Comparative investigation of the chemical composition and the water permeability of fruit and leaf cuticles

Vergleichende Untersuchung zur chemischen Zusammensetzung und zur Wasserpermeabilität der Kutikula von Früchten und Blättern

Doctoral thesis for a doctoral degree
at the Graduate School of Life Sciences,
Julius-Maximilians-Universität Würzburg,
Section Integrative Biology

submitted by

Hua Huang
from
Yibin, Sichuan, China

Würzburg 2017

Submitted on: ……………………………………………………………

Office stamp

Members of the *Promotionskomitee*:

Chairperson: Prof. Dr. Christian Janzen

Primary Supervisor: Prof. Dr. Markus Riederer

Supervisor (Second): Prof. Dr. Thomas Müller

Supervisor (Third): Dr. Adrian Friedmann

Supervisor (Fourth): Dr. Markus Burghardt

Date of Public Defence: …………………………………………….…

Date of Receipt of Certificates: ………………………………………

Table of contents

Table of contents I
Summary V
Zusammenfassung VII
Introduction 1
1 Composition and structure 2
1.1 Cutin polymers 3
1.2 Cuticular waxes 4
1.3 Other lipid barrier related constituents 6
2 Biosynthesis and regulation 7
3 Cuticle structure and barrier properties 9
3.1 Effect of wax load and cuticle thickness 9
3.2 Effect of cuticular components 10
3.3 Effect of environment factors 13
3.3.1 Temperature 13
3.3.2 Hydration (humidity) 14
3.3.3 Water deficit 15
3.4 Mechanical properties of the cuticle 16
4 Motivations and aims of the present work 18
Materials and methods 21
1 Plant material, growth conditions, fruit and leaf harvesting 21
2 Fruit and leaf characteristics 22
2.1 Saturation of fruit and leaf samples 22
2.2 Determination of surface area 22
2.3 Scanning electronic microcopy 23
3 Characterization of cuticular transpiration 23
4 Measurement of the minimum conductance 25
5 Chemical analysis of the cuticular components 26
5.1 Isolation of cuticular membranes 26
5.2 Cuticular wax extraction for gas chromatographic analysis 26
5.3 Cutin depolymerization for gas chromatographic analysis 27

5.4 Chemical analysis by gas chromatography 28
6 Statistical analysis 29
Results 30
1 Cuticular permeance and minimum conductance 30
2 Chemical analysis of cuticular waxes from fruits and leaves 33
2.1 Cuticular waxes of *Olea europaea* L. cv. 'Arbequina' 38
2.1.1 Cuticular waxes of ripe fruits and fully expanded leaves 38
2.1.2 Cuticular waxes of fruit in different developmental stages 44
2.2 Cuticular waxes of *Olea europaea* subsp. *europaea* var. *sylvestris* 48
2.3 Cuticular waxes of *Ligustrum vulgare* L. 52
2.4 Cuticular waxes of *Averrhoa carambola* L. 56
2.5 Cuticular waxes of *Coffea arabica* L. 60
2.6 Cuticular waxes of *Crataegus pedicellata* Sarg. 64
2.7 Cuticular waxes of *Malus domestica* L. cv. 'Topaz' 69
2.8 Cuticular waxes of *Prunus avium* L. 74
2.9 Cuticular waxes of *Prunus cerasifera* Ehrh. 78
2.10 Cuticular waxes of *Prunus domestica* L. subsp. *syriaca* Janich. 82
2.11 Cuticular waxes of *Prunus domestica* subsp. *insititia* (L.) 86
2.12 Cuticular waxes of *Prunus persica* L. 90
2.13 Cuticular waxes of *Vitis vinifera* L. cv. 'Nelly' 94
2.14 Cuticular waxes of *Vitis vinifera* L. cv. 'Silvana' 98
2.15 Cuticular waxes of *Cornus officinalis* Siebold & Zucc. 102
2.16 Cuticular waxes of *Ficus carica* L. 106
2.17 Cuticular waxes of *Capsicum annuum* L. cv. 'Kalocsai' 110
3 Morphological characteristics of fruit and leaf surfaces 114
Discussion 116
1 The transpiration barrier properties of fruits and leaves 116
2 Wax compositions of fruits and leaves 120
3 Cuticular waxes and the transpiration barrier properties 124
3.1 Correlations between wax load and transpiration barrier properties 125
3.2 Effect of cyclic waxes on the transpiration barrier properties 129
3.3 Effect of aliphatic waxes on the transpiration barrier properties 130
4 Cuticular waxes and the mechanical properties of cuticle 137

4.1 Attribution of wax load to the integrity of cuticle 137
4.2 Attribution of cyclic waxes to the mechanical properties of cuticle 139
4.3 Attribution of aliphatic waxes to the mechanical properties of cuticle..........140
5 Conclusions and Outlook.. 142
References.. 144
Annex 162
Acknowledgements 180
Publications and Presentations 181
Curriculum Vitae 182
Affidavit... 183
Eidesstattliche Erklärung 183

Summary

The plant cuticle is a continuous extracellular protective layer covering the outermost surfaces of higher plants that are in contact with the surrounding atmosphere. The primary function of the cuticular lipid membrane, which is mainly composed of biopolymer cutin and cuticular waxes, is to protect the plant organs against uncontrolled water loss. The chemical composition and the biophysical properties of cuticular waxes affect the rate of water diffusion across the cuticle. Fruit transpiration plays an important role in the development and the maintenance of fruit quality. The fruit has been suggested to present better dehydration stress tolerance than the leaf. However, the differences in transpiration and the chemical composition of cuticular waxes between fruit and leaf have yet to be comprehensively investigated.

The present study aims to investigate the water permeability and cuticular wax composition of fruit and leaf cuticles of a wide range of plant species and to elucidate the different roles of the cuticular wax components in the transpiration barrier. To address these objectives, fruit and leaf samples from 17 species were investigated. The cuticular transpiration of intact fruits and astomatous adaxial leaf surfaces and the minimum leaf conductance obtained by leaf drying curves for intact leaves were gravimetrically determined for a variety of plant species. The chemical composition of cuticular waxes of fruits and leaves was thoroughly analysed by gas chromatography with flame ionization and mass spectrometry.

The water permeability of fruits ranged from 3.7 x 10^{-5} m s^{-1} (*Prunus domestica* subsp. *syriaca*) to 37.4 x 10^{-5} m s^{-1} (*Coffea arabica*), whereas permeability for leaves varied between 1.6 x 10^{-5} m s^{-1} (*Cornus officinalis*) and 4.5 x 10^{-5} m s^{-1} (*Prunus domestica* subsp. *syriaca* (L.)). The interspecies range of water permeability of fruits was significantly higher than that of leaves. Chemical analyses of the cuticular waxes demonstrated that fatty acids, primary alcohols, *n*-alkanes, aldehydes and alkyl esters were the predominant very-long-chain aliphatic compound classes of fruit and leaf surfaces. Sterols, such as β-sitosterol and campesterol, and triterpenoids, such as oleanolic acid, ursolic acid, α-amyrin and ß-amyrin, were the major cyclic compound classes in the cuticular wax membrane.

The amount and composition of cuticular waxes of both fruits and leaves varied at an intraspecific level. There were no significant correlations between the total cuticular wax load or the individual cuticular wax composition and the water permeability of fruits

or leaves independently or together. After combining the fruit and leaf data set, a significant correlation between the average chain length of very-long-chain aliphatic compounds and permeabilities was detected, i.e. the longer the average chain length, the lower the water permeability.

Interestingly, *n*-Nonacosane (C_{29}) was abundantly detected in fruit waxes of Rosaceae species. These fruits exhibited a relatively low transpiration level, which was very close to their leaf cuticular permeability. The present study suggests that the lower cuticular permeability of leaves, in comparison to that of fruits, may be attributed to the longer average chain length of aliphatic compounds. The accumulation of total wax, triterpenoids and aliphatic compounds may not contribute to the transpiration barrier directly. The present results are highly consistent with the previous model assumptions for the cuticular structure and transport barrier. Furthermore, this comparative study on leaf and fruit cuticles provides further insights linking the cuticular wax chemistry to the physiological properties of the plant cuticle.

Zusammenfassung

Die pflanzliche Kutikula ist eine kontinuierliche extrazelluläre Schutzschicht, welche die oberirdischen primären Abschlussgewebe höherer Pflanzen bedeckt, die in Kontakt mit der umgebenden Atmosphäre stehen. Die primäre Funktion der lipophilen Kutikularmembran, die hauptsächlich aus dem Biopolymer Kutin und kutikulären Wachsen aufgebaut ist, besteht darin, die Pflanzenorgane vor unkontrolliertem Wasserverlust zu schützen. Die chemische Zusammensetzung und die biophysikalischen Eigenschaften von kutikulären Wachsen beeinflussen weitgehend die Geschwindigkeit der Wasserdiffusion über die Kutikula. Die Transpiration von Früchten spielt eine wichtige Rolle in der Ausbildung und Beständigkeit von Fruchtqualitätsmerkmalen. Unterschiede in der Transpiration und der chemischen Zusammensetzung der kutikulären Wachse zwischen Frucht und Blatt sollten untersucht werden.

Die vorliegende Studie zielt darauf ab, die Wasserpermeabilität und die kutikuläre Wachszusammensetzung von Früchten und Blättern aus einem breiten Spektrum von Pflanzenarten zu untersuchen und die verschiedenen Rollen der kutikulären Wachskomponenten in den Transpirationsbarriereeigenschaften aufzuklären. Um diesen Zielen näherzukommen, wurden Frucht- und Blattproben von 17 Arten untersucht. Die kutikuläre Transpiration von intakten Früchten und astomatären adaxialen Blattoberflächen ausgewählter Arten sowie der minimale Leitwert von deren Blättern, ermittelt durch Austrocknungskurven mit intakten Blättern, wurden gravimetrisch bestimmt. Die chemische Zusammensetzung der kutikulären Wachse von Früchten und Blättern wurde durch Gaschromatographie mit Flammenionisation und Massenspektrometrie nachgewiesen.

Die Wasserdurchlässigkeit von Früchten reichte von 3,7 x 10^{-5} m s^{-1} (*Prunus domestica* subsp. *syriaca*) bis 37,4 x 10^{-5} m s^{-1} (*Coffea arabica*), während die Werte für Blätter zwischen 1,6 x 10^{-5} m s^{-1} (*Cornus officinalis*) und 4,5 x 10^{-5} m s^{-1} variierten (*Prunus domestica* subsp. *syriaca*). Der interspezifische Vergleich der Wasserdurchlässigkeit von Früchten war deutlich höher als die der Blätter. Chemische Analysen der kutikulären Wachse zeigten, dass Fettsäuren, primäre Alkohole, *n*-Alkane, Aldehyde und Alkylester die häufigsten sehr langkettigen aliphatischen Verbindungsklassen für Früchte und Blätter waren. Sterole wie β-Sitosterol und Campesterol und Triterpenoide zum Beispiel Oleanolsäure, Ursolsäure, α-Amyrin und

ß-Amyrin, waren die wichtigsten zyklischen Verbindungsklassen in den kutikulären Wachsmischungen. Die Menge und Zusammensetzung der kutikulären Wachse, sowohl von Früchten als auch von Blättern, variierte auf intraspezifischer Ebene. Es waren keine signifikanten Korrelationen zwischen der Menge der kutikulären Wachsablagerung oder der kutikulären Wachszusammensetzung und der Wasserdurchlässigkeit von Frucht- und/oder Blattoberflächen zu erkennen. Wurden die Frucht- und Blattdatensätze zusammen untersucht, so war eine signifikante Korrelation zwischen der durchschnittlichen Kettenlänge von sehr langkettigen aliphatischen Verbindungen und der Permeabilität festzustellen, ging eine längere durchschnittliche Kettenlänge mit geringerer Wasserdurchlässigkeit einher.

Interessanterweise wurden große Mengen an *n*-Nonacosan in Fruchtwachsen der untersuchten Rosaceae-Arten nachgewiesen. Diese Früchte zeigten ein relativ niedriges Transpirationsniveau, das sehr nahe an der Permeabilität ihrer Blattkutikeln lag. Die vorliegende Studie liefert weitere Belege dafür, dass der im Allgemeinen niedrigere minimale Leitwert von Blättern auf die – im Vergleich zur Kutikula von Früchten – längere durchschnittliche Kettenlänge der aliphatischen Verbindungen zurückzuführen ist. Die Anhäufung von Gesamtwachs, Triterpenoiden oder aliphatischen Verbindungen trägt nicht direkt zur Transpirationsbarriere bei. Die vorliegenden Ergebnisse decken sich in hohem Maße mit den bisherigen Modellannahmen zur Struktur der Kutikula und der von ihr vermittelten Funktion als Transpirationsbarriere. Darüber hinaus gibt diese Vergleichsstudie über die Kutikula von Früchten und Blättern zahlreiche Einblicke, die dabei helfen können, die kutikuläre Wachschemie mit den physiologischen Eigenschaften der pflanzlichen Kutikula zu verknüpfen.

Introduction

The appearance of the first land pants occurs in the mid-Palaeozoic era between 480 and 360 million years ago (Kenrick and Crane, 1997; Wellman et al., 2003). To adapt to the desiccation of territorialised habitats the outermost epidermis layer developed a hydrophobic skin, the cuticle. This development can be interpreted as a specialized lipid modification of epidermal cell wall in order to restrict dehydration (Yeats and Rose, 2013; Fernández and Khayet, 2015). Consequently, the extracellular cuticular membrane covering the outer surface of fruits, leaves, flowers, and other aerial primary plant organs is in continuous contact with the surrounding atmosphere. The occurrence of the cuticle is one of the pivotal developments during the land colonization of plant (Kenrick and Crane, 1997; Bateman et al., 1998).

The plant cuticle plays a dynamic and multifunctional role in protecting organs against biotic and abiotic stresses (Riederer and Müller, 2008; Barthlott et al., 2017). One of the important functions of the cuticle is the transport barrier against non-stomatal uncontrolled water loss from the interior tissues or foliar uptake (Riederer and Schreiber, 1995; Riederer and Schreiber, 2001). The cuticle also plays a role as an interface between the plant surface and the habitat environment. For instance, cuticular components play important roles in the penetration of fungi (Hansjakob et al., 2010) and the resistance of microbial infection (Serrano et al., 2015). The outermost surface cuticular crystals function in reflectance of ultraviolet (UV) radiation (Holmes and Keiller, 2002; Pfündel et al., 2008); sliding of insects (Gorb et al., 2005; Scholz et al., 2010); self-cleaning ('lotus effect'), and water repellence (Barthlott and Neinhuis, 1997; Neinhuis and Barthlott, 1997). The cuticle has also been found to provide a boundary to prevent organ fusion during development (Smirnova et al., 2013; Mazurek et al., 2017). Another key role of the cuticle is the biophysical properties (viscoelastic) that are involved in the maintenance of the structural integrity of fruit, leaf, and other organs during development e.g. fruit and leaf extension, fruit cracking, and leaf shrinkage (Matas et al., 2004; Bargel and Neinhuis, 2005; Edelmann et al., 2005; Ríos et al., 2015); or suffering stresses e.g. wind, rainfall (Bargel et al., 2006).

As the extracellular cuticular membrane covers the outer epidermis cells, the multiple physical and ecological functions have been thought largely attributed by their chemical compositions and structural arrangements in the cuticular layer.

1 Composition and structure

The plant cuticle is independent from the polysaccharide rich epidermis cell wall, which can be isolated by digestion with an enzyme solution. The plant cuticle is composed of an insoluble polymer matrix (cutin, dominated by C_{16} and C_{18} hydroxyl fatty acids and their derivatives) impregnated by and covered with solvent-soluble lipids, termed as ‘waxes’. The cutan, an aliphatic biopolymer that is highly resistant to degradation, has been demonstrated in drought-adapted plants (Boom et al., 2005). As the overlaying connection of epidermis cell wall and the cuticle, some polysaccharides and phenolics distribute or incorporate with cutciular compounds to be part of the cuticle components. The cuticle is a heterogeneous membrane. The fine developed cuticular layer can be chemically and structurally distinguished into two spatially distinct layers: the external cuticular layer covering the outer surface the internal cuticular layer connect to cell wall (Jeffree, 2006; Buschhaus and Jetter, 2011). The internal cuticular layer is composed of intracuticular waxes embedded within the cutin matrix as well as some polysaccharides and phenolics, while the external cuticular includes an epicuticular wax film or wax crystals mixture (Figure 1).

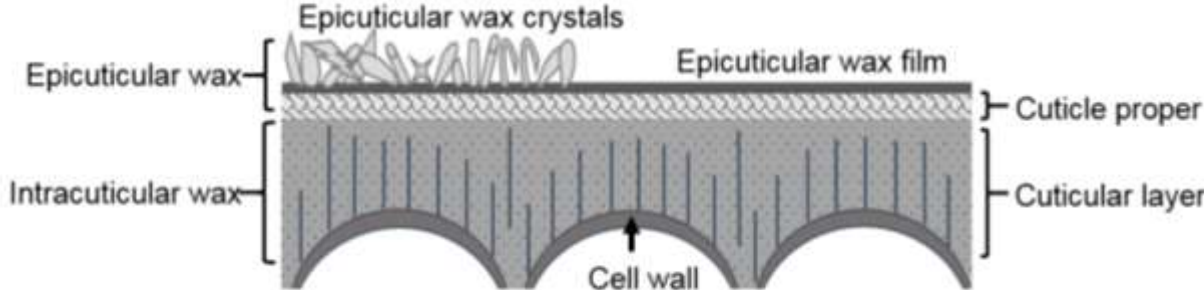

Figure 1. Schematic of the cross-section of plant cuticle for highlighting the main structural features (not drawn in scale). The plant cuticle is heterogeneous accumulation into various chemical fractions and different layers (according to Müller and Riederer, 2005; Jeffree, 2006).

1.1 Cutin polymers

The cutin polymers are predominantly composed of C_{16} and C_{18} fatty acids with midchain groups. The polymers typically contain a terminal hydroxyl group (ω-OH) with one or more midchain hydroxyl, epoxy and oxo groups (Table 1). Dicarboxylic acids with midchain hydroxyl groups, fatty acids, phenolics and glycerol exist in a small amount (Holloway, 1982; Beisson et al., 2012; Fich et al., 2016). In addition, fatty aldehydes, *n*-alkanes, and primary alcohols have been detected in some cases. However, whether these components are affiliated to cutin polymers or part of the non-extractable waxes remains uncertain.

Table 1. Typical cutin monomers and the major functional groups.

C_{16} family	C_{18} family
16-Hydroxyhexadecanoic acid	18-Hydroxyoctadec-9-enoic acid
10, 16-Dihydroxyhexadecanoic acid	10, 18-Dihydroxyoctadecanoic acid
16-Hydroxy-10-oxo-hexadecanoic acid	18-Hydroxy-9, 10-epoxy-octadecanoic acid
16-Hydroxy-10-oxo-hexadecanedioic acid	9, 10, 18-Trihydroxyoctadecanoic acid
Coumaric acid	Glycerol

Hydroxyl	Carboxyl	oxo	Epoxy
—OH	$-COOH$	O=	(epoxide ring)

The cutin monomer type varies across different species, organs and developmental stages (Holloway, 1982; Franke et al., 2005; Leide et al., 2007). The general monomer profiles are predominated by C_{16}, C_{18} or mixture of C_{16} and C_{18}. The structural properties are proposed based upon their functional group which primary form into ester cross-linking networks. The main molecular structure of cutin polymers are classified into three types: linear chain by esterified between terminal carboxylic and hydroxyl groups, dendritic structure esterified by the midchain hydroxyls and cross-linking between cutin chains by incorporation of glycerol esterified with dicarboxylic acid monomers (Fich et al., 2016). NMR studies have indicated that the esterification of mid-chain hydroxyls is rare cases and primarily primary hydroxyl groups are esterified (Deshmukh et al., 2003). Thus, the existence of glycerol, dicarboxylic acids, primary alcohols and free fatty acids provide the potential for various structural arrangements of cutin monomers. In addition, cutin polymers are covalently connected to polysaccharides to create tight associations between the cutin matrix and the epidermis cell wall (Fang et al., 2001).

1.2 Cuticular waxes

The cuticular waxes are complex mixtures of very-long-chain (VLC) fatty acids as well as their derivatives termed as ‘aliphatics’ and cyclic compounds (Jetter et al., 2008). The mixtures in one species can be up to 150 compounds including different compound classes as well as their homologous in each class (Leide, 2008). Overall, the main aliphatics are VLC fatty acids, primary alcohols, *n*-alkanes, aldehydes and alkyl esters (Table 2). Methyl esters, secondary alcohols, alcohol acetates, mono- & di-ketones and *n*-alkenes are detected in some cases. The carbon chain length of fatty acids, primary alcohols and aldehydes can be between C_{20} to C_{38} and are dominated by even-numbered chains. The odd numbered chains dominate for *n*-alkanes, secondary alcohols and ketones, which are usually distributed from C_{23} to C_{35}. The alkyl esters that esterified between fatty acids and primary alcohols are distinguished by a carbon chain length of C_{36} to C_{56}.

The non-aliphatic components are often detected as another main group of lipid compounds in cuticular waxes. The most common cyclics are pentacyclic triterpenoids, namely either oleane type with oleanolic acid, δ/β-amyrin, and erythrodiol; ursane type with ursolic acid, α-amyrin, and uvaol; and lupane type with lupeol, betulinic acid *etc.* Sterols, including β-sitosterol, campesterol, and stigmasterol *etc.*, are detected in a

small amount from different tissues. Other cyclic compounds, such as tocopherols and phenylmethyl esters, have also been detected in some cases (Jetter et al., 2008).

Table 2. Common cuticular wax components. Aliphatics and cyclics are the two main groups of cuticular wax. The major and typical chain length and cyclic compounds are shown.

Aliphatics	Cyclics
N-alkane (C_{29})	Oleanolic acid
Fatty acid (C_{28})	
Primary alcohol (C_{28})	Ursollic acid
Aldehyde (C_{28})	β-sitosterol
Alkyl ester (C_{42})	

The diversity of wax compositions varies largely across species, organs, ontogeny development and genetic background (Jetter and Schäffer, 2001; Leide et al., 2007; Jetter et al., 2008; Kosma et al., 2010; Szakiel et al., 2012). On the other hand, the growing environmental factors, such as humidity, temperature, light quality, water resources, and geographical locations have been detected to apparently shift the accumulation of cuticular wax amount but not affect the compositions for the same species (Riederer and Schneider, 1990; Grammatikopoulos et al., 1998; Koch et al., 2006; Leide, 2008; Kosma et al., 2009; Szakiel et al., 2012).

The cuticular waxes are constituted structurally and chemically distinct in regard to plant cell expansion and wax accumulation by two continuous layers: the intra- and the epicuticular wax layer (Jetter et al., 2008; Buschhaus and Jetter, 2011). It has been

demonstrated by microscopic (Jeffree, 1996; Barthlott et al., 1998) and selective removal of epi- and intracuticular waxes (Jetter et al., 2000; Jetter and Schäffer, 2001).

Thus, it must be noted that the previous reports described the soluble cuticular waxes obtained by organic solvents as 'epicuticular waxes', while actually referring to the total cuticular waxes (Bianchi et al., 1992; Vichi et al., 2015; Vichi et al., 2016). As far as data collected from different organs (leaf, fruit, and petal), cyclic compounds, such as pentacyclic triterpenoids, steroids, aromatic compounds and other cyclics, and some aliphatics are found to be embedded in the intracuticular wax layer. Whereas the epicuticular waxes are almost solely dominated by aliphatic compounds (Jetter and Schäffer, 2001; Vogg et al., 2004; Buschhaus and Jetter, 2011; Buschhaus et al., 2015; Jetter and Riederer, 2016; Zeisler and Schreiber, 2016).

Detection of intra- and epicuticular waxes on Prunus lauracerasus leaf during development indicates that the intracuticular waxes remain constant while the epicuticular waxes shift quantitatively and qualitatively (Jetter and Schäffer, 2001). A highly dynamic process of self-assembly of epicuticular waxes on living plant surfaces has been further observed by atomic force microscopy (AFM) (Koch et al., 2004). The dynamic deposition of epicuticular waxes on organism surfaces form into multiple microstructures as wax crystals or wax films. The epicuticular waxes has been comprehensively classified into 23 types. The typical and common epicuticular waxes are in films, layers, crusts and crystalloids e.g. granules, plates, platelets, tubules, rodlets etc (Barthlott et al., 1998). The microstructures of different wax types are largely related to their major compounds, e.g. primary alcohols for plates and platelets, n-nonacosanol or diketones for tubules, ketones or *n*-alkanes for rodlets (Barthlott et al., 2017).

1.3 Other lipid barrier related constituents

The aliphatic biopolymer cutan, which is highly resistant to degradation, has been demonstrated to extend constituents of plant cuticle. The cutan polymers are detected to be a series of long chain *n*-alkenes, *n*-alkanes, hydroxyl fatty acids, and benzenecarboxylic acids in fossil leaves, *Agave americana*, *Clivia miniata* and several drought–adapted plants (Nip et al., 1986; McKinney et al., 1996; Boom et al., 2005; Deshmukh et al., 2005; Gupta et al., 2006). Fourier transform infrared (FTIR), NMR, and X-ray diffraction studies suggest that an ether-linked amorphous three-dimensional network occurrs in cutan polymers (Schouten et al., 1998; Villena et al.,

1999). However, limited information about the chemical and structure of cutan polymers has so far been obtained.

Suberin is an extracellular lipid polymer located between the primary cell wall and the plasma membrane, prior to secondary cell wall formation (Kolattukudy, 1980; Pollard et al., 2008). The suberin polymers occur in the external periderm of secondary roots, stems (bark), cotton fibers, in internal tissues of root endodermis, the bundle sheaths of monocots and at abscission zones. Suberin contains a polyaliphatics domain (dicarboxylic acids, hydroxyl fatty acids, fatty alcohols *etc.*), a polyphenolic (hydroxycinnamic acids derived from ferulic acid) domain and glycerol-linking polyesters (Kolattukudy, 1981; Bernards et al., 1995; Graça and Pereira, 2000; Bernards, 2002; Franke et al., 2005). Suberin is considered to deposit as a barrier in response to wound healing, boundary between tissues, water and nutrient uptake for root and pathogen stress (Fahn, 1986; Lulai and Corsini, 1998; Franke and Schreiber, 2007; Leide et al., 2012).

2 Biosynthesis and regulation

The biosynthesis of both cutin and VLC fatty acids and their derivatives begin with C_{16} or C_{18} fatty acid precursors that originate from *de novo* fatty acid synthesis catalysed by fatty acid synthase (FAS) in the plastids of epidermis cell (Haslam and Kunst, 2013; Yeats and Rose, 2013; Delude et al., 2016). Generally, free C_{16} or C_{18} fatty acids are firstly esterified by Long-Chain-acyl-CoA Synthetase (LACS) iso-enzymes. Three LACSs, LACS1, LACS2, and LACS4, are revealed to be important for wax synthesis (Lü et al., 2009; Jessen et al., 2011). The C_{16}/C_{18}-CoA esters are transferred to the endoplasm reticulum (ER) for the synthesis of waxes and cutin monomers.

