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Original article

Anatomy and chemical composition of *Pinus pinea* L. bark

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Abstract – The secondary phloem of *Pinus pinea* L. bark has sieve cells and axial and radial parenchyma, but no fibres. Resin ducts are present in fusiform rays. Styloid crystals, starch granules and tannins occur inside sieve and parenchyma cells. The rhytidome of *P. pinea* bark has a variable number of periderms forming scale-type discontinuous layers over expanded parenchyma cells. The phellem comprises two to four layers of thick-walled cells and the phelloderm a layer of two or three thin-walled cells with inclusions and sometimes a layer of expanded cells. Ash content of *P. pinea* bark is low and the pH is slightly acidic. Total extractives amount to 19.1 % and tannins to 7.2 % of o.d. weight. Content of lignin and unhydrolysable phenolic acids is 37.5 % and of polysaccharides 36.8 %, with the following monosaccharide composition: glucose 44.6 %, mannose 18.2 %, xylose 20.7 %, galactose 7.6 % and arabinose 8.9 %. (© Inra/Elsevier, Paris.)

***Pinus pinea* L. / bark / anatomy / chemical composition**

Résumé – Anatomie et composition chimique de l'écorce de *Pinus pinea* L. Le phloème secondaire de *Pinus pinea* L. contient des cellules criblées, du parenchyme axial et radial mais pas de fibres. Les canaux résinifères apparaissent dans les rayons fusiformes. Des cristaux styloïdes, des granules d'amidon et des tanins ont été observés dans les cellules criblées et le parenchyme. Le rhytidome renferme un nombre variable de péridermes qui forment des couches discontinues en écailles sur des cellules de parenchyme élargies. Le phellème comprend de deux à quatre couches de cellules à paroi épaisse et le phelloderme une couche de deux à trois cellules à paroi mince avec inclusions et, parfois, une couche de cellules élargies.
La teneur minérale de l' écorce de *Pinus pinea* est faible et le pH légèrement acide. Les extractibles correspondent à 19,1 % et les tanins à 7,2 % (masse anhydre). La teneur en lignine et en acides phénoliques non hydrolysables est de 37,5 %, tandis que celle des polysaccharides est de 36,8 % avec la composition suivante: glucose 44,6 %, mannose 18,2 %, xylose 20,7 %, galactose 7,6 % et arabinose 8,9 %. (© Inra/Elsevier, Paris.)

***Pinus pinea* L. / écorce / anatomie / composition chimique**

1. Introduction

Pinus pinea L. (stone pine) is a pine species of economic importance, growing in southern Europe especially in the western Mediterranean countries, namely in the Iberian peninsula and in France. *P. pinea* is a species of ecological importance in these regions and its protective role for other species, e.g. cork-oak and holm-oak, is acknowledged. In Portugal, the area forested with stone pine is approximately 80 000 ha

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and it is expanding due to the choice of this species for many afforestation projects. The stone pine is used mainly for the production of the edible pine nut but also for timber and the trees may be tapped for resin. The importance of nut production has increased with the recent use of grafting, allowing fruit production after 6 years.

The bark of *P. pinea* has not previously been characterised, although the anatomy and chemical composition of its wood has already been studied [2, 6, 12]. Detailed descriptions of the bark of other *Pinus* species, e.g. *P. echinata, P. taeda, P. palustris* and *P. rigida*, have been made [7, 8] and data on pine bark chemical composition have been summarised [5]. A description of *P. pinaster* bark anatomy and chemical composition has recently been published [9].

In this paper we describe the anatomy and chemical composition of *P. pinea* bark.

2. Materials and methods

The material used for analysis was obtained from commercial *P. pinea* plantations from one site in the south of Portugal (Albufeira). Ten trees approximately 25 years old were randomly selected and bark samples were taken at breast height.

For determination of the chemical composition, a composite sample was prepared by mixing aliquots of the bark of each tree sampled. The composite sample was milled and the granulometric fraction 40–60 mesh was used for analysis. The chemical composition was determined using standard methods for wood analysis [15] with adaptations reported for cork chemical analysis [11] and used previously for the analysis of maritime pine bark [9].

Extractives were determined using successive extractions with dichloromethane, ethanol and water. Suberin content was determined on extractive-free bark: 1.5 g were reacted under reflux with a sodium methoxide solution in methanol (250 mL methanol and 2.7 g Na) for 3 h and further with 100 mL methanol for 15 min after filtration; the combined filtrates were acidified to pH 6 with a 2 M sulphuric acid solution in methanol, evaporated to dryness and the residue suspended in water; the alcoholysis products were extracted three times with 200 mL chloroform, dried over sodium sulphate and evaporated to dryness. The desuberinised residue was hydrolysed and the hydrolysate used for separation and quantification of monosaccharides as alditol acetates by gas chromatography. The residue of hydrolysis was weighted as lignin and phenolic acids.

