistent with those described in the literature [6,29]. In particular, cluster structures inside the heartwood vessels similar to tyloses were observed, which Baas [6] attributes to the presence of chemical deposits for which further investigations would have been necessary in the future. Indeed, the particular configuration of parenchyma-vessel pits, small and bordered, would not be suitable for tylose formation [6]. The literature is very poor regarding studies on olive wood anatomical features. It is therefore difficult to compare the detailed results found in this study with other, similar research. Indeed, when the results of the produced research were analysed, only general information about the anatomy of olive wood was found because the purpose of the studies was different. For example, Santos-Rufo et al. [30] undertook a quantitative analysis of the olive wood anatomy of two cultivars to investigate whether anatomical differences could be related to the differential resistance response to the fungus *Verticillium dahlia*. Terral and colleagues [5,7] studied the anatomical differences between wild and cultivated olive wood in relation to climatic changes [5], using wood and charcoals. The results of qualitative analysis of both wood types were in accordance with the findings of this study, with the only difference concerning the rays. The authors describe them as uni-biseriate, without specifying the type of cells they were composed of, while in this study, one to three cell rays were observed and the type of ray cells described. Differences could be genetic or could be a response to growth conditions [31]. A recent study by Alhaithloul et al. [31] analysed the genotyping and morphological differences between eight olive cultivars growing in Saudi Arabia, but the authors took into account stem anatomy and not cell anatomy.

Concerning the presence of starch in the parenchyma cells of both cultivars, it is well known by plant physiologists [32], but, to the authors' knowledge, its presence has not been highlighted by wood technologists. The presence of starch may play a role in supporting the growth and ripening of fruits [33], and could play a crucial part in the olive trees' survival strategy, helping the tree to recover in the semiarid Mediterranean climate [34]. From a technological point of view, radial cracks can occur in parenchymal rays [35]. How might the presence of starch affect the occurrence of radial fractures? From an initial and only qualitative observation, it emerged that the Ottobratica cultivar seems to have greater starch deposits than the Sinopolese, but a more in-depth analysis is necessary through a quantitative analysis conducted on more specimens to demonstrate this hypothesis.

A recent study [18] compared the physical and mechanical properties of stem wood of "Sinopolese" and "Ottobratica" trees, and in general, the "Sinopolese" cultivar showed higher values. The authors justified these results by the differences obtained in wood density between the cultivars or by some intrinsic characteristics [19]. For these reasons, knowledge about olive wood's anatomical characteristics is desirable to favour its use in multiple sectors.

4. Conclusions

This study reported a detailed and in-depth qualitative anatomical characterisation of two cultivars (Ottobratica and Sinopolese) of olive wood. Based on the results obtained, the

wood of both cultivars exhibited a complex anatomical structure, often irregular, but in line with what is described in the scientific literature on olive wood. Only a small difference was found, and it concerns the parenchymal rays. In this study, some of them were composed of one to three rows, visible in tangential sections, instead of one to two as reported in other studies. In both cultivars, the presence of starch in parenchyma cells has been found, and from the comparison of the two cultivars, the Ottobratica cultivar seems to have a greater amount of granular and other deposits. Furthermore, tylose-like structures seem to be more numerous in the Ottobratica than the Sinopolese cultivar. Despite what has been observed, these differences could be attributed to the variability of wood and therefore may not be sufficient to distinguish the two cultivars. The findings of this qualitative analysis of the anatomy of olive wood need to be verified through a quantitative analysis and, if it is not sufficient, it must be further investigated with a genetic study. Because of the lack of studies addressing the anatomical investigation of centuries-old olive trees, this study, and those that will follow it, will contribute to the understanding of *Olea europaea* L. and the valorisation and use of its wood.

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