

Different surface characteristics of primary and secondary needles of *Pinus canariensis*

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Summary

Surface characteristics of primary and secondary needles of *Pinus canariensis* were investigated using scanning electron microscopy and gas chromatography to study structure and composition of epicuticular wax, cuticle micromorphology and the structure of stomata.

Tubular waxes could be observed on the whole needle surface of the glaucous primary needles whereas on secondary needles they were restricted to the lower surface of young needles. Recrystallization resulted in comparable wax tubes and, additionally, plate like structures recrystallized from primary needle wax. Isolated cuticles of primary needles were tender and showed a simple stomata complex with six subsidiary cells whereas the cuticles of secondary needles were massive and revealed 9–12 subsidiary cells. In contrast to the cuplike epistomatal chamber of the primary needles that of the secondary needles was larger and often irregularly formed. Main constituents of the cuticular wax were ω -hydroxy-n-alkanoic acids, 10-nonacosanol and n-alkanoic acids with no differences in the qualitative composition between primary and secondary needles but with some differences in the quantitative pattern.

The possible role of the investigated cuticular features in adaptive strategies of the needles to avoid light and water stress is discussed.

Key words: Canary Island pine, *Pinus canariensis*, primary and secondary needles, epicuticular wax, wax chemistry, cuticle micromorphology

Introduction

Pinus canariensis Chr. Sm. ex DC in Buch is an endemic tree species of the Canary Archipelago whose natural distribution area is restricted to the highest islands. In Tenerife it grows spontaneously from near the sea level up to about 2.200 m (de l'Arco et al. 1990). It colonizes volcanic soil and is able to live from mesic places in the North to very dry and rocky sites in the

south of the island. Outside its natural area this pine species grows very well in the North and South of Africa, and under good environmental conditions in different Mediterranean countries, South of USA, South America, Australia and New Zealand (Schütt et al. 1995).

The typical pine needles, the secondary needles, are up to 30 cm in length and are grouped in fascicles of three on short shoots. Recently some structural

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investigations showing a high degree of xeromorphosis were published (Jiménez et al. 2000; Grill et al. 2004). In the development of the seedling the secondary needles are preceded by short glaucous primary needles growing on long shoots. *P. canariensis* has a high ability of regeneration, e.g. following forest fires. Highly damaged branches and stems are able to form long shoots from abundant adventitious buds bearing in the first phase glaucous primary needles (Schütt et al. 1995).

As a result of high solar irradiation, air, and soil drought pine forests in the Canary Islands experience a high evaporative demand (Wieser et al. 2002; Peters et al. 2003). Drought stress is a key factor in Mediterranean type ecosystems with pronounced summer droughts and is accompanied by development of oxidative stress (Smirnoff 1993; Jiménez et al. 1997; Tausz et al. 2001). One way to survive and thrive in such an environment are structural adaptations on the level of the needle surface (Larcher 2001). Stomata characteristics and cuticular features play an important role for adaptation to drought, high photon flux densities and UV radiation (Robinson et al. 1993; Barnes & Cardoso-Vilhena 1996; Grace & van Gardingen 1996; Holmes & Keiller 2002; Long et al. 2003). These functions are connected with the composition and the structure of the cuticular wax (Riederer 1989; Kolattukudy 1996).

In the presented work differences in the structure and composition of cuticular waxes, cuticle micromorphology and stomatal features of primary and secondary needles of *P. canariensis* were investigated with the aim to identify different strategies of adaptation to an environment with frequent water shortage and high solar radiation.

Materials and Methods

Plant materials and sampling

Secondary needles were sampled from mature trees growing in the South of Tenerife (Canary Islands) and primary needles from one year old seedlings growing in pots in the nursery of the University La Laguna (Tenerife). Needles were stored in paper bags and air dried. Some of the needles were dipped in chloroform for several seconds to remove the epicuticular wax. Longitudinal and cross sections of rehydrated needles were prepared by a cryotome (Leica CM 3000; Nussloch, Germany).

For studies of wax chemistry and wax recrystallization primary and secondary needles were sampled from young plants growing in pots in the greenhouse of the Institute of Plant Physiology Graz (Austria).

Cuticle isolation

Needles were cut into sections and immersed in 20% chromium trioxide solution for 96 h (Stockey & Ko 1990). Cuticles were washed in distilled water and prepared for SEM.

Extraction of cuticular wax

Fresh needles (primary and secondary) were immersed in chloroform (reagent grade) for 30–60 seconds; the solution was filtered and concentrated by vacuum evaporation. For analysis of wax chemistry see below.

