Chemistry and Morphology of Epicuticular Waxes from Various Organs of *Jojoba* (*Simmondsia chinensis* [Link] Schneider)

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The surface of leaves and seed coats of *Jojoba* is covered with similar amounts of epicuticular waxes per surface area. The composition of these waxes was analysed by chemical methods, whereas their microscopic structures *in situ* were revealed by scanning electron microscopy. Differences in the chemical structure and composition of the complex mixtures cause differences in the morphological appearance of wax on the surface of both organs. The predominance of saturated and long chain components in leaf wax results in a crystalline wax layer on this organ. In contrast, the seed coat carries a cover of non-crystalline fluid consistency. The lower melting point of this wax mixture is caused by shorter chain and unsaturated compounds predominating on this organ.

Introduction

The compositions of epicuticular waxes from the surface of aerial parts of land plants differ from species to species [1]. In addition organ specific wax compositions are observed [2–7]. The chemical nature and composition of the epicuticular waxes correlate to the morphological structure of the plant surface and imply a liquid or solid coverage. The aerial surfaces of all higher plants carry a continuous thin layer of amorphous wax. These amorphous layers are frequently superimposed by crystalline formations. Several crystalline structures like plates, tubes, rods, riblons, needles, filiaments, dendrites etc. are observed on leaves [8–13]. Environmental conditions can modify the crystalline wax structures [14]. The following study shows epicuticular wax structures obtained by scanning electron microscopy in correlation to the chemial composition of wax components from *Jojoba* leaves and seed coats.

Materials and Methods

Jojoba fruits, leaves and stems for this study were the same air dried materials as described earlier [15, 16]. The native *Jojoba* materials were directly sputtered with gold (about 600 Å) and then brought in the scanning electron microscope, Philips PSME 500. The pictures were shooting with a polaroid camera.

The extraction of epicuticular waxes was carried out with chloroform for leaves and stems [16] and with hexane for pericarp and seed coats [15].

Results and Discussion

The crystalline fine structure of plant epicuticular waxes as shown by scanning electron microscopy (SEM) is often discussed as being a function of the preparation procedures [17]. Because of this uncertainty, we have applied several shadowing methods. First, the air dried plant material of *Jojoba* was directly sputtered with gold to stabilize the original epicuticular wax layer at room temperature. These preparation gave SEM micrographs with reproducible surface structures. *Jojoba* leaves (Figs. 1, 3, 4) and stems (Fig. 5) were densely covered by thin wax plates with fringed edges while the seed coats (Fig. 7, 9) and pericarp (Fig. 6) showed a liquid wax layer.

In addition we have rapidly frozen the plant samples by immersion in liquid nitrogen (minus 196 °C) followed by sputtering with gold. By this method we could observe arteficial crystals,

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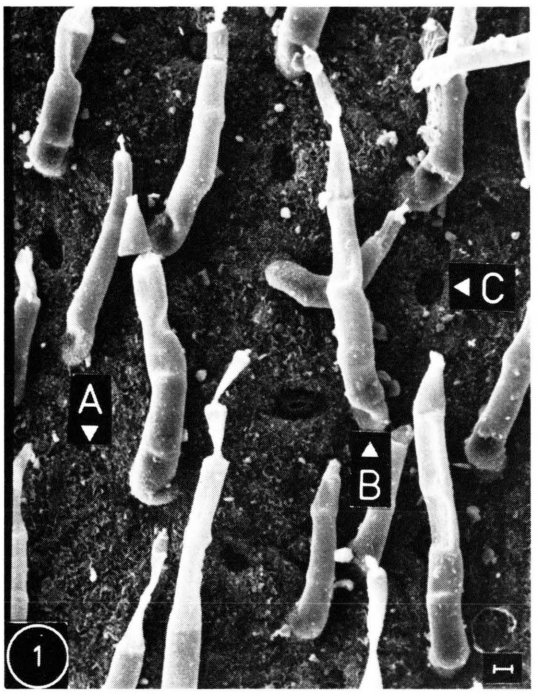

Fig. 1. *Jojoba* leaf surface, dence coverage with (A) thin plates with fringed edges, (B) many long trichomes and (C) stomata. Bar = 10 µm.

