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Studies on the *Quercus* hybrids in Sicily: leaf micromorphology and xylem structure in *Q. ×fontanesii* Guss.

Abstract

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In order to find new suitable characters in the taxonomic delimitation of the genus *Quercus*, whose hardness is well known, *Quercus ×fontanesii*, endemic to Sicily, has been studied in comparison with its parents, i.e. *Q. gussonei*, which is close to *Q. cerris*, and *Q. suber*. These taxa are here delimited as far as taxonomy, ecology and Sicilian distribution are concerned. Furthermore, the results of SEM microstructure analysis of leaves and xylem anatomy are illustrated and commented.

The analysis of the leaf microstructures shows that there is a remarkable similarity between the stomata and stellate trichomes of *Q. ×fontanesii* and both the parental species, as the trichome rays are numerically intermediate in the nothotaxon. Also xylem anatomy in *Q. ×fontanesii* results intermediate between both the parents. In particular, the qualitative analysis of the transverse sections on earlywood and latewood shows that the vessel distribution in *Q. ×fontanesii* gives origin to a structure with intermediate features between a diffuse porous wood (*Q. suber*) and a ring porous wood (*Q. gussonei*). The quantitative analysis, based on the maximum and minimum diameter, surface and shape factor, confirms the conclusion stated above. In particular, the minimum diameter of the wood vessels is the most significant parameter. The frequency distribution shows an increase in the number of vessels in *Q. ×fontanesii*, from earlywood to latewood, differently from the two parental species.

Introduction

Between the 19th century and the beginning of the 20th, the remarkable polymorphism of Sicilian deciduous oaks and their adaptation to different ecological conditions were stressed by the description of a high number of distinct taxa at specific, subspecific and varietal rank.

At present, literature attributes to most of them values ranging from simple individual forms to nothotaxonomic ranks. According to Pignatti (1982) 7 specific taxa occur in Sicily, while Greuter & al. (1986) and Schwarz (1993) report 10 (5 doubtful) and 8 taxa, respectively. Brullo & al. (1998-1999) record 9 deciduous species, re-evaluating some species described by Gussone (1843) in the 19th century.

Such discordance puts in evidence that several taxonomic problems are still to be settled. This, with particular regard to the *Q. pubescens* group, and in general within the whole genus *Quercus,* since it is very frequently involved in intensive interbreeding processes which make hard the delimitation of the taxa when only morphological and phenotypic characters are taken into account (Rushton 1993).

On the other hand, interspecific hybridization is even increased by the strong anthropic environmental modifications (Di Noto & al. 1998) that favoured the forest fragmentation and, consequently, increased the genetic dispersal. It is therefore advisable to find more and more distinctive characters.

With the purpose of contributing to this complex matter some years ago an investigation was started on some *Quercus* populations of uncertain hybrid status.

In this paper leaf micro-morphology and xylem structure are taken into account in order to clarify the relationship between *Q. ×fontanesii* Guss. and both *Q. gussonei* (Borzì) Brullo and *Q. suber* L., that are its postulated parents (Brullo & Marcenò 1985).

Taxonomy, ecology and distribution of these taxa are outlined as follows, leaving out *Q. suber* that is well known (Schwarz 1993, Pignatti 1982, Jalas & Suominen 1976).

Taxonomy, ecology and distribution of *Quercus ×fontanesii* and *Q. gussonei*

Taxonomy

- *Quercus fontanesii* Guss., Index Sem. Hort. Boccadifalco 1825: 10 (1825).

This taxon was originally distinguished from *Q. pseudosuber* G. Santi only by having deciduous leaves. Then Gussone (1843) provided additional characters of bark, branches, leaves and fruits from plants collected in the Ficuzza Wood, South of Palermo. Later on, plants known in Italy with the vernacular name of "sugherella minore" were referred to the same taxon by Bertoloni (1854). First Parlatore (1867) noted the hybrid nature of *Q. ×fontanesii* and included it within *Q. pseudosuber*, considering insignificant the winter loss of leaves. Subsequently, Strobl (1878), Tornabene (1887), Borzì (1911) Lojacono Pojero (1904) and also Fiori (1923) and Camus (1936-1938) treated *Q. ×fontanesii* as the hybrid between *Q. cerris* and *Q. suber*. More recently *Q. ×fontanesii* has been included by Pignatti (1982) within *Q. cerris* L., as a variety with dentate leaves, occurring in the area of Salerno (Campania, S Italy) and in Sicily while, according to Greuter & al. (1986), it is doubtful.

Brullo & Marcenò (1985), instead, regard it as a natural hybrid between *Quercus gussonei* (Borzì) Brullo and *Q. suber* L., morphologically correlated to *Q. ×crenata* Lam. and occurring in the Ficuzza Wood, as well as on the northern slope of Nebrodi Mts., where it is more frequent.

- *Quercus gussonei* (Borzì) Brullo in Brullo & Marcenò, Not. Fitosoc. 19 (1): 205 (1985).

Jalas & Suominen (1976), Schwarz (1993), Greuter & al. (1986), and Pignatti (1982) disregard this taxon that in the past was considered either as a distinct species (Gussone, 1843 sub *Q. haliphleos;* Lojacono, 1903) or included in *Q. cerris* L. at variety rank (Borzì 1911). According to Brullo & Marcenò (1985), *Q. gussonei* differs from *Q. cerris* by having bigger and much larger leaves as well as bulky acorns with bigger cupules. Ecology also differs in the two taxa.

