INSTITUTO
SUPERIOR DE
AGRONOMIA
Universidade de Lisboa

Hinc Patriam Sustinet

CORK OAK AND CLIMATE CHANGE: INFLUENCE OF DROUGHT ON CORK GROWTH

AND CHEMICAL COMPOSITION

CARLA DO NASCIMENTO SANTOS LEITE

SCIENTIFIC ADVISORS: PhD Helena Margarida Nunes Pereira.

PhD Isabel Maria Silva Sanches de Miranda

PhD Vanda Cristina Paiva Tavares de Oliveira

THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
FORESTRY ENGINEERING AND NATURAL RESOURCES

2020

U LISBOA | UNIVERSIDADE DE LISBOA

CORK OAK AND CLIMATE CHANGE: INFLUENCE OF DROUGHT ON CORK GROWTH AND CHEMICAL

COMPOSITION

CARLA DO NASCIMENTO SANTOS LEITE

SCIENTIFIC ADVISORS: PhD Helena Margarida Nunes Pereira.

PhD Isabel Maria Silva Sanches de Miranda

PhD Vanda Cristina Paiva Tavares de Oliveira

THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN

FORESTRY ENGINEERING AND NATURAL RESOURCES

Jury:

President: Doutora Maria Margarida Branco de Brito Tavares Tomé, Professora Catedrática do Instituto Superior de Agronomia da Universidade de Lisboa.

Members: Doutor Francisco Javier Vázquez Piqué, Professor Titular da Universidade de Huelva, Espanha;

Doutora Helena Margarida Nunes Pereira, Professora Catedrática Jubilada do Instituto Superior de Agronomia da Universidade de Lisboa;

Doutora Maria Helena Reis de Noronha Ribeiro de Almeida, Professora Associada Aposentada do Instituto Superior de Agronomia da Universidade de Lisboa;

Doutora Maria Cristina Amaral Penas Nabais dos Santos, Professora Auxiliar da Faculdade de Ciências e Tecnologia da Universidade de Coimbra;

Doutora Carla Maria Alexandre Pinheiro, Professora Auxiliar Convidada da Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa.

This page intentionally left blank

Climate scenarios in the Mediterranean predict raising temperatures and more frequent and extreme drought. We focused on analysing the effect of drought on cork growth and chemical composition to evaluate if more frequent and severe forthcoming droughts will compromise the performance of cork as a sealant for wine bottles.

A few species can produce cork in a significant proportion. We initiated this research on gathering the available information to put cork oak and its cork under this general panorama. We concluded that the cork from *Quercus suber* is presently the only raw-material with the characteristics necessary for production of solid cork products and focused our research on it.

To study the response of cork-growth to drought and the effect of phellogen age we used a dendroecological methodology and a components resilience analysis, confirming that drought reduces growth and provided extra knowledge on this subject: cork oak is very tolerant and resilient to extreme droughts but more severe droughts correspond to higher decrease of growth and more trees affected although to greater recovery performance. Nevertheless, there are other factors involved in the response: site, tree and the age of the phellogen.

Regarding chemical composition the research developed brings insights into the effect of drought on the proportion of its main components. Our results show that, in general, drought does not affect it, with some exceptions. Nevertheless, the variability associated to the tree is much more relevant than the effect of drought conditions and affects all the parameters analyzed.

In practical terms, the potential increased occurrence of droughts arising from climatic changes will not compromise the performance of cork as a sealant for wine bottles but cork debarking rotations should be enlarged in order to have the necessary cork plank width, namely if the drought occurs in the first 2 years.

Keywords: *Quercus suber;* cork, dendroecology; chemical composition; Mediterranean climate change mitigation.

Os cenários climáticos preveem, para o Mediterrâneo, um aumento da temperatura e secas mais frequentes e extremas. A nossa análise incidiu no efeito da seca no crescimento e na composição química da cortiça, no sentido de avaliar se as secas irão comprometer o desempenho da cortiça enquanto vedante de garrafas de vinho.

Apenas um conjunto restrito de espécies produz cortiça em quantidades significativas. Começámos a nossa investigação recolhendo a informação disponível por forma a colocar o sobreiro e a sua cortiça no panorama geral, concluindo que a cortiça de *Quercus suber* é no momento a única matéria-prima com as características necessárias para a produção de produtos de cortiça sólida e focámos a nossa investigação nela.

Para estudar a resposta do crescimento da cortiça à seca utilizámos uma metodologia dendroecológica e uma análise das componentes da resiliência, confirmando que a seca reduz o crescimento e fornecendo conhecimento adicional sobre este assunto: o sobreiro é muito tolerante e resiliente à seca extrema, mas secas mais extremas conduzem a maiores reduções no crescimento, mais árvores afectadas mas também maior capacidade de recuperação. No entanto, existem outros factores envolvidos: local, árvore e idade do felogénio.

No que respeita à composição química, a investigação desenvolvida traz conhecimento sobre o efeito da seca na proporção dos componentes principais. Os nossos resultados demonstram que, em geral, a seca não a afecta havendo algumas excepções. No entanto, a variabilidade associada à árvore é muito mais relevante afectando todos os parâmetros analisados.

Na prática, o potencial aumento de ocorrência de secas associado às alterações climáticas não irá comprometer o desempenho da cortiça como vedante de garrafas de vinho, mas as rotações de descortiçamento devem ser alargadas no sentido de produzir pranchas com a espessura necessária, nomeadamente se a seca ocorrer nos primeiros dois anos.

Palavras-chave: *Quercus suber;* cortiça, dendroecologia; composição química; mitigação das alterações climáticas no Mediterrâneo.

Os cenários climáticos para a região da bacia mediterrânica indicam um aumento da temperatura e condições de seca cada vez mais frequentes e severas. O sobreiro é uma espécie cuja área de distribuição natural ocorre na zona ocidental desta região do globo, ocupando cerca de 22% dos espaços florestais em Portugal (ICNF, 2019). A cortiça tem uma importância económica grande, constituindo a base para o fabrico de muitos produtos, com diversas aplicações, sendo as rolhas o produto mais conhecido e com maior valorização económica, representando cerca de 70% do valor dos produtos fabricados a partir desta matéria-prima.

Neste trabalho começámos por reunir a informação disponível sobre outras espécies para além do sobreiro, que produzem cortiça em quantidade significativa, por forma a permitir a sua utilização industrial. De forma integrativa apresentamos uma descrição da formação, dos tipos de cascas e do desenvolvimento da cortiça em cada uma das espécies, numa perspectiva comparativa com a cortiça de sobreiro. Adicionalmente são, por um lado, identificadas as lacunas de conhecimento, e por outro apresentadas e discutidas as linhas futuras de investigação e as utilizações potenciais deste material em cada espécie.

Concluímos que apesar da cortiça de outras espécies ter potencial para algumas utilizações industriais, a cortiça de sobreiro é a única matéria-prima com as características necessárias para a manufactura de produtos de cortiça sólida (e.g. rolhas de cortiça natural) sendo a sua trituração e utilização na produção de aglomerados linhas de produção complementares.

Em seguida, procurou avaliar-se o efeito da seca na cortiça, nomeadamente no seu crescimento e na proporção relativa dos seus constituintes químicos: suberina, lenhina, polissacáridos e extractivos, nos monómeros da suberina e dos polissacáridos, com o objectivo de avaliar se as cada vez mais intensas e frequentes secas que se preveem para a região do Mediterrâneo irão comprometer a utilização da cortiça como vedante natural das garrafas de vinho.

Desta forma, o trabalho posterior à recolha de informação, dividiu-se em duas grandes tarefas –análise do efeito da seca a) no crescimento da cortiça e b) na proporção dos constituintes químicos - que se complementam, uma vez que a utilização que se faz da cortiça advém da sua espessura (que resulta do seu crescimento) e das suas propriedades que resultam da composição química.

No âmbito da análise do efeito da seca e da idade do felogénio no crescimento da cortiça realizámos, inicialmente, um estudo preliminar em amostras de cortiça produzida em três locais na região de Coruche (a mais vasta região de produção de cortiça em Portugal). Os dados correspondentes ao crescimento anual da cortiça possibilitaram-nos construir uma cronologia entre 1999 e 2011 em que todas as amostras apresentavam 8 anos completos de crescimento, permitindo-nos dispor de três conjuntos de amostras em que a seca de 2005 ocorreu em três idades do felogénio diferentes – um com dois anos, um com 5 e outro com 7 anos. Com esta análise dendrocronológica não só corroboramos os resultados obtidos anteriormente por outros autores, confirmando que a seca reduz a quantidade de cortiça produzida, como concluímos também que a resposta do crescimento da cortiça é independente da idade do felogénio, isto é, o decréscimo de crescimento (estandardizado) devido à seca e a recuperação após a seca não parecem ser afectados pela idade do felogénio. Verificámos, no entanto, que em valor absoluto, as reduções de crescimento observadas são maiores para felogénios mais jovens.

Após este estudo preliminar, e no sentido de confirmar os resultados obtidos, construímos uma série cronológica com dados de crescimento ao longo de 30 anos (entre 1986 e 2015) incluindo diversos episódios de seca, com várias intensidades e, pela primeira vez nesta espécie, recorrendo a uma metodologia dendroecológica conjugada com uma análise das componentes da resiliência. Esta série foi também construída com dados de crescimento de cortiça de amostras com 8 anos completos, provenientes de 12 locais na região de Coruche. Os nossos resultados confirmaram, mais uma vez, que a seca reduz o crescimento da cortiça e que quanto maior a severidade da seca maior a sua redução (e.g. no ano com seca mais severa – 2005 – a redução do crescimento foi de cerca de 46% quando comparado com os dois anos precedentes) mas, pela primeira vez, constatou-se que quanto mais intensa é a seca maior o número de árvores afectadas: em 2005, 82% das árvores apresentaram uma redução no crescimento da cortiça de pelo menos 30% em relação à média dos dois anos anteriores. No entanto, nestas circunstâncias foi também maior a capacidade de recuperação (1.62 em 2005).

Os nossos resultados confirmaram também que, tal como referido por outros autores, a resistência e a recuperação dependem da quantidade de reservas (nomeadamente açúcares) previamente armazenadas uma vez que menores valores de resistência corresponderam a maiores valores de recuperação. Com efeito, uma resistência baixa indica um consumo de reservas durante a seca, mas também que, havendo recuperação depois da seca, há um efeito positivo da recuperação da actividade fotossintética após a seca.

Adicionalmente, os valores médios de resiliência nos anos de seca foram em geral superiores a 0.80, demonstrando que o crescimento da cortiça após a seca é em geral semelhante ao crescimento antes deste evento (resiliência unitária indica que os valores de crescimento antes e após a seca são iguais), indicando, mais uma vez, uma grande capacidade de recuperação por esta espécie. Desta forma, verificámos que o impacto da seca no crescimento da cortiça nos anos subsequentes à seca é moderadamente baixo, suportando a hipótese de que o sobreiro é bastante tolerante e resiliente às secas extremas.

Neste estudo, confirmámos também as constatações de outros autores relativamente ao facto de a precipitação no inverno, primavera e anual serem determinantes para o crescimento da cortiça. No entanto, a nossa análise estatística com recurso a modelos mistos demonstrou que existem outros factores envolvidos na resposta do crescimento da cortiça, durante e após a seca, em particular: as condições intrínsecas do local, a árvore e a idade do felogénio. Tal contraria o estudo preliminar referido anteriormente dado que nos 2 primeiros anos e nos 2 últimos anos do ciclo de crescimento da cortiça os efeitos da seca são mais pronunciados do que no período intermédio, sendo este factor determinante na recuperação, na resistência e na resiliência mas não na resiliência relativa – e.g as diferenças mais pronunciadas manifestaram-se ao nível da recuperação que em amostras com felogénio com idade inferior a três anos aquando da seca, apresentaram valores 17% mais baixos do que amostras com felogénio com idades compreendidas entre 3 e 6 anos. Adicionalmente, o facto da resiliência relativa não ser afectada pela idade do felogénio sugere que a capacidade de recuperação do crescimento da cortiça não é afectada pela idade do felogénio. No entanto, a nossa análise incidiu em dados provenientes de amostras com felogénio com idades compreendidas entre 1 e 8 anos e, por isso, deve haver alguma prudência em relação às conclusões obtidas.

Importa salientar que não existe ainda informação suficiente sobre o limite mínimo de precipitação para a recuperação do crescimento da cortiça, ou seja, ainda não é possível determinar quais as condições de precipitação capazes de comprometer a recuperação posterior da capacidade meristemática do felogénio.

Em condições de seca, os resultados obtidos sugerem que, para garantir a produção de pranchas de cortiça com espessura suficiente para a produção de rolhas, poderá ser necessário prolongar os períodos entre descortiçamentos para além dos habituais 9 anos. Adicionalmente, atendendo às previsões de alterações climáticas para a bacia mediterrânea, poderá ser conveniente que novas plantações sejam instaladas em regiões mais húmidas do que as actuais regiões características do sobreiro.

Relativamente à composição química da cortiça, o nosso estudo incidiu na análise do efeito da seca na proporção dos componentes da cortiça (suberina, lenhina, polissacáridos e extractivos) e nos monómeros da suberina e dos polissacáridos. Para o efeito analisámos três conjuntos de 10 amostras provenientes do mesmo local, no concelho de Coruche. Num conjunto as amostras cresceram sem nenhum evento de seca, noutro conjunto com duas secas e no outro as amostras cresceram sob efeito da seca de 2005. Os resultados obtidos sugerem que a seca não afecta a proporção da maior parte dos constituintes químicos da cortiça, dos monómeros da suberina ou dos polissacáridos, havendo, no entanto, algumas poucas excepções. Com efeito, o teor de extractivos em etanol e a proporção de xilose nos polissacáridos aumentam ligeiramente com a seca e a arabinose nos polissacáridos diminui. A análise estatística, com recurso a modelos mistos, permitiu verificar que a variabilidade associada à árvore, provavelmente resultado da informação genética, é muito mais relevante na composição química da cortiça afectando todos os parâmetros analisados.

Desta forma, a análise efetuada no âmbito deste trabalho, sugere que as secas mais severas e mais frequentes que se preveem para a bacia do Mediterrâneo no âmbito das alterações climáticas não deverão comprometer o desempenho deste material natural enquanto vedante das garrafas de vinho, sendo a variabilidade genética o factor que mais contribui para esta característica.

Palavras-chave: *Quercus suber;* cortiça, dendroecologia; composição química; mitigação dos efeitos das alterações climáticas no Mediterrâneo.

Introduction

Climate change is an unquestionable and challenging issue by now, either in a mitigation or an adaptation perspective (Stott et al., 2016). Changes in climate and, in particular, weather extremes will regionally conditionate forest structure, including tree species composition with consequent negative economic impacts (Kätzel and Höppner, 2011). Moreover, the continuity of the forest ecosystems is constrained by the adaptive capacity of species (Bräuning et al., 2017) under its two mechanisms: the intrinsic adaptive capacity of trees and forests, and the socio-economic factors determining the capability to perform planned adaptation measures (Lindner et al., 2010).

Under this framework and although tree species can adjust to new environmental conditions, the course is too slow and there is not much knowledge about the processes involved (Lindner et al., 2010). In the meantime, forest managers must adapt to this new reality, so they can satisfy the needs of all their stakeholders, from the public in general to a rather competitive and demanding forest industry and, therefore, the scientific community must give inputs for this adaptation/mitigation process.

All climatic change scenarios for Mediterranean ecosystems predict increasing temperatures, water deficits and periodicity of extreme events, namely severe droughts, heat waves, heavy rainfall and fewer cold days (IPCC, 2014; Lindner et al., 2010). Additionally, due to summer water deficits, it is expectable to have productivity losses and changes in the species distribution (Gea-Izquierdo et al., 2013; Piayda et al., 2014; Santos and Miranda, 2006). In fact, ground water availability constrains survival and tree growth (Kelly et al., 2002) as when evaporation is greater than soil moisture uptake, stomata close to reduce water loss decreasing carbon uptake and consequently growth.

Cork oak forests are distributed in the Mediterranean basin, in an area of approximately 2.2 million ha corresponding to an annual production of up to 200 thousand tons of cork supplying an important industry (APCOR, 2018). These forests also have an outstanding ecological role, namely, against desertification and in maintaining animal and plant biodiversity, in their restricted area of occurrence, in most of the western Mediterranean countries.

Portugal, with about 34 % of the world's cork oak area (APCOR, 2018) is the leader producer of raw cork and cork products namely cork stoppers for the wine industry and boards (Pereira, 2007). According to the foreign trade data from the National Statistics Institute (INE), Portugal exported 986 million euros (197 thousand tons of cork) in 2017. Cork stoppers lead the Portuguese cork exports, accounting for about 70 % of their total value (711 million euros), followed by cork building materials with 25 % (APCOR, 2018).

The production of cork is based on a sustainable periodical removal of the cork layer from the stem. In the main producing regions, the period between consecutive cork removals is usually 9 years, enabling to have a cork plank with the required thickness (> 27 mm) for the production of cork stoppers (the cork product with the major added value that supports all the cork segment).

Cork is a natural material with outstanding properties, namely, low density, very little permeability to liquids and gases, chemical and biological inertia, mechanical elasticity, high friction, good insulation and high damping capacity (Pereira, 2007). These characteristics largely justify the interest of cork as a raw material for multiple usages from sealants to agglomerates and composites, apt for diverse utilizations (Pereira, 2015, 2007; Silva et al., 2005), such as insulation materials, surfacing panels for construction and aeronautics, pollutants absorbers, clothing, decorative articles and the one with the most important economic income – cork stoppers.

The above-mentioned restricted cork availability, both geographically and in quantity, conditions the development of the cork industrial sector. Therefore, the study of other species with barks containing a high cork proportion is a promising research line. Several authors report species whose bark has high cork content, and a few have been used to replace cork from the cork oak but usually for niche markets or in times or regions with restricted access to *Q. suber* cork. However, the number of species that may have potential to be a source of cork and therefore enlarge the cork supply to the industry is not very high and little information regarding them is available.

The cork thickness, and the related cork annual growth that defines it, determines its suitability for specific products, namely for the production of natural cork stoppers (Costa et al., 2002; Ferreira et al., 2000). In fact, given its importance, the cork plank thickness is normalized by caliper classes (NP 298:1993 and ISO 1219:1998). Thicker corkboards are less suitable for the production of stoppers because of their weaker performance in the bottle (Lauw et al., 2018) e.g. oxygen permeability, an important property related to the behavior of cork stoppers as wine

bottle sealants, is different when the stoppers are produced from thinner (27–32 mm) or thicker (45–54 mm) corkboards (Faria et al., 2011; Oliveira et al., 2013).

In addition to the cork thickness, the cellular structure and chemical composition are also decisive characteristics in the behavior and properties of this material. In fact, most of the properties of cork that are used in the above identified products arise from its structure and chemical composition, namely, high compressibility, flexibility under compression, permeability and chemical and biological inertness (Pereira, 2015, 2007).

Objectives and Overview

Under the climate change scenarios for the Mediterranean region framework, the research carried out in this work aimed to:

1. compare the characteristics and properties of cork produced by the cork oak with the ones from other species that produce cork in substantial amounts, providing a general integrative appraisal of the formation and types of barks and of cork development as well as the identification of knowledge gaps, potential interesting research lines and the utilization perspectives.

2. contribute to a deeper understanding on the effect of drought on cork from cork oak[1] growth and to evaluate, for the first time, the effect of this extreme climatic event on the cork chemical composition, namely if drought induces any changes on the proportion of the main chemical constituents of cork (suberin, lignin, polysaccharides, extractives) and on the suberin and polysaccharide monomeric composition.

3. evaluate if drought compromises the utilization of cork for the production of cork stoppers, regarding their thickness and their chemical composition, and to propose forest management advices to mitigate the effects of the forthcoming more frequent droughts in the Mediterranean basin.

To achieve this overall target, the specific objectives were:

Objective 1 Gather the information available for most of the species with barks containing a substantial amount of cork, concerning the development, structural and chemical

---

[1] From now on and unless otherwise mentioned, "cork" refers to the cork from cork oak

characteristics of the cork component, as well as their potential usage for cork-based bioproducts.

Objective 2 Study the effect of drought on cork growth, through the establishment of a 30-year chronology and applying a dendroecological approach.

Objective 3 Assess the effect of the phellogen age on the response of cork growth to drought.

Objective 4 Evaluate the effect of drought on the proportion of the main chemical constituents of cork and on the suberin and polysaccharide monomeric composition.

This thesis is organized in chapters, with the results presented as internationally refereed papers published in scientific journals.

The current chapter intends to be a brief introduction of the thesis with a description of the main objectives and the list of publications that came from the development of the PhD program.

The second chapter presents the state of the art related to the subjects dealt within this work. It gives a general overview of barks and of cork in particular, followed by a general description of the cork oak tree and the sustainable management for cork production, reviewing the formation, structure and chemical composition of this material.

Because the work focused on the effect of drought on cork growth, a state of art about drought and cork growth is also presented.

The third chapter presents the original research starting with an outline, followed by the material and methods used in this work, and the results that encompass a total of one book chapter and three articles published in international journals, all with referees. In sequence, an integrative discussion of all the results is presented, and at last the conclusions are highlighted with the perspective of future works.

List of Publications and Presentations

This thesis is based on the following peer-reviewed publications:

1. Leite C and Pereira H. 2017. Cork-Containing Barks—A Review. Front. Mater. 3:63. doi: 10.3389/fmats.2016.00063

2. Leite C., Oliveira V., Lauw A., Pereira H. 2018. Effect of a drought on cork growth along the production cycle. In Alves, F., Leal Filho, W. Azeiteiro, U. (Ed) Theory and Practice of Climate Adaptation, Springer, Berlin. DOI: 10.1007/978-3-319-72874-2_7

3. Leite C., Oliveira V., Lauw A., Pereira H. 2019. Cork rings suggest how to manage *Quercus suber* to mitigate the effects of climate changes. Agricultural and Forest Meteorology, 266-267:12-19. DOI: 10.1016/j.agrformet.2018.11.032

4. Leite, C., Oliveira, V., Miranda, I. *et al.* 2020. Cork oak and climate change: Disentangling drought effects on cork chemical composition. Scientific Reports **10,** 7800. DOI: 10.1038/s41598-020-64650-9

All publications are reproduced here with the permission of the publishers.

The results from this work were also accepted for oral presentations in the following conferences:

1. Leite C., Oliveira V., Lauw A., Pereira H. 2019. Cork growth under drought conditions – results from a 30-year resilience components analysis. TRACE 2019– Tree-rings in Archaeology, Climatology and Ecology, 7-10 May, San Leucio - Caserta, Italy.

2. Leite C., Oliveira V., Lauw A., Pereira H. 2018. Effect of a drought on cork growth along the production cycle – 10th WorldDendro – International tree ring conference, 10 - 15 June, Timphu, Bhutan

   https://www.geog.cam.ac.uk/events/worlddendro2018/

3. Leite C., Oliveira V., Lauw A., Pereira H. 2017. Effect of a drought on cork growth along the production cycle. 2nd World Symposium on Climate Change Adaptation (WSCCA-2017), 6-8 September, Coimbra, Portugal, https://www.haw-hamburg.de/en/ftz-nk/events/portugal-wscca-2017.html

4. Leite C., Oliveira V., Pereira H. 2017. Impact of climatic events on cork growth and production. Encontro Ciência 2017, 3-5 July, Lisbon, Portugal. http://www.encontrociencia.pt/2017/home/

Bark structure and formation

Bark is a heterogeneous cellular material, resulting from the activity of the two radial meristems: the vascular cambium and the phellogen (Evert, 2006). The vascular cambium encircles the stem of plants and produces xylem cells inwards and phloem cells to the outside (Figure 1). Phloem is the main food-conducting tissue and includes a functional layer near the cambium and a non-functional layer to the outside. Functional and non-functional phloem are also called, respectively, non-collapsed and collapsed phloem. The phellogen originates phellem (cork) cells to the outside and phelloderm cells to the interior. Together, phellem, phellogen and phelloderm form the periderm, as represented in Figure 1. In most species, the phellogen has a limited lifespan, and after its death a new one is formed inside the phloem. The successive periderms, which are separated by layers of phloem are called rhytidome.

Therefore, bark consists of phloem, periderm and rhytidome and its macroscopic appearance and properties will depend on the structure of these tissues, their extent and relative proportion (Huang et al., 2006).

Figure 1. Schematic drawing of a cross section of a tree stem showing: (A) the xylem (wood), the phloem (functional and non-functional) and the periderm; and (B) the xylem (wood), the phloem (functional and non-functional), the periderm and the rhytidome with successive periderms and phloem layers between them (adapted from Sen et al., 2015).

Periderm development

The periderm is a protective tissue formed in most dicotyledons and gymnosperms to replace the epidermis when this tissue no longer is able to accommodate radial growth and cracks. Also, in the case of an injury, a traumatic periderm may form to protect from exposure and infection.

The phellogen initials result from the differentiation (i.e. return to a meristematic function) of mature parenchyma cells. The first phellogen can arise in different locations: in most situations it is formed below the epidermis, but in some cases it appears in the epidermis or in the phloem (Evert, 2006; Pereira, 2007). The phellogen mother-cells start their meristematic activity by periclinal division: the inner cell differentiates as phelloderm; the outer cell undergoes another periclinal division and originates to the exterior a phellem cell (cork) and inwards the initial phellogen that continues this meristematic activity. Sometimes, cork cells occur immediately by the first division and no phelloderm cell is formed (Fahn, 1990; Pereira, 2007). In general, plants produce more phellem cells than phelloderm; in many cases, there is only one layer of phelloderm and several layers of phellem, although in a few species the phelloderm may be up to six layers thick (Beck, 2010; Fahn, 1990).

The first phellogen can be initiated uniformly around the stem or in localized areas and acquires continuity as the result of lateral spread due to meristematic activity (Evert, 2006). Timing and location of phellogen initiation is influenced by several factors, namely, genetics, physiology and environment (Lev-Yadun, 2011). The phellogen has only one kind of cells that appear in transverse sections as a tangentially disposed layer of rectangular cells, in radial section they appear flattened and in tangential view they show a polygonal structure, sometimes rather irregular (Evert, 2006; Pereira, 2007). There are no intercellular spaces between the phellogen cells, except where lenticels arise.

The phellogen activity, like that of the vascular cambium, is seasonal with periods of dormancy and of activity depending on environmental conditions, namely, light, water and temperature (Evert, 2006; Fahn, 1990). The number of cork layers is very variable between species and with plant age, and may be very large, as in the cork oak. In fact, the longevity and activity of the phellogen are decisive factors to determine the thickness and homogeneity of the cork tissue. There is also a large variability between species about the duration of the first phellogen and in some species, like the cork oak, the first phellogen is active throughout the entire life of the plant (Fahn, 1990; Pereira, 2007).

When one periderm ceases its functional activity and dies, it is substituted by a new functioning periderm, each time forming deeper inside the living tissues. Therefore the first formed periderm is the outermost in the rhytidome, while the newest one (and active) is the innermost (Fahn, 1990). These successive periderms may completely encircle the stem with a cylindrical shape, or not, e.g. with lens-shaped or shell-like portions, partially overlapping each other (Beck, 2010). Trees from temperate zones usually produce more sequential periderms than tropical ones.

As phellem and phelloderm cells result from periclinal divisions of the phellogen, i.e. parallel to the tangential direction, they are disposed in well-defined radial rows. To allow for diameter increment, the phellogen cells also perform occasional anticlinal divisions thereby increasing the number of radial rows (Beck, 2010; Pereira, 2007).

The phelloderm cells are living cells with non-suberized walls that resemble parenchyma cells but identified by their arrangement in radial rows under the phellogen initials. The phellem cells are dead cells, characterized by a cell wall containing suberin that is internally deposited onto the primary cell wall. Subsequently the phellem cells lose their protoplasm and the cell lumen becomes empty (Pereira, 2007).

Rhytidome

In most woody species in temperate climates, the initial periderm is only functional for a few years and is replaced, in the interior, by a new functional periderm. Consequently, bark accumulates to the outside of the functioning periderm layers of dead non-functional periderms and phloem tissues between them, forming the so-called rhytidome (Evert, 2006). The term outerbark is also commonly used to designate these non-living layers, and innerbark the living tissues between cambium and the active phellogen (Pereira, 2007). Figure 1B shows a schematic diagram of a bark containing successive periderms in the rhytidome.

Along time, there is a noticeable diametric expansion of the stem because the cambium produces many xylem and phloem cell layers. Consequently, there is a compression of the outer phloem and also a substantial tangential tensile stress on the bark, leading to cracking, splitting and wrinkling in the most external layers of the rhytidome (Beck, 2010). The structure of the rhytidome e.g. the number of different periderms and their cellular features and development, and the cellular composition and arrangement of the phloem tissues e.g. the proportion and

arrangement of fibers, directly influence the surface morphology of the bark, and often give the unique features of particular species, like depth and direction of wrinkling and the kind of exfoliation (Beck, 2010; Roth, 1981). These characteristic external features of bark can be very useful for taxonomy, especially for tropical trees.

As rhytidome is the result of the development of successive periderms, barks that have only one periderm do not have rhytidome (Evert, 2006). For instance, *Quercus suber*, *Q. variabilis* and *Kielmeyera coriacea* do not have rhytidome and are some of the species analyzed in this work.

Cork-rich barks

The barks may be classified in two groups in relation to periderm characteristics: those that have only one superficial periderm and do not have rhytidome (Figure 1A); and those that have rhytidome (Figure 1B). This distinction is of particular relevance when a potential exploitation of the cork layer is envisaged. When only one periderm is present, the cork layer is radially and tangentially homogenous and if its thickness is adequate, it may be used for production of solid cork products e.g. cork stoppers. In the case of a rhytidome, the cork layers of the successive periderms are separated by phloemic layers; therefore, the recovery of cork will require trituration of the rhytidome and fractionation of the cork component, thereby obtaining it in a granulated form that only allows use in cork agglomerated products.

The present main commercial provider of cork is the cork oak, *Quercus suber*, that has only one periderm and a substantial production of cork. The Chinese cork is also commercially used: it is obtained from *Quercus variabilis*, a tree that also has only one periderm. Other species were referred as having been used for production of cork or as having potential for it. Natividade (1950) points out *Q. variabilis*, *Phellodendron amurense*, *Ulmus campestris auct.* var. *suberosa*, as having been industrially used in a similar way as *Q. suber*. This author also refers that *Pseudotsuga menziesii* bark and the rhytidome of *Abies lasiocarpa* var. *arizonica, Abies concolor* and *Erythrina* spp. were used in agglomerates. Further he identified *Pithecolobium incuriale, Enterolobium ellipticum, Kielmeyera coriacea, Aspidosperma tomentosum, Zeyheria montana, Connarus suberosus* as Brazilian cork producing species with a potential value. *Melaleuca leucadendron* spongy and impermeable bark was also mentioned as a possible substitute to cork from the cork oak.

Rizzini and Mors (1995) referred that *Agonandra brasiliensis, Pisonia tomentosa, Aspidosperma dasycarpum, Erythrina mulungu, Symplocos lanceolata* produce enough cork to justify their commercial exploitation. Abramovay (1999) suggested *Erythrina crista-galli, Pithecolobium incuriale, Stryphnodendron adstringens* and *Anona coriacea* as promising cork species. Pereira (1988) showed that *Calotropis procera* has a suberous bark. Sen et al. (2010, 2011a,b) studied *Quercus cerris* rhytidome and its cork to evaluate its potential for agglomerates. Bhat (1982) observed the bark structure and some physical properties of *Betula pendula*, identifying several cork layers in the rhytidome. Recently, Mota et al. (2016) studied the cork of *Plathymenia reticulata* from the Brazilian cerrado and Sen et al. (2018) *Beaucarnea recurvata* cork.

As far as we know, only *Q. suber*, *Q. variabilis*, *Q. cerris*, *Kielmeyera coriacea*, *Pseudotsuga menziesii, Betula pendula, Plathymenia reticulata, Abies lasiocarpa* var *arizonica* and *Abies concolor* have been studied at variable degree for their bark and potential cork utilization. Table 1 lists these species dividing them in Gymnosperm and Angiosperm and classifies them regarding their bark characteristics i.e. bark with or without rhytidome.

	Bark with rhytidome	Bark without rhytidome
Gymnosperm	*Pseudotsuga menziesii* *Abies lasiocarpa* var. *arizonica* *Abies concolor*	
Angiosperm	*Quercus cerris* *Betula pendula*	*Quercus suber* *Quercus variabilis* *Kielmeyera coriacea* *Plathymenia reticulata*

Table 1 - List of gymnosperm and angiosperm species that have been studied in relation to their cork-rich barks, classified according to their bark structure (presence/absence of rhytidome).

The sustainable management of cork production

Cork oak spreads along the western part of the Mediterranean basin with economic and ecological importance in the Mediterranean countries. It covers around 2 million hectares of which Portugal has 736 thousand hectares (34% of the world's area and 23% of the national forest). The world cork production is more than 200 thousand ton., with Portugal being responsible for around 50% of it. (APCOR, 2018).

Ecologically, cork oak forests have several functions: environmental protection against soil erosion and desertification; unique and valuable landscape role; support to high levels of biodiversity; aesthetic and identity values and attractiveness for recreation and environmental balance (Bugalho et al., 2011; Costa et al., 2009; Pinto-Correia et al., 2011; Surová et al., 2011; Surová and Pinto-Correia, 2008).

Moreover, cork oak forests are a multifunctional agro-forestry-pastoral system called "*montado*" that is considered by the European Environmental Agency as a High Nature Value Farming System (Pinto-Correia et al., 2011). These ecosystems are also recognized as habitats of conservation value in the Habitats Directive (Catry et al., 2012).

Under this framework, cork harvesting is still the foremost economic activity, and cork the most valuable (non-wood forest) product. The entire cork chain from the forest to the consumer relies on the regular and sustainable production of cork. To maintain cork production and provide the above-mentioned environmental services, cork oak forests need to be properly and sustainably managed (Pereira, 2007).

The exploitation of the cork oak as a cork producer relies on the periodical removal of the cork from the stem and branches in an adequate degree with the preservation of the tree in good physiological conditions (Pereira, 2007). Cork production yields depend not only on the tree growth and cork growth, but also on management decisions such as the intensity of cork extraction and the interval between strippings which are regulated by strict rules under the Portuguese legislation (Decreto-Lei n.º 155/2004).

In fact, cork oaks are debarked every 9 years (the legal minimum allowed) or more and the decision of longer production cycles is often needed in order to achieve a minimum cork plank thickness. This means that in cork management decisions cork growth is probably one of the most important criteria to consider since it defines the thickness of the cork plank that is available for industrial processing, which is mostly oriented for the production of wine stoppers (requiring a minimum thickness of 27 mm after boiling).

Cork biology

Cork formation

Cork is a protective tissue located in the outer bark of trees, as described previously (Figure 1). The cork oak produces a periderm with special characteristics of development, regularity, growth intensity and longevity, as well as with regeneration capacity after removal that have made this species very unique. The phellogen initials result from the dedifferentiation (i.e. return to a meristematic function) of mature parenchyma cells (Pereira, 2007). The phellogen mother-cells start their meristematic activity by periclinal division: the inner cell differentiates as phelloderm; the outer cell undergoes another periclinal division and originates to the exterior a phellem cell (cork) and inwards the phellogen initial that continues this meristematic activity. In the cork oak, the first phellogen maintains its activity year after year, producing successive layers of cork. The phellogen may be functional for many years, probably during the lifetime of the tree, but the intensity of its activity decreases with age (Pereira, 2007).

Each time cork is removed from the cork oak, in a procedure called cork stripping, a new phellogen is formed inside the phloem forming a traumatic periderm and its subsequent cork layer. The first cork produced is called virgin cork and the subsequent second cork. If the second cork is removed, the process is replicated forming a new phellogen and a new cork layer (reproduction cork). After the stripping of this reproduction cork, the procedure is periodically repeated, along the entire life of the tree allowing for the continuous (every 9 years or more) removal of the reproduction cork.

The phellogen activity, like that of the vascular cambium, is seasonal with periods of dormancy and of activity depending on environmental conditions, namely light, water and temperature (Evert, 2006; Fahn, 1990). In fact, due to the Mediterranean climatic conditions, the phellogen is functional between April and September/October, with a maximum activity in June and a minimal growth in the summer (Pereira, 2007). Therefore, it is possible to observe annual rings that are marked by the presence of a layer of latecork cells at the end of the growth season. These cells are darker, smaller, with thicker cell walls than the earlycork cells (Fortes and Rosa, 1992). The rate and duration of the meristematic activity of the phellogen largely determines the width of a cork ring (Pereira, 2007).

The structure of cork

To better describe cork structure, we need to place it in relation to its original position in the tree (Figure 2) and the usual plant anatomist nomenclature will be used here. The transverse

section is perpendicular to the axial direction (*x-y* plane); the tangential section is perpendicular to a radial direction (*x-z* plane); and the radial section contains the axial direction and is perpendicular to the tangential direction (*z-y* plane).
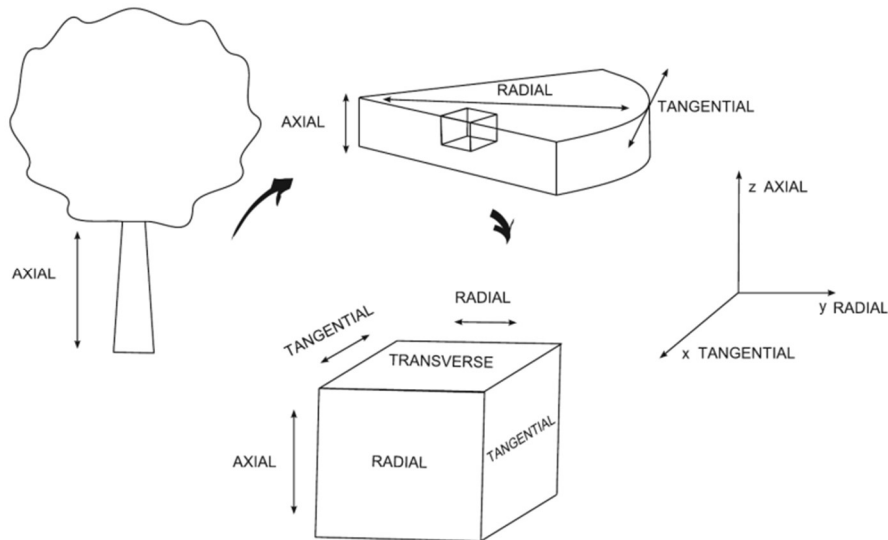
Figure 2. Diagram for the spatial description of cork structure showing the axis system and sections nomenclature as used in plant anatomy (Pereira, 2007).