Wax recrystallization

Best results for wax recrystallization gave the following device (modified to Jeffree et al. 1975): A glass-fibre wick fitting into a 5 ml glass vial was immersed in the wax solution and on the other end contacted a PTFE membrane (polytetrafluoroethylene; pore diameter 0.45 µm). The wax solution evaporated on the surface of the membrane that was prepared for SEM.

Scanning electron microscopy (SEM) and image analysis

The material (air dried needles, dewaxed needles, isolated cuticles, PTFE membranes with recrystallized waxes) was mounted on aluminium stubs with carbon tabs, sputter coated with gold (Agar Sputter Coater B7340; Essex, UK) and investigated with a Philips XL30 ESEM (FEI; Eindhoven, the Netherlands) in the high vacuum mode with 10–20 kV acceleration voltage. Images from dewaxed samples were analysed for opening area of the Florin ring using digital image analysis software (Optimas® 6.1).

Analysis of wax chemistry

Sample preparation

Quantitative analysis of the cuticular lipid composition was completed with gas chromatography (GC) without prior class separation. After addition of 0.6 mg heptadecanoic acid (internal standard), the samples were refluxed with 2 mol l⁻¹ anhydrous methanolic HCl containing 0.3% 2,6-di-tert-butyl-4-methylphenol (= butylated hydroxytoluene, BHT) for 2 h at 65 °C. Esters and estolides were hydrolyzed by this treatment. BHT acted as antioxidant to avoid sample deterioration. The lipids were transferred to toluene in a separatory funnel, washed three times with water and dried over anhydrous sodium sulphate. The toluene phase was taken to dryness in a rotary evaporator and the lipids were redissolved with 1 ml methylene chloride.

Gas chromatography (GC)

GC was performed using a Varian CP-3800 gas chromatograph (Varian Chromatography Systems; Walnut Creek, California, USA) equipped with a 1079 universal capillary injector operating at 300°C in the split mode (1 : 20), and a flame ionisation detector (FID) running at 310°C. The samples were analysed on a fused silica 50 m Permabond SE 54 (5% diphenyl-95%-dimethylpolysiloxan) capillary column (Macherey-Nagel; Düren, Germany) with an internal diameter of 0.25 mm and a film thickness of 0.27 µm. The hydrogen carrier gas had a delivery rate of 1.8 ml min⁻¹ (controlled constant flow). Oven temperature programming was 100°C during injection, and then increased from 100 to 310°C at the rate of 5°C min⁻¹, and 310°C held for additional 15 minutes.

GC-MS was performed on a Hewlett Packard (Hewlett Packard; Palo Alto, California, USA) G1800A GCD system (electron impact voltage: 70 eV, injector temperature: 300°C, detector temperature: 310°C, foreline pressure: 4 Pa, mass range 30–425 amu). The samples were analysed on a HP-5 (5% diphenyl-95%-dimethylpolysiloxan; 30 m × 0.25 mm i.d.; film thickness 0.25 µm) capillary column (Hewlett Packard). The helium carrier gas had a delivery rate of 1 ml min⁻¹ (controlled constant flow) and the oven temperature programming was 100°C during injection, and then increased from 100 to 310°C at the rate of 5°C min⁻¹, and 310°C held for additional 15 minutes.

Compounds were identified using both chromatographic and mass spectroscopic criteria. The Wiley275 data base was used for automatic identification of GC-MS peaks, and linear retention indices (van den Dool & Kratz 1963) were compared with published data. Whenever possible, mass spectra and retention indices were also compared with data obtained from authentic compounds (Sigma, Merck). Quantitative results were achieved from GC-FID profiles using the internal standard method without consideration of calibration factors (i.e. F = 1.0) for all compounds.

Results

Epicuticular wax structures

The primary needles of *P. canariensis* (Fig. 1a) were characterized by a glaucous appearance due to a dense layer of tubular waxes covering the whole needle surface (Fig. 1b, c) with no difference in appearance on the different sides of the needle. The stomata were arranged in rows (Figs. 1a, d) and the Florin ring, a surface feature produced by the cells encircling the stomata (Yoshie & Sakai 1985), raised outwards and was densely covered by wax (Fig. 1b, c). It had a volcanic shape and the opening area was more or less circular (Fig. 1d). The size of the opening was variable over a wide range with a mean opening area of 194.92 µm² (Table 1). Also the inner edge of the opening was densely covered by tubular wax (Fig. 1b). Very often a change in appearance could be observed due to mechanical impact resulting in a change from tubular to amorphous wax and very often recrystallized wax tubes could be observed (not shown). Only very rarely plate like wax structures could be seen on areas with a strong degradation of tubular waxes combined with a high dust load on the surface (Fig. 3d).