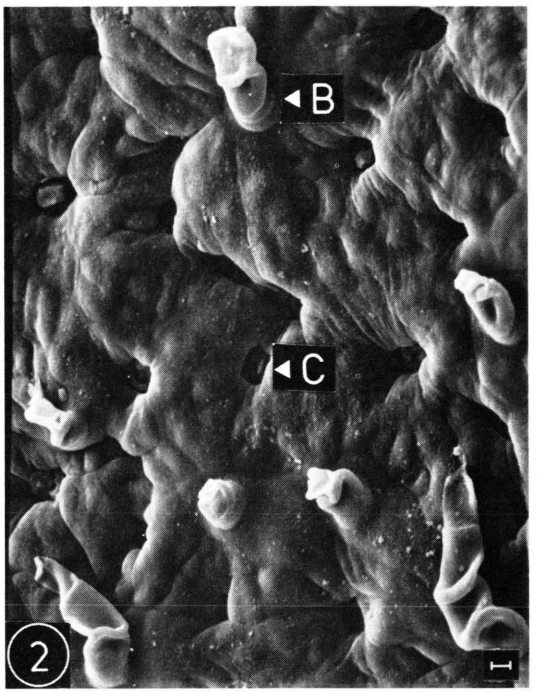

Fig. 2. *Jojoba* leaf surface extracted with chloroform, all epicuticular wax plates are washed up. Bar = 10 µm.

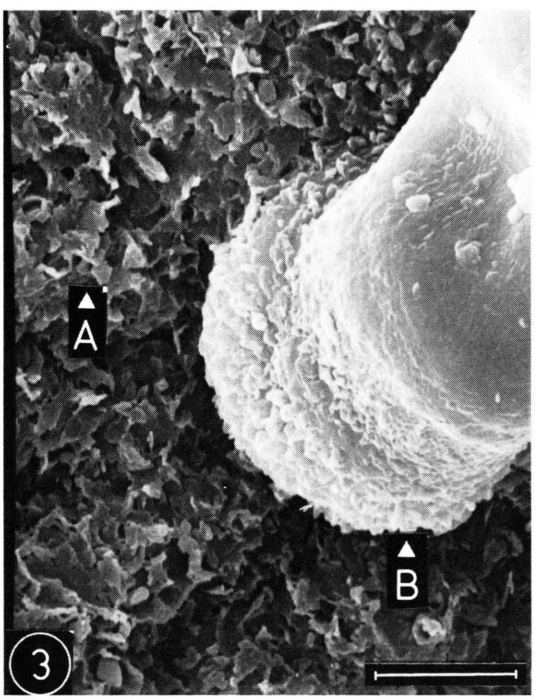

Fig. 3. *Jojoba* leaf surface with the basis of a trichome (B) and dense irregular plate structures of epicuticular waxes (A). Bar = 10 µm.

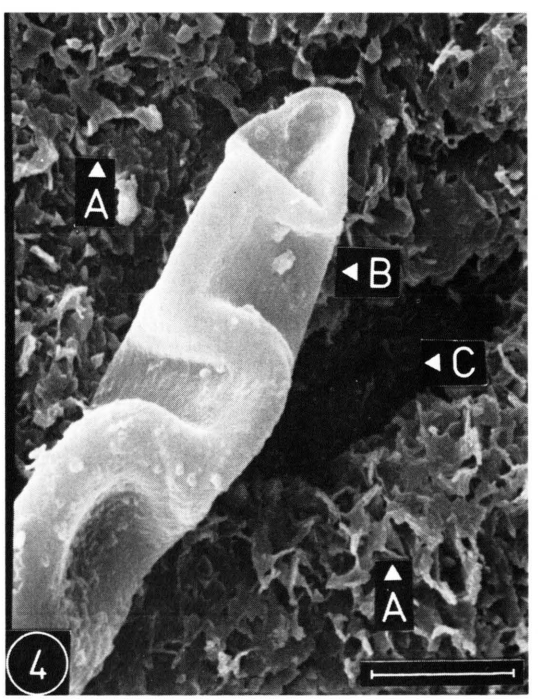

Fig. 4. *Jojoba* leaf surface with the top of a trichome (B) and a stomata (C). Bar = 10 µm.

