

Surface Structure and Chemical Composition of Leaf Waxes from *Quercus robur* L., *Acer pseudoplatanus* L. and *Juglans regia* L.

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Dedicated to Professor Wilhelm Menke on the occasion of his 80th birthday

Quercus robur, *Acer pseudoplatanus*, *Juglans regia*, Waxes, Surface Structure

The leaves of three deciduous broadleaf trees namely *Quercus robur* L. (oak), *Acer pseudoplatanus* L. (maple) and *Juglans regia* L. (walnut) have different wax surface structures with different chemical compositions. On both the upper and lower side of the oak leaf, crystalloid platelets with sharp fringed edges were observed. Maple and walnut leaf surfaces showed quite different crystalloid arrangements on the upper and the lower side of the leaves. On the lower side of the maple leaf crystalloid platelets in form of lumps were found, whereas the upper side showed no crystalloids. Walnut leaves exhibited dense platelets with rounded edges on the upper side but not on the lower side.

Introduction

All aerial organs of higher plants are covered primarily with a thin continuous wax layer. These waxes consist of a complex mixture of homologous series of very long chained lipids. Often triterpenoids were also present either free or esterified or in form of their ketones.

The surface waxes on the seed coats [1–3], petals, anthers [4] and young leaves [5, 6] had a layer of waxy or fluid consistency. The reason for this may be the melting point depression of the wax lipids resulting from the solubility of the different lipid classes or their individual components in each other. But in contrast to this, crystalloids with very different and manifold crystal structures were observed in mature leaves [1, 3, 7–13]. In the present paper an attempt was made to correlate the surface wax structures obtained by scanning electron microscope with the chemical composition of the leaf wax layers from the deciduous broadleaved trees of *Quercus robur*, *Acer pseudoplatanus* and *Juglans regia*.

Materials and Methods

Leaves of deciduous broadleaf trees were harvested in July from *Q. robur* L., *A. pseudoplatanus* L. and *J. regia* L., cultivated in the garden of the

Botany Institute of the University of Cologne. The extraction and chemical analysis of these leaf waxes were described recently [14–16]. The patterns for each tree in Fig. 4 were described in peak area percent of the gas chromatograms. Fresh and air-dried leaves were prepared for scanning electron microscopy by sputtering them with gold using a Semcoat sputter coater (A 1231) and examined in a Hitachi scanning electron microscope (S-405 A).

Results and Discussion

Scanning electron microscope pictures from the leaf surfaces showed structures of the wax layers which were differing from tree to tree. Moreover, on the upper or the lower leaf sides wax structures differing in form and arrangement were observed. Oak leaves have nearly the same crystalline surface structures on the upper and lower side (Fig. 1, A–D). Maple leaves were found to contain crystalloid structures only on the lower leaf side but not on the upper side (Fig. 2, A–D). Walnut leaves show again different upper and lower surface structures. In this case only the upper leaf side has dense crystalline wax platelets (Fig. 3, A–D).

The three broadleaf trees studied in the present investigation showed different qualitative and quantitative leaf wax lipid compositions and therefore different arrangements in the wax surface structures. The distribution patterns of the wax lipids are summarized in Fig. 4.