In the wax biosynthetic pathway, the C_{16}/C_{18}-CoA precursors are firstly formed into VLC acyl-CoAs catalysed by Fatty Acid Elongases (FAEs). Four subunits of FAEs are important: β-ketoacyl-CoA synthase (KCS), β-ketoacyl-CoA reductase (KCR), β-hydroxyacyl-CoA dehydratase (HCD) and enoyl-CoA reductase (ECR) (Joubes et al., 2008). The VLC acyl-CoAs are then used as precursors to synthesize primary alcohols by CER4 (*cer*, *eceriferum*) (Jenks et al., 1995; Rowland and Domergue, 2012). The wax esters are subsequently catalysed (wax synthase/diacylglycerol acyltransferases 1, WSD1) from primary alcohols (Lardizabal et al., 2000; Li et al., 2008). An additional independent pathway for the synthesis of *n*-alkanes from VLC acyl-CoAs uses aldehydes as an intermediate catalysed independently or co-functionally by CER1 or

CER3 (Bourdenx et al., 2011; Bernard et al., 2012; Bernard and Joubès, 2013). Loss of function of CER6 in tomato fruit significantly hinders the synthesis VLC *n*-alkanes (> C28), and induces petal fusion (Vogg et al., 2004; Leide, 2008; Smirnova et al., 2013). The secondary alcohols and ketones are formed by consecutive oxidation of *n*-alkanes (Greer et al., 2007).

Non-aliphatic compounds, such as triterpenoids and sterols, are biosynthetically derived from the cytosolic mevalonic acid (MVA) pathway (Phillips et al., 2006; Thimmappa et al., 2014). The skeletons of triterpenes and phytosterols are cyclized from 2,3-oxidosqualene by oxidosqualene cyclases (OSCs). These backbones further undergo various modifications (oxidation, substitution and glycosylation), which are mediated by cytochrome P450-dependent monooxygenases, acyltransferases, and other enzymes (Haralampidis et al., 2002; Augustin et al., 2011).

The cutin monomers are synthesized from C_{16}/C_{18}-CoA precursors catalysed by cytochrome P450 members (CYPs) (Kandel et al., 2006; Fich et al., 2016). Generally, the C_{16}/C_{18}-CoA precursors are hydroxylated for terminal carbon reactions catalysed by CYP86A8, CYP86A2, and CYP86A4 (Wellesen et al., 2001; Xiao et al., 2004; Li-Beisson et al., 2009). The oxidoreductase HOTHEAD (HTH) may involve in synthesis of dicarboxylic acid (DCA) (Kurdyukov et al., 2006). The midchain hydroxyl reactions have been found to be involved in in-chain hydroxylase CYP77A6 (Li-Beisson et al., 2009).

The terminal hydroxyl acid or DCA are acylated by LACS1 and LACS2 and the acyl-CoAs precursors are subsequently esterified following the incorporation of glycerol in the *sn*-2 positon: The key enzymes for this step are glycerol-3-phosphate acyltransferases (GPATs) such as GPAT4 and GPAT8 in leaves and stems (Li et al., 2007), and GPAT6 in petals (Li-Beisson et al., 2009). These enzymes catalysis produces 2-monoacylglycerols (Yang et al., 2012). Meanwhile, the phenolic components are incorporated by BAHD-type acyltransferases (Rautengarten et al., 2012). The extracellular polymerization is found to be related to cutin synthases, such as GDSL1/CD1, and its ortholog LTL1 (Girard et al., 2012; Yeats et al., 2012; Yeats et al., 2014).

The regulation of cuticle biosynthesis is complex and depends upon the genetic background of different plant species, organs, tissues, the developmental stages, environmental factors, such as light, temperature, pathogen responses, and plant hormones (ABA) (Shepherd and Wynne Griffiths, 2006; Yeats and Rose, 2013).

Transcriptional regulation has also been widely performed on tomato fruit and *Arabidopsis* leaf, flower and stem. Different transcription factors, such as TOMATO AGAMOUS-LIKE 1 (TAGL1), SHN1/WIN1, SHN2, SHN3, MYB41, MYB94, MYB96, and MYB106 *etc.*, are indicated to be involved in the regulation of the production of wax or cutin monomers (Yeats and Rose, 2013; Lee and Suh, 2014; Delude et al., 2016).

The cuticle compounds are produced in the ER and moved to the plasma membrane via Golgi-derived secretory vesicles (McFarlane et al., 2014). The plasma membrane cuticle compounds are then exported to accumulate on the plant surface by ATP-binding cassette transporters (ABCs). The ABCG11 and ABCG12 act as half transporters for wax or cutin monomers export, while the ABCG11/ABCG12 heterodimers co-function in wax export (Pighin et al., 2004; Bird et al., 2007; McFarlane et al., 2010). Two other ABCG transporters, ABCG13 and ABCG32/PEC1, are found to be involved in the formation of cuticle compounds in flowers or leaves (Panikashvili et al., 2011; Fabre et al., 2016). Recently, the glycosylphosphatidylinositol-anchored LTPs (LTPGs) function in accumulation wax or cutin monomers has been isolated (DeBono et al., 2009; Kim et al., 2012).

3 Cuticle structure and barrier properties

The transport barrier for water transpiration and solutes diffusion has been putatively reported to be predominantly constructed of cuticular wax (Schönherr, 1976; Schönherr and Riederer, 1989; Riederer and Schreiber, 1995; Riederer and Schreiber, 2001). The cutin polymers allow for the accumulation of waxes, maintenance of cuticle integrity and function as a pathogen barrier in the hydrophobic scaffolding (Isaacson et al., 2009; Fich et al., 2016). Therefore, the coverage amount, chemical compositions, spatial arrangement and chain length distributions of waxes may play important roles for the barrier properties.

3.1 Effect of wax load and cuticle thickness

The total wax coverage and thickness of the cuticle vary depending on the species and organs. Wax coverage on different leaf cuticles ranges from less than 1 µg cm^{-2} (*Arabidopsis thaliana*, *Morus nigra* L. etc.) (Aharoni et al., 2004; Mamrutha et al., 2010) up to over 300 µg cm^{-2} (*Nerium oleander* L., *Agave americana* L.) (Wattendorff and Holloway, 1982; Schreiber and Riederer, 1996; Schuster, 2016). Fruit cutcicle

accumulates relative high amount of wax ranging between 4 μg cm^{-2} (Satsuma mandarin, *Citrus unshiu* Marc) (Wang et al., 2014) and 8700 μg cm^{-2} (bayberry, *Myrica pensylvanica* L.) (Simpson and Ohlrogge, 2016).

Most leaf cuticles have thickness ranging from 0.1–10 μm in size (Riederer and Schreiber, 2001; Semerdjieva et al., 2003). The fruit cuticle thickness is generally 2–25 μm thicker than that of leaf (Sterling, 1953; King et al., 1987; Biles et al., 1993; Demirsoy and Demirsoy, 2004; Bargel and Neinhuis, 2005; Hammami and Rapoport, 2012; Konarska, 2015). However, few species, such as *Arabidopsis*, form a very thin cuticle, ranging from 22 nm at leaf blades to 45 nm at petioles (Franke et al., 2005). A distinctively thick cuticle of 225 μm has also been found on the *Ariocarpus fissuratus* stem (Loza-Cornejo and Terrazas, 2003).

As the total wax load and cuticle thickness vary from different organs or species, cuticular water permeability is not correlated to the cuticular wax coverage or cuticle thickness, as tested on different species, organs, and tissues (Riederer and Schneider, 1990; Schreiber and Riederer, 1996; Riederer and Schreiber, 2001; Jetter and Riederer, 2016). Moreover, the shift of wax quantity has also not been found to be important for the transpiration properties (Premachandra et al., 1991; Ristic and Jenks, 2002; Leide, 2008).

3.2 Effect of cuticular components

Based on the molecular constituents, the structure of the cuticular wax has been investigated using light microscopy, nuclear magnetic resonance (NMR), Differential scanning calorimetry (DSC) and X-ray diffraction studies. Similar to other artificial wax (Le Roux, 1980; Basson and Reynhardt, 1988), the cuticular wax is shown to mainly contain three distinct fractions: hydrocarbon chains for Zone A, adjacent between hydrocarbon chain ends for Zone B, and short hydrocarbon chains together with cyclic compounds for Zone C (Reynhardt and Riederer, 1991, 1994). The hydrocarbon chains are assembled into highly regular orthorhombic or hexagonal crystalline lattices, which are perpendicular to the cuticle surface.

This crystalline zone is solely dependent on aliphatic compounds and tightly packed into laterally extended structures, such as platelets or flakes (Figure 2, *Zone A*). For the chain-ends of hydrocarbon chains, a solid amorphous zone occurs between two adjacent flakes. Some chain-ends may extend from one flake across adjacent zones to the other crystalline. The size of this solid amorphous zone largely depends upon

the varieties of chain length distribution in a volume fraction for the chain-ends between two adjacent crystalline layers (Figure 2, *Zone B*). In addition to the crystalline zone and the adjacent solid amorphous zone, aliphatics have relatively short-chain lengths (and subsequently a low melting point) together with some less ordered cyclic compounds form the mobile amorphous zone (Figure 2, *Zone C*). This zone exhibits high mobility and is sensitive to environmental factors, especially to temperature.

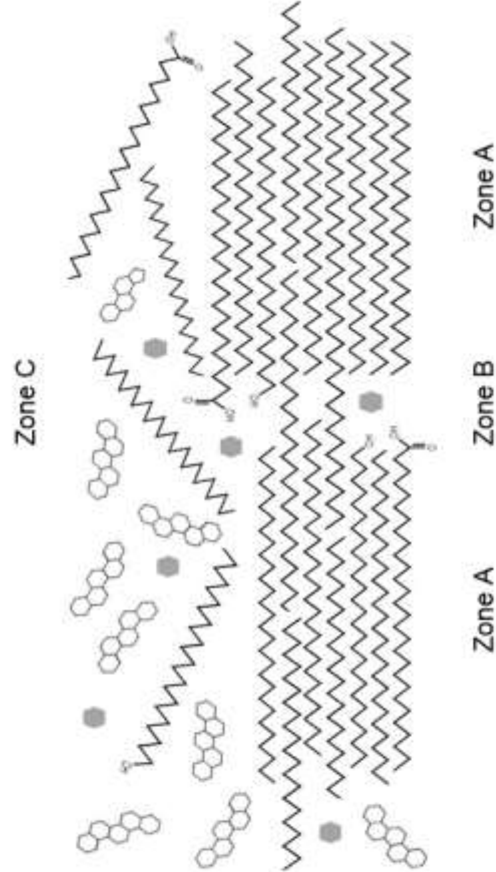

Figure 2. Schematic of the proposed molecular structure of cuticular waxes. The major fractions with different wax components are shown (not drawn in scale). The very-long-chain aliphatic compositions packed tightly (*Zone A*), the adjacent of aliphatic crystals (*Zone B*), and short chain-length aliphatic component together with cyclic compounds (*Zone C*, according to Riederer and Schreiber 1995).

As a consequence, the cuticle structure is proposed to be mainly in terms of crystalline and amorphous zones (Riederer and Schneider, 1990; Riederer and Schreiber, 1995). The crystalline zone is packed by VLC hydrocarbons, which are tightly aligned to form impermeable flake obstacles. The molecules can only be transported through the amorphous zones. Consequently, the regularly packed crystalline flakes embedded in cuticular wax and cutin polymers, which are spaced like

a 'brick wall' and function as a transport barrier (Figure 3). This makes the diffusion of molecules tortuous; thus, extending the length of the diffusion pathway in the cuticle (Riederer and Schreiber, 1995; Baur et al., 1996; Buchholz, 2006).

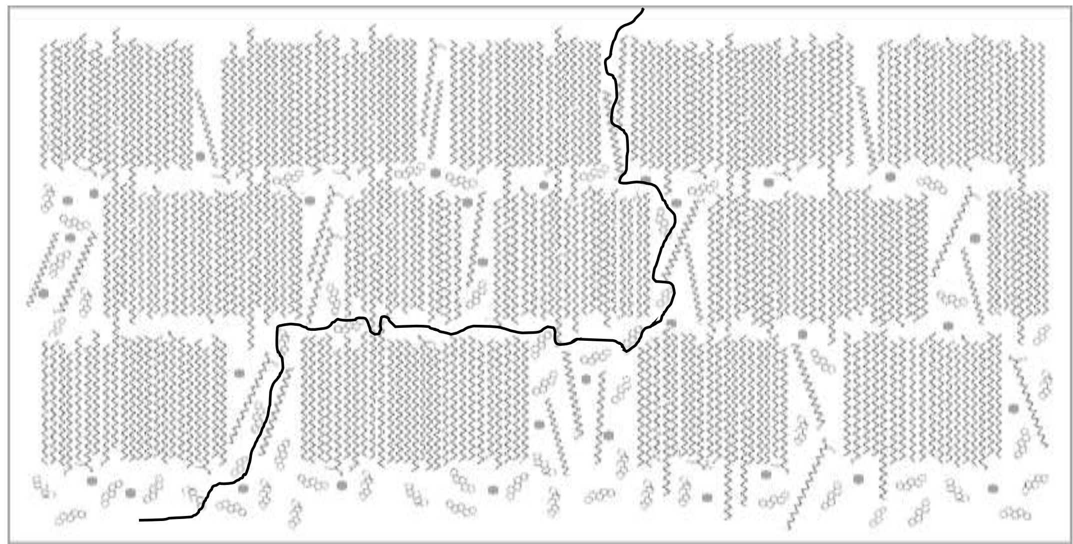

Figure 3. The proposed structural and transport barrier-membrane model of plant cuticle. The impermeable flakes of crystalline *Zone A* embed within the amorphous fraction of *Zone C* and form the adjacent between flakes as *Zone B.* The tight packed flakes force the water or other molecules diffusion as tortuous paths through cuticular membranes (according to Riederer and Schreiber 1995).

The tomato and *Arabidopsis* plants have been viewed as model organisms for fruit and leaf cuticle investigation for the last several decades. Only rare examples for the investigation of the relations between the wax and functional aspects have been obtained. One example with the tomato fruit is the absence of β-ketoacyl-CoA synthase (CER6) activity, a specific enzyme for wax synthesis, reduces the accumulation of *n*-alkanes remarkably, especially in carbon chain length above C_{28} (Leide, 2008). The phenotype of modified tomato fruit exhibits shrinkage in the ripe stage (Vogg et al., 2004). Compared to wild-type, an eight times greater permeability is observed in mutant fruits (Leide et al., 2007). Another example is the genetic modification of *Arabidopsis* leaf to overproduce β-amyrin which leads to an increase in the accumulation of triterpenoids in the intracuticular wax layer and reduces the effectiveness of the transpiration barrier for leaf (Buschhaus and Jetter, 2012). However, the relationship between the chemical characteristics and the transpiration barrier properties have yet to be fully understood.

3.3 Effect of environment factors

3.3.1 Temperature

The proposed wax structure with mobile amorphous zones constructed by short-chain aliphatics with relative low melting point, are largely affected by temperatures (Basson and Reynhardt, 1992; Riederer and Schreiber, 1995). Water permeability of evergreen and deciduous fresh leaves and isolated cuticles increase by a factor between 12 and 264 with a temperature increase from 10 °C to 55 °C. The occurrence of phase transition of transpiration at 35 °C is argued to be related to a structural shift under high temperatures (Schreiber and Schönherr, 1990; Schreiber, 2001). Meanwhile, the rheological properties of the cuticle are dynamically modified by the abiotic factors, especially temperature. A phase transition of mechanical behavior in the cutin polymer matrix has been tested on isolated tomato fruit cuticles between 23 °C and 35 °C (Matas et al., 2004; Matas et al., 2005). The changes of strength and stiffness of the cuticle are seemed to be largely determined by its chemical composition and molecular structure (Edelmann et al., 2005).

Recently, a study on desert plant leaves found no phase transition for transpiration, but the permeability increases by a factor of 2.4 under temperatures ranging between 15 °C and 55 °C. In this study, a high amount of leaf wax was dominated by triterpenoids (85%), which have been described as perfect fillers to protect the cuticle from structural shift for adaptation to a high temperature climate (Schuster et al., 2016). The investigation of Paraffinic Fischer-Tropsch waxes shows that the mobile amorphous zone is formed only after it has been filled by short-chain alipahtics (Basson and Reynhardt, 1992). This suggests that the low level of aliphatic compounds in the leaf wax reduces the possibility to form mobile amorphous zones, while the cyclic compounds with a relative high melting point are reinforced into the cutin matrix to strengthen the cuticle (Schuster et al., 2016).

In addition, water permeability of evergreen leaves increases much more slowly than that for deciduous leaves under temperature increase from 10 °C to 55 °C. The permeability of leaves from different growing areas or plant types show a tendency increase over evergreen leaves, followed by xeromorphic plants in the Mediterranean, deciduous leaves and is the highest for desert plants (including shrubs, and grasses) (Schreiber and Riederer, 1996; Riederer and Schreiber, 2001; Schuster et al., 2016). Meanwhile, the attempt to establish the relationship between the melting point of wax

in different species and their permeablities has yet to be fully understood, but a tendency decrease in transpiration with an increase of wax melting points has been observed (Schreiber and Riederer, 1996).

3.3.2 Hydration (humidity)

Studies on the citrus leaf or tomato fruit grown under different relative humidity conditions showed no significant effect on the change of cuticular composition (Riederer and Schneider, 1990; Leide, 2008). The transport barrier constructed by waxes consisting of relative stable compositional set, therefore, the barrier properties are not affected by the growing environments. Nonetheless, the comprehensive analysis of cuticular wax amount and composition from *lecer6* fruit and leaf, shows the wax deposition may also affect cuticle structure and integrity for the shrinkage phenotype of *lecer6* (Leide, 2008). Thus, the change of water availability conditions does not affect the intrinsic wax compositions, while a shift of amount may affect the mechanical properties of cuticle.

The water sorption of isolated CMs of tomato fruit rises with an increase in humidity, especially above 60% of RH (Chamel et al., 1991; Luque et al., 1995; Domínguez and Heredia, 1999). Removal of the waxes does not modify the water sorption of cuticle, while the sorption capacity reduces drastically for hydrolyzed cutin matrix (Chamel et al., 1991). Two main configurations of H_2O molecules, *volatile* and *embedded,* have been distinguished by Fourier-transformation and (near) infrared spectroscopy (Maréchal and Chamel, 1996; Maréchal and Chamel, 1997). The *volatile* water molecules connect with the hydroxyl groups through one weak hydrogen bond, mainly in the polysaccharide fraction of the cuticle. This helps the cuticle to be in equilibrium with the outer-atmospheric moistures. On the other hand, the *embedded* water molecules are held by two strong or three weak hydrogen bonds between cutin and polysaccharides in the cuticle. The latter configuration molecules are difficult to evaporate even in temperatures above 100 ^{o}C (Maréchal and Chamel, 1996; Heredia-Guerrero et al., 2014). This indicates that polysaccharides and phenolics with hydroxyl groups in cuticle may play a crucial role for the interaction between water and the cuticle. The cuticular membranes *in situ* are tightly accumulated on the fully saturated epidermis cell wall; therefore, the *embedded* water configuration will not be largely affected by outer environments. The various relative humidity conditions provide a

hydration status for the cuticle between the interior of plant cells and the atmosphere, which may affect the configuration of *volatile* water molecule in the cuticle.

Meanwhile, it has been demonstrated that the cuticle strength and stiffness decrease following an increase in the degree of hydration of CMs (Edelmann et al., 2005; Matas et al., 2005). Studies on the mechanical properties of tomato-isolated CMs reveals that the stiffness of the cuticle is primarily contributed by the polysaccharides fraction, while the cutin matrix provides the plasticity for the cuticle (López-Casado et al., 2007). Hence, the water molecules act as a plasticizer, which may modify the biomechanical behavior for the cuticle. Under relatively low humidity (< 40%), the cuticle displays a conversion of stiffness into elasticity and sorbs water slowly to fulfill the *volatile* water configuration. On the other hand, at relatively high humidity (> 60%) or wet conditions (liquid water), water plasticizes to reduce the stiffness of the cuticle. For instance, the presence of water droplets on the surface of fruit, which form following rainfall or from water condensed by large differences in day and night temperature, induces fruit cracking (Emmons and Scott, 1997; Aloni et al., 1998; Matas et al., 2005). Application of surfactants to help hydrate and plasticize the cuticle putatively modify the permeation of water-soluble active ingredients between the outer environments and the plant cells (Matas et al., 2004; Asmus et al., 2016).

3.3.3 Water deficit

Previous reports have indicated that drought (dehydration) stress induces an apparently increase accumulation of wax coverage or single *n*-alkanes on surface of leaves (Aharoni et al., 2004; Cameron et al., 2006; Kim et al., 2007; Kosma et al., 2009; Seo et al., 2011; Ni et al., 2012; Zhu and Xiong, 2013; Al-Abdallat et al., 2014) and fruits (Baker and Procopiou, 1997). The wild-type and genetic modified plants exhibited a comparable shift in the expression of wax synthesis-related genes, such as CERs, KCR, and KCSs *etc.* following drought stress. Thus, the modulate wax accumulation through wax biosynthesis could be a way to enhance drought tolerance (Aharoni et al., 2004; Seo and Park, 2011; Lü et al., 2012). However, how the increased accumulation of cuticular wax amount or *n*-alkanes affects the cuticular barrier properties remains unclear.

The response and regulation of stomatal function by ABA following water deficit has been comprehensively studied (Lee and Luan, 2012), while the direct function of ABA for the regulation of cuticle biosynthesis is still not clear. Very recently, ABA action

influences cuticle formation in an organ-dependent manner with leaf expansion, and by drought induced (Martin et al., 2017). Another study modified the formation of the stomatal outer cuticular ledge and demonstrated that the stomatal pores were entirely covered by continuous cuticle. The modification of cuticle fusion on the stomatal pores reduced the leaf transpiration and improved the drought tolerance (Hunt et al., 2017).

Beside the cuticle, other factors that are involved in drought or other abiotic stress tolerance have also been shown to be important. The adaptation of plant following the abiotic stresses, such as uncontrolled water loss, water deficit, pathogen infection, UV light radiation, insect interaction *etc.,* can be found throughout the whole plant development process (Jenks and Hasegawa, 2008; Gucci et al., 2012). Take *olea europaea* L. as an example, in order to better adapt the drought environment, high densities of trichrome and stomata on the leaf surfaces (a small leaf area), decline of epidermal cell size and numbers, and activating metabolic processes to produce substances have been observed (Dichio et al., 2003; Ennajeh et al., 2006; Bacelar et al., 2009; Guerfel et al., 2009; Boughalleb and Hajlaoui, 2010). They also demonstrated that the stomatal density, stomatal index and trichome number of the epidermis play important roles for leaf pathogen defence (Stenglein et al., 2005). A low number of pores, a thick epidermis and an external hypodermis with numerous cell layers are detected to be important for pathogen defence for grape berries (Ficke et al., 2002; Gabler et al., 2003). In addition, chemical substances of plant tissues, such as phenolics and flavonoids, have been detected to be important for UV radiation defence on pea and wheat (Alexieva et al., 2001; Winkel-Shirley, 2002; Doupis et al., 2016).

3.4 Mechanical properties of the cuticle

Most cuticles share similar compositions: for example, they differ in percentage for each fraction based upon organs, species and growing environments. Thus, the mechanical properties might be largely dependent on the different concentration of the diversity components. The rheological properties of some cuticular constituents: the cutin polymers, phenolic compounds and polysaccharides, have been comprehensively studied. The polysaccharides are important for linear elasticity, while the cutin polymers are related to the viscoelastic behaviors of cuticle (López-Casado et al., 2007; Espana et al., 2014). The cutin polymers and polysaccharides have been indicated as crucial fractions for interaction between the cuticle and water (Maréchal and Chamel, 1996). They may play major role in against pathogens, while less effective

as a barrier against transpiration (Isaacson et al., 2009). Meanwhile, the phenolic compounds are hypothesized to be correlated with the rigidity of the cutin matrix in ripe tomato fruit (Bargel et al., 2006). As a result, the cutin matrix is more likely providing a physical support for the accumulation of waxes or other cuticular components (Saladié et al., 2007; Fich et al., 2016).

Following drought stresses, one of the main strategies to enhance the efficiency of water-use and to limit water loss is to decrease leaf area (Blum, 1996; Bacelar et al., 2007; Farooq et al., 2009). As a result, the accumulation of wax increases the cuticle thickness. Though no correlations have been found between wax accumulation or cuticle thickness and the permeability characteristics, the cuticle thickness is putatively related to the mechanical properties of the cuticle (Matas et al., 2004). For instance, the crack of susceptibility of different cultivars of cherry tomato and sweet cherry fruits is suggested to be related to their cuticle thickness (Demirsoy and Demirsoy, 2004; Matas et al., 2004). With the development of the sweet cherry fruit, the fruit surface expansion, strain and formation of micro-cracks in the CMs is implicated to be related to the lack of deposition of waxes in the cuticle membrane (Alkio et al., 2012). These correlations have only been found between different cultivars of same species; this relationship remains uncertain for different species and organs.

4 Motivations and aims of the present work

The cuticular permeance of leaves has been investigated in different species and different habitats (Riederer and Schreiber, 2001; Jetter and Riederer, 2016; Schuster et al., 2016). The cuticular permeability of the leaf ranges with a 2.5 order of magnitude between 3.6×10^{-7} m s^{-1} (*Vanilla planifolia*) and 1.4×10^{-4} m s^{-1} (*Abies alba*). However, there is a wide range in overlap of permeabilities. A general tendency of evergreen leaves displays the lowest cuticular permeability, increased by Mediterranean evergreen or deciduous leaves, and temperate deciduous leaves. Recently, the cuticular permeance of desert plant leaves, including the woody plants, shrubs and grasses, has been detected to be comparable to temperate deciduous leaves (Schuster et al., 2016). Very limit analyses of the cuticular permeance of fruits, ranging from 0.9×10^{-5} m s^{-1} (*Solanum lycospersicum* L. cv. 'Micro-Tom') to 2.0×10^{-4} m s^{-1} (*Capsicum annuum* L.) have been performed (Riederer and Schreiber, 2001; Leide et al., 2007). Interestingly, the overall cuticular permeance for fruits might be larger than that for leaves. However, more plant species and/or cultivars and different type of fruits are needed to be investigated for further confirming this tendency of cuticular permeance.

Since the molecular structure of cuticle wax layers has been proposed (Riederer and Schreiber, 1995), few specific examples have been studied to confirm it. So far, only one systematic study showed a wide range of plant leaves from different species to link between the chemical compositions of cuticular waxes and the cuticular water permeance has been reported (Schuster, 2016). The coverage of VLC aliphatic wax compounds is found to be pivotal for the cuticular barrier function. The genetic modification in the synthesis of VLC wax components, especially the *n*-alkanes (> C_{28}) on tomato fruit affects the transpiration barrier properties and texture features (Vogg et al., 2004).

A common distribution of odd numbered chain length between C_{27} and C_{33} for *n*-alkanes in leaves has been detected. This pattern is even stimulated under dehydration stresses (Cameron et al., 2006; Kosma et al., 2009). VLC *n*-alkanes have also been deduced to be crucial for preventing fruit cracking (Ríos et al., 2015). Though small portion of the triterpenoids in *Arabidopsis* leaves, the alteration of ß-amyrin accumulation shifts transpiration (Buschhaus and Jetter, 2012). Whereas the cyclic compounds, which dominated the total cuticular waxes of *Rhazya stricta*, is thought to be important for the mechanical properties (Schuster et al., 2016). Based on these

conflicting reports, it is necessary to give a comprehensively comparable study on fruit and leaf from different species and/or cultivars, and to observe the different roles of components in the cuticular wax mixture.

The present study aims to characterize the water permeability of fruits and leaves to quantify the contribution of cuticular components to the transpiration barrier properties:

1) the characteristics of the cuticular transpiration of fruits and leaves,
2) the total cuticular wax load and composition of fruits and leaves,
3) the chain-length distribution of aliphatic components of fruits and leaves,
4) the cyclic compounds distribution of fruits and leaves,
5) the effect of wax amount and composition and carbon chain length distribution on the cuticular transpiration.

