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Bark anatomy of *Quercus cerris* L. var. *cerris* from Turkey

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Abstract: *Quercus cerris* L. var. *cerris* has a conspicuous bark which is generally thicker than that of other *Quercus* L. species in Turkey. The present study aimed to provide detailed anatomical characterisation of the bark of this species, which is necessary to assess its potential. The anatomical studies were conducted on the bark samples of nine 70- to 80-year-old trees growing in 3 sites of Andırın district from Kahramanmaraş province in Turkey. For microscopic observation transverse and longitudinal sections were prepared and individual specimens were taken for maceration. The bark is composed of phloem, periderm, and a very substantial rhytidome. The rhytidome has sequential periderms with phloem tissue between them, and includes compact nodules of sclerified tissues. The phellem has typical cork cells arranged regularly in radial rows and showing rings. The phelloderm is poorly developed. The phloem is layered regularly from cambium until the last formed periderm in successive tangential bands of fibres and groups of sclereids alternated with axial parenchyma and sieve tubes. Uniseriate phloem rays transverse the fibre groups, and fused phloem rays originate conspicuous broad rays. The dilatation growth showed large and conspicuous sclereids. Numerous crystals and druses in axial parenchyma cells were also observed. Full illustration of this species is given.

Key words: Phloem, periderm, rhytidome, *Quercus cerris*

Türkiye'deki *Quercus cerris* L. var. *cerris*'in kabuk anatomisi

Özet: *Quercus cerris* L. var. *cerris* Türkiye'deki diğer *Quercus* L. türlerinden genel olarak daha kalın olan belirgin bir kabuğa sahiptir. Bu çalışmada, bu kabuğun kullanım potansiyelini değerlendirmek üzere gerekli olan, kabuğun detaylı anatomik karakterizasyonunun sağlanması amaçlanmıştır. Anatomik çalışmalar Türkiye'de Kahramanmaraş'ın Andırın ilçesindeki üç ayrı bölgeden alınan 70-80 yaşlarındaki dokuz kabuk örneğinde gerçekleştirilmiştir. Mikroskobik gözlem için enine ve boyuna kesitler hazırlanmış ve bireysel örnekler maserasyon için ayrılmıştır. Kabuk, floem, periderm ve önemli miktarda ritidomdan oluşmaktadır. Ritidom aralarında floem dokusu bulunan sıralı peridermlere sahiptir ve sklereid hücrelerine dönüşmüş dokulardan oluşan kompakt nodüller içermektedir. Fellem, radyal sıralar halinde düzenli olarak sıralanmış karakteristik mantar hücrelerine sahiptir ve gelişim halkaları görülmektedir. Felloderm gelişimi zayıftır. Floem, kambiyumdan en son oluşan periderme kadar, ardışık teğet sıralı lif ve sklereid grupları ve bunlar ile yer değiştiren boyuna paranşim ve kalburlu borular ile düzenli olarak tabakalıdır. Tek sıralı floem ışınları lif grupları arasından geçmekte, birleşik floem ışınları da belirgin geniş ışınları oluşturmaktadır. Genişleme bölgesinde büyük ve belirgin sklereidler görülmektedir. Boyuna paranşim hücrelerinde aynı zamanda çok sayıda kristal ve druzlar gözlenmiştir. Bu türün tüm tanım resimleri sunulmaktadır.

Anahtar sözcükler: Floem, periderm, ritidom, *Quercus cerris*

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Introduction

Quercus L. is one of the most important woody genera in the northern hemisphere, namely in North America, Europe, and especially in Eastern Asia, where the highest diversity can be found with about 250 species (Özcan, 2007).

In Turkey, *Quercus* species have a natural distribution of about 6.5 million ha area including many subspecies, varieties, and natural hybrids (Özcan & Bayçu, 2005). Hedge and Yaltırık (1982), who classified the *Quercus* species existing in Turkey, considered a total number of 18 species, which was a reduction from the previously accepted 35 *Quercus* species. However, nomenclatural and typification problems are still unresolved (Borazan & Babaç, 2003), because widespread hybridisation and introgression have much obscured specific limits (Hedge & Yaltırık, 1982).

Turkey oak (*Quercus cerris* L.) grows naturally from central and south-eastern Europe to Asia Minor. In Turkey, except in the eastern and north-eastern areas, it grows in all the regions with 2 varieties, *Q. cerris* var. *cerris* and *Q. cerris* var. *austriaca* (Wild.) Louden (Yaltırık, 1984). The distribution area for *Q. cerris* var. *cerris* is unknown after its new classification (previously *Q. cerris* var. *pseudocerris*) (Hedge & Yaltırık, 1982; Borazan & Babaç, 2003). In the Andırın district the distribution area of *Q. cerris* var. *cerris* is about 235,000 ha (Mıhçıoğlu, 1942).

Q. cerris especially *Q. cerris* var. *cerris* in the southern region of Anatolia has a conspicuous bark which is generally thicker than that of other *Quercus* species in Turkey. Morphological and anatomical studies were recently conducted to compare endemic Turkish species (Cabi, 2010; Güvenç & Duman, 2010).

The bark of *Q. cerris* var. *cerris* contains in its rhytidome substantial, albeit not continuous, regions of cork tissue that are clearly visible to the naked eye. Because of this and due to cork products shortage, *Q. cerris* bark was used in Turkey as an alternative to cork from *Q. suber* L. for production of agglomerates for insulation during World War II (Mıhçıoğlu, 1942), and later for production of bottle stoppers.