In the transverse and radial sections, the structure is a brick-wall type with the cells cut parallel to their prism axis and appearing with a rectangular form. In the tangential section the cork cells appear polygonal, mostly as hexagons with a honeycomb structure (Figure 3).
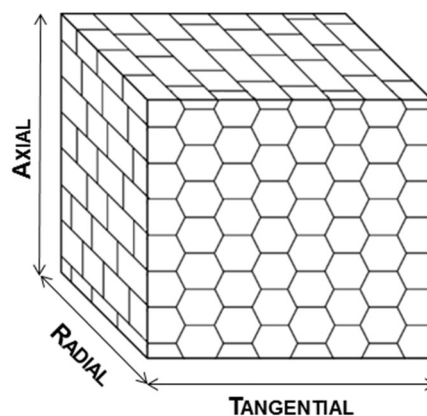
Figure 3. Schematic representation of the cellular structure of cork (Oliveira et al., 2014)**.**

The cork is a tissue with a compact structure and a very regular arrangement of the individual cells without intercellular spaces. The cells are in general hexagonal prisms stacked base-to-base in radial rows, and the rows aligned in parallel; in adjacent rows the prism bases often in staggered positions. The structure of cork observed by scanning electron microscopy in the three principal sections is shown in Figure 4.
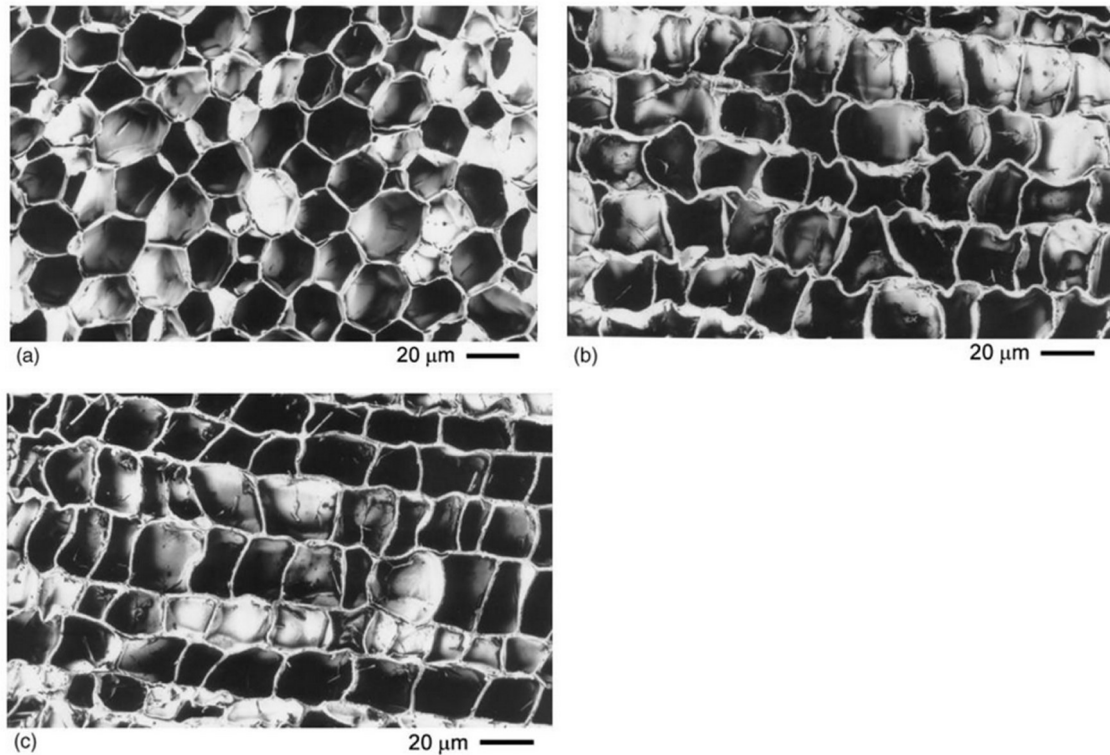
Figure 4. Scanning electron micrographs of sections of reproduction cork: (a) tangential; (b) radial; and (c) transverse sections (Pereira, 2007).

The cell volume is on average 1.7×10^{-5} mm^3, and the solid cell-wall content 10%. The cork cell-walls, especially those that constitute the lateral prism faces, show *ab initio* some bending and undulations of varying intensity that can attain strong corrugation derived from constraints during cork growth in the tree (Fortes and Rosa, 1992; Pereira et al., 1987). Observing the sides of the cells from the tangential section corrugations are not visible but some buckling may take place (Pereira, 2007).

Cork cell-walls are composed of a suberinic secondary wall and are flexible enough to undulate or corrugate with variable intensity under compression without fracture. The smaller and thick-walled latecork cells are much more rigid and stronger when compared to earlycork cells, and do not show any undulations. When the phellogen starts its meristematic activity at the beginning of the growing season, the first cork cells formed are pushed against the existing cork layers and compressed against the previous year's latecork cells causing the undulation of the

cell walls (Pereira, 2007). A brief summary of the average earlycork and latecork cells dimensions is presented in Table 2.

	Earlycork	Latecork
Prism height	30-40 µm	10-15 µm
Prism base edge	13-15 µm	13-15 µm
Average base area	$4x10^{-6}$ to $6x10^{-6}$ cm^2	$4x10^{-6}$ to $6x10^{-6}$ cm^2
Cell face thickness	1-1.5 µm	2-3 µm
Number of cells per cm³	$4x10^7$ to $7x10^7$	$10x10^7$ to $20x10^7$

Table 2 – Dimensions of cork cells (Pereira et al., 1987)

Chemical composition

Cork is chemically very different from other plant tissues, namely from wood and phloem. It is out singled by the presence of suberin as a major cell wall structural component. Suberin is a large biopolymer of lipid nature formed by the esterification of glycerol and long chain fatty acids, α,ω-diacids and ω-hydroxyacids, either saturated or with an unsaturation, epoxy or vicinal diol substitution at mid-chain chain (Graça and Pereira, 1997). Suberin also includes a few aromatic monomers in most cases ferulic acid (Graça and Pereira, 1998; Marques et al., 2015). Suberin confers impermeability to water and gases, resistance to acids and contributes to compressibility (Pereira, 2015, 2007).

Lignin is the second most important structural component of cork. This macromolecule is a cross-linked aromatic polymer with strong covalent bonds disposed as a 3D-network that confers strength to the cell wall (Pereira, 2007). Lignin is usually defined as a polymer of phenylpropane units with three different aromatic units - *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) - and the lignins are classified according to their H/G/S ratios. Lignin structural composition of barks, namely of corks, is largely unknown except for a few cases that showed that cork lignin is composed mainly of guaiacyl units with a low proportion of syringyl units (Marques et al., 2006, 1999, 1996, 1994, 2015; Marques and Pereira, 2013).

The structural polysaccharides of cell walls are cellulose and hemicelluloses. While in wood they represent up to 80% of the structural components of the cell wall, in cork they have a much lower importance and correspond to about 20% of cork (Pereira, 2007; Silva et al., 2005) Xylans are the most important hemicelluloses in cork (Pereira, 1988b).

Cork also contains non-structural components that are soluble in different solvents. Lipophilic extractives including fatty acids and alcohols, sterols and terpenes, as well as polar compounds of phenolic nature are present in substantial amounts (Pereira, 2007).

The inorganic materials content, determined as ash, is usually below 3% (Pereira, 1988b; Ponte-e-Sousa and Neto-Vaz, 2011).

Much effort has been undertaken to study the variability of *Q. suber* cork in relation to chemical composition because this characteristic is responsible for many of its properties (Bento et al., 2001; Pereira, 2013, 1988b; Sen et al., 2016). In fact, it is the cell structure and chemical composition that determine cork properties e.g. the solid volume ratio and the material's density that influence elasticity and mechanical strength, as well as cork performance in insulation (Pereira, 2015). Of all mechanical properties, compression behavior is the one that has attracted most attention, due to the importance of compression in the world-known use of cork as stoppers for wine bottles (Anjos et al., 2014, 2008; Oliveira et al., 2014).

Cork growth and climate

From a physiological point of view, water deficit situations cause, for example, a decrease in photosynthesis, stomatal closure, and reduced leaf area. The cork oak is sensitive to the amount and the moment when spring rains occur, recovering quickly after a year of extreme dryness (Besson et al., 2014). Moreover, this species manifest resilience to interannual variability of rainfall, since it can use the water stored in the soil in greater depth. In relation to cork oak growth, cumulative precipitation between January and June (growth season) and in the previous autumn-winter is the most important positive influencing factor (Leal et al., 2008). The monthly temperature has a less significant effect, found to be negatively correlated with the summer growth and positively at the beginning of the growing season (Costa et al., 2002).

Regarding cork growth, the results showed that rainfall, summer drought and temperature are determining factors. In particular, water availability is the most limiting factor for growth (Caritat et al., 2000; Cherubini et al., 2003; Oliveira et al., 2016). In fact, as shown by Pizzurro et al. (2010), the rain period from May to September strongly influences phellogen activity. Cork growth is extremely affected by drought conditions in short time scales (from 2 to 11 months) namely regarding spring precipitation. Nevertheless, cork growth rapidly recovers when drought conditions end (Oliveira et al., 2016). Temperature has also some influence in phellogen activity,

namely the absolute minimum temperature in June and the absolute maximum temperature in September show a positive correlation (Pizzurro et al., 2010). Also, as reported by Oliveira et al. (2016), it has a positive influence on growth at the beginning of phellogen activity post-dormancy (until April) but shows a negative influence during the growth period (from May to August).

It is key to note that in the Mediterranean conditions the access to water resources and the relationship to soil-site conditions are crucial factors for cork oak development (Costa et al., 2008). Soils with low depth and high compactness have a negative influence on the development of the cork oak deep root system, thereby diminishing the access to direct ground water resources, namely during summer drought (Costa et al., 2008; David et al., 2013, 2007).

Nevertheless, the climate-cork growth relationship is not straightforward. In fact, as resumed by Paulo et al. (2017), several factors/variables (e.g. tree; management; site; climatic conditions and intra specific competition) affect cork growth.

Taking into consideration that future climate scenarios predict a reduction of spring precipitation and greater severity of droughts in the Iberian Peninsula, a cork growth decrease is expected, with narrower annual rings and a consequent decrease of cork thickness. This will have implications regarding the raw-material adequacy to the cork industry, and the need for a potential adjustment of the silvicultural cork management.

This page intentionally left blank

Research outline

The research carried out in this work aimed at undertaking an analysis on the effect of drought on cork growth with an increased depth and detail in relation to the studies performed so far on this matter, and also at evaluating, for the first time, the effect of this climatic event on the cork chemical composition, in order to assess if the more frequent forthcoming drought conditions, associated to climate change in the Mediterranean region, may compromise the utilization of cork as a sealant for wine bottles. To achieve this objective several tasks were outlined.

The first task consisted in gathering information about species whose bark has high cork content, concerning the development, structural and chemical characteristics of the cork component, as well as their potential usage for cork-based products, to contextualize the cork from the cork oak and its characteristics under the general panorama of corks.

The second task involved a dendroclimatological exploratory study about the effect of a severe drought in different moments of the cork 9-year production cycle, e.g. beginning, middle or end of the cycle, to evaluate if the age of the phellogen had any influence on cork growth under and after drought conditions. The results obtained in this study were published in a book chapter and orally presented in two international congresses.

This assessment was complemented by a more detailed understanding on the response of cork production under drought conditions, through a component resilience analysis, considering not only the effect of the phellogen age on that response but also the effect of site and tree on it. This was our third task and a publication with the obtained results was written as well as an oral presentation in an international congress. Forest management advices were also suggested to mitigate drought effects under climate change.

The fourth task consisted in analyzing the cork chemical composition under no drought conditions, one drought event and two drought events and compare them to assess if drought induces any change on the relative amounts of suberin, lignin, polysaccharides, extractives, monosaccharides and suberin monomers produced by cork oak. These results were submitted to a scientific journal.

Material and Methods

Study area

The study area consisted in 12 sites each corresponding to private *montado* estates located in the Coruche municipality, in an area considered to be one of the main continuous production areas for the cork oak. The dominating soils are litholic, non-humic, little unsaturated, derived from coarse consolidated sands (sandstones) with low water storage capacity.

The climate is Mediterranean with Atlantic Ocean influence, which means, temperate with dry summers. According to the climatic data obtained in the 30-year period from 1980 to 2010, by the Climate Change Research Group of Faculdade de Ciências da Universidade de Lisboa, Coruche has an annual average precipitation of 647 mm from which 83 % is concentrated from October to April. The mean annual temperature is 16.6 °C but the highest temperatures occur in the summer when the precipitation is the lowest.

The cork oak stands in this region are characterized by a multifunctional use of the land, combining agriculture with silvopastoral activities. Therefore, trees are planted at wide spacings into grazed pastures.

The stands in which the cork samples were collected had a mean density of 83 trees/ha, ranging from 63 to 106 trees/ha and an average tree diameter at breast height between 32 and 75 cm. Management practices are similar in all stands with natural pasture and extensive grazing of cows under coppice in most of them.

Cork samples

The cork samples used in this research were provided by Associação de Produtores Florestais de Coruche (APFC) and resulted from the annual cork quality sampling procedure performed by this forest producer association. The samples were collected during several years between 1994 and 2016 and in most of the sites it was possible to have two consecutive strippings and in two of them three.

These allowed us to have cork growth data (complete years) between 1986 and 2015 building a 30-year chronology with a variable sample depth (Figure 5). It should be noticed that the striping years are not coincident in all the sites.
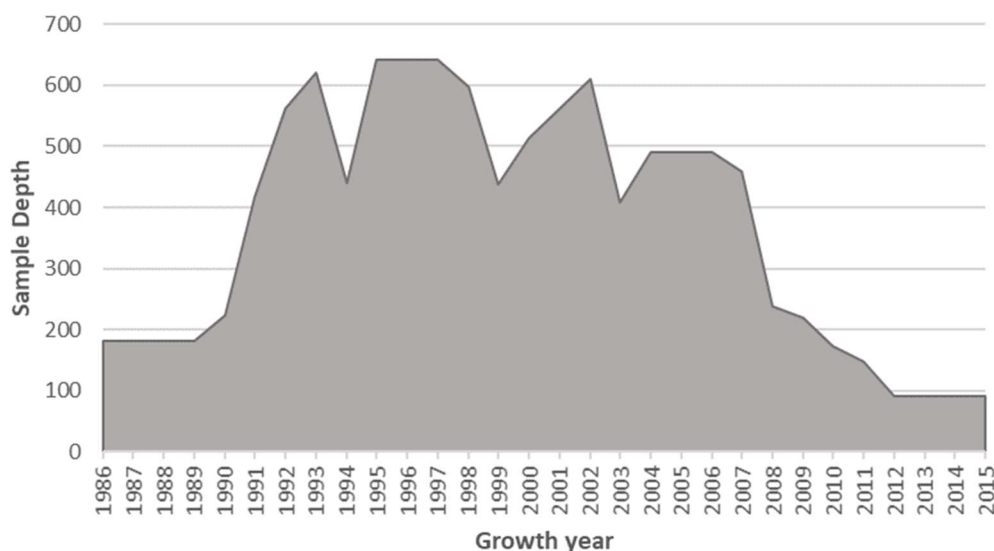
Figure 5 – Sample depth of each debarking year.

The samples were collected at breast height (1.30m) during the stripping season on mature cork oak trees under cork production. The trees were randomly selected, and the number of trees sampled per site was proportional to the total number of trees under stripping on the site. The cork production cycle was of 9 years in all the sites, corresponding to 8 complete years of cork growth as stripping interrupts the cork growth of that year.

The samples (with approximately 15×15 cm^2) were boiled in water for one hour and left to air dry until equilibrium in a similar procedure as performed by cork industry in raw corkboards.

In terms of cork growth analysis, the samples collected by APFC can be divided in two groups - a set of 1584 samples was collected between 1994 and 2010, corresponding to a 24-year chronology, was previously measured and analyzed by Oliveira et al. (2016), and a second set of 497 samples stripped between 2012 and 2016 that was just used under this research. The cork growth analysis object of this work included these two sets of data, corresponding to a growth period from 1986 to 2015.

The samples used for the chemical analysis belonged to one of the sites with three consecutive strippings, allowing the comparison of the chemical composition of cork produced in three different growth periods – one without drought effects, one with the effect of one severe drought and one when two droughts occurred. For each growth period, 10 samples were randomly selected and used for the chemical analysis.

Climate data

The droughts occurring from 1986 to 2015, were characterized by the drought index Standard Precipitation Evapotranspiration Index (SPEI) developed by Vicente-Serrano et al. (2010) because it is one of the most generalized approaches for drought analysis, and droughts in the Iberian Peninsula are better detected with SPEI than with other indices like the Standard Precipitation Index (SPI) (Páscoa, et al., 2017).

In fact, SPEI is multi-scalar drought index based on a monthly climatic water balance (precipitation minus potential evapotranspiration) and not only on precipitation data as the SPI, therefore representing a simple climatic water balance. Furthermore, as it includes temperature data, it accounts for the warming-related drought impacts on diverse ecological, hydrological and agricultural systems (Vicente-Serrano et al., 2010). Also, SPEI can be used to analyze drought at different time scales (Beguería et al., 2013 - http://spei.csic.es/home.html). Furthermore, it accounts for the accumulation of deficits/overplus at diverse timescales (Drew et al., 2013). SPEI values are negative in situations with water stress while humid periods correspond to positive values of SPEI. The SPEI data were downloaded from the Global SPEI database (http://sac.csic.es/spei/database.html), with a 0.5° spatial resolution for Coruche municipality. The values used are based on the FAO-56 Penman-Monteith estimation of potential evapotranspiration which is advised for most situations including long-term climatological analysis.

Moreover, to climatically categorize the years of our chronology, we used the classification proposed by (Agnew, 2000) and followed by Páscoa, et al. (2017) that orders drought indices according to four classes: no drought if SPEI>-0.84; moderate if -0.84 > SPEI>-1.28; severe, if -1.28>SPEI>-1.65 and extreme, if -1.65 > SPEI.

Cork-ring measurements

For a more precise observation of the growth rings, the transversal sections were polished and two radial strips per sample with approximately 1 cm thickness were cut and fixed on a microscope slide (Figure 6) and digitalized in a laser-based fluorescence imager (Fluoro Image Analyzer FLA -5100, Fujifilm, Life Science USA, Stamford, CT 06092) according to the methodology developed by Surový et al. (2009) (Figure 7). Cork ring thickness was measured with AnalySIS® image processing software (Analysis Soft Imaging System GmbH, Münster, Germany, version 3.1) and, whenever possible, along two transects in the radial direction with an accuracy of 0.01 mm. For each tree the average of the two measurements was determined.

Figure 6 – Preparation of the cork samples to measure cork rings.
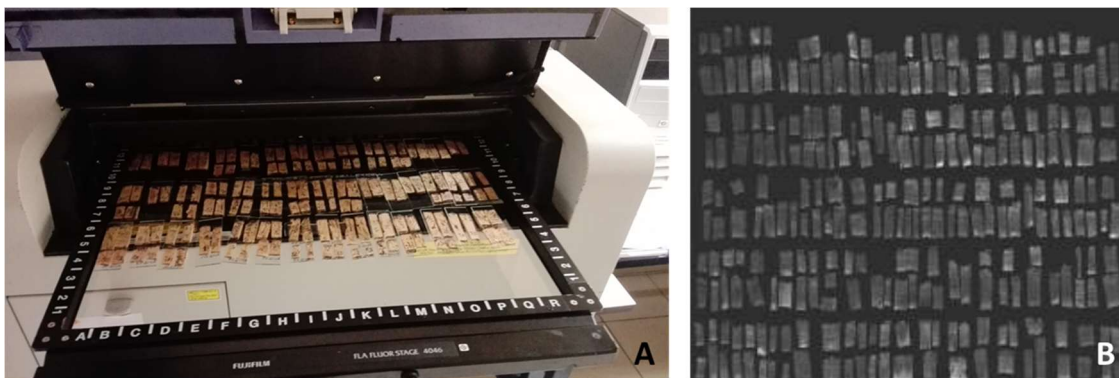
Figure 7 – [A] Fluorescence imager with cork samples and [B] image obtained for a set of 90 microscope slides.

The initial and final half rings corresponding to the years of the strippings were not considered for analysis as they are not complete growth years (Figure 8 - A). As recognized earlier by several authors (Caritat et al., 1996; Oliveira et al., 2016; Surový et al., 2009), the delimitation of annual rings was not unequivocal in all the samples (Figure 8 - B), and only in 67 % of the samples (1403 samples) cork rings were measured and dated. Each cork ring chronology was plotted and visually checked with TSAP-Win™ software (Rinntech, Heidelberg, Germany).
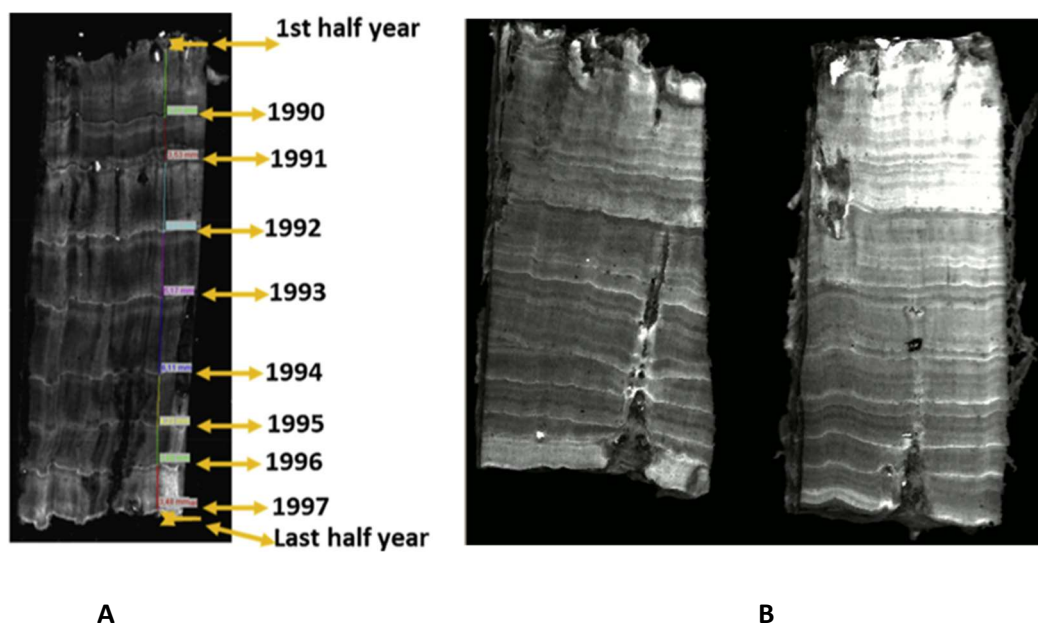
Figure 8 – A - Cork sample measured and dated showing two incomplete years B – cork samples where annual ring measurement was not possible.

Chemical analysis

Each sample was cut in small pieces with a chisel and the back (outermost bark layer of phloemic tissues) and the belly (innermost cork layer) removed to avoid contamination with other materials. The small pieces of cork were first milled with a knife mill (Retsch SM 2000) passing through a 2x2 mm^2 sieve and, afterwards, with an ultra-centrifugal mill (Retsch ZM 200). The material was granulometric separated with a vibratory sieve (Retsch AS 200basic) for 10 minutes. The fractions that passed the 60 mesh screen (0.250 mm) were discarded to avoid contamination with lenticular material or woody inclusions that are chemically different from cork and the particles between 40 (0.425 mm) and 60 mesh were used, as usually performed for cork chemical analysis (Pereira, 2007).

The chemical summative analyses included determination of extractives soluble in dichloromethane, ethanol and water, suberin, klason and acid soluble lignin and the monomeric composition of polysaccharides. All the results were quantified as percent of dry cork mass.

Extractives determination was adapted from TAPPI 204 cm-97 procedure, in a Soxhlet system using successively dichloromethane (6 h), ethanol (16 h) and water (16 h). The extractives solubilized by each solvent were determined from the mass solid residue after drying for two days at 60ºC and *over-night* at 105ºC.

The suberin content was determined in the extractive-free residue after methanolysis for depolymerization (Pereira, 1988b). A sample of 1.5g of extractive-free material was refluxed with a 3 % (m/v) solution of sodium methoxide in methanol (100 ml) for 3 h. After that, the sample was filtrated and washed with methanol, and the filtrated residue refluxed again with 100 ml methanol for 15 min and filtrated again. Both filtrates were acidified to pH 6 with sulfuric acid 2 M and evaporated to dryness. The residues were suspended in water (50ml) and the products recovered with dichloromethane in three successive extractions of 50 ml each. The combined extracts were dried over anhydrous sodium sulfate, and the solvent evaporated to dryness. The suberin extracts were quantified after drying at 60ºC *over-night* and at 105ºC for two hours.

Klason and acid-soluble lignin, and carbohydrates contents were determined on the extracted and desuberinized materials. 3.0 ml of sulphuric acid (72 %) were added to 0.35 g of that material and the mixture placed in a water bath at 30 °C for 1 h. The solution was watered down until the sulfuric acid concentration was 3% and then autoclaved at 121 °C for 30 min. After cooling down, the insoluble fraction was separated by filtration and the Klason lignin was weighed after drying at 105 °C. The acid-soluble lignin was measured through the UV absorption at 206 nm using an extinction coefficient of 110 l g^{-1}cm^{-1} (UV-Vis spectrometer 160A, Shimadzu, Nakagyo-ku, Kyoto, Japan).

The polysaccharides content was determined by quantification of the content in neutral monossacharides (rhamnose, arabinose, xylose, galactose, mannose and glucose) and uronic acids (galacturonic and glucuronic acids released by the total acid hydrolysis used for lignin determination, after derivatization as alditol acetates. The sugar monomers were determined using a high-performance anion exchange chromatography (HPAEC) with Aminotrap plus CarboPac PA10 column (250 x 4 mm). The content of acetic acid was also determined in the hydrolysate using a High-Pressure Ion exclusion Chromatography with a UV/Visible detector (HIPCE-UV). The compounds were separated in a Thermo Finnigan Surveyor installed with a Biorad Aminex 87H column (300 x 7.8 mm). The carbohydrate composition was expressed in percent of total monosaccharides.

Extractives analysis was done in triplicate aliquots, determination of suberin and lignin were done in duplicate aliquots, and monomeric composition of polysaccharides was determined in one sample/tree.

Regarding the analysis of the monomeric composition of the suberin, one aliquot/tree of the methanolic extracts (5 ml) from the suberin depolymerization was taken and evaporated under

nitrogen flow and dried under vacuum at r.t. overnight. The samples were evaporated, derivatized by trimethysilylation and immediately analyzed by GC-MS, with the following Zebron conditions: Zebron 7HGG015-02 column (Phenomenex, Torrance, CA, USA) (30 m, 0.25 mm; ID, 0.1 µm film thickness), injector 400°C, oven temperature program: 50°C (held 1 min), 10°Cmin^{-1} to 150°C, 5°C min^{-1} to 200°C, 4°C min^{-1} to 300°C, 10°C min^{-1} to 380°C (held 5 min). The MS source was kept at 220ºC and the electron impact mass spectra (EIMS) taken at 70 eV of energy.

The area of peaks in the total ion chromatograms of the GC–MS analysis was integrated, and their relative area proportions expressed as percentage for semi-quantitative analysis. Compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data (Graça, 2015; Graça and Pereira, 2000a,b; Pereira, 2007), reference compounds, ion fragmentation patterns, and/or retention times.

It is key to note that the experimental procedure used for the suberin compositional determination does not allow the quantification of glycerol but only of the long-chain fatty components, alcohols and the other monomers that are soluble in dichloromethane.

Publication I

Cork-Containing Barks—A Review

Cork-Containing Barks—A Review

Carla Leite* and Helena Pereira

Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

Tree barks are among the less studied forest products notwithstanding their relevant physiological and protective role in tree functioning. The large diversity in structure and chemical composition of barks makes them a particularly interesting potential source of chemicals and bioproducts, at present valued in the context of biorefineries. One of the valuable components of barks is cork (phellem in anatomy) due to a rather unique set of properties and composition. Cork from the cork oak (*Quercus suber*) has been extensively studied, mostly because of its economic importance and worldwide utilization of cork products. However, several other species have barks with substantial cork amounts that may constitute additional resources for cork-based bioproducts. This paper makes a review of the tree species that have barks with significant proportion of cork and on the available information regarding the structural and chemical characterization of their bark. A general integrative appraisal of the formation and types of barks and of cork development is also given. The knowledge gaps and the potential interesting research lines are identified and discussed, as well as the utilization perspectives.

INTRODUCTION

Trees are externally covered on their stems and branches by the bark that represents 9 to 15% of the stem volume (Harkin and Rowe, 1971). The bark is composed of several types of tissues and cells with different functions: translocation and storage of organic materials, water storage, wound healing, protection from herbivores, pathogens, and environmental factors (e.g., irradiation, desiccation, wind, flooding, hail, snow, fire), and photosynthesis in shoots (Lev-Yadun, 2011). Barks are very variable in thickness, color, and texture depending on species, age, and growing conditions, among other factors. The bark often gives a species its characteristic appearance and may be used for taxonomic purposes.

The radial growth of woody plants results from the activity of two meristems: the vascular cambium, which gives rise to the xylem (wood) and to the secondary phloem, and the cork cambium or phellogen that produces the phelloderm and phellem (cork), which together constitute the system named periderm. Bark can be defined as all the tissues formed to the outside of the vascular cambium, therefore including the phloem and the periderm (Trockenbrodt, 1990; Junikka, 1994; Richter et al., 1996; Evert, 2006). A schematic diagram of a tree stem cross section is given in **Figure 1A**.

Barks have been used since ancient times for several purposes: medicine, construction, chemistry, clothing, and energy. More recently, they are viewed as a potential raw material for biorefineries, given their complex structure and rich chemistry, as well as large availability (Şen et al., 2015). In fact, roundwood world production was about 3.591 million m^3 in 2013 (FAO, 2015), generating over 300 million m^3 of bark that are largely concentrated at processing sites and industrial mills. Nevertheless, the timber economy usually treats barks as a residue, and their main use is as fuel. Consequently, the effort undertaken to study bark development, structure, and chemistry is quite

FIGURE 1 | Schematic drawing of a cross section of a tree stem showing (A) the xylem (wood), the phloem (functional and non-functional), and the periderm and (B) the xylem (wood), the phloem (functional and non-functional), the periderm, and the rhytidome with successive periderms and phloem layers between them [adapted from Şen et al. (2015)].
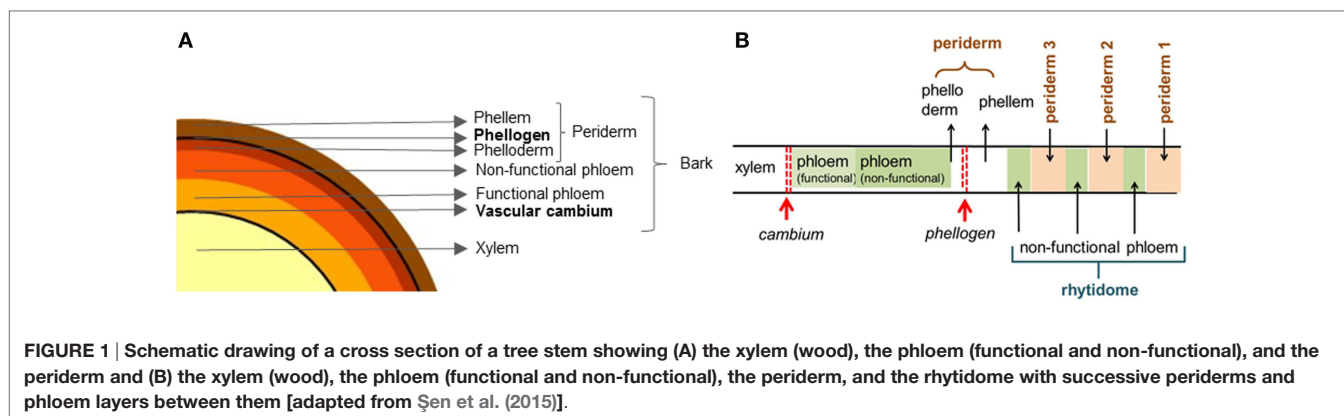
limited, e.g., only a small portion of the one given to wood (Lev-Yadun, 2011).

One exception is the bark produced by the cork oak (*Quercus suber* L.), and this is because of cork, a material that has attracted the curiosity of mankind for many centuries and is now the basis of an economic relevant industry. Cork is a cellular material with an outstanding set of properties, namely, low density, very little permeability to liquids and gases, chemical and biological inertia, mechanical elasticity, high friction, good insulation, and high damping capacity (Pereira, 2007). These characteristics largely justify the interest of cork as a raw material for multiple usages (Pereira, 2015). Cork is used for many products, from sealants to agglomerates and composites, suitable for diverse purposes, such as bottle stoppers for the wine industry, insulation, and surfacing panels for construction and aeronautics, pollutants absorbers, clothing, and decorative articles (Fortes et al., 2004; Pereira, 2007; Gil, 2009, 2015; Duarte and Bordado, 2015).

The cork oak produces a periderm with special characteristics of development, regularity, growth intensity, and longevity, as well as with regeneration capacity after removal, which has made this species very unique. Cork oaks have a distribution restricted to the western Mediterranean basin, with the largest areas located in Portugal and Spain, and an annual total production of cork is limited to about 200,000 tons (APCOR, 2015).

This restricted cork availability, both geographically and in quantity, conditions the development of the cork industrial sector. Therefore, the study of other species with barks containing a high cork proportion is a promising research line. Several authors report species, whose bark has high cork content, and a few have been used to replace cork from the cork oak but usually for niche markets or in times or regions with restricted access to *Q. suber* cork. However, the number of species that may have potential to be a source of cork and, therefore, enlarge the cork supply to the industry is not very high, and little information regarding them is available.

The present review presents a general overview of barks and of cork in particular and gathers the information available for some of the species with barks containing a substantial amount of cork, concerning the development and structural and chemical characteristics of the cork component, as well as their potential usage for cork-based bioproducts.

BARK STRUCTURE AND FORMATION

Bark is a heterogeneous cellular material, resulting from the activity of the two radial meristems: the vascular cambium and the phellogen (Evert, 2006).

The vascular cambium encircles the stem of plants and produces xylem cells inwards and phloem cells to the outside (**Figure 1**). Phloem is the main food-conducting tissue and includes a functional layer near the cambium and a non-functional layer to the outside. Functional and non-functional phloem are also called, respectively, non-collapsed and collapsed phloem. The phellogen originates phellem (cork) cells to the outside and phelloderm cells to the interior. Together, phellem, phellogen, and phelloderm form the periderm, as represented in **Figure 1**. In most species, the phellogen has a limited lifespan, and after its death a new one is formed inside the phloem. The successive periderms, which are separated by layers of phloem are called rhytidome.

Therefore, bark consists of phloem, periderm, and rhytidome, and its macroscopic appearance and properties will depend on the structure of these tissues, their extent, and relative proportion (Huang et al., 2006).

Periderm Development

The periderm is a protective tissue formed in most dicotyledons and gymnosperms to replace the epidermis when this tissue no longer is able to accommodate radial growth and cracks. Also, in the case of an injury, a traumatic periderm may form to protect from exposure and infection.

The phellogen initials result from the dedifferentiation (i.e., return to a meristematic function) of mature parenchyma cells. The first phellogen can arise in different locations: in most situations it is formed below the epidermis, but in some cases it appears in the epidermis or in the phloem (Evert, 2006; Pereira, 2007). The phellogen mother-cells start their meristematic activity by periclinal division: the inner cell differentiates as phelloderm; the outer cell undergoes another periclinal division and originates to the exterior a phellem cell (cork) and inwards the phellogen initial that continues this meristematic activity. Sometimes, cork cells occur immediately by the first division, and no phelloderm cell is formed (Fahn, 1990; Pereira, 2007). In general, plants produce more phellem cells than phelloderm; in many cases, there is only

one layer of phelloderm and several layers of phellem, although in a few species the phelloderm may be up to six layers thick (Fahn, 1990; Beck, 2010).

The first phellogen can be initiated uniformly around the stem or in localized areas and acquires continuity as the result of lateral spread due to meristematic activity (Evert, 2006). Timing and location of phellogen initiation is influenced by several factors, namely, genetics, physiology, and environment (Lev-Yadun, 2011). The phellogen has only one kind of cells that appear in transverse sections as a tangentially disposed layer of rectangular cells; in radial section, they appear flattened and in tangential view they show a polygonal structure, sometimes rather irregular (Evert, 2006; Pereira, 2007). There are no intercellular spaces between the phellogen cells, except where lenticels arise.

The phellogen activity, like that of the vascular cambium, is seasonal with periods of dormancy and of activity depending on environmental conditions, namely, light, water, and temperature (Fahn, 1990; Evert, 2006). The number of cork layers is very variable between species and with plant age and may be very large, as in the cork oak. In fact, the longevity and activity of the phellogen are decisive factors to determine the thickness and homogeneity of the cork tissue. There is also a large variability between species about the duration of the first phellogen, and in some species like the cork oak and others, the first phellogen is active throughout the entire life of the plant (Fahn, 1990; Pereira, 2007).

When one periderm ceases its functional activity and dies, it is substituted by a new functioning periderm, each time forming deeper inside the living tissues. Therefore, the first formed periderm is the outermost in the rhytidome, while the newest one (and active) is the innermost (Fahn, 1990). These successive periderms may completely encircle the stem with a cylindrical shape, or not, e.g., with lens-shaped or shell-like portions, partially overlapping each other (Beck, 2010). Trees from temperate zones usually produce more sequential periderms than tropical ones.

As phellem and phelloderm cells result from periclinal divisions of the phellogen, i.e., parallel to the tangential direction, they are disposed in well-defined radial rows. To allow for diameter increment, the phellogen cells also perform occasional anticlinal divisions, thereby increasing the number of radial rows (Pereira, 2007; Beck, 2010).

The phelloderm cells are living cells with non-suberized walls that resemble parenchyma cells but identified by their arrangement in radial rows under the phellogen initials. The phellem cells are dead cells, characterized by a cell wall containing suberin that is internally deposited onto the primary cell wall. Subsequently the phellem cells lose their protoplasm, and the cell lumen becomes empty (Pereira, 2007).

Rhytidome

In most woody species in temperate climates, the initial periderm is only functional for a few years and is replaced, in the interior, by a new functional periderm. Consequently, bark accumulates to the outside of the functioning periderm layers of dead non-functional periderms and phloem tissues between them, forming the so-called rhytidome (Evert, 2006). The term outerbark is also commonly used to designate these non-living layers, and

innerbark the living tissues between cambium and the active phellogen (Pereira, 2007). **Figure 1B** shows a schematic diagram of a bark containing successive periderms in the rhytidome.

Along time, there is a noticeable diametric expansion of the stem because the cambium produces many xylem and phloem cell layers. Consequently, there is a compression of the outer phloem and also a substantial tangential tensile stress on the bark, leading to cracking, splitting, and wrinkling in the most external layers of the rhytidome (Beck, 2010). The structure of the rhytidome, e.g., the number of different periderms and their cellular features and development, and the cellular composition and arrangement of the phloem tissues, e.g., the proportion and arrangement of fibers, directly influence the surface morphology of the bark and often give the unique features of particular species, like depth and direction of wrinkling and the kind of exfoliation (Roth, 1981; Beck, 2010). These characteristic external features of bark can be very useful for taxonomy, especially for tropical trees.

As rhytidome is the result of the development of successive periderms, barks that have only one periderm do not have rhytidome (Evert, 2006). For instance, *Q. suber*, *Quercus variabilis*, and *Kielmeyera coriacea* do not have rhytidome and are some of the species analyzed in this review.

CORK STRUCTURE AND CHEMICAL COMPOSITION

Cork is formed by cells with empty lumens and suberized cell walls. The presence of suberin is the specific characteristic of cork and often used to identify cork cells in plant anatomy by applying specific suberin staining, e.g., sudan dye. Suberin confers impermeability to water and gases and resistance to acids and contributes to compressibility (Pereira, 2007, 2015).

The cork structure is compact with a very regular arrangement of the individual cells and without intercellular spaces. The cells are in general hexagonal prisms that are stacked base-to-base in radial rows, and the rows aligned in parallel; in adjacent rows, the prism bases often lay in staggered positions. When observed two-dimensionally, i.e., in sections, the arrangement has a different appearance. In the transverse section (the plane perpendicular to the plant axis), the structure is a brick-wall type with the cells cut parallel to their prism axis and appearing with a rectangular form. The radial section (the plane that contains the plant axis and a diameter) is very similar. In the tangential section (the plane perpendicular to a radius), the cork cells appear polygonal, mostly as hexagons with a honeycomb structure (**Figure 2**).

It is often possible to identify growth increments in cork. Macroscopically they are distinguished by the darker color of the cell layers formed at the end of the growing season, that have thicker walled cells and smaller in the radial direction (latecork cells) in contrast to the thinner walls and radially longer cells of the beginning and core of the growing season (earlycork cells) (Pereira, 2007).

The cork cells may have evenly or unevenly thickened walls, e.g., some have U-shaped wall thickenings of the inner or outer tangential wall (Evert, 2006). In some species, the phellem

FIGURE 2 | Schematic representation of the cellular structure of cork.
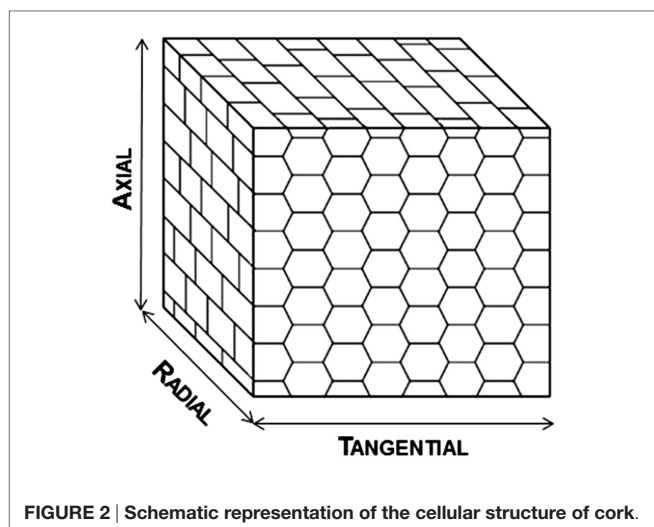
contains also non-suberized cells, the phelloids, which have thick or thin cell walls and differentiate as sclereids.