The secondary needles were green and had a length of up to 30 cm. The surface of the upper needle side was smooth and covered by an amorphous wax layer (Fig. 2a). Structural waxes could only be observed in the epistomatal chamber, covering the inner edge of the Florin ring but not on the ridges of the Florin ring itself (Fig. 2b). On the lower needle side structural waxes could also be seen on the surface and not only in the epistomatal chamber (Fig. 2c, d). However, a high degree

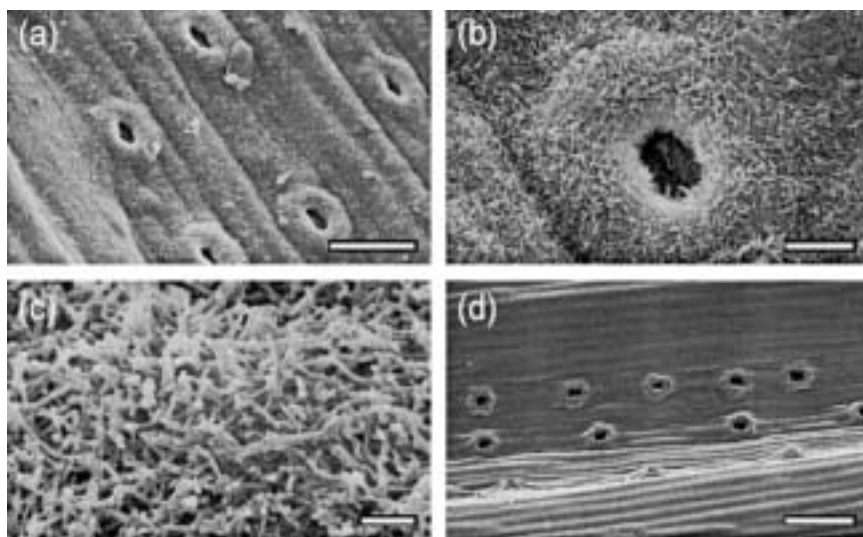


Fig. 1. SEM micrographs of primary needles of *P. canariensis*. (a) Overview; bar = 50 µm. (b) Florin ring and needle surface densely covered by tubular wax structures; bar = 10 µm. (c) Tubular wax structures on the Florin ring; bar = 2 µm. (d) Dewaxed needle; bar = 100 µm.