In general, the bark anatomy of *Q. cerris* has received little attention. The bark of *Q. cerris* var. *cerris* was briefly studied in the beginning of the 20th century and compared with *Q. suber*, leading to reports that it was of inferior quality (Mıhçıoğlu, 1942; Telgeren, 1976). Babos (1979a) also analysed the bark structure of *Q. cerris* var. *cerris* and compared it with *Q. cerris* var. *austriaca* from Hungary. However, such studies remained insufficient to enable a realistic evaluation of *Q. cerris* cork properties and potential uses. Overall there is a limited number of bark anatomical studies in other *Quercus* species (e.g. Howard, 1977; Trockenbrodt, 1991, 1994, 1995a, 1995b). An exception is the knowledge and the research effort made on *Q. suber* bark, which constitutes a model for cork in barks (e.g. Natividade, 1950; Pereira et al., 1987, 1992; Costa et al., 2002; Graça & Pereira, 2004). Recently, Pereira (2007) compiled the available knowledge on cork of this species in a review book.

This study presents a detailed analysis on the structure and anatomical characteristics of *Q. cerris* var. *cerris* bark from Turkey. This characterisation of *Q. cerris* var. *cerris* bark is a first step within the knowledge development that is necessary to assess its potential exploitation and products.

Materials and methods

The anatomical studies were conducted on the barks of 9 *Quercus cerris* var. *cerris* trees, growing in 3 sites of the Andırın district from Kahramanmaraş province in Turkey, with an altitude of 1000 m, 662.2 mm annual rainfall, and 16.5 °C mean temperature: Tiril (37°35′49″N, 36°18′32″E), Okçu (37°43′48″N, 36°22′1″E) and Armutkuyusu (37°32′1″N, 36°16′7″E). Three trees were randomly selected in each site, with the following age and mean diameter at breast height: 75 years and 45 cm in Tiril, 70 years and 35 cm in Okçu, and 80 years and 40 cm in Armutkuyusu.

The bark samples were collected at 1.30 m of height from the main trunks of *Q. cerris* var. *cerris* trees. The samples were impregnated with DP 1500 polyethylene glycol and transverse and longitudinal microscopic sections of approximately 17 μm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval (Quilhó et al., 1999). The sections were stained with malachite green and hematoxylin, as well as with a triple staining of chrysodine/acridine red and astra blue. The sections were mounted on

glycerine Kaiser and after 24 h drying, the lamellas were submerged in xylol for 30 min to remove the Tesafilm adhesive, dehydrated on alcohol 96% and alcohol 100%, and mounted on Eukitt. Sudan 4 was used for selective staining of suberin.

Individual specimens were taken sequentially from the cambium towards the periphery and macerated in a solution of 30% H_2O_2 and CH_3COOH 1:1 at 60 °C for 48 h and stained with astra blue.

Light microscopic observations were made using Leica DM LA and photomicrographs were taken with a Nikon Microphot-FXA.

The terminology follows mainly Trockenbrodt (1990) and Richter et al. (1996).

Results and discussion

All the *Q. cerris* var. *cerris* trees that were analysed revealed a similar bark structure and the description here is therefore integrative. The bark is thick (3-7 cm) with a brown greyish colour, hard to the touch, and longitudinally furrowed with short deep furrows as in other *Quercus* species (Withmore, 1962; Howard, 1977). This results from the formation and growth of several periderms, the spatial development of phellem, and the amount of tissues cut off by each periderm (Junika, 1994).

The bark is composed of phloem and a very substantial rhytidome that can be clearly distinguished in transverse sections of the stem (Figure 1).

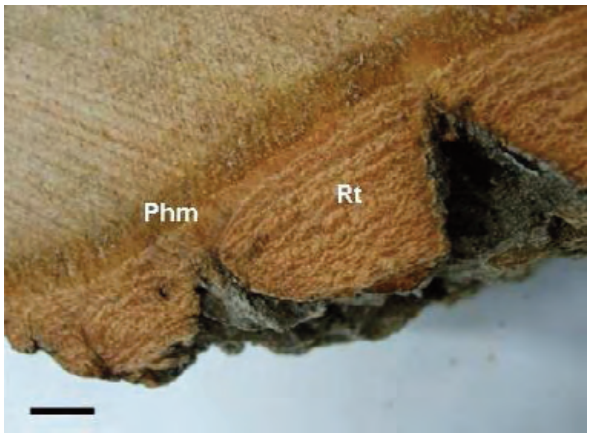

Figure 1. Phloem (Phm) and rhytidome (Rt) of *Quercus cerris* var. *cerris*. Scale bar = 25 mm.

Rhytidome and periderms

The rhytidome of *Q. cerris* var. *cerris* (Figure 2a, b) consists of various sequential periderms (3 to 4) with dead phloem tissue between them (Figure 2a). Taking into account the tree age of 70-80 years, we estimate a phellogen lifespan in *Q. cerris* var. *cerris* of about 25 years in accordance with a rhytidome formation at 30 years of age (Babos, 1979a) or the 25-35 years reported for oaks (Roth, 1981; Trockenbrodt, 1994).