In addition to the typical hollow, thin-walled, and radially widened cork cells, the cork layer may include thick-walled and radially flattened cells, often filled with dark resins or tannins that occur in some species in alternating tangential bands (Fahn, 1990).

Cork is chemically very different from other plant tissues, namely, from wood and phloem. It is out-singled by the presence of suberin as a major cell wall structural component. Suberin is a large biopolymer of lipid nature formed by the esterification of glycerol and long-chain fatty acids, α,ω-diacids and ω-hydroxyacids, either saturated or with an unsaturation, epoxy, or vicinal diol substitution at mid-chain (Graça and Pereira, 1997). Suberin also includes a few aromatic monomers in most cases ferulic acid (Graça and Pereira, 1998; Marques et al., 2016). The specific composition of suberin, i.e., the proportion of monomers varies between species, as detailed in the following sections.

Lignin is the second most important structural component of cork. This macromolecule is a cross-linked aromatic polymer with strong covalent bonds disposed as a 3D-network that confers strength to the cell wall (Pereira, 2007). Lignin is usually defined as a polymer of phenylpropane units with three different aromatic units—*p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)—and the lignins are classified according to their H/G/S ratios. Lignin structural composition of barks, namely of corks, is largely unknown except for a few cases that showed that cork lignin is composed mainly of guaiacyl units with a low proportion of syringyl units (Marques et al., 1994, 1996, 1999, 2006, 2016; Marques and Pereira, 2013).

The structural polysaccharides of cell walls are cellulose and hemicelluloses. While, in wood they represent up to 80% of the structural components of the cell wall, in cork they have a much lower importance and correspond to about 20% of cork (Silva et al., 2005; Pereira, 2007). Xylans are the most important hemicelluloses in cork (Pereira, 1988).

Cork also contains non-structural components that are soluble in different solvents. Lipophilic extractives including fatty acids and alcohols, sterols, and terpenes, as well as polar compounds of phenolic nature are present in substantial amounts. The proportion and the composition of cork extractives differ substantially between species (Ferreira et al., 2015a,b, 2016a,b; Mota et al., 2016; Sen et al., 2016a).

The inorganic materials content, determined as ash, is usually below 3% (Pereira, 1988; Sen et al., 2010; Ponte-e-Sousa and Neto-Vaz, 2011; Ferreira et al., 2015a).

Much effort has been undertaken to study the variability of *Q. suber* cork in relation to chemical composition because this characteristic is responsible for many of its properties (Pereira, 1988, 2013; Bento et al., 2001; Sen et al., 2016a). For corks of other species, there is no systematic study of natural chemical variation.

Together, the cell structure and chemical composition determine cork properties, e.g., the solid volume ratio and the material's density that influence elasticity and mechanical strength, as well as cork performance in insulation (Pereira, 2015). Of all mechanical properties, compression behavior is the one that has attracted most attention, due to the importance of compression in the world-known use of cork as stoppers for wine bottles (Anjos et al., 2008, 2014; Oliveira et al., 2014).

CORK-RICH BARKS

The barks may be classified in two groups in relation to periderm characteristics: those that have only one superficial periderm and do not have rhytidome (**Figure 1A**); and those that have rhytidome (**Figure 1B**). This distinction is of particular relevance when a potential exploitation of the cork layer is envisaged. When only one periderm is present, the cork layer is radially and tangentially homogenous and if its thickness is adequate, it may be used for production of solid cork products, e.g., cork stoppers. In the case of a rhytidome, the cork layers of the successive periderms are separated by phloemic layers; therefore the recovery of cork will require trituration of the rhytidome and fractionation of the cork component, thereby obtaining it in a granulated form that only allows use in cork agglomerated products.

The present main commercial provider of cork is the cork oak, *Q. suber*, which has only one periderm and a substantial production of cork. The Chinese cork is also commercially used: it is obtained from *Q. variabilis*, a tree that also has only one periderm. Other species were referred as having been used for production of cork or as having potential for it. Natividade (1950) points out *Q. variabilis*, *Phellodendron amurense*, and *Ulmus campestris* auct. var. *suberosa*, as having been industrially used in a similar way as *Q. suber*. This author also refers that *Pseudotsuga menziesii* bark and the rhytidome of *Abies lasiocarpa* var. *arizonica*, *Abies concolor*, and *Erythrina* spp. were used in agglomerates. Further, he identified *Pithecolobium incuriale*, *Enterolobium ellipticum*, *K. coriacea*, *Aspidosperma tomentosum*, *Zeyheria montana*, and *Connarus suberosus* as Brazilian cork-producing species with a potential value. *Melaleuca leucadendron* spongy and impermeable bark was also mentioned as a possible substitute to cork from the cork oak.

Rizzini and Mors (1995) referred that *Agonandra brasiliensis*, *Pisonia tomentosa*, *Aspidosperma dasycarpum*, *Erythrina mulungu*, and *Symplocos lanceolata* produce enough cork to justify their commercial exploitation. Abramovay (1999) in Rios (2007) suggested *Erythrina crista-galli*, *P. incuriale*, *Stryphnodendron adstringens*, and *Anona coriacea* as promising cork species. Pereira (1988) showed that *Calotropis procera* has a suberous bark. Sen et al. (2010, 2011a,b) studied *Quercus cerris* rhytidome and its cork to evaluate its potential for agglomerates. Bhat (1982) observed the bark structure and some physical properties of *Betula pendula*, identifying several cork layers in the rhytidome. Recently, Mota et al. (2016) studied the cork of *Plathymenia reticulata* from the Brazilian cerrado.

As far as we know, only *Q. suber*, *Q. variabilis*, *Q. cerris*, *K. coriacea*, *P. menziesii*, *B. pendula*, *P. reticulata*, *A. lasiocarpa* var *arizonica*, and *A. concolor* have been studied at variable degree for their bark and potential cork utilization. **Table 1** lists these species dividing them in gymnosperm and angiosperm and classifies them regarding their bark characteristics, i.e., bark with or without rhytidome.

In the following sections, a brief explanation is given for each species about distribution area and economic importance, as well as a review of the information available about their periderm and cork characteristics, and a brief discussion about prospective utilizations.

CHARACTERIZATION OF SOME CORK-RICH BARKS

There are few works on the barks that were identified as having a high cork content and on the characteristics of their cork component. One exception is the cork oak, *Q. suber*, the present main provider of commercial cork used by an integrated industrial chain of high economic relevance in the production regions. It has been extensively studied, and the rich array of publications was compiled in a reference book (Pereira, 2007). The barks of the other species listed in **Table 1** have been much less studied, and in some cases only a few notes on bark development are available. The features of *Q. suber* cork, therefore, benchmark the general characterization of cork materials.

In the following sections, a synthesis of the existing knowledge on the cork of the different barks is made, organized by species, including, when available, details on periderm or rhytidome, cork cellular and chemical features, and use potential. Summary

tables were prepared for comparison of corks from the different species: **Table 2** summarizes cell dimensions, **Table 3** summarizes chemical composition, **Table 4** summarizes polysaccharide composition, and **Table 5** summarizes suberin composition.

Quercus suber

The cork oak (*Q. suber*) is a species native to the western Mediterranean basin, with the largest cork-producing areas situated in Portugal and Spain. The *Q. suber* trees are of median height (15–20 m) and may reach up to 25 m, with a broad crown and a very conspicuous bark (**Figure 3**).

Cork from the cork oak has high economic importance, mainly as a raw material for wine stoppers and also for surfacing and insulating materials. The tree is exploited using a sustainable management with periodic removals of the cork layer under a silvicultural system that has been perfected along time, integrating a multifunctional agro-forest system called *montado* (Natividade, 1950; Pereira and Tomé, 2004).

Periderm Development

In the cork oak, the phellogen forms a continuous layer surrounding stem and branches and may live as long as the tree, although the intensity of its activity decreases with age (Natividade, 1950).

The phellogen activity begins in the first year of the shoot; the first cork cells keep the tangential form of the phellogen initial and build up radially aligned rows (Graça and Pereira, 2004). Besides the periclinal divisions of the phellogen cells, some anticlinal divisions also occur that increase the number of phellogen initials, and therefore of the radial rows of cork cells.

The cork in the first periderm is called virgin cork (**Figure 3A**). It shows numerous and deep cracks that run mostly longitudinally due to the radial enlargement of the tree (Pereira, 2007).

If the initial phellogen is destroyed, as it happens by the stripping of the cork layer, a new (traumatic) phellogen is formed in

TABLE 1 | List of gymnosperm and angiosperm species that have been studied in relation to their cork-rich barks, classified according to their bark structure (presence/absence of rhytidome).

	Bark with rhytidome	Bark without rhytidome
Gymnosperm	*Pseudotsuga menziesii* *Abies lasiocarpa* var. *arizonica* *Abies concolor*	
Angiosperm	*Quercus cerris* *Betula pendula*	*Quercus suber* *Quercus variabilis* *Kielmeyera coriacea* *Plathymenia reticulata*

TABLE 2 | Cellular biometry of cork of *Quercus suber*, *Quercus variabilis*, *Kielmeyera coriacea*, *Plathymenia reticulata*, *Quercus cerris*, and *Pseudotsuga menziesii*.

Species		Prism height (µm)	Prism base edge (µm)	Cell wall thickness (µm)
Q. suber[a]	Earlycork Latecork	30–40 10–15	13–15 13–15	1–1.5 2–3
Q. variabilis[b]	Earlycork	22.7	13.6	n.d.
K. coriacea[c]		40–70	24	1.5–2
P. reticulata[d]	Earlycork Latecork	21 12	24 n.d.	1.2 1.4
Q. cerris[e]	Earlycork Latecork	25 14	16 16	2–3 3–5
P. menziesii[f]		55	25	1.3

[a]*Pereira et al. (1987), reproduction cork.*
[b]*Ferreira et al. (2016a), reproduction cork.*
[c]*Rios et al. (2014).*
[d]*Mota et al. (2016).*
[e]*Sen et al. (2011a).*
[f]*Cardoso et al. (2016).*
n.d., no data available.

TABLE 3 | Chemical composition (% total dry mass) of the cork of *Quercus suber*, *Quercus variabilis*, *Kielmeyera coriacea*, *Plathymenia reticulata*, *Quercus cerris*, *Betula pendula*, and *Pseudotsuga menziesii*.

Species		Extractives	Suberin	Lignin	Polysaccharides
Q. suber[a]		16.2 (8.6–32.9)	42.8 (54.2–23.1)	22.0 (17.1–36.4)	19.0[b]
Q. variabilis[c]	Virgin cork	9.2	37.4	27.6	24.7[d]
	Reproduction cork	9.1	38.1	29.4	22.8[d]
K. coriacea[e]		14.2–23.0	16.1–30.3	43.6–55.3	10.5–16.6
P. reticulata[f]		12.7	24.7	34.5	20.9
Q. cerris[g]		16.7	28.5	28.1	16.5
B. pendula[h]		32.2	36.2	14.3	10.3
P. menziesii[i]		29.2	36.2	16.8	16.9

[a]Calculated from Pereira (2013), reproduction cork.
[b]Calculated by difference to 100%.
[c]Ferreira et al. (2016a).
[d]Calculated by difference to 100% after accounting for the ash content.
[e]Calculated from Rios et al. (2014).
[f]Mota et al. (2016).
[g]Sen et al. (2010).
[h]Ferreira et al. (2016b).
[i]Ferreira et al. (2015a).

TABLE 4 | Polysaccharides composition (% of total neutral monosaccharides) of the cork of *Quercus suber*, *Quercus variabilis*, *Kielmeyera coriacea*, *Plathymenia reticulate*, *Quercus cerris*, *Betula pendula*, and *Pseudotsuga menziesii*.

Species		Glucose	Mannose	Galactose	Rhamnose	Xylose	Arabinose
Q. suber	Virgin cork[a]	50.6 (48.9–51.7)	2.8 (2.3–3.7)	3.6 (3.2–4.3)	1.7 (1.6–1.8)	35.0 (32.9–37.3)	7.0 (6.2–7.7)
	Reproduction cork[b]	46.1 (53.6–41.8)	3.0 (12.4–2.1)	7.3 (10.4–5.2)	0.5 (1.1–0.0)	25.1 (31.7–21.4)	18.0 (24.4–12.7)
Q. variabilis[c]	Virgin cork	51.6	2.9	5.7	1.4	28.0	10.5
	Reproduction cork	51.7	2.7	6.2	1.3	27.9	8.2
K. coriacea[d]		55.5–64.6	1.8–5.7	5.5–9.5	0.6–1.7	19.7–26.2	6.0–8.0
P. reticulat5[e]		51.9	8.1	8.1	n.d.	18.8	13.1
Q. cerris[f]		49.7	2.4	7.3	1.2	27.9	11.5
B. pendula[g]		41.7	4.8	8.1	n.d.	19.1	26.3
P. menziesii[h]		55.4	10.1	10.3	n.d.	13.3	10.9

[a]Bento et al. (2001).
[b]Pereira (2013).
[c]Ferreira et al. (2016a).
[d]Rios et al. (2014).
[e]Mota et al. (2016).
[f]Sen et al. (2010).
[g]Ferreira et al. (2016b).
[h]Ferreira et al. (2015a).
n.d., no data available.

the inner tissues of the phloem and begins its meristematic activity in the same way as it happened in the first periderm, thereby forming a new regular cylindrical layer of cork cells around the tree; this cork is called reproduction cork. This new periderm is covered externally by the tissues that remained to the outside of where the phellogen was formed; therefore they include the first phelloderm (formed by the initial phellogen) and a layer of the non-functional phloem. As these tissues become exposed to air, they dry out and develop thin fissures building up what is called the cork back (**Figure 3B**) (Taco et al., 2003).

The first reproduction cork produced by this second phellogen (also called second cork) is often longitudinally fissured due to the still high tangential growth stress of the young tree (**Figure 3B**). When the second cork is removed, the process is repeated as described above, and the new cork layers no longer fissure since the tangential growth stress is much smaller (**Figure 3C**).

In the commercial exploitation of cork oaks, this process is repeated successively and a new phellogen and periderm are formed as described. This ability to develop each time a new periderm is the basis for the sustainability of the cork production and cork oak exploitation.

Cork Cellular Structure

The cork cells are mostly hexagonal prisms that are stacked by their bases in radially aligned rows disposed in parallel without intercellular voids (**Figure 4**). Therefore the cork cells appear as a honeycomb structure in the tangential section (**Figure 4A**) and as a brick-wall structure in transverse (**Figure 4B**) and radial sections (**Figure 4C**). On average the cell prism height is 30–40 μm and the cell wall thickness 1–1.5 μm (**Table 2**). It is possible to observe annual rings that are marked by the presence of a layer of latecork cells at the end of the growth season with a shorter prism

TABLE 5 | Suberin composition (mass % of the total compounds detected by GC) of the cork of *Quercus suber*; *Quercus variabilis*, *Kielmeyera coriacea*, *Plathymenia reticulata*, *Quercus cerris*, *Betula pendula*, and *Pseudotsuga menziesii*.

Species		ω-Hydroxyacids	α,ω-Diacid	Fatty acids	*n*-Alkanols	Ferulic acid	Other compounds	Unidentified
Q. suber	Virgin cork[a]	47.0	11.7	5.3	2.0	6.0	1.0	27.0
	Reproduction cork[b]	30.6	53.0	n.d.	0.5	0.6	11.6	3.7
Q. variabilis[c]	Virgin cork	62.8	21.7	5.2	1.7	3.6	4.9	0.1
	Reproduction cork	54.5	34.4	3.6	1.5	3.0	3.0	0.1
K. coriacea[d]		54.2	31.6	1.4	2.8	1.7	–	8.3
P. reticulata[e]		48.6	30.1	10.5	2.7	3.9	1.8	2.4
Q. cerris[f]		85.9	7.4	n.d.	1.4	0.7	–	2.7
B. pendula[g]		74.6	15.9	n.d.	0.2	1.9	2.3	5.1
P. menziesii[h]		36.2	18.6	6.2	6.2	1.4	6.8	24.6

[a]*Calculated from Bento et al. (2001).*
[b]*Calculated from Graça and Pereira (2000).*
[c]*Ferreira et al. (2016a).*
[d]*Calculated from Rios et al. (2014).*
[e]*Mota et al. (2016).*
[f]*Sen et al. (2010).*
[g]*Ferreira et al. (2016b).*
[h]*Ferreira et al. (2015a).*
n.d., no data available.
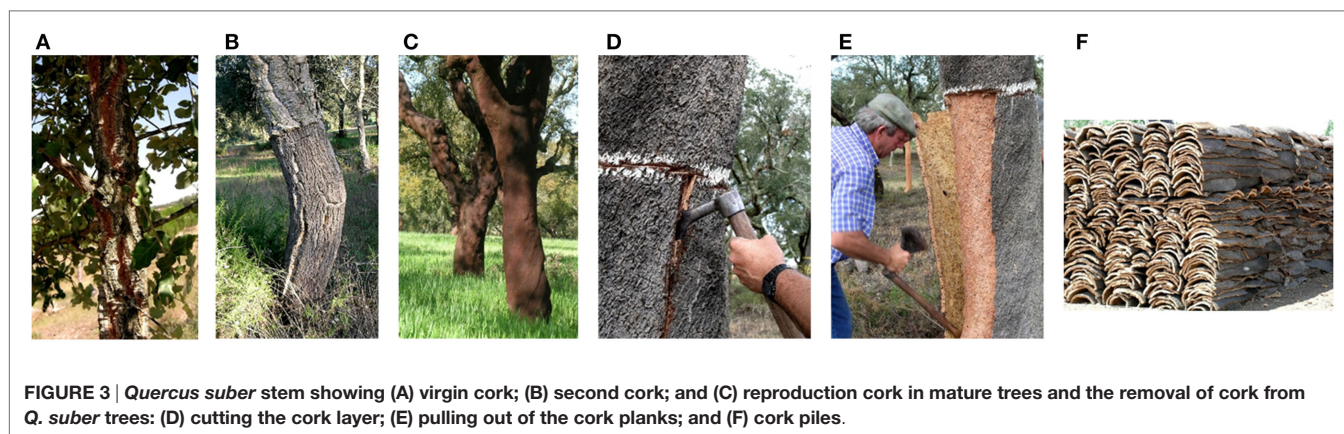
FIGURE 3 | *Quercus suber* stem showing (A) virgin cork; (B) second cork; and (C) reproduction cork in mature trees and the removal of cork from *Q. suber* trees: (D) cutting the cork layer; (E) pulling out of the cork planks; and (F) cork piles.
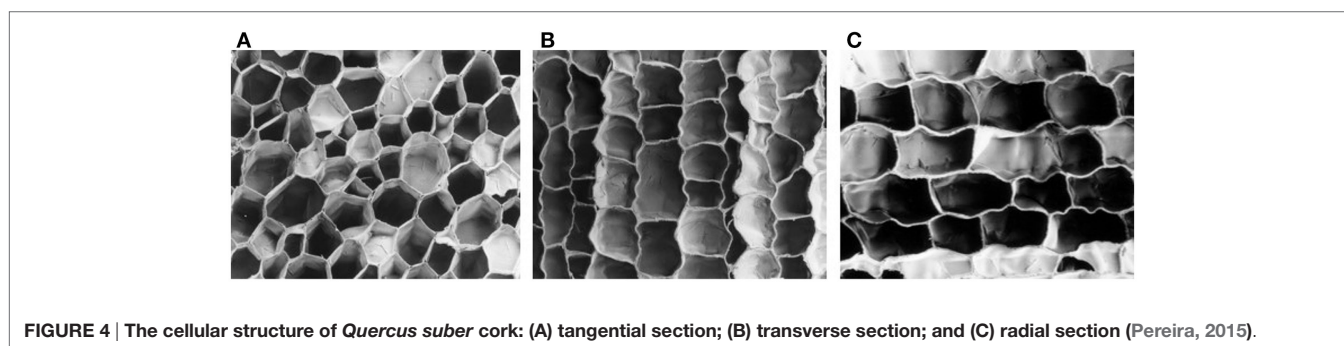
FIGURE 4 | The cellular structure of *Quercus suber* cork: (A) tangential section; (B) transverse section; and (C) radial section (Pereira, 2015).

height (10–15 μm) and a thicker cell wall (2–3 μm) in comparison with the earlycork cells.

The solid fraction in the cork is 8–9% in the earlycork and 15–22% in the latecork region (Pereira, 2007), which justifies the low density of cork.

An important structural characteristic is the corrugation of the radial aligned cell walls that arise from compression stresses during cork growth, i.e., the new cell layers compress the already existing cork cells by pushing them toward the exterior. Sometimes the cell wall corrugation may be strong, especially in virgin cork (Pereira et al., 1987).

Lenticels are present and develop as lenticular channels that cross radially the cork layers; they cause the so-called porosity of cork that appears as more or less circular in tangential sections

of cork and as thin strips in the other sections. Cork porosity has been extensively characterized because it is a visual quality parameter that defines the commercial quality of cork stoppers (Pereira et al., 1996; Costa and Pereira, 2007; Oliveira et al., 2012, 2015).

Chemical Composition

Reproduction cork has on average 16% extractives, 43% suberin, 22% lignin, and 19% cellulose and hemicelluloses (**Table 3**). The extractives include non-polar compounds that are extracted by solvents such as dichloromethane (representing on average 5.8% of cork) and polar compounds solubilized by ethanol and water (5.9 and 4.5%, respectively) (Pereira, 2013).

Suberin is the most important component of *Q. suber* cork. Its composition regarding the long-chain lipid monomers is shown in **Table 5**. In reproduction cork, the α,ω-diacids are the most abundant monomers (53.0% of the monomers), followed by ω-hydroxyacids (30.6%). The most abundant single monomer is the 9-epoxyoctadecanedioic acid (26.7%), followed by 22-hydroxydocosanoic acid (9.2%), 9,10-dihydroxyoctadecanodioic acid (9.0%), and 9-epoxy-18-hydroxyoctadecanoic acid (8.5%). In terms of chain length, most fatty acids have 18 and 22 carbons, representing, respectively, 66.2 and 14.4% of the total monomers. Suberin also contains, as a major monomer, glycerol that accounts on average to 8.5 or 14.2% of the suberin (Graça and Pereira, 2000; Pereira, 2015).

Lignin is the second most important component in the cork cell wall. Cork lignin is a G-type lignin composed by 95% guaiacyl units, 3% syringyl units, and 2% 4-hydroxyphenyl units (Marques et al., 2006; Marques and Pereira, 2013) with 80% of the inter-unit linkages as β-*O*-4-alkyl-aryl ether bond (Marques et al., 2016).

The hemicelluloses are mainly composed of xylose and arabinose, with smaller amount of galactose and mannose (**Table 4**).

There is a substantial chemical variability of *Q. suber* cork regarding between-tree and between-site differences that has been addressed in several studies (Pereira, 1988, 2013; Conde et al., 1998; Bento et al., 2001; Sen et al., 2016a). The content of suberin is the most important chemical attribute of cork since it is its chemical fingerprint and is directly related to most of its typical properties (Pereira, 2015).

Utilizations

Cork oak forests extend to about 2.2 million ha and produce annually up to 200,000 tons of cork that feed an important industry (APCOR, 2015). Portugal is the main producer of raw cork and of cork products that include mainly cork stoppers for the wine industry and insulation and surfacing boards (Pereira, 2007). The production of cork is based on the periodical removal of the cork layer by cutting large planks that are pulled out from the stem (**Figures 3D–F**). The cork removal is made usually in June–July, when the tree and the phellogen are active and allow an easy separation of the cork. The period between cork removals is usually 9 years in the major producing regions, which allows a thickness of the cork plank suitable to produce the cork stoppers (>24 mm). The cork oak silvicultural system is directed for cork production and is regulated, e.g., the minimum period between

cork removals or the extension of the debarking, aiming at maintaining the overall sustainability of the forests.

The cork planks are primarily directed for the production of natural cork stoppers that are bored out from the planks in the axial direction (Costa and Pereira, 2010). If the cork planks are too thin to allow this, they are directed to produce cork discs to be used for sparkling wine stoppers. The residues of these production lines, as well as other unsuitable cork pieces (for instance, virgin cork) are triturated, and the cork granules are agglomerated with suitable adhesives for production of technical wine stoppers, insulation boards, flooring and surfacing boards, joint sealants, and damping and shock absorption layers, among other usages (Pereira, 2007).

The cork of *Q. suber* has been comprehensively studied. Nevertheless, areas of interest remain where more knowledge and research are needed, e.g., fundamental studies on the topochemistry and 3D architecture of the cork cell wall, the extent and causes of natural variability in chemical composition and cellular features, and how these relate with properties, as well as innovative approaches to product development and new uses.

Quercus variabilis

Quercus variabilis (Chinese cork oak) is a species with a distribution area in China, Korea, and Japan; in China, it is found mostly in the Shaanxi province and in neighboring western Hubei and eastern Sichuan provinces (Zhang and Lu, 2002; Zhou et al., 2010). In China, it is exploited for cork production but with less importance than the cork oak in Europe, although cork production has increased in the last years to approximately 50,000 tons in conjunction with the marketing of some cork products (Zhao et al., 2013; Ferreira et al., 2016a). The tree is medium-sized to large, growing up to 25–30 m, and the bark forms a continuous periderm around the stem with a thick layer of cork, as in the cork oak, and the stem appearance of these two species is similar (**Figure 5**).

Some research about this species can be found, mainly in China, Japan, and Korea as demonstrated by several authors [e.g., Kim et al. (1990), Kim (1993), Wei et al. (2007), YaFang et al. (2009, 2012), Zhang et al. (2009), and Miranda et al. (2013)], but with the limitation that most of it is not written in English. Recently, Ferreira et al. (2016a) analyzed the virgin and reproduction cork, comparing their chemical and cellular characteristics with those of the cork oak.

Cork Cellular Structure

The cork cells from *Q. variabilis* are polygonal in a honeycomb-like arrangement in the tangential section, while the transverse and radial sections show a brick-wall structure with an alignment in parallel rows (**Figure 2**) (YaFang et al., 2009; Miranda et al., 2013; Ferreira et al., 2016a). Cork cells are prismatic, and the cell walls present corrugations, especially the earlycork cells that form against the previous latecork cells (Miranda et al., 2013; Ferreira et al., 2016a).

Like in the cork oak, the Chinese cork has rings with larger earlycork cells and with thinner walls than latecork cells. The average ring width of *Q. variabilis* is significantly smaller than in *Q. suber* cork (0.82 vs. 2.06 mm), and each ring has two to
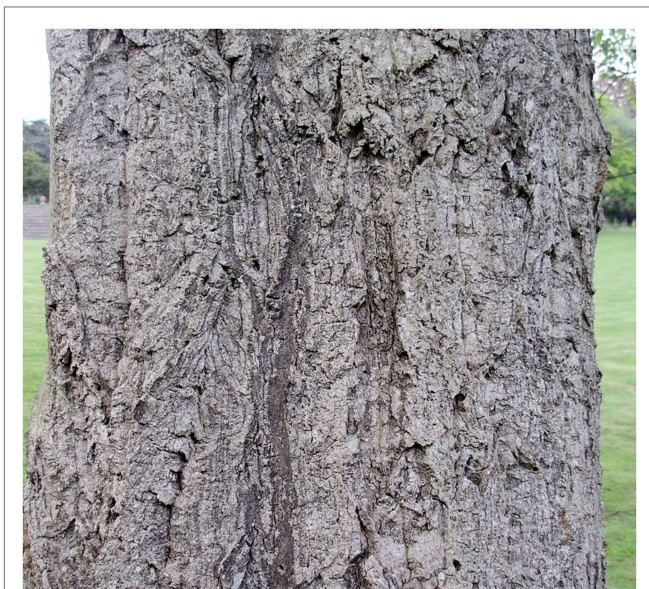
FIGURE 5 | *Quercus variabilis* stem—"Chinese cork oak bark" by **Velela—own work**. Licensed under Public Domain *via* Commons https://commons.wikimedia.org/wiki/File:Chinese_cork_oak_bark.jpg#/media/File:Chinese_cork_oak_bark.jpg.

six latecork cells representing 14% of the ring width (Kim, 1993; Miranda et al., 2013; Ferreira et al., 2016a).

Prism height in the earlycork cells of the reproduction cork of *Q. variabilis* is smaller than in *Q. suber* (23 vs. 30–40 μm, **Table 2**). The solid fraction in earlycork is, therefore, higher in the Chinese cork than in *Q. suber* cork (13 vs. 8–9%) (Miranda et al., 2013; Ferreira et al., 2016a). Consequently, *Q. variabilis* cork has higher density than *Q. suber*, what constitutes a disadvantage as an insulating material (Kim, 1993; Miranda et al., 2013). Another difference between these two corks is the appearance of the cell wall: the lumen side of the cork surface of *Q. variabilis* is rough with deposits of several dimensions, while in *Q. suber*, it is smooth and with only occasional deposits (Miranda et al., 2013).

Like in the cork oak, lenticular channels cross radially the cork layer with a circular or elliptical shape in the tangential section and with filling tissue (Kim, 1993; Ferreira et al., 2016a).

Chemical Composition

Virgin and reproduction cork of *Q. variabilis* are chemically very similar: on average 0.9% ash, 9.2% extractives, 37.8% suberin, and 28.5% lignin. The composition is within the variation range of reproduction cork from *Q. suber* (**Table 3**) although with less extractives, with lower levels of non-polar compounds extracted by dichloromethane (YaFang et al., 2009; Miranda et al., 2013; Ferreira et al., 2016a). The ratio suberin/lignin is 1.4 (virgin cork) and 1.3 (reproduction cork), lower than the average of 2.0 in *Q. suber* (Ferreira et al., 2016a). Given that most of the properties of cork result from the joint presence of suberin and lignin in the cell wall and their relative proportion, the higher proportion of lignin will give *Q. variabilis* cork higher compressive strength than *Q. suber* (Pereira, 2013).

Polysaccharides (**Table 4**) show a large presence of xylan-based hemicelluloses and a comparable proportion of cellulose (glucose amounts to 52% of the neutral sugars).

Suberin composition (**Table 5**) shows a substantial difference in relation to the reproduction cork from cork oak: *Q. variabilis* cork has more ω-hydroxyacids and less α,ω-diacids. The main monomers in *Q. variabilis* suberin are 22-hydroxydocosanoic acid (35.1–21.4%), 18-hydroxy-9-octadecenoic acid (22.0–17.1%), and 9,10-epoxy-18-hydroxyoctadecanoic acid (10.0–10.2%) (Ferreira et al., 2016a). Epoxyacids have lower contents (13.3–14.4%, Ferreira et al., 2016a) than in *Q. suber* suberin (30%, Graça and Pereira, 2000). The relation between the saturated and the substituted acids in *Q. variabilis* suberin is 1.0–0.7 (0.4 in *Q. suber)* (Ferreira et al., 2016a). Therefore, it is to be expected that suberin spatial development may be more compact in *Q. variabilis* cork. Ferulic acid is also present in the suberin extracts (3.6–3.0%), enforcing the conclusions about its function in the cross-linking between suberin and lignin (Marques et al., 2016).

Utilizations in Perspective

Cork of *Q. variabilis* is at present already harvested, industrially processed, and commercialized. Due to the characteristics of the raw material, it cannot be used for production of solid cork products, namely for wine stoppers; instead, it is granulated and used in agglomerates for various surfacing and insulation applications.

The cork from *Q. variabilis* presents lower quality than that from *Q. suber*, namely, higher density, compressive strength, and elasticity. However, this cork presents characteristics that are compatible with its use as an insulating, sealing, and energy absorption material and may be considered as a complementary raw material for cork from cork oak (Miranda et al., 2013; Ferreira et al., 2016a). The cork from *Q. variabilis* can also be a potential source of friedelin or of betulinic acid, due to the high levels of these triterpenes in the extractives (Ferreira et al., 2016a).

The exploitation of this species as a cork provider is far from being developed and optimized as it is for *Q. suber* and its cork, e.g., the development and improvement of *Q. variabilis* silviculture and forest management as well as of the respective cork industry may overcome the present constrains. More knowledge and research are certainly needed in the tree physiology related to the bark and cork formation that would complement applied silvicultural studies. The variation of cork characteristics between trees and geographical locations as well as with tree age is also important for improving products and industrial processes.

Kielmeyera coriacea

Kielmeyera coriacea is natural from the savannah-type ecosystems of the Brazilian *cerrado*. The trees are in general 1–4 m high (Rios, 2007) and the stem is covered by a conspicuous cork bark (**Figure 6**).

The bark can be several centimeters thick and has a periderm with 1.1–1.8 cm of cork and only a few inclusions of phloem (Rios et al., 2011). The cork tissue may be detached from the stem and the tree has a high regeneration capacity of the periderm and its

FIGURE 6 | *Kielmeyera coriacea* tree in the Brazilian cerrado (Minas Gerais) and its cork bark.

cork; this has led to suggestions of exploitation with successive cork removals at 5- to 6-year rotations (Souza, 1974; Lima and Marcati, 1989).

The phloem inclusions appear as thin bands, approximately tangentially oriented and parallel to the phellogen, representing from 10 to 25% in area. Lenticels are present and correspond from less than 1 to 15% of the cork cross section. Together phloem inclusions and lenticels represent between 12 and 40% of the outer bark area (Rios, 2011).

Cork Cellular Structure

The cellular structure of *K. coriacea* cork is similar to that of *Q. suber* cork. The cork cells are hexagonal prisms, disposed in a honeycomb-like arrangement (**Figure 2**); cell wall thickness is between 1.5 and 2 μm, and cell length in the radial direction is significantly higher than in cork oak cork (**Table 2**).

Chemical Composition

The chemical analysis of *K. coriacea* virgin cork, shown in **Table 3**, includes not only the cork tissue but also its phloem inclusions. The content of suberin is from 16 to 30%, and this species presents higher contents of lignin than cork oak cork with 43.6–55.3% (Rios et al., 2014).

Table 4 shows that xylans are the most important hemicelluloses in the virgin cork from *K. coriacea*; the glucose proportion is higher than in cork of *Q. suber* and of all the other reported species.

Kielmeyera coriacea cork suberin (**Table 5**) is chemically composed mainly by ω-hydroxyacids and α,ω-diacids have a lower proportion (Rios et al., 2014). The main ω-hydroxyacids are the C18 9,10 mid-chain substituted ones, namely, with an unsaturated

group (18-hydroxyoctadec-9-enoic-acid) and a vicinal diol group (9,10,18-trihydroxyoctadecanoic acid). The α,ω-diacids include the octadec-9-ene-1,18-dioic acid and 9,10-dihydroxyoctadecane-1,18-dioic acid. This suberin does not contain acid monomers with saturated chains, either ω-hydroxyacids, α,ω-diacids, or *n*-alkanoic acids (Rios et al., 2014).

Utilizations in Perspective

There is no industrial utilization of *K. coriacea* bark as a raw material for cork products. However, its exploitation as a cork provider has been already suggested (Natividade, 1950), namely, for the production of cork agglomerates (Rios et al., 2014) since the homogeneity of the cork layer does not seem suitable as a raw material for solid cork products. The cellular and chemical characteristics of *K. coriacea* cork are compatible with the common uses of cork, namely, as an insulation and surfacing material after trituration and agglomeration. Its use as a source for bio-based chemicals was also suggested, e.g., xantones and long-chain fatty acids from the extractives, or the bifunctional ω-hydroxyacids and α,ω-diacids monomers from suberin (Rios et al., 2014). However, any further considerations on the potential use of *K. coriacea* cork will require studies on its characterization as well as on tree-related aspects of cork development.

Plathymenia reticulata

Plathymenia reticulata is a species native to South America that vegetates in the Atlantic forest and, as also *K. coriacea*, in the *cerrado*, a particular savannah-type ecosystem. It grows up to 30 m but in the *cerrado* it is generally smaller reaching only 5 m. Its most important applications are relative to the wood which

is rot-resistant and widely used as a structural timber and in high-end carpentry (Carvalho, 2009). The stem has a cork bark, as shown in **Figure 7**.

The only study on the cork of this species is reported here, giving data on chemical composition and cellular structure (Mota et al., 2016).

Periderm Development

The bark of this species presents an internal thick brown layer of phloem that is covered by a periderm containing a conspicuous light brown layer of cork with deep fissures that result from the tangential growth stress. The periderm develops continuously around the stem with a lifespan probably equal to the tree age and with an annual regular activity of the phellogen (Mota et al., 2016).

Cork Cellular Structure

Plathymenia reticulata cork cellular structure has no substantial difference from the one of *Q. suber* cork. The cork cells are polygonal, in a honeycomb-like arrangement (as in **Figure 2**), cell wall thickness is within the variation range observed for *Q. suber* but prism base edge is much higher than in cork oak (**Table 2**).

The cork from *P. reticulata* shows growth rings, with fewer and smaller latecork cells (two to four cells in a radial row) and more and larger earlycork cells (six to nine cells), although the differences between earlycork and latecork are not so pronounced as in the cork oak cork, leading to a more homogeneous material (Mota et al., 2016). Large variations of size between cells were observed, either in the tangential or in the non-tangential sections. Like in *Q. suber,* the cork cells of this species have undulations in the non-tangential sections as well lenticular channels, sometimes with filling tissue.

FIGURE 7 | Stem cross section of *Plathymenia reticulata,* a tree from the Brazilian cerrado (Minas Gerais).

Chemical Composition

The summative chemical composition is included in **Table 3** (Mota et al., 2016). The important feature is that lignin is the major structural component and not suberin (34.5 and 24.7%, respectively). This indicates a low value of the suberin/lignin ratio of 0.7; this was also found by Rios et al. (2014) for the other *cerrado* species *K. coriacea*. In comparison with the cork of *Q. suber* although there is a large variability in the chemical composition values, the ratio suberin:lignin is quite above this value (Pereira, 2013). This chemical difference certainly will bring differences in the chemical behavior, as discussed in Pereira (2015).

In *P. reticulata* cork, xylan-based hemicelluloses prevail with a considerable proportion of arabinose (**Table 4**), including acetyl substitutions and uronic acid monomers (Mota et al., 2016).

The suberin composition (**Table 5**) shows more ω-hydroxyfatty acids and less α,ω-diacids than in *Q. suber* reproduction cork suberin. The main individual suberin monomers are the 18-hydroxyoctadecanoic acid (saturated and substituted), the octadecanodioic acid, the tetracosanoic acid, and the hexacosanoic acid while only minor values of the 9-epoxyoctadecandioic acid (<1%) were found (Mota et al., 2016), contrarily to what happens in the suberin from cork oak where it is a main component.

Utilizations in Perspective

The cellular and chemical characteristics of *P. reticulata* cork are compatible with the common uses of cork, namely, as an insulation and surfacing material after trituration and agglomeration (Mota et al., 2016). Other potential utilizations were identified, namely, as a biosorbent and as a raw material for extraction of valuable compounds like lupeol, fatty acids, and terpenoids.

However, in order to fully develop the utilization of *P. reticulata* cork as a raw material for cork products under a sustainable cork production management, further research is needed to understand how periderm regenerates after cork removal and the characteristics of the subsequent cork layers. Should the cork be obtained as a by-product of tree exploitation for timber, i.e., the bark is produced as a residue, then its valorization for cork agglomerates is possible straightaway. In any case, studies on P. properties, e.g., density, compression, and insulation properties are needed.

Quercus cerris

Quercus cerris L. (Turkey oak) is a medium-sized tree that can reach up to 30 m. It has a distribution area from central and south-eastern Europe to Asia Minor (Sen et al., 2011b).

Quercus cerris bark (**Figure 8**) is thick (3–7 cm) with a brown grayish color, hard to the touch, and with short deep longitudinal furrows. It is composed of phloem, periderm, and a considerable proportion of rhytidome. The rhytidome has sequential periderms with phloem tissue between them. The cork is clearly distinguished forming patches of variable radial thickness that are non-continuous tangentially and axially. The external periderms of the rhytidome do not shed (Sen et al., 2011b).

There are some records on the utilization of *Q. cerris* cork as a substitute from *Q. suber* cork for agglomerates and stoppers (Sen et al., 2011b). Nowadays, neither the wood nor the bark of

FIGURE 8 | *Quercus cerris* **logs (Turkey) and bark cross section**.

this species is economically exploited, and until recently, very little research was made on the characterization of its cork.

Periderm Development

Quercus cerris has a conspicuous cork presence in the rhytidome as layers with a radial width from about 1 to 10 mm, while the phelloderm is composed of a layer of only two or three thin-walled cells (Sen et al., 2011b).