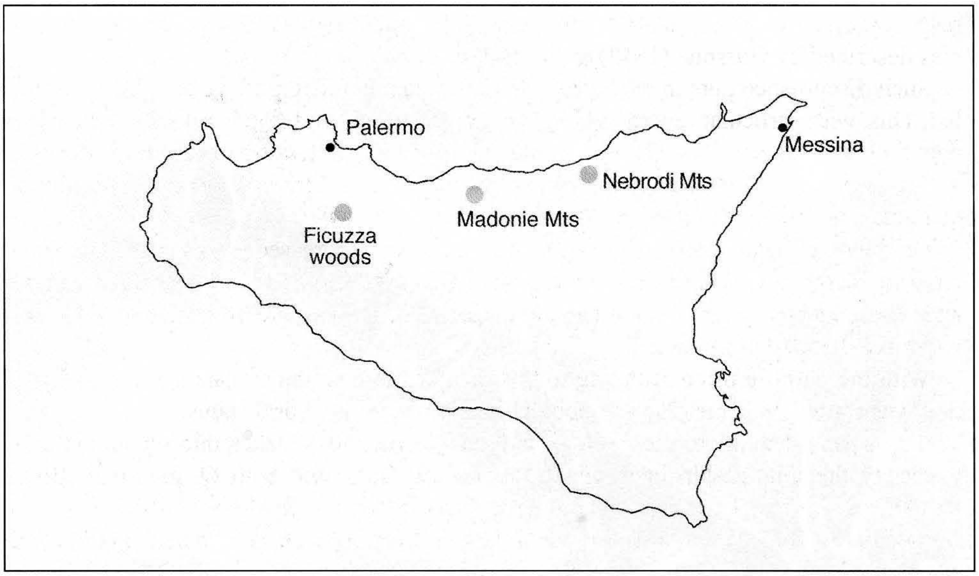


Fig. 1. Distribution of *Quercus ×fontanesii* and *Q. gussonei* in Sicily.

Ecology and distribution

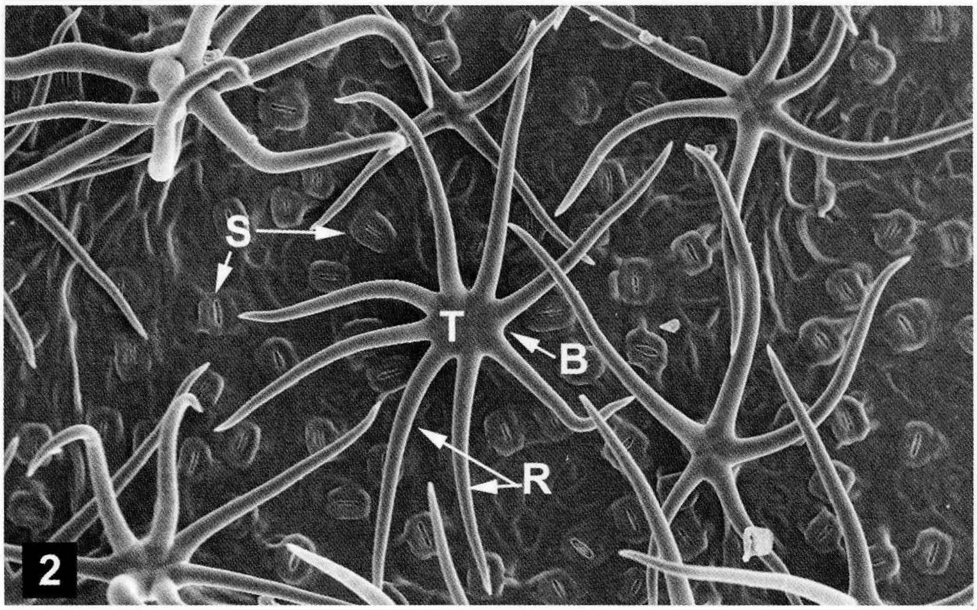
According to Brullo & Marcenò (1985) and Brullo & al. (1999) *Q. gussonei* is endemic to Sicily (Fig. 1). It occurs in the northern mountains, more frequent in the Nebrodi Mts. (NE Sicily) and in Ficuzza Wood (NW Sicily), rather rare in the Madonie Mts. (NC Sicily). In these areas it ranges between 300 and 900 m in altitude within the meso-mediterranean bioclimate with ombrotype tendentially sub-humid superior, mainly on subacid sandy soils evolved on flysch, schists and quartziferous sandstones. It is more thermophilous than *Q. cerris* which in Sicily occurs above 900 m a.s.l. *Q. gussonei* frequently forms more or less pure woods in the Nebrodi Mts., while in other localities it occurs in mixed woods together with evergreen (*Q. ilex* L., *Q. suber*) and deciduous oaks of the polymorphic group of *Q. pubescens* Willd. s.l. Such formations belong to *Quercetalia* and *Quercetea ilicis* Br.-Bl.

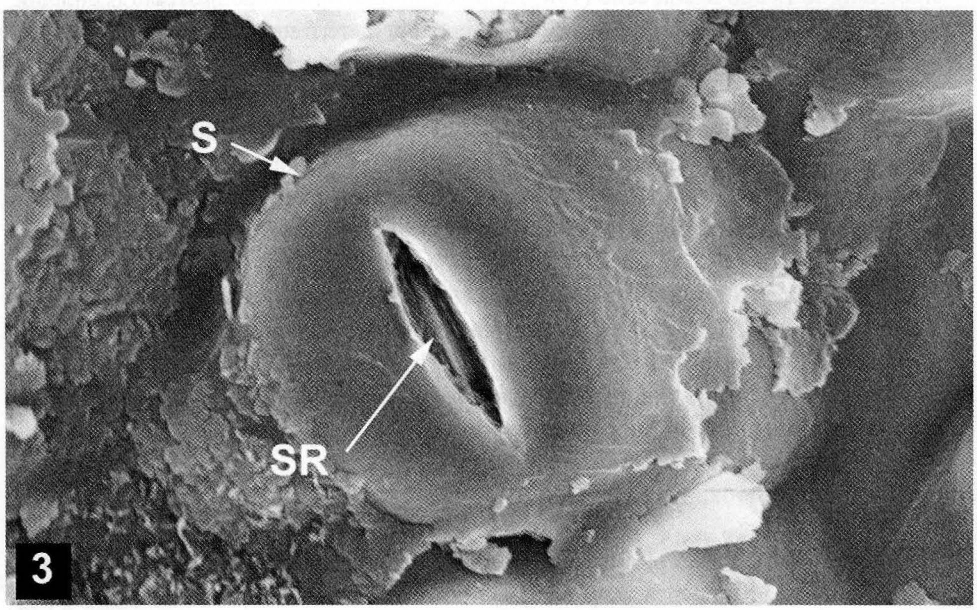
The distribution of *Q. ×fontanesii* is the same of its parent *Q. gussonei*, i.e. the Nebrodi and Madonie Mts. and the Ficuzza Wood as well. In these areas small groups or isolated individuals of the hybrid, some of them reaching more than 20 m in height, have always been recorded in presence of the parents.