No shedding of the outermost periderms of the rhytidome was observed. Therefore thickness of rhytidome increased with tree age and can provide tree protection, namely against extreme temperatures and fire (Roth, 1981; Dickison, 2000). The rhytidome shows a dark coloration resulting from abundant deposits of materials (Figure 2b).

The periderms of *Q. cerris* are very conspicuous due to the presence of prominent phellem layers (Figure 2a) that are clearly identified macroscopically as cork tissues.

The phellem layer curved slightly, forming discontinuous arching layers in cross section (Figure 2a). The phellogen is composed of rectangular and thin-walled cells in transverse section but is difficult to distinguish in the periderm. Figure 3a shows the differentiation of phellogen cells by tangential cell division. A narrow phelloderm formed by 2 or 3 cell layers of rectangular to round cells is developed, sometimes with thickened walls (Figure 3b) that often cannot be distinguished from the neighbouring parenchyma cells unless by their evident radial alignment. Roth (1981) stated that a very thick cork usually excludes a thick phelloderm and vice versa.

Rings were visible in the phellem layer in each periderm, which included 2-5 growth rings and each ring was composed of 6-12 layers of phellem cells with a more or less radial alignment without intercellular voids. The phellem cells are suberised and have thin walls with a uniform thickness in the tangential and radial walls, and sometimes are radially flattened. At the beginning of a growth ring the phellem cells are larger and have thinner walls in contrast to the narrow and thicker walled phellem cells of the end of the growth ring, as also occur in *Q. suber* (Pereira et al., 1987). However, both species differ markedly on the intensity of phellem growth. In *Q. suber* each

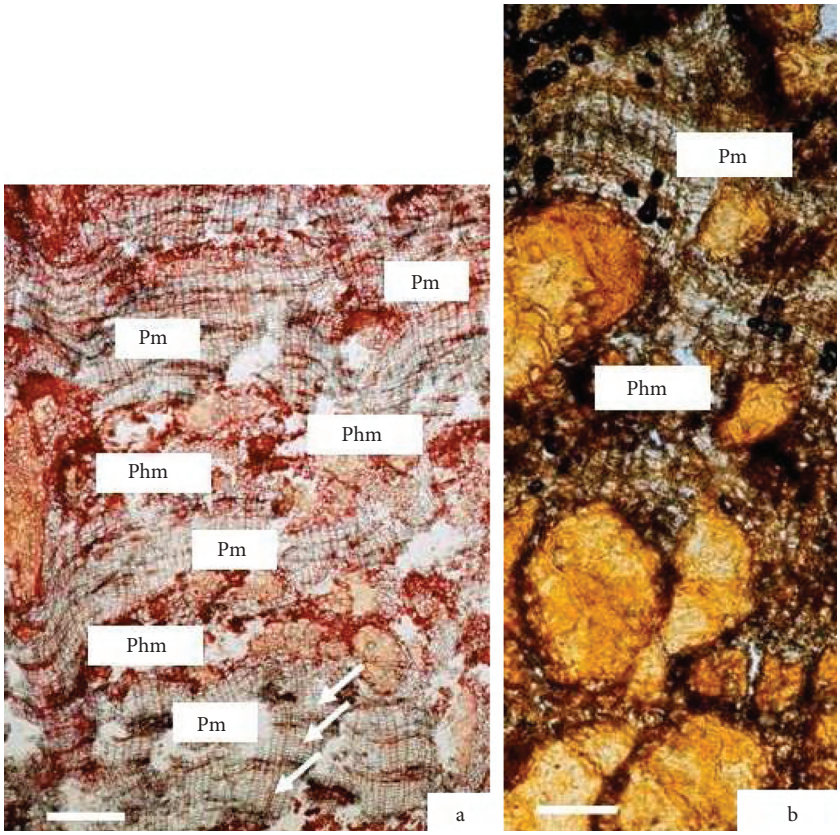

Figure 2. Transverse section of rhytidome of *Quercus cerris* var. *cerris*. a - Various sequential periderms conspicuous due to the presence of wide phellem layers (Pm) with phloem tissue (Phm) between them. Phellem with growth rings (arrows). b - phellem layers (Pm); phloem tissue (Phm). Scale bars; a = 125 µm; b = 50 µm.