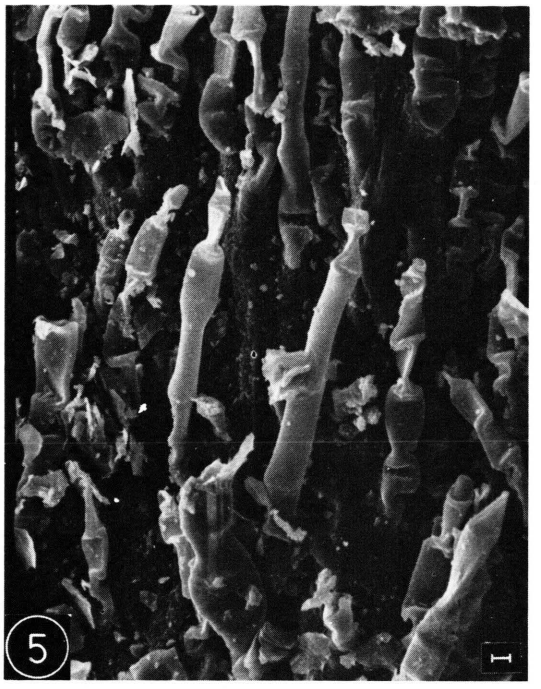

Fig. 5. *Jojoba* stem with many trichomes and irregular wax plates. Bar = 10 μm.

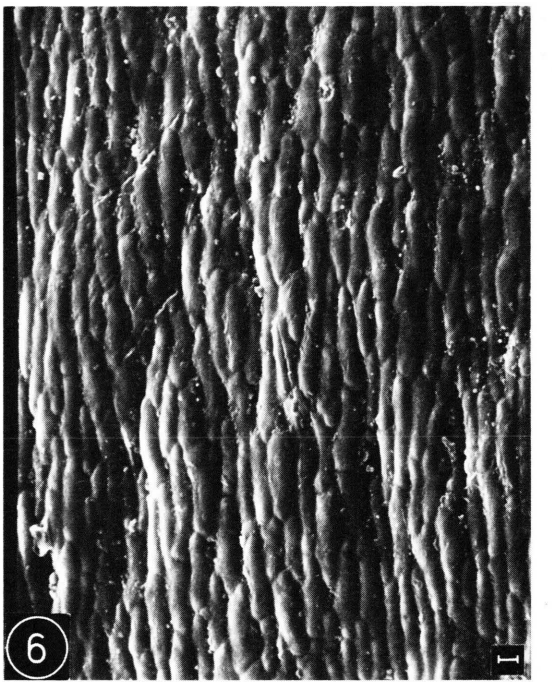

Fig. 6. *Jojoba* pericarp with a liquid wax layer. Bar = 10 μm.

crystallising out from the originally fluid and waxy *Jojoba* seed coat surface (Fig. 8) as a consequence of the applied low temperatures. This second preparation method was not suitable and therefore not used for this study. The SEM studies of the morphology of epicuticular waxes from numerous species of Angiosperms (5000 plants) by Barthlott and Wollenweber [9, 10] are also based on fresh or air dried plant material directly sputtering with gold or platin.

The SEM micrographs demonstrate that the surface of *Jojoba* leaf is covered with a dense layer of crystalline wax plates having irregular fringed edges and numerous long trichomes with meanderlike folded walls (Figs. 1, 3, 4). The wax plates could be completely removed with chloroform, exposing the outer surface of epidermal cell walls (Fig. 2). The composition and patterns of the extracted epicuticular waxes are shown in Fig. 11 [16]. *Jojoba* stems have a similar surface structure, dense wax plates and numerous trichomes.

The surface structure of a *Jojoba* seed coat is quite different [18]. The epidermal layer is composed of long macrosclerides (papillae) and covered with a mat of trichomes. The wax layer is of liquid consistency, no crystalline fine structures could be observed (Figs. 7, 9) at normal temperature. The liquid wax layer was removed already with hexane (Figs. 10) [15]. The surface structure of pericarp (Fig. 6) showed also a liquid wax layer.

