Cork oak wood: a hard material to sample and to measure

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Abstract

Different studies within the Suberwood project required measurements of wood growth in cork oak trees (*Quercus suber* L.), including tree ring analysis, as a basis to analyse the influence on tree growth of stress effects, namely those deriving from climatic conditions (i.e. water deficit) or from the cork extraction. The research partners have experienced serious difficulties in these tasks and some examples are presented and discussed.

The first difficulty regards sampling because cork oak harvesting is forbidden in Portugal and advantage has to be taken from legally authorised tree fellings. The available material therefore consists mainly in thinning material (young trees), dead or diseased trees, and trees with damaged stems due to improper cork harvesting, which are useless for such studies. In most cases the information concerning the stand management is lacking, namely regarding the time of cork extraction and of other silvicultural operations (i.e. pruning). Therefore the experimental design regarding treatments and repetitions often cannot follow the conventional statistical guidelines.

The cork oak wood also has anatomical and structural features that bring considerable complexity for ring definition. The wood has a semi-ring porosity with poor growth ring definition and it is very difficult, and frequently impossible, to accurately identify growth rings and to link measurements to a certain year. Additionally cork extraction disturbs wood growth and anatomy.

Different observation approaches were tested: overall microscopical observations, continuous radial measurement of vessel dimensions and density, continuous radial microdensitometric profiles, cambial wounding. The use of non-destructive samples (i.e. cores or small wood samples) adds to the uncertainty encountered. Stem discs offer a better observation but differences among different radii are often very large.

Keywords: *Quercus suber* L., sampling, growth rings, anatomy, wood growth

Introduction

In any study involving tree sampling, finding the adequate trees to harvest is always a key aspect both in number and in quality. In the case of cork oak this aspect is of particular importance since cork oak harvesting is forbidden by law. This was one risky aspect in the implementation of the Suberwood project, or of any other project involving the procurement of cork oak trees for wood evaluation and industrial processing, since legal restrictions to harvest mature and healthy cork oak trees are strictly enforced.

Besides of the difficulties arising from legal restrictions, cork oak wood has several specific characteristics that make its study a difficult task. Wood growth is very hard to quantify because of poor growth ring definition and cork harvesting disturbs the wood anatomy. Therefore the following of conventional experimental design and statistical guidelines are not possible in studies of mature cork oaks under cork production.

For the wood growth observation, different approaches were tested: overall microscopical observations, continuous radial measurement of vessel dimensions, continuous radial microdensitometric profiles, cambial wounding. Due to the uniqueness of the tree and its wood, all the approaches offered several difficulties and required special care. As these were a large component of our results, it seemed useful and important to report and discuss them so that these particularities and difficulties can be taken into account in future work.

This paper is a review of the difficulties encountered by the research partners on different studies on cork oak. A more detailed description of each of these studies is given in the corresponding specific papers presented at this conference.

Sampling limitations

The first limitation to sampling comes from the fact that cork oak harvesting is forbidden in Portugal. Tree harvesting is allowed only when the trees are dead or diseased, and need to be removed; for thinning purposes; or when there is any construction of public interest, for example roads or dams, that crosses a cork oak stand. In these cases, the entity interested in the harvesting has to apply for a legal authorisation. Legal authorities will verify in the field the conformity of the information given and decide if the request is accepted. In order to get cork oak wood for our study we had to take advantage of these authorised fellings.

Second limitation comes from the quality of the available material. Dead and unhealthy trees or thinning material are useless for our studies and most of the other trees have damaged stems due to improper cork harvesting (Fig. 1).

Figure 1. Examples of stem damages resulting from improper cork harvesting.

Third limitation is the lack of information concerning the stand management. The owners do not know, in most cases, how old are the trees, and when and how often cork was harvested. Age estimation (with sufficient accuracy) by ring counting is practically impossible due to the frequent poor growth ring definition.

A constant survey for wood material was maintained by keeping in contact with the legal authorities, providing harvesting authorisations, and with cork oak owners. Also due to the legal restrictions, the selection of sites and number of trees to sample was very limited and made according to what was available. Several potential sampling sites were visited but did not offer trees with the appropriate characteristics (e.g. healthy trees). In the end the procurement of trees for wood evaluation could be performed with success. However due to the particular characteristics of the cork oak stands and to the reasons for felling authorisation, it was never possible to follow a classical experimental design (e.g. with control plots, different treatments, repetitions).