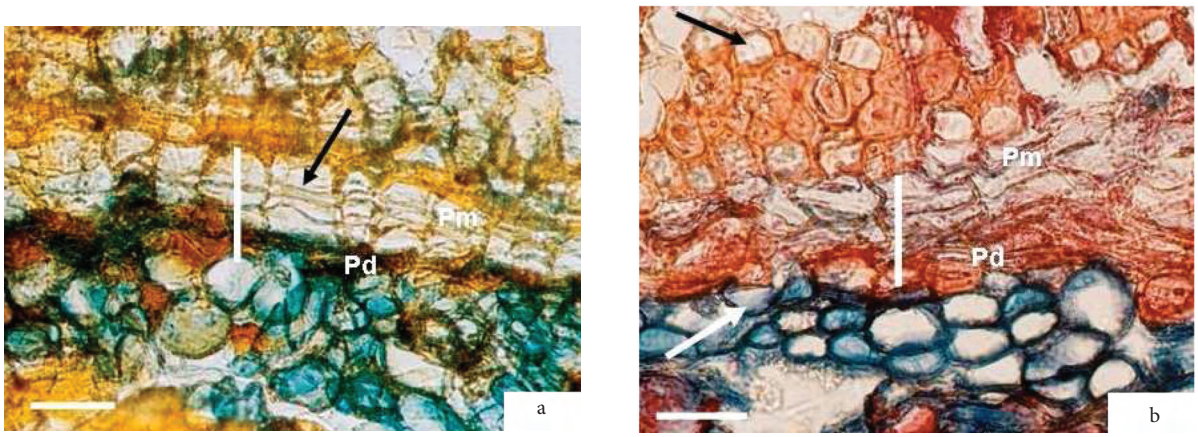

Figure 3. Transverse section of bark of *Q. cerris* var. *cerris*. Vertical bar showing one periderm with phellem (Pm) and phelloderm cells (Pd). a - differentiation of phellogen cells, tangential divisions (black arrow). b - Sc = sieve tubes and phloem parenchyma cells (white arrow). F = fibres and crystals (black arrow). Scale bars; a and b = 25 µm.

phellogen mother cell produces annually about 10-20 phellem cells in young plants (Graça & Pereira, 2004) and many more (up to about 100 cells) in mature trees (Pereira et al., 1992). In *Q. cerris* var. *cerris* the phellogen meristematic activity was much smaller with production in 1 ring of only 6-12 phellem cells in each radial row. The variation in phellem ring width that was found in our samples may be related with external conditions in parallel to what has been reported for *Q. suber* cork, where drought or temperature, or both, can limit cork growth (Costa et al., 2002).

A few (1-2 layers) of the phellem cells in the limit of each growth ring could thicken up to heavily lignified cells. Figure 4 shows lignified phellem cells. This feature was not noted in *Q. suber* cork although lignified thickened cells in phellem are observed in other species, i.e. *Eucalyptus globulus* Labill. (Quilhó et al., 1999) and various tropical barks (Roth, 1981).

Dark stained material was observed in the phellem cells of all the trees (Figure 2b), presumably phenolic compounds. Alonso and Machado (2008) also reported phenolic compounds in phellem cells in tropical species.

The occurrence of lenticular channels crossing radially the periderm was rare, and observed only in one sample of *Q. cerris* var. *cerris* and without filling material (Figure 5). This clearly differs from *Q. suber* cork, where lenticular channels are numerous, variable in number and size, and a key feature for the visual appreciation and quality classification of cork (Pereira, 2007). Their ontogeny was studied by Graça and Pereira (2004). The fact that the cork layers in *Q. cerris* var. *cerris* do not build a continuous cylindrical envelop around the tree stem, as is the case in *Q. suber* where cork makes up a tight impermeable coating, may be the reason why gas exchange between the living tissues underneath and the exterior does not require such a channel system.

In many cases, and especially near the last formed periderm, it was possible to recognise the regular and organised structure of the phloem, i.e. stratified tangential bands of fibres, sclereids, and broad phloem rays.

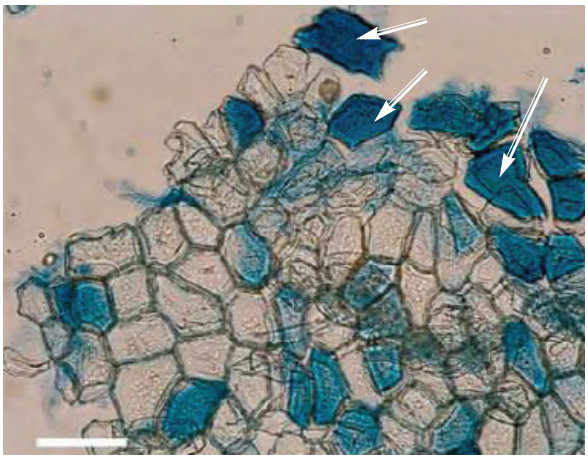

Figure 4. Thick-walled and heavily lignified phellem cells in macerated bark of *Q. cerris* var. *cerris* (arrows). Scale bar = 25 µm.

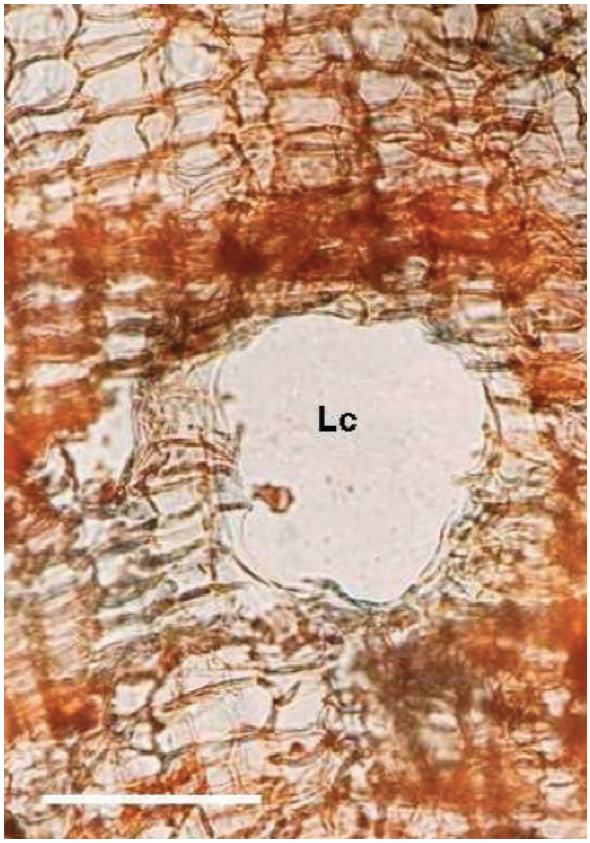

Figure 5. Tangential section of bark of *Q. cerris* var. *cerris*. a - lenticular channel (Lc) without filling material in the phellem of one periderm. Scale bar = 25 µm.