The phellem layers present 2–5 growth rings, and each ring is composed of about 6–12 layers of phellem cells more or less radially aligned without intercellular voids. The phellem cells are suberized, with thin walls with a uniform thickness in the tangential and radial walls and are sometimes radially flattened. Like in *Q. suber,* at the beginning of the growth ring, cells are larger with thin walls, while at the end of the growth ring, they are smaller and have thicker walls. Despite this similarity, both species have different intensities of phellem growth. In fact, in turkey oak, each phellogen cell produces only 6–12 phellem cells/ring in each radial row (Sen et al., 2011b), and in *Q. suber*, this figure is about 10–20 in young plants (Graça and Pereira, 2004) and up to 100 in mature trees (Pereira et al., 1992). It is also to remark that in this species, but not in *Q. suber*, one or two layers of the cork cells, in the limit of each growth ring, can thicken up and become very lignified (Sen et al., 2011b).

Lenticular channels crossing radially the periderm are rare and without filling material. This feature can be explained because in turkey oak cork layers are not continuous around the stem, as it happens in cork oak, and therefore, gas exchange between the innermost living tissues and the exterior does not require the development of these channels (Sen et al., 2011b). The cork tissue also includes lignified phloem cells (fibers and sclereids).

In what concerns phellogen longevity, it is probable that its lifespan is around 25 years (Sen et al., 2011b) but, the fact that each periderm presents from two to five rings suggests that phellogen activity is not annual and that the rings cannot be considered as successive annual rings (Sen et al., 2011a).

Cork Cellular Structure

The cork cellular structure of *Q. cerris* is very similar to the one presented in Section "Cork Structure and Chemical Composition" for *Q. suber* cork (**Figure 2**). In fact, the cork cells form a

bidimensional network of edges and vertices without intercellular voids. The cells are mostly hexagonal prisms, stacked base-to-base disposed in parallel rows aligned in the radial direction, in a compact space-filling arrangement. Frequently, in adjacent rows prism bases coincide with each other or lay in staggered positions (Sen et al., 2011a).

The cell walls also present corrugations. The intensity of corrugation is greater in *Q. cerris* than in *Q. suber*, possibly due to the thicker cell walls and to the more irregularity in the stress radial-growth distribution along the cork tissue as a consequence of the presence of phloem between the cork layers (Sen et al., 2011a).

Cork ring width is on average 201 µm and contains seven to nine earlycork cells and two to four latecork cells in each radial row (Sen et al., 2011a). Dimensions of earlycork and latecork cells are presented in **Table 2**. Prism base edge and cell wall thickness are higher in *Q. cerris* cork than in *Q. suber* cork, and therefore, *Q. cerris* cork has a higher density (Sen et al., 2011a).

Chemical Composition

The chemical composition of *Q. cerris* cork is given in **Table 3**. Suberin is the major cell wall structural component of the cork from *Q. cerris* but almost in the same proportion as lignin; the suberin:lignin ratio is 1.0. In the polysaccharides, that represent almost 16.7%, glucose is the major monosaccharide (**Table 4**) but important contents of xylose, arabinose, and galactose are also present. This composition suggests that the main hemicelluloses are xylans (Sen et al., 2010).

In *Q. cerris* cork suberin, ω-hydroxyacids represent almost 90% of all long-chain monomers (**Table 5**). The single most abundant compound is the vic-diol mid-chain substituted ω-hydroxyacid in C18: the 9,10,18-trihydroxyoctadecanoic acid represents about 54% of all long-chain monomers. The saturated ω-hydroxyacid in C22 represents 20% and the unsaturated ω-hydroxyacid 18-ω-hydroxyoctadec-9-enoic acid 11%. The α,ω-diacids are present in less than 8% of the total suberin and are constituted mostly by vic-diol substituted 9,10-dihydroxyoctadecanedioic acid with 5% of the long-chain monomers (Sen et al., 2010). Alkanoic acids in C16 and C18 as well as alkanols in C20, C22, and C24 represent a small part of the suberin (Sen et al., 2010). The C18 monomers are the most abundant, followed by C22 monomers, and their relative amounts are not very different from the ones found in

cork oak (Sen et al., 2010). The major quantitative difference between *Q. cerris* suberin and *Q. suber* reproduction cork suberin lies on the relative proportion of α,ω-diacids and ω-hydroxyacids which is much lower in the first one (**Table 5**).

Utilizations in Perspective

Due to the characteristics of *Q. cerris* bark, the industrial utilization of its cork requires previous trituration and fractioning by size and density in order to obtain pure cork or cork-enriched fractions. Therefore, the use of cork is limited to granules and the production of cork agglomerates. A pilot-scale fractioning of *Q. cerris* bark was already made (Sen et al., 2016b): a yield of 8.4% cork pure granulates suitable for agglomerated stoppers was obtained as well as 18.5% of cork-rich fractions usable in the production of surfacing and other cork agglomerates.

Considering the comparative cost advantage of the *Q. cerris* cork, it seems economically interesting to integrate it into the present cork industrial production lines, thereby allowing to enlarge the cork raw-material supply base. However, further studies are required for process optimization and for the development of *Q. cerris*-specific cork-based products.

Betula pendula

Birch (*B. pendula*) covers a large part of the Eurasian continent, from the Atlantic to eastern Siberia (except Iceland, the Iberian Peninsula, and Greece) but is present mainly in northern Europe where it is one of the most important species for pulp production (Hultén and Fries, 1986). Birch is a deciduous medium-sized tree, usually 15–25 m high but can reach 30 m.

In 2013, the European pulp and paper industry consumed about 19.6 million m³ of birch wood, thereby generating a large amount of bark (CEPI, 2013). Given a content of outer bark at 3.4% (Jensen, 1948), an annual estimate potential availability of birch outer bark at about 900,000 tons may be made.

The outer bark of birch has been used traditionally in small handicrafts, e.g., boxes, for canoe building, and for the still in-use roof coverings, but it is at present mainly used for energy.

Periderm Development

Betula pendula bark has a rhytidome with numerous tightly packed periderm layers that consist of thick-walled cork cells inwards and thin-walled cells outwards (Schönherr and Ziegler, 1980), that resemble annual increments produced in the early and late season of the growth period (Ferreira et al., 2016b). The birch periderm contains alternate layers of strongly suberized cells (five layers of cells with tangential walls up to 2 μm thickness) with a variable number of layers with little or no suberization (Schönherr and Ziegler, 1980). The cells are mostly flattened in the radial direction and stretched in the tangential direction and show many cell wall deposits on the lumen side (Ferreira et al., 2016b). **Figure 9** shows SEM photographs of the cross section of birch outerbak.

Birch bark is described as having a rhytidome composed by periderms with successive cork layers and phloem layers with several sclerenchymatic tissues (fibers, sclereids, cortical, and parenchyma cells) and showing conspicuous lenticels that contain suberized and non-suberized cells disposed in stratified layers (Bhat, 1982; Trockenbrodt, 1991; Ferreira et al., 2016b). Suberized layers are continuous across the lenticels and maintain in between the loose tissue together, breaking up successively as a new phellogen is formed. Intercellular spaces with diameters up to 5 μm are seen in the tangential section across the lenticels that represent about 3% of the total periderm area (Schönherr and Ziegler, 1980).

Chemical Composition

There are a few studies on *B. pendula* bark chemical composition made in the 70s and 80s of last century [e.g., Holloway (1972, 1983), Holloway and Deas (1973), Ekman (1983), and Ekman and Eckerman (1985)] and more recently on suberin composition [e.g., Gandini et al. (2006) and Ferreira et al. (2013, 2016b)]. It should be noticed that several of the studies were made with the whole birch outer bark instead of only the cork fraction, thereby including the non-suberized cells.

Betula pendula cork contains a large amount of extractives of about 32% (**Table 3**) mostly corresponding to lipophilic extractives, i.e., 98% of the total extractives are dichloromethane solubles. Their composition shows the striking dominance of triterpenoids (90–97% of all compounds) where betulin corresponds on average to 71% of the extract; the other compounds are lupeol (on average 14%) and betulinic acid (4%) (Ferreira et al., 2016b).

Suberin represents 36% of cork (Ferreira et al., 2016b) corresponding to 53% on extractive-free cork that are in agreement with previous reported values of 59% (Holloway, 1983) and 51% (Holloway and Deas, 1973). Lignin amounts are 14% of the cork, and therefore, the suberin:lignin ratio is 2.5.

Cellulose and hemicelluloses represent only 10% of *B. pendula* cork with most hemicelluloses formed by arabinose and xylose (**Table 4**).

The suberin from *B. pendula* (**Table 5**) contains aliphatic ω-hydroxyacids of chain lengths between C16 and C26, with a large proportion of components in C18 (73% of all long-chain monomers) and C22 and with smaller quantities of fatty acids and neutral constituents (Holloway, 1972). In comparison with *Q. suber* reproduction cork, birch cork suberin contains much more ω-hydroxyfatty acids and much less α,ω-diacids. In fact, the main individual suberin monomers are the 9,10-epoxy-18-hydroxyoctadecanoic acid (30% of total monomers), the 22-hydroxydocosanoic acid (13%), the 9,10-dihydroxyoctadecandioic acid (12%), and the 18-hydroxyoctadecanoic acid (12%) (Ferreira et al., 2016b).

Utilization in Perspective

Birch cork does not have the structural and cellular characteristics that impart the properties that have made cork so interesting as a material. The cell biometry, e.g., the radially flattened cells and the cork thickness of the periderms, the weak tangential cohesion, and the heavy inclusion of lumen deposits do neither allow a prospective utilization for stoppers nor for the most common applications of cork as a cellular material (Ferreira et al., 2016b). Consequently, this cork main utilization and valorization will be as a chemical source.

Due to its high content in suberin, birch outer bark has been proposed as a source of monomers, e.g., of ω-hydroxyfatty acids,
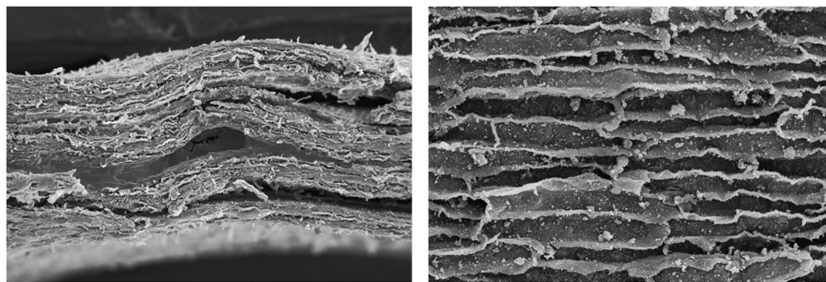
FIGURE 9 | SEM photograph of a transverse section of *Betula pendula* outerbark and of cork cells.

FIGURE 10 | *Pseudotsuga menziesii* stem, cross section of the bark and SEM photograph of a transverse section of cork.

α,ω-dicarboxylic acids, and homologous mid-chain dihydroxy or epoxy derivatives for production of novel macromolecular materials (Gandini et al., 2006), polyesters (Olsson et al., 2007; Sousa et al., 2007), polyols (Evtiouguina et al., 2000, 2002), polyurethanes (Cordeiro et al., 1997, 1999; Evtiouguina et al., 2001) and has also been considered as a source of extractives, namely, of triterpenoids and in particular of betulinol (Pinto et al., 2009). Some patents have already addressed the possibility of using birch bark as a source of chemicals (Krasutsky et al., 2003, 2005, 2009).

Pseudotsuga menziesii

Douglas-fir (*P. menziesii*) is a conifer native from western North America, occupying approximately 14.4 million ha in the USA and 4.5 million ha in Canada (Weiskittel et al., 2012) and introduced into many parts of the temperate regions of the world, including Europe (Lavender and Herman, 2014). It is a very important species for timber production in its natural distribution area and also in Europe. The height of *P. menziessi* mature

trees depends on site location, but it can reach up to 100 m (Lavender and Herman, 2014).

The bark contains a substantial proportion of cork from 25 to almost 50% (Kurth, 1953; Ross and Krahmer, 1971; Krahmer and Wellons, 1973), and cork formation occurs at a relatively early age (Hergert and Kurth, 1952). Douglas-fir bark presents a great variety in thickness, density, chemical composition, and cork content according to site quality, tree age, and axial position on the tree (Kurth and Kiefer, 1950; Hergert and Kurth, 1952; Ross and Krahmer, 1971).

The stem appearance is shown in **Figure 10**. The rhytidome contains alternated bands of phloem (dark brown) with cork tissues (light cream brown) very well distinguishable to the naked eye.

Periderm Development

The development of the first periderm in *P. menziesii* is similar to the process reported in Section "Bark Structure and Formation"

and when it dies, a new phellogen forms inside the phloem (Grillos, 1956). The new phellogen does not form a whole circumference around the tree, but only in short portions around and along the length of the stem; therefore, the cork layer is interspersed with small parts of inactive crushed phloem (Grillos, 1956; Krahmer and Wellons, 1973). Douglas-fir rhytidome is composed by approximately 49% (in volume) of dead phloem and 51% of periderms (Patel, 1975).

Although the first phellogen can be functional for 25 to 35 years, only the deeper phellogens produce enough cork that show distinct increments (Grillos, 1956).

Most of the periderm is formed by cork cells disposed in well-oriented radial rows and the phelloderm layer is between one and three cells thick (Chang, 1954; Grillos, 1956; Krahmer and Wellons, 1973). The cork thickness is very variable between trees, in average 1.8 cm at breast height ranging from 2 cm to less than 1 mm at root collars and from 5 to 40 cell layers (Kurth, 1953; Grillos, 1956; Ross and Krahmer, 1971). At any particular tree height, the number of cork growth increments is inferior to the age of the tree (Ross and Krahmer, 1971).

One striking feature of Douglas-fir cork is the major presence of bands of compressed cork cells that build up a compact layer of crushed cells due to a severe folding of the radial cell walls (Ross and Krahmer, 1971; Cardoso et al., 2016). These bands of high solid content appear as dark layers, with a successive tangential alignment, separated by light colored layers. These layers have been called as growth increments (Chang, 1954; Ross and Krahmer, 1971; Krahmer and Wellons, 1973).

Cork Cellular Structure

Pseudotsuga menziesii cork cells are in general thin-walled and uniform in thickness. Some thick-walled cells with small pores or pits are occasionally observed either disposed in bands or scattered (Chang, 1954; Grillos, 1956); when their proportion is high the cork is referred as "woody cork" (Grillos, 1956; Krahmer and Wellons, 1973).

Cork cells are more or less isodiametric, symmetrical in shape, and disposed without intercellular voids. On the transverse (**Figure 10**) and radial directions, they are similar, square to rectangular (if the cells have not been crushed); on the tangential direction, the cells are mostly pentagonal or hexagonal (Krahmer and Wellons, 1973; Cardoso et al., 2016).

Douglas-fir cork cell dimensions are presented in **Table 2**. The cell prism height and base edge are high and cell wall thickness is low; the radial walls are wrinkled due to growth stresses after the cell walls are formed (Grillos, 1956; Krahmer and Wellons, 1973; Cardoso et al., 2016); 60% of the cells observed by Cardoso et al. (2016) were crushed.

Chemical Composition

Pseudotsuga menziesii cork has a high content of extractives (**Table 3**) that are mostly polar compounds soluble in ethanol and water (23.5% of the cork) (Ferreira et al., 2015a). The lipophilic extracts (5.4% of the cork) contain as major component catechin (49% of the total), as well as pinoresinol and β-sitosterol (Ferreira et al., 2015a).

The cell walls are composed mainly of suberin (Krahmer and Wellons, 1973; Graça and Pereira, 2000). Suberin represents 36.2% of the Douglas fir cork and lignin 16.8% (Ferreira et al., 2015a). The suberin:lignin ratio is 2.2, a value similar to the one in *Q. suber* cork (Pereira, 2013).

The monosaccharide composition (**Table 4**) shows that hemicelluloses contain arabinoxylans and also galactoglucomannans, e.g., galactose and mannose represented 20.4% of the sugars, and xylose and arabinose 24.2%. This differs from the compositions of the other corks and is associated to the fact that Douglas-fir is a softwood (Ferreira et al., 2015a).

Suberin composition (**Table 5**) shows that the most important monomers are the ω-hydroxyacids, representing 36.2% of the total, followed by the α,ω-diacids (18.6%); chain lengths of C16 and C22 are more relevant (49% of the identified long-chain monomers) (Ferreira et al., 2015a).

When comparing with *Q. suber* cork suberin, the Douglas-fir suberin contains more saturated α,ω-diacids (hexadecanedioic and octadecanedioic acids), less unsaturated α,ω-diacids, and no epoxyacids (Graça and Pereira, 2000; Ferreira et al., 2015a).

Utilizations in Perspetive

The potential of Douglas-fir bark as a raw material, namely, of its cork component, was recognized since the 50s of last century and several studies were performed (Kurth and Kiefer, 1950; Hergert and Kurth, 1952; Kurth, 1953; Grillos, 1956).

Although there is a considerable proportion of cork in Douglas-fir bark, the small dimensions of the cork layers and their discontinuous distribution with interspersed phloem restrict its direct use and require previous bark milling and particle size separation (Ferreira et al., 2015a,b). An integrated bark valorization approach should contemplate the separation of cork-rich fractions to be further purified and used as a raw material for cork-based products (e.g., composites), while phloem-rich fractions can be used for the extraction of polar soluble compounds (e.g., phenols and polyphenols), while the polysaccharide–lignin matrix may be considered for biorefineries (Ferreira et al., 2015b).

The utilization of the Douglas-fir cork granulates in products that rely on the properties given by the typical cork cellular structure is hindered by the high proportion of crushed cells in this cork (Cardoso et al., 2016). Studies on structural improvement, e.g., cell expansion might therefore increase its utilization potential.

Abies lasiocarpa var. *arizonica*

Abies lasiocarpa var. *arizonica* (corkbark fir) is a gymnosperm that occurs naturally in the United States of America, namely, in the Arizona, Colorado, and New Mexico at about 2400–3400 m of altitude. The wood is used for general structural purposes and pulp manufacture. This species is a medium-sized tree growing up to 20 m, exceptionally to 40–50 m.[1] The bark forms a rhytidome and has a rough aspect.

---

[1]http://www.conifers.org/pi/Abies_lasiocarpa.php.

The only works found on the cork from the corkbark fir were from the 50s and the 60s of last century (Chang, 1954; Mogensen, 1968).

Periderm Development

The first phellogen initiates in the outermost layer of the cortex below the epidermis. It is long lived and remains active, producing a relatively large amount of cork before a new one is formed, deeper inside the phloem, initiating the formation of a rhytidome. The rhytidome starts at 43–88 years of age, depending on sun exposure (Mogensen, 1968). Before rhytidome formation, the external bark has a soft and spongy nature given by the phellem but becomes rough and hard to the touch after the formation of the rhytidome. The cork layers do not form a complete circumference around the stem and crack due to diameter growth, and the outer layers may wear away (Mogensen, 1968).

Within the cork, there are periodic rows of cells with thick and sclerified outer tangential walls that mark the separation between the growth increments which, in young stems, can be correlated with the age of the stem. The other cork cells are thin-walled and usually slightly elongated radially.

Utilizations in Perspective

The use of this species as a raw material for cork products seems possible since the initial phellogen remains functional for a long period and produces a large quantity of cork. However, little is known on the structure, chemical composition, and properties of this cork, and research is needed to evaluate its potential utilization.

Abies concolor

Abies concolor (white fir) is a gymnosperm that occurs throughout much of the mountainous western North America and, more scattered, in Mexico (Farjon, 2013). The trees are in general 12–15 m high and are exploited for lumber (Gilman and Watson, 2014). The bark in mature trees may be 10–15 cm thick at the stem base and is deeply furrowed, hard, and resistant to fire.

The outerbark contains salmon-colored corky layers interspersed with areas of dark red phloem; cork represents from 25 to 55%, depending on age and location on the stem (Hergert, 1958). Only a few studies were performed in the 50s of last century on white fir bark and on its cork fraction that was separated from air-dried pulverized bark by suspending in 60–85°C water and skimming off the cork particles (Hergert and Kurth, 1953; Hergert, 1958).

The chemical analyses of cork gave the following composition: 26% extractives removed with methylethylketone:water and water, 18.8% hydroxy fatty acids; 26.7% polyphenolic acids, 4.7% low-molecular-weight phenols mainly ferulic acid, 8.2% polysaccharides (mainly cellulose, with glucose amounting to 58% of the neutral monosaccharides), and 15.6% lignin (Hergert, 1958). It should be noted that these results were obtained with a chemical protocol that markedly differs to the more recent summative chemical analysis.

Utilizations in Perspective

The potential commercial value of the cork fraction of white fir bark was recognized early (Hergert, 1958). However, the knowledge is very scarce, and further research should be carried out to characterize this cork and its potential application value.

CONCLUDING REMARKS

This review shows that there are some species with a high content of cork in their barks which should be scrutinized, given the importance of cork as a raw material. The species with cork-containing barks may be classified as those with only one periderm and a continuous cork layer, e.g., *Q. suber*, *Q. variabilis*, and the *cerrado* species *K. coriacea* and *P. reticulata*, and those who form a rhytidome, therefore with cork layers interspersed with phloem, e.g., *P. menziesii* and *Q. cerris*.

The present cork industrial chain is based on the sustainable exploitation of cork from *Q. suber*, a species for which a dedicated silviculture and management have been fine-tuned along the centuries allowing a sustainable exploitation of the raw material; in succession, the cork industry has developed with sound and innovative technologies, and the body of knowledge on cork and the cork oak science is very large.

The cork from *Q. suber* is presently the only raw material that has the characteristics necessary for production of solid cork products, e.g., of wine natural cork stoppers, and for which the trituration and production of cork agglomerates are complementary production lines.

A new cork raw material has been brought recently to the market, the Chinese cork from *Q. variabilis*. Presently used only in triturated form, this raw material appears to have an interesting place given the already large amounts that are harvested as well as the existing forest potential, now still far from an adequate cork-targeted management. Research is under away, and it is foreseeable that in a few years the body of knowledge on this cork will increase substantially.

The valuable properties of cork, as benchmarked by the *Q. suber* cork, require an adequate combination of structural and cellular features with the chemical composition. This is met by the corks of several species, e.g., *K. coriacea*, *P. reticulata*, and *Q. cerris*, that despite species specificities regarding cell biometry and chemistry, have characteristics that allow forecasting "corkish" properties and uses. It is clear that knowledge on the raw material and on the species has to be gathered before any utilization attempts. This knowledge is scarce for most of the cases, with the exception of *Q. cerris* cork that has been recently under study and for which a pilot scale test has demonstrated the feasibility of cork separation and use.

The specific cellular characteristics may hinder or limit the use of cork as a cellular material. This is the case of *B. pendula* cork mainly due to cell biometry, and of *P. menziesii* due to the large proportion of heavily corrugated or collapsed cells. The use of such corks as a chemical source is, therefore, a promising valorization route. In fact, the extractives are a chemical component group that is receiving a lot of attention in research and development in various fields, including biomedical and

health care. Also suberin, the main structural component of cork cell walls, is a macromolecule with an unusual composition of long-chain fatty acids with different functional groups, e.g., hydroxyl, epoxide, and unsaturation, that is species specific and a potential source of chemical intermediates.

The available information gathered in this review on different species with cork-containing barks clearly shows that knowledge is still very limited or inexistent. Research on such barks, namely, regarding their structural and chemical characteristics, is, therefore, a first step toward their prospective valorization.

AUTHOR CONTRIBUTIONS

All the authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

Abramovay, R. (1999). *Moratória para os cerrados. Elementos para uma estratégia de agricultura sustentável*. São Paulo: Departamento de Economia e Programa de Ciência Ambiental da USP.

Anjos, O., Pereira, H., and Rosa, M. E. (2008). Effect of quality, porosity and density on the compression properties of cork. *Holz. Roh. Werkst.* 66, 295–301. doi:10.1007/s00107-008-0248-2

Anjos, O., Rodrigues, C., Morais, J., and Pereira, H. (2014). Effect of density on the compression behaviour of cork. *Mater. Des.* 53, 1089–1096. doi:10.1016/j.matdes.2013.07.038

APCOR. (2015). *Anuário de cortiça 2015*. Santa Maria de Lamas: Associação Portuguesa da Cortiça.

Beck, C. (2010). *An Introduction to Plant Structure and Development – Plant Anatomy for the Twenty-First Century*. Cambridge: Cambridge University Press.

Bento, M. F. S., Pereira, H., Cunha, M. Á, Moutinho, A. M. C., Van den Berg, K. J., and Boon, J. J. (2001). A study of variability of suberin composition in cork from *Quercus suber* L. using thermally assisted transmethylation GC–MS. *J. Anal. Appl. Pyrol.* 57, 45–55. doi:10.1016/S0165-2370(00)00093-0

Bhat, K. M. (1982). Anatomy, basic density and shrinkage of birch bark. *IAWA J.* 3, 207–213. doi:10.1163/22941932-90000841

Cardoso, S., Ferreira, J. P. A., Quilhó, T., and Pereira, H. (2016). Cork of Douglas-fir bark: impact of structural and anatomical features on usage. *Ind. Crop. Prod.* (in press).

Carvalho, P. (2009). "Vinhático, *Plathymenia reticulata*," in *Embrapa Florestas* (São Paulo: Comunicado técnico), 231.

CEPI. (2013). *Key Statistics 2013*. European Pulp and Paper Industry.

Chang, Y. P. (1954). *Bark Structure of North American Conifers*. Washington, DC: US Dept. of Agriculture.

Conde, E., Cadahía, E., Garcia-Vallejo, M. C., and González-Adrados, J. R. (1998). Chemical characterization of reproduction cork from Spanish *Quercus suber*. *J. Wood Chem. Technol.* 18, 447–469. doi:10.1080/02773819809349592

Cordeiro, N., Belgacem, M. N., Gandini, A., and Neto, C. P. (1997). Urethanes and polyurethanes from suberin: 1. kinetic study. *Ind. Crop. Prod.* 6, 163–167. doi:10.1016/S0926-6690(96)00212-9

Cordeiro, N., Belgacem, M. N., Gandini, A., and Neto, C. P. (1999). Urethanes and polyurethanes from suberin 2: synthesis and characterization. *Ind. Crop. Prod.* 10, 1–10. doi:10.1016/S0926-6690(98)00029-6

Costa, A., and Pereira, H. (2007). Influence of vision systems, black and white, colored and visual digitalization, in natural cork stopper quality estimation. *J. Sci. Food Agric.* 87, 2222–2228. doi:10.1002/jsfa.2947

Costa, A., and Pereira, H. (2010). Influence of cutting direction of cork planks on the quality and porosity characteristics of natural cork stoppers. *For. Syst.* 19, 51–60. doi:10.5424/fs/2010191-01166

ACKNOWLEDGMENTS

FUNDING

Duarte, A. P., and Bordado, J. C. (2015). Cork – a renewable raw material: forecast of industrial potential and development priorities. *Front. Mater.* 2:2. doi:10.3389/fmats.2015.00002

Ekman, R. (1983). The suberin monomers and triterpenoids from the outer bark of *Betula verrucosa* Ehrh. *Holzforschung* 37, 205–211. doi:10.1515/hfsg.1983.37.4.205

Ekman, R., and Eckerman, C. (1985). Aliphatic carboxylic acids from suberin in birch outer bark by hydrolysis, methanolysis and alkali fusion. *Paperi ja Puu* 67, 255–273.

Evert, R. F. (2006). *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development*. New Jersey: John Wiley & Sons, Inc.

Evtiouguina, M., Barros, A. M., Cruz-Pinto, J. J., Neto, C. P., Belgacem, N., Pavier, C., et al. (2000). The oxypropylation of cork residues: preliminary results. *Bioresour. Technol.* 73, 187–189. doi:10.1016/S0960-8524(99)00158-3

Evtiouguina, M., Barros-Timmons, A., Cruz-Pinto, J. J., Neto, C. P., Belgace, M. N., and Gandini, A. (2002). Oxypropylation of cork and the use of the ensuing polyols in polyurethane formulations. *Biomacromolecules* 3, 57–62. doi:10.1021/bm010100c

Evtiouguina, M., Gandini, A., Neto, C. P., and Belgacem, N. M. (2001). Urethanes and polyurethanes based on oxypropylated cork. 1. Appraisal and reactivity of products. *Polym. Int.* 50, 1150–1155. doi:10.1002/pi.760

Fahn, A. (1990). *Plant anatomy*. Oxford: Pergamon Press.

FAO. (2015). *FAOSTAT – Forestry Production and Trade*. Food and Agriculture Organization of the United Nations.

Farjon, A. (2013). *Abies concolor. The IUCN Red List of Threatened Species 2013: e.T42276A2969061*. doi:10.2305/IUCN.UK.2013-1.RLTS.T42276A2969061.en

Ferreira, J. P., Miranda, I., Sen, A., and Pereira, H. (2016a). Chemical and cellular features of virgin and reproduction cork from *Quercus variabilis*. *Ind. Crop. Prod.* 94, 638–648. doi:10.1016/j.indcrop.2016.09.038

Ferreira, J. P., Quilhó, T., and Pereira, H. (2016b). Characterization of *Betula pendula* outer bark regarding cork and phloem components at chemical and structural levels in view of biorefinery integration. *J. Wood Chem. Technol.* 1–16. doi:10.1080/02773813.2016.1224248

Ferreira, J. P. A., Miranda, I., Gominho, J., and Pereira, H. (2015a). Chemical characterization of cork and phloem from Douglas fir outer bark. *Holzforschung* 70, 475–483. doi:10.1515/hf-2015-0119

Ferreira, J. P. A., Miranda, I., Gominho, J., and Pereira, H. (2015b). Selective fractioning of *Pseudotsuga menziesii* bark and chemical characterization in view of an integrated valorization. *Ind. Crop. Prod.* 74, 998–1007. doi:10.1016/j.indcrop.2015.05.065

Ferreira, R., Garcia, H., Sousa, A. F., Freire, C. S., Silvestre, A. J., Rebelo, L. P. N., et al. (2013). Isolation of suberin from birch outer bark and cork using ionic liquids: a new source of macromonomers. *Ind. Crop. Prod.* 44, 520–527. doi:10.1016/j.indcrop.2012.10.002

Fortes, M., Rosa, M., and Pereira, H. (2004). *A Cortiça*. Lisboa: IST Press.

Gandini, A., Neto, C. P., and Silvestre, A. J. (2006). Suberin: a promising renewable resource for novel macromolecular materials. *Prog. Polym. Sci.* 31, 878–892. doi:10.1016/j.progpolymsci.2006.07.004

Gil, L. (2009). Cork composites: a review. *Materials* 2, 776–789. doi:10.3390/ma2030776

Gil, L. (2015). Cork: sustainability and new applications. *Front. Mat.* 1:38. doi:10.3389/fmats.2014.00038

Gilman, E., and Watson, D. (2014). *Abies concolor. ENH160 Environmental Horticulture, University of Florida/IFAS Extension*, Florida.

Graça, J., and Pereira, H. (1997). Cork suberin: a glyceryl based polyester. *Holzforschung* 51, 225–234. doi:10.1515/hfsg.1997.51.3.225

Graça, J., and Pereira, H. (1998). Feruloyl esters of ω-hydroxyacids in cork suberin. *J. Wood Chem. Technol.* 18, 207–217. doi:10.1080/02773819809349577

Graça, J., and Pereira, H. (2000). Methanolysis of bark suberins: analysis of glycerol and acid monomers. *Phytochem. Anal.* 11, 45–51. doi:10.1002/(SICI)1099-1565(200001/02)11:1<45:AID-PCA481>3.0.CO;2-8

Graça, J., and Pereira, H. (2004). The periderm development in *Quercus suber*. *IAWA J.* 25, 325–335. doi:10.1163/22941932-90000369

Grillos, S. (1956). *Structure and Development of the Bark of Douglas-fir, Pseudotsuga menziesii [PhD. Dissertation]*, Oregon State University.

Harkin, J. M., and Rowe, J. W. (1971). *Bark and Its Possible Uses*. Madison: Research Note Forest Products Laboratory 091.

Hergert, H. L. (1958). Chemical composition of cork from white fir bark. *For. Prod. J.* 8, 335–339.

Hergert, H. L., and Kurth, E. F. (1952). The chemical nature of the cork from Douglas-fir bark. *Tappi* 35, 59–66.

Hergert, H. L., and Kurth, E. F. (1953). The chemical nature of the extractives of white fir bark. *Tappi* 36, 137–144.

Holloway, P. J. (1972). The composition of suberin from the corks of *Quercus suber* L. and *Betula pendula* Roth. *Chem. Phys. Lipids* 9, 158–170. doi:10.1016/0009-3084(72)90011-4

Holloway, P. J. (1983). Some variations in the composition of the suberin from the cork layers of higher plants. *Phytochemistry* 22, 495–502. doi:10.1016/0031-9422(83)83033-7

Holloway, P. J., and Deas, A. H. B. (1973). Epoxyoctadecanoic acids in plant cutins and suberins. *Phytochemistry* 12, 1721–1735. doi:10.1016/0031-9422(73)80393-0

Huang, Z. K., Zheng, C. H., Du, J. X., and Wan, Y. Y. (2006). "Bark classification based on textural features using artificial neural networks," in *International Symposium on Neural Networks* (Berlin, Heidelberg: Springer), 355–360. doi:10.1007/11760023_52

Hultén, E., and Fries, M. (1986). *Atlas of North European Vascular Plants (North of the Tropic of Cancer)*, Vol. I-III. Oberreifenberg: Koeltz Scientific Books.

Jensen, W. (1948). *Om ytskiktsavfallet vid framst¨ allning avbj¨ orkfaner (About the Surface Layer Waste from Production of Birch Veneer) [PhD Dissertation]*, Acta Acad. Aboensis, Math. Phys. XVI. Abo.

Junikka, L. (1994). Survey of English macroscopic bark terminology. *IAWA J.* 15, 3–45. doi:10.1163/22941932-90001338

Kim, B. (1993). Studies on the physical and mechanical properties of imported and domestic corks. *J. Korean Wood Sci. Technol.* 21, 45–54 (in Korean with abstract in English).

Kim, B. R., Mishiro, A., Sugiyama, J., and Okano, T. (1990). The physical properties of virgin and reproduction corks of *Quercus variabilis* Blume. *Bull. Tokyo Univ. For.* 82, 199–217 (in Japanese).

Krahmer, R. L., and Wellons, J. D. (1973). Some anatomical and chemical characteristics of Douglas-fir cork. *Wood Sci.* 6, 97–105.

Krasutsky, P., Carlson, R., and Kolomitsyn, I., Inventors; University of Minnesota, Assignee. (2003). *Isolation of Natural Products from Birch Bark*. United States patent US 2003109727.

Krasutsky, P., Carlson, R., Kolomitsyn, I., and Krasutsky, D., Inventors; University of Minnesota, Assignee. (2009). *Depolymerization Extraction of Compounds from Birch Bark*. United States patent US 20090182158.

Krasutsky, P., Carlson, R., Nesterenko, V., Kolomitsyn, I., and Edwardson, C., Inventors; University of Minnesota, Assignee. (2005). *Birch Bark Processing and the Isolation of Natural Products from Birch Bark*. United States patent US20050158414.

Kurth, E. F. (1953). Chemicals from Douglas-fir bark. *Tappi* 36, 119A–122A.

Kurth, E. F., and Kiefer, H. J. (1950). Wax from Douglas-fir bark. *Tappi* 33, 183–186.

Lavender, D. P., and Herman, R. K. (2014). *Douglas-Fir: The Genus Pseudotsuga*. Corvallis OR: Forest Research Publications Office.

Lev-Yadun, S. (2011). "Bark," in *Encyclopedia of Life Sciences* (London: Nature Publishing Group). Published in print in: 2007. *Handbook of Plant Science*, Vol. 1, ed. K. Roberts (Chichester: John Wiley & Sons, Ltd.), 153–156.

Lima, J. T., and Marcati, C. R. (1989). "Anatomia da madeira de *Kielmeyera coriacea* Mart. (Pau-santo) – Guttiferae," in *Abstract presented at the XI Congresso Nacional de Botânica* (Cuiabá, Brazil), 301.

Marques, A. V., and Pereira, H. (2013). Lignin monomeric composition of corks from the barks of *Betula pendula*, *Quercus suber* and *Quercus cerris* determined by Py–GC–MS/FID. *J. Anal. Appl. Pyrol.* 100, 88–94. doi:10.1016/j.jaap.2012.12.001

Marques, A. V., Pereira, H., Meier, D., and Faix, O. (1994). Quantitative analysis of cork (*Quercus suber* L.) and milled cork lignin by FTIR spectroscopy, analytical pyrolysis, and total hydrolysis. *Holzforschung* 48, 43–50. doi:10.1515/hfsg.1994.48.s1.43

Marques, A. V., Pereira, H., Meier, D., and Faix, O. (1996). Isolation and characterization of a guaiacyl lignin from saponified cork of *Quercus suber* L. *Holzforschung* 50, 393–400. doi:10.1515/hfsg.1996.50.5.393

Marques, A. V., Pereira, H., Meier, D., and Faix, O. (1999). Structural characterization of cork lignin by thioacidolysis and permanganate oxidation. *Holzforschung* 53, 167–174. doi:10.1515/HF.1999.028

Marques, A. V., Pereira, H., Rodrigues, J., Meier, D., and Faix, O. (2006). Isolation and comparative characterization of a Björkman lignin from the saponified cork of Douglas-fir bark. *J. Anal. Appl. Pyrol.* 77, 169–176. doi:10.1016/j.jaap.2006.03.003

Marques, A. V., Rencoret, J., Gutiérrez, A. C., del Río, J. C., and Pereira, H. (2016). Ferulates and lignin structural composition in cork. *Holzforschung* 70, 275–289. doi:10.1515/hf-2015-0014

Miranda, I., Gominho, J., and Pereira, H. (2013). Cellular structure and chemical composition of cork from Chinese cork oak (*Quercus variabilis*). *J. Wood Sci.* 59, 1–9. doi:10.1007/s10086-012-1300-8

Mogensen, H. L. (1968). Studies on the bark of the cork bark fir: *Abies lasiocarpa* var. *Arizonica* (Merriam) Lemmon. I. Periderm ontogeny. *J. Ariz. Acad. Sci.* 5, 36–40. doi:10.2307/40022822

Mota, G. S., Sartori, C. J., Ferreira, J., Miranda, I., Quilhó, T., Mori, F. A., et al. (2016). Cellular structure and chemical composition of cork from *Plathymenia reticulata* occurring in the Brazilian Cerrado. *Ind. Crop. Prod.* 90, 65–75. doi:10.1016/j.indcrop.2016.06.014

Natividade, J. V. (1950). *Subericultura*. Lisboa: Ministério da Economia, Direcção Geral dos Serviços Florestais e Aquícolas.

Oliveira, V., Knapic, S., and Pereira, H. (2012). Natural variability of surface porosity of wine cork stoppers of different commercial classes. *J. Int. Sci. Vigne Vin* 46, 331–340.

Oliveira, V., Lopes, P., Cabral, M., and Pereira, H. (2015). Influence of cork defects in the oxygen ingress through wine stoppers: insights with X-ray tomography. *J. Food Eng.* 165, 66–73. doi:10.1016/j.jfoodeng.2015.05.019

Oliveira, V., Rosa, M. E., and Pereira, H. (2014). Variability of the compression properties of cork. *Wood Sci. Technol.* 48, 937–948. doi:10.1007/s00226-014-0651-2

Olsson, A., Lindström, M., and Iversen, T. (2007). Lipase-catalyzed synthesis of an epoxy-functionalized polyester from the suberin monomer cis-9,10-epoxy-18-hydroxyoctadecanoic acid. *Biomacromolecules* 8, 757–760. doi:10.1021/bm060965w

Patel, R. N. (1975). Bark anatomy of radiata pine, corsican pine, and Douglas fir grown in New Zealand. *New Zeal. J. Bot.* 13, 149–167. doi:10.1080/0028825X.1975.10430317

Pereira, H. (1988). Chemical composition and variability of cork from *Calotropis procera* (Ait.) R. Br. *IAWA J.* 9, 53–58. doi:10.1163/22941932-90000468

Pereira, H. (2007). *Cork: Biology, Production and Uses*. Amsterdam: Elsevier Publications.