To address these above items, a wide range of plant species and/or cultivars were employed in the present study. They include five evergreen or evergreen/semi-evergreen plant species: three from Oleaceae family, one species from Oxalidaceae family, and one species from Rubiaceae family. The other elven species of deciduous plants are seven species and/or sub-species from Rosaceae family, two cultivars of grape berry from Vitaceae family, one species from Cornaceae family, one species from Moraceae family, and one species from Solanaceae family.

For this purpose, comparable analyses of water permeability, total wax load, aliphatic and cyclic compounds accumulation, carbon chain length distribution of aliphatic compounds between fruits and leaves were conducted. The correlation between the chemical composition of the cuticular waxes and the transpiration barrier properties was discussed.

Materials and methods

1 Plant material, growth conditions, fruit and leaf harvesting

Olive trees of *Olea europaea* L. cv. ‘Arbequina’ (Oleaceae) were grown in El Soleràs, Lleida, Spain (41°24'48.71"N, 0°40'50.05"E). The trees were non-irrigated and only rain-fed. The fruits in different development stages of green, turning and black were harvested during the ripening periods in November 2014. Fully expanded leaves were collected from the same olive trees as the fruits. The black ripe fruit was also sampled in November 2015. In addition, cv. ‘Arbequina’ fruits in green, turning and black stages, and fully expanded leaves were obtained from trees cultivated at experimental orchards located at the research center, IRTA-Mas de Bover, Constantí (Tarragona), Spain (41°10'11.46", N 1°10'9.61"E) in December 2016. The orchards received supported irrigation during fruit development.

Leaves from *Olea europaea* subsp. *europaea* var. *sylvestris* (Oleaceae) were collected in the Botanical Garden, University of Würzburg (49°45'58.10"N, 9°56'11.11"E) in December of 2015, and 2016 during the ripening period of the fruits, respectively.

The macroclimatic data were available from the international climate address (http://www.weatheronline.de/). In the last five years (2012-2016), the mean annual temperature was 22.1°C and the monthly mean of the maximum temperature was 33.9°C during July in Lleida, Spain. From 2012 to 2016, the mean annual rainfall was 440.3 mm, the mean annual temperature was 23.3°C and the monthly mean of the maximum temperature was 35.2°C during July in Constantí (Tarragona), Spain. The mean annual rainfall was 487.1 mm, the mean annual temperature was 15°C and the monthly mean of the maximum temperature was 26.4°C during July in Würzburg (2012-2016).

The fruits and leaves of 7 species belonging to the Rosaceae were obtained in Würzburg. *Malus domestica* L. cv. ‘Topaz’ (apple), *Prunus persica* L. (nectarine) and *Crataegus pedicellata* Sarg. (Scarlet hawthorn) were grown at outdoor sites with regular irrigation in the Botanical Garden. The fruits and leaves were sampled in July (nectarine), September (scarlet hawthorn and apple) in 2015. *Prunus avium* L. (sweet cherry), *Prunus cerasifera* Ehrh. (cherry plum), *Prunus domestica* L. subsp. *syriaca* Janich. (mirabelle plum) and *Prunus domestica* subsp. *insititia* (L.) (European plum) were grown in a wild field without regular irrigation. The field was close to the University of Würzburg. Fruits and leaves were obtained at the ripening period in July and August for cherry plum and mirabelle plum in 2015, respectively. The leaf

and fruit samples for sweet cherry and European plum were obtained in June and in August in 2016, respectively.

Vitis vinifera L. cv. 'Nelly' and cv. 'Silvana' (Vitaceae) were grown at an outdoor site with regular irrigation in the Botanical Garden. The leaf and berry samples were harvested in September of 2015.

Fruits and leaves of *Ficus carica* L. (figs, Moraceae) and *Cornus officinalis* Siebold & Zucc. (Cornaceae, dogwood) were picked in July of 2015. The plants were grown at outdoor sites with regular irrigation in the Botanical Garden. Fruit and leaf samples of *Averrhoa carambola* L. (Oxalidaceae, star fruit, in July of 2015), and *Coffea arabica* L. (Rubiaceae, in April of 2016) were obtained from the green house of the Botanical Garden.

The fruit and leaf samples of *Capsicum annuum* L. cv. 'Kalocsai' (pepper) were harvested from cultivated plants in the experimental green house of the Chair of Botany II of the University of Würzburg.

All the plant materials studied here were listed in Table 25. Intact fruits and leaves, with no mechanical damage, were picked carefully by detaching at the pedicle or petiole base for each sample. The fresh fruit and leaf samples were carefully packed in plastic bags, to avoid mechanical damage and water loss, and immediately transported to the laboratory for further experiments.

2 Fruit and leaf characteristics

2.1 Saturation of fruit and leaf samples

Fresh fruit and leaf samples were rehydrated in humid chambers (relative humidity 100%). The cut pedicels of the fruits and petioles of the leaves were submerged in water. The rehydration period was achieved following the previous reports (Garnier et al., 2001; Schuster, 2016). All fruit and leaf samples saturated overnight at ambient room temperature.

2.2 Determination of surface area

The fruit patterns of the two equatorial diameters and the polar diameter were measured. The fruit surface areas were determined by assuming the fruit surface area was estimated from the value of the polar and the equatorial diameter under the assumption of a spherical or other specifically shapes, e.g. one intact or two halves of ellipsode, cone (Clayton et al., 1995). Some fruits such as star fruit, pepper were cut into small pieces and scanned with a flatted scanner. The leaf surface area was obtained by scanning the fresh leaves with a flatbed

scanner. The experimental surface areas were calculated based on a standard area. The total leaf area was calculated as the double of the projected leaf area.

2.3 Scanning electronic microcopy

For the microscopic observation of surface characteristics, the fruit and leaf samples were cut into small pieces (3 mm x 5 mm). The small fruit and leaf pieces were mounted on aluminum stubs using a conductive double-sided adhesive tape (Plannet Plano) and then allowed to air-dry. The dry samples were coated with gold: palladium (60:40) at 25 mA using a Bal-Tec SCD 005 sputter coater (300 s; Balzers), depositing an alloy of approximately 20 nm thickness. The characteristics of the sample surfaces were examined under a field emission scanning electron microscope (JEOL JSM-7500F) at 5 kV accelerating voltage and 10 mm working distance.

3 Characterization of cuticular transpiration

The transpiration of water from intact fruits of different developmental stages was determined gravimetrically. Before the measurement, the attachment sites of the fruit pedicels were sealed with paraffin wax (melting point 65°C, Roth, Karlsruhe, Germany). As no stomata were detected on the adaxial leaf surfaces, the stomatous abaxial leaf surfaces were covered by one-sided adhesive aluminum foil tape (tesa) to guarantee the water transpiration solely from the astomatous adaxial leaf surfaces. The cut petioles were sealed with paraffin wax. Fruit or leaf samples were placed in sealed boxes above dry silica gel (Applichem). Under this condition, the samples were surrounded by a water vapor concentration of nearly zero. The boxes were placed in an incubator (IPP 110, Memmert) to control the surrounding temperature (25°C).

The amount of water transpired from intact fruits or adaxial leaf surfaces versus time (at least five to six data points per individual sample) was measured using an analytic electronic balance with a precision of 0.1 mg (Sartorius MC-1 AC210S, Göttingen, Germany). The air temperature was measured with a digital thermometer (Testoterm 6010, Lenzkirch, Germany) and the actual fruit and leaf temperatures were measured with an infrared laser thermometer (Harbor Freight Tools, Pittsburgh, USA).

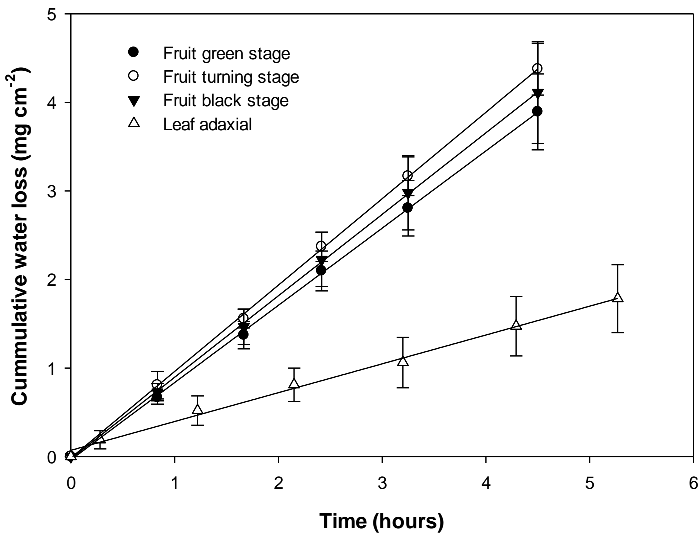

Figure 4. Cumulative water loss of olive fruits (three developmental stages) and adaxial leaf surfaces (*Olea europaea* L. cv. 'Arbequina'). Correlation of water loss and time exhibited a linear regression for the fruits in the green (r^2 = 0.999), turning (r^2 = 0.999), black (r^2 = 0.999) stage, as well as for the leaf surfaces (r^2 = 0.995). Data were given as mean values ± standard deviation (n = 12).

For the astomatous water transpiration, the plotted cumulative water loss against the time was linear (Figure 4). The transpiration rate (T, g m^{-2} s^{-1}) was obtained from the change of the weight of the samples (*ΔW*, g) per time (*Δt*, s) and per surface area (A, m^2):

$$T = \frac{\Delta W}{\Delta t \cdot A}$$

The permeance (P, m s^{-1}) was calculated from the transpiration rate (T) divided by the driving force (Δc) according to the equation:

$$P = \frac{T}{\Delta c} = \frac{T}{c_{wv}^{*}\,(a_s - a_{air})}$$

The driving force (Δc) is the water vapor concentration difference between the samples and the surrounding atmosphere. The water activity of fruit or leaf samples (a_s) was assumed to be unity (Burghardt and Riederer, 2003). As the relative humidity surrounding the samples was controlled by dry silica gel, the water activity of air (a_{air}) was nearly zero. The water vapour saturation concentration (water vapour content of air at saturation, c_{wv}) at 25°C is 23.07 g m^{-3} (Nobel, 2009). The cuticular transpiration rate and, thus, the permeance were corrected for the differences between sample and air temperature.

4 Measurement of the minimum conductance

The transpiration of stomatous leaf surfaces from different plant species was determined gravimetrically in a temperature chamber at 25°C. Before the measurement, the cut site of leaf petioles was sealed with paraffin wax. The leaf blades were randomly exposed to the surrounding air in a climate incubator. The temperature and relative humidity were measured with a digital thermometer (Testoterm 6010, Lenzkirch, Germany). The actual leaf temperature was measured with an infrared laser thermometer.

The leaf water loss is determined repeatedly with leaf desiccation. The transpiration rate is the water loss per time and per leaf area. From the transpiration rate the conductance is obtained by dividing with the driving force (Δc):

$$g = \frac{T}{\Delta c} = \frac{T}{c_{wv}^{*}\left(a_{leaf} - a_{air}\right)}$$

The water vapour saturation concentration (water vapour content of air at saturation, c_{wv}) was derived from Nobel (2009) for the corresponding temperature. The water activity of the leaf interior (a_{leaf}) was assumed to be unity. Air water activity (a_{air}) was derived from the measured relative humidity. The transpiration rate and, thus, the conductance were corrected for the differences between sample and air temperature.

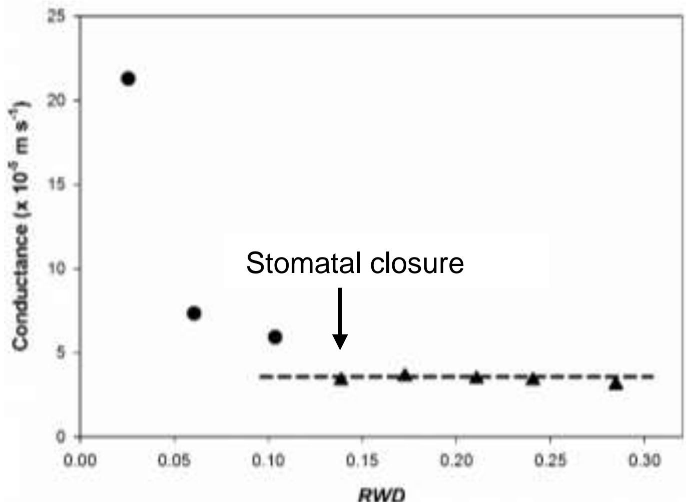

Figure 5. Leaf drying curve of a representative leaf of *Averrhoa carambola* L. at 25°C. The leaf conductance was plotted against the relative water deficit. The initial leaf conductance was very high (black dots), after reach a certain RWD, the change of conductances was constant and linear (black triangles). The transition point indicates the point of maximum stomatal closure for dehydration.

The relative water deficit (RWD) was calculated at each measurement point based on the actual fresh weight (FW), the saturation fresh weight (FW_s) and the dry weight (DW):

$$RWD = 1 - \frac{FW - DW}{FW_s - DW}$$

The saturation fresh weight (FW_s) was measured after rehydration of the fresh samples. For the dry weight (DW) determination, samples were kept at 90°C until a constant dry weight was reached.

Plotting the conductance versus the relative water deficit, an initial high conductance indicates stomatal transpiration influencing the conductance. At a certain RWD, the conductance was constant and linear. The transition point is the point of maximum stomatal closure under desiccation (leaf drying curve, Figure 5). The linear conductance values were defined as the minimum conductance (g_{min}, Burghardt and Riederer, 2003).

5 Chemical analysis of the cuticular components

5.1 Isolation of cuticular membranes

Cuticular membranes (CMs) of fruits and leaves were isolated enzymatically. The punched-out fruit and leaf discs (with diameter of 12 mm and 20 mm), were immersed in 20 mM citrate buffer (pH 3.0; citric acid monohydrate, Applichem) containing pectinase (1%, Trenolin, Erbslöh) and cellulase (1%, Celluclast, NCBE). Additionally, 1 mM sodium azide (Sigma-Aldrich) was added to avoid growth of microorganism (Schönherr and Riederer, 1986). The enzyme solution was changed every 5 to 7 days (storage at room temperature) until the tissue was largely dissolved and separated from the cuticular membranes. The isolated cuticular membranes were washed in aqueous borax buffer at least for 24 h (10 mM; pH 9.0, Applichem). This treatment released the extraneous lipophilic substances that sorbed to the cuticles during isolation (Schönherr and Riederer, 1986). Afterwards, the isolated cuticular membranes were washed by deionized water and subsequently dried under a gentle stream of pressurized air which helped to flatten the cuticles. The cuticular membranes were stored at room temperature in plastic petri dishes for further experiments.

5.2 Cuticular wax extraction for gas chromatographic analysis

To extract the cuticular waxes, the CMs isolated from fruit or leaves were immersed in high purity chloroform (≥ 99.8%, Roth, Karlsruhe, Germany) for 30-60 s. The samples from which the cuticular membranes could not be isolated enzymatically, the cuticular waxes were

extracted directly by dipping the samples in chloroform. The extraction sample types were listed in Table 25. To avoid a contact between the solvent with the pedicels/petioles, approximately 90% of the sample surface was vertically dipped into chloroform for 30-60 s. To extract the cuticular wax from the adaxial and abaxial leaf surface separately, leaf blades were placed on a flat plate equipped with a flexible rubber mat. A 10 mm diameter of glass cylinder was fixed and the height was controlled by the rubber mat (Jetter et al., 2000). Approximately 4 mL of chloroform were added into the glass cylinder to extract the cuticular waxes (30-60 s).

To ensure a complete extraction of the cuticular waxes, each CM or fresh sample of both fruit and leaf samples was extracted three times consecutively (Figure 6). Amounts of 5 or 10 µg *n*-tetracosane (1 µg mL^{-1}, > 99.5%, Sigma-Aldrich, Steinheim, Germany) was immediately added into the extracts as internal standard. The solvent was then evaporated at maximally 50°C under a gentle stream of nitrogen till dryness.

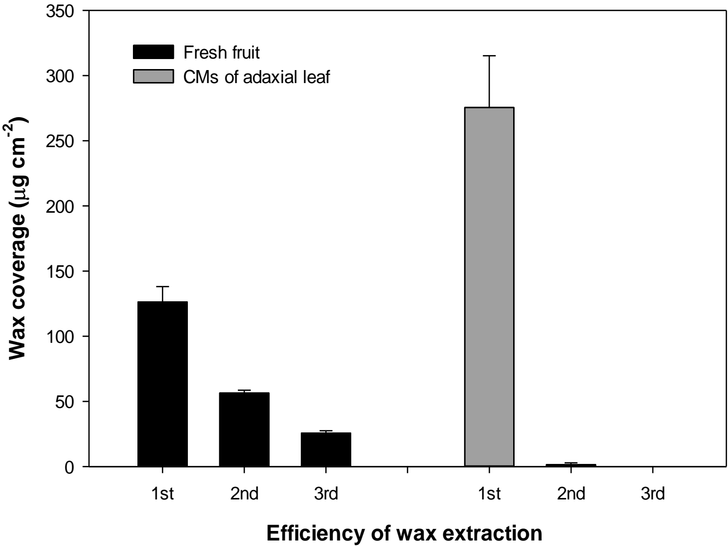

Figure 6. Extraction efficiency of the cuticular waxes from fresh fruit and CMs of adaxial leaf of *Olea europaea* L. cv. 'Arbequina'. The total wax amount from olive fruits and leaf CMs were extracted by chloroform for three times consecutively. The extracted wax amount decreased with extract times. Small portions of the total wax could be extracted in the third extraction from olive fruits (12%) and leaf CMs (traces). It indicates that most of the waxes have been extracted after three times of consecutive extraction. Results were given as mean values ± standard deviation (n = 5).

5.3 Cutin depolymerization for gas chromatographic analysis

For the cutin analysis, the air-dried isolated cuticular membranes were immersed in chloroform to remove the total cuticular waxes. The wax-free polymer matrix membranes

were depolymerized with BF_3-methanol (1.3 M boron trifluoride in methanol; Fluka) at 70°C overnight. 10 µg *n*-Dotriacontane (1 µg mL^{-1}, ≥ 98.0%, Sigma-Aldrich) as an internal standard was added to all extracts. Subsequently, a saturated aqueous NaCl solution (Applichem) was added, and the mixtures were extracted three times with chloroform. The collected extracts were dried with sodium sulfate (anhydrous; Applichem) and the organic solvent was gently evaporated under a continuous flow of nitrogen.

5.4 Chemical analysis by gas chromatography

Prior to the gas chromatographic analysis, the wax and cutin samples were derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Macherey-Nagel, Düren, Germany) in pyridine (Roth, Karlsruhe, Germany) for 30 min at 70°C. For the quantification of the cutin monomers and the cuticular waxes, a capillary gas chromatograph with flame ionization detector (6850N, GC-System; Agilent Technologies) and on-column injection with a capillary column (30 m × 0.32 mm, DB-1 ms, 0.1 µm film; J&W Scientific, Agilent Technologies) was used. For separation of the wax components, injection took place at 50°C followed by 2 min at 50°C, temperature raise by 40°C min^{-1} to 200°C, held for 2 min at 200°C, raise by 3°C min^{-1} to 320°C, and held for 30 min at 320°C. For separation of the cutin monomers, samples were injected at 50°C, followed by 1 min at 50°C, temperature raise by 10°C min^{-1} to 150°C, held for 2 min at 150°C, raise by 3°C min^{-1} to 320°C, and held for 30 min at 320°C.

Qualitative analysis was carried out with a gas chromatograph (6890N, Agilent Technologies) equipped with a mass spectrometric detector (m/z 50-750, MSD 5973; Agilent Technologies) under the same gas chromatographic conditions except that helium was used as carrier gas. Cuticular components were identified using authentic standards, a chemical database (NIST) and literature data.

The component coverage (C_s) was quantified against the amount of internal standard (M_{is}) by integrating the peak area of the component (A_s) and the peak area of internal standard (A_{is}), and dividing by the extracted area (A_{ea}).

$$C_s = \frac{A_s \cdot M_{is}}{A_{is} \cdot A_{ea}}$$

The weighted average of carbon chain length (ACL) of aliphatic compounds including or excluding the very-long-chain alkyl esters (≥ C_{36}) was calculated from the chain length (L_i,) and the mass fraction (M_i) of the component (i):

$$ACL = \frac{\sum_i L_i \cdot M_i}{\sum_i M_i}$$

Based on the ACL, the root-mean-square deviation of the average chain length (ΔACL) as a measurement of dispersion of chain length was calculated:

$$\Delta ACL = \sqrt{\frac{\sum_i M_i \cdot (ACL - L_i)^2}{\sum_i M_i}}$$

6 Statistical analysis

Statistical analysises were performed using SPSS Statistics 23 (IBM) and SigmaPlot 13 (Systat Software, California). Normal distribution of data was tested with Kolmogorow-Smirnow normality test (*P* value to reject 0.05). The significant differences between two independent group samples were tested by Student's *t*-test or Mann-Whitney U-test (level of significance $P < 0.05$). When comparing more than two group data, the analyses were performed by one way analysis of variance (ANOVA) or Kruskal-Wallis. Correlation coefficients between the water permeability and amount of wax load, variety of compositions, and ACL were carried out by correlation analysis of Pearson or Spearman Rank Order Correlation. All the graphs were performed by SigmaPlot 13.

Results

1 Cuticular permeance and minimum conductance

The cuticular water transpiration via intact fruit, leaf, or adaxial leaf surfaces from 17 species were determined gravimetrically under room temperature around 25 ^oC (Table 3). The overall permeances for water transpiration ranged from 7.18 × 10^{-5} to 9.91 × 10^{-5} m s^{-1} for olive (*Olea europeae* L. cv. 'Arbequina' and *Olea europaea* subsp. *europaea* var. *sylvestris*) fruits and from 1.88 × 10^{-5} m s^{-1} to 3.68 × 10^{-5} m s^{-1} for leaves that grown in different years and places. The cuticular water permeance of cv. 'Arbequina' fruit were 9.19 × 10^{-5}, 9.91 × 10^{-5}, and 9.45 × 10^{-5} m s^{-1}, respectively, in green, purple, and black developmental stages that sampled in 2014. No significant differences of fruit permeabilities among the different developmental stages were detedcted. In comparison to the permeabilitiies of black ripe fruit, the permeance for water via adaxial leaf surfaces was much lower, about one fourth of fruit permeability (2.64 × 10^{-5} m s^{-1}). The permeability of fruit in black stage was 7.18 × 10^{-5} m s^{-1} that sampled in 2015. Meawhile, very similar water permeabilities were detected for green (7.28 × 10^{-5}), turning (7.89 × 10^{-5}), and black (7.99 × 10^{-5} m s^{-1}) mature fruit, respectively, which were sampled in 2016. The water transpiration via adaxial leaf surfaces was 1.88 × 10^{-5} m s^{-1}, which exhibited a 4-fold lower than that of fruits.

The leaf water transpiration via adaxial surfaces of *Olea europaea* subsp. *europaea* var. *sylvestris* was 3.68 × 10^{-5} m s^{-1} that grown in 2015. It was 2.25 x 10^{-5} m s^{-1} as sampled in 2016. Similar as the difference of permeabilities between cv. 'Arbequina' fruit and leaf, about 4-fold higher permeability of 9.89 × 10^{-5} m s^{-1} was found for var. *sylvestris* fruit that sampled in 2016, when compared to its leaf transpiration.

As the occurrence of stomata on the ab- or adaxial leaf surfaces, the leaf minimum conductance based on the leaf drying curves at the maximum stomatal closure were determined. The minimum conductance of *Ligustrum vulgare* L. (oleaceae) leaf was 1.67 × 10^{-5} m s^{-1}. The transpiration of fruit was up to 10-fold (17.39 × 10^{-5} m s^{-1}) higher than leaf minimum conductance.

The water transpiration of fruit and leaf minimum conductance of 7 species belong to plant family of Rosaceae were determined. The transpiration of fruit and leaf minimum conductance of *Crataegus pedicellata* Sarg., showed a significant difference for fruit of 3.99 × 10^{-5} m s^{-1}, and 3.24 × 10^{-5} m s^{-1} for leaves, respectively, though they

seemed like very similar. The transpiration of *Malus domestica* L. cv. 'Topaz' fruit (3.99 × 10^{-5} m s^{-1}) was close to its leaf minimum conductance (3.08 × 10^{-5} m s^{-1}). Very similar water permeabilities were also obtained between fruit (3.93 × 10^{-5} m s^{-1}) and leaf (3.88 × 10^{-5} m s^{-1}) of *Prunus cerasifera* Ehrh. The cuticular transpiration of *Prunus domestica* L. subsp. *syriaca* Janich. fruit (3.67 × 10^{-5} m s^{-1}) was lower than that of leaf (4.45 × 10^{-5} m s^{-1}). The high transpiration of 14.82 × 10^{-5} m s^{-1}, 15.87 × 10^{-5} m s^{-1}, and 29.08 × 10^{-5} m s^{-1} were found for sweet cherry (*Prunus avium* L.), European plum (*Prunus domestica* subsp. *Insititia* (L.)), nectarine (*Prunus persica* L.) fruit, respectively. The leaf minimum conductance of these 3 species were similar and much lower than their fruit by 1.82 × 10^{-5} m s^{-1}, 2.73 × 10^{-5} m s^{-1}, and 2.07× 10^{-5} m s^{-1}, respectively.

The water transpiration of two cultivars of grape berry were very similar, being 7.48 × 10^{-5} m s^{-1} for *Vitis vinifera* L. cv. 'Nelly', and 7.93 × 10^{-5} m s^{-1} for *Vitis vinifera* L. cv. 'Silvana'. The leaf minimum conductance of these two grape berries were significantly lower than their fruit transpiration, which were 4.38 × 10^{-5} m s^{-1}, and 3.81 × 10^{-5} m s^{-1}, respectively.

The high transpiration of *Coffea arabica* L. and *Ficus carica* L. were detected by 3.74 × 10^{-4} m s^{-1}, and 3.62 × 10^{-4} m s^{-1}, respectively. The leaf conductance of these two species were nearly by 2.90 × 10^{-5} m s^{-1}, and 2.96 × 10^{-5} m s^{-1}, respectively. The water transpiration of *Averrhoa carambola* L. was 6.76 × 10^{-5} m s^{-1}, and a 2-fold lower of the leaf minimum conductance (3.00 × 10^{-5} m s-1) was detected. A 3-fod higher of transpiration for *Cornus officinalis* (Siebold & Zucc.) fruit (5.74 × 10^{-5} m s^{-1}) was found, when compared to the leaf minimum conductance of 1.57 × 10^{-5} m s^{-1}. The water transpiration of pepper *Capsicum annuum* L. cv. 'Kalocsai' fruit was significantly higher (7.53 × 10^{-5} m s^{-1}) than its leaf minimum conductance (1.86 × 10^{-5} m s^{-1}).

Table 3. The water transpiration of fruits and minimum conductance of leaves. The statistical analyses for comparing the interspecies differences of transpiration between fruits and leaves (*P*) were conducted. Data were given as mean values ± SD (x 10^{-5} m s^{-1}, n=7-24).