Q. robur leaf surface waxes were isolated in amounts of 1.3% of dry weight with a wax layer of

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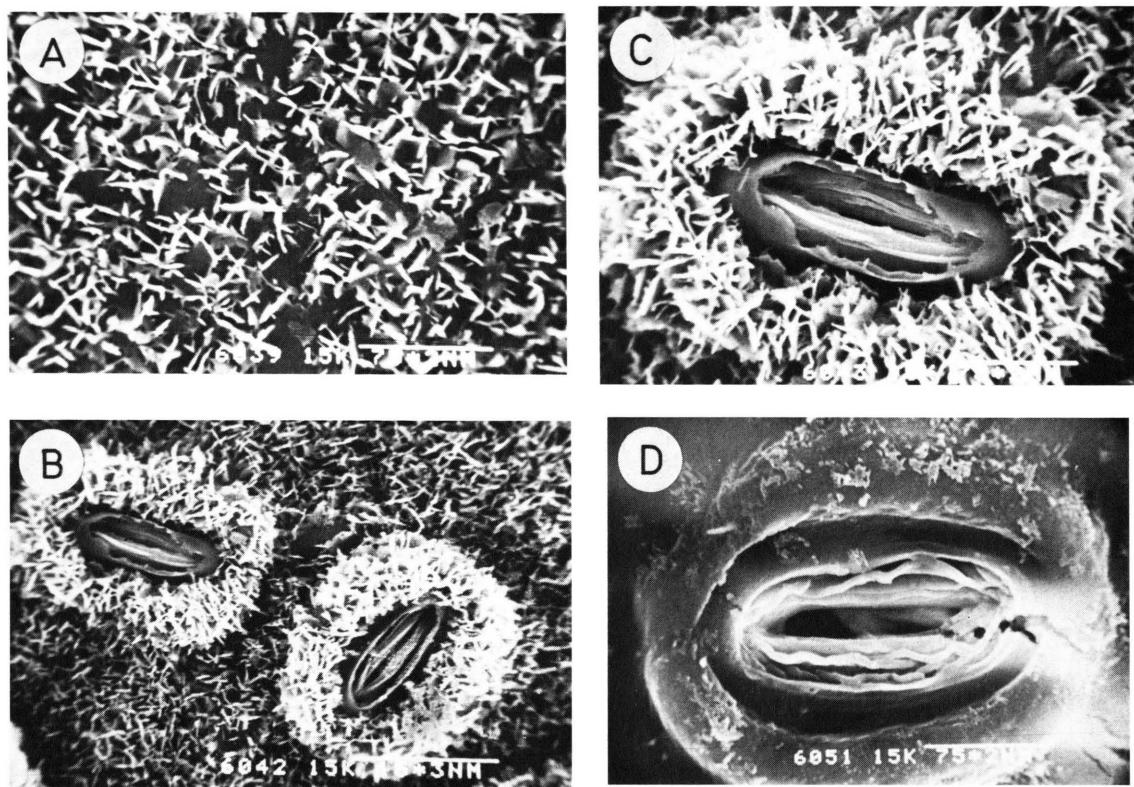


Fig. 1. *Quercus robur* leaf surface. A, upper leaf side, crystalloid platelets are distributed continuously, arising from a fluid wax layer. Bar = 15 μ m. B, lower leaf side, dense crystalloid platelets are observed similar as on the upper side, but with a concentrated orientation around the stomata. Bar = 45 μ m. C, lower leaf side, similar to B, but in a magnification like A. Bar = 15 μ m. D, lower leaf side, the wax layer was extracted with CHCl_3 . Bar = 15 μ m.

59 $\mu\text{g}/\text{cm}^2$ leaf area. These waxes consisted of hydrocarbons (6.4% wax), wax esters (1.1%), aldehydes (38.8%), alcohols (36%), fatty acids (6.1%) and triterpenoids (Fig. 4). Taraxerol, β -amyrin, α -amyrin and lupeol were found free (3.6%) and esterified (0.5%) with long chain fatty acids. The homologous series of aldehydes and alcohols with steep distribution patterns were dominating in this leaf wax [14]. These chemical wax compositions resulted in a continuous wax layer of dense crystalloid platelets with irregular sharp fringed edges arising from a fluid wax layer, both on the upper and lower side of the oak leaf. Around the stomata on the lower side a defined orientation of these platelets was observed (Fig. 1, A–D).