Phloem

The phloem included the non-collapsed phloem and the collapsed phloem layered regularly from the vascular cambium towards the periphery with growth rings marked by non lignified cells (Figure 6). Babos (1979a) referred to the occurrence of phloem rings in *Q. cerris* var. *cerris* and *Q. cerris* var. *austriaca*. Growth rings were also observed in the phloem of *Q. robur* by Trockenbrodt (1991).

At the beginning of each growth ring sieve tubes and axial parenchyma were generally formed followed by tangential bands of fibres and groups of sclereids, while at the end of the ring only a narrow layer of axial parenchyma was formed. According to Barlow and Luck (2006), the tangential banding of the different cell types within the phloem indicates synchrony of cellular development.

Therefore, the phloem of *Q. cerris* var. *cerris* is characterised in the transverse section by the occurrence of successive tangential bands of fibres and groups of sclereids arranged parallel to the cambium, and alternated with axial parenchyma and sieve tubes until the last formed periderm. Uniseriate phloem rays transverse the fibre groups (Figure 6) and fused phloem rays were observed forming conspicuous broad rays (Figure 7).

This structure remained more or less unaltered towards the periderm, mainly in the non-collapsed phloem, which is responsible for the active conduction and represents only a narrow band close to the vascular cambium as in other *Quercus* spp., i.e. *Q. velutina*, *Q. rubra*, *Q. alba*, and *Q. coccinia* (Howard, 1977).

The collapsed phloem in *Q. cerris* var. *cerris* starts not far from the cambium, and shows collapsed sieve tube cells that ceased their conductive function and a disorganised tissue arrangement, as also described in

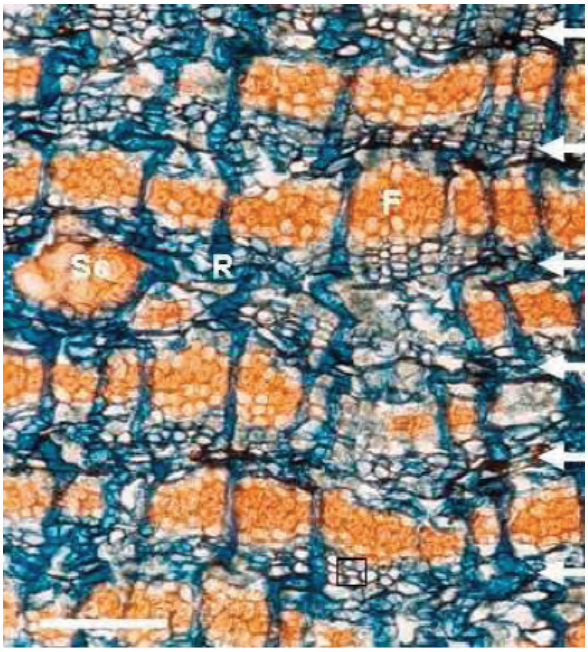

Figure 6. Transverse section of phloem of *Q. cerris* var. *cerris*. Growth rings marked by non lignified cells (arrows). Successive tangential bands of fibres (F) and groups of sclereids (Sc) arranged parallel to the cambium, alternated with axial parenchyma and sieve tubes (¤). Uniseriate phloem rays (R) undulated with moderate dilatation. Scale bar = 50 µm.

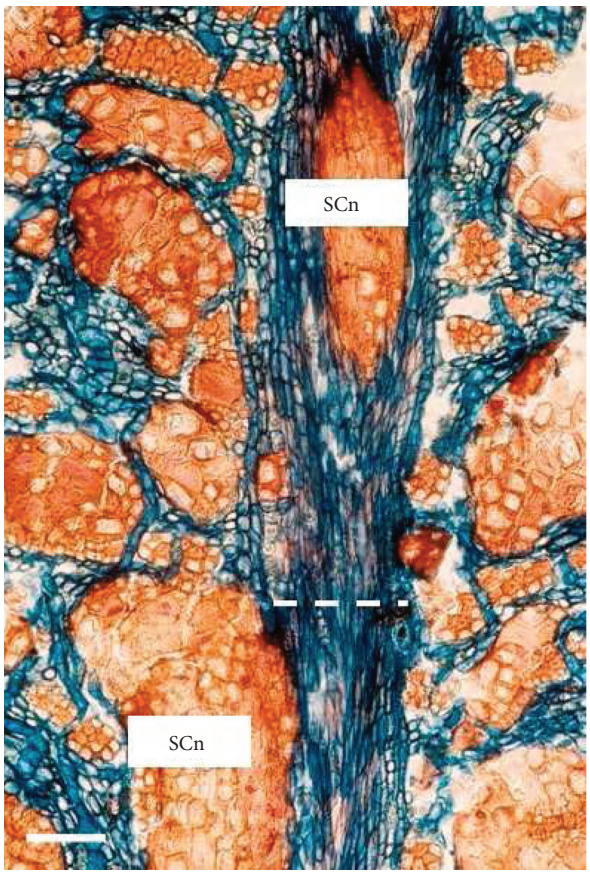

Figure 7. Transverse section of bark of *Q. cerris* var. *cerris*. Fused phloem rays (—). Sclerification of radial parenchyma cells, formation of nodules of sclereids (SCn) within and near the broad rays. Scale bar = 50 µm.