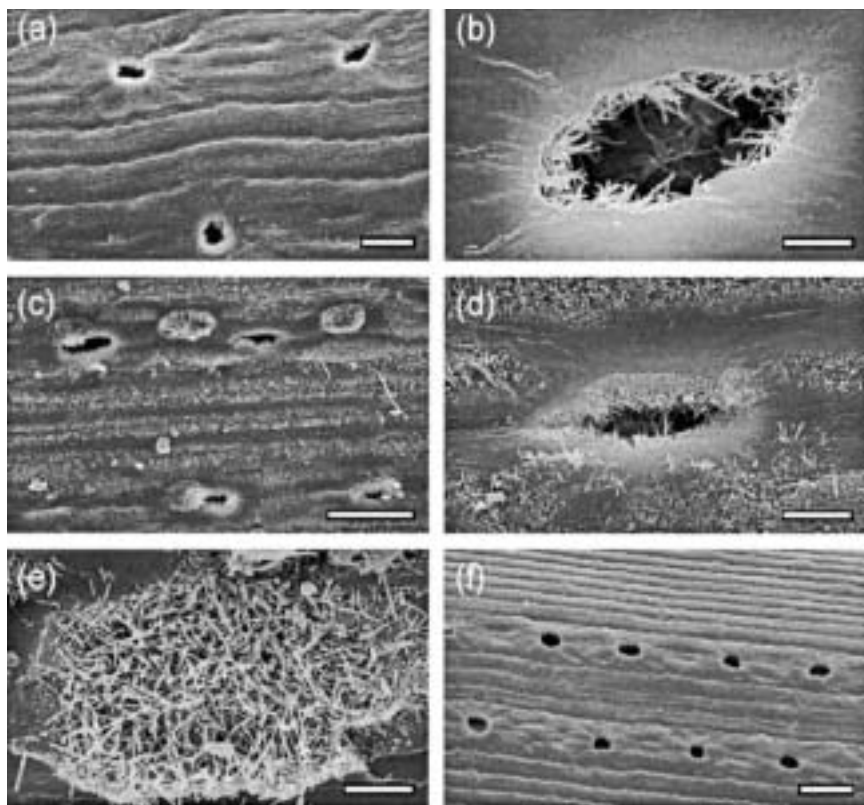


Fig. 2. SEM micrographs of secondary needles of *P. canariensis*. (a) Upper side; bar = 25 μm . (b) Upper side, opening area of the epistomatal chamber; bar = 5 μm . (c) Lower side; bar = 50 μm . (d) Lower side, opening area of the epistomatal chamber; bar = 10 μm . (e) Undisturbed tubular waxes in a small dent on the needle surface; bar = 5 μm . (f) Upper side of a de-waxed needle; bar = 50 μm .

of mechanical degradation and a shift from a crystalline to an amorphous appearance could be observed on all convex areas, above all on the ridges of the Florin ring (Fig. 2d) – wax recrystallization occurred regularly in these areas. In small dents on the surface, however, the crystalline structure remains for a longer time (Fig. 2e). The Florin ring rose gently as a low ridge on the general surface (2f). Its shape was more often circular to elliptical on the upper side (Fig. 2a, f) whereas on the lower side it was oblong (Fig. 2c, d). The mean opening area on the lower needle side was with 182.51 μm^2 comparable to those of the primary needles whereas the mean opening area on the upper side (221.18 μm^2) was significantly larger (Mann-Whitney U-test: $p < 0.001$; Table 1).

To study recrystallization cuticular wax was removed from the needles with chloroform and reorganisation occurred on the surface of a PTFE membrane. Long and thin wax tubes could be observed after recrystallization of chloroform extracts from primary as well as from secondary needles (Fig. 3a, b). While this was the only wax type recrystallized from secondary needle wax extracts plates were additionally formed after evaporation of the solvent from primary needle wax extract (Fig. 3c). This observation was accompanied

Table 1. Opening size of the Florin ring (μm^2) on the upper (adaxial) and lower (abaxial) needle side of primary and secondary needles of *P. canariensis* showing the mean, minima and maxima, standard deviation (SD) and number of stomata measured (n).

	n	Mean	Minimum	Maximum	SD
Secondary needle; adaxial	97	222.18	85.93	387.68	65.93
Secondary needle; abaxial	128	182.51	76.86	333.65	55.89
Primary needle	83	194.92	91.94	302.06	47.47

by slight differences in the appearance of the wax extracts. The chloroform extract of the secondary needles was more or less colourless and could easily be evaporated to dryness. By contrast, that of the primary needles was slightly greenish and after evaporation an oily residue remained. Plates could only be observed on top of an amorphous layer formed on the surface of the recrystallization device (PTFE membrane) after solvent evaporation while the tubular wax structures could be detected on the direct surface of the membrane.

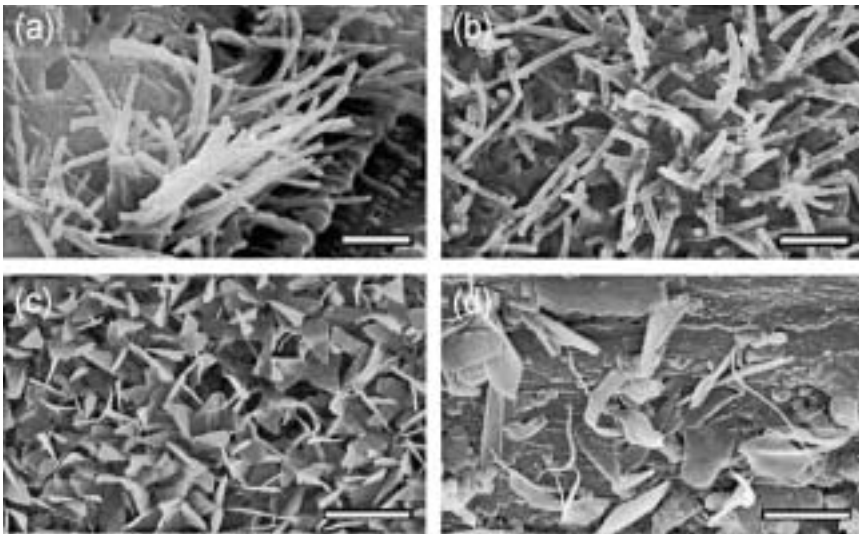


Fig. 3. (a)–(c) SEM micrographs of wax recrystallized on the surface of a PTFE membrane. (a) Primary needle wax extract, wax tubes; bar = 2 μm . (b) Secondary needle wax extract, wax tubes; bar = 2 μm . (c) Primary needle wax extract, platelets; bar = 5 μm . (d) SEM micrograph of a native surface of a primary needle showing platelets; bar = 5 μm .