Materials and methods

The research started in 1996. Firstly in *Q. ×fontanesii* the macroscopic characters of leaves, buds, fruits and branches were analysed on both herbarium specimens (PAL!) and living individuals in nature; in these latter samples the trunk bark was surveyed, too.

Later on, the micro-morphologic analysis of the leaf structure and the wood anatomy was carried out in both *Q. ×fontanesii* and its parents *Q. suber* and *Q. gussonei*.





Figs. 2-3 - Terms used to describe the main micromorphologic features of the lower leaf blade. Fig. 2 (*Quercus gussonei*): T = trichome; B = base; R = rays; S = stomata; Fig. 3 (*Q. ×fontanesii*): S = stomata; SR = stomatal rim.

Leaf microstructures

The investigation mainly deals with the qualitative parameters, because the quantitative ones are directly influenced by the micro-ecologic conditions existing in the growing sites (Gellini & al. 1992, Bussotti & Grossoni 1997).

The microstructures here considered are stomata, hairs of the leaf blade, waxes, type and arrangement of trichomes (Figs. 2-3).

For the terms used, reference is made to Hardin (1979) for trichomes and Safou & al. (1988) for stomata.

For each sample, leaf fragments, measuring 10-15 mm^2, were removed from the internerval zones of the blade. According to Huttunen & Laine (1983) methodology, the leaves were previously dried at room temperature. Fragments were set up on circular metal supports and subsequently gold-coated with an agar auto sputter coater. Observations were carried out with a SEM Leica S420 at 15 kV.

Micromorphological analysis of the wood

The analysis of wood tissues was carried out on 24 mature individuals located on the Nebrodi Mts. and in the Ficuzza Wood. Of these individuals, 12 belong to *Quercus ×fontanesii*, 6 to *Q. gussonei* and 6 to *Q. suber*.

The analysis was performed on radial cores, with a diameter of 5 mm, extracted by

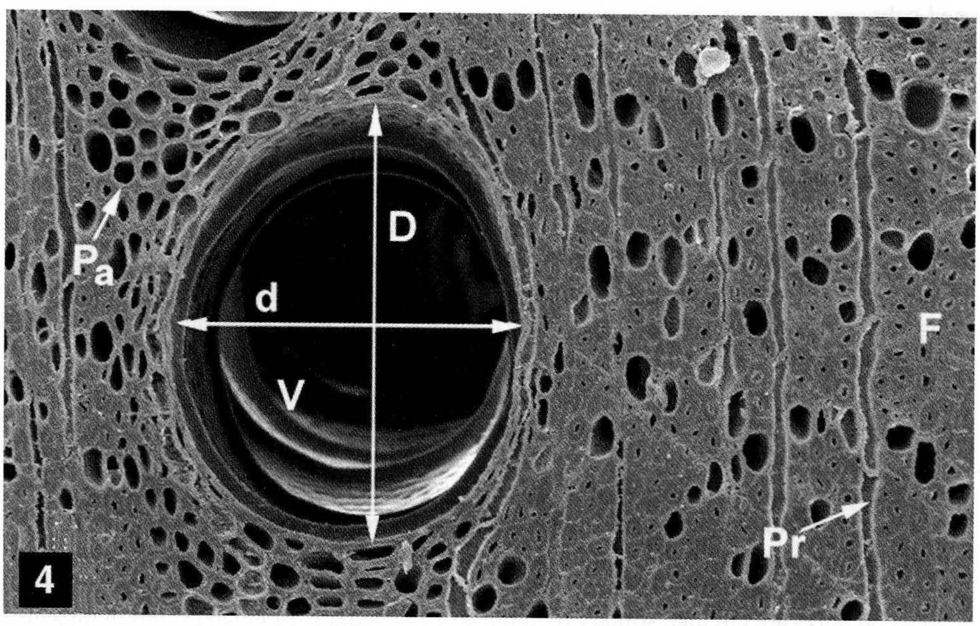


Fig. 4 - Main anatomical features of wood cross section. Symbols: V = Vessel; D = maximum diameter (radial); d = minimum diameter (tangential); Pr = radial parenchyma; Pa = axial parenchyma; F = fibres.

means of a Pressler corer, in order to damage the tree as less as possible. For cores extraction at BH, only straight trees were chosen in order to avoid the reaction (tension) wood.

On each core only the rings n° 20, 21, 22 (from the pith) were selected to avoid, as much as possible, the variation of anatomical characteristics pertaining to juvenile wood and climatic influence. The core portions, including selected growing years, were boiled in water, to soften wood and eliminate air bubbles, and cut to prepare the cross section for anatomical measures.

Each small cylinder was then set up on aluminium stabs and gold coated (Balzers SCD 004) and observed through a Scanning Electron Microscope (SEM Philips XL 20).

Since the most differentiating elements of the wood are the vessels, their dimensions and radial distribution along the growth layers, the anatomical analysis concerned just the cross sections of the samples.