A. pseudoplatanus leaf surface waxes were extracted in amounts of 1% of dry weight with a wax layer of 34 $\mu\text{g}/\text{cm}^2$ of leaf area. These waxes con-

sisted of hydrocarbons (6.9% wax), wax esters (5.5%), benzyl acyl esters (2.1%), aldehydes (38.1%), alcohols (10.2%), fatty acids (17.1%) and triterpenoids (19.3%). β -Sitosterol, β -amyrin and 24-methylene-cycloartenol were found predominantly in form of their acetates in considerable amounts (14.4%). β -Amyrin was observed also free (4.9%) and esterified with long chain fatty acids (~0.7%) (Fig. 4). The aldehydes are the dominating lipid class in the maple leaf wax [15]. The upper side of the maple leaf shows a fluid consistency without any crystals, but with very dense arrangements of cuticular folds (Fig. 2, C–D). The lower maple leaf side has a continuous wax layer with numerous crystalloid lumps arising from the wax layer. The single lumps consisted of a very dense arrangement of platelets which also covered the stomata (Fig. 2, A–B). The upper and

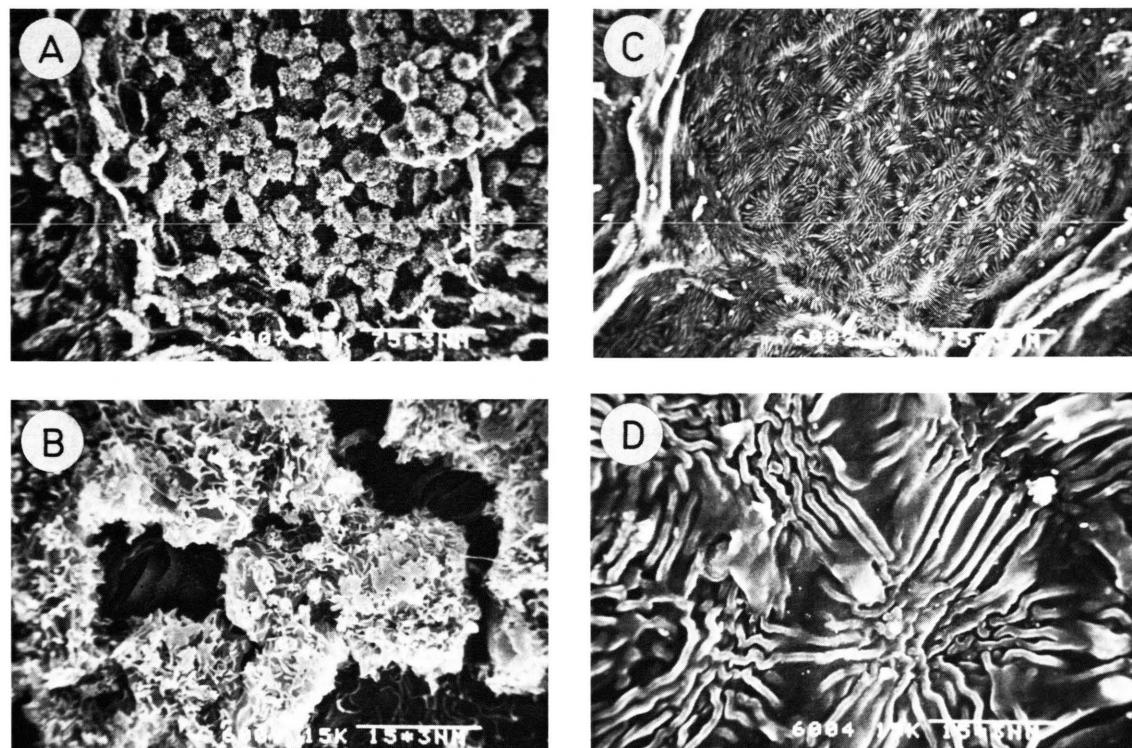


Fig. 2. *Acer pseudoplatanus* leaf surface. A, lower leaf side, crystalloid lumps arising from a fluid wax layer with irregular distribution. Bar = 225 µm. B, lower leaf side, the crystalloid platelets in form of lumps are arranged in a dense and concentrated manner around stomata. Bar = 45 µm. C, upper leaf side, the epidermal cells show a very close and dense arrangement of cuticular folds. Bar = 225 µm. D, upper leaf side, magnification of C, the waxes are continuously distributed as a fluid layer without crystalline structure over the epidermal cells. Bar = 45 µm.

the lower maple leaf surface showed a quite different structure. Therefore, it seemed possible that the two leaf sides of maple had each a different wax lipid composition and that the analyzed wax lipids were a mixture or an average of these two leaf wax layers.