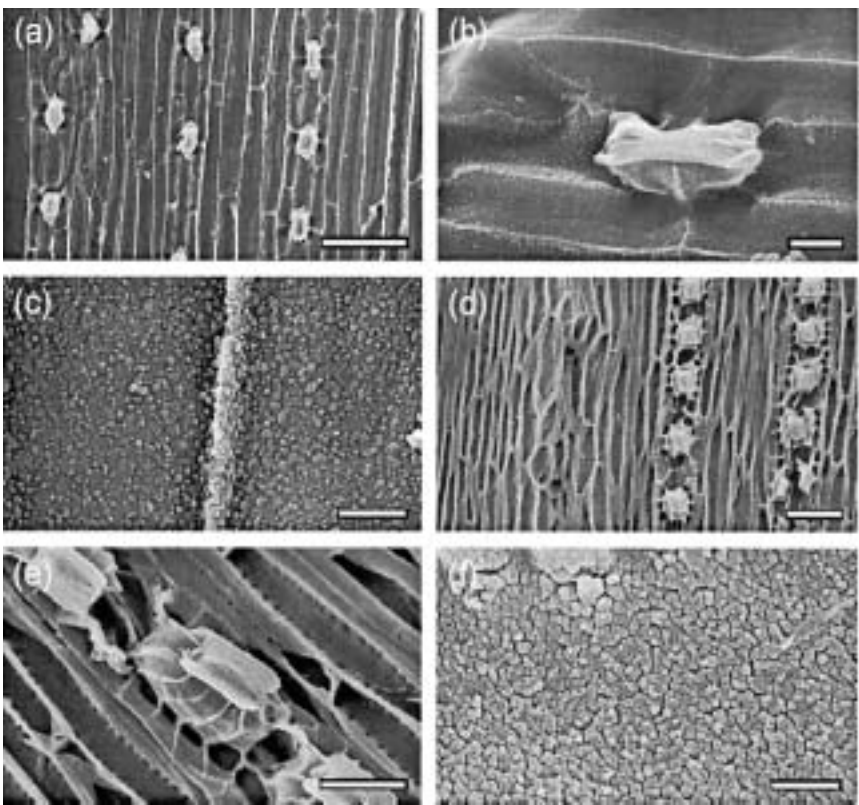


Fig. 4. SEM micrographs of isolated cuticles showing the micromorphology of the inner cuticle. (a)–(c) Primary needles. (a) Overview; bar = 100 μm . (b) Stomatal complex with 6 subsidiary cells; bar = 10 μm . (c) Periclinal surface; bar = 5 μm . (d)–(f) Secondary needles. (d) Overview; bar = 100 μm . (e) Stomatal complex; bar = 50 μm . (f) Periclinal surface; bar = 5 μm .

Cuticle micromorphology and epistomatal chamber

The inner surface of the isolated cuticle gave a clear impression of the size and shape of the epidermal cells and the structure of the stomatal apparatus and was differently developed on secondary and primary needles

(Fig. 4). The epidermal cells of the primary needles were long ($>200 \mu\text{m}$) and regularly shaped with the end walls being vertical or oblique (Fig. 4a). The periclinal surface of the cuticle was fine granular (Fig. 4c) and the anticlinal wall imprints were only weakly developed (Fig. 4a). The stomatal apparatus was composed of the guard cell and six subsidiary cells with no distinct

differences in size between the lateral and polar subsidiary cells (Fig. 4b). The anticlinal projections of the inner cuticles of the secondary needles were heavily developed, the cells were not regularly shaped and partly tapering (Fig. 4d). The periclinal surface of the inner cuticle was coarse granular (Fig. 4f). The stomatal apparatus revealed a large epistomatal chamber and 9–12 subsidiary cells with the polar subsidiary cell often being larger than the lateral ones (Fig. 4d, e).