Q. ×fontanesii is an hybrid between *Q. suber*, an evergreen species, and *Q. gussonei*, a deciduous species; *Q. suber* is a diffuse - porous species, while *Q. gussonei* is a typically ring - porous oak. The analysis of cross section could allow obtaining a clear differentiation between the two anatomical typologies.

Images obtained from SEM were then elaborated through an image analysis software (ANALYSIS 2.1), in order to measure on each ring and on each sample the following parameters: number of the vessels (a total number of 3.486 vessels were measured), maximum and minimum diameter of each vessel (Fig. 4), vessel surface and shape factor.

Data obtained from each growth ring were then elaborated through statistical software (SPSS 7.5) to point out the statistical reliability of the difference between the three examined oaks.

Results

Quercus ×fontanesii

- Leaf microstructures

SEM observations (Schicchi & al. 1998) show waxy "encrustations" of various shape in the lower blade and many stellate trichomes (Fig. 5), similar to those of *Q. suber* in the shape and the number of rays, which cover the stomata. Rays (8-14) are about 150-200 µm long and start from the external part of the trichome basis, whose central part is generally free and more or less concave. Stomata (Fig. 6) are raised, elliptical, their rima (23.2 ×17.19 µm) being elliptical and more or less similar to the parents. The upper leaf blade is sparsely covered by stellate trichomes about 4-8 rayed and by bulbose trichomes.

- Xylem microstructure

Transverse section is characterised by distinct growth ring boundaries, with a pore distribution from semi-ring to diffuse–porous (Fig. 11). These characteristics could be related to the persistence of the leaves: some individuals are evergreen, while others are quite deciduous. Earlywood pores are circular-oval, irregularly distributed, often in radial multiples. Latewood pores are circular, solitary or in more or less radially oriented groups, with a gradually diminishing diameter to the final wood. The transition from earlywood to latewood zones is gradual, i.e. pore size decreases gradually. Earlywood vessels of heartwood show thin walled tyloses. Libriform fibres are thick walled and disposed in regular patches.

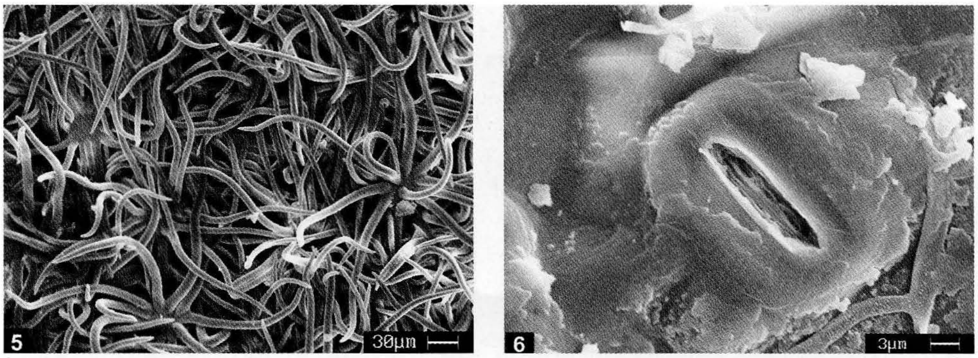


Fig. 5-6. *Quercus ×fontanesii*: trichomes and stomata.

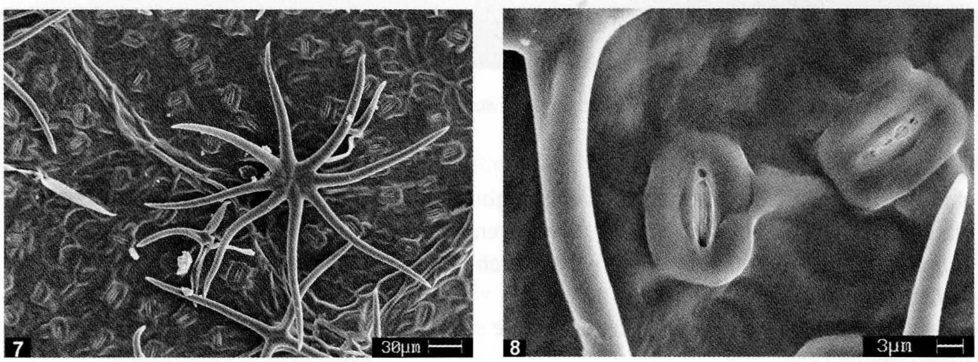


Fig. 7-8. *Quercus gussonei*: trichomes and stomata.

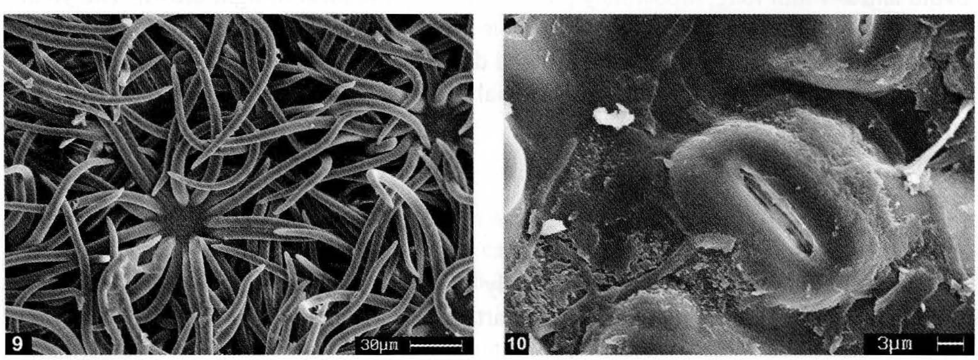


Fig. 9-10. *Quercus suber*: trichomes and stomata.