Total phenols and tannins were determined in the extracts obtained by successive extractions with ethanol and water and spectrophotometric measurement at 765 nm after reaction with Folin-Ciocalteu reagent using gallic acid for calibration [11]. The determination of tannin content used methylcellulose as absorbent: 10 mL extract were added to 10 mL of a 0.04 % solution of methylcellulose of high substitution degree, 8 mL of a saturated ammonium sulphate solution and 25 mL water; after 20 min this was filtered and the phenol content in the filtrate determined as previously. Tannin content was calculated as the difference between total phenols and phenols remaining after tannin absorption. Total nitrogen was determined by the Kjeldahl method. The pH was measured in a suspension of 2 g bark in 100 mL distilled water.

The anatomy studies were carried out using the bark of the individual trees sampled. Microscopic sections of 10 μm in thickness were prepared for optical microscopy using a Reichter sliding microtome after penetration with DP 1500 polyethylene glycol [14], and stained with a triple staining with chrysodine pyronine and astra blue. Sudan 4 was used for suberin detection. Observations were also made by light microscopy on dissociated elements. The samples were macerated in acetic acid and hydrogen peroxide 1:1 at 60 °C for 48 h and the macerated material was stained with astra blue. The terminology used for the anatomical description mainly followed Trockenbrodt [17].

3. Results and discussion

The stone pine (*Pinus pinea* L.) has a thick scale bark of a strong brown reddish colour.

The bark anatomy of *Pinus pinea* L. shows three structural layers from cambium to the outside (*figure 1a, b*): the secondary phloem, the innermost periderm and the rhytidome (which includes a variable number of periderms). The anatomical characteristics observed were similar for all trees. The anatomy is similar to that of the bark of *P. pinaster* [9] and other *Pinus* spp. [7, 8].The secondary phloem includes sieve cells (Sc), axial parenchyma (p) and rays (r) (*figure 1a, b*). No fibres were found and this agrees with general observations for the genus *Pinus* [4].

Sieve cells are elongated with unlignified thin walls and with many lateral sieve areas (*figure 2*). The sieve cells nearest to the cambium are radially aligned but this alignment is subsequently lost by distortion towards the outside through cell collapse due to loss of turgidity [7]. This distortion is particularly obvious near the innermost periderm.