The different morphological wax structures on the surface of *Jojoba* leaves and seed coats should be primarily a function of the chemical nature, the composition and distribution patterns of the epicuticular waxes (Fig. 11). Each *Jojoba* organ has characteristic and specific wax components and patterns. The crystalline wax fine structure of leaves results from a predominance of long chained and saturated wax components, especially wax esters, free fatty acids and alcohols. The liquid wax layer of seed coats, on the other hand, results from the presence of many unsaturated compounds (wax esters, fatty acids), branched alkanes and a shift to shorter chain lengths for the individual components (alkanes, wax esters, aldehydes, fatty acids and alcohols) in contrast to leaf wax components. Acetates and specific esters are found only on leaves, on seed coats sterols are found. Thus at

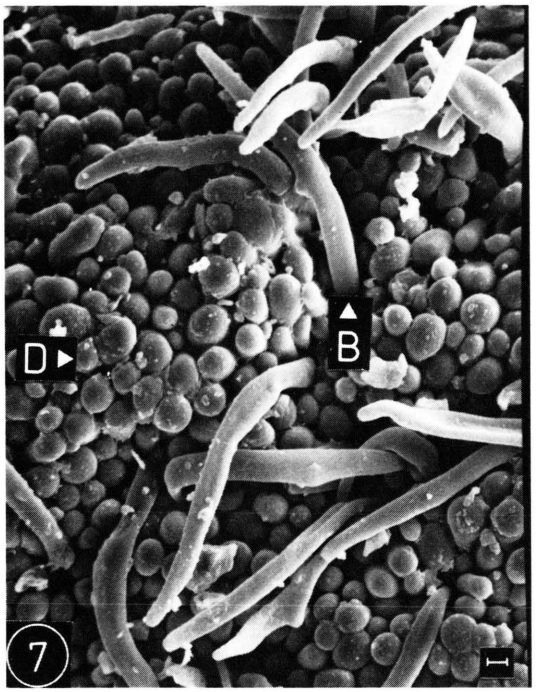

Fig. 7. *Jojoba* seed coat surface with many papillae (D) and trichomes (B). Bar = 10 µm.

Fig. 8. *Jojoba* seed coat surface treated with fluid nitrogen, arteficial wax crystals. Bar = 10 µm.

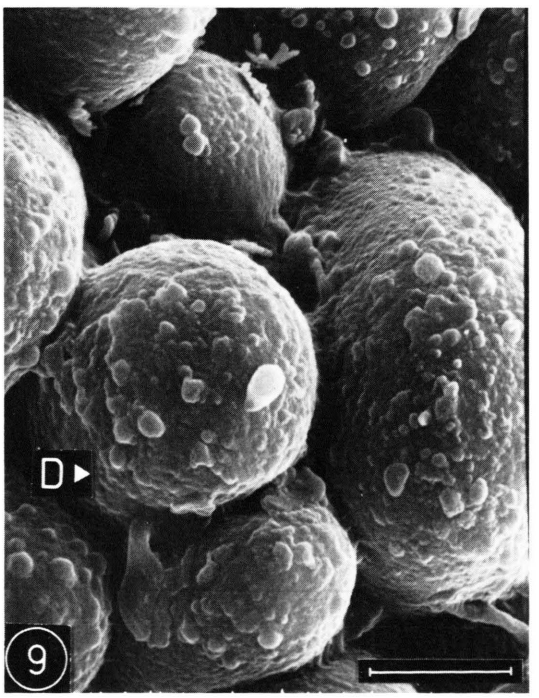

Fig. 9. *Jojoba* seed coat surface, papillae (D) are covered with a liquid epicuticular wax layer. Bar = 10 µm.

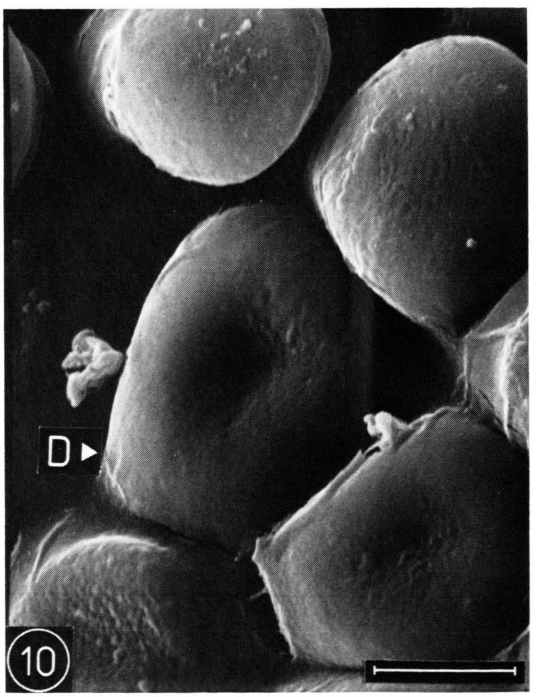

Fig. 10. *Jojoba* seed coat surface extracted with hexane, all epicuticular waxes are washed up. Bar = 10 µm.