Needle sections demonstrated clear differences in the architecture of the epistomatal chamber of primary and secondary needles (Fig. 5). In the primary needles it was regularly shaped, cup-like, the opening was centred above the guard cells and the walls were covered by a dense layer of tubular waxes (Fig. 5a, b). The epistomatal chamber of the secondary needles was much larger, forming an irregularly shaped cave. The opening was often not centred above the guard cells (Fig. 5c, d). Comparable to the primary needles the walls of this cavity were also covered by a dense layer of tubular wax (Fig. 5d).

Wax chemistry

The wax yield was significantly higher for the primary needles than for the secondary needles (% needle dry weight; primary needles: 2.35 ± 0.17 , $n = 6$; secondary needles: 1.68 ± 0.21 , $n = 5$; Mann-Whitney U-test: $p < 0.01$).

The composition of the transmethylated cuticular wax of the primary and secondary needles of *P. canariensis* is shown in Table 2. Unidentified fatty acids (methyl esters) were characterised on the basis of their mass spectral data and their retention behaviour (m/z 74, 87, 98, 143; Christie 1989). The shorter chain length com-

pounds were probably transesterification products of estolides. Main components were ω -hydroxy-*n*-alkanoic acids followed by the secondary alcohol 10-nonacosanol and *n*-alkanoic acids. The applied methodology and equipment did not allow an unambiguous identification of nonacosanediols. Although the main wax compounds were the same in both needle classes, there were characteristic differences in their quantitative pattern. The concentration of 10-nonacosanol was 4 times higher in secondary needles as compared to primary needles. The wax of the primary needles was characterized by higher amounts of compounds with shorter chain lengths (C_{12} , C_{14}), whereas the amount of compounds with longer chain lengths was significantly higher in the wax of secondary needles. Diterpene acids could also be detected and their amount was also significantly higher in the wax of secondary needles.

Discussion

Primary and secondary needles of *P. canariensis* differ distinctly in various surface characteristics.

The surface of primary needles of *P. canariensis* is completely covered by tubular wax crystals resulting in a glaucous appearance. The production of copious quantities of highly reflective structural wax on the surface of leaves render increased protection against UV-radiation and also against high photon flux densities (Robinson et al. 1993; Barnes & Cardoso-Vilhena 1996; Holmes & Keiller 2002; Long et al. 2003). For *Cotyledon orbiculata* it was shown that removal of the waxy coating by brushing resulted in photoinhibitory damage (Robinson et al. 1993). Since the natural environment of *P. canariensis* is characterised by high solar radiation (Wieser et al. 2002) it can be supposed

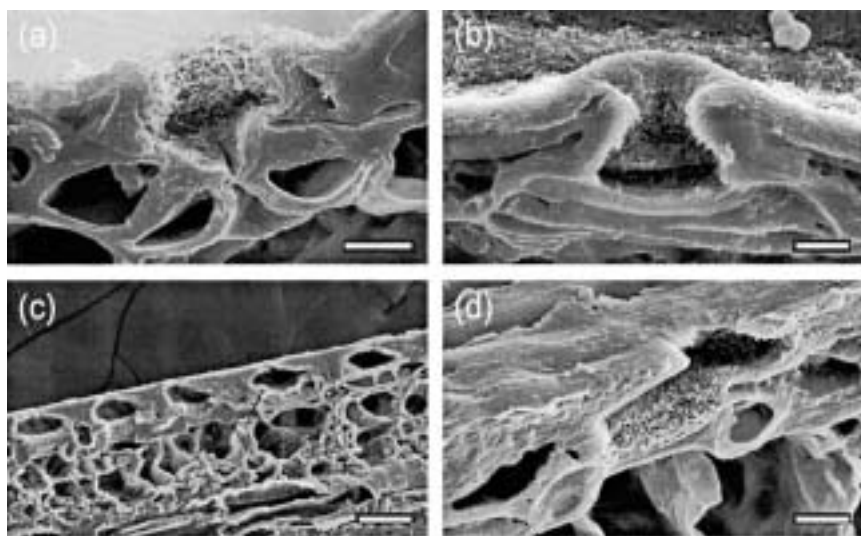


Fig. 5. SEM micrographs of sections of primary and secondary needles showing the architecture of the epistomatal chamber. (a)–(b) Primary needles. (a) Cross section; bar = 10 μ m. (b) Longitudinal section; bar = 10 μ m. (c)–(d) Longitudinal sections of secondary needles. (c) Overview; bar = 50. (d) Detail of one stomatal complex; bar = 10 μ m.