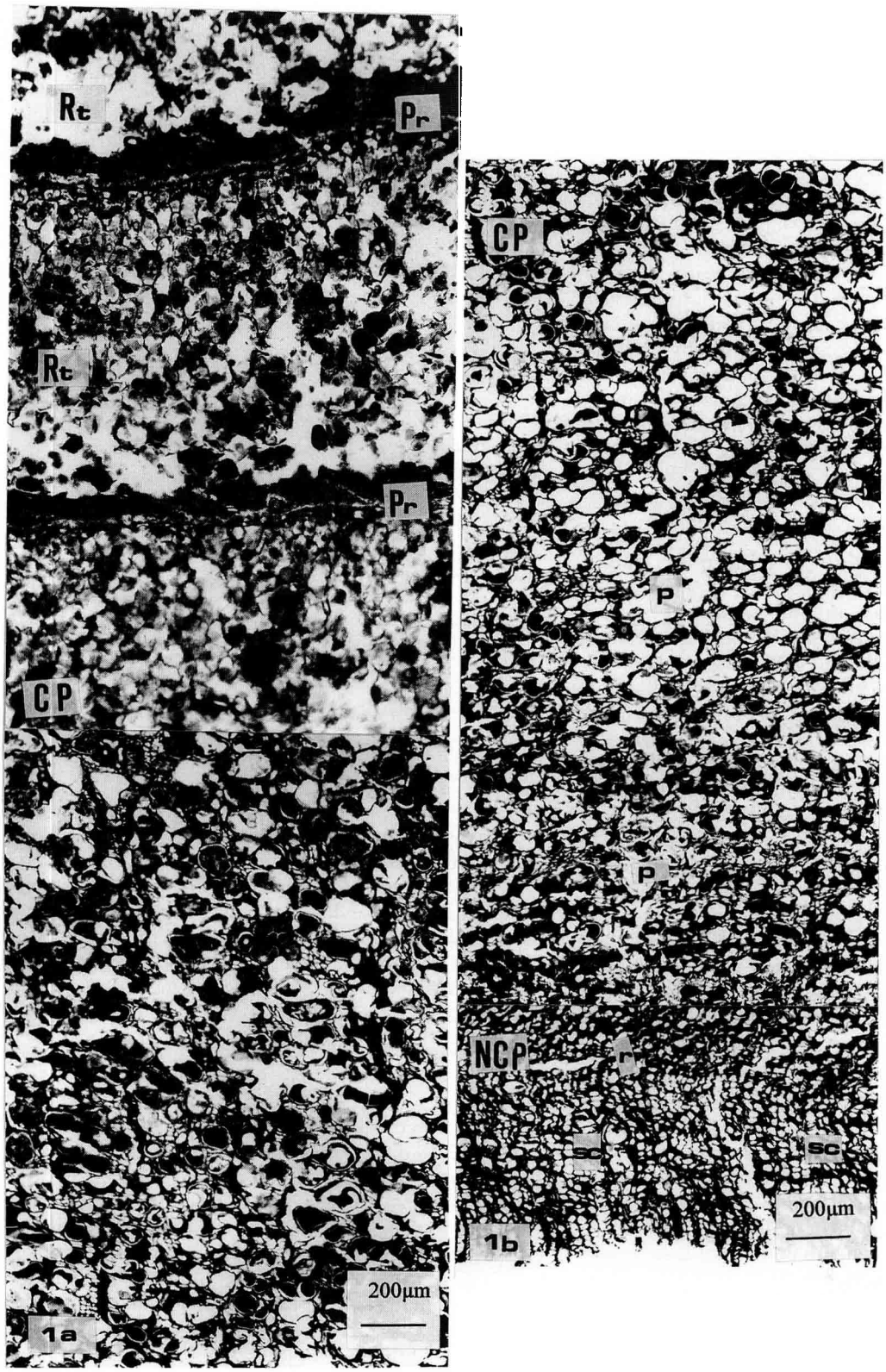

Figure 1. *Pinus pinea* bark (transverse section). a) Rhytidome (Rt), periderm (Pr) and collapsed phloem (CP). b) The secondary phloem, collapsed phloem (CP) and non-collapsed phloem (NCP). Sieve cells (sc); axial parenchyma (p) and rays (r).

The parenchyma cells (p) are thin-walled, approximately circular in cross section and scattered. They have styloid crystals (c) (*figure 3*) and starch granules. It has been reported for southern pine [7] that styloid crystals are composed of calcium oxalate and that they are abundant throughout the innerbark and rhytidome, deposited as a metabolic by-product.

The phloem rays (r) are similar to the xylem rays, uniseriate and homocellular. Albuminous cells form the margins of some rays. Fusiform rays with resin ducts are also observed (*figure 4*). Rays extend linearly through the non-collapsed phloem, but near the periderm the alignment is lost. The transition from non-collapsed to collapsed phloem is gradual and signalled by the collapse of sieve cells and loss of their radial and tangential alignment, the distortion of rays and the expansion of parenchyma cells. These changes represent the adjustment of the secondary phloem to the radial tree growth, as reported for *P. pinaster* [9] and for other species of the genus *Pinus* [7, 8] as well as for other genera [1, 13, 16].

The periderm (Pr) is made up of phellem (Pm), phellogen and phelloderm (Pd) (*figures 1a, 5*). In each periderm, the samples showed a variable number of cells in the phellem and phelloderm layers. The phellem includes layers of two to four radially aligned thick-walled and sclerified cells (Scl), sometimes with many inclusions (*figure 5*). A band of suberised cells alternating with thick-walled cells was not observed, as described for other *Pinus* spp. [7]. The phelloderm, located to the interior of the phellogen, has two types of cells: near the phellogen, two to three layers of thin-walled cells with numerous inclusions; then one layer of thin-walled, radially expanded cells (*figure 5*, arrow) and may be confused with cells from the expanded parenchyma (ex) (*figure 5*). The variable number of cells in the phellem and phelloderm layers in each periderm agrees with Howard [7] who found that in the bark of southern pine the amount of tissue in the periderm may vary within a single sample.

In *P. pinea*, similar to *P. pinaster* and other *Pinus* spp., the periderm is discontinuous, following an irregular path around the stem. The periderms form layers with edges curving outwards to merge with older periderms; the phloem is thus isolated in a scale-type pattern and the outer bark forms a scalebark. The rhytidome (Rt) has a variable number of periderms (on average three periderms) that overlap enclosing a phloem tissue that is mainly made up by expanded parenchyma cells many times larger than their original diameters (*figure 5*). This type of cell change at the outside of the innermost periderm has been observed in *P. pinaster* [9], in southern pines [7] and also in other genera [3]. These expanded parenchyma cells (ex) occupy a large volume fraction of the rhytidome and contribute to its porous structure; they are sensitive to fracture and determine the external morphology of the rhytidome, which is kept together by the sclerified cells (Scl) of the periderm. The rhytidome of *P. pinea* has a high amount of material deposited in the lumens, mainly tannins, which gives the bark its strong reddish brown colour.

Table I. Average chemical composition of *Pinus pinea* bark.

Component	Percentage of oven-dried weight
Ash	2.3
Extractives	
Total	19.1
Dichloromethane	2.1
Ethanol	12.1
Water	4.9
Suberin	2.5
Lignin and polyphenols	37.5
Polysaccharides	36.8

Table II. Total phenolics and tannins of *Pinus pinea* bark.