Pereira, H. (2013). Variability of the chemical composition of cork. *BioResources* 8, 2246–2256. doi:10.15376/biores.8.2.2246-2256

Pereira, H. (2015). The rationale behind cork properties: a review of structure and chemistry. *BioResources* 10, 6207–6229. doi:10.15376/biores.10.3.Pereira

Pereira, H., Graça, J., and Baptista, C. (1992). The effect of growth rate on the structure and compressive properties of cork. *IAWA Bull.* 13, 389–396. doi:10.1163/22941932-90001294

Pereira, H., Lopes, F., and Graça, J. (1996). The evaluation of the quality of cork planks by image analysis. *Holzforschung* 50, 111–115. doi:10.1515/hfsg.1996.50.2.111

Pereira, H., Rosa, M. E., and Fortes, M. A. (1987). The cellular structure of cork from *Quercus suber* L. *IAWA Bull.* 8, 213–218. doi:10.1163/22941932-90001048

Pereira, H., and Tomé, M. (2004). "Cork oak and cork," in *Encyclopedia of Forest Sciences*, eds J. Burley, J. Evans, and J. A. Youngquist (Oxford: Elsevier), 613–620.

Pinto, P. C., Sousa, A. F., Silvestre, A. J., Neto, C. P., Gandini, A., Eckerman, C., et al. (2009). *Quercus suber* and *Betula pendula* outer barks as renewable sources of oleochemicals: a comparative study. *Ind. Crop. Prod.* 29, 126–132. doi:10.1016/j.indcrop.2008.04.015

Ponte-e-Sousa, J. C. A. C. C., and Neto-Vaz, A. M. (2011). Cork and metals: a review. *Wood Sci. Technol.* 45, 183–202. doi:10.1007/s00226-009-0288-8

Richter, H. G., Mazzoni-Viveiros, S. C., Alves, E. S., Luchi, A. E., and Costa, C. G. (1996). Padronização de critérios para a descrição anatômica da casca: lista de características e glossário de termos. *IF Série Registros São Paulo* 16, 1–25.

Rios, P., Cabral, V., Santos, S., Mori, F., and Graça, J. (2014). The chemistry of *Kielmeyera coriacea* outer bark: a potential source of cork. *Eur. J. Wood Prod.* 72, 509–519. doi:10.1007/s00107-014-0811-y

Rios, P., Mori, F., and Barbosa, A. (2011). Morphological characterization of *Kielmeyera coriacea* Mart. Cork from Brazilian Cerrado. *Cerne, Lavras* 17, 387–392. doi:10.1590/S0104-77602011000300013

Rios, P. D. (2007). *Technological Characterization and Production of Boards of the Cork from Kielmeyera coriacea Mart. (pau-santo). [MSc Dissertation]*, Universidade Federal de Lavras, Minas Gerais, Brazil.

Rios, P. D. (2011). *Anatomy and Chemical Characterization of Kielmeyera coriacea Mart. Cork [Ph.D. Dissertation]*, Universidade Federal de Lavras, Minas Gerais, Brazil.

Rizzini, C. T., and Mors, W. B. (1995). *Botânica econômica brasileira*. Rio de Janeiro: Âmbito Cultural.

Ross, W. D., and Krahmer, R. L. (1971). Some sources of variation in structural characteristics of Douglas-fir bark. *Wood Fiber* 3, 35–46.

Roth, I. (1981). *Structural Patterns of Tropical Tree Barks*. Berlin: Schweizerbart – Science Publishers Borntraeger.

Schönherr, J., and Ziegler, H. (1980). Water permeability of *Betula* periderm. *Planta* 147, 345–354. doi:10.1007/BF00379844

Sen, A., Miranda, I., Santos, S., Graça, J., and Pereira, H. (2010). The chemical composition of cork and phloem in the rhytidome of *Quercus cerris* bark. *Ind. Crop. Prod.* 31, 417–422. doi:10.1016/j.indcrop.2010.01.002

Şen, A., Pereira, H., Olivella, M. A., and Villaescusa, I. (2015). Heavy metals removal in aqueous environments using bark as a biosorbent. *Int. J. Environ. Sci. Technol.* 12, 391–404. doi:10.1007/s13762-014-0525-z

Sen, A., Quilhó, T., and Pereira, H. (2011a). The cellular structure of cork from *Quercus cerris* var *cerris* bark in a material's perspective. *Ind. Crop. Prod.* 34, 929–936. doi:10.1016/j.indcrop.2011.02.015

Sen, A., Quilhó, T., and Pereira, H. (2011b). Bark anatomy of *Quercus cerris* L. var *cerris* from Turkey. *Turk. J. Bot.* 35, 45–55. doi:10.3906/bot-1002-33

Sen, A., Zhianski, M., Ferreira, J., Glushova, M., Petkova, K., and Pereira, H. (2016a). Chemical composition and cellular structure of corks from *Quercus suber* trees planted in Bulgaria and Turkey. *Wood Sci. Technol.* 50, 1261–1276. doi:10.1007/s00226-016-0836-y

Sen, A., Leite, C., Lima, L., Lopes, P., and Pereira, H. (2016b). Industrial valorization of *Quercus cerris* bark: pilot scale fractionation. *Ind. Crop. Prod.* 92, 42–49. doi:10.1016/j.indcrop.2016.07.044

Silva, S. P., Sabino, M. A., Fernandes, E. M., Correlo, V. M., Boesel, L. F., and Reis, R. L. (2005). Cork: properties, capabilities and applications. *Int. Mater. Rev.* 50, 345–365. doi:10.1179/174328005X41168

Sousa, A. F., Pinto, P. C. R. O., Silvestre, A. J. D., Gandini, A., and Neto, C. P. (2007). "Novel biopolyesters from suberin monomers," in *Proceedings of the First International Conference on Biodegradable Polymers and Sustainable Composites, BIOPOL-2007* (Alicante, Spain).

Souza, F. P. (1974). *Tecnologia de Produtos Florestais*. Rio de Janeiro: Nacional.

Taco, C., Lopes, F., and Pereira, H. (2003). La variation dans l'arbre de l'épaisseur du liège et du dos des planches de liège pour des chênes-lièges en pleine production. *Anais Instituto Superior de Agronomia* 49, 209–221.

Trockenbrodt, M. (1990). Survey and discussion of the terminology used in bark anatomy. *IAWA Bull.* 11, 141–166. doi:10.1163/22941932-90000511

Trockenbrodt, M. (1991). Qualitative structural changes during bark development in *Quercus robur*, *Ulmus glabra*, *Populus tremula* and *Betula pendula*. *IAWA Bull.* 12, 5–22. doi:10.1163/22941932-90001199

Wei, X., Xiang, S., and Zhou, W. (2007). Chemical composition and its effect on properties of cork from three geographical locations. *China Wood Ind.* 6, 17–18 (in Chinese with abstract in English).

Weiskittel, A. R., Crookston, N. L., and Rehfeldt, G. E. (2012). Projection of Douglas-fir future habitat and productivity in western North America. *Schweiz. Z. Forstwes.* 163, 70–78. doi:10.3188/szf.2012.0070

YaFang, L., Ting, J., and Xiaozhou, S. (2012). Chemical composition of cork from *Quercus variabilis*. *Wood Fiber Sci.* 44, 214–219.

YaFang, L., YanZhen, L., Wei, Z., and JingFeng, Z. (2009). The microstructure of cork from *Quercus variabilis*. *Scientia Silvae Sinicae* 45, 167–170 (in Chinese with abstract in English).

Zhang, L., Lei, Y., and Chang, Y. (2009). Contents of the main chemical components of cork from *Quercus variabilis*. *J. Northwest For. Univ.* 24, 163–165 (in Chinese with abstract in English).

Zhang, W. H., and Lu, Z. J. (2002). A study on the biological and ecological property and geographical distribution of *Quercus variabilis* population. *Xibei Zhiwu Xuebao* 22, 1093–1101 (in Chinese with abstract in English).

Zhao, J., Feng, D., Lei, Y., Zhang, W., and Zhang, Y. (2013). Cell structure and chemical components of sclereids and lenticels from *Quercus variabilis* cork. *J. Northwest A & F Univ. (Nat. Sci. Ed.)* 7, 22 (in Chinese with an abstract in English).

Zhou, J. Y., Lin, J., He, J. F., and Zhang, W. H. (2010). Review and perspective on *Quercus variabilis* research. *J. Northwest For. Univ.* 25, 43–49 (in Chinese with an abstract in English).

Publication II

Effect of a Drought on Cork Growth Along the Production Cycle

Effect of a Drought on Cork Growth Along the Production Cycle

Carla Leite, Vanda Oliveira, Alexandra Lauw and Helena Pereira

Abstract Cork oak (*Quercus suber* L.) grows in the western Mediterranean region for which the most recent climatic scenarios predict higher temperatures and lower precipitation than usual values. Cork, the tree's outerbark, is obtained under a sustainable management system and has a considerable economic importance for forest producers and industry. Cork's specific set of properties allows multiple usages, from cork stoppers to insulating materials. This paper presents the first results of a dendroclimatological exploratory study about the effect of a severe drought in different moments of the cork 9-year production cycle, e.g. beginning, middle or end of the cycle. The results showed that the response of the phellogen (cork cambium) to the severe drought of 2004–2006 is independent of its age. In a mitigating strategy for the impact of the forthcoming more frequent drought events, and since cork growth decreases due to the reduction of water availability, forest managers should extend cork growth cycles and/or water cork oak stands. This is a way to ensure the production of cork with enough thickness to produce stoppers, thereby contributing to the overall sustainability of the cork sector in a climate change context.

Keywords Drought · Cork oak · Mediterranean region · Climate change

Introduction

Climate change is one of our most challenging issues in the 21st century[1] either regarding mitigation or adaptation strategies. The long term maintenance of forests is greatly determined by the adaptive capacity of species[2] under its two

---

[1]Stott et al. (2016).
[2]Braeuning et al. (2017).

C. Leite (✉) · V. Oliveira · A. Lauw · H. Pereira
Centro de Estudos Florestais, Instituto Superior de Agronomia,
Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal
e-mail: cleite@isa.ulisboa.pt

components: the intrinsic adaptive capacity of trees and forests, and the socio-economic factors determining the capability to perform planned adaptation measures.[3] Although tree species can adjust to new environmental conditions, there is not much expertise about the processes involved.[4] In the meanwhile, forest managers do need to adapt their practices to the new reality in order to satisfy the needs of all their stakeholders, from the public in general to a rather competitive and demanding forest industry.

All climatic scenarios for Mediterranean ecosystems predict increasing temperatures, water deficits and periodicity, intensity and duration of extreme events, namely severe droughts, heat waves, heavy rainfall and fewer cold days.[5,6] Tree growth is constrained by water availability e.g. when evaporation is greater than soil moisture uptake, stomata close to reduce water loss decreasing carbon uptake and growth. For the Mediterranean basin, due to summer water deficits, it is expectable to have productivity losses and changes in species distribution.[7,8,9]

Cork oak forests cover an area of approximately 2.2 million ha and produce annually up to 200 thousand tons of cork that supply an important industry. [10] Portugal is the main producer of raw cork and cork products namely cork stoppers for the wine industry and boards.[11] The production of cork is based on the periodical removal of the cork layer from the stem. In the main producing regions, the period between consecutive cork removals is usually 9 years, enabling to have a cork plank with the desirable thickness (>24 mm) for the production of cork stoppers (the cork product with the major added value that supports all the cork segment).

The cork oak is ecologically plastic, adapting physiology and phenology to a changing environment, namely to summer drought and higher temperatures. This species vegetates well with mean annual precipitations of 600–800 mm, with 500 mm considered to be the minimum annual precipitation for a balanced tree development.[12] and [13] Furthermore it is sensitive to the amount and the moment when rains occur, namely precipitation in late spring has more influence on cork oak growth than the total annual precipitation, while it recovers quickly after a year of extreme dryness.[14]

---

[3]Lindner et al. (2010).

[4]See footnote 3.

[5]IPCC (2014).

[6]See footnote 3.

[7]Santos and Miranda (2006).

[8]Gea-Izquierdo et al. (2013).

[9]Piayda et al. (2014).

[10]APCOR (2016).

[11]Pereira (2007).

[12]Natividade (1950).

[13]See footnote 11.

[14]Besson et al. (2014)

In what relates to cork growth, results showed that rainfall, summer drought and temperature are determining factors; in particular, water availability is the most limiting factor for growth. [15,16,17] In fact, the rain period from May to September strongly influences activity of the phellogen (the cork cambium).[18] Cork growth is also extremely affected by drought conditions in short time scales (from 2 to 11 months) namely regarding spring precipitation. Nevertheless, cork growth rapidly recovers when drought conditions end.[19]

Similar to wood, it is possible to observe annual growth rings in cork as they are marked by the presence of a layer of darker latecork cells at the end of the growth season; the width of a cork ring is largely determined by the rate and duration of the meristematic division of the phellogen[20] which is seasonal with the environmental conditions of light and temperature as the main drivers for begin and end of dormancy.[21]

In spite of all the research performed so far about cork growth and its relation with climate (e.g. temperature and precipitation), we identified a gap of knowledge about the effect on cork growth of drought occurring in different moments of the 9-year cork growth cycle e.g. beginning, middle and end. Furthermore, as pointed out by[22] the present challenge is to translate the ecophysiology and forest ecology scientific research into forest management prescriptions useful for forest owners and managers.

This paper is an effort to overcome these shortcomings. With cork samples collected in three sites, located in one of the largest continuous forest areas of cork oak, we compared cork growth during the 2004–2006 drought that[23] considered as the driest event in the Iberian Peninsula over the last 140 years. The decrease of cork growth and also the recovery after the drought were analyzed. The complete growth (8 years) was also analyzed because the production of cork stoppers requires to have planks with more than 24 mm thickness. The sites were selected in order to have this drought event occurring in different moments along the growth cycle (see Table 1), allowing to study its effect according to the age of the phellogen in 2005 (the year in which the growth is negatively affected by the drought). Moreover, the results obtained are converted in forest management advices in order to adapt or mitigate the effects of the forthcoming more frequent droughts in the Mediterranean basin.

---

[15]Cherubini et al. (2003)

[16]Caritat et al. (2000)

[17]Oliveira et al. (2016)

[18]Pizzurro et al. (2010).

[19]See footnote 17.

[20]See footnote 11.

[21]Evert (2006).

[22]See footnote 2

[23]García-Herrera et al. (2007).

Table 1 Number of samples collected in each site, number of samples measured in this research, stripping year, civil years of the chronology (8 years) and age of the phellogen in 2005

	Number of samples collected	Number of samples measured	Stripping year	Civil years of the chronology	Age of the phellogen in 2005 (years)
Site 1	37	32	2007	1999–2006	7
Site 2	84	60	2009	2001–2008	5
Site 3	92	57	2012	2004–2011	2

Methods

Study Area

The cork samples used in this research were collected in three *montado* estates located in central-west of Portugal, in the Coruche municipality. The sites are set in the watershed of the Tagus river, in one of the main production areas of cork oak, where the Mediterranean climate prevails. In all the sites forest management is characterized by low tree density, with the trees being exploited for cork production and, in site 1, the herbaceous layer is used for extensive grazing.

Data Acquisition

A set of 224 cork samples was collected at breast height (1.30 m) during the stripping season of adult productive cork oak trees. The trees were randomly selected in the sites and a proportion of the total trees under striping was sampled. Table 1 shows some characteristics of the 3 sites sampled. All cork samples presented 8 complete years of growth with the previous debarking occurring 9 years before (the stripping is performed when phellogen is active thereby interrupting the cork growth in that year).

After debarking, the samples ($\pm 15 \times 15$ cm^2) were boiled in water for 1 h at atmospheric pressure and left to air-dry until equilibrium. For a more accurate observation of the growth rings the transversal sections were polished and two opposite radial strips with approximately 1 cm thickness were cut, fixed on a microscope slide (Fig. 1a) and digitalized in a laser based fluorescence imager (Fluoro Image Analyzer FLA -5100, Fujifilm, Life Science USA, Stamford, CT 06092).[24] Cork ring thickness was measured with AnalySIS® image processing software (Analysis Soft Imaging System GmbH, Münster, Germany, version 3.1) and along two transects in the radial direction with an accuracy of 0.01 mm. For each tree the media of the two measurements was determined.

---

[24]Surový et al. (2009).
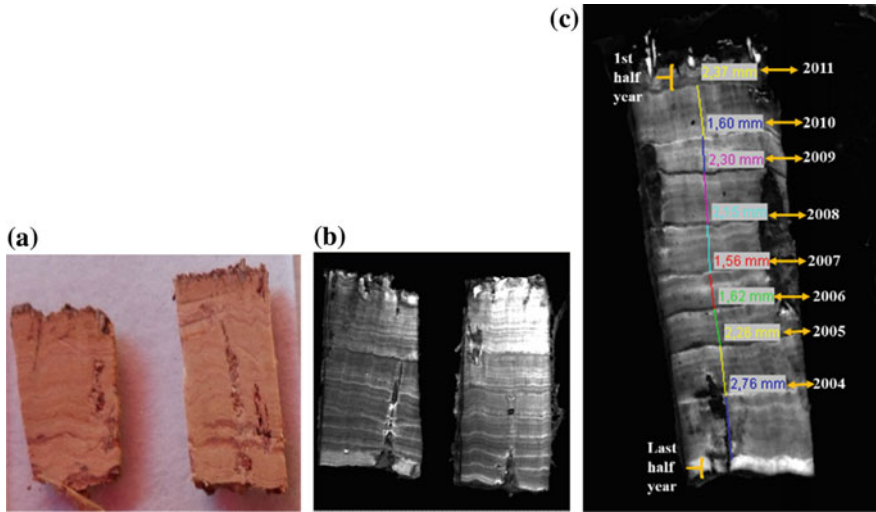
Fig. 1 a Slides with cork samples **b** example of a sample in which the delimitation of the 8 complete growth years is not possible **c** example of a sample from site 3 measured and dated

The initial and final half rings corresponding to the years of the present and last strippings were not considered for analysis as they are not complete growth years. As recognized earlier by several authors,[25,26] and[27] the delimitation of annual rings was not unequivocal in all the samples (Fig. 1b), and only in 70% (149) of the samples cork rings width were measured and cork rings dated (Fig. 1c).

Data Analysis

In order to take a brief characterization of our raw data a boxplot for each site was performed, allowing us to compare the sites and evaluate the respective variability.

As usually done in dendroclimatic studies,[28] each cork-growth curve was plotted and standardized in order to remove systematic changes of growth associated with phellogen age and maximize the inter-annual fluctuations due to weather. Due to the low age of the phellogen and in a similar approach as performed by[29] and[30] we used a negative exponential curve in the standardization process (detrend) through

---

[25]Caritat et al. (1996).

[26]See footnote 24.

[27]See footnote 17.

[28]Fritts (1976).

[29]See footnote 17.

[30]Costa et al. (2001).

the dendrochronology program library (dplr) of the R software[31] and[32]. This procedure fits the following model:

$$G_t = ae^{-bt} + k$$

where G_t is the growth trend and is estimated as a function of time t, being a, b and k, the coefficients. If that nonlinear model could not be fitted, then a standard linear model was adjusted $G_t = b_0 + b_1 t$ where b_0 is the intercept and b_1 the slope.[33]

After this procedure, for each tree, RWI—ring width indexes (or Cork Growth Indexes) were calculated dividing the real cork growth (R_t) by the expected growth at time t (G_t).

We initially performed the Shapiro-Wilk Normality Test (shapiro.test) in R, and then, as it failed at a significance level of 0.05, a non-parametric test—the Kruskal-Wallis rank sum test (kruskal.test) was performed for the RWI of 2005 (year which growth is affected by the drought) and for the difference between RWI of 2004 and 2005 (RWI_{2005}–RWI_{2004}) to analyze the effect of the drought in reducing RWI and between RWI of 2005 and 2006 (RWI_{2006}–RWI_{2005}) to evaluate the phellogen recovery.

Furthermore, as the total growth of the cork determines its industrial utilization, we also tested if the total growth was different according to the moment in which the drought occurred. The test was performed with the same methodology as before.

Results

The cork growth of the 8 complete years in each site is presented in Fig. 2. The minimum total growth is very similar in all the sites (approximately 12 mm) but the media and the third quartile of site 3 are lower than those of the other sites.

The mean series of annual cork growth indices suggest one strong climatic signal, and 2005 can be considered a negative pointer year in all the chronologies. In general, the cork ring indices from the same year have a similar behavior when compared to the preceding/next year, in all the mean chronologies (Fig. 3).

The Shapiro-Wilk Normality Test performed for the RWI for the year 2005 gave a p-value below 0.05 (3.71×10^{-6}—see Table 2) and we performed a non-parametric approach through the Kruskal-Wallis rank sum test. We concluded that the 2005-RWI is not different between all the sites as the p-value is under the significance level of 5% (Table 2).

We also compared RWI_{2005}–RWI_{2004} and RWI_{2006}–RWI_{2005} for the 3 sites. The normality was again rejected and the results from the Kruskal-Wallis rank sum test
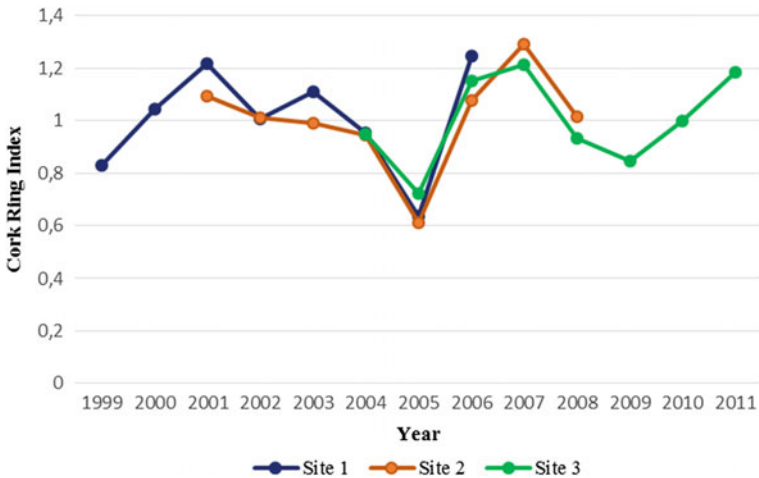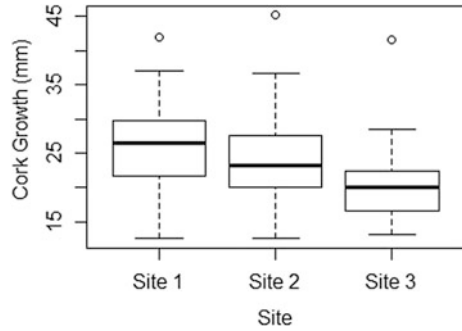
---

[31]Bunn (2008).

[32]R Core Team (2017).

[33]See fotenote 31.

Fig. 2 Boxplot for the cork growth of 8 complete years of the 3 sites considered in this research

Fig. 3 Mean cork growth chronologies for each site

suggest that the phellogen behavior is the same (not different) in the 3 sites i.e. independent of its age. However the total cork growth (of the 8 years) showed differences between the sites (Table 2).

As there were differences between the sites, we calculated the average growth for each site: site 1 = 25.57 mm; site 2 = 24.18 mm and site 3 = 20.08 mm. By performing the Wilcoxon test (non-parametric) we concluded (with a significance level of 5%) that site 3 has less total growth than the other 2 sites, which are not different from each other.

Table 2 Results (p-values) of the Shapiro-Wilk Normality Test and Kruskal-Wallis rank sum test performed for the Cork Ring Index for the year 2005, for the differences of the indexes for the years 2005–2004 and 2006–2005 and for the growth of the 8 complete years

	Shapiro-Wilk normality test	Kruskal-Wallis rank sum test
RWI_{2005}	3.71×10^{-6}	0.0681
$RWI_{2005}-RWI_{2004}$	2.69×10^{-6}	0.5932
$RWI_{2006}-RWI_{2005}$	0.004727	0.1972
Total growth	0.000188	5.19×10^{-6}

Discussion

The values obtained for the cork growth of 8 complete years in each site are below the average values found by[34] in similar locations. The differences of the growth of the 8 complete years obtained for one of the sites are difficult to explain as several factors affect growth, namely climate, age of the trees, the site, competition and other disturbances.[35]

Analyzing the cork-growth chronology, the results show that in all the sites, the 2004–2006 drought negatively affected cork growth and that 2005 can be considered a negative pointer year in all the chronologies. This effect of drought on cork growth is consistent with results obtained by several authors.[36,37] The trees recovered well when precipitation returned to values similar to the long term mean, which is also in accordance of the findings of several authors[38] and.[39]

Moreover, our results indicate that the age of the phellogen has no influence on the intensity of growth decline in the drought pointer year, i.e. the ring width index was similar for all the sites regardless of its occurrence during the cork growth cycle. Also there was no effect of the age of the phellogen neither on the decreasing of growth due to the drought nor on the recovery after the drought.

In practical terms however, and considering the absolute values of cork ring in the successive years along the growth cycle, it is expected to have a stronger impact on the total 8-year cork growth if the drought event occurs in the beginning of the cycle.

---

[34]Costa et al. (2016).

[35]Cook and Kairiukstis (1989).

[36]See footnote 17.

[37]See footnote 34.

[38]See footnote 17.

[39]See footnote 14.

Conclusion

The results of this exploratory study suggest that the age of the phellogen has no effect on the response of the tree to a drought event and on its recovery. Consequently, forest managers shall adapt their management practices to drought events independently of the moment of its occurrence along the cork growing cycle.

The results once again confirmed that drought decreases cork growth, which may hinder the possibility of producing, in the usual 9-year cycle, a cork thick enough for the manufacture of cork stoppers, especially if occurring in the first part of the growth cycle. This is a strong signal for forest managers to consider the feasibility to water the cork oak trees and/or to enlarge the cork growing cycles of cork.

Nevertheless, the results obtained should be confirmed with a larger sample and number of chronologies in order to analyze the severe drought of 2004–2006 with samples for more sites as well as to evaluate the effects of other severe droughts.

References

APCOR. (2016). *Anuário de cortiça 2016 APCOR*. site accessed 9th Mar 2017. http://www.apcor.pt/wp-content/uploads/2016/09/Boletim-estatistico-2016.pdf.

Besson, C., Lobo-do-Vale, R., Rodrigues, M., Almeida, P., Herd, A., Grant, O., et al. (2014). Cork oak physiological responses to manipulated water availability in a Mediterranean woodland. *Agricultural and Forest Meteorology, 184,* 230–242. https://doi.org/10.1016/j.agrformet.2013.10.004.

Braeuning, A., Bolte, A., Nabais, C., Rossi, S., & Sass-Klaassen, U. (2017). Studying tree responses to extreme events. *Frontiers in Plant Science, 8,* 506. https://doi.org/10.3389/fpls.2017.00506.

Bunn, A. (2008). A dendrochronology program library in R (dplR). *Dendrochronologia, 26*(2), 115–124. https://doi.org/10.1016/j.dendro.2008.01.002.

Caritat, A., Gutiérrez, E., & Molinas, M. (2000). Influence of weather on cork-ring width. *Tree Physiology, 20*(13), 893–900. https://doi.org/10.1093/treephys/20.13.893.

Caritat, A., Molinas, M., & Gutierrez, E. (1996). Annual cork-ring width variability of *Quercus suber L.* in relation to temperature and precipitation (Extremadura, southwestern Spain). *Forest Ecology and Management, 86*(1), 113–120. https://doi.org/10.1016/S0378-1127(96)03787-5.

Cherubini, P., Gartner, B., Tognetti, R., Bräker, O., Schoch, W., & Innes, J. L. (2003). Identification, measurement and interpretation of tree rings in woody species from Mediterranean climates. *Biological Reviews, 78*(1), 119–148. https://doi.org/10.1017/S1464793102006000

Cook, E., & Kairiukstis, L. (1989). *Methods of dendrochronology: Applications in the environmental sciences*. Dordrech: Springer Science & Business Media. ISBN 978-90-481-4060-2.

Costa, A., Barbosa, I., Roussado, C., Graça, J., & Spiecker, H. (2016). Climate response of cork growth in the Mediterranean oak (*Quercus suber L.*) woodlands of southwestern Portugal. *Dendrochronologia, 38,* 72–81. https://doi.org/10.1016/j.dendro.2016.03.007.

Costa, A., Pereira, H., & Oliveira, A. (2001). A dendroclimatological approach to diameter growth in adult cork-oak trees under production. *Trees, 15*(7), 438–443. https://doi.org/10.1007/s004680100119.

Evert, R. F. (2006). *Esau's plant anatomy: Meristems, cells, and tissues of the plant body: Their structure, function, and development*. London: Wiley. ISBN 0-470-04737-2.

Fritts, H. (1976). *Tree rings and climate* (1st ed.). London: Academic Press. ISBN 978-0-323-14528-2.

García-Herrera, R., Hernández, E., Barriopedro, D., Paredes, D., Trigo, R., Trigo, I., et al. (2007). The outstanding 2004/05 drought in the Iberian Peninsula: Associated atmospheric circulation. *Journal of Hydrometeorology, 8*(3), 483–498. https://doi.org/10.1175/JHM578.1.

Gea-Izquierdo, G., Fernández-de-Uña, L., & Cañellas, I. (2013). Growth projections reveal local vulnerability of Mediterranean oaks with rising temperatures. *Forest Ecology and Management, 305,* 282–293. https://doi.org/10.1016/j.foreco.2013.05.058.

IPCC. (2014). In C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, et al. (Eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., et al. (2010). Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management, 259,* 698–709. https://doi.org/10.1016/j.foreco.2009.09.02.

Natividade, J. (1950). *Subericultura*. Lisboa: Ministério da Economia–Direcção Geral dos Serviços Florestais e Aquícolas.

Oliveira, V., Lauw, A., & Pereira, H. (2016). Sensitivity of cork growth to drought events: Insights from a 24-year chronology. *Climatic Change, 137*(1), 261–274. https://doi.org/10.1007/s10584-016-1680-7.

Pereira, H. (2007). *Cork: Biology, production and uses*. Amsterdam: Elsevier. ISBN 0-08-047686-4.

Piayda, A., Dubbert, M., Rebmann, C., Kolle, O., Silva, F., Correia, A., et al. (2014). Drought impact on carbon and water cycling in a Mediterranean *Quercus suber L.* woodland during the extreme drought event in 2012. *Biogeosciences, 11*(24), 7159–7178. https://doi.org/10.5194/bg-11-7159-2014.

Pizzurro, G., Maetzke, F., & Veca, D. (2010). Differences of raw cork quality in productive cork oak woods in Sicily in relation to stand density. *Forest Ecology and Management, 260*(5), 923–929. https://doi.org/10.1016/j.foreco.2010.06.013.

R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/.

Santos, F. D., & Miranda, P. (2006). Alterações climáticas em Portugal. *Cenários, Impactos E Medidas de Adaptação–Projeto SIAM II*. ISBN: 989-616-081-3.

Stott, P., Christidis, N., Otto, F., Sun, Y., Vanderlinden, J. P., van Oldenborgh, G., et al. (2016). Attribution of extreme weather and climate-related events. *Wiley Interdisciplinary Reviews: Climate Change, 7*(1), 23–41. https://doi.org/10.1002/wcc.380.

Surový, P., Olbrich, A., Polle, A., Ribeiro, N. A., Sloboda, B., & Langenfeld-Heyser, R. (2009). A new method for measurement of annual growth rings in cork by means of autofluorescence. *Trees, 23*(6), 1237. https://doi.org/10.1007/s00468-009-0363-7.

Publication III

Cork rings suggest how to manage *Quercus suber* to mitigate the effects of climate changes
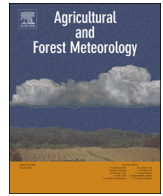
Cork rings suggest how to manage *Quercus suber* to mitigate the effects of climate changes

Carla Leite*, Vanda Oliveira, Alexandra Lauw, Helena Pereira

Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal

ABSTRACT

Climate scenarios in the Mediterranean region predicts raising temperatures and more frequent and extreme drought conditions. Cork oak is a Mediterranean species with a large distribution in Portugal from which cork is extracted in a sustainable way and mainly used as the raw material for cork stoppers and insulating materials. To study the response of cork oak to drought and the effect of phellogen age on that response we examined cork growth from a 30-year chronology of trees from 12 sites in the main Portuguese cork oak production area. For the first time in cork, a components resilience study was performed. The research confirmed that drought reduces cork growth and provided extra knowledge on the responses of cork oak to drought: more severe droughts correspond to higher decrease of cork growth and more trees affected but to greater recovery performance. Moreover, cork oak is very tolerant and resilient to extreme droughts. Nevertheless, there are other factors that affect cork growth during and after drought, namely site, tree and the age of the phellogen. In fact, in the first 2 years and in the last 2 years of the production cycle the effects of drought on growth are more pronounced than in the middle of the cycle. The age of the phellogen is significant in the recovery, resistance and resilience but not in the relative resilience. The most noticeable differences occurred in the recovery for phellogen under 3 years (17% lower than that for phellogen with 3 to 6 years of age). Moreover, under drought conditions, there is a strong evidence that forest managers should enlarge debarking rotations, namely if drought occurs in the first 2 years of the production cycle and/or establish new cork oak stands in more humid areas, namely, in higher latitudes than the actual species distribution area.

1. Introduction

Climate change is one of the worldwide main and challenging issues of the XXIst century requiring either a mitigation or an adaptation perspective (Stott et al., 2016). Changes in climate and, in particular, weather extremes will condition forest structure and tree species composition (Kätzel and Höppner, 2011) and consequently forest management strategies.

Although tree species can adjust to new environmental conditions, there is not much knowledge about the processes involved (Lindner et al., 2010). Nevertheless, forest managers need to adjust to this new reality and meet stakeholders needs, from a more demanding public to a more competitive and exigent forest industry.

All climatic scenarios for the Mediterranean region foresee raising temperatures, water deficits and more intense, frequent and long extreme events, in particular, severe droughts, heat waves, heavy precipitation and fewer cold days (IPCC, 2014; Lindner et al., 2010). It is predictable that this region will have a decrease in productivity and

changes in species distribution as a consequence of summer water deficits (Kelly et al., 2002; Santos and Miranda, 2006; Gea-Izquierdo et al., 2013). Albeit we are only at an initial step in the forecasted tendencies of global warming, ecological responses to recent climate change are by now clearly noticeable (Walther et al., 2002) and a deeper understanding on tree response strategies should be a scientific priority (Anderegg et al., 2015; Allen et al., 2010).

One of the Mediterranean forest systems that may severely suffer from climate changes is the cork oak (*Quercus suber*) based agro-forestry system (Santos and Miranda, 2006). Cork oak forests are distributed in the Mediterranean basin, in an area of approximately 2.2 million hectares directed to an annual production of up to 200 thousand tons of cork that supply an important industry (APCOR, 2016). Portugal is the leader producer of raw cork and processed cork products namely cork stoppers for the wine industry, the most important product in all the cork segment, with the greatest added value (Pereira, 2007). The production of cork is based on a sustainable periodical stripping (usually with 9 year interval) of the cork (outer bark of the cork oak) from the

---

* Corresponding author.
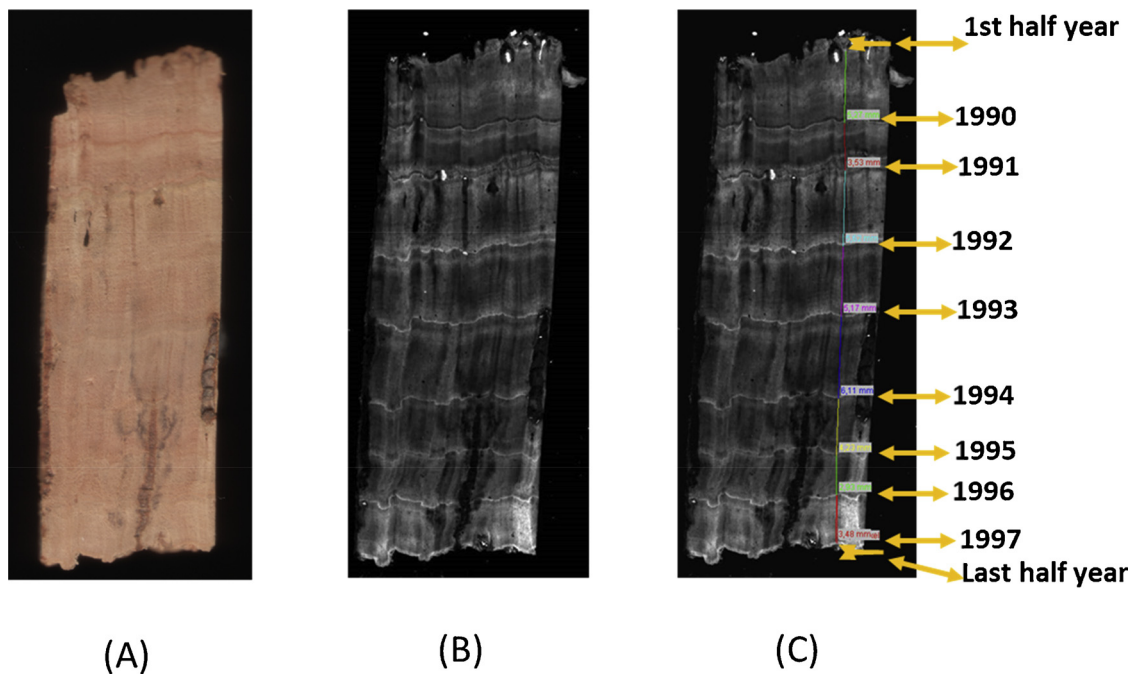 *E-mail address:* cleite@isa.ulisboa.pt (C. Leite).

Fig. 1. A - Transverse view of a radial strip cut from a cork sample B – Fluorescence image of the cork radial strip C - Sample measured and dated, showing the two incomplete growth rings.

stem, allowing to have a plank with a thickness required for the manufacture of cork stoppers (> 24 mm).

Ecologically *Q. suber* grows well with mean annual precipitations from 600 mm to 800 mm, with 500 mm considered to be the minimum for a balanced tree development (Natividade, 1950; Pereira, 2007). However, it is susceptible to the amount and moment of precipitation, and late spring precipitation has more influence on cork oak growth than the entire annual precipitation, while it recovers quickly after a year of extreme dryness (Besson et al., 2014).

In respect to cork growth, rainfall, summer drought and temperature are determining factors and, in particular, water availability is the most constraining growth factor (Cherubini et al., 2003; Caritat et al., 2000; Costa et al., 2016; Oliveira et al., 2016) with a great effect of the precipitation from May to September on the activity of the phellogen (Pizzurro et al., 2010). Cork growth is conditioned by water availability in short time scales (from 2 to 11 months), with spring precipitation having a major effect. Moreover, cork growth rapidly recuperates when drought circumstances end (Oliveira et al., 2016).

As well as wood, cork has two different annual growth periods (spring and autumn) and annual growth rings are detectable due to the presence of a noticeable layer of darker latecork cells at the end of the growth period (Pereira et al., 1988). The rate and duration of the meristematic activity of the phellogen largely determines the width of a cork ring (Pereira, 2007).

In spite of all the research conducted so far about the relation between cork growth and climate (e.g. Caritat et al., 1996, 2000; Costa et al., 2016; Oliveira et al., 2016), there is little knowledge about the effect on cork growth of drought occurring in different moments of the 9-year cork growth cycle (e.g. beginning, middle and end), corresponding to different phellogen ages. Only a preliminary study of Leite et al. (2018) addressed this issue. Therefore, the focus of the present research is the investigation of the influence of phellogen age on the cork-growth responses to drought. This goal is addressed through a pointer year analysis followed by a resilience components study using a mixed model approach.

This publication is, as far as we know, the first analysis of resilience components on cork growth and, furthermore, the first dendroclimatic study on cork using a mixed model methodological approach. The

results obtained are converted in forest management advices to mitigate the effects of the forthcoming more frequent droughts in the Mediterranean basin. This is of great importance because one of the present challenges is to transpose the ecophysiology and forest ecology scientific research into forest management prescriptions suitable for forest owners and managers (Bräuning et al., 2017).

2. Material and methods

2.1. Study area

The cork samples used in this research were collected in 12 Portuguese central-west *montado* estates placed in one of the largest continuous forest areas of cork oak and located in the Coruche municipality, Portugal. *Montado* is a multifunctional system characterized by a forest with low density trees combined with agriculture and/or pastoral activities. The sampling sites are set in the Tagus river basin, in one of the main production areas for the cork oak, characterized by a Mediterranean climate influenced by the Atlantic Ocean, as previously described in detail in Oliveira et al. (2016).