Non-destructive sampling, like tree coring, has been tried out but proved to be inadequate for this species, besides that cores are extremely difficult to extract due to the wood's high hardness (Gourlay and Pereira 1998). It is therefore necessary to have wood discs. Cork oak wood has an irregular anatomy with large radial parenchyma and a radial disposition of vessels that does not really follow a straight radial line from pith to bark. These aspects are only visible in wood discs.

Measurements on the wood

Cork oak wood shows a semi-ring-porosity with solitary vessels, in general with a circular cross-section and arranged in a radial pattern (Fig. 2). Ring growth definition is usually better in young trees, during the first 10-15 years of age, becoming very poor onwards. Accurate identification of growth rings is, therefore, often impossible, which does not allow to link measurements to a certain calendar year.

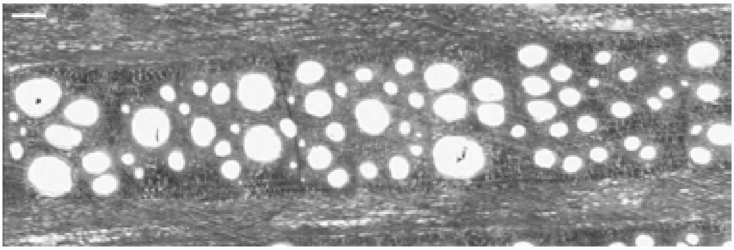

Figure 2. Cork oak wood transverse section observed under low power microscope and showing semi-ring porosity with poor growth ring definition. Vessels were highlighted with white wax.

Cork harvesting disturbs wood growth and anatomy adding extra difficulties to the identification of rings boundaries. Wood growth is reduced during the first and second years following cork removal and wood porosity becomes confusing and ring definition less clear (Leal and Pereira 2008).

Accidental wounding of the cork cambium during cork removal causes the formation of callus areas in the wood tissue, making it even harder to identify regular wood growth (Fig. 3). If the wounding did not occur in recent years the disturbance will not be visible on the tree stem, which may result in sampling inappropriate trees. Moreover, if cork has been extracted a long time ago this is also not recognisable without felling the tree (González-Adrados and Gourlay 1998).

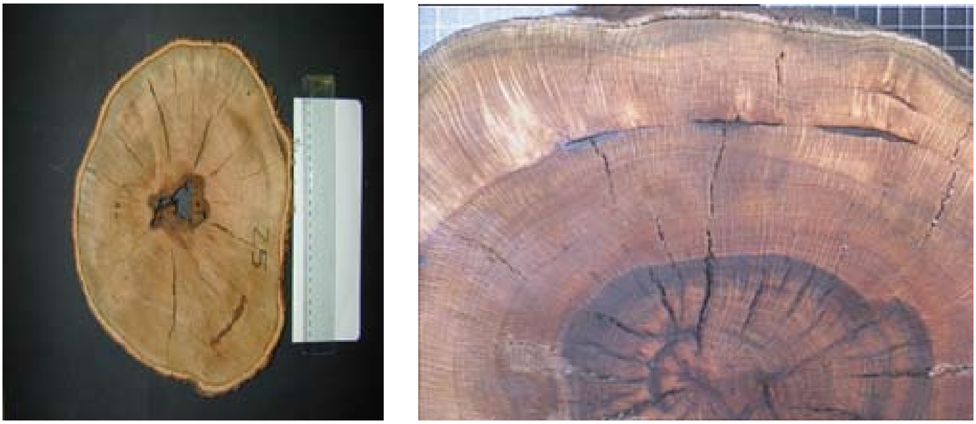

Figure 3. Stem discs showing internal scars resulting from accidental wounding during cork harvesting.

It has been possible to measure and crossdate cork oak wood rings in discs from a set of 30 young trees never submitted to cork harvesting (Nunes 1996). Although young trees present a better ring definition, it was only possible to use 14 out of the 30 trees. Our several preliminary attempts to measure and crossdate tree rings in mature trees have not been successful. The attempts made to identify rings on the wood cross-section (without and with magnification) do not have sufficient confidence of accuracy and ring growth differs markedly between trees and different radii of a same tree. Tree discs proved to be preferable to cores because of the large growth differences encountered within a tree. Analysis using larger sample sizes with trees growing under homogeneous conditions is desirable. We keep a constant survey for such trees.

Vessels were measured continuously along the radial direction, on fine sanded transverse surfaces, using image analysis (Leal *et al.* 2008). A good surface preparation is essential for the observation of wood vessels limits. Fine sanding with a P400 grid followed by impregnation of pores with white wax, in order to highlight the vessels, proved to be an adequate surface treatment. The natural radial orientation of vessels, in transverse surfaces does not follow a straight radial line.