Family	Species	Year	Fruit	Leaf	*P*
Oleaceae					
	Olea europaea L. cv. 'Arbequina'	2014	9.19 ± 0.87 [a]		
	Olea europaea L. cv. 'Arbequina'	2014	9.91 ± 0.67 [b]		
	Olea europaea L. cv. 'Arbequina'	2014	9.45 ± 1.21	2.64 ± 0.82 [c]	< 0.01
	Olea europaea L. cv. 'Arbequina'	2015	7.18 ± 0.68		
	Olea europaea L. cv. 'Arbequina'	2016	7.28 ± 0.76 [a]		
	Olea europaea L. cv. 'Arbequina'	2016	7.89 ± 0.72 [b]		
	Olea europaea L. cv. 'Arbequina'	2016	7.99 ± 1.33	1.88 ± 1.24 [c]	< 0.01
	Olea europaea subsp. *europaea* var. *sylvestris*	2015		3.68 ± 1.13 [c]	
	Olea europaea subsp. *europaea* var. *sylvestris*	2016	9.89 ± 1.25	2.25 ± 0.47 [c]	< 0.01
	Ligustrum vulgare L.	2016	17.39 ± 1.51	1.67 ± 0.26	< 0.01
Oxalidaceae					
	Averrhoa carambola L.	2015	6.76 ± 1.70	3.00 ± 0.63	< 0.01
Rubiaceae					
	Coffea arabica L.	2016	37.41 ± 8.07	2.90 ± 0.70	< 0.01
Rosaceae					
	Crataegus pedicellata Sarg.	2015	3.99 ± 0.35	3.24 ± 0.79	< 0.05
	Malus domestica L. cv.'Topaz'	2015	3.99 ± 0.86	3.08 ± 1.60	0.125
	Prunus avium L.	2016	14.82 ± 4.24	1.82 ± 0.22	< 0.01
	Prunus cerasifera Ehrh.	2015	3.93 ± 0.48	3.88 ± 1.16	0.095
	Prunus domestica L. subsp. *syriaca* Janich.	2015	3.67 ± 0.33	4.45 ± 1.04	< 0.01
	Prunus domestica subsp. *insititia* (L.)	2016	15.87 ± 2.65	2.73 ± 0.64	< 0.01
	Prunus persica L.	2015	29.08 ± 10.59	2.07 ± 1.00	< 0.01
Vitaceae					
	Vitis vinifera L. cv. 'Nelly'	2015	7.48 ± 0.97	4.38 ± 2.00	< 0.01
	Vitis vinifera L. cv. 'Silvana'	2015	7.93 ± 0.92	3.81 ± 0.89	< 0.01
Cornaceae					
	Cornus officinalis Siebold & Zucc.	2015	5.74 ± 1.51	1.57 ± 0.26	< 0.01
Moraceae					
	Ficus carica L.	2015	33.30 ± 8.46	2.96 ± 0.66 [c]	< 0.01
Solanaceae					
	Capsicum annuum L. cv. 'Kalocsai'	2016	7.53 ± 2.19	1.86 ± 0.31	< 0.01

a, permeances for water were measured for the green mature fruits;

b, permeances for water were measured for the turning mature fruit;

c, permeances for water were measured via adaxial leaf surfaces.

2 Chemical analysis of cuticular waxes from fruits and leaves

The overall cuticular wax load and compositions of fruit and leaf of different species or cultivars were determined by gas chromatograph equipped with a flame ionization detector and the qualitative analysis by gas chromatograph equipped with a mass spectrometric detector. The cuticular wax of fruit and leaf of 3 species from oleaceae family, 7 species from rosaceae family, 2 cultivars of *vitis vinifera* L. (Vitaceae), and 5 other species from different plant families were analyzed. Overall, the total wax load on different fruit surfaces ranged between 15.48 µg cm^{-2} (*Coffea arabica* L.) and 451.05 µg cm^{-2} (*Crataegus pedicellata* Sarg.). The coverage of aliphatic wax ranged between 3.11 µg cm^{-2} (*Coffea arabica* L.) and 203.84 µg cm^{-2} (*Prunus domestica* subsp. *insititia* (L.)). The cyclic compounds deposited between 2.06 µg cm^{-2} (*Ficus carica* L.) and 258.61 µg cm^{-2} (*Crataegus pedicellata* Sarg., Table 4).

The leaf surfaces were covered by a wide range of wax load varied between 5.17 µg cm^{-2} (*Capsicum annuum* L. cv. 'Kalocsai') and 277.10 µg cm^{-2} (*Olea europaea* L. cv. 'Arbequina'). The aliphatic components ranged between 4.29 µg cm^{-2} (*Ligustrum vulgare* L.) and 22.24 µg cm^{-2} (*Prunus persica* L.). The cyclic compounds in leaves ranged between trace of 0.08 µg cm^{-2} (*Vitis vinifera* L. cv. 'Nelly') and 243.12 µg cm^{-2} for (*Olea europaea* L. cv. 'Arbequina', Table 5).

Subsequence, the ratio of aliphatics over cyclics varied from 0.13 (nectarine) to 38.59 (European plum) for fruits, while a bigger range between 0.04 (Olea europaea L.) and 123.02 (cv. Nelly) for leaves. Overall, the deposition of cuticular waxes appears to be vary in a species-specific manner, therefore, the contribution of each component to the barrier properties may be subject into various ways.

Based on the coverage of aliphatic compounds in each class, the weighted average carbon chain length (ACL) of aliphatic compounds was calculated. The ACL values of aliphatic compounds in fruits ranged between 25.80 (green mature fruit of *Olea europaea* L. cv. 'Arbequina') and 30.12 (*Vitis vinifera* L. cv. 'Nelly'). The leaf ACL value of aliphatics ranged between 28.79 (*Prunus cerasifera* Ehrh.) and 33.85 (*Prunus persica* L., Table 6).

Table 4. The amount of total wax, aliphatic, and cyclic components of fruits. Data were given as mean values ± SD (in μg cm^{-2}, n=5).

Family	Species	Year	Fruits			
			Total wax	Aliphatics	Cyclics	Aliphatics/cyclics
Oleaceae						
	Olea europaea L. cv. ‘Arbequina’	2014	194.61 ± 22.84	41.77 ± 5.85	145.17 ± 19.32	0.29 ± 0.04
	Olea europaea L. cv. ‘Arbequina’	2014	201.61 ± 19.65	52.65 ± 6.18	137.12 ± 13.07	0.38 ± 0.03
	Olea europaea L. cv. ‘Arbequina’	2014	208.64 ± 17.94	61.55 ± 5.94	130.68 ± 12.09	0.47 ± 0.01
	Olea europaea L. cv. ‘Arbequina’	2015	219.01 ± 28.66	64.26 ± 9.20	143.82 ± 22.70	0.47 ± 0.03
	Olea europaea L. cv. ‘Arbequina’	2016	153.76	33.34	112.15	0.30 [a]
	Olea europaea L. cv. ‘Arbequina’	2016	148.24	31.48	109.31	0.29 [a]
	Olea europaea L. cv. ‘Arbequina’	2016	149.20 ± 12.79	44.55 ± 3.34	94.13 ± 9.48	0.47 ± 0.03
	Olea europaea subsp. *europaea* var. *sylvestris*	2016	169.85 ± 25.22	48.53 ± 6.52	110.22 ± 19.39	0.44 ± 0.03
	Ligustrum vulgare L.	2016	148.09 ± 11.44	18.37 ± 4.64	115.76 ± 13.80	0.16 ± 0.05
Oxalidaceae						
	Averrhoa carambola L.	2015	47.32 ± 6.93	32.37 ± 5.42	2.76 ± 0.29	11.76 ± 1.62
Rubiaceae						
	Coffea arabica L.	2016	15.48 ± 2.25	3.11 ± 0.54	5.07 ± 1.03	0.65 ± 0.23
Rosaceae						
	Crataegus pedicellata Sarg.	2015	451.05 ± 60.57	155.19 ± 16.48	258.61 ± 46.81	0.61 ± 0.09
	Malus domestica L. cv.'Topaz'	2015	230.21 ± 12.61	137.02 ± 14.23	78.23 ± 17.98	1.88 ± 0.72
	Prunus avium L.	2016	37.52 ± 7.43	7.46 ± 1.43	25.53 ± 6.22	0.30 ± 0.06
	Prunus cerasifera Ehrh.	2015	205.64 ± 6.90	59.68 ± 4.77	127.80 ± 11.66	0.47 ± 0.09
	Prunus domestica L. subsp. *syriaca* Janich.	2015	212.07 ± 12.27	99.52 ± 10.31	98.31 ± 8.04	1.02 ± 0.18
	Prunus domestica subsp. *insititia* (L.)	2016	243.26 ± 21.90	203.84 ± 20.20	5.35 ± 0.66	38.59 ± 5.82
	Prunus persica L.	2015	288.04 ± 32.60	31.49 ± 5.84	234.53 ± 25.71	0.13 ± 0.01
Vitaceae						
	Vitis vinifera L. cv. 'Nelly'	2015	257.90 ± 22.08	111.55 ± 4.91	127.37 ± 30.81	0.92 ± 0.25
	Vitis vinifera L. cv. 'Silvana'	2015	168.20 ± 27.16	62.37 ± 16.76	89.45 ± 13.99	0.70 ± 0.18
Cornaceae						
	Cornus officinalis Siebold & Zucc.	2015	82.11 ± 14.35	25.19 ± 3.44	54.72 ± 10.40	0.47 ± 0.09
Moraceae						
	Ficus carica L.	2015	40.29 ± 7.98	33.81 ± 6.24	2.06 ± 0.76	17.63 ± 4.52
Solanaceae						
	Capsicum annuum L. cv. 'Kalocsai'	2016	28.40 ± 4.63	3.49 ± 0.77	8.58 ± 0.78	0.41 ± 0.07

a, the amount of total wax, aliphatic and cyclic components were extracted from one sample as references.

Table 5. The amount of total wax, aliphatics, and cyclics of leaves. Data were given as mean values ± SD (in µg cm^{-2}, n=5).

Family	Species	Year	Leaves			
			Total wax	Aliphatics	Cyclics	Aliphatics/cyclics
Oleaceae						
	Olea europaea L. cv. ‘Arbequina’	2014	277.10 ± 40.66	11.93 ± 1.75	243.12 ± 36.89	0.05 ± 0.01
	Olea europaea L. cv. ‘Arbequina’	2016	144.39 ± 16.97	6.19 ± 0.49	130.93 ± 16.87	0.05 ± 0.01
	Olea europaea subsp. *europaea* var. *sylvestris*	2015	242.2 ± 38.48	15.97 ± 4.65	216.8 ± 32.81	0.04 ± 0.01
	Olea europaea subsp. *europaea* var. *sylvestris*	2016	252.61 ± 17.56	8.27 ± 1.73	206.38 ± 20.96	0.07 ± 0.01
	Ligustrum vulgare L.	2016	26.18 ± 4.39	4.29 ± 0.68	19.13 ± 3.31	0.23 ± 0.01
Oxalidaceae						
	Averrhoa carambola L.	2015	19.35 ± 5.67	16.58 ± 5.02	0.24 ± 0.06	79.69 ± 15.38
Rubiaceae						
	Coffea arabica L.	2016	7.00	5.33	0.95	5.43 [a]
Rosaceae						
	Crataegus pedicellata Sarg.	2015	30.56 ± 10.35	12.05 ± 5.40	15.87 ± 4.37	0.68 ± 0.15
	Malus domestica L. cv.'Topaz'	2015	39.26 ± 4.29	6.24 ± 0.61	7.97 ± 1.15	0.26 ± 0.07
	Prunus avium L.	2016	22.82 ± 3.17	7.13 ± 1.73	12.60 ± 4.18	0.65 ± 0.34
	Prunus cerasifera Ehrh.	2015	46.40 ± 8.03	7.21 ± 2.04	32.48 ± 7.06	0.22 ± 0.05
	Prunus domestica subsp. *syriaca* Janich.	2015	39.62 ± 2.41	7.52 ± 2.34	29.57 ± 3.55	0.36 ± 0.15
	Prunus domestica subsp. *insititia* (L.)	2016	23.70 ± 1.68	7.38 ± 0.50	12.38 ± 1.11	0.66 ± 0.25
	Prunus persica L.	2015	47.29 ± 12.44	22.24 ± 6.04	20.21 5.88	1.31 ± 0.42
Vitaceae						
	Vitis vinifera L. cv. 'Nelly'	2015	11.04 ± 2.32	9.16 ± 2.00	0.08 ± 0.04	123.02 ± 10.21
	Vitis vinifera L. cv. 'Silvana'	2015	16.36 ± 1.80	12.31 ± 0.93	0.77 ± 0.16	15.36 ± 2.83
Cornaceae						
	Cornus officinalis Siebold & Zucc.	2015	18.42 ± 3.91	4.83 ± 0.44	10.84 ± 3.25	0.50 ± 0.31
Moraceae						
	Ficus carica L.	2015	8.60 ± 1.69	4.87 ± 0.65	0.71 ± 0.28	7.53 ± 2.48
Solanaceae						
	Capsicum annuum L. cv. 'Kalocsai'	2016	5.17 ± 1.66	4.69 ± 2.58	0.25 ± 0.16	43.90 ± 5.08

a, the amount of total wax, aliphatic and cyclic components were extracted from one sample as reference.

Table 6. The ACL value of the aliphatic wax components, and the standard deviation (ΔACL) as a measurement of dispersion based on the molar mass of different aliphatic compounds.

Family	Species	Year	Fruit			Leaf		
			ACL	ΔACL	ΔACL ACL^{-1}	ACL	ΔACL	ΔACL ACL^{-1}
Oleaceae								
	Olea europaea L. cv. 'Arbequina'	2014	26.07	2.57	0.10 [a]			
	Olea europaea L. cv. 'Arbequina'	2014	26.55	3.92	0.15 [b]			
	Olea europaea L. cv. 'Arbequina'	2014	27.27	5.07	0.19	30.06	2.68	0.09 [c]
	Olea europaea L. cv. 'Arbequina'	2015	26.36	4.62	0.18			
	Olea europaea L. cv. 'Arbequina'	2016	26.04	3.54	0.14 [a]			
	Olea europaea L. cv. 'Arbequina'	2016	26.96	5.01	0.19 [b]			
	Olea europaea L. cv. 'Arbequina'	2016	27.21	5.60	0.21	29.94	2.81	0.09 [c]
	Olea europaea subsp. *europaea* var. *sylvestris*	2015				30.30	2.46	0.08 [c]
	Olea europaea subsp. *europaea* var. *sylvestris*	2016	26.91	2.01	0.07	30.32	2.73	0.09 [c]
	Ligustrum vulgare L.	2016	28.46	3.57	0.13	30.01	2.97	0.10
Oxalidaceae								
	Averrhoa carambola L.	2015	26.22	5.55	0.21	30.85	1.42	0.05
Rubiaceae								
	Coffea arabica L.	2016	29.24	3.29	0.11	30.73	1.82	0.06
Rosaceae								
	Crataegus pedicellata Sarg.	2015	29.95	5.37	0.18	29.23	0.36	0.01
	Malus domestica L. cv.'Topaz'	2015	29.20	3.74	0.13	33.29	7.61	0.22
	Prunus avium L.	2016	27.88	2.94	0.11	33.79	8.82	0.26
	Prunus cerasifera Ehrh.	2015	29.63	4.69	0.16	28.79	2.72	0.09
	Prunus domestica L. subsp. *syriaca* Janich.	2015	29.15	2.79	0.10	28.85	2.52	0.09
	Prunus domestica subsp. *insititia* (L.)	2016	29.68	4.70	0.16	30.87	6.99	0.23
	Prunus persica L.	2015	29.92	6.16	0.21	33.85	8.77	0.26
Vitaceae								
	Vitis vinifera L. cv. 'Nelly'	2015	30.12	6.85	0.23	31.18	7.05	0.22
	Vitis vinifera L. cv. 'Silvana'	2015	28.20	5.04	0.18	30.49	3.04	0.10
Cornaceae								
	Cornus officinalis Siebold & Zucc.	2015	28.86	3.61	0.13	29.63	4.20	0.14

Table 6. continued

Moraceae							
Ficus carica L.	2015	26.28	2.79	0.11	30.90	3.34	0.11 [c]
Solanaceae							
Capsicum annuum L. cv. 'Kalocsai'	2016	25.99	4.31	0.17	30.38	3.09	0.10

a, The ACL, ΔACL, and ACL/ΔACL values were calculated based on aliphatic waxes from the green mature fruits;

b, The ACL, ΔACL, and ACL/ΔACL values were calculated based on aliphatic waxes from the turning mature fruit;

c, The ACL, ΔACL, and ACL/ΔACL values were calculated based on aliphatic waxes from adaxial leaf surfaces.

2.1 Cuticular waxes of *Olea europaea* L. cv. 'Arbequina'

2.1.1 Cuticular waxes of ripe fruits and fully expanded leaves

The wax coverage on fruit of *Olea europaea* L. cv. 'Arbequina' were 208.64 ± 17.94 µg cm^{-2}, 219.01 ± 28.66 µg cm^{-2}, and 149.20 ± 12.79 µg cm^{-2} of black ripe stage that sampled in 2014, 2015, and 2016, respectively. The adaxial leaf surfaces were covered by 277.10 ± 40.66 µg cm^{-2}, and 144.39 ± 16.97 µg cm^{-2} that sampled in 2014 and 2016, respectively (Table 7).

Very similar compositions and the proportion of each wax class were detected for both fruits and leaves that sampled in different years and places. The fruit wax composed of a higher proportion of cyclic components (63.7%, averaged in three years), in comparison to a lower content of very-long-chain aliphatics (29.6%, averaged in three years). The major leaf wax was cyclic compounds (89.2%, averaged in 2014 and 2016), and a minor portion of aliphatics (4.3%, averaged in 2014 and 2016).

In fruit, the primary alcohols were the most abundant aliphatic compounds (12.2% averaged in three years), followed by fatty acids (8.4%, averaged in three years), alkyl esters (2.8%, averaged in three years), and aldehydes (2.1%, averaged in three years). Very small amount of additional aliphatic components, e.g. *n*-alkanes, unsaturated alkyl esters, diacylglycerols, and methyl esters were detected (Figure 7 A-C). The distribution of carbon chain length of very-long-chain aliphatics ranged from C_{20} to C_{42}, the most abundant chain lengths were C_{24}, C_{26}, and C_{28}, which were dominated by primary alcohols and fatty acids (Figure 7 D-F). The ACL of aliphatics were 27.27, 26.36, and 27.37 sampled from 2014, 2015, and 2016, respectively (Table 6).

The major aliphatic components of adaxial leaf wax was *n*-alkanes (2.8% averaged in 2014 and 2016), followed by very small amount of primary alcohols, fatty acids, and methyl esters (Figure 7 A-C). The distribution of carbon chain lengths ranged from C_{20} to C_{33}, the most abundant chain lengths were C_{31} and C_{33}, which were dominated by *n*-hentriacotanes and *n*-tritriacotanes (Figure 7 D-F). The ACL value of leaves were 30.06 and 29.94 sampled in 2014 and 2016, respectively (Table 6).

The triterpenoids were the prominent cyclic compounds in both of fruit and adaxial leaf cuticular wax. The triterpenoids were dominated by oleanolic acid (33%, averaged from three years in fruit; 59.5% averaged from year of 2014 and 2016 in adaxial leaf), and maslinic acid. Ursolic acid was only detected in adaxial leaf surfaces. Other cyclic

components such as β-amyrin, erythrodiol, and uvaol; and small amount of sterols were also detected into relative small amount for both fruit and adaxial leaf waxes (Table 7).

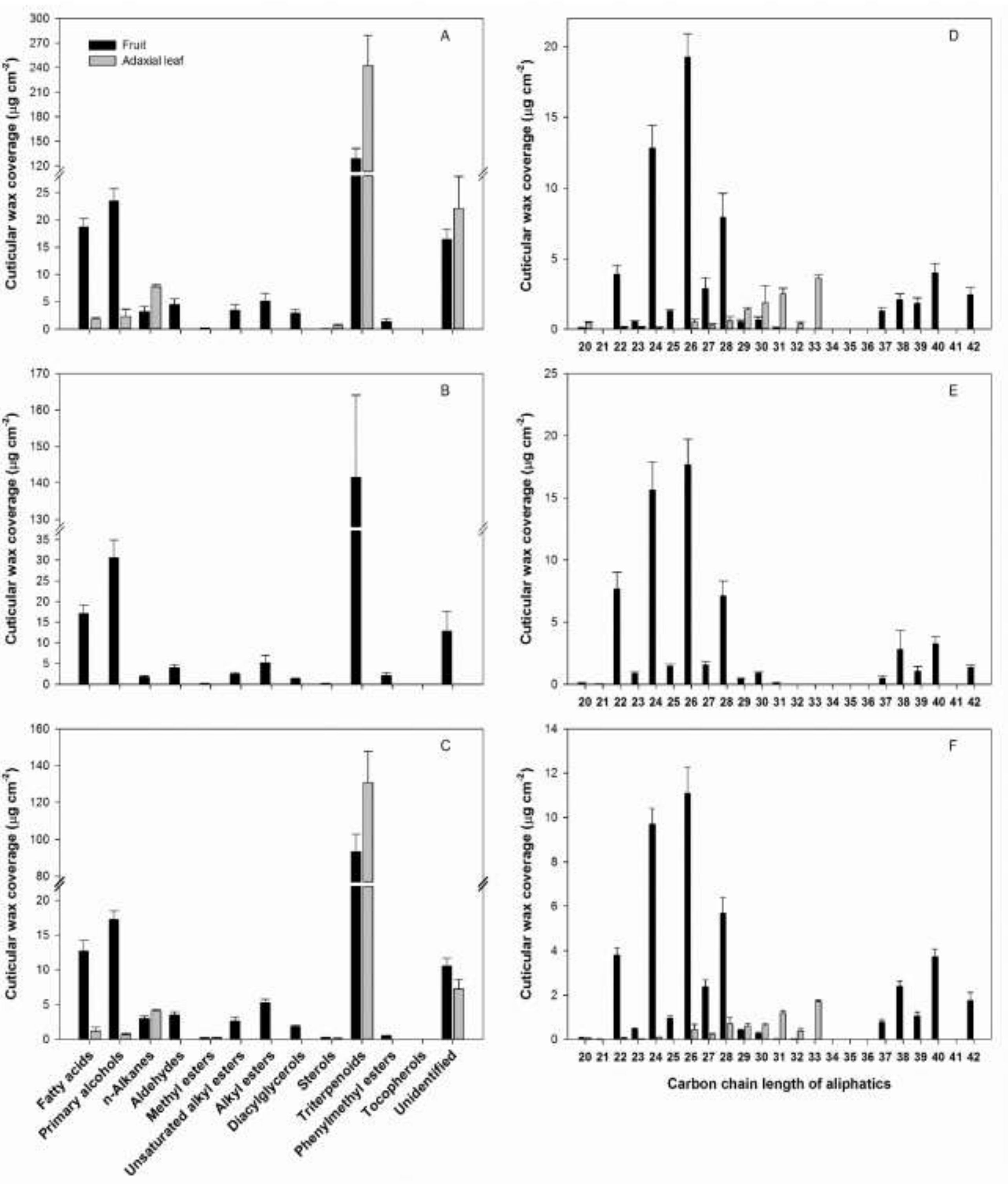

Figure 7. Cuticular wax compositions from *Olea europaea* L. cv. 'Arbequina' fruits and adaxial leaf surfaces. Cuticular wax compsoitions of fruits and leaves that sampled in (A) 2014, (B) 2015, and (C) 2016; the carbon chain length distribution of aliphatics sampled in (D) 2014, (E) 2015, and (F) 2016. Waxes were extracted from fresh fruit and adaxial leaf surfaces (mean values ± SD, n = 5).

Table 7. The cuticular wax load and compositions of *Olea europaea* L. cv. 'Arbequina' fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit			Adaxial leaf	
	2014	2015	2016	2014	2016
Fatty acids					
20	0.06 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	0.39 ± 0.04	0.04 ± 0.00
21					0.03 ± 0.03
22	0.77 ± 0.17	0.93 ± 0.14	0.54 ± 0.02	0.13 ± 0.05	
23	0.16 ± 0.03	0.23 ± 0.08	0.14 ± 0.03	0.00 ± 0.00	
24	3.92 ± 0.37	4.14 ± 0.41	2.61 ± 0.28	0.12 ± 0.03	0.04 ± 0.01
25	0.39 ± 0.11	0.45 ± 0.02	0.28 ± 0.05	0.00 ± 0.00	0.06 ± 0.05
26	9.07 ± 0.67	7.49 ± 1.15	5.66 ± 0.77	0.34 ± 0.13	0.25 ± 0.19
27	0.35 ± 0.09	0.30 ± 0.04	0.22 ± 0.03		0.04 ± 0.04
28	3.64 ± 1.34	3.13 ± 0.62	3.17 ± 0.42	0.40 ± 0.28	0.37 ± 0.25
29	0.08 ± 0.01	0.11 ± 0.10	0.06 ± 0.02	0.42 ± 0.13	
30	0.16 ± 0.11	0.21 ± 0.06			
Primary alcohols					
20	0.02 ± 0.00	0.03 ± 0.02	0.02 ± 0.01	0.09 ± 0.02	0.01 ± 0.01
21	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.01		
22	3.01 ± 0.53	6.64 ± 1.27	3.06 ± 0.32		0.03 ± 0.01
23	0.23 ± 0.04	0.48 ± 0.06	0.20 ± 0.01		
24	7.91 ± 1.11	10.77 ± 1.94	6.54 ± 0.44		0.05 ± 0.02
25	0.43 ± 0.02	0.63 ± 0.17	0.25 ± 0.01		
26	7.79 ± 0.66	8.03 ± 0.74	4.64 ± 0.42	0.15 ± 0.07	0.13 ± 0.04
27	0.42 ± 0.04	0.45 ± 0.05	0.21 ± 0.02		0.08 ± 0.06
28	3.29 ± 0.55	3.02 ± 0.43	2.12 ± 0.27	0.20 ± 0.09	0.15 ± 0.14
29	0.05 ± 0.03				
30	0.25 ± 0.16	0.43 ± 0.09	0.18 ± 0.06	1.88 ± 1.22	0.18 ± 0.03
n-Alkanes					
23	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.12 ± 0.05	0.03 ± 0.01
25	0.46 ± 0.06	0.36 ± 0.03	0.40 ± 0.05		
26			0.04 ± 0.00		
27	2.10 ± 0.66	0.83 ± 0.21	1.92 ± 0.29	0.25 ± 0.12	0.11 ± 0.03
28			0.05 ± 0.02		
29	0.40 ± 0.13	0.28 ± 0.09	0.34 ± 0.05	0.99 ± 0.05	
30			0.02 ± 0.00		0.19 ± 0.09

Table 7. continued

31	0.11 ± 0.03	0.12 ± 0.03	0.04 ± 0.01	2.50 ± 0.40	
32			0.04 ± 0.01	0.37 ± 0.17	0.37 ± 0.11
33				3.58 ± 0.25	1.70 ± 0.06
Aldehydes					
20	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01		
22	0.12 ± 0.05	0.08 ± 0.02	0.19 ± 0.03		
24	0.99 ± 0.26	0.72 ± 0.18	0.47 ± 0.12		
25			0.03 ± 0.01		
26	2.23 ± 0.46	1.96 ± 0.33	0.63 ± 0.11		
27			0.02 ± 0.01		
28	0.97 ± 0.26	0.95 ± 0.25	0.34 ± 0.07		
30	0.23 ± 0.11	0.25 ± 0.01	0.04 ± 0.01		
Methyl esters					
24			0.09 ± 0.04		0.07 ± 0.01
26	0.17 ± 0.09	0.18 ± 0.03	0.12 ± 0.02		0.13 ± 0.02
28					0.07 ± 0.01
Unsaturated alkyl esters					
40	1.65 ± 0.27	1.27 ± 0.31	1.06 ± 0.28		
42	1.82 ± 0.97	1.16 ± 0.16	1.52 ± 0.37		
Alkyl esters					
38	2.08 ± 0.44	2.82 ± 1.53	2.39 ± 0.23		
39	0.11 ± 0.07	0.29 ± 0.24	0.00 ± 0.00		
40	2.34 ± 0.40	1.97 ± 0.42	2.66 ± 0.27		
42	0.61 ± 0.85	0.17 ± 0.04	0.22 ± 0.02		
Diacylglycerols					
37	1.27 ± 0.28	0.50 ± 0.16	0.77 ± 0.11		
39	1.71 ± 0.37	0.76 ± 0.17	1.06 ± 0.17		
Sum aliphatic components					
	61.55 ± 5.94	64.26 ± 9.20	44.55 ± 3.34	11.93 ± 1.75	6.19 ± 0.49
	29.5%	**29.3%**	**29.9%**	**4.3%**	**4.3%**
Sterols					
cholesterol			0.04 ± 0.02		
ß-sitosterol	0.08 ± 0.03	0.19 ± 0.09	0.22 ± 0.02	0.69 ± 0.23	0.12 ± 0.04
Triterpenoids					
ß-amyrin	0.19 ± 0.07	0.43 ± 0.12	0.13 ± 0.03	0.39 ± 0.07	0.16 ± 0.04

Table 7. continued

α-amyrin					0.14 ± 0.04
erythrodiol	2.84 ± 0.37	2.46 ± 0.60	2.60 ± 0.45	21.55 ± 13.17	8.90 ± 2.60
uvaol	0.72 ± 1.48	1.05 ± 0.31	0.68 ± 0.17	24.48 ± 9.23	11.45 ± 3.27
oleanolic acid	72.84 ± 8.71	67.27 ± 13.32	50.01 ± 5.77	160.11 ± 23.33	88.40 ± 7.87
betulinic acid	1.39 ± 0.22	1.73 ± 0.46	0.90 ± 0.21	1.26 ± 0.17	1.53 ± 0.19
ursolic acid				25.88 ± 3.32	11.53 ± 2.38
maslinic acid	27.59 ± 8.89	18.86 ± 15.07	20.34 ± 2.71	3.69 ± 1.32	4.58 ± 1.64
other triterpenoids	23.61 ± 8.73	49.78 ± 11.96	18.69 ± 1.63	5.07 ± 2.24	4.12 ± 1.19
Phenylmethyl esters					
26	0.86 ± 0.29	1.03 ± 0.50	0.14 ± 0.05		
28	0.55 ± 0.22	1.04 ± 0.85	0.34 ± 0.02		
Tocopherols					
α-tocopherol			0.03 ± 0.00		
Sum cyclic components					
	130.68 ± 12.09	143.82 ± 22.70	94.13 ± 9.48	243.12 ± 36.89	130.93 ± 16.87
	62.6%	**65.5%**	**63.0%**	**87.7%**	**90.6%**
Unidentified					
	16.41 ± 1.92	12.82 ± 4.80	10.53 ± 1.16	22.05 ± 5.87	7.27 ± 1.32
Total wax	208.64 ± 17.94	219.01 ± 28.66	149.20 ± 12.79	277.10 ± 40.66	144.39 ± 16.97

2.1.2 Cuticular waxes of fruit in different developmental stages

The total wax coverage of *Olea europaea* L. cv. 'Arbequina' fruits in green and turning stages were sampled in 2014 and 2016 (Table 8). As a high accumulation of total wax on the black stage fruit (208.64 ± 17.94 μg cm^{-2}, Table 7), the coverage of wax was very similar on the fruit in mature green (194.61 ± 22.84 μg cm^{-2}), and turning (201.61 ± 19.65 μg cm^{-2}) stages sampled in 2014. Similarly, very closed coverage of wax on the mature green (153.76 μg cm^{-2}), turning (148.24 μg cm^{-2}) and black stage fruits (149.20 ± 12.79 μg cm^{-2}, Table 7) were detected in 2016.