Table 2. Composition of epicuticular wax of primary and secondary needles of *P. canariensis*; mean (mg 100⁻¹ mg⁻¹ sample), standard deviation; differences between primary and secondary needles, * -p = 0.01–0.05; ** -p = 0.001–0.01.

	Primary needles n = 6		Secondary needles n = 5		Significance (U-Test)
	mean	SD	mean	SD	
<i>n</i>-Alkanes					
C ₂₇	0.09	0.03	0.06	0.01	*
C ₂₉			0.09	0.01	
C ₃₀	0.01	0.01			
C ₃₁	0.01	0.02	0.01	0.01	
<i>n</i>-Alkanoic acids (methyl esters)					
C ₁₀	0.03	0.02	0.04	0.01	
C ₁₂	5.39	0.6	1.75	0.15	**
C ₁₃	0.01	0.01	0.02	0.00	
C ₁₄	0.73	0.11	0.56	0.03	
C ₁₅	0.01	0.01	0.02	0.01	
C ₁₆	0.21	0.04	0.43	0.05	**
C ₁₈	0.13	0.07	0.1	0.02	
C ₁₉	0.01	0.01	0.02	0.00	**
C ₂₀	0.16	0.04	0.23	0.02	**
C ₂₁			0.04	0.04	
C ₂₂	0.06	0.03	0.31	0.02	**
C ₂₃	0	0.01	0.05	0.00	**
C ₂₄	0.09	0.01	0.59	0.04	**
C ₂₅			0.02	0.01	
C ₂₆	0.03	0.02	0.3	0.03	**
C ₂₇			0.02	0.00	
C ₂₈	0.06	0.03	0.57	0.03	**
ω-Hydroxy-<i>n</i>-alkanoic acids (methyl esters)					
C ₁₂	13.55	1.12	3.52	0.41	**
C ₁₄	23.18	2.12	8.67	0.38	**
C ₁₆	12.55	1.03	11.04	0.83	
C ₁₈	0.45	0.04	0.44	0.04	
α,ω-Alkandioic acids (dimethyl esters)					
C ₁₂	0.03	0.02	0	0.00	**
C ₁₄	0.21	0.01	0.21	0.02	
C ₁₆	0.12	0	0.17	0.02	**
Ketones					
10-Nonacosanone	0.01	0.01	0.11	0.01	**
Primary alcohols					
1-Decanol	0.06	0.01	0.06	0.01	
1-Dodecanol	0.02	0.02	0.03	0.02	
1-Tetradecanol			0.05	0.02	
1-Octadecanol	0.07	0.04			
Secondary alcohols					
10-Nonacosanol	2.47	0.36	10.04	3.55	**
α,ω-Alkane diols					
C ₁₂	0.07	0.05	0.01	0.01	**
C ₁₄	0.97	0.13	0.06	0.05	**
C ₁₆	?		?		

Table 2. (continued)

	Primary needles n = 6		Secondary needles n = 5		Significance (U-Test)
	mean	SD	mean	SD	
Unidentified fatty acids (methyl esters)					
C _x	0.89	0.06	0.26	0.03	**
C _{x+2}	1.45	0.12	0.58	0.02	**
C _{x+4}	0.79	0.06	0.71	0.05	
C _{x+6}	0.02	0.02	0.03	0.00	
Diterpene acids (methyl esters)					
Dehydroabietic acid	tr		0.62	0.12	
Abietic acid	0.18	0.16	0.64	0.13	**
unknown terpene	0.08	0.06	0.46	0.09	**
unknown terpene			0.02	0.02	

that the wax layer on the surface of the tender primary needles serve as part of a photoprotection system.

In contrast crystalline waxes on the secondary needles can only be observed within the stomatal bands on the lower side of young needles whereas the upper side is bald. With increasing age these crystalline waxes vanish soon (Jiménez et al. 2000). Mechanical degradation of epicuticular wax resulting in a shift from a crystalline to an amorphous appearance is often observed on conifers and is supposed to be the main reason for the disappearance of crystalline waxes on needle surfaces (van Gardingen et al. 1991; Bermadinger-Stabentheiner 1995; Grace & van Gardingen 1996). Mechanical abrasion seems to be unavoidable with needles growing in fascicles of three where they always bang together.