J. regia leaf surface waxes were found in amounts of 1.64% of dry weight with a wax layer of 69 µg/cm² of leaf area. These waxes consisted of hydrocarbons (3.0% wax), wax esters (3.5%), aldehydes (5.5%), alcohols (41.6%), fatty acids (8.4%) and juglone (29.8%) (Fig. 4). The presence of juglone in very high amounts was unusual in this walnut leaf wax. The homologous alcohols in a steep distribution pattern were the main wax lipids [16].

The upper and the lower sides of walnut leaves showed again a quite different surface structure

but in a manner opposite to that of maple leaves. The lower side of a walnut leaf with the stomata has a continuous wax layer of fluid consistency. Only few single and isolated crystals were seen in some parts of the leaf surface (Fig. 3, A–B). The upper side of a walnut leaf contained very dense packed platelets with rounded edges distributed all over the upper surface (Fig. 3, C–D). Again in this case the leaf wax surface of both sides may have a different chemical wax composition. The higher concentration of alcohols, a relatively polar lipid, on the upper side of the leaf might have resulted in the wax platelets on this leaf side.

All wax analyses clearly demonstrate, that the plant kingdom shows a considerable diversity concerning the composition of epicuticular leaf wax layers. The trees studied have species-specific wax components differing in number, amount and