With the development of fruit, the accumulation of aliphatics increased slightly from 21.5% in green (26.1%, in turning stage) mature fruit to 29.5% in black fruit sampled in 2014. This was further indicated by the samples performed in 2016, which increased from 21.7% in green to 29.3% in black fruits. The accumulation of triterpenoids showed a slight decrease from green stage of 74.1% to 61.9% of black fruits that sampled in 2014. Similar change trend was found in the samples sampled in 2016, which decreased from green fruit of 73.2% to 62.5% in the black fruit (Figure 8 A and B).

The major aliphatic compounds, the primary alcohols increased from 9.6% to 11.6% in 2014 and from 9.2% to 11.2% in 2016. The fatty acids kept relative stable around 7.7% (averaged in three stages). The *n*-alkanes accumulated from 1.0% to 2.0% in 2014, and from 0.6% to 1.5% in 2016. The alkyl esters increased apparently with the development of fruit from 0.8% in green mature to 3.6% in black fruit. This change trend was also detected from 0.3% to 2.5% in the samples that performed in 2016.

The distribution of carbon chain length of very-long-chain aliphatics ranged from C_{20} to C_{42}, the most abundant chain lengths were C_{24}, C_{26}, and C_{28}, which were dominated by primary alcohols and fatty acids (Figure 2 C and D). The ACL value of aliphatics for green and turning stage fruit were 26.07, and 26.55 sampled in 2014, and were 26.04 and 26.96 sampled in 2016, respectively (Table 6).

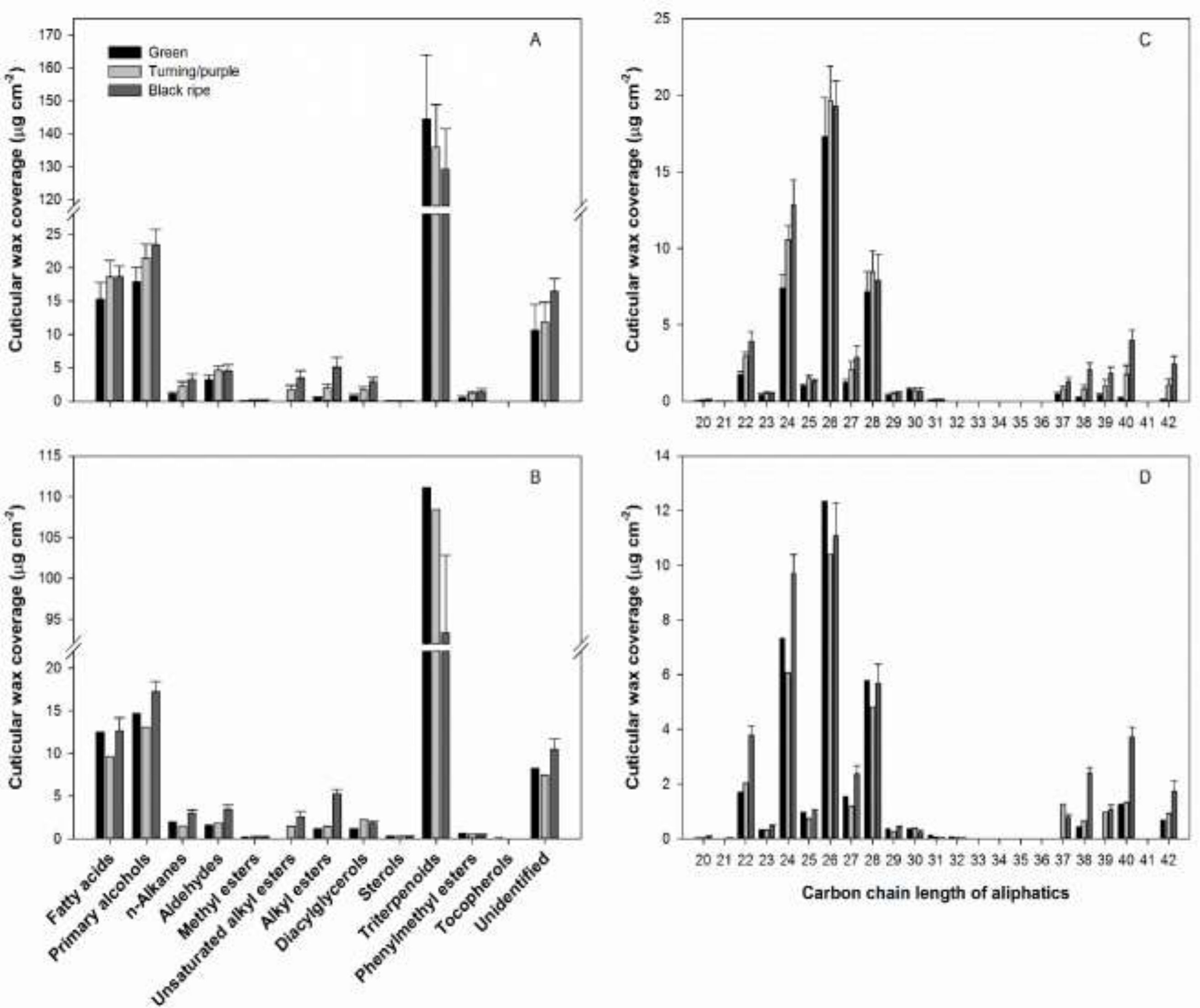

Figure 8. Cuticular wax compositions from olive (*Olea europaea* L. cv. 'Arbequina') fruits in green, turning, black developmental stages. Cuticular wax compound classes of fruits in different developmental stages that sampled in (A) 2014 and (B) 2016; the carbon chain length distribution of aliphatics sampled in (C) 2014, and (D) 2016. Waxes were extracted from fresh fruit and adaxial leaf surfaces (mean values ± SD, n = 5).

Table 8. The cuticular wax load and compositions of *Olea europaea* L. cv. ‘Arbequina’ fruits in green and turning developmental stages that sampled in 2014 and 2016. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	2014		2016	
	Green	Turning	Green [a]	Turning [a]
Fatty acids				
20	0.04 ± 0.01	0.04 ± 0.01	0.02	0.01
22	0.31 ± 0.09	0.58 ± 0.07	0.32	0.38
23	0.11 ± 0.02	0.16 ± 0.05	0.12	0.12
24	2.87 ± 0.35	3.54 ± 0.35	2.38	1.93
25	0.36 ± 0.07	0.47 ± 0.06	0.27	0.23
26	7.80 ± 1.27	9.00 ± 1.21	6.14	4.53
27	0.20 ± 0.10	0.37 ± 0.10	0.19	0.17
28	3.28 ± 0.80	4.07 ± 0.81	2.99	2.21
29	0.06 ± 0.02	0.15 ± 0.15	0.07	0.05
30	0.20 ± 0.05	0.23 ± 0.18		
Primary alcohols				
20	0.01 ± 0.01	0.02 ± 0.02	0.01	0.01
21	0.02 ± 0.00	0.02 ± 0.00	0.01	0.02
22	1.35 ± 0.22	2.36 ± 0.14	1.34	1.57
23	0.22 ± 0.10	0.23 ± 0.04	0.14	0.12
24	4.17 ± 0.58	6.33 ± 0.75	4.56	3.77
25	0.45 ± 0.07	0.62 ± 0.21	0.28	0.23
26	7.67 ± 0.95	8.11 ± 0.79	5.56	4.79
27	0.58 ± 0.16	0.43 ± 0.04	0.26	0.23
28	3.08 ± 0.37	3.12 ± 0.44	2.27	2.06
30	0.33 ± 0.15	0.18 ± 0.10	0.29	0.29
n-Alkanes				
23	0.05 ± 0.01	0.12 ± 0.06	0.06	0.08
25	0.21 ± 0.04	0.45 ± 0.06	0.30	0.24
26			0.03	0.02
27	0.45 ± 0.12	1.26 ± 0.49	1.06	0.78
28			0.03	0.05
29	0.32 ± 0.06	0.33 ± 0.08	0.29	0.22
30			0.04	0.02
31	0.09 ± 0.02	0.10 ± 0.03	0.13	0.05
32			0.06	0.04
Aldehydes				
20				0.01
22	0.04 ± 0.02	0.07 ± 0.03	0.05	0.08
24	0.38 ± 0.17	0.67 ± 0.12	0.31	0.24
25			0.10	0.04
26	1.73 ± 0.44	2.34 ± 0.43	0.54	0.91
27			0.05	0.01
28	0.81 ± 0.22	1.26 ± 0.18	0.51	0.50
30	0.23 ± 0.08	0.32 ± 0.07	0.04	0.07
Methyl esters				
24			0.08	0.12
26	0.10 ± 0.02	0.20 ± 0.03	0.08	0.15
Unasturated alkyl esters				
40		0.81 ± 0.30		0.64
42		0.92 ± 0.35		0.82

Table 8. continued

Alkyl esters								
38	0.20	±	0.08	0.81	±	0.25	0.43	0.67
39				0.12	±	0.11		
40	0.22	±	0.03	0.95	±	0.30	0.61	0.68
42	0.11	±	0.02	0.16	±	0.02	0.14	0.11
Diacylglycerols								
37	0.44	±	0.15	0.83	±	0.19	0.64	1.27
39	0.38	±	0.11	0.88	±	0.42	0.54	0.97
Sum aliphatic components								
	41.77	±	5.85	52.65	±	6.18	33.34	31.48
		21.5%			26.1%		21.7%	21.2%
Sterols								
cholesterol							0.07	0.05
ß-sitosterol	0.08	±	0.04	0.06	±	0.03	0.24	0.24
Triterpenoids								
ß-amyrin	0.17	±	0.12	0.12	±	0.05	0.29	0.21
erythrodiol	3.56	±	1.18	3.64	±	0.90	4.24	3.42
uvaol	0.68	±	0.47	0.50	±	0.58	0.50	0.58
oleanolic acid	72.51	±	10.37	70.11	±	8.98	59.60	57.99
betulinic acid	1.48	±	0.17	1.64	±	0.21	1.02	0.91
maslinic acid	27.76	±	8.63	20.11	±	3.71	36.90	25.88
other triterpenoids	38.29	±	8.66	39.71	±	5.95	8.60	19.47
Phenylmethyl esters								
26	0.37	±	0.15	0.71	±	0.17	0.18	0.18
28	0.18	±	0.09	0.53	±	0.13	0.41	0.35
Tocopherols								
α-tocopherol							0.10	0.03
Sum cyclic components								
	145.17	±	19.32	137.12	±	13.07	112.15	109.31
		74.5%			68.0%		72.9%	73.7%
Unidentified								
	10.69	±	3.82	11.84	±	2.97	8.26	7.45
Total wax	194.61	±	22.84	201.61	±	19.65	153.76	148.24

a, the waxes of fruit in green and turning stages growing in 2016 were extracted once as reference.

2.2 Cuticular waxes of *Olea europaea* subsp. *europaea* var. *sylvestris*

The total wax coverage of *Olea europaea* subsp. *europaea* var. *sylvestris* fruit was 169.85 ± 25.22 µg cm^{-2} that sampled in 2016. The adaxial leaf surfaces were covered by 242.16 ± 38.48 µg cm^{-2} sampled in 2015, and by 252.61 ± 17.56 µg cm^{-2} sampled in 2016 (Table 9). Similar as waxes of *Olea europaea* L. cv. 'Arbequina', triterpenoids dominated the waxes of fruit (64.7%, 110.22 ± 19.39 µg cm^{-2}) and adaxial leaf surfaces (81.6%, 206.38 ± 20.96 µg cm^{-2} in 2016; and 89.6%, 216.80 ± 32.81 µg cm^{-2} in 2015). The minor portion of aliphatics was 28.7% (48.53 ± 6.52 µg cm^{-2}) in fruit, and were 3.3% (8.27 ± 1.73 µg cm^{-2}) and 6.5% (15.97 ± 4.65 µg cm^{-2}) in adaxial leaf surfaces that sampled in 2016 and 2015, respectively.

The main aliphatic components were fatty acids (9.4%) and primary alcohols (8.6%), followed by aldehydes (6.1%), *n*-alkanes (2.3%), and alkyl esters (1.8%) in olive fruit. Very small amount of additional aliphatic components, e.g. unsaturated alkyl esters, diacylglycerols and methyl esters were detected (Figure 9 A and B). The distribution of carbon chain length of aliphatics ranged from C_{20} to C_{42}, the most abundant chain lengths were C_{24}, C_{26}, and C_{28}, which were dominated by octacosanoic acid and hexacosanol (Figure 9. C and D). The ACL value of aliphatics were 26.91 (Table 6).

The major aliphatic components of adaxial leaf wax was *n*-alkanes (2.7% in 2016 and 5.3% in 2015), followed by very small amount of primary alcohols, fatty acids, and methyl esters (Figure 9. A and B). The distribution of carbon chain lengths ranged from C_{20} to C_{33}, the most abundant chain lengths were C_{31}, and C_{33}, which were dominated by *n*-hentriacontane and *n*-tritriacontane (Figure 9. C and D). The ACL value of aliphatics from leaves were 30.30 and 30.32 sampled in 2015 and 2016, respectively (Table 6).

Triterpenoids were the prominent cyclic compounds in both of fruit and leaf wax. The triterpenoids were dominated by oleanolic acid (32.2%, 54.76 ± 12.94 µg cm^{-2} for fruits; 51%, 137.46 ± 21.52 µg cm^{-2} for leaves sampled in 2016; and 54%, 151.87 ± 29.47 µg cm^{-2} for leaves sampled in 2015). Ursolic acid (4% to 5% of total wax) was detected in leaf waxes and only traces were detected for fruit waxes. Very small amount of triterpenoid alcohols such as β-amyrin, erythrodiol, and uvaol were detected in both of fruit and adaxial leaf surfaces. Small amount of tocopherols, and phenylmethyl esters were only found in fruit waxes (Table 9).

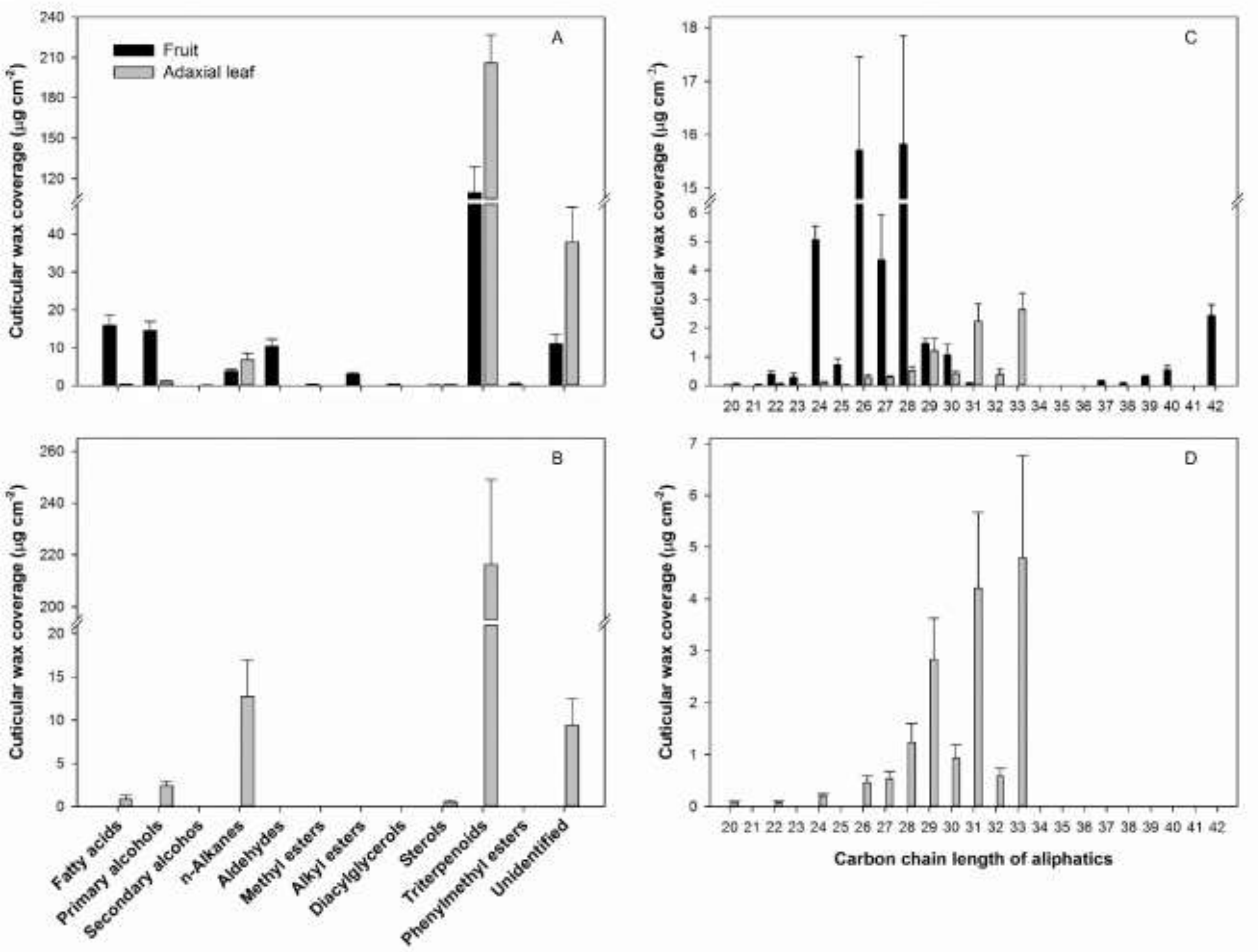

Figure 9. Cuticular wax compositions from *Olea europaea* subsp. *europaea* var. *sylvestris* fruits and adaxial leaves. Cuticular wax compositions of fruits and leaves sampled in (A) 2016, and (B) 2015; the carbon chain length distribution of aliphatics sampled in (C) 2016, and (D) 2015. Waxes were extracted from fresh fruit and adaxial leaf surfaces (mean values ± SD, n = 5).

Table 9. The cuticular wax load and compositions of *Olea europaea* subsp. *europaea* var. *sylvestris* fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Adaxial leaf	
	2016	2016	2015
Fatty acids			
20	traces	0.06 ± 0.01	0.04 ± 0.01
21	traces	traces	
22	0.14 ± 0.07		
23	0.26 ± 0.12		
24	1.63 ± 0.27	0.04 ± 0.02	0.06 ± 0.03
25	0.37 ± 0.02	0.02 ± 0.02	
26	6.21 ± 1.01	0.15 ± 0.10	0.21 ± 0.11
27	0.39 ± 0.05	0.05 ± 0.01	0.12 ± 0.04
28	6.83 ± 0.92	traces	0.43 ± 0.28
29	0.14 ± 0.08		
30	0.17 ± 0.09		
Primary alcohols			
20		traces	0.05 ± 0.01
22	0.20 ± 0.04	0.06 ± 0.01	0.08 ± 0.03
23	0.03 ± 0.02		
24	1.70 ± 0.26	0.07 ± 0.03	0.14 ± 0.04
25	0.20 ± 0.07		
26	5.87 ± 0.65	0.13 ± 0.03	0.25 ± 0.17
27	1.33 ± 1.14	0.15 ± 0.07	0.23 ± 0.10
28	4.84 ± 0.56	0.51 ± 0.14	0.81 ± 0.22
29			0.31 ± 0.08
30	0.41 ± 0.22	0.08 ± 0.02	0.54 ± 0.18
Secodary alcohols			
29 (pos.2)		0.07 ± 0.04	
n-Alkanes			
23	0.05 ± 0.01	traces	0.02 ± 0.02
25	0.25 ± 0.02		
26	0.02 ± 0.01		
27	2.35 ± 0.36	0.08 ± 0.03	0.21 ± 0.05
29	1.11 ± 0.14	1.14 ± 0.42	2.53 ± 0.73
30		0.33 ± 0.08	0.40 ± 0.09
31	0.08 ± 0.02	2.23 ± 0.60	4.21 ± 1.46
32		0.39 ± 0.20	0.59 ± 0.14
33		2.64 ± 0.57	4.79 ± 1.98
Aldehydes			
22	0.07 ± 0.01		
24	1.74 ± 0.21		
26	3.30 ± 0.90		
27	0.37 ± 0.13		
28	4.14 ± 0.84		
29	0.21 ± 0.07		
30	0.48 ± 0.17		
Methyl esters			
26	0.18 ± 0.03		
Unsaturated alkyl esters			
40 (:1)	0.29 ± 0.06		
42 (:2)	1.48 ± 0.24		
42 (:1)	0.87 ± 0.18		

Table 9. continued

Alkyl esters									
38	0.07	±	0.02						
40	0.27	±	0.07						
42	0.07	±	0.03						
Diacylglycerols									
37 (R1 16+R2 18:1)	0.15	±	0.04						
39 (R1 18:1+R2 18:1)	0.31	±	0.05						
Sum aliphatic components									
	48.53	±	6.52	8.27	±	1.73	15.97	±	4.65
		28.7%			**3.3%**			**6.5%**	
Sterols									
β-Sitosterol	0.18	±	0.03	0.28	±	0.04	0.50	±	0.23
Triterpenoids									
β-amyrin	0.15	±	0.03	0.50	±	0.37	0.57	±	0.16
α-amyrin				0.26	±	0.06			
erythrodiol	4.15	±	4.64	25.18	±	7.73	18.56	±	7.53
uvaol	3.82	±	2.21	14.28	±	7.31	22.88	±	15.59
oleanolic acid mthylester	0.83	±	0.50	2.87	±	2.36			
oleanolic acid	54.76	±	12.94	137.46	±	21.52	151.87	±	29.47
betulinic acid	0.72	±	0.29				2.37		1.03
ursolic acid	0.37	±	0.12	10.15	±	1.24	12.28	±	2.50
maslinic acid	27.98	±	10.06	5.93	±	3.89	7.50	±	4.00
other triterpenoids	16.79	±	12.69	9.46	±	7.47	0.28	±	0.10
Phenylmethyl esters									
26	0.27	±	0.12						
28	0.20	±	0.01						
Total	0.47	±	0.11						
Tocopherols									
α-tocopherol	traces								
Sum cyclic components									
	110.22	±	19.39	206.38	±	20.96	216.80	±	32.81
		64.7%			**81.6%**			**89.6%**	
Unidentified									
	11.11	±	2.40	38.70	±	9.19	9.38		3.09
Total wax	169.85	±	25.22	252.61	±	17.56	242.16	±	38.48

2.3 Cuticular waxes of *Ligustrum vulgare* L.

The total wax coverage of *Ligustrum vulgare* L. fruit was 148.09 ± 11.44 μg cm^{-2}. The overall coverage of wax on leaf surfaces was 26.18 ± 4.39 μg cm^{-2}. The leaf waxes were distributed by 29.09 ± 6.51 μg cm^{-2} and 23.27 ± 2.80 μg cm^{-2} on the ab- and adaxial surfaces, respectively (Table 10). Cyclic components were the main waxes on both fruit (78%, 115.76 ± 13.80 μg cm^{-2}) and leaf (72.5%, 19.13 ± 3.31 μg cm^{-2}) surfaces. The minor portion of aliphatics was 12.5% (18.37 ± 4.64 μg cm^{-2}) for fruit wax, and was 16.5% (4.29 ± 0.68 μg cm^{-2}) for leaf wax.

The main aliphatic components were *n*-alkanes (8.8%, 13.02 ± 3.31 μg cm^{-2} for fruit, 9.8%, 2.57± 0.42 μg cm^{-2} for leaf) followed by fatty acids (2.7% for fruit, 2.9% forleaf) and primary alcohols (0.9% for fruit, 3.1% for leaf). Small amount of secondary alcohols, aldehydes, and iso- and anteiso *n*-alkanes were detected on adaxial leaf but not on fruit surfaces (Figure 10 A). Carbon chain lengths ranged from C_{20} to C_{35}, the most abundant chain lengths were C_{31} for fruit, and were C_{31} and C_{33} for leaf aliphatic waxes (Figure 10 B). The ACL value of aliphatics was 28.46 for fruit, and was 30.01 for leaf (Table 6).