other *Quercus* spp. (Howard, 1977; Trockenbrodt, 1991). A slight distortion of rays was observed that was accompanied by dilatation tissue resulting from expanded axial and radial parenchyma cells and subsequent sclerification. The sclereids form prominent nodules near the fibres and the wide rays. Such structural alterations in the phloem are a consequence of stem radial growth (Quilhó et al., 1999) as described in the genus *Quercus* by Whitmore (1962), Howard (1977), and Trockenbrodt (1991).

Nevertheless, a structural pattern is recognised in the collapsed phloem of *Q. cerris* var. *cerris* with parallel bands of sclerified tissues, i.e. secondary fibres, gross nodules of cluster sclereids, and broad rays with ray sclerified cells, probably providing mechanical support of the tissue. The occurrence of sclerenchyma as a mechanical barrier against collapse of living cells was mentioned in the phloem of *Q. suber* (Quilhó et al., 2003) and in tropical species (Machado et al., 2005).

Sieve tubes, companion cells and sieve plates are of the same type of those described for *Quercus* spp. (Howard, 1977). Sieve tubes with companion cells are solitary or in groups of 2-3 elements with a tangential arrangement. The companion cells are difficult to recognise in transverse and longitudinal sections. The sieve elements have a round to irregular shape in transverse section and the cell walls are thin and unlignified, and may frequently be confused with axial parenchyma. Sieve plates are inclined, compound, and scalariform with 4-8 sieve areas per plate (Figure 8) and numerous sieve pores; several lateral sieve areas are also present.

Fibres are arranged parallel to the vascular cambium in continuous tangential bands, about 3 to 4 cells wide, sometimes interrupted by groups of sclereids (Figure 6). The fibres are slender with tapered overlapping end with narrow lumens (Figure 9a), occasionally forked. They are usually thick walled, lignified, accompanied by chambered and crystalliferous parenchyma cells of approximately equal length at their inner and outer sides (Figure 9b).

The fibre morphology and arrangement are in accordance with observations by Babos (1979a, b) while various fibre arrangements occur in other *Quercus* spp. i.e. fairly continuous, regular, tangential layers of fibres in *Q. robur,* scattered sparse clusters in *Q. encleisocarpa* (Whitmore, 1962) or fibres in small groups in widely spaced, discontinuous tangential bands (Howard, 1977).

Axial parenchyma cells are round to rectangular in transverse section (Figure 3b). They have thin walls and round pit fields and occur near the sieve elements making tangential bands of 2-3 cells in width. In the outer portion of the phloem close to the periderm, these cells proliferated and expanded forming the tissue of dilatation growth.

Prismatic crystals occur profusely in chambered parenchyma cells as strands of up to 10 cells near the fibres (Figure 9b). The strands of crystal-bearing parenchyma along the margins of the fibre band were also described by Howard (1977) and Trockenbrodt (1991) and illustrated in *Q. suber* by Quilhó et al. (2003). The abundance of druses and polygonal crystals (Figure 10), probably of calcium oxalate, found in the phloem of *Q. cerris* var. *cerris* is also found in the bark of oaks as a by product of metabolism (Howard, 1977; Trockenbrodt, 1991, 1995a, 1995b) although depending on environment and plant development (Marcati & Angyalossi, 2005).

Uniseriate rays (Figure 11a) are about 3 cells high, but frequently up to 10 cells, with procumbent cells (Figure 11b). Rays are undulated at the beginning of

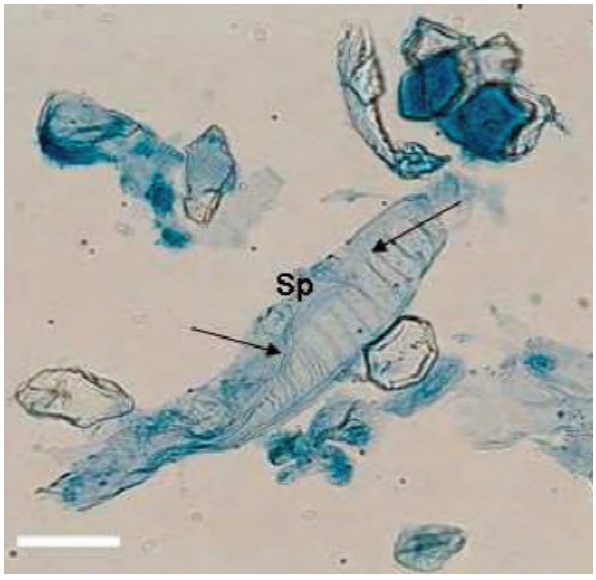

Figure 8. Sieve element of *Q. cerris* var. *cerris* with sieve plates (Sp) and sieve areas (arrow). Scale bar = 25 μm.