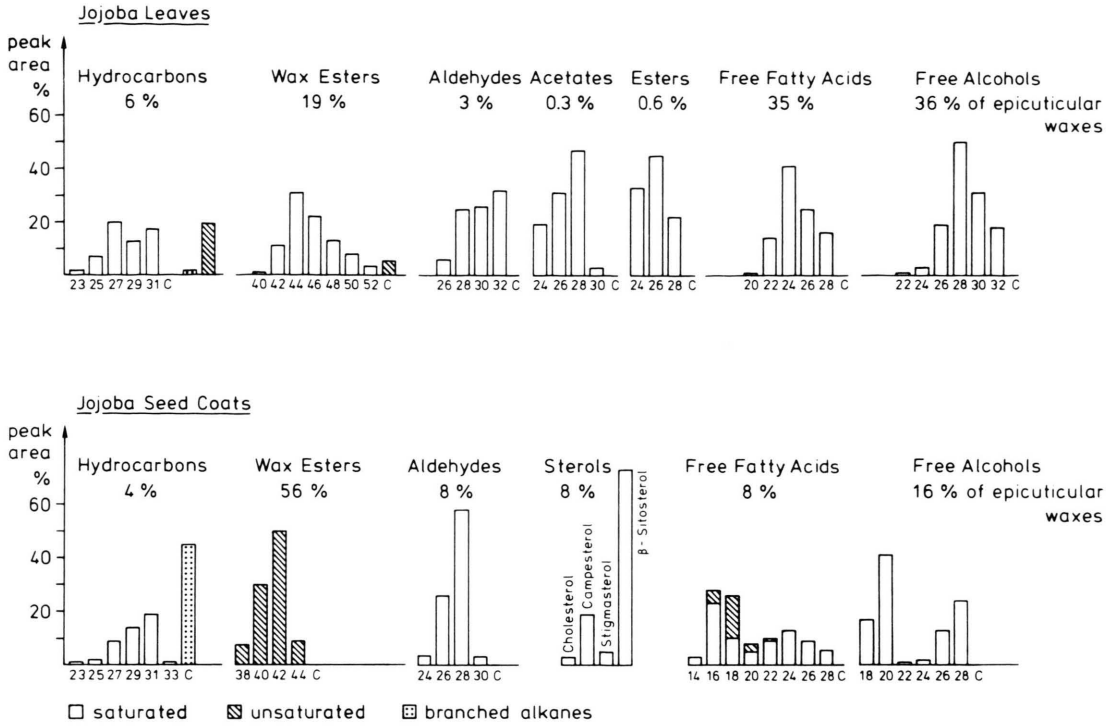

Fig. 11. Composition and distribution patterns of epicuticular waxes from *Jojoba* leaves and seed coats.

normal temperature, the specific chemical nature and composition of epicuticular waxes from *Jojoba* leaf result in a crystalline fine structure of these waxes (plates) on the epidermal cells of this organ. On the other hand, epicuticular waxes from *Jojoba* seed coat consist of numerous unsaturated and branched compounds, and most of the components are of shorter chain length than those from leaves. The chemical nature and compositions of these substances cause a melting point depression of the wax mixture with the consequence of a liquid wax layer on the seed coat.

The amounts of epicuticular waxes from *Jojoba* leaves are 0.34% of dry weight and from seed coats 0.03% of dry weight. Calculated per surface area of leaf or seed coat, the proportions of epicuticular waxes are in the same dimensions, 0.079 mg waxes/cm^2 for leaf and 0.057 mg waxes/cm^2 for seed coat. Different *Jojoba* organs are covered with similar amounts of wax per surface area but due to differences in composition these wax coats differ in their morphological structure.

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