Radial samples were X-rayed perpendicularly to the transverse section and their image scanned for microdensitometric analysis (Louzada *et al.* 2008). Radial profiles of variation in wood vessels size and in density (Fig. 4) did not contribute significantly to improvements in the identification of ring boundaries. The measurements could not be analysed in terms of their annual variation.

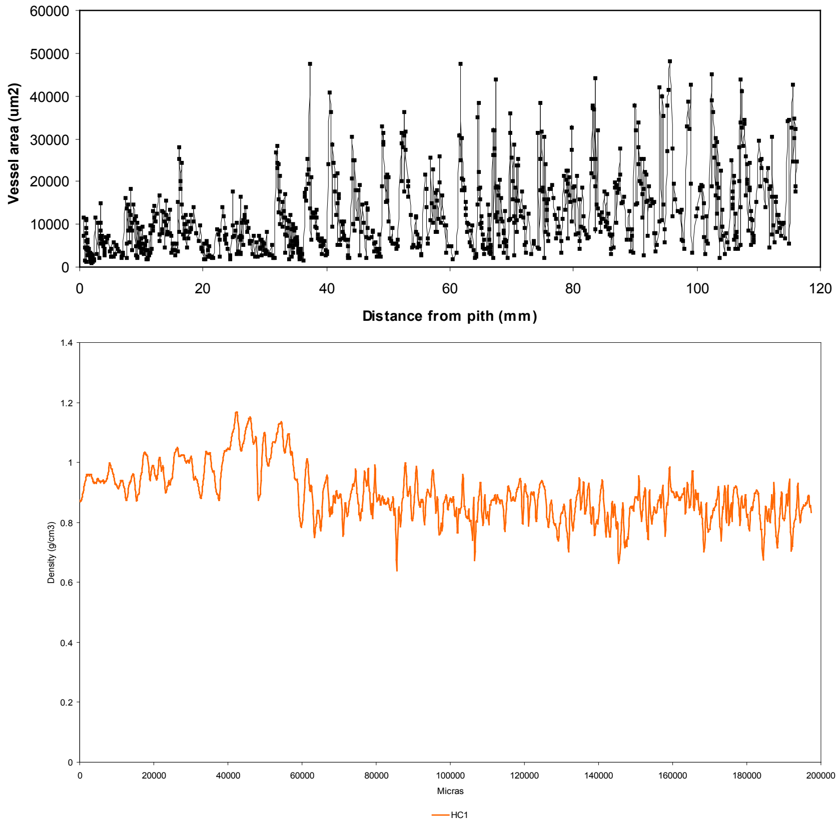

Figure 4. Radial profiles of variation in wood vessel area (top) and microdensity (bottom), for two wood radius.

Density and vessel measurements should be complemented with visual inspection of the samples. Ring boundaries identification through the observation of vessel size or density peaks offers doubts. The radial variations in vessel size and density are very gradual and differ markedly depending on the radius taken for the measurements. Other peaks, not corresponding to ring boundaries, may also appear.

Measurements on the living tree

The outer bark, or periderm, of a tree is formed by a cambium, the phelogene. The phelogene divides, with periclinal and anticlinal divisions, to the outside originating suber and to the inside forming pheloderm (Graça and Pereira 2004). The periderm is rich in suberin, a natural polymer that provides isolation from the outer environment. Some characteristics of the cork oak phelogene make it unique. Usually the phelogene dies after a variable number of years but the same does not happen for the cork oak. Unless the cork is harvested, the same phelogene maintains its activity throughout the tree's life. The phelogene activity is much more intense in cork oak than in other species, originating a large amount of suber, the cork, in a comparatively short time.

When the phelogene is in its peak of activity, between May and July, there is one layer of very fragile adjacent cells. During this time of the year, the cork can be easily harvested by separating it through these cells. Every cork removal results in the death of the phelogene and in the formation of a new one, a traumatic phelogene, therefore allowing a sustained cork production during the tree's lifetime.

The first cork extraction is performed when the cork oak tree has around 25-30 years. Each subsequent extraction can be performed with a minimum of nine years of difference. This is a legally imposed period considered to be enough for tree recovery and for the formation of cork with a thickness that allows the production of stoppers.

Radial growth of trees under full cork production is being followed with band dendrometers in a trial with 40 trees. Measurements of tree diameter were collected every month during one cycle of cork production, from 1991 to 2000 (Costa *et al* 2002) and continue to be collected, after cork harvesting, during another cycle, from 2000 to 2009. The main problem of this methodology is how to distinguish cork growth from wood growth, since the radial increase is the sum of both increments. Moreover, wood and cork growths develop differently along one cycle of cork production: cork shows higher growth rates during the first 3 years while wood growth is reduced during the first and second years.