Triterpenoids were the prominent cyclic components in both of fruit (77.9%, 115.33 ± 13.80 μg cm^{-2}) and leaf (72.8%, 19.07 ± 3.27 μg cm^{-2} of leaf) waxes. The triterpenoids were dominated by ursolic acid (37.9%, 56.19 ± 8.37 μg cm^{-2} for fruit; 47.2%, 12.36 ± 1.96 μg cm^{-2} for leaf), and oleanolic acid (16.9%, 25.08 ± 4.39 μg cm^{-2} for fruit; 15.4%, 4.03 ± 0.88 μg cm^{-2} for leaf). Maslinic acid and corosolic acid were only detected in fruit waxes. Very small amount of α-amyrin, erythrodiol, uvaol, betulic acid, and β-sterols were also found in both of fruit and leaf waxes (Table 10).

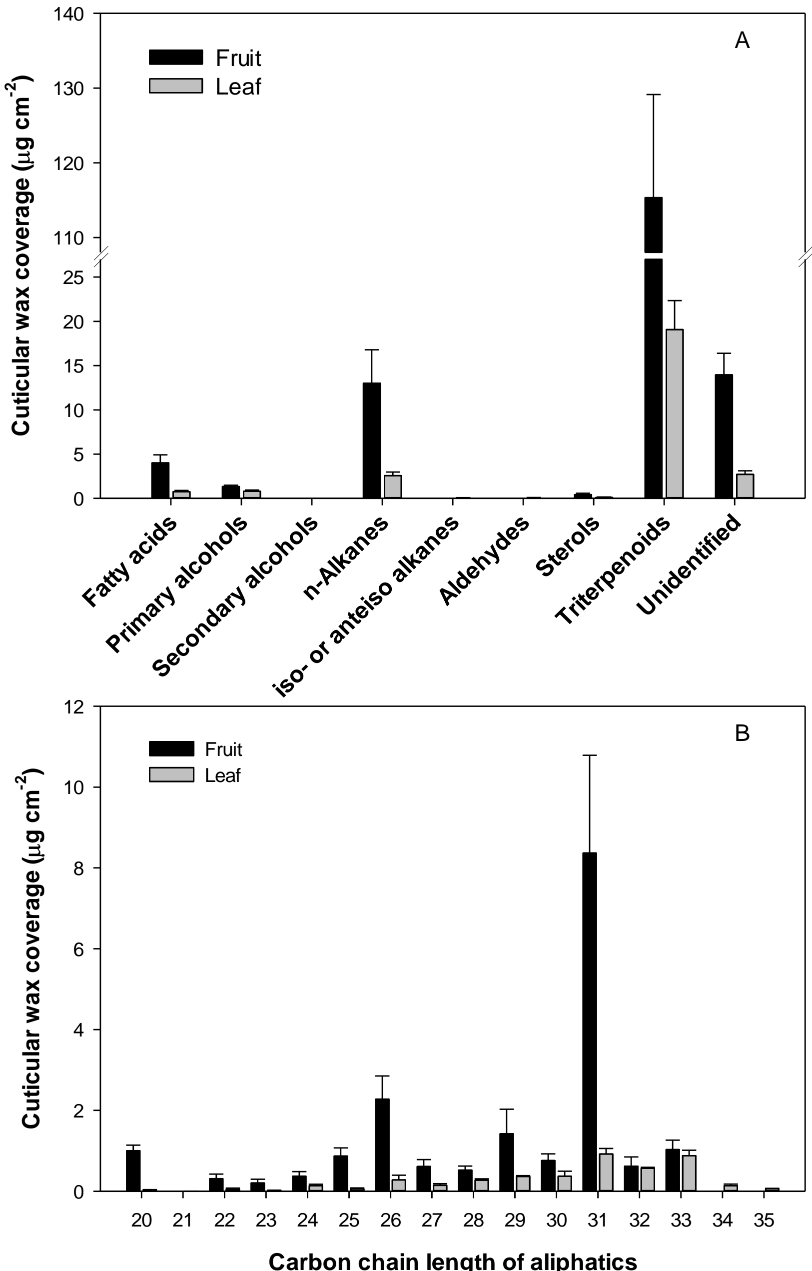

Figure 10. Cuticular wax compositions from *Ligustrum vulgare* L. fruits and leaves. (A) Cuticular wax compositions of privet fruits and leaves; (B) the carbon chain length distribution of aliphatics. Fruit waxes were extracted from isolated cuticular membranes. Leaf waxes were extracted from fresh ad- and abaxial leaf surfaces (mean values ± SD, n = 5).

Table 10. The cuticular wax coverage and compositions of *Ligustrum vulgare* L. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	1.00 ± 0.14	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.02
21		traces	traces	
22	0.17 ± 0.04	0.03 ± 0.03	0.01 ± 0.00	0.07 ± 0.05
23		traces	traces	
24	0.21 ± 0.06	0.07 ± 0.04	0.09 ± 0.03	0.07 ± 0.04
25	0.28 ± 0.13	0.01 ± 0.00	0.01 ± 0.00	
26	1.87 ± 0.44	0.16 ± 0.07	0.14 ± 0.06	0.18 ± 0.10
27	0.12 ± 0.04	0.01 ± 0.00	0.01 ± 0.00	
28	0.26 ± 0.07	0.16 ± 0.04	0.12 ± 0.04	0.21 ± 0.05
29		0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
30		0.12 ± 0.04	0.07 ± 0.04	0.18 ± 0.08
31		0.01 ± 0.00	0.02 ± 0.01	
32	0.15 ± 0.08	0.14 ± 0.04	0.10 ± 0.05	0.19 ± 0.04
33		0.01 ± 0.00	0.01 ± 0.00	
34		0.01 ± 0.01	0.02 ± 0.02	
Primary alcohols				
20		traces	0.01 ± 0.00	
22	0.13 ± 0.08	0.02 ± 0.00	0.03 ± 0.01	
24	0.16 ± 0.06	0.07 ± 0.03	0.03 ± 0.02	0.11 ± 0.06
25	0.13 ± 0.08	traces	0.01 ± 0.00	
26	0.26 ± 0.11	0.12 ± 0.06	0.09 ± 0.02	0.15 ± 0.13
27	0.15 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	
28	0.27 ± 0.06	0.11 ± 0.01	0.10 ± 0.02	0.12 ± 0.03
29		0.01 ± 0.00	0.02 ± 0.01	
30	0.29 ± 0.03	0.10 ± 0.02	0.10 ± 0.03	0.09 ± 0.01
31		0.01 ± 0.00	0.03 ± 0.01	
32		0.28 ± 0.02	0.23 ± 0.05	0.33 ± 0.06
33		0.01 ± 0.00	0.02 ± 0.01	
34		0.06 ± 0.02	0.02 ± 0.01	0.11 ± 0.04
Secondary alcohols (pos.2)				
27		traces	0.01 ± 0.00	
29		traces	0.01 ± 0.00	
33		0.01 ± 0.00	0.01 ± 0.00	
n-Alkanes				
23	0.20 ± 0.09	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
25	0.46 ± 0.19	0.05 ± 0.03	0.09 ± 0.05	
26	0.14 ± 0.06			
27	0.40 ± 0.13	0.13 ± 0.06	0.07 ± 0.05	0.19 ± 0.10
28		traces	0.01 ± 0.00	
29	1.42 ± 0.61	0.33 ± 0.05	0.46 ± 0.05	0.21 ± 0.05
30	0.47 ± 0.16	0.14 ± 0.05	0.10 ± 0.03	0.18 ± 0.12
31	8.37 ± 2.42	0.88 ± 0.14	1.11 ± 0.27	0.65 ± 0.07
32	0.52 ± 0.17	0.10 ± 0.02	0.08 ± 0.02	0.11 ± 0.04
33	1.03 ± 0.23	0.84 ± 0.17	1.02 ± 0.20	0.67 ± 0.14
34		0.02 ± 0.00	0.03 ± 0.01	
35		0.06 ± 0.01	0.13 ± 0.02	
iso- & anteiso alkanes				
29		traces	0.01 ± 0.00	
31		0.02 ± 0.00	0.04 ± 0.01	
32		0.02 ± 0.00	0.04 ± 0.00	
33		0.02 ± 0.00	0.04 ± 0.00	
34		0.02 ± 0.00	0.04 ± 0.00	

Table 10. continued

Aldehyded				
30		0.01 ± 0.00	0.02 ± 0.01	
32		0.03 ± 0.02	0.06 ± 0.04	
34		0.02 ± 0.01	0.05 ± 0.03	
Sum aliphatic components				
	18.37 ± 4.64	4.29 ± 0.68	4.80 ± 0.86	3.79 ± 0.53
	12.5%	**16.5%**	**16.7%**	**16.3%**
Sterols				
ß-sitosterol	0.43 ± 0.15	0.11 ± 0.06	0.04 ± 0.02	0.18 ± 0.10
Triterpenoids				
α-amyrin	0.23 ± 0.20	0.05 ± 0.03		0.10 ± 0.05
erythrodiol	0.33 ± 0.08	0.24 ± 0.06	0.35 ± 0.10	0.12 ± 0.02
uvaol	1.03 ± 0.28	0.20 ± 0.06	0.18 ± 0.04	0.21 ± 0.10
oleanolic acid methyl ester		0.11 ± 0.03	0.23 ± 0.05	
oleanolic aldehyde		0.51 ± 0.28	1.28 ± 0.65	
oleanolic acid	25.08 ± 4.39	4.03 ± 0.88	5.17 ± 1.68	2.90 ± 0.30
betulinic acid	0.44 ± 0.13	0.31 ± 0.05	0.35 ± 0.10	0.27 ± 0.09
ursolic acid	56.19 ± 8.37	12.36 ± 1.96	13.90 ± 3.55	10.83 ± 1.43
maslinic acid	5.74 ± 2.40			
corosolic acid	17.04 ± 7.20			
other triterpenoids	9.24 ± 5.58	1.26 ± 0.52	1.01 ± 0.50	1.51 ± 0.57
Sum cyclic components				
	115.76 ± 13.80	19.13 ± 3.31	22.24 ± 5.43	16.02 ± 1.88
	78.0%	**72.5%**	**76.2%**	**68.9%**
Unidentified				
	13.96 ± 2.44	2.71 ± 0.41	2.06 ± 0.32	3.36 ± 0.52
Total wax	148.09 ± 11.44	26.18 ± 4.39	29.09 ± 6.51	23.27 ± 2.80

2.4 Cuticular waxes of *Averrhoa carambola* L.

The total wax coverage of *Averrhoa carambola* L. fruit was 47.31 ± 6.92 μg cm^{-2}. The overall coverage of waxes on leaf surfaces was 19.35 ± 5.67 μg cm^{-2}. The leaf waxes were distributed by 22.60 ± 9.16 μg cm^{-2} and by 16.09 ± 2.82 μg cm^{-2} on the ad- and abaxial surfaces, respectively (Table 11). The aliphatic compounds dominated the fruit wax (68.3%, 32.37 ± 5.42 μg cm^{-2}) and leaf waxes (85.2%, 16.58 ± 5.02 μg cm^{-2}). Very small amount of cyclic components were detected in either of fruit (5.9%, 2.76 ± 0.29 μg cm^{-2}) or leaf (1.2%) waxes.

The main aliphatic components of fruit waxes were *n*-alkenes (25.3%, 12.27 ± 4.80 μg cm^{-2}) followed by *n*-alkanes (19.2%, 9.02 ± 0.77 μg cm^{-2}), primary alcohols (8.0%), fatty acids (5.9%), unsaturated alkyl esters (4.7%), alkyl esters (4.0%), and aldehydes (3.2%). The predominant leaf aliphatic components were *n*-alkanes (39.2%, 8.62 ± 4.72 μg cm^{-2}) followed by primary alcohols (34.1%, 5.88 ± 0.81 μg cm^{-2}), aldehydes (5.9%), fatty acids (3.1%), and alkyl esters (2.9%) (Figure 11 A). Carbon chain lengths ranged from C_{20} to C_{50} for fruit and to C_{52} for leaf aliphatic waxes. The most abundant aliphatics were 9/12-tricosene (C_{23}, 9.6%, 3.93 ± 0.56 μg cm^{-2}) and 9/12-pentacosene (C_{25}, 11.7%, 5.53 ± 2.10 μg cm^{-2}) for fruit wax, and were 1-triacontanol (C_{30}, 22.7%, 4.40 ± 0.65 μg cm^{-2}) and *n*-hentriacontane (C_{31}, 27.9%, 5.40 ± 3.03 μg cm^{-2}) in leaf wax (Figure 11 B). The ACL value of aliphatics was 26.22 for fruit, and was 30.85 for leaf (Table 6).

The cyclic compounds were distributed by small amount of α-amyrin, β-sitosterol and campesterol in both of fruit and leaf wax (Table 11).

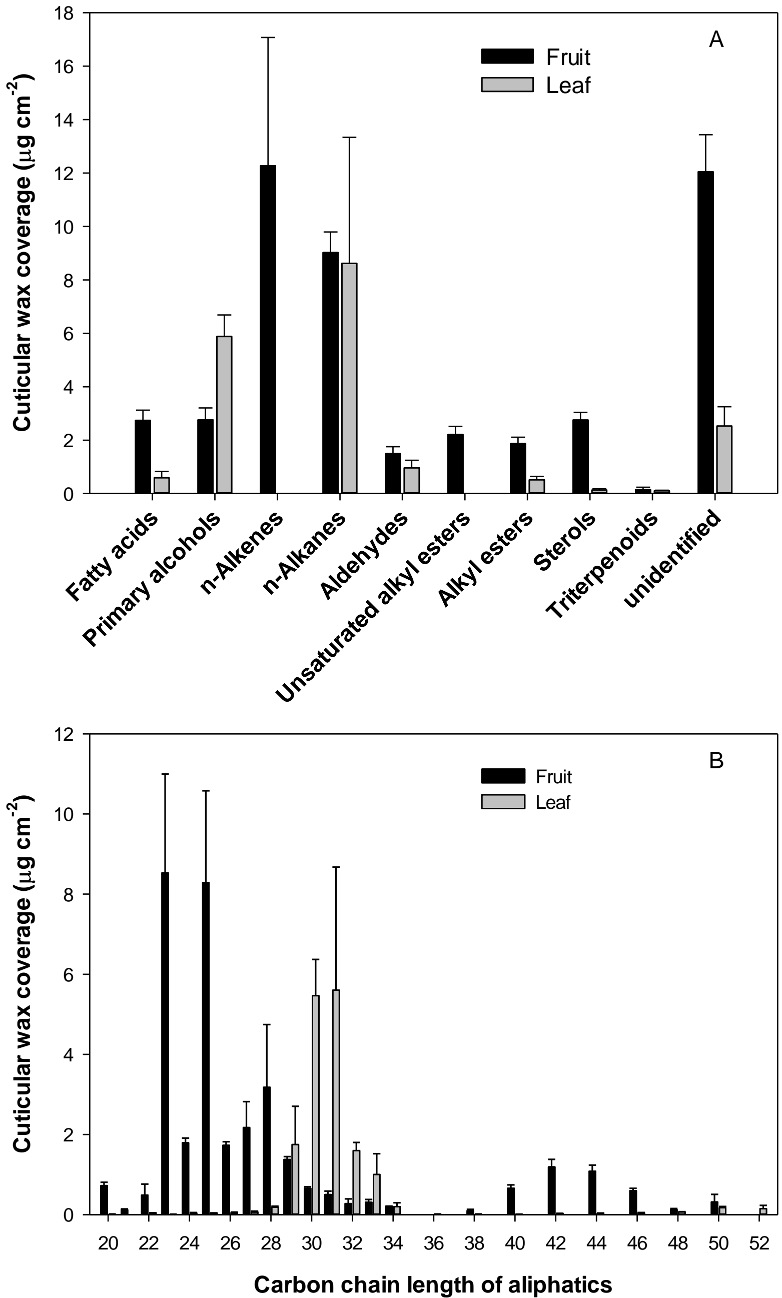

Figure 11. Cuticular wax compositions from *Averrhoa carambola* L. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics (mean values ± SD, n = 5). Fruit wax was extracted from isolated cuticular membranes, the leaf wax was extracted from the fresh ad- and abaxial leaf surfaces (mean values ± SD, n = 5).

Table 11. The cuticular wax coverage and compositions of *Averrhoa carambola* L. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	0.72 ± 0.08	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
21	0.12 ± 0.02			
22	0.38 ± 0.26	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.02
23	0.07 ± 0.02			
24	0.69 ± 0.04	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
25	0.14 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	
26	0.24 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
27	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
28	0.18 ± 0.05	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
29		0.02 ± 0.01	0.04 ± 0.02	
30	0.17 ± 0.05	0.20 ± 0.15	0.18 ± 0.08	0.21 ± 0.26
31		0.08 ± 0.02	0.05 ± 0.02	0.11 ± 0.04
32		0.11 ± 0.04	0.11 ± 0.09	0.10 ± 0.03
33		0.01 ± 0.00	0.03 ± 0.01	
34		0.02 ± 0.01	0.03 ± 0.02	
Primary alcohols				
20	0.03 ± 0.01			
22	0.11 ± 0.04			
24	0.54 ± 0.20	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
25	0.08 ± 0.01			
26	0.72 ± 0.16	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.00
27	0.05 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01
28	0.76 ± 0.13	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
29	0.16 ± 0.04	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
30	0.33 ± 0.04	4.40 ± 0.65	2.75 ± 0.66	6.04 ± 1.08
31		0.12 ± 0.04	0.11 ± 0.06	0.13 ± 0.03
32		0.83 ± 0.14	0.58 ± 0.15	1.09 ± 0.31
33		0.09 ± 0.03	0.12 ± 0.05	0.06 ± 0.02
34		0.18 ± 0.09	0.28 ± 0.10	0.09 ± 0.11
36		0.01 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
n-Alkenes				
23 (9/12)	4.53 ± 1.92			
24 (9)	0.26 ± 0.11			
25 (9/12)	5.53 ± 2.10			
27 (9/12)	1.68 ± 0.61			
29 (9/12)	0.27 ± 0.09			
n-Alkanes				
23	3.93 ± 0.56	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
25	2.56 ± 0.25	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
27	0.41 ± 0.05	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.02
28		0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.01

Table 11. continued

29	0.95 ± 0.07	1.66 ± 0.94	2.96 ± 1.59	0.36 ± 0.34
30	0.16 ± 0.01	0.19 ± 0.09	0.31 ± 0.15	0.08 ± 0.04
31	0.50 ± 0.09	5.40 ± 3.03	8.79 ± 5.34	2.02 ± 1.00
32	0.17 ± 0.09	0.37 ± 0.16	0.57 ± 0.28	0.16 ± 0.05
33	0.31 ± 0.07	0.90 ± 0.49	1.48 ± 0.87	0.32 ± 0.16
34	0.21 ± 0.00			
Aldehydes				
24	0.30 ± 0.04			
26	0.77 ± 0.16			
28	0.31 ± 0.15			
30		0.67 ± 0.25	0.28 ± 0.15	1.06 ± 0.44
32	0.10 ± 0.04	0.29 ± 0.10	0.17 ± 0.07	0.41 ± 0.23
Alkyl esters				
38	0.10 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
40	0.46 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
42	0.45 ± 0.08	0.03 ± 0.01	0.01 ± 0.00	0.04 ± 0.01
44	0.30 ± 0.03	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
46	0.12 ± 0.03	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.01
48	0.13 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.09 ± 0.01
50	0.32 ± 0.19	0.17 ± 0.03	0.12 ± 0.04	0.22 ± 0.03
52		0.15 ± 0.08	0.17 ± 0.06	0.12 ± 0.12
Sum aliphatic components				
	32.37 ± 5.42	16.58 ± 5.02	19.92 ± 8.53	13.24 ± 1.86
	68.3%	**85.2%**	**87.6%**	**82.8%**
Sterols				
ß-sitosterol	2.22 ± 0.26	0.12 ± 0.04	0.15 ± 0.05	0.09 ± 0.05
campesterol	0.53 ± 0.05	0.01 ± 0.00	0.02 ± 0.00	
Triterpenoids				
α-amyrin	0.15 ± 0.09	0.10 ± 0.02	0.15 ± 0.05	0.05 ± 0.01
Sum cyclic components				
	2.76 ± 0.29	0.24 ± 0.06	0.32 ± 0.08	0.15 ± 0.05
	5.9%	**1.2%**	**1.6%**	**0.9%**
Unidentified				
	12.05 ± 1.39	2.53 ± 0.73	2.35 ± 0.65	2.71 ± 1.00
Total wax	47.32 ± 6.93	19.35 ± 5.67	22.60 ± 9.16	16.09 ± 2.82

2.5 Cuticular waxes of *Coffea arabica* L.

The total wax coverage of *Coffea arabica* L. fruit was 15.48 ± 2.25 μg cm^{-2}. The overall coverage of waxes on leaf surfaces was 7.00 μg cm^{-2} (Table 12). The fruit wax composed similar portion of aliphatic compounds (20.1%, 3.11 ± 0.54 μg cm^{-2}) and cyclics (33.4%, 5.07 ± 1.03 μg cm^{-2}). The leaf wax composed a major portion of aliphatic components (76.1%, 5.33 μg cm^{-2}) and a minor portion of cyclics (13.6%, 0.95 μg cm^{-2}).

The main aliphatic components of fruit waxes were primary alcohols (7.3%, 1.11 μg cm^{-2}) followed by *n*-alkanes (6.0%, 0.92 μg cm^{-2}), alkyl esters (3.0%), fatty acids (2.5%), aldehydes (1.0%), and very small amount of hydroxyl fatty acids (Figure 12 A). The predominant leaf aliphatic components were primary alcohols (45.9%, 3.21 μg cm^{-2}) followed by *n*-alkanes (21.3%, 1.49 μg cm^{-2}), fatty acids (3.3%), aldehydes (2.8%), and ketones (1.5%). Additional small amount of secondary alcohols, alcohol acetates, and hydroxyl fatty acids were detected (Figure 12 A). Carbon chain lengths ranged from C_{20} to C_{50} for fruit and to C_{38} for leaf wax. The most abundant chain lengths were C_{30} and C_{32} for fruit wax, and were from C_{29} to C_{32} for leaf wax (Figure 12 B). The ACL value of aliphatics was 29.24 for fruit, and was 30.73 for leaf (Table 6).

The cyclic compounds were dominated by ursolic acid (24.5%, 3.79 ± 0.79 μg cm^{-2} for fruit; 10.9%, 0.76 μg cm^{-2} for leaf wax) in both of fruit and leaf wax. Additional small amount of β-sitosterol, cholesterol, oleanolic acid, tocopherols were also detected in both of fruit and leaf waxes (Table 12).

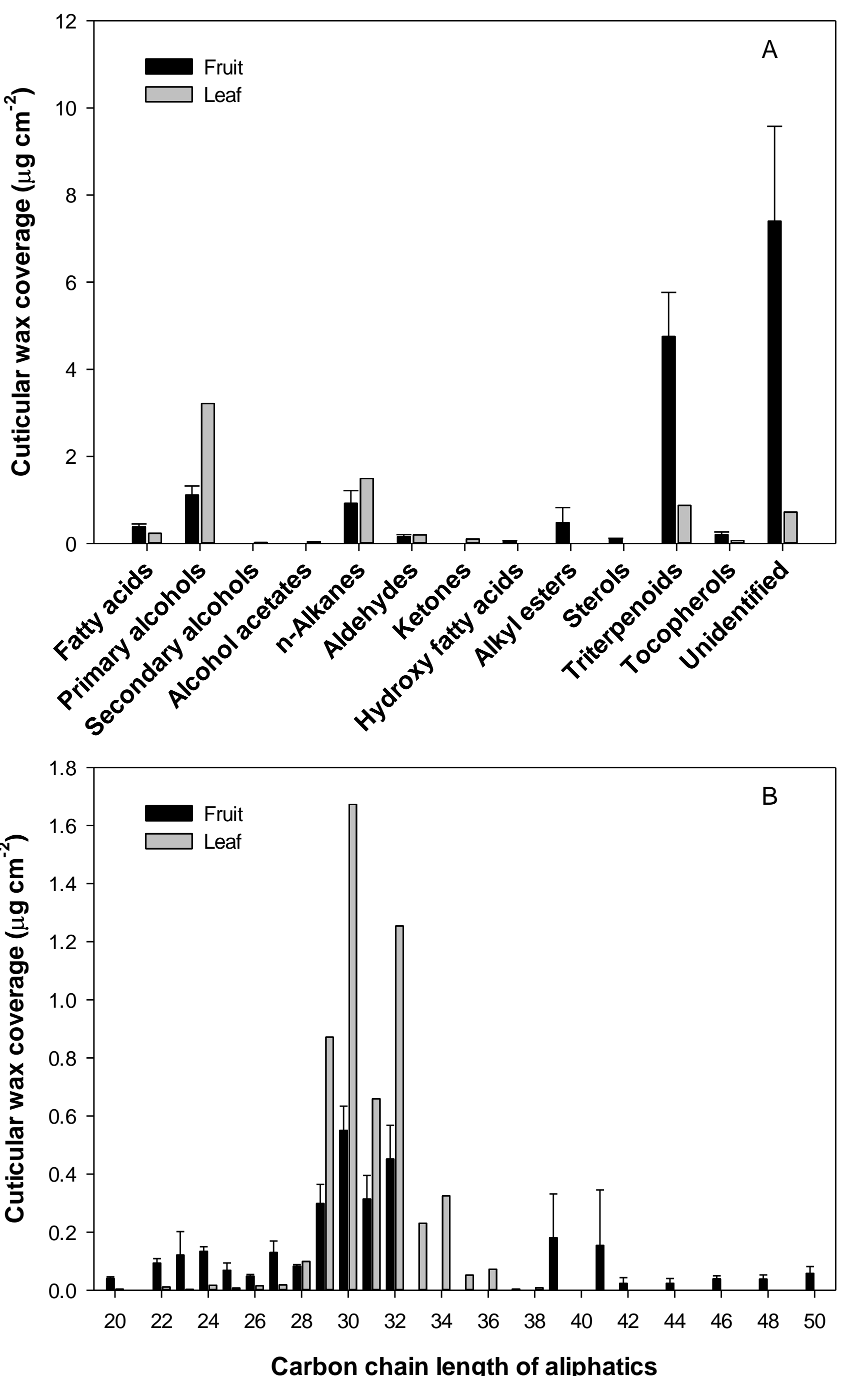

Figure 12. Cuticular wax compositions of *Coffea arabica* L. fruits and leaves. (A) Cuticular wax compound classes of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from fresh fruits and intact leaves (mean values ± SD, n = 5).

Table 12. The cuticular wax coverage and compositions of *Coffea arabica* L. fruits and leaves.