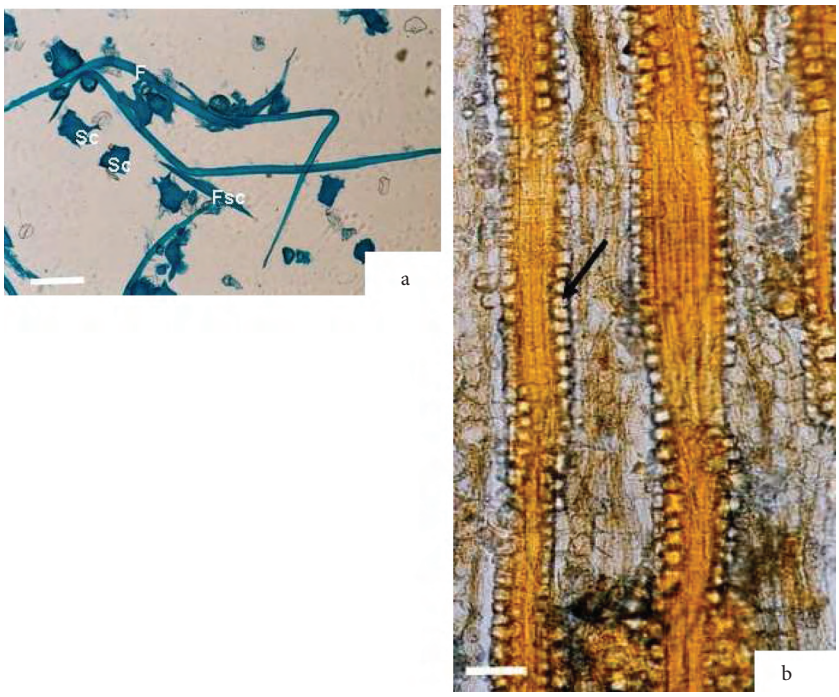

Figure 9. Bark of *Q. cerris* var. *cerris* bark. a - fibres (F) fibrosclereid (Fsc) and sclereids (Sc) in maceration. b - radial section of phloem with fibres (F) accompanied by chambered and crystalliferous parenchyma cells (arrow). Scale bars; a and b = 50 μm.

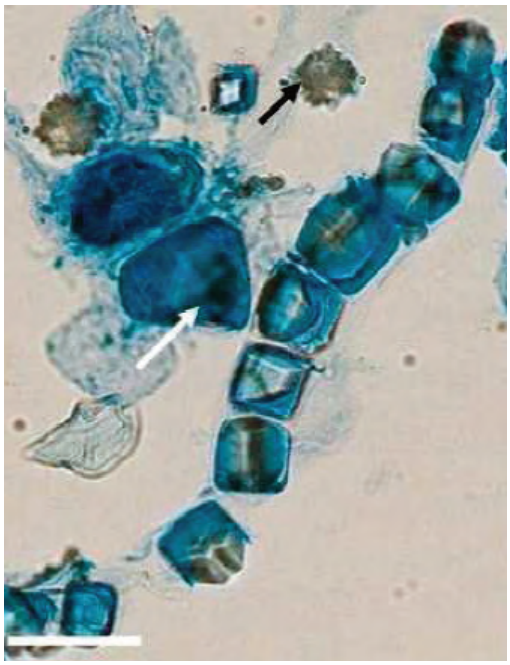

Figure 10. Maceration of *Q. cerris* var. *cerris* bark; crystalliferous parenchyma cells (arrow); druses (black arrow) and a sclerified cell filled with a crystal (white arrow). Scale bar = 125 μm.

the growth ring and show a moderate dilatation (Figure 6) towards the periderm due mainly to the tangential expansion and sometimes division of their cells. In the outer phloem close to the periderm, the ray dilatation tissue may be confused with axial parenchyma cells. Some cells develop thick, lignified secondary walls (Figure 11c).

Sclerification of radial parenchyma cells occurred near the cambium and groups of sclereids accompanied the broad rays (Figure 7) forming radial groups of sclereids (e.g. Figure 12). This was described by Babos (1979a) in his work on *Q. cerris* var. *cerris* as rays with a so-called "palm" formation. The sclerification of phloem ray cells is also present in *Q. suber* (Graça & Pereira, 2004) and in other *Quercus* spp. (Howard, 1977).

Sclereids (Sc) are abundant and occur mostly in clusters. A high proportion of sclereids was observed in all the bark samples. In general sclereids are isodiamteric (Figure 9a), although they attain various shapes and sizes with thickened and polylamellate walls transversed by minute pit channels. They