Component	Percentage of oven-dried weight
Total phenolics	7.5
Tannins	7.2

Table III. Average carbohydrate composition of cell wall polysaccharides in *Pinus pinea* bark.

Component	Percentage of total monosaccharides
Glucose	44.6
Mannose	18.2
Galactose	7.5
Xylose	20.7
Arabinose	8.9

The chemical composition of pine bark is summarised in *table I*. Extractives amount to 19,1 % and include mainly polar compounds extracted by ethanol and water (14.0 % of o.d. bark); approximately half of the extractives (7.2 % of o.d. bark) are of phenolic character and correspond to tannins which amount to 96 % of total phenolics (*table II*). The microscopic observations show that tannins are deposited in the cell lumens of the rhytidome (*figure 5*). The suberin content is low, in accordance with the small number of phellem cells found in the rhytidome and the fact that they were thick-walled and sclerified (*figure 5*). Consequently, lignin and unhydrolysable polyphenol content is high, amounting to

Figure 2. Sieve cell (sc) and sieve areas (arrow) in surface view of a lateral wall (tangential section).

Figure 3. Styloid crystals (c) in the secondary phloem of *Pinus pinea* (tangential section).

Figure 4. Uniseriate and homocellular rays (r); fusiform ray showing a resin duct (arrow).

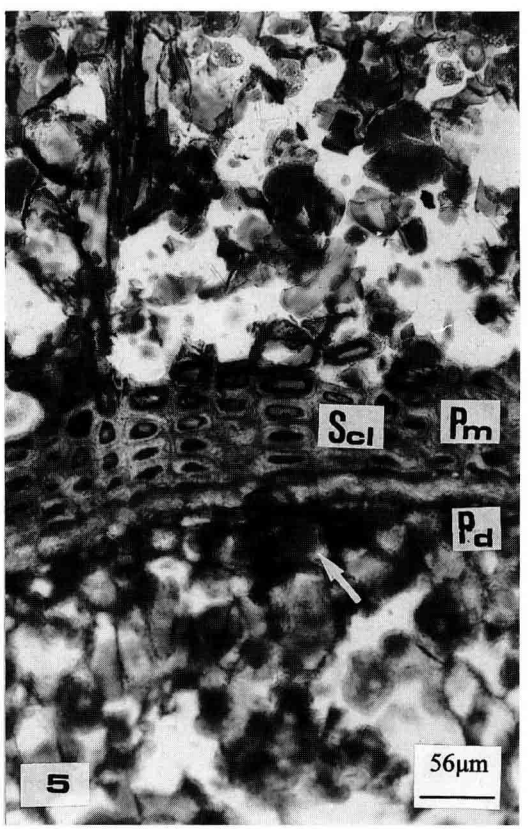

Figure 5. Transverse section of *Pinus pinea* bark. Periderm with phellem (Pm), and phelloderm (Pd). Phellem includes layers of two to four thick-walled and sclerified cells (Scl); phelloderm with two to three cells in radial direction with many inclusions and a layer of thin-walled cells, sometimes radially expanded (arrow).

38 % of the o.d. bark. The monomeric composition of polysaccharides (*table III*), which correspond on average to approximately 37 % of pine bark, shows a predominance of glucose. In the hemicelluloses, xylans and mannans have almost similar importance, 20.7 and 18.2 %, respectively, of total monosaccharides. Arabinose is present in considerable amounts (9 % of monosaccharides).

In comparison to the chemical composition of other *Pinus* species [5, 9], *P. pinea* bark shows a content of extractives similar to *P. sylvestris* and *P. taeda* (20.7 and 18.3 %, respectively), higher than *P. pinaster* (11.4 %) and lower than *P. elliotti* (35.8 %). The content of total polysaccharides is lower than in *P. pinaster* and *P. sylvestris*. The proportion of xylans is higher than in *P. sylvestris* (12.6 % of total monosaccharides) and lower than in *P. pinaster* (26.1 %).

The bark of stone pine is slightly acidic (pH 4.5) and total nitrogen content is 0.76 %.

4. Conclusions

The bark anatomy of *P. pinea* L. is similar to that of *P. pinaster* and other *Pinus* spp. The secondary phloem of *P. pinea* has sieve cells and axial and radial parenchyma, but no fibres. Resin ducts are present in fusiform rays and styloid crystals, starch granules and tannins occur inside sieve and parenchyma cells. The rhytidome of *P. pinea* has a variable number of periderms as scale-type discontinuous layers over expanded parenchyma cells. The phellem has a small number of thick-walled and small suberised cells.

The chemical composition of *P. pinea* bark shows a considerable amount of tannins and a relatively low content of polysaccharides.

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