Compound	Fruit			Whole leaf [a]
Fatty acid				
20	0.04	±	0.01	
22	0.04	±	0.01	
24	0.11	±	0.02	0.01
25	0.01	±	0.00	
26	0.01	±	0.00	0.01
27	0.02	±	0.01	
28	0.04	±	0.01	0.03
29	0.03	±	0.01	0.02
30	0.09	±	0.02	0.06
32				0.05
33				0.02
34				0.01
36				0.01
Primary alcohols				
22	0.03	±	0.01	
24	0.02	±	0.01	
25	0.00	±	0.00	
26	0.01	±	0.00	
27	0.00	±	0.00	
28	0.04	±	0.01	0.05
29	0.04	±	0.01	0.06
30	0.41	±	0.09	1.37
31	0.18	±	0.07	0.13
32	0.38	±	0.11	1.11
33				0.08
34				0.31
35				0.01
36				0.07
37				
38				0.01
Secondary alcohols				
28 (pos.3)				traces
29 (pos.2)				traces
32 (pos.3)				0.02
Alcohol acetates				
30				0.05
n-Alkane				
23	0.12	±	0.07	traces
25	0.07	±	0.02	
26	0.03	±	0.01	traces
27	0.12	±	0.04	0.01
28	0.06	±	0.01	0.02
29	0.27	±	0.08	0.79
30	0.06	±	0.02	0.06
31	0.16	±	0.07	0.53
32	0.05	±	0.02	0.02
33				0.06
Aldehydes				
28	traces			
30	0.13	±	0.04	0.14
32	0.03	±	0.01	0.06

Table 12. continued

Hydroxy fatty acids				
22	0.04	±	0.01	traces
24	0.01	±	0.01	traces
Ketones				
33 (pos.2)				0.07
35 (pos.2)				0.04
Alkyl esters				
39	0.16	±	0.14	
41	0.13	±	0.08	
42	0.02	±	0.02	
43	0.02	±	0.00	
44	0.02	±	0.01	
46	0.04	±	0.01	
48	0.04	±	0.01	
50	0.06	±	0.02	
Sum aliphatic components				
	3.11	±	0.54	5.33
	20.1%			**76.1%**
Sterols				
cholesterol	0.02	±	0.01	traces
stigmasterol	0.04	±	0.01	
ß-sitosterol	0.03	±	0.01	traces
fucosterol	0.02	±	0.01	
Triterpenoids				
erythrodiol	0.02	±	0.01	
uvaol	0.07	±	0.04	
oleanolic acid	0.82	±	0.20	0.11
betulic acid	0.05	±	0.02	
ursolic acid	3.79	±	0.79	0.76
Tocopherols				
δ-tocopherol	0.05	±	0.01	0.00
ß-tocopherol	0.07	±	0.02	0.01
γ-tocopherol	0.05	±	0.03	0.05
α-tocopherol	0.03	±	0.02	0.01
Sum cyclic components				
	5.07	±	1.03	0.95
	33.4%			**13.6%**
Unidentified				
	7.40	±	2.18	0.72
Total wax	15.48	±	2.25	7.00

a, Data were given as mean values ± SD (in μg cm^{-2}, n = 5) for fruit, while the waxes from leaf were extracted from one leaf sample as reference.

2.6 Cuticular waxes of *Crataegus pedicellata* Sarg.

The total wax load of *Crataegus pedicellata* Sarg. fruit was 451.05 ± 60.57 μg cm^{-2}. The overall coverage of wax on leaf surfaces was 30.56 ± 10.35 μg cm^{-2}. The leaf waxes were distributed by 20.60 ± 14.35 μg cm^{-2} and 40.52 ± 7.35 μg cm^{-2} on the ad-, and abaxial surfaces, respectively (Table 13). Cyclic components were the main waxes for both of fruit (57.1%, 258.61 ± 46.81 μg cm^{-2}) and leaf (54.5%, 15.87 ± 4.37 μg cm^{-2}). The minor portion of aliphatics was 34.6% (155.19 ± 16.48 μg cm^{-2}) for fruit, and was 35.7% (12.05 ± 5.40 μg cm^{-2}) for leaf waxes.

The main aliphatic components were secondary alcohols (12.6%, 56.67 ± 8.97 μg cm^{-2}) followed by *n*-alkanes (10.8%, 48.68 ± 6.27 μg cm^{-2}), alkyl esters (6.0%), and fatty acids (3.9%). Very small amount of ketones, primary alocohos, and aldehydes were also detected in fruit waxes. The leaf aliphatic components were dominated by *n*-alkanes (28.3%, 8.65 ± 4.19 μg cm^{-2}) followed by primary alcohols (4.5%), and fatty acids (3.4%). Small amount of secondary alcohols, aldehydes, and alkyl esters were detected on leaf surfaces (Figure 13 A). Carbon chain lengths ranged from C_{20} to C_{52} for fruit and to C_{50} for leaf waxes. The most abundant chain lengths were C_{29} and C_{30} for fruit wax, and were C_{29} and C_{31} for leaf wax (Figure 13 B). The ACL value of aliphatics was 29.95 for fruit, and was 29.23 for leaf (Table 6).

Triterpenoids were the prominent cyclic compounds in both of fruit (56.9%, 256.54 ± 46.87 μg cm^{-2}) and leaf (48.8%, 14.92± 4.36 μg cm^{-2} of leaf) waxes. The triterpenoids were dominated by ursolic acid (37.1%, 167.52 ± 31.00 μg cm^{-2} for fruit; 29.9%, 9.13 ± 2.86 μg cm^{-2} for leaf), and oleanolic acid (8.8%, 39.90 ± 5.20 μg cm^{-2} for fruit; 7.6%, 2.32 ± 0.93 μg cm^{-2} for leaf). Small amount of betulic acid, erythrodiol, uvaol, and sterols were also found in both of fruit and leaf waxes. Traces of stigmasterol and tocopherols were detected in leaf waxes (Table 13).

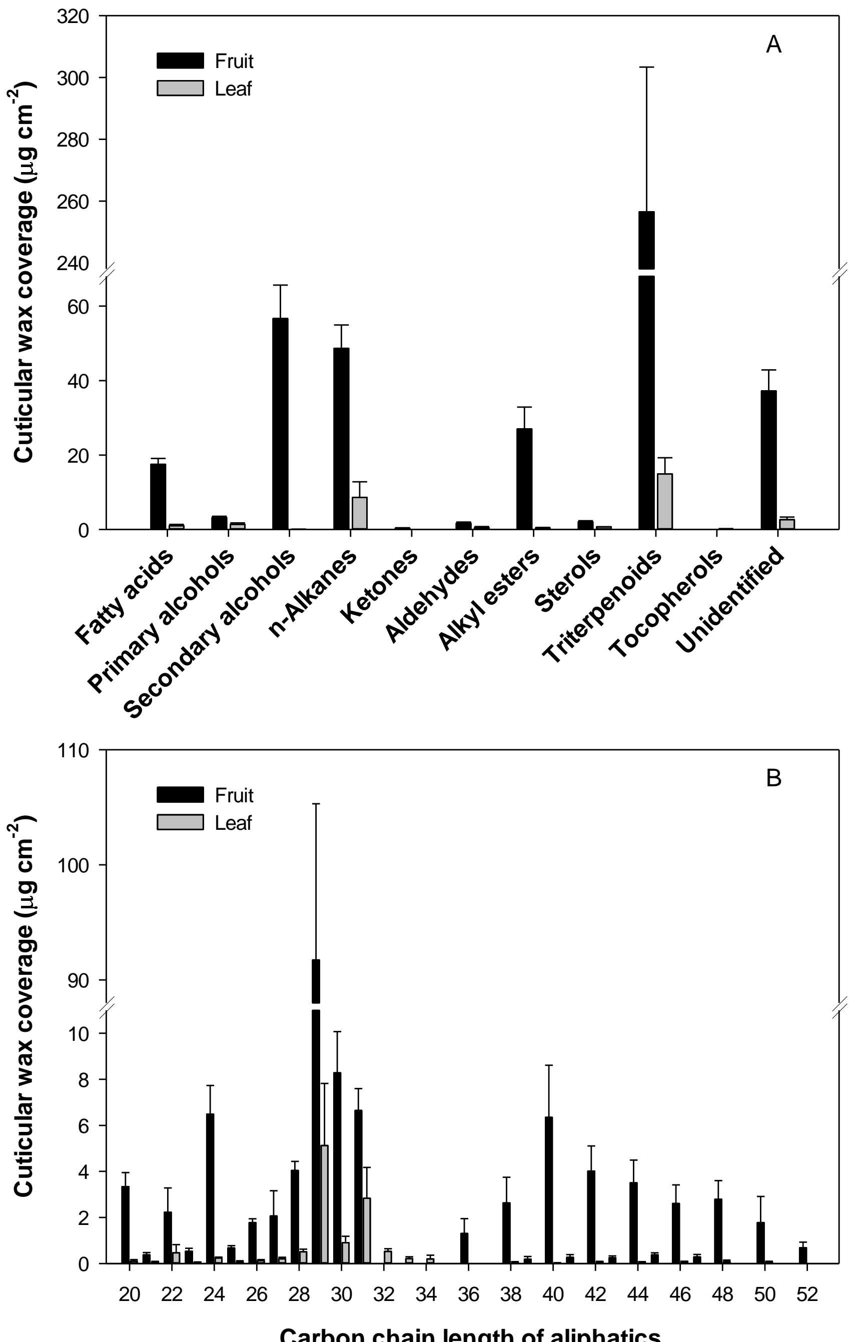

Figure 13. Cuticular wax compositions from *Crataegus pedicellata* Sarg. fruits and leaves. (A) Cuticular wax compostions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Fruit waxes were extracted from isolated cuticular membranes and leaf waxes were extracted from fresh ad- and abaxial leaf surfaces (mean values ± SD, n = 5).

Table 13. The cuticular wax coverage and compositions of *Crataegus pedicellata* Sarg. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	3.18 ± 0.61	0.07 ± 0.03	0.05 ± 0.06	0.10 ± 0.01
21	0.17 ± 0.07	0.03 ± 0.01	0.04 ± 0.00	0.03 ± 0.01
22	1.94 ± 1.05	0.25 ± 0.17	0.13 ± 0.25	0.36 ± 0.12
23	0.19 ± 0.08	0.02 ± 0.01	0.04 ± 0.03	0.02 ± 0.01
24	5.53 ± 1.11	0.11 ± 0.05	0.13 ± 0.10	0.10 ± 0.02
25	0.12 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.00
26	1.27 ± 0.19	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.01
27	0.14 ± 0.12	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
28	1.53 ± 0.16	0.08 ± 0.01	0.06 ± 0.05	0.09 ± 0.04
29	0.35 ± 0.09	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
30	3.09 ± 1.13	0.30 ± 0.16	0.17 ± 0.13	0.42 ± 0.27
32		0.10 ± 0.04	0.06 ± 0.04	0.15 ± 0.06
Primary alcohols				
20	0.15 ± 0.07			
21		0.03 ± 0.02	0.06 ± 0.03	0.03 ± 0.01
22	0.29 ± 0.07	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.03
23	0.01 ± 0.02	traces	0.01 ± 0.00	
24	0.47 ± 0.06	0.06 ± 0.05	0.10 ± 0.09	0.03 ± 0.01
25	0.10 ± 0.07	0.04 ± 0.06	0.10 ± 0.14	0.03 ± 0.01
26	0.51 ± 0.05	0.10 ± 0.04	0.06 ± 0.05	0.13 ± 0.03
27	0.28 ± 0.09	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01
28	0.70 ± 0.17	0.20 ± 0.06	0.18 ± 0.11	0.21 ± 0.03
29		0.05 ± 0.03	0.02 ± 0.01	0.08 ± 0.05
30	0.72 ± 0.15	0.31 ± 0.04	0.24 ± 0.06	0.37 ± 0.03
31		0.05 ± 0.02	0.04 ± 0.02	0.06 ± 0.03
32		0.30 ± 0.09	0.25 ± 0.09	0.35 ± 0.11
34		0.20 ± 0.17	0.14 ± 0.08	0.25 ± 0.28
Secondary alcohols				
28 (pos.9/10)	0.29 ± 0.07			
28 (diol)	0.45 ± 0.12			
29 (pos.9/10)	48.58 ± 8.23	0.08 ± 0.04	0.06 ± 0.06	0.10 ± 0.03
29 (10,13-diol)	2.86 ± 0.58			
30 (pos.9/10)	2.90 ± 0.33			
31 (pos.10/11)	1.48 ± 0.24			
n-Alkanes				
21	0.20 ± 0.07			
23	0.33 ± 0.07	0.03 ± 0.01	0.02 ± 0.02	0.03 ± 0.02
25	0.45 ± 0.06			
27	1.64 ± 1.17	0.17 ± 0.06	0.17 ± 0.14	0.18 ± 0.04
28	0.45 ± 0.06	0.10 ± 0.03	0.07 ± 0.05	0.13 ± 0.02
29	39.42 ± 5.17	4.96 ± 2.62	2.60 ± 1.72	7.32 ± 1.81

Table 13. continued

30	1.02 ± 0.44	0.25 ± 0.10	0.11 ± 0.16	0.40 ± 0.10
31	5.16 ± 0.72	2.78 ± 1.32	1.18 ± 0.94	4.38 ± 0.75
32		0.12 ± 0.05	0.04 ± 0.03	0.20 ± 0.05
33		0.21 ± 0.08	0.10 ± 0.05	0.32 ± 0.07
34		0.02 ± 0.03		0.10 ± 0.06
Ketones				
29 (pos.10)	0.36 ± 0.12			
Aldehydes				
20		0.03 ± 0.03	0.02 ± 0.04	0.05 ± 0.02
21		0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
22		0.20 ± 0.18	0.04 ± 0.02	0.24 ± 0.15
24	0.49 ± 0.16	0.06 ± 0.04	0.07 ± 0.03	0.06 ± 0.05
28	0.61 ± 0.27	0.13 ± 0.07	0.14 ± 0.11	0.12 ± 0.06
29	0.16 ± 0.04			
30	0.46 ± 0.10	0.04 ± 0.05	0.05 ± 0.05	0.04 ± 0.04
Alkyl esters				
36	1.31 ± 0.64			
38	2.63 ± 1.11	0.05 ± 0.03	0.06 ± 0.03	0.04 ± 0.04
39	0.19 ± 0.12			
40	6.35 ± 2.27	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
41	0.27 ± 0.11			
42	4.02 ± 1.09	0.06 ± 0.03	0.05 ± 0.05	0.07 ± 0.03
43	0.25 ± 0.08			
44	3.50 ± 0.99	0.06 ± 0.02	0.05 ± 0.04	0.06 ± 0.02
45	0.37 ± 0.09			
46	2.60 ± 0.81	0.07 ± 0.03	0.06 ± 0.04	0.08 ± 0.02
47	0.28 ± 0.11			
48	2.79 ± 0.81	0.09 ± 0.07	0.08 ± 0.08	0.10 ± 0.06
50	1.78 ± 1.13	0.07 ± 0.03	0.04 ± 0.01	0.10 ± 0.06
52	0.68 ± 0.24			
Sum aliphatic components				
	155.19 ± 16.48	12.05 ± 5.40	7.03 ± 3.44	17.07 ± 3.68
	34.6%	**35.7%**	**29.3%**	**42.0%**
Sterols				
ß-sitosterol	2.07 ± 0.30	0.69 ± 0.05	0.53 ± 0.12	0.85 ± 0.07
stigmasterol		0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.04
Triterpenoids				
erythrodiol	0.91 ± 0.42	0.19 ± 0.11	0.20 ± 0.10	0.17 ± 0.16
uvaol	4.69 ± 1.25	0.01 ± 0.02		0.06 ± 0.00
oleanolic acid metyl ester	8.63 ± 2.92	0.14 ± 0.29		0.70 ± 0.88
oleanolic acid	39.90 ± 5.20	2.32 ± 0.93	1.32 ± 1.06	3.33 ± 1.30
betulic acid	5.86 ± 2.19	0.12 ± 0.03	0.10 ± 0.02	0.14 ± 0.05
ursolic acid	167.52 ± 31.00	9.13 ± 2.86	6.51 ± 3.98	11.74 ± 2.65
maslinic acid	1.59 ± 0.25	0.34 ± 0.11	0.36 ± 0.13	0.32 ± 0.12
other triterpenoids	27.44 ± 8.80	2.68 ± 0.46	2.13 ± 0.46	3.22 ± 0.61

Table 13. continued

Tocopherols												
δ-tocopherol				0.11	±	0.03	0.04	±	0.07	0.19	±	0.02
γ-tocopherol				0.10	±	0.01	0.05	±	0.03	0.14	±	0.03
α-tocopherol				0.01	±	0.02				0.05	±	0.04
Sum cyclic components												
	258.61	±	46.81	15.87	±	4.37	11.27	±	5.72	20.48	±	3.97
		57.1%			**54.5%**			**58.5%**			**50.5%**	
Unidentified												
	37.23	±	5.65	2.65	±	0.72	2.32	±	1.02	2.99	±	0.55
Total wax	451.05	±	60.57	30.56	±	10.35	20.60	±	14.35	40.52	±	7.35

2.7 Cuticular waxes of *Malus domestica* L. cv. 'Topaz'

The total wax of *Malus domestica* L. cv. 'Topaz' fruit was 230.21 ± 12.61 μg cm^{-2}. The overall wax load of leaf surfaces was 39.26 ± 4.29 μg cm^{-2}. The leaf waxes were distributed by 34.74 ± 6.68 μg cm^{-2} and 43.79 ± 5.55 μg cm^{-2} on the ad- and abaxial surfaces, respectively (Table 14). Aliphatic components deposited into a higher level (59.6%, 137.02 ± 14.23 μg cm^{-2}), when compared to the coverage of cyclic compounds (33.9%, 78.23 ± 17.98 μg cm^{-2}). The leaf wax composed a major portion of cyclic compounds (73.1%, 29.33 ± 4.40 μg cm^{-2}) and a minor portion of aliphatics (17.1%, 6.24 ± 0.61 μg cm^{-2}).

The main aliphatic components of fruit waxes were *n*-alkanes (31.5%, 72.53 ± 7.69 μg cm^{-2}) followed by secondary alcohols (12.5%, 28.85 ± 6.36 μg cm^{-2}), fatty acids (6.0%), alkyl esters (3.4%), and primary alcohols (2.7%). Very small amount of ketones, unsaturated alkyl esters, *n*-alkenes and aldehydes were also detected (Figure 14 A). The leaf aliphatic components were dominated by *n*-alkanes (4.1%, 1.59 ± 0.16 μg cm^{-2}) and alkyl esters (4.7%, 1.83 ± 0.30 μg cm^{-2}) followed by primary alcohols (3.9%), and fatty acids (2.9%). Small amount of aldehydes were detected from adaxial leaf waxes, and methyl esters were detected on abaxial leaf surfaces. Carbon chain lengths ranged from C_{20} to C_{52} in botn fruit and leaf waxes. The most abundant chain lengths were C_{29} and C_{30} for fruit waxes, and were C_{30} and C_{31} for leaf waxes (Figure 14 B). Nonacosan-10-ol (10.8%, 24.87 ± 6.05 μg cm^{-2}) and *n*-nonacosane (27.8%, 64.01± 7.67 μg cm^{-2}) dominated the aliphatics of fruit wax. The ACL value of aliphatics was 29.67 for fruit, and was 33.29 for leaf (Table 6).

Triterpenoids were the main cyclic compounds for both of fruit (33.6%, 77.26 ± 17.90 μg cm^{-2}) and leaf (72.2%, 28.99 ± 4.36 μg cm^{-2} for leaf) waxes. The triterpenoids were dominated by ursolic acid (16.2%, 37.28 ± 7.93 μg cm^{-2} for fruit; 35.6%, 13.96 ± 2.23 μg cm^{-2} for leaf), and oleanolic acid (6.2%, 14.16 ± 2.90 μg cm^{-2} for fruit; 7.0%, 2.75 ± 0.36 μg cm^{-2} for leaf). Very small amount of erythrodiol, uvaol, and sterols were also found in both of fruit and leaf waxes. Traces of phenylmethyl esters and tocopherols were detected in leaf waxes (Table 14).

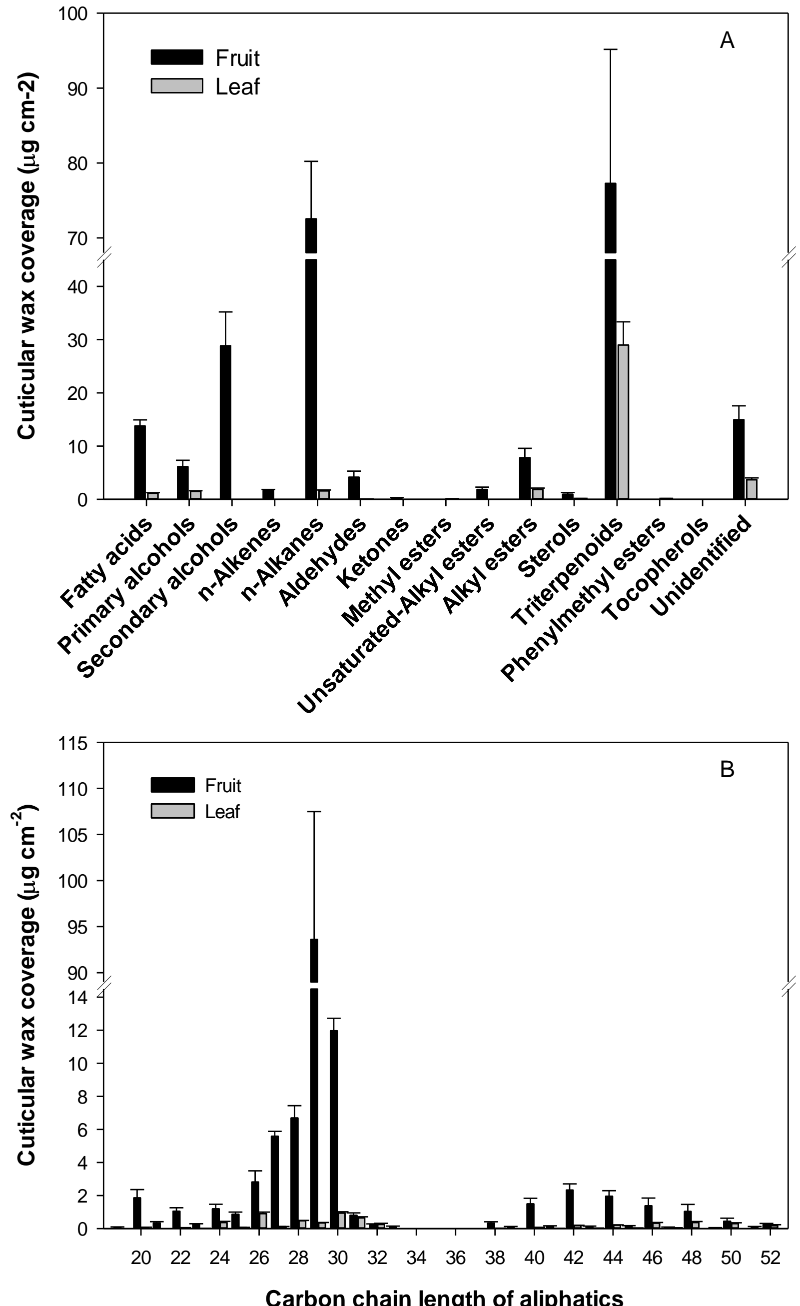

Figure 14. Cuticular wax compositions from *Malus domestica* L. cv. 'Topaz' fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 14. The cuticular wax coverage and compositions of *Malus domestica* L. cv. 'Topaz' fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf abaxial	Leaf adaxial
Fatty acids				
19	0.08 ± 0.02			
20	1.85 ± 0.50	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
22	0.72 ± 0.19	0.04 ± 0.01	0.06 ± 0.01	0.03 ± 0.02
23	0.09 ± 0.02			
24	0.49 ± 0.10	0.22 ± 0.02	0.39 ± 0.04	0.06 ± 0.06
25	0.10 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	
26	0.62 ± 0.13	0.21 ± 0.01	0.38 ± 0.02	0.04 ± 0.03
27	0.32 ± 0.07	0.01 ± 0.00	0.02 ± 0.01	
28	2.09 ± 0.19	0.15 ± 0.01	0.20 ± 0.02	0.10 ± 0.04
29	0.80 ± 0.25	0.03 ± 0.01	0.06 ± 0.02	
30	6.60 ± 0.59	0.28 ± 0.06	0.40 ± 0.08	0.16 ± 0.07
32		0.11 ± 0.06	0.22 ± 0.12	
Primary alcohols				
21	0.21 ± 0.08			
22	0.32 ± 0.04			
24	0.59 ± 0.14	0.13 ± 0.06	0.23 ± 0.12	0.03 ± 0.01
25	0.11 ± 0.06	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.01
26	1.42 ± 0.44	0.69 ± 0.09	1.29 ± 0.17	0.10 ± 0.02
27	0.22 ± 0.05	0.03 ± 0.00	0.06 ± 0.00	0.01 ± 0.00
28	1.24 ± 0.52	0.29 ± 0.01	0.42 ± 0.03	0.15 ± 0.02
29	0.77 ± 0.22	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.03
30	1.26 ± 0.08	0.29 ± 0.04	0.38 ± 0.06	0.20 ± 0.02
Secondary alcohols				
28 (pos. 3)	0.26 ± 0.09			
28 (pos. 10/11)	0.26 ± 0.09			
29 (pos. 2)	0.50 ± 0.40			
29 (pos. 9/10)	24.87 ± 6.05			
29 (10,13-diol)	1.43 ± 0.36			
30 (pos. 10/11)	0.83 ± 0.18			
31 (pos. 10/11)	0.69 ± 0.22			
n-Alkenes				
26	0.38 ± 0.10			
27	0.14 ± 0.04			
28	1.14 ± 0.15			
n-Alkanes				
21	0.13 ± 0.06			
23	0.16 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
25	0.64 ± 0.13			
26	0.19 ± 0.04			
27	4.90 ± 0.30	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
28	0.83 ± 0.21			
29	64.01 ± 7.67	0.27 ± 0.02	0.33 ± 0.06	0.21 ± 0.07

Table 14. continued

30	0.59 ± 0.09	0.33 ± 0.06	0.58 ± 0.13	0.07 ± 0.03
31	0.80 ± 0.14	0.64 ± 0.07	1.08 ± 0.19	0.20 ± 0.07
32	0.20 ± 0.07	0.14 ± 0.03	0.26 ± 0.06	0.02 ± 0.02
33	0.11 ± 0.05	0.13 ± 0.03	0.27 ± 0.07	
Ketones				
29 (pos.10)	0.24 ± 0.10			
Aldehydes				
24	0.10 ± 0.12	0.01 ± 0.01		0.02 ± 0.01
26	0.19 ± 0.13			
28	0.87 ± 0.31	0.01 ± 0.01		0.02 ± 0.01
29	0.21 ± 0.04			
30	2.83 ± 0.80	0.03 ± 0.01		0.07 ± 0.03
Methylesters				
26		0.07 ± 0.04	0.13 ± 0.09	
28		0.03 ± 0.01	0.06 ± 0.02	
Unsaturated alkyl esters				
40 (:1)	0.17 ± 0.07			
42 (:1)	0.43 ± 0.10			
44 (:1)	0.57 ± 0.09			
46 (:1)	0.33 ± 0.08			
48 (:1)	0.34 ± 0.22			
Alkyl esters				
38	0.32 ± 0.09	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02
39	0.08 ± 0.04			
40	1.32 ± 0.31	0.05 ± 0.02	0.07 ± 0.03	0.04 ± 0.01
41	0.13 ± 0.03	0.01 ± 0.01	0.02 ± 0.01	
42	1.89 ± 0.30	0.18 ± 0.01	0.14 ± 0.03	0.22 ± 0.03
43	0.10 ± 0.04	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
44	1.38 ± 0.25	0.21 ± 0.02	0.08 ± 0.02	0.34 ± 0.03
45	0.11 ± 0.05	0.04 ± 0.00	0.02 ± 0.01	0.06 ± 0.01
46	1.04 ± 0.40	0.32 ± 0.04	0.11 ± 0.06	0.54 ± 0.02
47	0.07 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.01
48	0.68 ± 0.26	0.37 ± 0.06	0.17 ± 0.09	0.57 ± 0.06
49		0.04 ± 0.02		0.07 ± 0.04
50	0.44 ± 0.19	0.30 ± 0.05	0.15 ± 0.07	0.45 ± 0.05
51		0.06 ± 0.06		0.12 ± 0.12
52	0.23 ± 0.08	0.18 ± 0.06	0.07 ± 0.06	0.28 ± 0.06
Sum aliphatic components				
	137.02 ± 14.23	6.24 ± 0.61	7.97 ± 1.15	4.50 ± 0.28
	59.6%	**17.1%**	**23.7%**	**10.4%**
Sterols				
ß-sitosterol	0.97 ± 0.32	0.17 ± 0.02	0.17 ± 0.03	0.17 ± 0.03
Triterpenoids				
α-amyrin		0.03 ± 0.01		0.06 ± 0.02

Table 14. continued

erythrodiol	3.23 ± 0.93		0.23 ± 0.09		0.20 ± 0.07		0.27 ± 0.14	
uvaol	1.37 ± 0.76		0.31 ± 0.05		0.11 ± 0.02		0.52 ± 0.08	
lupeol	0.33 ± 0.00							
gypsogenin	0.36 ± 0.12		0.09 ± 0.04				0.18 ± 0.08	
oleanolic acid methyl ester			0.10 ± 0.07		0.04 ± 0.02		0.16 ± 0.14	
oleanolic aldehyde			0.79 ± 0.41				1.58 ± 0.83	
oleanolic acid	14.16 ± 2.90		2.75 ± 0.36		1.56 ± 0.44		3.94 ± 0.55	
betulinic acid	2.28 ± 0.68		0.45 ± 0.19		0.35 ± 0.16		0.55 ± 0.26	
ursolic acid	37.28 ± 7.93		13.96 ± 2.23		10.10 ± 2.59		17.83 ± 4.73	
maslinic acid			0.26 ± 0.08		0.53 ± 0.16			
corosolic acid			1.16 ± 0.33		2.31 ± 0.65			
other triterpenoids	18.51 ± 7.35		8.85 ± 2.19		6.79 ± 3.18		10.92 ± 2.13	
Phenylmethyl esters								
26			0.16 ± 0.04		0.33 ± 0.08			
Tocopherols								
δ-tocopherol			0.01 ± 0.01				0.02 ± 0.01	
Sum cyclic components								
	78.23 ± 17.98		29.33 ± 4.40		22.48 ± 6.92		36.19 ± 4.42	
	33.9%		**73.1%**		**63.5%**		**83.0%**	
unidentified								
	14.96 ± 2.61		3.69 ± 0.34		4.29 ± 0.38		3.09 ± 1.00	
Total wax	230.21 ± 12.61		39.26 ± 4.29		34.74 ± 6.68		43.79 ± 5.55	

2.8 Cuticular waxes of *Prunus avium* L.

The total wax coverage of *Prunus avium* L. fruit was 37.52 ± 7.43 µg cm^{-2}. The overall coverage of wax on leaf surfaces was 22.82 ± 3.17 µg cm^{-2}. The leaf waxes were distributed by 21.90 ± 1.84 µg cm^{-2} and 23.59 ± 3.98 µg cm^{-2} on the ad- and abaxial surfaces, respectively (Table 15). Cyclic components dominated for both fruit (67.6%, 25.53 ± 6.22 µg cm^{-2}) and leaf (54.5%, 12.60 ± 4.18 µg cm^{-2}) waxes. The minor portion of aliphatic compounds was 20.1% (7.46 ± 1.43 µg cm^{-2}) for fruit, and was 31.7% (7.13 ± 1.73 µg cm^{-2}) for leaf waxes.