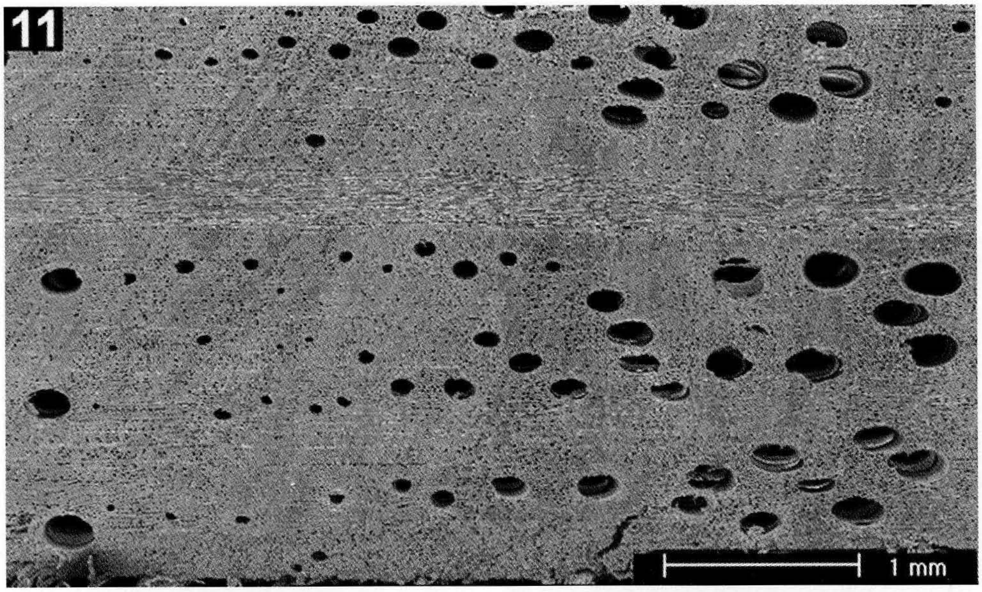


Fig. 11. *Quercus ×fontanesii*, transverse section: semi-ring porous distribution of earlywood pores.

Axial parenchyma is paratracheal discontinuous and apotracheal either diffuse or in uniseriate diagonal and tangential bands. Ray parenchyma is characterised by straight rays uni- to multiseriate, homogeneous; fibres are often mixed in radial ray tissues.

- Macromorphology

As the macromorphological characters are concerned, some individuals are almost evergreen, while others are quite deciduous. The leaves are polymorphic, varying both in size (4-15 × 2-6.5 cm) and in shape and showing a transition gradient between the two parental species. They are oblong, petiolate (5-16 mm), with coriaceous blade, rough in the upper part and pubescent in the lower one. The bark is suberose, grey-coloured when mature, and longitudinally deeply fissured, showing the reddish phellogen. Buds are globose-ovoid and 2-4 mm long, moderately pubescent, brown-reddish or light brown. The young branches are pubescent, brown-greyish; their section is roundish. The corn, oblong-ellipsoidal, is variable in length (25-40 mm) and diameter (15-26 mm). The cupule covers the fruit from 1/3 to 1/2 of its length, its proximal scales are curved-patent and the distal ones are straight or slightly curved.

Quercus gussonei

- Leaf microstructures

The lower blade is covered by small scaly waxes, together with several stellate trichomes (Fig. 7), similar to those of *Q. cerris*, partially hiding the stomata. The rays (4-9) start from a raised basis and are more or less flattened over the blade. Some bulbose trichomes are also present. The stomata (Fig. 8), raised, have more or less elliptic shape and rima,

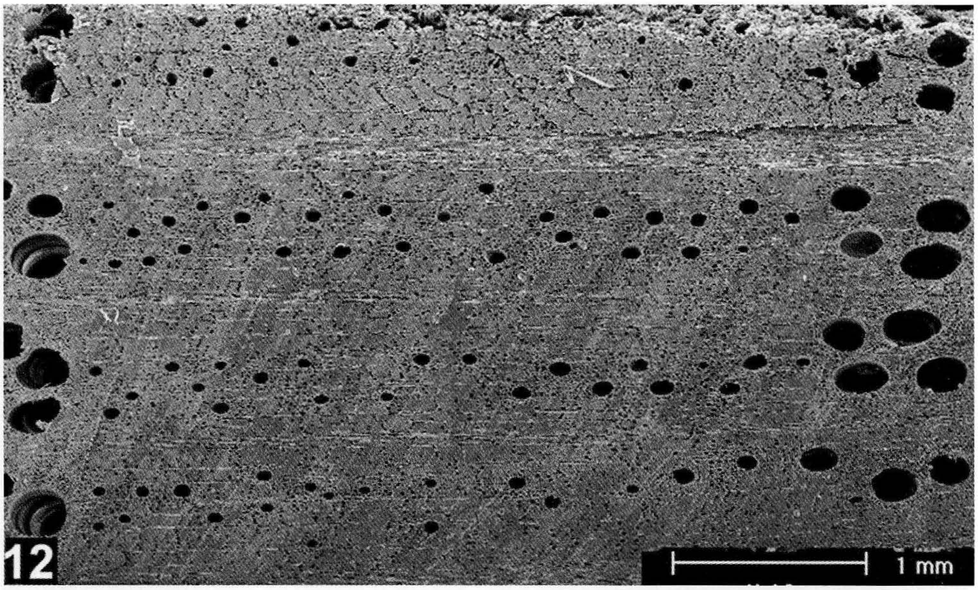


Fig. 12. *Quercus gussonei*, transverse section: ring porous distribution of earlywood pores.

although they are slightly crushed at the poles. The upper leaf blade has stellate trichomes about 4-8 rayed, mostly near the nervations.