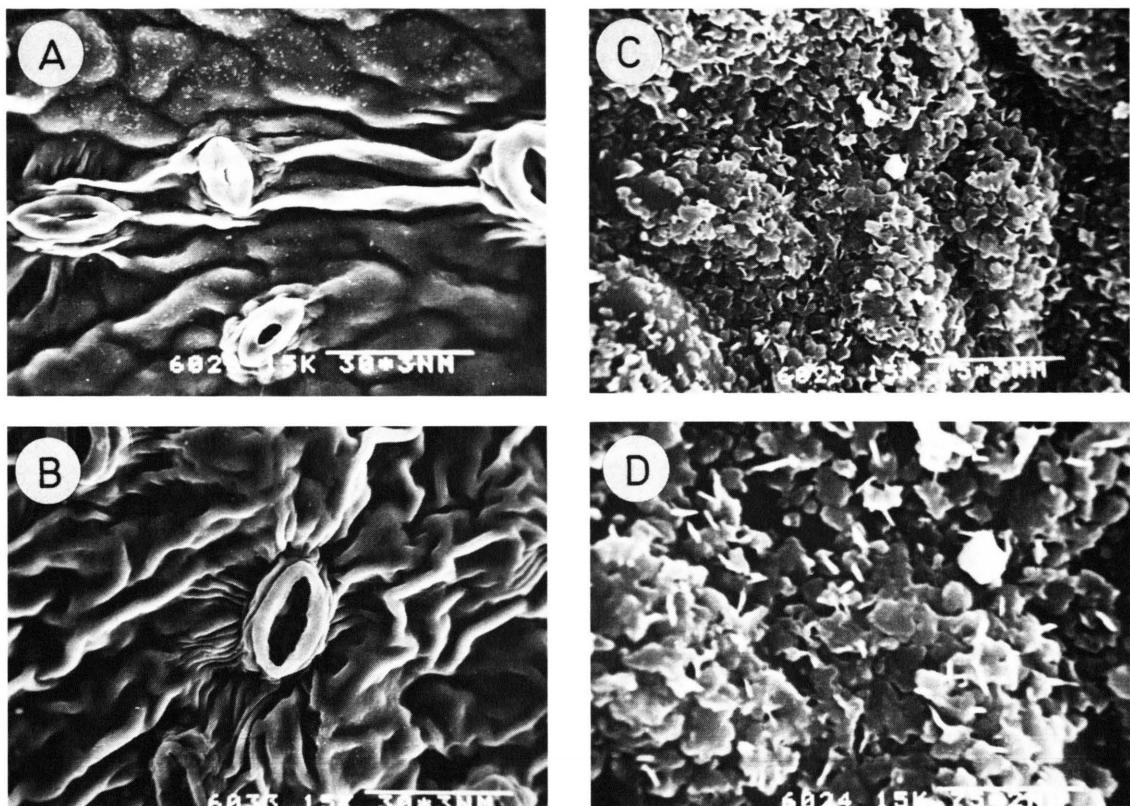


Fig. 3. *Juglans regia* leaf surface. A, lower leaf side, the wax layer shows a fluid consistency with only very little crystals, scattered on the surface. Bar = 30 µm. B, lower leaf side, the wax layer extracted with CHCl_3 , the folded epidermal cells and a stoma is clearly visible. Bar = 90 µm. C, upper leaf side, very dense packed crystalline platelets all over the surface are seen. Bar = 45 µm. D, upper leaf side, magnification of C shows the complex arrangement of the crystalloids with rounded edges. Bar = 15 µm.

their patterns of the homologous series of lipids in different surface structures. Each of three broad-leaf trees studied exhibit a different arrangement of leaf wax surface structures on the upper and the lower leaf side. Nevertheless, these different and species-specific wax compositions have only the one and same function: to form a nearly impermeable cuticular membrane.

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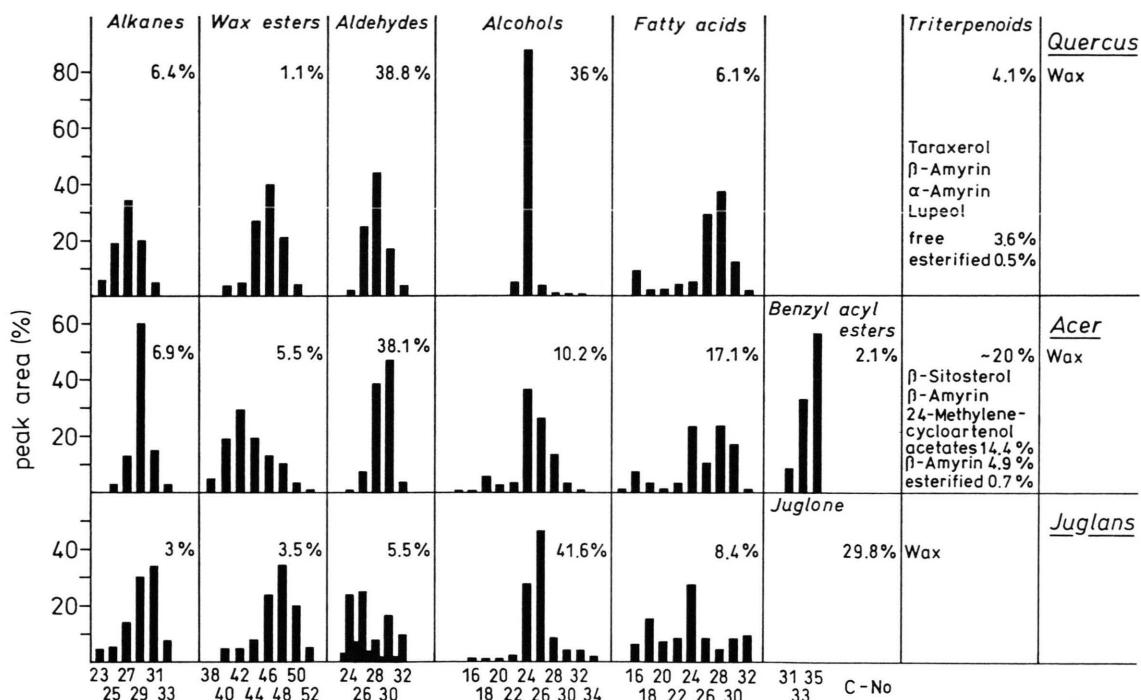


Fig. 4. Composition and distribution patterns of epicuticular waxes from leaves of the trees *Q. robur*, *A. pseudoplatanus* and *J. regia*.

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