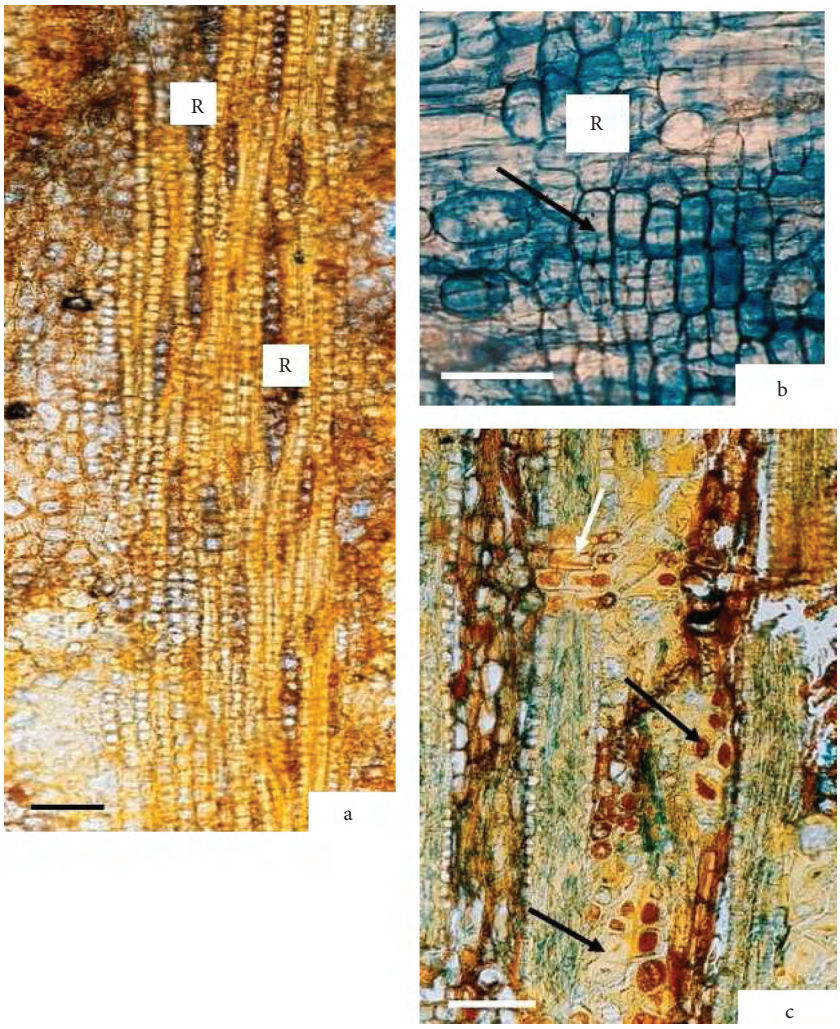

Figure 11. Rays in bark of *Quercus cerris* var. *cerris*. a - Tangential section of secondary phloem; uniseriate rays (R). b - Radial section of secondary phloem; ray (R) with procumbent cells (arrow). c - Sclerified radial parenchyma cells (white arrow) and sclerified axial parenchyma cells (black arrow) with phenolic compounds. Scale bars; a and c = 50 µm; b = 25 µm.

frequently include large prismatic crystals (Figure 10), and phenolic compounds (Figure 11c). Groups of sclereids sometimes form prominent nodules with large tangential or radial diameters. In the transverse section, nodules of clustered sclereids have a tangential or radial arrangement: they are adjacent to the fibre groups, or form radial bands near or even within the broad rays (Figures 7, 12). Fibre sclereids (Fs) are similar to the fibres but shorter and were only distinguish in macerated bark (Figure 9a).

Sclereids originate from axial and radial parenchyma, which gradually enlarge and thicken their radial and tangential cell walls and compact masses of sclereids are clearly visible on cut surfaces of *Quercus* barks (Trockenbrodt, 1991; Howard, 1977; Graça & Pereira, 2004). These cells give bark rigidity and brittleness but cause substantial problems during processing (Roth, 1981) and are one of the causes for the reported inferior quality of *Q. cerris* var. *cerris* bark in comparison with *Q. suber* (Mıhçıoğlu, 1942).

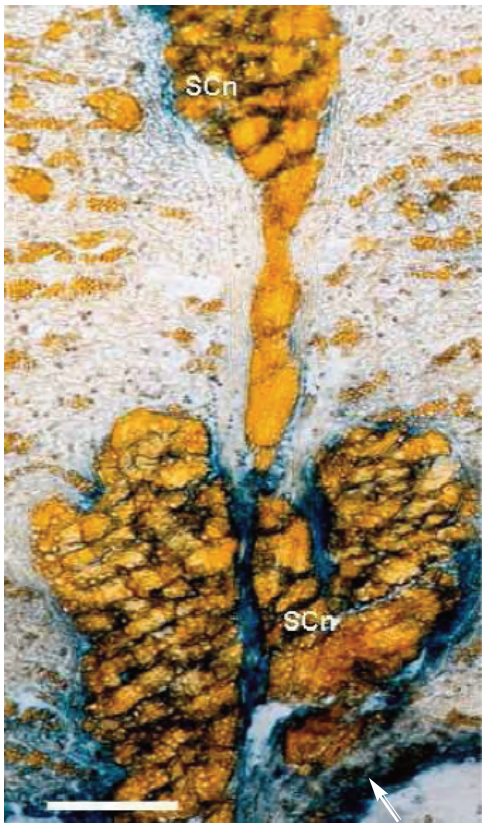

Figure 12. Nodules of sclereids (SCn) in bark of *Q. cerris* var. *cerris*. Transverse section of secondary phloem near the vascular cambium. A narrow band close to the vascular cambium, responsible for the active conduction (arrow). Scale bars = 125 μm.

Conclusions

The findings in this study enhance our understanding of *Q. cerris* var. *cerris* bark and show that the tissue structural complexity is a key feature that has to be taken into account when envisaging bark uses. A careful fractioning of the non-phellemic tissue will be a requirement whenever cork is the targeted component to be valued. The presence of numerous sclereids and crystal inclusions is an important characteristic that lowers the quality of *Q. cerris* var. *cerris* cork and calls for an adequate processing design.

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