- Xylem microstructures

The xylem structure in *Q. gussonei* is very similar to that of *Q. cerris*. The transverse section shows growth ring boundaries clearly distinct due to the ring-porous pore distribution (Fig. 12). The earlywood pores are circular-oval, with a wide lumen and make a ring with one or more rows of pores, more or less compact. Latewood pores are circular, with lower diameter and thicker walls, solitary or in radially oriented groups. In wide growth rings pore groups are radial to dendritic. Earlywood vessels of heartwood show thin walled tyloses. Libriform fibres are in regular, radial rows. Axial parenchyma is apotracheal either diffuse or in uniseriate diagonal and tangential bands, rarely paratracheal discontinuous. Broad rays are visible to the naked eye. Rays are from uni- to multiseriate, straight, homogeneous, sometimes with square cells in uniseriate rays. Sometimes fibres are mixed in radial ray tissue.

Quercus suber

- Leaf microstructures

According to Gellini & al. (1992) and Bussotti & Grossoni (1997), a dense tomentum covers the lower leaf blade so that stomata cannot be observed. Trichomes (Fig. 9) have 10-18 rays, grouped at their base, about 150-200 µm long. The stomata (Fig. 10), raised, have elliptical shape and rima. On the upper leaf blade, sporadic stellate and bulbose trichomes are present.

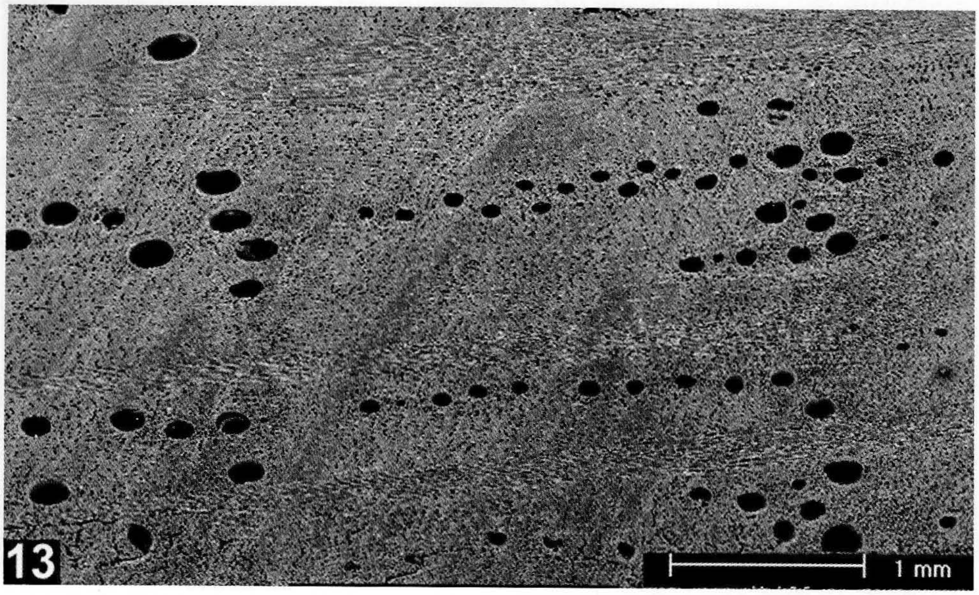


Fig. 13. *Quercus suber*, transverse section: diffuse porous distribution of earlywood pores.

- Xylem microstructures

The transverse section (Fig. 13) shows distinct growth ring boundaries. Pore distribution is diffuse to semi-ring porous. Earlywood pores are circular-oval, and show thin-walled tyloses in heartwood, the transition from earlywood to latewood zones is gradual, i.e. pore size gradually decreases, latewood pores are circular and thick-walled. Vascular tracheids are present. Fibres are very thick-walled. Axial parenchyma is abundant, apotracheal and paratracheal. Rays are uni- to multiseriate, homogeneous. Fibres are often mixed in radial ray tissue (Cambini1967, Giordano 1981, Schweingruber 1991).

Qualitative and quantitative comparison between xylem structures of *Q.* ×*fontanesii* and parental species (*Q. gussonei* and *Q. suber*)

The analysis of maximum diameter, minimum diameter, surface and shape factor of the vessels was performed in order to evaluate the distintictive anatomical characteristics between *Quercus* ×*fontanesii* and its parents *Q. gussonei* and *Q. suber*.

Results show, both for Nebrodi and Ficuzza provenances, that the most distinctive parameter is the vessel minimum diameter (in the tangential direction), while the maximum (in radial direction) varies depending on the individual growing speed. The other two parameters are less significant.

Fig. 14 shows the frequency distribution of the minimum diameters for the different diametric classes.

The distribution of *Q.* ×*fontanesii* is mostly intermediate between those of *Q. gussonei* and *Q. suber*, showing the most gradual transition pattern between the different diametric

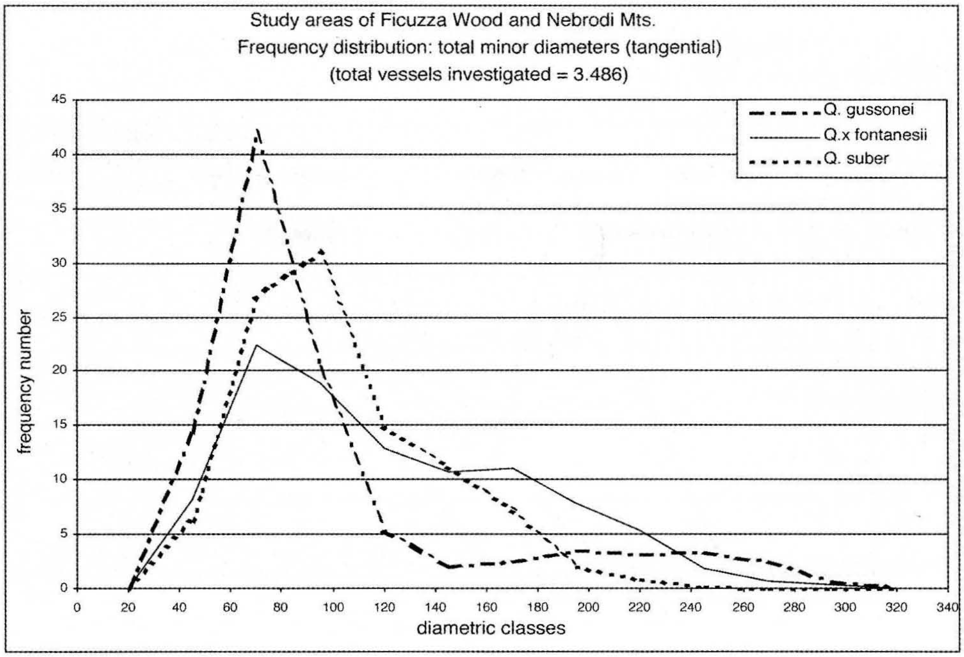


Fig. 14. Per cent frequency distribution of the minimum diameter (μ) of the vessels.