2.2. Data acquisition

A total set of 2081 cork samples, of which 1584 from Oliveira et al. (2016), was collected at breast height (1.30 m) during the stripping season in adult productive cork oak trees. The trees were randomly selected in the sites as a proportional part of the total trees under stripping. All the cork samples included 8 complete years of growth with the preceding debarking taking place 9 years before (the stripping is performed when phellogen is active leading to an incomplete cork ring). The stripping years occurred between 1994 and 2016, allowing the study of cork growth in the time span between 1986 and 2015, corresponding to a 30-year chronology, which includes several drought events occurring in different moments along the growth cycle of cork, i.e. corresponding to different ages of the phellogen.

The collected samples (± 15 × 15 cm²) were boiled in water for 1 h at atmospheric pressure and left to air-dry until equilibrium, in a procedure similar to the one performed by the cork industry in raw

corkboards. For a more precise observation of the growth rings, the transversal sections were polished with fine sandpaper and two radial strips per sample with approximately 1 cm thickness were cut (Fig. 1A), fixed on a microscope slide and digitalized in a laser-based fluorescence imager (Fluoro Image Analyzer FLA -5100, Fujifilm, Life Science USA, Stamford, CT 06092) according to the methodology developed by Surový et al. (2009) – (Fig. 1B). Cork ring thickness was measured (Fig. 1C) with AnalySIS® image processing software (Analysis Soft Imaging System GmbH, Münster, Germany, version 3.1) and, whenever possible, along two transects in the radial direction with an accuracy of 0.01 mm. For each tree the average of the two measurements was determined.

The initial and final half rings corresponding to the years of the strippings were not considered for analysis as they are not complete growth years (see Fig. 1C). As recognized earlier by several authors (Caritat et al., 1996; Surový et al., 2009; Oliveira et al., 2016), the delimitation of annual rings was not unequivocal in all the samples, and only in 67% of the samples (1403 samples) cork rings were measured and dated. Each cork ring chronology was plotted and visually checked with TSAP-Win™ software (Rinntech, Heidelberg, Germany).

2.3. Exploratory data analysis

A brief characterization of the raw data was performed with a boxplot for the cork growth in each site, allowing to compare the sites and evaluate the respective variability. As the research focus is not the analysis of these specific sites but, instead, the potential climatic signal they reflect, the performed characterization was only for a descriptive analysis purpose.

Ring width curves were plotted for visual inspection and the sign test Gleichläufigkeit (glk) calculated using the dendrochronology program library (dplr) of the R software (Bunn, 2008; R Core Team, 2017). Gleichläufigkeit (glk) is a measure of pairwise comparison of chronologies, testing if two chronologies are simultaneously increasing/decreasing in growth at the same time (Speer, 2010); it is therefore, a measure for the likeness of tree ring curves from different trees. Furthermore, glk is a statistic that assesses the homogeneity of the samples/sites (Schweingruber et al., 1990). If in the common intervals trees respond in the same way glk is one, if they disagree glk is zero.

As usually done in dendroclimatic studies (Fritts, 1976), each cork-growth curve was plotted and standardized in order to remove systematic changes of growth associated with tree age and maximize the inter-annual fluctuations caused by the weather. In a similar approach as performed previously (Caritat et al., 2000; Costa et al., 2001; Oliveira et al., 2016), a negative exponential curve was used in the standardization process (detrend) through the early mentioned dplr library. This selection is also in accordance to Cook (1987), as the *montado* system is characterized by trees growing in open-environments with negligible competition for light. Indexation equalizes all ring width curves to an unitarian mean value, so that a tree with a large average growth will not dominate over other trees with small growth when they are put together in a mean chronology (Douglass, 1919).

This procedure fits the following model:

$$G_t = ae^{-bt} + k$$

where G_t is the growth trend and is estimated as a function of time t, being a, b and k, the coefficients. If that nonlinear model could not be fitted, then a standard linear model was adjusted $G_t = b_0 + b_1t$ where b_0 is the intercept and b_1 the slope (Bunn, 2008).

After this procedure, for each tree, RWI - ring width indices (or cork growth indices) were calculated dividing the real cork growth (R_t) by the expected growth at time t (G_t). The division of R_t by G_t not only removes the trend in growth but also scales the variance so that it is approximately the same all over the entire period of the time series (Fritts, 1976). Furthermore, this approach also retains as much potential low-frequency climate information as possible (Fritts, 1976; Cook

et al., 1990; Cook and Kairiukstis, 1990; Briffa et al., 1992).

After detrending, the signal-to-noise ratio (SNR) was calculated also with the dplR package to assess the strength of the observed mutual signal between trees (Cook et al., 1990). Furthermore, a final chronology was created with the average of the indices of all the trees and plotted (sample depth is also showed). This final index chronology contains the environmental signal common to all trees, i.e. the population signal (Fritts, 1976).

2.4. Climate data

The droughts that occurred in the 30-year period of our chronology, were characterized by the drought index *Standard Precipitation Evapotranspiration Index* (SPEI) developed by Vicente-Serrano et al. (2010) because it is one of the most generalized approaches for drought analysis, and droughts in the Iberian Peninsula are better detected with SPEI than with the *Standard Precipitation Index* (SPI) (Páscoa et al., 2017). In fact, SPEI is based on a monthly climatic water balance (precipitation minus potential evapotranspiration) and not only on precipitation data as the SPI, therefore representing a simple climatic water balance. Furthermore, as it includes temperature data, it accounts for the warming-related drought impacts on diverse ecological, hydrological and agricultural systems (Vicente-Serrano et al., 2010). Also, SPEI can be used to analyze drought at different time scales (Beguería et al., 2013; http://spei.csic.es/home.html). Furthermore, it accounts for the accumulation of deficits/overplus at diverse timescales (Drew et al., 2013).

Cork has two different annual growth periods in spring and in autumn, and is affected mainly by drought conditions from two to eleven months (Oliveira et al., 2016). The highest Pearson correlations between SPEI and cork growth (index) were found, by these authors, for time scales comprising previous winter and spring of the growth year. So, to reproduce the previous winter growing conditions we used the accumulated SPEI of April with the previous seven months (SPEI Oct-Apr) and to reflect the spring growing conditions we used the accumulated SPEI of August with the previous five months (SPEI Apr-Aug). We also used the SPEI of the hydrologic year (from October of the previous year until September of the growth year) to reflect the drought conditions of the entire year (SPEI Oct-Sep). The three accumulated SPEI data were directly retrieved from the Global SPEI database (http://sac.csic.es/spei/database.html), with a 0.5° spatial resolution for the Coruche municipality. This index is based on the FAO-56 Penman-Monteith estimation of potential evapotranspiration that is one of the most recommended for long-term climatological analysis (http://spei.csic.es/home.html). A plot with the SPEI and RWI for the 30-year period was produced to better demonstrate the relation between growth and the diverse SPEI.

In order to climatically categorize the years of our chronology, we used the classification proposed by Agnew (2000) and followed by Páscoa et al. (2017) that orders drought indices according to four classes: no drought if SPEI > -0.84; moderate if -0.84 > SPEI > -1.28; severe, if -1.28 > SPEI > -1.65 and extreme, if -1.65 > SPEI.

2.5. Pointer years

As the main interest of our research is the study of the effect of drought on cork growth and the influence of the phellogen age on the cork oak response to those climatic events, a pointer years analysis was performed. In fact, pronounced pointer years are formed in years of climatic extremes (Matisons et al., 2013; Schweingruber et al., 1990). Pointer years are years with remarkable growth responses at the stand level (Schweingruber et al., 1990). To identify a pointer year, we used the package *pointRes* of the R software (van der Maaten-Theunissen et al., 2015; R Core Team, 2017) with a relative growth change approach. We considered that a pointer year occurred when at least 55% of the trees had 30% (or more) increase/decrease in cork growth when

compared with the average growth of the preceding 2 years. Only the previous 2 years were considered because cork growth is influenced mainly by the precipitation in the previous winter and February mean minimum temperature, and not by earlier weather conditions (Oliveira et al., 2016).

For the negative pointer years, the components of resilience were also calculated (Lloret et al., 2011). In fact, these authors preconize that these components of resilience analysis can be used to better understand the mechanisms underlying resilience patterns as the recovery after drought is an indicator of individual-level resilience. Therefore, this methodology considers the following indicators (components): 1) resistance, the opposite of the decrease in ecological performance during the disturbance, and estimated by the ratio between growth during and before the disturbance; 2) recovery, that is the recuperating capacity in relation to produced damage, and estimated by the ratio between the growth after and during the disturbance; 3) resilience, defined as the ability to return to the previous ecological performance, and assessed by the ratio between the growth after and before the disturbance; and 4) relative resilience, the resilience weighted by the growth decrease experienced during the disturbance – this index is based on the concept that the impact (in this case the reduction in cork growth) during the disturbance determines the ability to reach the pre-disturbance growth. However, the interpretation of this index, at the individual level, is not unambiguous since high values can mean either a higher recovering capacity (through, for example, mobilization of stored reserves) or the effect of the decrease in the competition (due to increased neighbor mortality) (Lloret et al., 2011).

2.6. Statistical analysis

To evaluate the effect of the age of the phellogen in the ecological performance of the trees when exposed to drought (disturbance), a mixed model approach was used (Pinheiro and Bates, 2000). Although we were not interested in analyzing the covariance structure induced by the grouping of the data, the account of that variability in the model allowed to better detect the effect of our variables of interest (phellogen age and drought) than if a fixed effects model was used (e.g. ANOVA, ANCOVA or other non-parametric models). Indeed, mixed models have several advantages when compared with fixed effects models (Brown and Prescott, 2014). As highlighted by (Paulo et al., 2017) this approach has been used in several analysis of cork oak relevant tree dendrometric variables (e.g. height-diameter relation; weight of de-barked cork; crown diameter and, by these authors, cork caliper). In our approach, we considered the age of the phellogen, SPEI Oct-Apr, SPEI Apr-Aug and SPEI Oct-Sep as fixed effects and site and tree nested on the site as random effects. The sites were considered random effects because they are a random sample of all the sites in the *Coruche* region, and also because we are not interested in studying the sites by themselves. The age of the phellogen was grouped in 3 classes – young (1–2 years); mean (3–6) and old (7–8).

The R package *nlme* was used for this analysis (Pinheiro et al., 2018; R Core Team, 2017), specifying the fitting method of maximum likelihood, as it is the only method that allows the estimation of the fixed effects estimators. The best model was selected with the R package *MuMln* (Bartón, 2018; R Core Team, 2017).

3. Results

3.1. Cork growth and pointer years

The average annual cork-ring width distribution is presented in Fig. 2. The minimum value occurred in site 2 (0.78 mm) and the maximum value in site 6 (8.22 mm), corresponding to an amplitude of 7.44 mm. If the possible outliers are not considered, the most heterogeneous sites are sites 1 and 2, and site 6 is the one with the lowest amplitude. The mean values are in the range from 2.60 mm (site 6) to
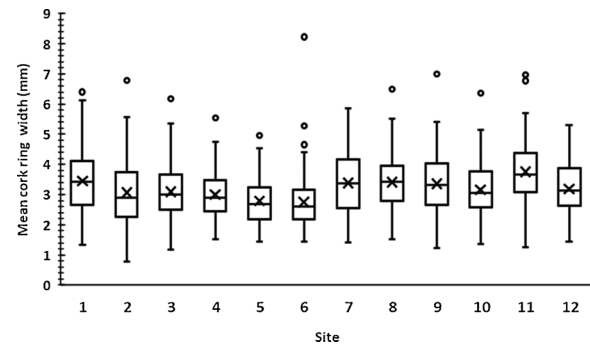
Fig. 2. Boxplot of the average annual cork ring width (mm) in each site.

3.67 mm (site 11), revealing a considerable homogeneity between sites. The mean value of the entire sample is 3.30 ± 1.44 mm.

In respect to the dendrochronological statistics, the sign test - Gleichläufigkeit (glk) presented a value of 0.656 representing a good homogeneity of the samples/sites. The SNR was considerably high – 144 times more signal than noise, the total number of trees was 1403 and the mean number of trees used to calculate SNR was 371, which reveals the robustness of the sample.

Fig. 3 shows the final mean chronology for the entire period and the respective sample depth. The mean sample depth is 370 and the minimum sample depth occurs in the years from 2012 to 2015 with almost 100 samples, also giving confidence to the results. A total of five pointer years were identified: positive (2007) and negative (1995, 1999, 2005 and 2012) pointer years are highlighted. It should be also pointed out that the cork growth index of the year immediately after a negative pointer year is always greater than 1, thereby revealing a great growth recovery i.e. growth in these years is above the mean value.

For the negative pointer years, Table 1 presents the percentage of trees that have a 30% deviation under the mean value of the growth of the previous 2 years and the mean deviation from the mean growth value of the 2 previous years. It should be noticed that the mean deviation from the mean growth in 2005 reaches almost -46% indicating a great decrease on growth due to the extreme drought conditions experienced in this year. Additionally, in all the negative pointer years more than 55% of the trees have a growth 30% under the mean value and in 2005 this value is greater than 80% revealing that the great majority of the trees were very affected by drought in this year.

Fig. 4 shows the relation between the cork-growth index mean chronology and the standard precipitation evapotranspiration index (SPEI) representative of the drought conditions in the winter before the growing season (SPEI Oct-Apr), in the spring of the growing season (SPEI Apr-Aug) and in the hydrologic year (SPEI Oct-Sep). In most of the years, there is a clear cause-effect relationship between the three SPEIs (e.g. 1986, 1987, 1988, 1995, 1996, 1999, 2002, 2005, 2007, 2009, 2010, 2011, 2014 and 2015), as to negative/positive SPEIs
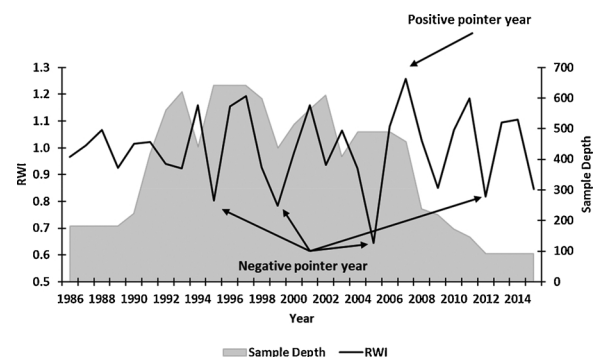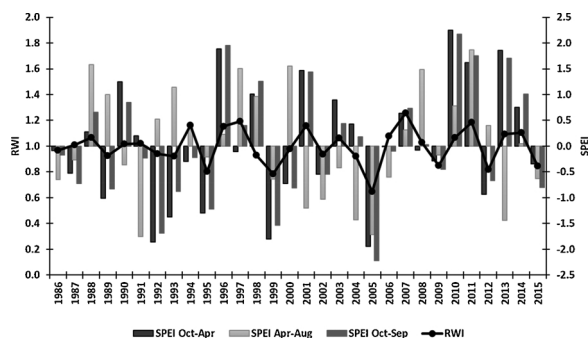
Fig. 3. Cork-growth index (RWI) chronology (in black) and sample depth of each year (grey area). Pointer years are highlighted by arrows.

Table 1

Characterization of the identified pointer years regarding the number and proportion of trees with growth decreases above 30% and mean growth deviation.

Year	Number of trees in the series	Proportion of trees with growth 30% under the mean growth of the 2 previous years (%)	Mean growth deviation from the mean growth of the 2 previous years (%)
1995	440	55.23	−30.51
1999	405	66.17	−35.26
2005	409	81.66	−45.64
2012	91	60.44	−33.36

Fig. 4. Relation between cork-growth index (RWI) chronology (line) and SPEI for different timescales reflecting spring, winter and hydrologic year wet conditions (bars).

correspond a decline/increase in cork growth indices. Nevertheless, in the years when there is not a synchrony between all the SPEIs and cork growth indices, this relation occurs in at least one of the SPEIs (1989–1994, 1997, 2000, 2001, 2003, 2004, 2008, 2012, 2013). In fact, only in 1998 did a decline on cork growth index correspond to positive SPEIs while in 2006 the inverse situation occurred. It should be noticed that large negative values of SPEIs in 1992 and 1993 did not correspond to great decreases on cork growth index as already pointed out by (Oliveira et al., 2016). Further, except in 2012, all the other pointer years experienced a clear situation of drought corresponding to at least two of the SPEIs. Also, some years (e.g. 1991 and 2013) had extreme or severe drought in spring (SPEI Apr-Aug) while the cork growth index showed an above the average growth; on the contrary, in 2004, wet conditions were prevailing, but the mean cork growth index was under the mean value. Furthermore, 1992 was not considered a pointer year even if the winter prior to the growing season (SPEI Oct-Apr) and the hydrological year (SPEI Oct-Sep) were considered to have extreme drought, according to the classification proposed by Agnew (2000), and the mean cork growth was below 1.

It should also be highlighted that several authors (e.g. García-Herrera et al., 2007 and Páscoa et al., 2017) considered that 2005 had the most severe drought in the Iberian Peninsula which is in accordance to the classification of extreme drought.

Overall the analysis of Fig. 4 suggests that drought reduces cork growth and that more severe drought conditions correspond to a greater decrease in growth. However, there should be other factors affecting cork growth, rather than climate conditions since this relation is not universal (e.g. 2012 and 2004).

3.2. Resilience components

The results presented in Table 2 show that 2005, the year with the most severe drought conditions (lowest SPEI for all the time spans), is the year with the lowest mean resistance (0.54), which means, that it is the year with the major cork growth loss due to drought but also the year with the greatest mean recovery (1.62), i.e. the year with the highest recovering capacity. However, this behavior is not transversal to all the years: for example, 1999 is a year of extreme drought in the winter and severe all over the year but has the lowest recovery value

(1.16), while 1995 with less severe drought conditions than 1999 in all the timespans has higher mean recovery (1.48 vs 1.16).

Moreover, the pointer year of 2012 with the highest SPEI for all the timespans does not stand out in any one of the calculated parameters; in fact, it has the 2nd lowest mean recovery (1.32) and relative resilience (0.16) and the 2nd highest average resistance (0.67) and resilience (0.82).

Furthermore, recovery is the component of resilience with the greatest heterogeneity (standard deviation values between 0.40 and 0.70) for all the pointer years and to the greater mean values (2005 and 1995) correspond greater variability.

It should also be pointed out that mean resilience is always at least 0.79 and in 2005 (year with most severe drought conditions) corresponds to the 2nd highest value (0.82).

The correlation between the components of resilience and SPEIs is shown in Table 3. Resistance (growth during vs. before drought) is highly and positively (from 0.72 to 0.88) correlated with less severe drought conditions (higher SPEI values); also, resilience (growth after vs. before drought) is positively (from 0.23 to 0.35) correlated with less severe drought conditions but less than resistance. It is also evident that recovery (growth after vs. during drought) and relative resilience (resilience weighted by reduction during drought) are positively correlated with more severe drought conditions (lower SPEI values).

3.3. Statistical analysis

The effect of the age of the phellogen in the ecological performance of the trees regarding cork growth when exposed to drought (disturbance) was evaluated by modelling the resilience components. The best linear mixed model for recovery and resilience was the full model, which means the model that included all the considered fixed effects (SPEI Apr-Aug, SPEI Oct-Apr, SPEI Oct-Sep and phellogen age), as well as the effects of site and tree, nested on site, that were considered random. The effect of the phellogen age was statistically significant in the recovery (p-value = 0.002) and in the resilience (p-value = 0.004).

The recovery of the cork growth when the age of the phellogen is less than 3 years was approximately 17% lower than when the phellogen is between 3–6 years old and when the age is above 6 years the recovery was 8% lower. The resilience when the phellogen age is above 6 years was 9% lower than when the phellogen age is between 3–6 years.

In what concerns the resistance, the best model did not include the SPEI Oct-Apr but included all the random effects. The effect of the phellogen age was statistically significant for this component (p-value = 4.93×10^{-14}). When the phellogen age was greater than 6 years the decrease was 14% compared to the phellogen with 3–6 years.

The best model for the relative resilience was the model that did not include the age of the phellogen but included all the SPEI's and all the random effects (site and tree, nested on site).

4. Discussion

The studies on cork growth and its relation with climate started about 20 years ago (Caritat et al., 1996) and continued since then (Caritat et al., 2000; Costa et al., 2001, 2016; Oliveira et al., 2016 and Leite et al., 2018). Also, the response of trees, communities and

Table 2

Drought conditions in winter (SPEI Oct-Apr), spring (SPEI Apr-Aug) and hydrologic year (SPEI Oct-Sep), mean and standard deviations of each one of the resilience components calculated for the negative pointer years (1995, 1999, 2005 and 2012).

Year	Drought conditions			Mean recovery	Mean resistance	Mean resilience	Mean relative resilience
	SPEI Oct-Apr	SPEI Apr-Aug	SPEI Oct-Sep				
1995	severe −1.30	no drought −0.21	moderate −1.22	1.48 ± 0.70	0.70 ± 0.22	1.01 ± 0.28	0.31 ± 0.33
1999	extreme −1.81	no drought −0.64	severe −1.54	1.16 ± 0.42	0.65 ± 0.28	0.79 ± 0.28	0.06 ± 0.26
2005	extreme −1.94	extreme −1.71	extreme −2.22	1.62 ± 0.61	0.54 ± 0.20	0.82 ± 0.23	0.27 ± 0.23
2012	moderate −0.94	no drought 0.40	no drought −0.66	1.32 ± 0.40	0.67 ± 0.23	0.82 ± 0.24	0.16 ± 0.19

ecosystems to short-term disturbances has been widely explored as described by Lloret et al. (2011). Nevertheless, this present research on cork growth is, as far as we know, the first one on disturbance analysis through a component resilience approach.

The mean values reported in this study for cork ring width (3.30 mm) are in accordance to the ones reported by several authors for the cork oak and also encompass the variability between trees and sites that is consistently reported. In fact, the values found for cork ring width by different authors are in the range from 2.8 to 3.6 mm (3.30 mm and 3.56 mm by Caritat et al., 2000; 3.8 mm by Costa et al., 2002; 3.5 mm by Pereira, 2007; 3.3 mm by Oliveira et al., 2016; 3.6 mm, 3.1 mm and 2.8 mm by Costa et al., 2016). In a large cork sampling covering all the production regions in Portugal, a mean value of cork ring width of 3.6 mm was reported ranging from site means of 1.6 mm to 4.6 mm (Lauw et al., 2018).

The high number of samples analyzed (1403, with a minimum of 91 samples/year, Fig. 3) allowed us to better account for the variability of the entire population of interest and therefore to have better estimations. This sampling intensity combined with the high value of the SNR (144) are strong evidences of the reliability of our results.

We confirmed the outcomes of several authors about the negative effect of drought on cork growth (Caritat et al., 1996; Costa et al., 2016 and Oliveira et al., 2016), reinforcing that the spring and winter rain strongly influences phellogen activity. Furthermore, our results (Table 1) demonstrated that more severe drought conditions correspond to greater decreases in cork growth and to more trees affected by drought, although, as pointed out by Oliveira et al. (2016) and Besson et al. (2014), cork oak rapidly recovers as water availability increases, achieving previous ecological performance (see Table 2 and Fig. 4).

Our analysis demonstrated that the age of the phellogen is a relevant factor on the recovery and on the resilience of cork oak regarding cork growth and that the younger the phellogen (up to 2 years) the greater the effect of the drought on growth. These findings are not in accordance to the previous results (Leite et al., 2018), that supported the hypothesis that the age of the phellogen has no influence on the magnitude of cork growth decline in response to drought. However this assumption was supported on data from a small sampling and number of drought events (3 sites, 149 samples and 1 drought event). The present study expanded the number of sites and samples (12 and 1403) and analyzed various drought events/pointer years (4) while applying a methodology of analysis of resilience components instead of cork

growth indices with a mixed model approach that better detect the effect of the variables of interest.

These findings reinforce the importance of the sampling size in evaluating biological/ecological processes namely when a large growth and response variability is present, such as it is the case of the cork oak and cork growth.

Additionally, the results (Table 2) suggest that more severe droughts correspond to lower resistance values, which means, greater the effects of the drought on the growth of the drought year. The trade-off between resistance and recovery after drought, as identified by (Galiano et al., 2011) and highlighted by (Lloret et al., 2011), that states that resistance and recovery depend on the quantity of stored reserves was confirmed here, with the lowest value of resistance corresponding to the highest recovery. A low resistance indicates reserve consumption during drought but if there is recovery after drought then there is a positive effect from the regaining of photosynthetic capacity after drought (Galiano et al., 2011).

Nevertheless, there is not a clear pattern between the severity of drought and the recovery, resistance or resilience behavior and there is a great variability on the performed recovery (Table 2 and statistical analysis). The same conclusions were achieved by (Lloret et al., 2011) who got miscellaneous results for the impact of drought on subsequent tree performance and concluded that tree responses to disturbance are extremely complex. These authors also referred that micro-site quality is a more determinant factor in response to disturbance than physiological or structural factors.

The mean resilience values were in general higher than 0.80 (Table 2) showing that post-drought cork growth is close to pre-drought (unitary resilience indicates similar growth values before and after drought) and demonstrating that cork oak has a great capacity to recuperate the previous ecological performance. Therefore, the impact of drought on cork growth of the subsequent years was moderately low, supporting the hypothesis that cork oak is very tolerant and resilient to extreme droughts. Similar results and conclusions were achieved regarding wood growth for other Mediterranean species (*Pinus nigra*, *P. sylvestris* and *Juniperus communis*) (Herrero and Zamora, 2014) although not for another oak (*Q. faginea*) (Granda et al., 2013). *P. sylvestris* needs more time than *Q. pyrenaica* to recover from drought (Gea-Izquierdo et al., 2014). Regarding this, species acclimated to more humid environments may be more susceptible to drought as reported for *Q. ilex* (Granda et al., 2013).

Table 3

Pearson correlation coefficients between the mean annual components of resilience and SPEI for different timescales reflecting spring, winter and hydrologic year wet conditions.

	Mean annual resistance	Mean annual recovery	Mean annual resilience	Mean annual relative resilience
SPEI Apr-Aug	0.88	−0.52	0.27	−0.24
SPEI Oct-Apr	0.72	−0.18	0.35	0.05
SPEI Oct-Sep	0.83	−0.49	0.23	−0.23

The results of this research also enforce that the occurrence of wet conditions in winter, spring and the whole year are determinant to cork growth, during and after drought, but there are other factors involved, namely the site, the tree and the age of the phellogen. This issue was raised by Sánchez-González et al. (2007) by stating that there is a relationship between cork growth and "unobservable site factors" rather than average climatic conditions.

Moreover, the ability to recover (expressed by the relative resilience) does not seem to be affected by the age of the phellogen. It should be stressed that the phellogen age range in the present research is only between 1–8 years, therefore not allowing to extrapolate the response for much older meristems. For instance Lloret et al. (2011) found no overall decrease in all the resilience components in old trees, supporting the hypothesis that old trees recover better from more recent events.

Also, there is still lack of information about the minimum threshold for the recovery of cork growth, which means that it is still unknown which are the drought conditions in which this species no longer is able to recover. As more frequent drought conditions are expected, further investigation is needed on the question if the response to drought conditions remains high after more repeated droughts.

Furthermore, it should also be interesting to analyze not only the effect of the age of the phellogen but also the effect of the age of the tree on the post-drought response. Other future research opportunities could also be related to the simultaneous analysis of these resilience components on the growth of the wood and of the cork, to have a deeper insight in the highly complex phenomena involved under cork oak drought responses. These issues could contribute to a better knowledge regarding cork oak vulnerability to drought and should be considered in a forest management strategy under a context of adaptation/mitigation of climate change effects in the Mediterranean region.

Transposing our dendroecological results to forest management we preconize that cork growth cycles should be enlarged in case of occurrence of drought and when the production records suggest that the reduction on growth due to drought may lead to obtain cork planks under 24 mm, and particularly if the drought occurs in the first two years of the production cycle. This management directive lies on the economic rationale of not compromising the technological possibility to produce cork stoppers (Lauw et al., 2018). The extension of the cork stripping rotation in order to mitigate the effect of climate change was also proposed by (Palma et al., 2015) and (Leite et al., 2018).

Moreover, the predicted scarcity of water in the actual distribution area of cork oak also suggests that new cork oak plantations should be shifted into more humid areas (higher latitudes) where, in the nearest future, the annual precipitation will be more adequate for the balanced cork oak development and thereby allowing the maintenance of the production of cork planks thick enough to manufacture cork stoppers with the usual 9-year cycle.

5. Conclusions

In a changing climate, Mediterranean ecosystems are expected to be under more severe, frequent and extreme drought conditions similar to the ones analyzed in this research. Our results show that dry spells have a strong impact on cork growth and the more severe the drought the greater the reduction on growth but also the greater the recovery performed. Moreover, although the effects of drought persist, the recovering capacity of cork oak is not affected even in drought conditions as severe as the ones from 2005. Therefore, we advise forest managers of the Mediterranean region to continue to produce cork as, according to our findings, it is expected that cork oak recovers from the growth reduction imprinted by drought.

Nevertheless, there are other factors that affect the performance of cork growth during and after drought, namely site, tree and the age of the phellogen, and have an important role on the responses of cork oak to drought and on its ability to recover. The effects of drought on

growth are more pronounced in the first two years and in the last two years of the cork production cycle than in the middle of the cycle.

The results of this study demonstrate that cork oak is very tolerant and resilient to droughts, has a great capacity to recover from water scarcity conditions, reflecting its high adaptation to the variability of water availability in the Mediterranean region. Our research also demonstrates that cork-ring data analyses may be adequate to detect signals of cork oak response to less water availability in a nearest future.

Furthermore, there is a strong signal for forest managers to extend the cork stripping rotation in case of drought, namely if the drought conditions occur in the beginning of the production cycle (first 2 years) and if there is the risk that cork planks are under 24 mm thickness as given by the past history of the site. Moreover, our results also suggest that in a context of more frequent and severe drought episodes, new cork oak plantations should be established in more humid zones (e.g. higher latitudes) to ensure the production, in a 9-year cycle, of cork planks thick enough to produce cork stoppers.

References

Agnew, C., 2000. Using the SPI to identify drought. Drought Netw. News 12, 6–12.
Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., (Ted) Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660–684. https://doi.org/10.1016/j.foreco.2009.09.001.
Anderegg, W.R.L., Flint, A., Huang, C., Flint, L., Berry, J.A., Davis, F.W., Sperry, J.S., Field, C.B., 2015. Tree mortality predicted from drought-induced vascular damage. Nat. Geosci. 8, 367.
APCOR, 2016. Anuário de cortiça 2016. APCOR.
Bartón, K., 2018. MuMIn: Multi-Model Inference v. 1.40.4.
Beguería, S., Vicente-Serrano, S., Reig, F., Latorre, B., 2013. Standardized precipitation evapotranspiration index (SPEI) revisited: parameter fitting, evapotranspiration models, tools, datasets and drought monitoring. Int. J. Climatol. 34, 3001–3023. https://doi.org/10.1002/joc.3887.
Besson, C., Lobo-do-Vale, R., Rodrigues, M., Almeida, P., Herd, A., Grant, O., David, T.S., Schmidt, M., Otieno, D., Keenan, T., 2014. Cork oak physiological responses to manipulated water availability in a Mediterranean woodland. Agric. For. Meteorol. 184, 230–242. https://doi.org/10.1016/j.agrformet.2013.10.004.
Bräuning, A., Bolte, A., Nabais, C., Rossi, S., Sass-Klaassen, U., 2017. Editorial: studying tree responses to extreme events. Front. Plant Sci. 8, 506. https://doi.org/10.3389/fpls.2017.00506.
Briffa, K.R., Jones, P.D., Bartholin, T.S., Eckstein, D., Schweingruber, F.H., Karlén, W., Zetterberg, P., Eronen, M., 1992. Fennoscandian summers from ad 500: temperature changes on short and long timescales. Clim. Dyn. 7, 111–119. https://doi.org/10.1007/BF00211153.
Brown, H., Prescott, R., 2014. Applied Mixed Models in Medicine. John Wiley & Sons, New York, NY.
Bunn, A., 2008. A dendrochronology program library in R (dplR). Dendrochronologia 26, 115–124. https://doi.org/10.1016/j.dendro.2008.01.002.
Caritat, A., Gutiérrez, E., Molinas, M., 2000. Influence of weather on cork-ring width. Tree Physiol. 20, 893–900. https://doi.org/10.1093/treephys/20.13.893.
Caritat, A., Molinas, M., Gutierrez, E., 1996. Annual cork-ring width variability of Quercus suber L. in relation to temperature and precipitation (Extremadura, southwestern Spain). For. Ecol. Manag. 86, 113–120. https://doi.org/10.1016/S0378-1127(96)03787-5.
Cherubini, P., Gartner, B., Tognetti, R., BRAeKER, O., Schoch, W., Innes, J.L., 2003. Identification, measurement and interpretation of tree rings in woody species from Mediterranean climates. Biol. Rev. 78, 119–148. https://doi.org/10.1017/

S1464793102006000.

Cook, E., Briffa, K., Shiyatov, S., Mazepa, V., 1990. Tree-ring standardization and growth-trend estimation. In: Cook, E., Kairiukstis, L. (Eds.), Methods of Dendrochronology: Applications in the Environmental Sciences. Kluwer/IIASA, Dordrecht, pp. 104–123.

Cook, E., Kairiukstis, L., 1990. Methods of Dendrochronology: Applications in the Environmental Sciences. Kluwer Academic Publishers, Dordrecht.

Cook, E.R., 1987. The decomposition of tree-ring series for environmental studies. Tree-Ring Bull. (1987), 37–59.

Costa, A., Barbosa, I., Roussado, C., Graça, J., Spiecker, H., 2016. Climate response of cork growth in the Mediterranean oak (Quercus suber L.) woodlands of southwestern Portugal. Dendrochronologia 38, 72–81. https://doi.org/10.1016/j.dendro.2016.03.007.

Costa, A., Pereira, H., Oliveira, A., 2002. Influence of climate on the seasonality of radial growth of cork oak during a cork production cycle. Ann. Sci. 59, 429–437. https://doi.org/10.1051/forest:2002017.

Costa, A., Pereira, H., Oliveira, A., 2001. A dendroclimatological approach to diameter growth in adult cork-oak trees under production. Trees 15, 438–443. https://doi.org/10.1007/s004680100119.

Douglass, A.E., 1919. Climatic Cycles and Tree-growth. Carnegie Institution of Washington.

Drew, D.M., Allen, K., Downes, G.M., Evans, R., Battaglia, M., Baker, P., 2013. Wood properties in a long-lived conifer reveal strong climate signals where ring-width series do not. Tree Physiol. 33, 37–47. https://doi.org/10.1093/treephys/tps111.

Fritts, H., 1976. Tree Rings and Climate, 1st edition. Academic Press, London.

Galiano, L., Martínez-Vilalta, J., Lloret, F., 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. New Phytol. 190, 750–759. https://doi.org/10.1111/j.1469-8137.2010.03628.x.

García-Herrera, R., Paredes, D., Trigo, R., Trigo, I., Hernandez, E., Barriopedro, D., Mendes, M., 2007. The outstanding 2004/05 drought in the Iberian peninsula: associated atmospheric circulation. J. Hydrometeorol. 8, 483–498. https://doi.org/10.1175/JHM578.1.

Gea-Izquierdo, G., Fernández-de-Uña, L., Cañellas, I., 2013. Growth projections reveal local vulnerability of Mediterranean oaks with rising temperatures. For. Ecol. Manag. 305, 282–293. https://doi.org/10.1016/j.foreco.2013.05.058.

Gea-Izquierdo, G., Viguera, B., Cabrera, M., Cañellas, I., 2014. Drought induced decline could portend widespread pine mortality at the xeric ecotone in managed mediterranean pine-oak woodlands. For. Ecol. Manag. 320, 70–82. https://doi.org/10.1016/j.foreco.2014.02.025.

Granda, E., Camarero, J.J., Gimeno, T.E., Martínez-Fernández, J., Valladares, F., 2013. Intensity and timing of warming and drought differentially affect growth patterns of co-occurring Mediterranean tree species. Eur. J. For. Res. 132, 469–480. https://doi.org/10.1007/s10342-013-0687-0.

Herrero, A., Zamora, R., 2014. Plant responses to extreme climatic events: a field test of resilience capacity at the Southern range edge. PLoS One 9, e87842. https://doi.org/10.1371/journal.pone.0087842.

IPCC, 2014. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, New York, NY 1132 pp.

Kätzel, R., Höppner, K., 2011. Adaptation strategies in forest management under the conditions of climate change in Brandenburg. Folia For. Pol. Ser. - For. 53, 43–51.

Kelly, P.M., Leuschner, H.H., Briffa, K.R., Harris, I.C., 2002. The climatic interpretation of pan-European signature years in oak ring-width series. Holocene 12, 689–694. https://doi.org/10.1191/0959683602hl582rp.

Lauw, A., Oliveira, V., Lopes, F., Pereira, H., 2018. Variation of cork quality for wine stoppers across the production regions in Portugal. Eur. J. Wood Wood Prod. 76, 123–132. https://doi.org/10.1007/s00107-017-1196-5.

Leite, C., Oliveira, V., Lauw, A., Pereira, H., 2018. Effect of a drought on Cork growth along the production cycle. In: Alves, F., Leal Filho, W., Azeiteiro, U. (Eds.), Theory and Practice of Climate Adaptation. Springer International Publishing, Cham, pp. 127–136. https://doi.org/10.1007/978-3-319-72874-2_7.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, a., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M.J., Marchetti, M., 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For. Ecol. Manag. 259, 698–709. https://doi.org/10.1016/j.foreco.2009.

09.02.

Lloret, F., Keeling, E.G., Sala, A., 2011. Components of tree resilience: effects of successive low-growth episodes in old ponderosa pine forests. Oikos 120, 1909–1920. https://doi.org/10.1111/j.1600-0706.2011.19372.x.

Matisons, R., Elferts, D., Brūmelis, G., 2013. Pointer years in tree-ring width and earlywood-vessel area time series of Quercus robur—Relation with climate factors near its northern distribution limit. Dendrochronologia 31, 129–139. https://doi.org/10.1016/j.dendro.2012.10.001.

Natividade, J., 1950. Subericultura. Ministério da Economia–Direcção Geral dos Serviços Florestais e Aquícolas, Lisboa.

Oliveira, V., Lauw, A., Pereira, H., 2016. Sensitivity of cork growth to drought events: insights from a 24-year chronology. Clim. Change 137, 261–274. https://doi.org/10.1007/s10584-016-1680-7.

Palma, J.H.N., Paulo, J.A., Faias, S.P., Garcia-Gonzalo, J., Borges, J.G., Tomé, M., 2015. Adaptive management and debarking schedule optimization of Quercus suber L. stands under climate change: case study in Chamusca, Portugal. Reg. Environ. Change 15, 1569–1580. https://doi.org/10.1007/s10113-015-0818-x.

Páscoa, P., Gouveia, C.M., Russo, A., Trigo, R.M., 2017. Drought trends in the Iberian peninsula over the last 112 years. Adv. Meteorol.(2017). https://doi.org/10.1155/2017/4653126. 13 pages.

Paulo, J.A., Pereira, H., Tomé, M., 2017. Analysis of variables influencing tree cork caliper in two consecutive cork extractions using cork growth index modelling. Agrofor. Syst. 91, 221–237. https://doi.org/10.1007/s10457-016-9922-2.

Pereira, H., 2007. Cork: Biology, Production and Uses. Elsevier, Amsterdam.

Pinheiro, J., Bates, D., 2000. Mixed-Effects Models in S and S-PLUS, 1st edition. Springer-Verlag New York, New York.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2018. Linear and Nonlinear Mixed Effects Models. R Package Version 3.

Pizzurro, G., Maetzke, F., Veca, D., 2010. Differences of raw cork quality in productive cork oak woods in Sicily in relation to stand density. For. Ecol. Manag. 260, 923–929. https://doi.org/10.1016/j.foreco.2010.06.013.

R Core Team, 2017. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Sánchez-González, M., Calama, R., Cañellas, I., Montero, G., 2007. Variables influencing cork thickness in spanish cork oak forests: A modelling approach. Ann. Sci. 64, 301–312. https://doi.org/10.1051/forest:2007007.

Santos, F.D., Miranda, P., 2006. Alterações climáticas em Portugal - Cenários, Impactos e Medidas de Adaptação–Projecto SIAM II. Lisboa, Portugal. .

Schweingruber, F.H., Eckstein, D., Serre-Bachet, F., Bräker, O.U., 1990. Identification, presentation and interpretation of event years and pointer years in dendrochronology. Dendrochronologia 8, 9–38.

Speer, J., 2010. Fundamentals of Tree-Ring Research. The University of Arizona Press, Tucson.