The epistomatal chamber of the primary needles is cup-like, the opening is centred above the guard cells and is comparable to other pine species, e.g. *P. sylvestris* (compare cross sections: van Gardingen et al. 1991; Bacic et al. 1992). In contrast to this the epistomatal chamber of the secondary needles is forming a spacious cave with an irregular shape where the opening is often not centred above the guard cells. This characteristic feature of the secondary needles was also shown recently as the result of a 3-D reconstruction of serial sections (Zellnig et al. 2002). In both needle types the walls of the epistomatal chamber are densely covered by tubular waxes. The epistomatal chamber plays an important role in gas exchange processes and especially in reduction of water loss (Riederer 1989). Jeffrey et al. (1971) estimated that the wax filled antechamber of *Picea sitchensis* contributed 66% and 32% of the entire resistance toward the diffusion of water vapour and CO₂, respectively. The epistomatal chambers of *P. canariensis* are not filled with a wax plug as is the case for *Picea sitchensis*, but the opening area is much

smaller. It is within the range given for *P. canariensis* by Yoshie & Sakai (1985: 175 ± 38 μm²). For *Picea sitchensis* it was roughly estimated to be at least 5 times larger (approximately 1000 μm²; measured from Fig. 3b in van Gardingen et al. 1991). Additionally, long and thin wax tubes protrude from the inner edge of the opening towards the centre thus further reducing the area available for gas exchange. In recent gas exchange measurements on *P. canariensis* the very low CO₂ concentrations at the mesophyll level (C_i) were explained as a result of numerous resistances that complicate the CO₂ path from the outer air to the interior mesophyll (Peters et al. 2003). The special appearance of the epistomatal chamber of the secondary needles certainly plays an important role as part of these resistances.

The micromorphology of isolated cuticles also reflects differences in both needle types where the isolated cuticles of the primary needles appear tender as compared to the massive cuticles of the secondary needles. Recently some papers dealing with cuticle micromorphology of several pine species were published to provide new taxonomic data for infrageneric classification (Kim et al. 1999; Ickert-Bond 2000; Whang et al. 2001). *P. canariensis* was not included in these studies. But *P. roxburghii*, the nearest relative of *P. canariensis*, was investigated (Kim et al. 1999) and its cuticle micromorphology with weakly developed anticlinal wall imprints and a simple stomatal complex with only a few subsidiary cells resembles that of the primary needles investigated in the presented paper. A comparable high number of 8–12 subsidiary cells for the secondary needles is only reported for *P. luchuensis* (Kim et al. 1999) and for *P. kesiya* (Ickert-Bond 2000).

Cuticular wax is mainly composed of long-chain aliphatic compounds derived from very long chain fatty acids (Kollatukudy 1996; Kunst & Samuels 2003).

Main constituents of the wax of *P. canariensis* are ω -hydroxy-n-alkanoic acids followed by the secondary alcohol 10-nonacosanol and n-alkanoic acids and are comparable to other coniferous species (Franich et al. 1978; Riederer 1989). There are no differences in the qualitative composition of the wax between primary and secondary needles but there are some differences in the quantitative pattern. The wax of the secondary needles is characterized by a significantly higher amount of 10-nonacosanol and compounds with longer chain lengths as compared to the wax of the primary needles.

The well known connection between structure and composition of cuticular waxes (Jeffree 1996) can also be seen here where a high amount of the secondary alcohol 10-nonacosanol in the wax extract is connected with the occurrence of tubular waxes on natural surfaces and after recrystallization of the wax extract. This capability of self organization can also be observed on native needle surfaces (van Gardingen et al. 1991; Bacic et al. 1994; Bermadinger-Stabentheiner 1995).

Additionally, recrystallization of extracts from primary needles resulted in plate like structures. However, plates could never be observed on undisturbed needle surfaces though morphologically similar structures could sometimes be found on surface areas with a high degree of wax degradation. According to Barthlott et al. (1998) platelets are the most common wax crystalloids and are found in all major plant groups – they are often dominated by primary alcohols. Primary alcohols are minor constituents of the wax of *P. canariensis* but there are no significant differences between primary and secondary needles that can explain the differences in recrystallization. These differences may possibly reflect differences between epi- and intracuticular waxes that have been recently demonstrated by selective physical removal and analysis of epicuticular wax, followed by analysis of remaining intracuticular wax (Ensikat et al. 2000; Jetter et al. 2000; Jetter & Schäfer 2001). This question will be part of further investigations.

In stress physiological investigations no distinct oxidative stress even on dry sites could be detected indicating that *P. canariensis* is well adapted to this environment dominated by light and water stress (Jiménez et al. 1997; Morales et al. 1999; Tausz et al. 2001). Needle characteristics certainly play an important part in this adaptation.

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