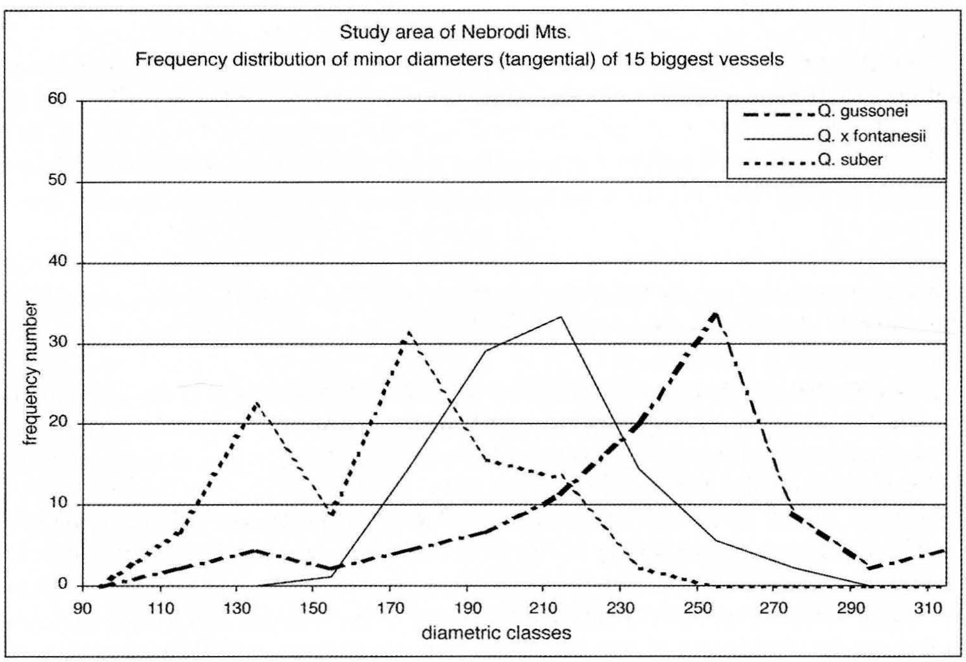


Fig. 15. Per cent frequency distribution of the minimum (tangential) diameters (μ) of the 15 biggest vessels (Nebrodi Mts.).

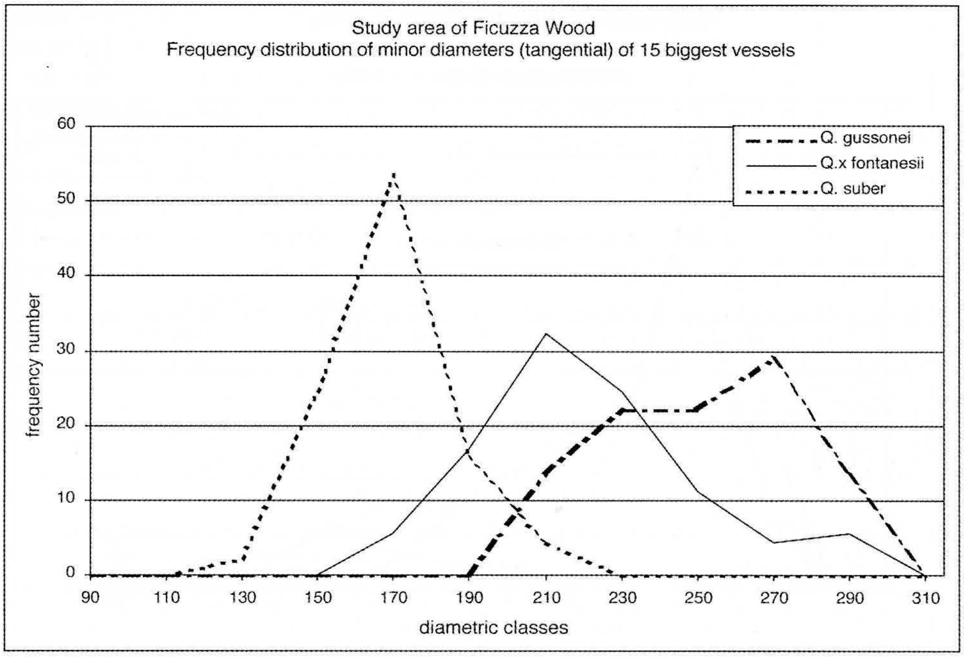


Fig. 16. Per cent frequency distribution of the minimum (tangential) diameters (μ) of the 15 biggest vessels (Ficuzza Wood).

classes. It is to be also underlined that the curve zones to be evaluated are the earlywood and the earlywood-latewood transition, while the latewood region is largely influenced by the growing speed.

Within the earlywood region, corresponding to the minimum vessel diameter ranging from 220 to 300 μ (Fig. 15), the frequency distribution of *Q.* ×*fontanesii* is distinctly intermediate.

Remarkable is the frequency distribution corresponding to the earlywood-latewood transition (diameters ranging from 140 to 220 μ), where *Q.* ×*fontanesii* shows an higher frequency of vessels, *Q. gussonei* clearly exhibits its ring-porous nature, and *Q. suber* its diffuse distribution of vessels.