Stott, P., Christidis, N., Otto, F., Sun, Y., Vanderlinden, J.P., van Oldenborgh, G., Vautard, R., von Storch, H., Walton, P., Yiou, P., Zwiers, F., 2016. Attribution of extreme weather and climate-related events. Wiley Interdiscip. Rev. Clim. Change 7, 23–41. https://doi.org/10.1002/wcc.380.

Surový, P., Olbrich, A., Polle, A., Ribeiro, N.A., Sloboda, B., Langenfeld-Heyser, R., 2009. A new method for measurement of annual growth rings in cork by means of autofluorescence. Trees 23, 1237. https://doi.org/10.1007/s00468-009-0363-7.

van der Maaten-Theunissen, M., van der Maaten, E., Bouriaud, O., 2015. pointRes: an R package to analyze pointer years and components of resilience. Dendrochronologia 35, 34–38. https://doi.org/10.1016/j.dendro.2015.05.006.

Vicente-Serrano, S.M., Beguería, S., López-Moreno, J.I., 2010. A multiscalar drought index sensitive to global warming: The Standardized Precipitation Evapotranspiration Index. J. Clim. 23, 1696–1718. https://doi.org/10.1175/2009JCLI2909.1.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389.

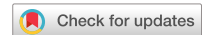## Web references

http://sac.csic.es/spei/database.html accessed on 10/04/2018
http://spei.csic.es/home.html accessed on 15/11/2018

Publication IV

Cork oak and climate change: Disentangling drought effects on cork chemical composition

SCIENTIFIC REPORTS

natureresearch

Check for updates

OPEN

Cork oak and climate change: Disentangling drought effects on cork chemical composition

Carla Leite ✉, Vanda Oliveira, Isabel Miranda & Helena Pereira

Climate change induces in the Mediterranean region more frequent and extreme events, namely, heat waves and droughts, disturbing forest species and affecting their productivity and product quality. The cork oak (*Quercus suber*) is present along the western Mediterranean basin and its outer bark (cork) is sustainably collected and used for several products, mainly wine bottle stoppers. Since most cork properties arise from its chemical composition, this research studies the effect of drought on cork chemical composition (suberin, lignin, polysaccharides and extractives) and on polysaccharide and suberin monomeric composition. Three sets of cork samples, from the same site, were examined: in one set the cork grew without drought; in another two drought events occurred during cork growth and in the third one drought event happened. The results show that, in general, drought does not affect the proportion of the main components of cork, the monomers of suberin or of polysaccharides, with few exceptions e.g. drought increased ethanol extractives and xylose in polysaccharides and decreased arabinose in polysaccharides. The variability associated to the tree is much more relevant than the effect of drought conditions and affects all the parameters analyzed. Therefore, our research suggests that the tree genetic information, or its expression, plays a much more important role on the chemical composition of cork than the drought conditions occurring during cork growth. In practical terms, the potential increased occurrence of droughts arising from climatic changes will not compromise the performance of cork as a sealant for wine bottles.

Climate change is happening and several authors agree that forest species are already being affected (e.g[1–3].) and will keep on being in the future[4–8]. The intensity and the way species are affected depends on the dimension of their present and future distribution area, their environmental tolerance, and capacity to disperse[9] while the scientific community and forest managers may have an active role in adapting and mitigating climate changes effects.

The Mediterranean region is considered to be a hotspot for climate change[10] for which the models predict an increase in the temperature and a pronounced decrease in the precipitation[10–13], corresponding to an intensification in frequency, intensity and duration of drought, mainly during the warm season[7,11,14,15]. Consequently, this region will experience high water stress conditions and reduced vegetation production.

The cork oak (*Quercus suber*) is an evergreen oak species distributed along the western Mediterranean basin, covering an area of approximately 2.1 million ha[16]. Its outer bark (cork) is periodically (usually every 9 years) removed on a sustainable procedure, corresponding to an annual production of up to 200 thousand tons[16]. This forest product is the second most important non wood forest product commercially exploited[11] and it is the raw material of an important industry with diversified products and applications, from sealants to agglomerates and composites used as insulation materials, surfacing panels for construction and aeronautics, pollutants absorbers, clothing and decorative articles, and the one with the most added value – cork stoppers for the wine industry, corresponding to 70% of the generated income of the cork industry[17–20].

Most of the characteristics of cork, namely high compressibility, flexibility under compression, low permeability and chemical and biological inertness come from its chemical composition[18,21]. Actually, many of the cork properties arise from the presence in cork cell walls of its main component – suberin[18,21]. Besides suberin (that represents on average 43%), cork is also constituted by lignin (22%), polysaccharides (19%) and extractives (16%)[19]. The monomeric composition of suberin was object of several analysis with different analytical methods (see[18] for a review on this subject). [22] identified ω-hydroxyacids (31% of the total monomers), α,ω-diacids (53%),

Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal. ✉e-mail: cleite@isa.ulisboa.pt

Stripping year	Years with drought	Annual SPEI of the drought year	Drought classification
1994	—	—	—
2003	1995	−1.22	Moderate
	1999	−1.54	Severe
2012	2005	−2.22	Extreme

Table 1. Cork stripping years, years with drought, annual SPEI and drought classification of drought years according to[35].

n-alkanols (<1%) and ferulic acid (<1%) (values calculated by[23]). The holocellulose of cork is mainly composed of glucose (50% of total monosaccharides) and xylose (35%), with smaller amounts of arabinose, mannose, galactose and rhamnose[21]. Nevertheless, there is a substantial variability in the chemical composition of *Q. suber* cork regarding between-tree and between site differences that has been evaluated in several research works[19,24–28].

The impact of drought on cork growth was addressed in numerous studies (e.g[29–34].) concluding that drought severely reduces cork growth but cork oak is very resilient and cork growth rapidly recovers when drought conditions end. To date and to our knowledge, there are no studies on the effect of drought on cork chemical composition. The objective of this study is to analyze the effect of drought on cork chemical composition. Our specific goals are to examine if drought induces any changes in the proportion of the main chemical constituents of cork and in the suberin and polysaccharide monomeric composition that could compromise the utilization of cork for the production of wine stoppers.

Material and Methods

Material. The cork samples used in this research were collected in a cork oak stand (*montado*) located in central west Portugal, in the Coruche municipality, inside the region that is considered to be one of the best production regions for the cork oak. The site has around 190 ha with 67 trees/ha and is located in the Tagus river basin where Mediterranean climate is influenced by the Atlantic Ocean.

The samples were randomly collected at breast height (1.3 m above ground), during the cork stripping season, in mature trees under exploitation. The analyzed samples were collected with different drought conditions in their timespans of the cork production cycle: 10 samples were collected in a timespan without any drought, 10 samples in a period when one drought occurred and 10 samples with two droughts.

Table 1 shows a brief description of the climatic framework for the samples - the years of cork striping (harvesting), the years of the occurrence of the droughts, the respective annual Standard Precipitation Evapotranspiration Index (SPEI) and the drought classification according to[35]. The Standard Precipitation Evapotranspiration Index (SPEI), developed by[36], is one of the most used indicators for drought analysis and droughts in the Iberian Peninsula are better detected with SPEI than with other indices like the Standard Precipitation Index (SPI)[35]. Furthermore, the classification of droughts proposed by[35] and followed by[37] categorizes drought indices according to four classes: no drought if SPEI > −0.84; moderate if −0.84 > SPEI > −1.28; severe, if −1.28 > SPEI > −1.65 and extreme, if −1.65 > SPEI.

The 30 samples (with approximately 15 × 15 cm^2) were boiled in water for one hour at atmospheric pressure and left to air-dry until equilibrium, in a procedure similar to the one usually performed by the cork industry for the production of cork stoppers. Each sample was cut in small pieces with a chisel and the back (outermost bark layer of phloemic tissues) and the belly (innermost cork layer) removed to avoid contamination with other materials. The small pieces of cork were first milled with a knife mill (Retsch SM 2000) passing through a 2 × 2 mm^2 sieve and, afterwards, with an ultra-centrifugal mill (Retsch ZM 200). The material was granulometric separated with a vibratory sieve (Retsch AS 200basic) for 10 minutes. The fractions that passed the 60 mesh screen (0.250 mm) were discarded to avoid contamination with lenticular material or woody inclusions that are chemically different from cork and the particles between 40 (0.425 mm) and 60 mesh were used, as usually performed for cork chemical analysis[21].

Chemical analysis. Summative chemical composition comprised the determination of extractives, suberin, Klason and acid-soluble lignin and the monomeric composition of polysaccharides. The analytical procedures were previously described by[38] and are here only briefly detailed. Extractives content was determined by successive Soxhlet extractions of cork samples with dichloromethane (6 h), ethanol (16 h) and water (16 h). The suberin content was determined in the extractive-free cork using methanolysis for depolymerization[38]. The suberin content (that corresponds to the fatty acids and alcohols derivatives resulting from suberin depolymerization) was quantified as percent of dry cork mass.

Klason and acid-soluble lignin were determined on the pre-extracted and desuberinized material using total hydrolysis with sulphuric acid.

The polysaccharides content was determined by quantification of the monosaccharide monomers released by the total acid hydrolysis used for lignin determination[38]. The sugar monomers were determined using a high-performance anion exchange chromatography (HPAEC) using Aminotrap plus CarboPac SA10 anion exchange columns. The carbohydrate composition was expressed in percent of total monosaccharides.

For each tree, extractives analysis was performed in 3 independent (sub)samples collected in the fraction of particles between 40 (0.425 mm) and 60 mesh (triplicate aliquots), determination of suberin and lignin were done in duplicate aliquots, and monomeric composition of polysaccharides was determined in one sample/tree.

Chemical parameter	1994 (no drought)	2003 (two droughts)	2012 (one drought)
Extractives total	12.05 ± 1.79	12.09 ± 1.22	12.52 ± 1.70
Dichloromethane	5.18 ± 1.04	5.33 ± 0.66	4.93 ± 0.82
Ethanol	3.00 ± 1.06	3.03 ± 0.99	4.12 ± 1.23
Water	3.87 ± 0.67	3.73 ± 0.69	3.47 ± 0.72
Suberin	36.56 ± 3.32	38.24 ± 3.67	35.97 ± 4.50
Lignin, total	27.93 ± 2.38	26.04 ± 2.71	27.74 ± 3.30
Klason	26.63 ± 2.36	24.72 ± 2.75	26.54 ± 3.18
Acid soluble	1.30 ± 0.26	1.32 ± 0.19	1.19 ± 0.24
Ratio suberin/total lignin	1.33 ± 0.22	1.49 ± 0.27	1.33 ± 0.33
Polysaccharide composition (% of total monosaccharides)			
Rhamnose	1.48 ± 0.50	0.82 ± 0.19	1.35 ± 0.47
Arabinose	19.22 ± 4.25	14.19 ± 2.41	17.82 ± 2.57
Galactose	8.92 ± 2.03	6.31 ± 0.79	8.04 ± 0.77
Glucose	40.73 ± 2.81	40.22 ± 0.96	39.24 ± 1.57
Xylose	20.11 ± 6.16	29.64 ± 3.79	23.78 ± 1.91
Mannose	1.95 ± 3.11	1.12 ± 1.20	1.59 ± 1.97
Galacturonic acid	5.73 ± 1.51	4.45 ± 0.36	5.89 ± 0.93
Glucuronic acid	1.18 ± 1.60	2.47 ± 0.16	1.11 ± 1.44
Acetyl	0.68 ± 0.22	0.78 ± 0.38	1.19 ± 0.76

Table 2. Chemical composition (mean and standard deviation) of the cork samples according to the year of debarking (and number of droughts), in % of total dry mass. Monosaccharide composition is expressed in % of total monosaccharides.

The monomeric composition of suberin was determined in aliquots from the methanolic extracts obtained after the depolymerization of suberin[38]. The samples were evaporated, derivatized by trimethysilylation and immediately analyzed by GC-MS, with the following Zebron conditions: Zebron 7HGG015-02 column (Phenomenex, Torrance, CA, USA) (30 m, 0.25 mm; ID, 0.1 μm film thickness), injector 400 °C, oven temperature program: 50 °C (held 1 min), 10 °C min^{-1} to 150 °C, 5 °C min^{-1} to 200 °C, 4 °C min^{-1} to 300 °C, 10 °C min^{-1} to 380 °C (held 5 min). The MS source was kept at 220 °C and the electron impact mass spectra (EIMS) taken at 70 eV of energy. The experimental procedure used for the suberin compositional determination does not allow the quantification of glycerol but only of the long-chain fatty components.

Statistical analysis.

All results were expressed as mean and standard deviation.

To evaluate the effect of the occurrence of drought(s) on the chemical composition of cork, a mixed model approach was used[39], considering the stripping year (that is directly connected to the number of droughts that occurred in the growing period of the cork - Table 1) as a factor of fixed effect and the tree, nested on the stripping year, as a factor with random effect. With this approach the variability associated to the tree is accounted for and, therefore, we can: (i) better estimate the (fixed) effect of the drought on the chemical composition and (ii) evaluate if there is variability associated to the tree. For this analyses the R package *nlme* was used[40,41] specifying the maximum likelihood as the fitting method, as it is the only method that allows the estimation of the fixed effects estimators. To validate the underlying distributional model assumptions[39], namely normality of the residuals and of the predictors of random effects, independence and homogeneity of variances, we used the traditional plots (trough the commands *plot* and *qqnorm)*, because they are considered to be the most useful methods for assessing the validity of the abovementioned assumptions[39].

For the monomeric analysis of the polysaccharides and of the suberin, an analysis of variance was performed. In this analysis the variability associated to the tree could not be accounted. The normality assumption for all the variables was confirmed with the Shapiro-Wilk test and the equality of variances validated with a F test. Whenever these tests failed, a non-parametric approach was used with the Kruskal-Wallis rank sum test and if differences occurred the Wilcoxon test was also applied. This statistical analysis was performed using the R programming language[41]. In all the statistic procedures the effects were considered as statistically significant when the *p*-value was less than or equal to 0.05.

Results

The summative chemical composition of the cork samples produced without drought and with one and two drought events during the cork growth period is shown in Table 2. The mean content of total extractives is 12.1% (no drought), 12.5% (one drought) and 12.1% (two droughts). The ethanol and water-soluble compounds accounted for about 60% of the total extractives and non-polar compounds soluble in dichloromethane for about 40%. Suberin content ranged between 36.0% (one drought) and 38.2% (two droughts), and total lignin between 27.9% (no drought) and 26.0% (two droughts).

The mixed model analysis, performed with maximum likelihood, revealed that the year of debarking i.e. the number of drought events during the cork growth period did not have a significant effect on the chemical

composition of cork, except in the content of extractives soluble in ethanol (p-value = 0.03), that was significantly higher in 2012 (see Table 2 and supplementary material).

Concerning the variability associated to the tree, all the chemical parameters (extractives, suberin and lignin) showed a significant variability (p-values between 1.4×10^{-3} and $<2.0 \times 10^{-16}$), meaning that the tree has a much more significant effect on the proportion of the chemical parameters than the drought conditions during the cork growth.

The mixed model assumptions were all confirmed as the performed graphics showed the "confirming" shapes and no outliers of random effects were seen. The random effects and residual variances are presented as supplementary material (Table S2). Their estimate was not our primary interest – we actually wanted to evaluate if the effect of the fixed factor (drought) was significant on the cork chemical composition and if there was variability associated to the tree. Therefore, the mixed model approach was used mainly to account for the variability associated to the random factor (tree) in the statistical analysis (which cannot be done with other linear models like the ANOVA as this variability is included in the error). Regarding the carbohydrate composition in proportion of the total monomers, glucose ranged between 39.2% (one drought) and 40.7% (no droughts), xylose between 20.1% (no drought) and 29.6% (two droughts), and arabinose between 14.2% (two droughts) and 19.2% (no drought). The cork polysaccharides also contained smaller amounts of other monomers: on average 1.2% rhamnose, 7.7% galactose, 1.6% mannose, 5.4% galacturonic acid, 1.6% glucuronic acid and 0.9% acetyl groups.

The analysis of variance showed that there is a significant effect of the drought conditions on the xylose and arabinose contents (p-value=1×10^{-5} and 5×10^{-3} respectively). Cork produced under two drought events had higher amounts of xylose and lower levels of arabinose than the cork produced without or with one drought event (p-values = $1.0 \times 10^{-3}/1.0 \times 10^{-4}$ and $4.3 \times 10^{-3}/4.0 \times 10^{-3}$ respectively) that are not different from each other. The glucose amounts didn't follow a normal distribution (p value= 0.04) neither the ratios between glucose and xylose and between glucose and the sum of xylose and arabinose (p-values of 8.0×10^{-4} and 5.8×10^{-5}, respectively). The non-parametric test revealed that glucose and the ratio between glucose and the sum of xylose and arabinose were not affected by drought but the ratio glucose/xylose showed an unclear pattern since cork debarked in 2012 had the highest ratio and cork grown under two drought events the lowest ratio (p-values between 0.02 and 7.5×10^{-4}). The amount of the other sugars did not seem to be affected by drought.

A graphical representation of most of the chemical parameters summarized in Table 2 can be found as supplementary material.

Suberin composition. The monomeric composition of the suberin of the samples produced without drought (1994), with two drought events (2003) and one drought event (2012) is presented in Table 3, in percent of the peak area in relation to the total peak chromatogram area, grouped by chemical families. The detailed composition by monomer is shown as supplementary data. The monomers identified by GC-MS were the same in all the samples.

The main monomers were the ω- hydroxyl alkanoic acids (representing between 37.7% and 43.1% of the total), namely the ω-hydroxyl alkanoic acids with mid-chain substitution, representing between 21.4% and 24.2% of the total monomers found in the suberin depolymerization products. Alkanoic acids represented between 23.8% and 25.8% of the total monomers and were mainly mid chain substituted. Alkanoic diacids ranged between 15.5% and 19.0% and showed a higher proportion of saturated acids. The other identified monomers presented much smaller values (e.g. 3.2% for glycerol and glycerides under two drought events).

The statistical analysis showed that there was no effect of drought on the proportion of the identified families of the suberin monomers.

Discussion

This research focused on evaluating if drought events occurring during the cork production cycle (i.e. the years comprised between two cork strippings) affect the chemical composition of cork, namely, if they modify the relative proportion of the chemical constituents in a way that could compromise the utilization of cork for wine stoppers. In fact, cork chemical composition is directly associated to the material's properties, namely to the permeation to gases and liquids and performance of cork stoppers in wine bottles[18].The present study on the chemical composition variation of cork was designed to have representativeness of samples while the timespan allowed to use a temporal control[42] and the use of a single site for the sampling reduced the effects of confounding variables rather than the drought. Also the sampling in all the cases at only one location within the tree (breast height) avoids any variability associated with a potential chemical variation along the cork oak stem[21].

There are a few species in addition to the cork oak that produce considerable amounts of cork, as reviewed by[23]. Most of the scientific works about those species were performed recently and, as far as we know, none has addressed the effect of climate on their cork chemical composition. Also, the effect of drought on the chemical composition of wood has not gathered much attention among the scientific community. Nevertheless[43], analyzed the effect of drought on the concentration of wood terpenoids in *Pinus sylvestris* and *Picea abies* seedlings, concluding that severe drought increased the concentration of several individual monoterpenes and resin acids (respectively 39 and 32% higher in Scots pine and 35 and 45% higher in Norway spruce). Therefore, drought may affect the chemical composition of secondary metabolites extractives, a study that was not made in present cork analysis.

Regarding the chemical composition of cork, it is known that there is a large natural variability[19] that can, at least partially, contribute to understand the diversity in the behavior of cork products, particularly of cork stoppers[38]. Several studies addressed the chemical variation of cork[19,25–28,44] but the emphasis of these studies has been on the analysis of the geographical variability rather than on the climate effect on the chemical composition. To our knowledge, this is the first research about the effect of drought on the chemical composition of cork.

Identified families	1994 (no drought)	2003 (two droughts)	2012 (one drought)
Alkanoic acids saturated	6.50 ± 1.46	7.79 ± 1.31	7-90 ± 1.60
Alkanoic acids with mid-chain substitution	17.34 ± 2.12	18.04 ± 1.36	17.41 ± 1.92
(Total alkanoic acids)	**23.83 ± 1.54**	**25.83 ± 1.36**	**25.31 ± 0.64**
Alkanoic α,ω-diacids saturated	14.37 ± 1.35	12.18 ± 1.31	12.95 ± 1.52
Alkanoic α,ω-diacids with mid-chain substitution	4.59 ± 0.94	3.35 ± 0.61	5.10 ± 1.10
(Total alkanoic α,ω-diacids)	**18.95 ± 1.58**	**15.53 ± 1.81**	**18.06 ± 2.58**
ω-Hydroxyl alkanoic acids saturated	19.02 ± 1.28	16.37 ± 1.50	17.18 ± 2.51
ω-Hydroxyl alkanoic acids with mid-chain substitution	24.05 ± 1.46	21.37 ± 2.05	24.19 ± 1.48
(Total ω-hydroxyl alkanoic acids)	**43.07 ± 2.50**	**37.74 ± 1.92**	**41.37 ± 3.52**
Alkanols	1.99 ± 0.30	2.84 ± 0.60	2.44 ± 0.57
Aromatics	2.12 ± 0.29	2.43 ± 0.53	2.10 ± 0.74
Sterols	0.10 ± 0.02	0.11 ± 0.01	0.08 ± 0.06
Glycerol and glycerides	1.81 ± 1.38	3.15 ± 1.04	2.73 ± 0.86
Terpenoids	0.67 ± 0.11	0.90 ± 0.14	0.62 ± 0.29
Identified	**92.55**	**88.53**	**92.71**
Unidentified	**7.45**	**11.47**	**7.29**

Table 3. Composition (by chemical family) of suberin from cork produced without any drought (1994), with two drought events (2003) and one drought event (2012), determined in the GC–MS chromatograms of the depolymerization extracts (percentual peak area and standard deviation).

Overall, the chemical composition of the cork samples that were analyzed (Table 2) are in the range of the results known for cork, as reported by several authors (as reviewed in[21]).

For instance, the average 12.2% extractives of which 40.0% are non-polar compounds soluble in dichloromethane (Table 2) are comprised in the range of values reported by e.g.[19,24,26,28]. The results show that drought does not seem to affect the total amount of extractives and that the variation that was found is more related with the genetic information of the tree.

This applies also to the non-polar extractives that are lipophilic compounds including long-chain fatty acids and alcohols, and triterpenes[19,21] and are related to the permeability of cork with lower amounts inducing higher permeability. Our results suggest that drought does not affect this component and that it is the tree that accounts for the existing variation. This natural variability is well recognized and translates, for instance, in different oxygen transfer through cork stoppers into the wine bottles[45].

Drought enhanced the amount of the polar extractives soluble in ethanol that may contribute to the organoleptic properties of the cork bottled wine. However, the tree was a much more significant factor for the variation of these extractives thereby overruling any drought induced changes in this wine characteristic.

The content of suberin is the most important chemical attribute of cork since it is its chemical fingerprint and directly related to most of its typical properties, namely those linked to the materials flexibility and hydrophobicity[18]. The mean values for suberin (36.0% to 38.2%, Table 2) are within the range of values reported by the existing studies (e.g[19,26,28].).

Climate conditions, namely drought, do not seem to induce any changes in the proportion of suberin in cork; on the contrary, it is the tree genetic information that has a very strong impact on suberin content. In fact[46], refer that suberin varies within the species according to its geographical location and the tree condition. Our samples were collected in the same site and the trees were all mature production trees in good phytosanitary conditions, therefore the high variability found in our results should come from the tree genetics.

Lignin is the second most abundant component of cork, giving mechanical support and rigidity to the cell walls[21]. Our samples contained mean values of total lignin between 26.0% and 27.9% (Table 2) which are in the range presented by[19] and[26]. Drought had not a significant effect on Klason lignin, acid soluble lignin or total lignin contents, but the tree had a very strong influence on the relative amount of these compounds.

The proportion between suberin and lignin defines cork's unique properties namely mechanical behavior, resilience and permeability[18]. Our samples showed a lower mean ratio suberin/lignin when compared to the mean results obtained by[19] and[26] but still within the range of their values. Drought did not influence the suberin-to-lignin ratio and again it is the tree genetics the important factor on the variation of this feature.

The monosaccharide composition of the cork samples (Table 2) is within the range of values reported for cork[18,28]. It should be highlighted that the ratio glucose/xylose was influenced by drought although without a clear pattern i.e. the cork produced under two droughts episodes (debarked in 2003) had significantly lower levels of glucose/xylose than the cork grown in the other two periods but the cork produced without drought had a lower ratio than the cork produced under one drought event. Arabinose content increased and xylose was negatively affected by drought but only if two droughts occur during the formation of the cork.

Regarding the monomeric composition of suberin obtained by GC-MS analysis (Table 3), our data shows that the main chemical families found were ω-hydroxyl alkanoic acids (37.7 to 43.1% of total monomers) especially with mid-chain substitution, representing between 24.1 and 24.2% of total monomers. Alkanoic α,ω-diacids (15.5 to 19.0%) and total alkanoic acids (23.8 to 25.8%) represented most of the remaining monomers. The proportion for the three main chemical families is somewhat different from that given by other authors, namely regarding

the content in alkanoic acids[22]: reported 53.0% of α,ω-diacids, 30.6% of ω-hydroxyl alkanoic acids, and less than 2% of alkanoic acids, and[47] referred 29.5% of α,ω-diacids, 52.9% of ω- hydroxyl alkanoic acids and less than 2% of alkanoic acids. This variation reinforces that there is a significant variability on the suberin monomeric composition, as already reported by[24], possibly controlled by the genetic information of the tree.

In fact, our results suggest that the tree is more important in the chemical composition of cork than the wet conditions underlying the development of the cork. This conclusion is in line with reports that genetics must be a much more relevant factor in cork chemical composition and performance than other factors like geographical origin[19,21,25,26]. The chemical composition of cork produced under drought conditions is well within the variation range found by several authors for cork. Moreover, our results show that drought does not trigger the production of different compounds and has only a minor effect on the proportion of the chemical constituents of cork.

Therefore, the occurrence of drought events during the cork growth cycle does not seem to compromise the behavior of cork, namely when it is used as sealant material in wine bottles. However a word of caution must be given since cork properties arise not only from chemical composition but also from the cellular structure, namely cell dimensions[18,21]. With drought leading to thinner cork rings and less and smaller cells[33,34,48], an analysis of the effect on cork cellular structure should be made in order to evaluate the full impact of drought on cork behavior. Nevertheless, the large variation found in cork ring width in commercial cork planks used for the production of wine stoppers[33,34] allows to consider that this will not be a critical factor.

Conclusions

It is well known that there is large natural variability on the chemical composition of cork but research has focused mostly on its geographic variability. This paper presents the first analysis on the climatic effects of drought on the chemical composition of cork, including the proportion and monomeric composition of the main chemical constituents and an experimental design allowing to discriminate the drought and the individual tree response.

The results show that drought has a negligible effect on the cork chemical constitution namely regarding its structural components proportion and composition, and specifically the suberin-to-lignin proportion and suberin composition that are the flagship characteristics of cork underlying its properties. The genetic package of the tree is the most important factor of chemical variation of cork that overrules any impact from drought conditions.

Therefore, the expected more frequent and severe forthcoming droughts in the Mediterranean region where the cork production areas are included will not compromise the cork properties related to its chemical composition, namely the performance of cork as a sealant for wine bottles.

References

1. Buras, A., Rammig, A. & Zang, C. S. Quantifying impacts of the drought 2018 on European ecosystems in comparison to 2003. *ArXiv Prepr. ArXiv190608605* (2019).
2. Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42 (2003).
3. Walther, G.-R. *et al.* Ecological responses to recent climate change. *Nature* **416**, 389 (2002).
4. Allen, C. D. *et al.* A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Adapt. For. For. Manag. Chang. Clim* **259**, 660–684 (2010).
5. Bakkenes, M., Alkemade, J. R. M., Ihle, F., Leemans, R. & Latour, J. B. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. *Glob. Change Biol.* **8**, 390–407 (2002).
6. Garzón, M., Sánchez de Dios, R. & Sainz Ollero, H. Effects of climate change on the distribution of Iberian tree species. *Appl. Veg. Sci.* **11**, 169–178 (2008).
7. Seidl, R. *et al.* Forest disturbances under climate change. *Nat. Clim. Change* **7**, 395 (2017).
8. Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T. & Prentice, I. C. Climate change threats to plant diversity in Europe. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 8245 (2005).
9. Ohlemüller, R., Gritti, E. S., Sykes, M. T. & Thomas, C. D. Quantifying components of risk for European woody species under climate change. *Glob. Change Biol.* **12**, 1788–1799 (2006).
10. Giorgi, F. & Lionello, P. Climate change projections for the Mediterranean region. *Mediterr. Clim. Trends Var. Change* **63**, 90–104 (2008).
11. Gauquelin, T. *et al.* Mediterranean forests, land use and climate change: a social-ecological perspective. *Reg. Environ. Change* **18**, 623–636 (2018).
12. Lionello, P., Özsoy, E., Planton, S. & Zanchetta, G. Climate Variability and Change in the Mediterranean Region. *Clim. Var. Change Mediterr. Reg* **151**, 1–3 (2017).
13. Somot, S., Sevault, F., Déqué, M. & Crépon, M. 21st century climate change scenario for the Mediterranean using a coupled atmosphere–ocean regional climate model. *Mediterr. Clim. Trends Var. Change* **63**, 112–126 (2008).
14. IPCC. *Climate Change 2014*: *Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C. B., V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, and L. L. White (eds.)]*. (2014).
15. Lindner, M. *et al.* Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *For. Ecol. Manag* **259**, 698–709 (2010).
16. Portuguese Cork Association, Santa Maria de Lamas & Portugal. APCOR. Cork - Anuário de cortiça 18/19 (2018).
17. Aroso, I. M., Araújo, A. R., Pires, R. A. & Reis, R. L. Cork: Current Technological Developments and Future Perspectives for this Natural, Renewable, and Sustainable Material. *ACS Sustain. Chem. Eng.* **5**, 11130–11146 (2017).
18. Pereira, H. The Rationale behind Cork Properties: A Review of Structure and Chemistry. *Bioresour. Vol 10 No 3 2015* (2015).
19. Pereira, H. Variability of the Chemical Composition of Cork. *Bioresour. Vol 8 No 2 2013* (2013).
20. Silva, S. P. *et al.* Cork: properties, capabilities and applications. *Int. Mater. Rev.* **50**, 345–365 (2005).
21. Pereira, H. *Cork: biology, production and uses.* (Elsevier, 2007).
22. Graça, J. & Pereira, H. Methanolysis of bark suberins: analysis of glycerol and acid monomers. *Phytochem. Anal.* **11**, 45–51 (2000).
23. Leite, C. & Pereira, H. Cork-Containing Barks—A Review. *Front. Mater* **3**, 63 (2017).
24. Bento, M. *et al.* A study of variability of suberin composition in cork from *Quercus suber* L. using thermally assisted transmethylation GC–MS. *J. Anal. Appl. Pyrolysis* **57**, 45–55 (2001).

25. Conde, E., Cadahía, E., Garcia-Vallejo, M. C. & González-Adrados, J. R. Chemical Characterization of Reproduction Cork from Spanish *Quercus suber*. *J. Wood Chem. Technol.* **18**, 447–469 (1998).

26. Jové, P., Olivella, À. & Cano, L. Study of the variability in chemical composition of bark layers of *Quercus suber* L. from different production areas. *BioResources* **6**, 1806–1815 (2011).

27. Pereira, H. Chemical composition and variability of cork from *Quercus suber* L. *Wood Sci. Technol.* **22**, 211–218 (1988).

28. Sen, A. *et al.* Chemical composition and cellular structure of corks from *Quercus suber* trees planted in Bulgaria and Turkey. *Wood Sci. Technol.* **50**, 1261–1276 (2016).

29. Caritat, A., Gutiérrez, E. & Molinas, M. Influence of weather on cork-ring width. *Tree Physiol.* **20**, 893–900 (2000).

30. Costa, A., Barbosa, I., Roussado, C., Graça, J. & Spiecker, H. Climate response of cork growth in the Mediterranean oak (*Quercus suber* L.) woodlands of southwestern Portugal. *Dendrochronologia* **38**, 72–81 (2016).

31. Costa, A., Pereira, H. & Oliveira, A. Influence of climate on the seasonality of radial growth of cork oak during a cork production cycle. *Ann Sci* **59**, 429–437 (2002).

32. Leite, C., Oliveira, V., Lauw, A. & Pereira, H. Effect of a Drought on Cork Growth Along the Production Cycle. In *Theory and Practice of Climate Adaptation* (eds. Alves, F., Leal Filho, W. & Azeiteiro, U.) 127–136 (Springer International Publishing, 2018)., 10.1007/978-3-319-72874-2_7.

33. Leite, C., Oliveira, V., Lauw, A. & Pereira, H. Cork rings suggest how to manage *Quercus suber* to mitigate the effects of climate changes. *Agric. For. Meteorol.* **266–267**, 12–19 (2019).

34. Oliveira, V., Lauw, A. & Pereira, H. Sensitivity of cork growth to drought events: insights from a 24-year chronology. *Clim. Change* **137**, 261–274 (2016).

35. Agnew, C. Using the SPI to identify drought. *Drought. Netw. News* **12**, 6–12 (2000).

36. Vicente-Serrano, S. M., Beguería, S. & López-Moreno, J. I. A multiscalar drought index sensitive to global warming: The Standardized Precipitation Evapotranspiration Index. *J. Clim.* **23**, 1696–1718 (2010).

37. Páscoa, P., Gouveia, C. M., Russo, A. & Trigo, R. M. Drought trends in the iberian peninsula over the last 112 years. *Adv. Meteorol.* **2017**, 13 pages (2017).

38. Ferreira, J., Miranda, I., Gominho, J. & Pereira, H. Chemical characterization of cork and phloem from Douglas fir outer bark. *Holzforschung* **70**, 475 (2015).

39. Linear Mixed-Effects Models: Basic Concepts and Examples. In Mixed-Effects Models in S and S-PLUS (eds. Pinheiro, J. C. & Bates, D. M.) 3–56, https://doi.org/10.1007/0-387-22747-4_1 (Springer New York, 2000).

40. Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. Linear and nonlinear mixed effects models. *R Package Version* **3** (2018).

41. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. (2019).

42. Altwegg, R., Visser, V., Bailey, L. & Erni, B. Learning from single extreme events. *Philos. Trans. R. Soc. B Biol. Sci* **372**, 20160141 (2017).

43. Turtola, S., Manninen, A., Rikala, R. & Kainulainen, P. Drought Stress Alters the Concentration of Wood Terpenoids in Scots Pine and Norway Spruce Seedlings. *J. Chem. Ecol* **29**, 1981–1995 (2003).

44. Dehane, B., Benrahou, A., Bouhraoua, R., Hamani, F. Z. & Belhoucine, L. Chemical composition of Algerian cork according the origin and the quality. *Int. J. Res. Environ. Stud.* **1**, 17–25 (2014).

45. Oliveira, V., Lopes, P., Cabral, M. & Pereira, H. Kinetics of Oxygen Ingress into Wine Bottles Closed with Natural Cork Stoppers of Different Qualities. *Am. J. Enol. Vitic.* **64**, 395 (2013).

46. García-Vallejo, M. C., Conde, E., Cadahía, E. & de Simón, B. Fernández. Suberin Composition of Reproduction Cork from Quercus suber. *Holzforsch. - Int. J. Biol. Chem. Phys. Technol. Wood* **51**, 219 (2009).

47. Marques, V., Rencoret, J., Gutiérrez, A., del Río, J. & Pereira, H. Ferulates and lignin structural composition in cork. *Holzforschung* **70**, 275 (2015).

48. Pereira, H., Graça, J. & Baptista, C. The Effect of Growth Rate on the Structure and Compressive Properties of Cork. *IAWA J.* **13**, 389–396 (1992).

Acknowledgements

Author contributions

C.L., V.O., I.M. and H.P. have made substantial contributions to the entire manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-64650-9.

Correspondence and requests for materials should be addressed to C.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This research focused on two main issues:

1. positioning the cork oak in relation to the formation, anatomy and chemistry and features of its cork, allowing to establish its value as a benchmark industrial raw material.

2. analyzing the effect of drought on cork growth and chemical composition, to evaluate if the forthcoming, more frequent and severe, drought events can compromise its utilization for the production of cork stoppers since most of the properties of cork that determine its use as a sealant for wine bottles are the result of its unique chemical composition in conjunction with its structural features. In parallel, the stoppage of cork planks requires a minimum cork width of 27 mm, which is determined by cork growth. This framework largely justifies the research conducted under this study.

In order to contextualize the characteristics and properties of cork (from cork oak) under the vaster set of corks produced by other species, the information available for some of them was gathered in a review about this subject (Publication I).

Our review points out that cork oak develops only one periderm and not successive periderms, which means that cork oak does not have rhytidome. When only one periderm is present, the cork layer is radially and tangentially homogenous and if its thickness is adequate, it may be used for production of solid cork products e.g. cork stoppers. In the case of a rhytidome, the cork layers of the successive periderms are separated by phloemic layers; therefore the recovery of cork will require trituration of the rhytidome and fractionation of the cork component, thereby obtaining it in a granulated form that only allows its use in cork agglomerated products. This is the case for instance of the species *Quercus cerris* (Sen et al., 2011a) and *Pseudotsuga menziesii* (Cardoso et al., 2017) that have rhytidomes with a substantial proportion of cork.

Concerning the cork anatomy of *Q. suber*, cork cells are mostly hexagonal prisms that are stacked by their bases in radially aligned rows disposed in parallel without intercellular voids (see Figure 4 of Publication I) with an average cell prism height of 30-40 µm and 1-1.5 µm cell wall thickness (Table 2 of Publication I). The solid fraction in the cork is 8-9% in the earlycork and 15-22% in the latecork region (Pereira, 2007), what justifies the low density of cork. This can also be an advantage when compared to other species (e.g. *Q. variabilis* - Kim, 1993; Miranda et al., 2013)

as many of the properties used in cork materials come from its low density. However in other species with considerable amount of cork in their barks the cork cell walls have pronounced corrugations mostly related to higher radial growth stress (e.g. *Q. cerris* - Sen et al., 2011b or *P. menziesii* Cardoso et al., 2017) which, can result in collapsed cells in some regions, increasing the total density of the material and hindering its use in applications where an unfirm cellular structure is a key element.

Relating to cork chemistry, the reproduction cork from the cork oak has on average 16% extractives, 43% suberin, 22% lignin and 19% cellulose and hemicelluloses (see Table 3, Publication I). Suberin is the most important component and its composition regarding the long chain lipid monomers is shown in Table 5 (Publication I). The presence of high amounts of suberin is the most important chemical attribute of cork since it is its chemical fingerprint and is directly related to most of its typical properties (Pereira, 2015). An important set of properties in cork arise from the joint presence of suberin and lignin in the cell wall and their relative proportion (Pereira, 2013); in cork oak, the ratio suberin/lignin is almost 2 but in most species addressed in this work the proportion of lignin is higher, resulting in corks with higher compressive strength than that of *Q. suber* which means lower quality e.g. for sealants (Pereira, 2013). Nevertheless, these corks are still compatible with uses as insulating, sealing?? and energy absorption material and may be considered as a complementary raw material for the cork from cork oak (e.g. *Q. variabillis* - Ferreira et al., 2016; Miranda et al., 2013).

Our review emphasized that cork oak is the only species whose cork is fully suitable as raw material for cork stoppers production. This is the result of years of dedicated improvement of subericulture in a joint venture with a devoted industry, seeking for the production of the best sealant material for wine bottles. The review also stressed that in some other species their cork can be used in the production of agglomerates and in almost all of them it can be used as chemical sources.

The tree that produces this outstanding material – the cork oak - has its distribution area along the western part of the Mediterranean basin, where, according to climate change predictions, it is forecasted to have more pronounced summer water deficits. Therefore, in this region it is expectable to have a decrease in the production due to the reduction of the photosynthetic activity in response to the stomata closure mechanism (Gea-Izquierdo et al., 2013; Kelly et al., 2002; Piayda et al., 2014; Santos and Miranda, 2006). Cork oak is not an exception and as reported by several authors (Caritat et al., 2000; Costa et al., 2016, 2001; Oliveira et al., 2016)

cork growth is negatively affected by drought, mainly in short time scales (from 2 to 11 months), even if cork growth rapidly recovers when drought conditions end (Oliveira et al., 2016).

There is still little knowledge about the effect on cork growth of drought occurring in different moments of the 9-year cork growth cycle, corresponding to different phellogen ages. This was the framework of a preliminary study of the effect of drought along the production cycle conducted with data from 3 sites (149 samples – Publication II).