To address this question, two point dendrometers per tree, one at 1.0 m height and the other at 1.6 m height, were placed in 10 cork oak trees to register continuously in high time resolution changes in radial dimensions, since April 2002 (Spiecker and Duncker 2005). The dendrometers at 1.0 m are measuring the radial increment of wood and cork. The dendrometers in 1.6 m on the other hand are measuring just the radial increment of the wood. Therefore, the cork and a part of the phellogene have been removed (a schematic illustration can be seen in Fig. 5.), using a milling cutter. The challenge was to destroy the phellogen without destroying the cambium. In two other sites in Huelva and Málaga (Spain) with remarcable differences in site conditions, a similar experimental disposition was used. In May

2003 and in two trees per site, two electronic point dendrometers were attached to each tree, one over cork and the other over cambium, trying to separate phellogen and cambium activity (Vázquez *et al.*, 2008). However, the regeneration of a traumatic phellogen within the phloem and the begin of its meristematic activity producing cork cells, again make measurements a combination of wood and cork growth.

Cambial wounding was done once per month during 15 months, using 1 mm needles on a subset of four of these trees, in order to further trace intra-annual tree ring development by microscopical analysis on cross sections (González-Pérez, pers. com.). Microcores were in the end extracted including the incision zone, polished on a diamond fly-cutter and observed under a high resolution microscope (Fig. 6). Observations made in wood discs proved to be more accurate than in cores. Surface treatment using the diamond fly-cutter allows a better visualisation of the wood cells and brings the possibility to observe larger areas than histological cuts. Microtome cuts with sufficient quality and size are hard to obtain.

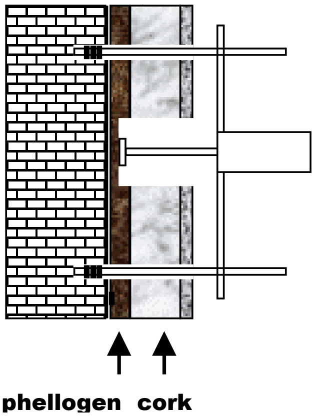

Figure 5. Schematic illustration of the insertion of a pointer dendrometer in the trunk at 1.6 m

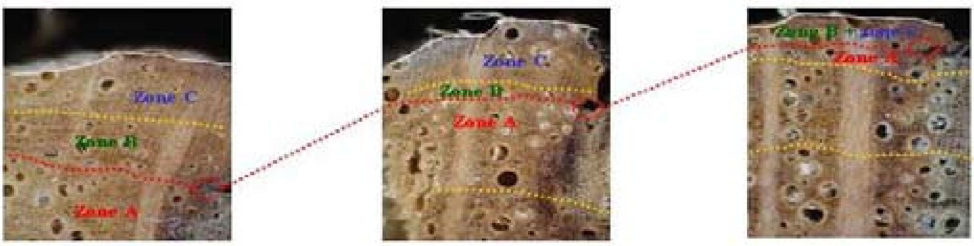

Figure 6. Wood growth observed in samples resulting from cambial wounding. Red line, callus line produced by the needle; yellow line, growth stops. Zone A: growth comprised between the last growth stop before needle insertion and the moment of insertion; Zone B: growth comprised between the moment of needle insertion and the next growth stop; Zone C: growth comprised between the last growth stop and sample extraction.

Several difficulties were encountered:

- It was not possible to identify the beginning of the callus formation on the level of single cells and, therefore, the increment after the wounding could only be measured in width.

- Cork removal induces cell disorganisation resulting in a non-homogeneous distribution of pore sizes, making it harder to distinguish early- from latewood.

- Annual radial growth is very low adding difficulty to the quantification of growth rates in shorter time periods.

Conclusions

Measuring cork oak wood growth offered several difficulties, with all the methods used;

Identification of ring boundaries is very difficult, especially after cork begins to be extracted. Dating of rings or linking measurements to a certain year is, therefore, often dubious, especially with long time-series;

Preliminary attempts to crossdate ring growth in mature trees have failed; however, for young trees, it was possible to crossdate tree rings and to relate growth to climate. Better results may be achieved with a larger and homogeneous sampling, which we were not yet able to obtain;

Ring analysis and analysis of samples resulting from cambial wounding are more accurate when done in wood discs. This brings extra difficulties because of the serious limitations concerning wood sampling.

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