These first results are better confirmed by the frequency distribution of the vessel minimum diameters corresponding to the earlywood regions. Analyses were performed on the minimum diameters of the 15 biggest vessels (the 5 biggest vessels from each growth ring) both from Nebrodi and Ficuzza provenances (Figs. 15-16).

From Figs. 15 and 16 we can also evaluate that the two provenances can have a significantly different growing speed.

The following tables (Tabs 1-4) show the descriptive values of minimum diameters of the 15 biggest vessels from each sample and the output of the Analysis of Variance (ANOVA) to evaluate the significance of zero hypothesis about the differences of the mean values of the three taxa, for the two provenances. The tables show that the highest significance is obtained by maximum diameters, mostly for the data from Ficuzza Wood, even if also for

Table 1. Descriptive statistic values for minimum diameters of the 15 biggest vessels – Nebrodi Mts.

Taxa	N	Mean	Standard deviation	Standard error	Confidence interval – 95 % of mean: Inferior limit	Confidence interval – 95 % of mean: Superior limit	Minimum	Maximum
Q. gussonei	45	160.2109	31.5764	4.7071	150.7243	169.6975	97.24	233.51
Q. ×fontanesii	90	198.8127	22.6119	2.3835	194.0767	203.5486	153.74	268.25
Q. suber	45	224.6416	42.8776	6.3918	211.7597	237.5234	112.63	305.11
All cases	180	195.6194	38.5136	2.8706	189.9548	201.2841	97.24	305.11

Table 2. ANOVA Nebrodi Mts.

	Sum of squares	Df	Mean of squares	F	Sig.
Between groups	95239.893	2	47619.947	49.502	.000
Within groups	170270.0	177	961.977		
Total	265509.9	179			

Table 3. Descriptive statistic values for minimum diameters of the 15 biggest vessels – Ficuzza Wood.

Taxa	N	Mean	Standard deviation	Standard error	Confidence interval – 95 % of mean: Inferior limit	Confidence interval – 95 % of mean: Superior limit	Minimum	Maximum
Q. gussonei	45	240.9609	24.3944	3.6365	233.6320	248.2898	201.50	289.76
Q. ×fontanesii	90	210.3557	28.4030	2.9939	204.4068	216.3046	165.08	283.94
Q. suber	45	160.9162	15.7029	2.3409	156.1985	165.6339	129.58	195.13
All cases	180	205.6471	37.8902	2.8242	200.0742	211.2201	129.58	289.76

Table 4. ANOVA Ficuzza Wood.

	Sum of squares	Df	Mean of squares	F	Sig.
Between groups	148151.5	2	74075.767	120.473	.000
Within groups	108832.4	177	614.872		
Total	256983.9	179			

Nebrodi Mts. the significance is very high.
Then the differences between *Q.* ×*fontanesii* and the two parental species can be evaluated from the analysis of the minimum diameters of the 15 biggest vessels.

Discussion and conclusions

The analysis of the leaf microstructures shows that the shape of the stomata and the stellate trichomes in *Q.* ×*fontanesii* is similar to the parental species. The high density of trichomes is more similar to *Q. suber*; while the number of the hybrid rays (8-13) is almost intermediate between *Q. gussonei* (4-9) and *Q. suber* (10-18). This investigation shows a remarkable affinity between *Q.* ×*fontanesii* and both the parental species. Nevertheless, it does not give meaningful distinctive characters from the taxonomic point of view, in agreement with Bussotti & Grossoni (1997) as far as subspecific and doubtful taxa are concerned.

The wood anatomy, carried out on the transversal sections, shows the intermediate features between the samples belonging to *Q.* ×*fontanesii* and those of the parental species.

The qualitative analysis of the transverse sections (Fig. 11) and the observation of the distribution type of the vessels on earlywood and latewood (Fig. 14), show that vessel distribution in the hybrids does not give origin to either a true diffuse porous wood (like in *Q. suber*), or a ring porous wood (like in *Q. gussonei*), but to a structure with intermediate features, instead. This structure, called "semi-ring porous" (Metcalfe & Chalk 1950, Woodcock 1989), shows a gradual variation in the vessel size along the growth ring. This could be explained taking into account that part of the leaves persist in *Q.* ×*fontanesii* for all the winter till the next renewal shoot. The quantitative analysis, based on the maximum and minimum diameter, surface and shape factor, confirmed the conclusion stated above. In particular, the minimum diameter of the wood vessels was the most significant parameter. The frequency distribution (Figs. 14-16) shows an increase in the number of vessels in *Q.* ×*fontanesii*, from earlywood to latewood, differently from the two parental species.

As regards the period of transition between earlywood and latewood, *Q.* ×*fontanesii* has more vessels than the parental species. In fact, *Q. gussonei* shows a drastic transition between spring and summer vessels, as evidenced by the typical ring porous structure. *Q. suber*, instead, being an evergreen species with diffuse porous wood, has a high quantity of minor vessels.

The results confirm the hybrid origin of *Q.* ×*fontanesii*, as already postulated by previous authors (Lojacono Pojero 1904, Fiori 1923, Camus 1936-1938, Brullo & Marcenò 1985).

One of the parents is *Q. suber* - occurring everywhere the hybrid grows - as the macro- and micromorphological data confirm. The other parental taxon is to be referred to *Q. gussonei*, a thermophilous deciduous oak close to *Q. cerris*. This latter species does not occur in the Ficuzza area, but grows in the mountain belt of Nebrodi Mts, between 900 and 1600 m *Q. cerris* and *Q. gussonei* mainly differ from the ecological and macromorphological point of view, while leaf microstructures and wood anatomy are quite similar. This suggests that the appropriate rank for *Q. gussonei* could be that of variety within *Q. cerris*, as stated by Borzì (1911).

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