Furthermore, the biological mechanisms underlying resilience patterns of cork growth under drought conditions were never addressed until the present research and this was the context for a more complete analysis on cork growth with extensive data (1403 samples) from 12 sites, corresponding to a 30-year chronology with multiple drought events and several phellogen ages (Publication III). Our study encompassed a detailed resilience components analysis, through a mixed model analysis.

Moreover and regarding cork chemical composition, for the first time, we examined if drought induces any changes in the proportion of the main chemical constituents of cork and in the suberin and polysaccharide monomeric composition that could compromise the utilization of cork for the production of wine stoppers (Publication IV). In fact, cork chemical composition is directly associated to this material's properties, namely to the permeation to gases and liquids and performance of cork stoppers in wine bottles (Pereira, 2015). Our study was designed to have representativeness of samples while the timespan allowed to use a temporal control (Altwegg et al., 2017) and the use of a single site for the sampling reduced the effects of confounding variables rather than the drought.

Our results of the preliminary evaluation of the phellogen age influence on the response of cork growth to drought suggested that the age of the phellogen has no influence on the response of this species to a drought event and on its recovery although, when regarding the total cork growth (9 year production cycle), the reduction of cork thickness is more pronounced if the drought event occurs in the beginning of the cycle. However, these assumptions were supported on data from a small sampling and number of drought events (3 sites, 149 samples and 1 drought event) and a more detailed analysis was necessary.

In the second and more complete analysis the total sampling showed a mean cork ring width of 3.30 mm which is in accordance to the values reported by several authors and encompassed the variability between trees and sites that is consistently reported. In fact, the values found for cork ring width by different authors are in the range from 2.8 to 3.8 mm (3.30 mm and 3.56 mm by

Caritat et al., 2000; 3.8 mm by Costa et al., 2002; 3.5 mm by Pereira, 2007; 3.3 mm by Oliveira et al., 2016; 3.6 mm, 3.1 mm and 2.8 mm by Costa et al., 2016). In a large cork sampling covering all the production regions in Portugal, a mean value of cork ring width of 3.6 mm was reported, ranging from site means from 1.6 mm to 4.6 mm (Lauw et al., 2018).

The high number of samples analyzed (1403, with a minimum of 91 samples/year, see Figure 3 of Publication II) allowed us to better account for the variability of the entire population of interest and therefore to have better estimations. This sampling intensity combined with the high value of the Signal to Noise Ratio (144) are strong evidences of the reliability of our results.

We confirmed the outcomes of several authors about the negative effect of drought on cork growth (Caritat et al., 1996; Costa et al., 2016; Oliveira et al., 2016), reinforcing that the spring and winter rain strongly influences phellogen activity. Furthermore, our results (see Table I of Publication III) demonstrated that more severe drought conditions correspond to greater decreases in cork growth and to more trees affected by drought, although, as pointed out by Oliveira et al. (2016) and Besson et al. (2014), cork oak rapidly recovers as water availability increases, achieving previous ecological performance (see Table 2 and Figure 4 of Publication III).

Our analysis demonstrated that the age of the phellogen is a relevant factor on the recovery and on the resilience of cork oak regarding cork growth and that the younger the phellogen (up to 2 years) the greater the effect of the drought on growth.

Additionally, the results (see Publication III - Table 2) suggest that more severe droughts correspond to lower resistance values, which means greater effects of the drought on the growth of the drought year. The trade-off between resistance and recovery after drought, as identified by Galiano L. et al. (2011) and highlighted by Lloret et al. (2011), stating that resistance and recovery depend on the quantity of stored reserves was confirmed, with the lowest value of resistance corresponding to the highest recovery. A low resistance indicates a reserve consumption during drought but if there is recovery after drought then there is a positive effect from the regaining of photosynthetic capacity after drought (Galiano L. et al., 2011).

Nevertheless, there is not a clear pattern between the severity of drought and the recovery, resistance or resilience behavior and there is a great variability on the performed recovery (Table 2 and Statistical Analysis, Publication III). The same conclusions were achieved by Lloret et al. (2011) who got miscellaneous results for the impact of drought on subsequent tree performance and concluded that tree responses to disturbance are extremely complex. These authors also

referred that micro-site quality is a more determinant factor in response to disturbance than physiological or structural factors.

The mean resilience values were in general higher than 0.80 (see Publication III - Table 2) showing that post-drought cork growth is close to pre-drought (unitary resilience indicates similar growth values before and after drought) and demonstrating that cork oak has a great capacity to recuperate the previous ecological performance. Therefore, the impact of drought on cork growth of the subsequent years was moderately low, supporting the hypothesis that cork oak is very tolerant and resilient to extreme droughts. Similar results and conclusions were achieved regarding wood growth for other Mediterranean species (*Pinus nigra*, *P. sylvestris* and *Juniperus communis*) (Herrero and Zamora, 2014) although not for another oak (*Q. faginea*) (Granda et al., 2013). *P. sylvestris* needs more time than *Q. pyrenaica* to recover from drought (Gea-Izquierdo et al., 2014). Regarding this, species acclimated to more humid environments may be more susceptible to drought as reported for *Q. ilex* (Granda et al., 2013).

The results of this research also enforce that the occurrence of wet conditions in winter, spring and the whole year are determinant to cork growth, during and after drought, but there are other factors involved, namely the site, the tree and the age of the phellogen. This issue was raised by Sánchez-González et al. (2007) by stating that there is a relationship between cork growth and "unobservable site factors" rather than average climatic conditions.

Moreover, the ability to recover (expressed by the relative resilience) does not seem to be affected by the age of the phellogen. It should be stressed that the phellogen age range in this research is only between 1-8 years, therefore not allowing to extrapolate the response for much older meristems. For instance, Lloret et al. (2011) found no overall decrease in all the resilience components in old trees, supporting the hypothesis that old trees recover better from more recent events.

In terms of forest management, our dendroecological results suggest that cork growth cycles should be enlarged in case of occurrence of drought and when the production records suggest that the reduction on growth due to drought may lead to obtain cork planks under 27 mm, and particularly if the drought occurs in the first two years of the production cycle. This management directive lies on the economic rationale of not compromising the technological possibility to produce cork stoppers (Lauw et al., 2018). The extension of the cork stripping rotation in order to mitigate the effect of climate change was also proposed by Palma et al. (2015).

Moreover, the predicted scarcity of water in the actual distribution area of cork oak also suggests that new cork oak plantations should be shifted into more humid areas (higher latitudes) where, in the nearest future, the annual precipitation will be more adequate for the balanced cork oak development and thereby allowing the maintenance of the production of cork planks thick enough to manufacture cork stoppers with the usual 9-year cycle.

Regarding cork chemical composition, our research focused on evaluating if drought events occurring during the cork production cycle (i.e. the years comprised between two cork strippings) affect the chemical composition of cork, namely, if they modify the relative proportion of the chemical constituents in a way that could compromise the utilization of cork for wine stoppers.

It is known that there is a large natural variability on the chemical composition of cork (Pereira, 2013) that can, at least partially, contribute to understand the diversity in the behavior of cork products, particularly of cork stoppers (Pereira, 2015). Several studies addressed the chemical variation of cork (Conde et al., 1998; Dehane et al., 2014; Jové et al., 2011; Pereira, 1988, 2013; Sen et al., 2016) but the emphasis of these studies has been on the analysis of the geographical variability rather than on the climate effect on the chemical composition. To our knowledge, this is the first research about the effect of drought on the chemical composition of cork.

Overall, the chemical composition of the cork samples that were analyzed (Table 2, Publication IV) are in the range of the results known for cork, as reported by several authors (as reviewed in Pereira, 2007).

The average 12.2% extractives of which 40.0% are non-polar compounds soluble in dichloromethane (Table 2, Publication IV) are comprised in the range of values reported by e.g. Jové et al. (2011); Pereira (2013); Bento et al. (2001) and Sen et al. (2016). The results show that drought does not seem to affect the total amount of extractives and that the variation that was found is more related with the genetic information of the tree.

This applies also to the non-polar extractives that are lipophilic compounds including long-chain fatty acids and alcohols, and triterpenes (Pereira, 2007, 2013) and are related to the permeability of cork with lower amounts inducing higher permeability. Our results suggest that drought does not affect these compounds and that it is the tree that accounts for the existing variation. This natural variability is well recognized and translates, for instance, in different oxygen transfer through cork stoppers into the wine bottles (Oliveira et al., 2013).

Drought enhanced the amount of the polar extractives soluble in ethanol that may contribute to the organoleptic properties of the cork bottled wine. However, the tree was a much more significant factor for the variation of these extractives, thereby overruling any drought induced changes in this wine characteristic.

The content of suberin is the most important chemical attribute of cork since it is its chemical fingerprint and directly related to most of its typical properties, namely those linked to the material's flexibility and hydrophobicity (Pereira, 2015). The mean values for suberin (36.0% to 38.2%, Table 2 – Publication IV) are within the range of values reported by the existing studies (e.g. Jové et al., 2011; Pereira, 2013; Sen et al., 2016).

Drought does not seem to induce any changes in the proportion of suberin in cork; on the contrary, it is the tree genetic information that has a very strong impact on suberin content. García-Vallejo et al. (2009) refer that suberin varies within the species according to its geographical location and the tree condition. Our samples were collected in the same site and the trees were all mature production trees in good phytosanitary conditions, therefore the high variability found in our results should come from the tree genetics.

Lignin is the second most abundant component of cork, giving mechanical support and rigidity to the cell walls (Pereira, 2007). Our samples contained mean values of total lignin between 26.0% and 27.9% (Table 2, Publication IV) which are in the range presented by Pereira (2013) and Jové et al. (2011). Drought had not a significant effect on Klason lignin, acid soluble lignin or total lignin contents, but the tree had a very strong influence on the relative amount of these compounds.

The proportion between suberin and lignin defines cork's unique properties namely its mechanical behavior, resilience and permeability (Pereira, 2015). Our samples showed a lower mean ratio suberin/lignin when compared to the mean results obtained by Pereira (2013) and Jové et al. (2011) but still within the range of their values. Drought did not influence the suberin-to-lignin ratio and again it is the tree genetics the important factor on the variation of this feature.

The monosaccharide composition of the cork samples (Table 2, Publication IV) is within the range of values reported for cork (Pereira, 2013; Sen et al., 2016). It should be highlighted that the ratio glucose/xylose was influenced by drought although without a clear pattern i.e. the cork produced under two droughts episodes (debarked in 2003) had significantly lower levels of glucose/xylose than the cork grown in the other two periods but the cork produced without

drought had a lower ratio than the cork produced under one drought event. Arabinose content increased and xylose was negatively affected by drought but only if two droughts occur during the formation of the cork.

Regarding the monomeric composition of suberin obtained by GC-MS analysis (Table 3, Publication IV), our data show that the main chemical families found were ω-hydroxyl alkanoic acids (37.7 to 43.1% of total monomers) especially with mid-chain substitution, representing between 24.1 and 24.2% of total monomers. Alkanoic α,ω-diacids (15.5 to 19.0%) and total alkanoic acids (23.8 to 25.8%) represented most of the remaining monomers. The proportion for the three main chemical families is somewhat different from that given by other authors, namely regarding the content in alkanoic acids: Graça and Pereira (2000) reported 53.0% of α,ω-diacids, 30.6% of ω-hydroxyl alkanoic acids, and less than 2% of alkanoic acids, and Marques et al. (2015) referred 29.5% of α,ω-diacids, 52.9% of ω-hydroxyl alkanoic acids and less than 2% of alkanoic acids. This variation reinforces that there is a significant variability on the suberin monomeric composition, as already reported by Bento et al. (2001), possibly controlled by the genetic information of the tree.

In fact, our results suggest that the tree genetic (ou as a distinctive factor) is more important in the chemical composition of cork than the wet conditions underlying the development of the cork. This conclusion is in line with reports that genetics must be a much more relevant factor in cork chemical composition and performance than other factors like geographical origin (Conde et al., 1998; Jové et al., 2011; Pereira, 2007, 2013). The chemical composition of cork produced under drought conditions is well within the variation range found by several authors for cork. Moreover, our results show that drought does not trigger the production of different suberinic compounds and has only a minor effect on the proportion of the chemical constituents of cork.

Therefore, the occurrence of drought events during the cork growth cycle does not seem to compromise the behavior of cork, namely when it is used as sealant material in wine bottles. However a word of caution must be given since cork properties arise not only from chemical composition but also from the cellular structure, namely cell dimensions (Pereira, 2007, 2015). With drought leading to thinner cork rings and less and smaller cells (Oliveira et al., 2016; Pereira et al., 1992), an analysis of the effect on cork cellular structure should be made in order to evaluate the full impact of drought on cork behavior. Nevertheless, the large variation found in cork ring width in commercial cork planks used for the production of wine stoppers (Oliveira et al., 2016) allows to consider that this will not be a critical factor.

The research carried out along this PhD project allowed to compile the disperse – and mostly scarce - available information about the characteristics of cork-rich barks and therefore to position the cork oak in relation to the formation, growth and features of its cork, and allowing to establish its value as an industrial raw-material. Also new knowledge was obtained on the effect of drought on cork growth and cork chemical composition and on their potential impact on the use for wine stoppers.

Under our specific objectives, the conclusions can be summarized as follows:

The cork from *Quercus suber* is presently the only raw material that has the characteristics necessary for production of solid cork products e.g. of wine natural cork stoppers, and for which the trituration and production of cork agglomerates are complementary production lines.

A new cork raw-material has been brought recently to the market, the Chinese cork from *Quercus variabilis* which appears to have an interesting place given the already large amounts that are harvested as well as the existing forest potential, now still far from an adequate cork-targeted management.

The valuable properties of cork, as benchmarked by the *Quercus suber* cork, require an adequate combination of structural and cellular features with the chemical composition. This is met by the corks of several species e.g. *Kielmeyera coriacea, Plathymenia reticulata, Quercus cerris*, that despite species specificities regarding cell biometry and chemistry, have characteristics that allow forecasting "corkish" properties and uses.

The specific cellular characteristics may hinder or limit the use of cork as a cellular material. This is the case of *Betula pendula* cork mainly due to cell biometry, and of *Pseudotsuga menziesii* due to the large proportion of heavily corrugated or collapsed cells. The use of such corks as a chemical source is therefore a promising valorization route. In fact, the extractives are a chemical component group that is receiving a lot of attention in research and development in various fields, including biomedical and healthcare. Also suberin, the main structural component of cork cell walls, is a macromolecule with an unusual composition of long chain fatty acids with different functional groups e.g. hydroxyl, epoxide, unsaturation, that is species specific, and a potential source of chemical intermediates.

Regarding the cork from cork oak, dry spells have a strong impact on cork growth and the more severe the drought the greater the reduction on growth but also the greater the recovery performed. Moreover, although the effects of drought persist, the recovering capacity of cork oak is not affected even in drought conditions as severe as the ones from 2005. Therefore, we advise forest managers of the Mediterranean region to continue to produce cork as, according to our findings, it is expected that cork oak recovers from the growth reduction imprinted by drought.

There are other factors that affect the performance of cork growth during and after drought, namely site, tree and the age of the phellogen, and have an important role on the responses of cork oak to drought and on its ability to recover. The effects of drought on growth are more pronounced in the first two years and in the last two years of the cork production cycle than in the middle of the cycle.

Cork oak is very tolerant and resilient to droughts, has a great capacity to recover from water scarcity conditions, reflecting its high adaptation to the variability of water availability in the Mediterranean region.

Cork-ring data analyses is adequate to detect signals of cork oak response to less water availability in a nearest future.

Drought has a negligible effect on the cork chemical constitution namely regarding its structural components proportion and composition, and specifically the suberin-to-lignin proportion and suberin composition that are the flagship characteristics of cork underlying its properties. The genetic package of the tree is the most important factor of chemical variation of cork that overrules any impact from drought conditions.

The expected more frequent and severe forthcoming droughts in the Mediterranean region where the cork production areas are included will not compromise the cork properties related to its chemical composition, namely the performance of cork as a sealant for wine bottles.

Nevertheless, in order to have cork planks thick enough to produce cork stoppers, there is a strong signal for forest managers to: a) extend the cork stripping rotation in case of drought, namely if the drought conditions occur in the beginning of the production cycle (first 2 years) and if there is the risk that cork planks are under 27 mm thickness as given by the past history of the site and b) establish new cork oak plantations in more humid zones (e.g. higher latitudes).

Regarding future research, we identified the following promising themes:

- ✓ structural and chemical characterization of the cork of other species that our review showed to be largely unknown as a first step towards their prospective valorization.
- ✓ establishment of a minimum threshold for the recovery of cork growth and verification if the recovery response after drought conditions remains high after more repeated droughts.
- ✓ combined analysis of the effect of the age of the phellogen and also the effect of the age of the tree on the post-drought response.
- ✓ simultaneous analysis of the resilience components on the growth of the wood and of the cork, in order to have a deeper insight in the highly complex phenomena involved under cork oak drought responses.
- ✓ evaluate if the chemical composition of the cork produced by the more resilient trees to drought encompasses the large variability usually found or if it corresponds to a more restrict range of values and if they are compatible with the utilization of cork as a sealant material.

Abramovay, R., 1999. Moratória para os cerrados—elementos para uma estratégia de agricultura sustentável.

Agnew, C., 2000. Using the SPI to identify drought. Drought Netw. News 12, 6–12.

Altwegg, R., Visser, V., Bailey, L., Erni, B., 2017. Learning from single extreme events. Philos. Trans. R. Soc. B Biol. Sci. 372, 20160141. https://doi.org/10.1098/rstb.2016.0141

Anjos, O., Pereira, H., Rosa, M.E., 2008. Effect of quality, porosity and density on the compression properties of cork. Holz Als Roh- Werkst. 66, 295. https://doi.org/10.1007/s00107-008-0248-2

Anjos, O., Rodrigues, C., Morais, J., Pereira, H., 2014. Effect of density on the compression behaviour of cork. Mater. Des. 53, 1089–1096. https://doi.org/10.1016/j.matdes.2013.07.038

APCOR, 2018. Cork - Anuário de cortiça 18/19.

Beck, C.B., 2010. An Introduction to Plant Structure and Development: Plant Anatomy for the Twenty-First Century, 2nd ed. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511844683

Beguería, S., Vicente-Serrano, S., Reig, F., Latorre, B., 2013. Standardized precipitation evapotranspiration index (SPEI) revisited: parameter fitting, evapotranspiration models, tools, datasets and drought monitoring. Int. J. Climatol. 34, 3001–3023. https://doi.org/10.1002/joc.3887

Bento, M., Pereira, H., Cunha, M.Á., Moutinho, A.M.C., van den Berg, K.J., Boon, J.J., 2001. A study of variability of suberin composition in cork from Quercus suber L. using thermally assisted transmethylation GC–MS. J. Anal. Appl. Pyrolysis 57, 45–55. https://doi.org/10.1016/S0165-2370(00)00093-0

Besson, C., Lobo-do-Vale, R., Rodrigues, M., Almeida, P., Herd, A., Grant, O., David, T.S., Schmidt, M., Otieno, D., Keenan, T., 2014. Cork oak physiological responses to manipulated water availability in a Mediterranean woodland. Agric. For. Meteorol. 184, 230–242. https://doi.org/10.1016/j.agrformet.2013.10.004

Bhat, K.M., 1982. Anatomy, Basic Density and Shrinkage of Birch Bark. IAWA J. 3.

Bräuning, A., Bolte, A., Nabais, C., Rossi, S., Sass-Klaassen, U., 2017. Editorial: Studying Tree Responses to Extreme Events. Front. Plant Sci. 8, 506. https://doi.org/10.3389/fpls.2017.00506

Bugalho, M.N., Caldeira, M.C., Pereira, J.S., Aronson, J., Pausas, J.G., 2011. Mediterranean cork oak savannas require human use to sustain biodiversity and ecosystem services. Front. Ecol. Environ. 9, 278–286. https://doi.org/10.1890/100084

Cardoso, S., Ferreira, J., Quilhó, T., Pereira, H., 2017. Cork of Douglas-fir bark: Impact of structural and anatomical features on usage. Ind. Crops Prod. 99, 135–141. https://doi.org/10.1016/j.indcrop.2017.02.001

Caritat, A., Gutiérrez, E., Molinas, M., 2000. Influence of weather on cork-ring width. Tree Physiol. 20, 893–900. https://doi.org/10.1093/treephys/20.13.893

Caritat, A., Molinas, M., Gutierrez, E., 1996. Annual cork-ring width variability of *Quercus suber* L. in relation to temperature and precipitation (Extremadura, southwestern Spain). For. Ecol. Manag. 86, 113–120. https://doi.org/10.1016/S0378-1127(96)03787-5

Catry, F.X., Moreira, F., Cardillo, E., Pausas, J.G., 2012. Post-Fire Management of Cork Oak Forests, in: Moreira, F., Arianoutsou, M., Corona, P., De las Heras, J. (Eds.), Post-Fire Management and Restoration of Southern European Forests. Springer Netherlands, Dordrecht, pp. 195–222. https://doi.org/10.1007/978-94-007-2208-8_9

Cherubini, P., Gartner, B.L., Tognetti, R., Bräker, O.U., Schoch, W., Innes, J.L., 2003. Identification, measurement and interpretation of tree rings in woody species from mediterranean climates. Biol. Rev. 78, 119–148. https://doi.org/10.1017/S1464793102006000

Conde, E., Cadahía, E., Garcia-Vallejo, M.C., Gonźalez-Adrados, J.R., 1998. Chemical Characterization of Reproduction Cork from Spanish *Quercus Suber*. J. Wood Chem. Technol. 18, 447–469. https://doi.org/10.1080/02773819809349592

Costa, A., Barbosa, I., Roussado, C., Graça, J., Spiecker, H., 2016. Climate response of cork growth in the Mediterranean oak (*Quercus suber* L.) woodlands of southwestern Portugal. Dendrochronologia 38, 72–81. https://doi.org/10.1016/j.dendro.2016.03.007

Costa, A., Madeira, M., Oliveira, Â.C., 2008. The relationship between cork oak growth patterns and soil, slope and drainage in a cork oak woodland in Southern Portugal. For. Ecol. Manag. 255, 1525–1535. https://doi.org/10.1016/j.foreco.2007.11.008

Costa, A., Pereira, H., Madeira, M., 2009. Landscape dynamics in endangered cork oak woodlands in Southwestern Portugal (1958–2005). Agrofor. Syst. 77, 83. https://doi.org/10.1007/s10457-009-9212-3

Costa, A., Pereira, H., Oliveira, A., 2002. Influence of climate on the seasonality of radial growth of cork oak during a cork production cycle. Ann Sci 59, 429–437. https://doi.org/10.1051/forest:2002017

Costa, A., Pereira, H., Oliveira, A., 2001. A dendroclimatological approach to diameter growth in adult cork-oak trees under production. Trees 15, 438–443. https://doi.org/10.1007/s004680100119

David, T.S., Henriques, M.O., Kurz-Besson, C., Nunes, J., Valente, F., Vaz, M., Pereira, J.S., Siegwolf, R., Chaves, M.M., Gazarini, L.C., David, J.S., 2007. Water-use strategies in two co-occurring Mediterranean

evergreen oaks: surviving the summer drought. Tree Physiol. 27, 793–803. https://doi.org/10.1093/treephys/27.6.793

David, T.S., Pinto, C.A., Nadezhdina, N., Kurz-Besson, C., Henriques, M.O., Quilhó, T., Cermak, J., Chaves, M.M., Pereira, J.S., David, J.S., 2013. Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow. For. Ecol. Manag. 307, 136–146. https://doi.org/10.1016/j.foreco.2013.07.012

Dehane, B., Benrahou, A., Bouhraoua, R., Hamani, F.Z., Belhoucine, L., 2014. Chemical composition of Algerian cork according the origin and the quality. Int. J. Res. Environ. Stud. 1, 17–25. https://doi.org/10.33500/ijres.2014.01.003

Drew, D.M., Allen, K., Downes, G.M., Evans, R., Battaglia, M., Baker, P., 2013. Wood properties in a long-lived conifer reveal strong climate signals where ring-width series do not. Tree Physiol. 33, 37–47. https://doi.org/10.1093/treephys/tps111

Evert, R.F., 2006. Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development, 3rd Edition. ed. John Wiley & Sons, Hoboken.

Fahn, A., 1990. Plant anatomy., 4th ed. ed. Pergamon Press.

Faria, D.P., Fonseca, A.L., Pereira, H., Teodoro, O.M.N.D., 2011. Permeability of Cork to Gases. J. Agric. Food Chem. 59, 3590–3597. https://doi.org/10.1021/jf200491t

Ferreira, A., Lopes, F., Pereira, H., 2000. Caractérisation de la croissance et de la qualité du liège dans une région de production. Ann Sci 57, 187–193. https://doi.org/10.1051/forest:2000169

Ferreira, J., Miranda, I., Şen, U., Pereira, H., 2016. Chemical and cellular features of virgin and reproduction cork from *Quercus variabilis.* Ind. Crops Prod. 94, 638–648. https://doi.org/10.1016/j.indcrop.2016.09.038

Fortes, M.A., Rosa, M.E., 1992. Growth stresses and strains in cork. Wood Sci. Technol. 26, 241–258. https://doi.org/10.1007/BF00200160

Galiano L., Martínez-Vilalta J., Lloret F., 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. New Phytol. 190, 750–759. https://doi.org/10.1111/j.1469-8137.2010.03628.x

García-Vallejo M. C., Conde E., Cadahía E., de Simón B. Fernández, 2009. Suberin Composition of Reproduction Cork from *Quercus suber*. Holzforsch. - Int. J. Biol. Chem. Phys. Technol. Wood 51, 219. https://doi.org/10.1515/hfsg.1997.51.3.219

Gea-Izquierdo, G., Fernández-de-Uña, L., Cañellas, I., 2013. Growth projections reveal local vulnerability of Mediterranean oaks with rising temperatures. For. Ecol. Manag. 305, 282–293. https://doi.org/10.1016/j.foreco.2013.05.058

Gea-Izquierdo, G., Viguera, B., Cabrera, M., Cañellas, I., 2014. Drought induced decline could portend widespread pine mortality at the xeric ecotone in managed mediterranean pine-oak woodlands. For. Ecol. Manag. 320, 70–82. https://doi.org/10.1016/j.foreco.2014.02.025

Graça, J., 2015. Suberin: the biopolyester at the frontier of plants. Front. Chem. 3, 62. https://doi.org/10.3389/fchem.2015.00062

Graça, J., Pereira, H., 2000a. Methanolysis of bark suberins: analysis of glycerol and acid monomers. Phytochem. Anal. 11, 45–51. https://doi.org/10.1002/(SICI)1099-1565(200001/02)11:1<45::AID-PCA481>3.0.CO;2-8

Graça, J., Pereira, H., 2000b. Suberin Structure in Potato Periderm: Glycerol, Long-Chain Monomers, and Glyceryl and Feruloyl Dimers. J. Agric. Food Chem. 48, 5476–5483. https://doi.org/10.1021/jf0006123

Graça, J., Pereira, H., 1998. Feruloyl Esters of ω-Hydroxyacids in Cork Suberin. J. Wood Chem. Technol. 18, 207–217. https://doi.org/10.1080/02773819809349577

Graça, J., Pereira, H., 1997. Cork Suberin: A Glyceryl Based Polyester. Holzforsch. - Int. J. Biol. Chem. Phys. Technol. Wood 51, 225. https://doi.org/10.1515/hfsg.1997.51.3.225

Granda, E., Camarero, J.J., Gimeno, T.E., Martínez-Fernández, J., Valladares, F., 2013. Intensity and timing of warming and drought differentially affect growth patterns of co-occurring Mediterranean tree species. Eur. J. For. Res. 132, 469–480. https://doi.org/10.1007/s10342-013-0687-0

Herrero, A., Zamora, R., 2014. Plant responses to extreme climatic events: A field test of resilience capacity at the Southern range edge. PLOS ONE 9, e87842. https://doi.org/10.1371/journal.pone.0087842

Huang, Z.-K., Zheng, C.-H., Du, J.-X., Wan, Y., 2006. Bark Classification Based on Textural Features Using Artificial Neural Networks, in: Wang, J., Yi, Z., Zurada, J.M., Lu, B.-L., Yin, H. (Eds.), Advances in Neural Networks - ISNN 2006. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 355–360.

ICNF, 2019. IFN6 –Principais resultados –relatório sumário. http://www2.icnf.pt/portal/florestas/ifn/resource/doc/ifn/ifn6/IFN6_Relatorio_completo-2019-11-28.pdf Accessed in November 2019.

IPCC, 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp., New York, NY.

Jové, P., Olivella, À., Cano, L., 2011. Study of the variability in chemical composition of bark layers of *Quercus suber* L. from different production areas. BioResources 6, 1806–1815.

References

Kätzel, R., Höppner, K., 2011. Adaptation strategies in forest management under the conditions of climate change in Brandenburg. Folia For. Pol. Ser. - For. 53, 43–51.

Kelly, P.M., Leuschner, H.H., Briffa, K.R., Harris, I.C., 2002. The climatic interpretation of pan-European signature years in oak ring-width series. The Holocene 12, 689–694. https://doi.org/10.1191/0959683602hl582rp

Kim, B., 1993. Studies on the physical and mechanical properties of imported and domestic corks. J Korean Wood Sci Technol 21, 45–54.

Lauw, A., Oliveira, V., Lopes, F., Pereira, H., 2018. Variation of cork quality for wine stoppers across the production regions in Portugal. Eur. J. Wood Wood Prod. https://doi.org/10.1007/s00107-017-1196-5

Leal, S., Nunes, E., Pereira, H., 2008. Cork oak (*Quercus suber* L.) wood growth and vessel characteristics variations in relation to climate and cork harvesting. Eur. J. For. Res. 127, 33–41. https://doi.org/10.1007/s10342-007-0180-8

Lev-Yadun, S., 2011. Bark, in: ELS. American Cancer Society. https://doi.org/10.1002/9780470015902.a0002078.pub2

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, a., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M.J., Marchetti, M., 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For. Ecol. Manag. 259, 698–709. https://doi.org/10.1016/j.foreco.2009.09.02

Lloret, F., Keeling, E.G., Sala, A., 2011. Components of tree resilience: effects of successive low-growth episodes in old ponderosa pine forests. Oikos 120, 1909–1920. https://doi.org/10.1111/j.1600-0706.2011.19372.x

Marques, A., Pereira, H., 2013. Lignin monomeric composition of corks from the barks of *Betula pendula*, *Quercus suber* and *Quercus cerris* determined by Py–GC–MS/FID. J. Anal. Appl. Pyrolysis 100, 88–94. https://doi.org/10.1016/j.jaap.2012.12.001

Marques, A., Pereira, H., Meier, D., Faix, O., 1999. Structural Characterization of Cork Lignin by Thioacidolysis and Permanganate Oxidation. Holzforschung 53, 167. https://doi.org/10.1515/HF.1999.028

Marques, A., Pereira, H., Meier, D., Faix, O., 1996. Isolation and Characterization of a Guaiacyl Lignin from Saponified Cork of *Quercus suber* L. Holzforsch. - Int. J. Biol. Chem. Phys. Technol. Wood 50, 393. https://doi.org/10.1515/hfsg.1996.50.5.393

Marques, A., Pereira, H., Meier, D., Faix, O., 1994. Quantitative Analysis of Cork (*Quercus suber* L.) and Milled Cork Lignin by FTIR Spectroscopy, Analytical Pyrolysis, and Total Hydrolysis. Holzforsch. - Int. J. Biol. Chem. Phys. Technol. Wood 48, 43. https://doi.org/10.1515/hfsg.1994.48.s1.43

Marques, A., Pereira, H., Rodrigues, J., Meier, D., Faix, O., 2006. Isolation and comparative characterization of a Björkman lignin from the saponified cork of Douglas-fir bark. J. Anal. Appl. Pyrolysis 77, 169–176. https://doi.org/10.1016/j.jaap.2006.03.003

Marques, V., Rencoret, J., Gutiérrez, A., del Río, J., Pereira, H., 2015. Ferulates and lignin structural composition in cork. Holzforschung 70, 275. https://doi.org/10.1515/hf-2015-0014

Miranda, I., Gominho, J., Pereira, H., 2013. Cellular structure and chemical composition of cork from the Chinese cork oak (*Quercus variabilis*). J. Wood Sci. 59, 1–9. https://doi.org/10.1007/s10086-012-1300-8

Mota, G.S., Sartori, C.J., Ferreira, J., Miranda, I., Quilhó, T., Mori, F.A., Pereira, H., 2016. Cellular structure and chemical composition of cork from *Plathymenia reticulata* occurring in the Brazilian Cerrado. Ind. Crops Prod. 90, 65–75. https://doi.org/10.1016/j.indcrop.2016.06.014

Natividade, J., 1950. Subericultura. Ministério da Economia–Direcção Geral dos Serviços Florestais e Aquícolas, Lisboa.

Oliveira, V., Lauw, A., Pereira, H., 2016. Sensitivity of cork growth to drought events: insights from a 24-year chronology. Clim. Change 137, 261–274. https://doi.org/10.1007/s10584-016-1680-7

Oliveira, V., Lopes, P., Cabral, M., Pereira, H., 2013. Kinetics of Oxygen Ingress into Wine Bottles Closed with Natural Cork Stoppers of Different Qualities. Am. J. Enol. Vitic. 64, 395. https://doi.org/10.5344/ajev.2013.13009

Oliveira, V., Rosa, M.E., Pereira, H., 2014. Variability of the compression properties of cork. Wood Sci. Technol. 48, 937–948. https://doi.org/10.1007/s00226-014-0651-2

Palma, J.H.N., Paulo, J.A., Faias, S.P., Garcia-Gonzalo, J., Borges, J.G., Tomé, M., 2015. Adaptive management and debarking schedule optimization of *Quercus suber* L. stands under climate change: case study in Chamusca, Portugal. Reg. Environ. Change 15, 1569–1580. https://doi.org/10.1007/s10113-015-0818-x

Páscoa, P., Gouveia, C.M., Russo, A., Trigo, R.M., 2017. Drought trends in the iberian peninsula over the last 112 years. Adv. Meteorol. 2017, 13 pages. https://doi.org/10.1155/2017/4653126

Paulo, J.A., Pereira, H., Tomé, M., 2017. Analysis of variables influencing tree cork caliper in two consecutive cork extractions using cork growth index modelling. Agrofor. Syst. 91, 221–237. https://doi.org/10.1007/s10457-016-9922-2

Pereira, H., 2015. The Rationale behind Cork Properties: A Review of Structure and Chemistry. Bioresour. Vol 10 No 3 2015.

Pereira, H., 2013. Variability of the Chemical Composition of Cork. Bioresour. Vol 8 No 2 2013.

Pereira, H., 2007. Cork: biology, production and uses. Elsevier, Amsterdam.

References

Pereira, H., 1988a. Structure and Chemical Composition of Cork from *Calotropis procera* (AIT.) R. BR. IAWA J. 9.

Pereira, H., 1988b. Chemical composition and variability of cork from *Quercus suber* L. Wood Sci. Technol. 22, 211–218. https://doi.org/10.1007/BF00386015

Pereira, H., Graça, J., Baptista, C., 1992. The Effect of Growth Rate on the Structure and Compressive Properties of Cork. IAWA J. 13, 389–396. https://doi.org/10.1163/22941932-90001294

Pereira, H., Rosa, M.E., Fortes, M.A., 1987. The Cellular Structure of Cork from *Quercus suber* L. IAWA J. 8.

Piayda, A., Dubbert, M., Rebmann, C., Kolle, O., Silva, F., Correia, A., Pereira, J.S., Werner, C., 2014. Drought impact on carbon and water cycling in a Mediterranean *Quercus suber* L. woodland during the extreme drought event in 2012. Biogeosciences 11, 7159–7178. http://dx.doi.org/10.5194/bg-11-7159-2014

Pinto-Correia, T., Ribeiro, N., Sá-Sousa, P., 2011. Introducing the montado, the cork and holm oak agroforestry system of Southern Portugal. Agrofor. Syst. 82, 99. https://doi.org/10.1007/s10457-011-9388-1

Pizzurro, G., Maetzke, F., Veca, D., 2010. Differences of raw cork quality in productive cork oak woods in Sicily in relation to stand density. For. Ecol. Manag. 260, 923–929. https://doi.org/10.1016/j.foreco.2010.06.013

Ponte-e-Sousa, J., Neto-Vaz, A., 2011. Cork and metals: a review. Wood Sci. Technol. 45, 183–202. https://doi.org/10.1007/s00226-009-0288-8

Rizzini, C., Mors, V., 1995. Botânica econômica brasileira - Revista e actualizada.

Roth, I., 1981. Structural patterns of tropical barks. Schweizerbart Science Publishers, Stuttgart, Germany.

Sánchez-González, M., Calama, R., Cañellas, I., Montero, G., 2007. Variables influencing cork thickness in Spanish cork oak forests: A modelling approach. Ann Sci 64, 301–312. https://doi.org/10.1051/forest:2007007

Santos, F.D., Miranda, P., 2006. Alterações climáticas em Portugal - Cenários, Impactos e Medidas de Adaptação–Projeto SIAM II. Lisboa, Portugal.

Sen, A., Miranda, I., Ferreira, J., Lourenço, A., Pereira, H., 2018. Chemical composition and cellular structure of ponytail palm (*Beaucarnea recurvata*) cork. Ind. Crops Prod. 124, 845–855. https://doi.org/10.1016/j.indcrop.2018.08.057

Sen, A., Miranda, I., Santos, S., Graça, J., Pereira, H., 2010. The chemical composition of cork and phloem in the rhytidome of *Quercus cerris* bark. Ind. Crops Prod. 31, 417–422. https://doi.org/10.1016/j.indcrop.2010.01.002

Sen, A., Pereira, H., Olivella, M.A., Villaescusa, I., 2015. Heavy metals removal in aqueous environments using bark as a biosorbent. Int. J. Environ. Sci. Technol. 12, 391–404. https://doi.org/10.1007/s13762-014-0525-z

Sen, A., Quilhó, T., Pereira, H., 2011a. Bark anatomy of *Quercus cerris* L. var. cerris from Turkey. Turk. J. Bot. 35(1), 45–55. https://doi.org/10.3906/bot-1002-33

Sen, A., Quilhó, T., Pereira, H., 2011b. The cellular structure of cork from *Quercus cerris* var. *cerris* bark in a materials' perspective. Ind. Crops Prod. 34, 929–936. https://doi.org/10.1016/j.indcrop.2011.02.015

Sen, A., Zhianski, M., Glushkova, M., Petkova, K., Ferreira, J., Pereira, H., 2016. Chemical composition and cellular structure of corks from *Quercus suber* trees planted in Bulgaria and Turkey. Wood Sci. Technol. 50, 1261–1276. https://doi.org/10.1007/s00226-016-0836-y

Silva, S.P., Sabino, M.A., Fernandes, E.M., Correlo, V.M., Boesel, L.F., Reis, R.L., 2005. Cork: properties, capabilities and applications. Int. Mater. Rev. 50, 345–365. https://doi.org/10.1179/174328005X41168

Stott, P., Christidis, N., Otto, F., Sun, Y., Vanderlinden, J.P., van Oldenborgh, G., Vautard, R., von Storch, H., Walton, P., Yiou, P., Zwiers, F., 2016. Attribution of extreme weather and climate-related events. Wiley Interdiscip. Rev. Clim. Change 7, 23–41. https://doi.org/10.1002/wcc.380

Surová, D., Pinto-Correia, T., 2008. Landscape preferences in the cork oak Montado region of Alentejo, southern Portugal: Searching for valuable landscape characteristics for different user groups. Landsc. Res. 33, 311–330. https://doi.org/10.1080/01426390802045962

Surová, D., Surový, P., de Almeida Ribeiro, N., Pinto-Correia, T., 2011. Integrating differentiated landscape preferences in a decision support model for the multifunctional management of the Montado. Agrofor. Syst. 82, 225–237. https://doi.org/10.1007/s10457-011-9373-8

Surový, P., Olbrich, A., Polle, A., Ribeiro, N.A., Sloboda, B., Langenfeld-Heyser, R., 2009. A new method for measurement of annual growth rings in cork by means of autofluorescence. Trees 23, 1237. https://doi.org/10.1007/s00468-009-0363-7

Vicente-Serrano, S.M., Beguería, S., López-Moreno, J.I., 2010. A multiscalar drought index sensitive to global warming: The Standardized Precipitation Evapotranspiration Index. J. Clim. 23, 1696–1718. https://doi.org/10.1175/2009JCLI2909.1