The main aliphatic components of fruit waxes were *n*-alkanes (16.5%, 6.10 ± 1.31 µg cm^{-2}) followed by fatty acids (2.9%), and small amount of primary and secondary alcohols. The leaf aliphatic components were dominated by alkyl esters (13.2%, 2.97 ± 0.38 µg cm^{-2}) followed by primary alcohols (7.6%), fatty acids (6.3%) and *n*-alkanes (4.7%) (Figure 15 A). Carbon chain lengths ranged from C_{20} to C_{31} for fruit and to C_{52} for leaf wax. The most abundant chain lengths were C_{27}, C_{29} and C_{30} for fruit wax, and were C_{28}, C_{29} and C_{30} for leaf wax (Figure 15 B). *N*-nonacosane (11.5%, 4.30 ± 0.96 µg cm^{-2}) dominated the aliphatics of fruit wax. The ACL value of aliphatics was 27.88 for fruit, and was 33.79 for leaf (Table 6).

Triterpenoids were the main cyclic compounds in both of fruit (67.0%, 25.15 ± 6.19 µg cm^{-2}) and leaf (54.2%, 12.52 ± 4.18 µg cm^{-2}) waxes. The triterpenoids were dominated by ursolic acid (50.8%, 19.07 ± 5.63 µg cm^{-2} for fruit; 44.2%, 10.09 ± 3.74 µg cm^{-2} for leaf), and oleanolic acid (7.6%, 2.87 ± 0.72 µg cm^{-2} for fruit; 7.1%, 1.62 ± 0.55 µg cm^{-2} for leaf). Very small amount of maslinic acid, uvaol, and sterols were also found in both of fruit and leaf waxes (Table 15).

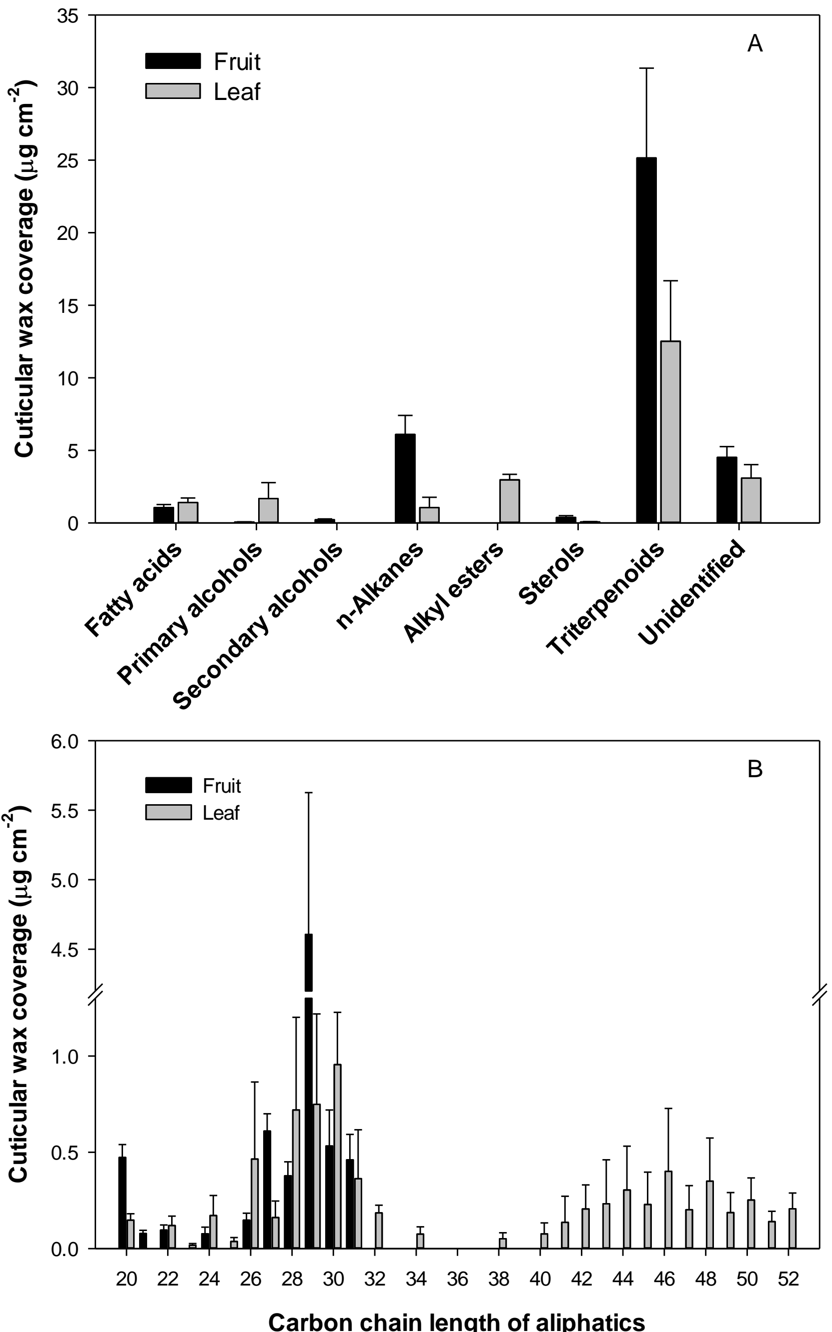

Figure 15. Cuticular wax compositions of *Prunus avium* L. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 15. The cuticular wax load and compositions of *Prunus avium* L. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit			Leaf			Leaf adaxial			Leaf abaxial		
Fatty acids												
20	0.47	±	0.07	0.15	±	0.03	0.12	±	0.02	0.17	±	0.02
21	0.08	±	0.02									
22	0.10	±	0.03	0.09	±	0.05	0.04	±	0.01	0.13	±	0.02
23				0.02	±	0.01	0.02	±	0.00	0.02	±	0.01
24	0.08	±	0.03	0.06	±	0.02	0.06	±	0.02	0.07	±	0.02
25				0.02	±	0.01	0.02	±	0.01			
26	0.08	±	0.03	0.09	±	0.06	0.12	±	0.07	0.06	±	0.01
27	0.00	±	0.00	0.03	±	0.02	0.04	±	0.02	0.02	±	0.00
28	0.08	±	0.04	0.31	±	0.15	0.42	±	0.14	0.21	±	0.07
29	0.09	±	0.04	0.08	±	0.02	0.08	±	0.02	0.07	±	0.02
30	0.09	±	0.06	0.54	±	0.13	0.58	±	0.11	0.50	±	0.15
32				0.05	±	0.02	0.06	±	0.02	0.04	±	0.01
Primary alcohols												
22				0.03	±	0.02	0.04	±	0.03	0.02	±	0.01
24				0.11	±	0.11	0.22	±	0.05	0.02	±	0.00
25				0.03	±	0.01	0.03	±	0.00	0.03	±	0.01
26				0.38	±	0.36	0.74	±	0.13	0.07	±	0.02
27				0.03	±	0.03	0.05	±	0.03	0.02	±	0.01
28				0.41	±	0.35	0.77	±	0.12	0.12	±	0.03
29				0.08	±	0.05	0.13	±	0.04	0.05	±	0.01
30	0.07	±	0.02	0.37	±	0.16	0.53	±	0.07	0.23	±	0.07
31				0.06	±	0.01	0.07	±	0.01	0.05	±	0.01
32				0.11	±	0.04	0.14	±	0.04	0.09	±	0.02
34				0.08	±	0.04	0.11	±	0.02	0.05	±	0.01
Secondary alcohols												
29 (pos.9/10)	0.22	±	0.07									
n-Alkanes												
26	0.06	±	0.02									
27	0.61	±	0.09	0.10	±	0.06	0.14	±	0.07	0.06	±	0.02
28	0.30	±	0.05									
29	4.30	±	0.96	0.59	±	0.42	0.94	±	0.37	0.29	±	0.11
30	0.37	±	0.17	0.05	±	0.03	0.07	±	0.03	0.03	±	0.01
31	0.46	±	0.13	0.30	±	0.24	0.53	±	0.19	0.12	±	0.03
32				0.04	±	0.02				0.04	±	0.02
Alkyl esters												
38				0.05	±	0.03	0.08	±	0.03	0.03	±	0.01
39				0.08	±	0.06	0.07	±	0.09	0.08	±	0.02
40				0.14	±	0.14	0.25	±	0.13	0.04	±	0.02
41				0.21	±	0.13	0.12	±	0.14	0.27	±	0.05
42				0.23	±	0.23	0.43	±	0.21	0.07	±	0.02
43				0.30	±	0.23	0.07	±	0.02	0.50	±	0.07
44				0.23	±	0.17	0.40	±	0.06	0.09	±	0.01

Table 15. continued

45		0.40 ± 0.33	0.06 ± 0.01	0.68 ± 0.04
46		0.20 ± 0.12	0.33 ± 0.04	0.10 ± 0.03
47		0.35 ± 0.22	0.12 ± 0.03	0.54 ± 0.07
48		0.19 ± 0.10	0.29 ± 0.02	0.10 ± 0.05
49		0.25 ± 0.11	0.14 ± 0.06	0.34 ± 0.04
50		0.14 ± 0.05	0.18 ± 0.04	0.10 ± 0.03
52		0.21 ± 0.08	0.13 ± 0.03	0.27 ± 0.03
Sum aliphatic components				
	7.46 ± 1.43	7.13 ± 1.73	8.71 ± 1.15	5.82 ± 0.58
	20.1%	**31.7%**	**39.8%**	**25.1%**
Sterols				
ß-sitosterol	0.38 ± 0.12	0.08 ± 0.03	0.08 ± 0.03	0.07 ± 0.04
Triterpenoid alcohols				
uvaol	0.23 ± 0.04	0.11 ± 0.03	0.09 ± 0.02	0.13 ± 0.03
gypsognein	0.46 ± 0.00			
oleanolic acid	2.87 ± 0.72	1.62 ± 0.55	1.21 ± 0.20	1.96 ± 0.51
betulinic acid	0.23 ± 0.08			
ursolic acid	19.07 ± 5.63	10.09 ± 3.74	6.96 ± 1.06	12.70 ± 3.02
maslinic acid	0.41 ± 0.10	0.36 ± 0.15	0.47 ± 0.08	0.26 ± 0.12
corosolic acid	1.28 ± 0.72			
oleanolic acid methyl ester	0.44 ± 0.30			
other triterpenoids	0.62 ± 0.30	0.35 ± 0.29	0.50 ± 0.38	0.22 ± 0.09
Sum cyclic components				
	25.53 ± 6.22	12.60 ± 4.18	9.32 ± 1.42	15.33 ± 3.68
	67.6%	**54.5%**	**42.7%**	**64.4%**
Unidentified				
	4.53 ± 0.73	3.09 ± 0.93	3.87 ± 0.79	2.44 ± 0.36
Total wax	37.52 ± 7.43	22.82 ± 3.17	21.90 ± 1.84	23.59 ± 3.98

2.9 Cuticular waxes of *Prunus cerasifera* Ehrh.

The total wax coverage of *Prunus cerasifera* Ehrh. fruit was 205.54 ± 6.88 μg cm^{-2}. The overall coverage of wax on leaf surfaces was 46.40 ± 8.03 μg cm^{-2}. The leaf waxes were distributed by 51.30 ± 8.28 μg cm^{-2} and 41.51 ± 8.86 μg cm^{-2} on the ad- and abaxial surfaces (Table 16). Cyclic components were the main waxes for both of fruit (64.3%, 132.02 ± 5.06 μg cm^{-2}) and leaf (76.3%, 34.66 ± 5.54 μg cm^{-2}). The minor portion of aliphatic compounds was 29.0% (59.68 ± 4.77 μg cm^{-2}) for fruit, and was 14.3% (7.21 ± 2.04 μg cm^{-2}) for leaf wax.

The main aliphatic components of fruit waxes were *n*-alkanes (18.1%, 37.18 ± 3.87 μg cm^{-2}) followed by fatty acids (4.1%), alkyl esters (3.8%), primary alcohols (2.1%), and small amount of aldehydes. The leaf aliphatic components were dominated by fatty acids (5.1%, 2.49 ± 0.67 μg cm^{-2}) and *n*-alkanes (4.9%, 2.50 ± 1.16 μg cm^{-2}) followed by primary alcohols (2.4%). The small amount of secondary alcohols, methyl esters, and alkyl esters were found on adaxial leaf waxes (Figure 16 A). Carbon chain lengths ranged from C_{20} to C_{46} for fruit and leaf waxes, the most abundant chain lengths were C_{28}, C_{29} and C_{30} for fruit waxes, and were C_{26}, C_{28}, C_{29} and C_{31} for leaf waxes (Figure 16 B). *N*-nonacosane (17.0%, 34.97 ± 3.77 μg cm^{-2}) dominated the aliphatics of fruit wax, and *n*-hentriacontane (2.7%, 1.24 ± 0.50 μg cm^{-2}) was the predominant aliphatics of leaf wax. The ACL value of aliphatics was 29.63 for fruit, and was 28.79 for leaf (Table 6).

Triterpenoids were the prominent cyclic components in both of fruit (53.5%, 110.08 ± 14.04 μg cm^{-2}) and leaf (76.3%, 34.66 ± 5.54 μg cm^{-2}) waxes. The triterpenoids were dominated by ursolic acid (19.6%, 40.33 ± 7.02 μg cm^{-2} for fruit; 53.0%, 24.60 ± 6.16 μg cm^{-2} for leaf), and oleanolic acid (19.7%, 40.56 ± 15.01 μg cm^{-2} for fruit; 10.8%, 5.00 ± 1.01 μg cm^{-2} for leaf). Very small amount of betulic acid, maslinic acid, erythrodiol, uvaol, oleanolic acid methyl ester, and oleanolic aldehyde were also detected in both of fruit and leaf waxes (Table 16).

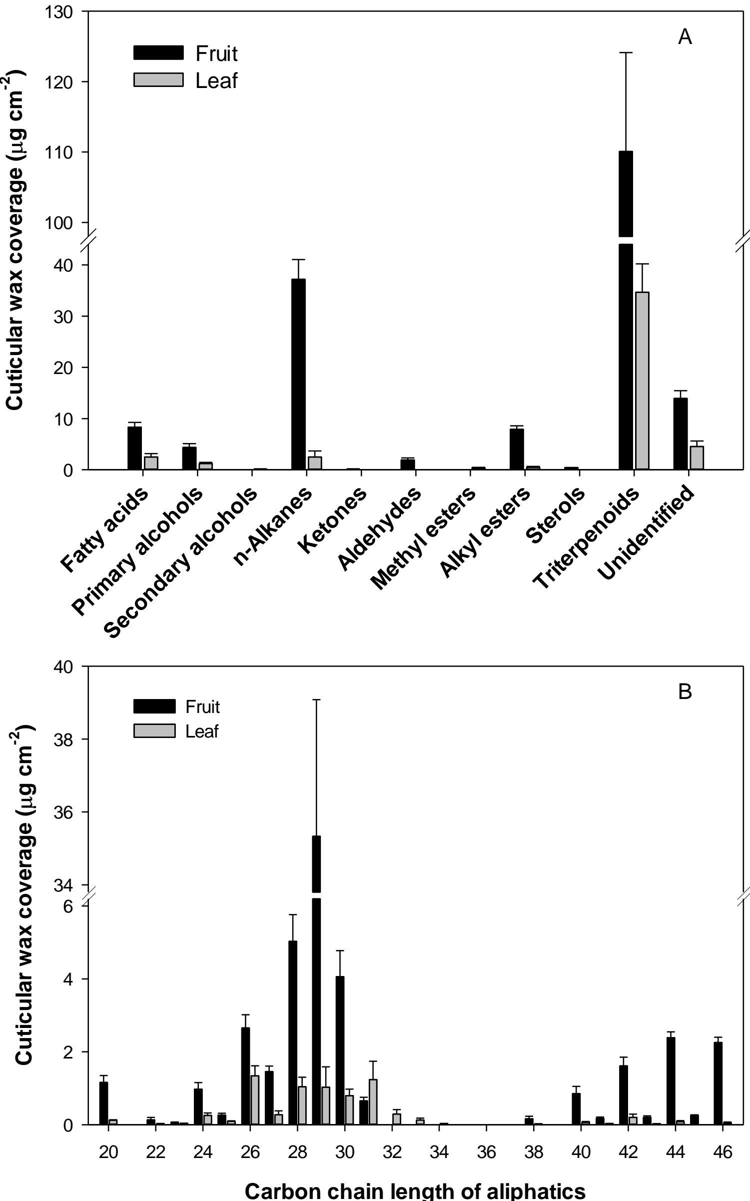

Figure 16. Cuticular wax compositions from *Prunus cerasifera* Ehrh. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 16. The cuticular wax coverage and compositions of *Prunus cerasifera* Ehrh. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	1.16 ± 0.19	0.12 ± 0.01	0.17 ± 0.02	0.08 ± 0.01
22	0.13 ± 0.07	0.03 ± 0.00	0.06 ± 0.01	
23		0.03 ± 0.00	0.05 ± 0.01	
24	0.55 ± 0.16	0.20 ± 0.07	0.41 ± 0.13	
25	0.17 ± 0.03	0.06 ± 0.01	0.13 ± 0.03	
26	1.59 ± 0.25	0.47 ± 0.16	0.89 ± 0.33	0.05 ± 0.02
27	0.15 ± 0.03	0.07 ± 0.03	0.13 ± 0.05	
28	2.52 ± 0.37	0.53 ± 0.17	0.91 ± 0.35	0.14 ± 0.08
29	0.19 ± 0.04	0.04 ± 0.03	0.08 ± 0.06	
30	1.88 ± 0.45	0.60 ± 0.14	0.74 ± 0.21	0.46 ± 0.15
32		0.29 ± 0.12	0.34 ± 0.23	0.25 ± 0.09
34		0.04 ± 0.03	0.08 ± 0.05	
Primary alcohols				
24	0.43 ± 0.09	0.05 ± 0.01	0.10 ± 0.03	
25	0.10 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	
26	1.00 ± 0.18	0.55 ± 0.09	1.05 ± 0.17	0.05 ± 0.02
27	0.10 ± 0.03	0.04 ± 0.01	0.09 ± 0.03	
28	1.48 ± 0.26	0.36 ± 0.08	0.52 ± 0.09	0.20 ± 0.12
29	0.17 ± 0.03	0.02 ± 0.01	0.05 ± 0.02	
30	1.09 ± 0.17	0.14 ± 0.03	0.19 ± 0.04	0.09 ± 0.03
34		0.03 ± 0.01	0.06 ± 0.01	
Secondary alcohols				
26 (pos.2)		0.15 ± 0.04	0.29 ± 0.08	
n-Alkanes				
23	0.06 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	
27	1.21 ± 0.12	0.16 ± 0.10	0.33 ± 0.20	
29	34.97 ± 3.77	0.97 ± 0.54	1.61 ± 1.01	0.32 ± 0.20
30	0.29 ± 0.05			
31	0.65 ± 0.10	1.24 ± 0.50	2.37 ± 0.99	0.10 ± 0.04
33		0.13 ± 0.05	0.26 ± 0.11	
Ketones				
29 (pos.10)	0.10 ± 0.09			
Aldehydes				
26	0.06 ± 0.06			
28	1.03 ± 0.21			
30	0.80 ± 0.17			
Methylesters				
26		0.17 ± 0.04	0.34 ± 0.07	
28		0.16 ± 0.08	0.31 ± 0.15	
30		0.05 ± 0.01	0.11 ± 0.03	
Alkyl esters				
38	0.16 ± 0.07	0.02 ± 0.00	0.04 ± 0.01	
40	0.85 ± 0.20	0.07 ± 0.02	0.13 ± 0.04	

Table 16. continued

41	0.17 ± 0.04	0.03 ± 0.01	0.06 ± 0.01	
42	1.61 ± 0.24	0.20 ± 0.09	0.40 ± 0.18	
43	0.20 ± 0.04	0.03 ± 0.01	0.05 ± 0.01	
44	2.39 ± 0.16	0.08 ± 0.03	0.17 ± 0.06	
45	0.26 ± 0.02			
46	2.26 ± 0.14	0.05 ± 0.02	0.11 ± 0.04	
Sum aliphatic components				
	59.68 ± 4.77	7.21 ± 2.04	12.69 ± 3.84	1.74 ± 0.53
	29.0%	**14.3%**	**24.4%**	**4.2%**
Sterols				
ß-sitosterol	0.40 ± 0.03			
Triterpenoids				
erythrodiol	1.53 ± 0.92	0.67 ± 0.21	0.68 ± 0.21	0.66 ± 0.49
uvaol	0.95 ± 0.08	0.34 ± 0.00		0.34 ± 0.00
oleanolic acid methyl ester	6.46 ± 4.94	0.47 ± 0.34	0.65 ± 0.44	0.29 ± 0.26
oleanolic aldehyde	5.57 ± 5.89	1.71 ± 1.29	2.96 ± 2.07	0.46 ± 1.03
oleanolic acid	40.56 ± 15.01	5.00 ± 1.01	4.79 ± 1.70	5.21 ± 0.69
betulinic acid	2.91 ± 1.34	0.52 ± 0.31	0.21 ± 0.07	0.82 ± 0.60
ursolic acid	40.33 ± 7.02	24.60 ± 6.16	21.39 ± 4.97	27.82 ± 8.66
maslinic acid	7.12 ± 4.26	0.20 ± 0.08	0.41 ± 0.16	
corosolic acid	4.65 ± 2.24			
other triterpenoids	17.31 ± 8.51	1.45 ± 0.20	0.89 ± 0.19	2.01 ± 0.38
Sum aliphatic components				
	132.02 ± 5.06	34.66 ± 5.54	31.98 ± 5.06	37.34 ± 8.10
	64.3%	**76.3%**	**62.6%**	**90.0%**
Unidentified				
	13.83 ± 1.48	4.54 ± 1.07	6.64 ± 1.67	2.44 ± 0.66
Total wax	205.54 ± 6.88	46.40 ± 8.03	51.30 ± 8.28	41.51 ± 8.86

2.10 Cuticular waxes of *Prunus domestica* L. subsp. *syriaca* Janich.

The total wax coverage of *Prunus domestica* L. subsp. *syriaca* Janich. fruit was 212.07 ± 12.27 μg cm^{-2}. The overall wax coverage of leaf surfaces was 39.62 ± 2.41 μg cm^{-2}. The leaf waxes were distributed by 36.89 ± 3.66 μg cm^{-2} and 42.35 ± 5.91 μg cm^{-2} on the asa- and abaxial surfaces, respectively (Table 17). The fruit wax composed similar portion of aliphatic (46.9%, 99.52 ± 10.31 μg cm^{-2}) and cyclic (46.4%, 98.31 ± 8.04 μg cm^{-2}) components. The leaf wax composed a major portion of cyclic components (73.7%, 29.57 ± 3.55 μg cm^{-2}) and a minor portion of aliphatic compounds (19.7%, 7.52 ± 2.34 μg cm^{-2}).

The main aliphatic components of fruit wax were *n*-alkanes (36.5%, 77.27 ± 9.49 μg cm^{-2}) followed by fatty acids (4.7%), aldehydes (2.2%), alkyl esters (1.7%), primary alcohols (1.6%), and small amount of secondary alcohols. The leaf aliphatic components were dominated by *n*-alkanes (9.8%, 3.85 ± 1.87 μg cm^{-2}), followed by fatty acids (4.0%) and primary alcohols (2.5%). Small amount of methyl esters, aldehydes and alkyl esters were only found on adaxial leaf surfaces (Figure 17 A). Carbon chain lengths ranged from C_{20} to C_{48} for fruit and leaf wax. The most abundant chain lengths were C_{28}, C_{29} and C_{30} for fruit wax, and was C_{31} for leaf wax (Figure 17 B). *N*-nonacosane (34.2%, 72.59 ± 8.98 μg cm^{-2}) dominated the aliphatics of fruit wax, and *n*-hentriacontane (5.4%, 2.12 ± 1.02 μg cm^{-2}) was the main aliphatics of leaf wax. The ACL value of aliphatics was 29.15 for fruit, and was 28.85 for leaf (Table 6).

Triterpenoids were the prominent cyclic components in both of fruit (46.1%, 97.58 ± 8.16 μg cm^{-2}) and leaf (73.6%, 29.50 ± 3.53 μg cm^{-2}) waxes. The triterpenoids were dominated by ursolic acid (23.9%, 50.75 ± 5.38 μg cm^{-2} for fruit; 53.3%, 21.13 ± 3.51 μg cm^{-2} for leaf), and oleanolic acid (11.8%, 25.03 ± 0.92 μg cm^{-2} for fruit; 10.5%, 4.15 ± 0.64 μg cm^{-2} for leaf). Small amount of maslinic acid, uvaol, β-amyrin, and α-amyrin were also found in both of fruit and leaf waxes (Table 17).

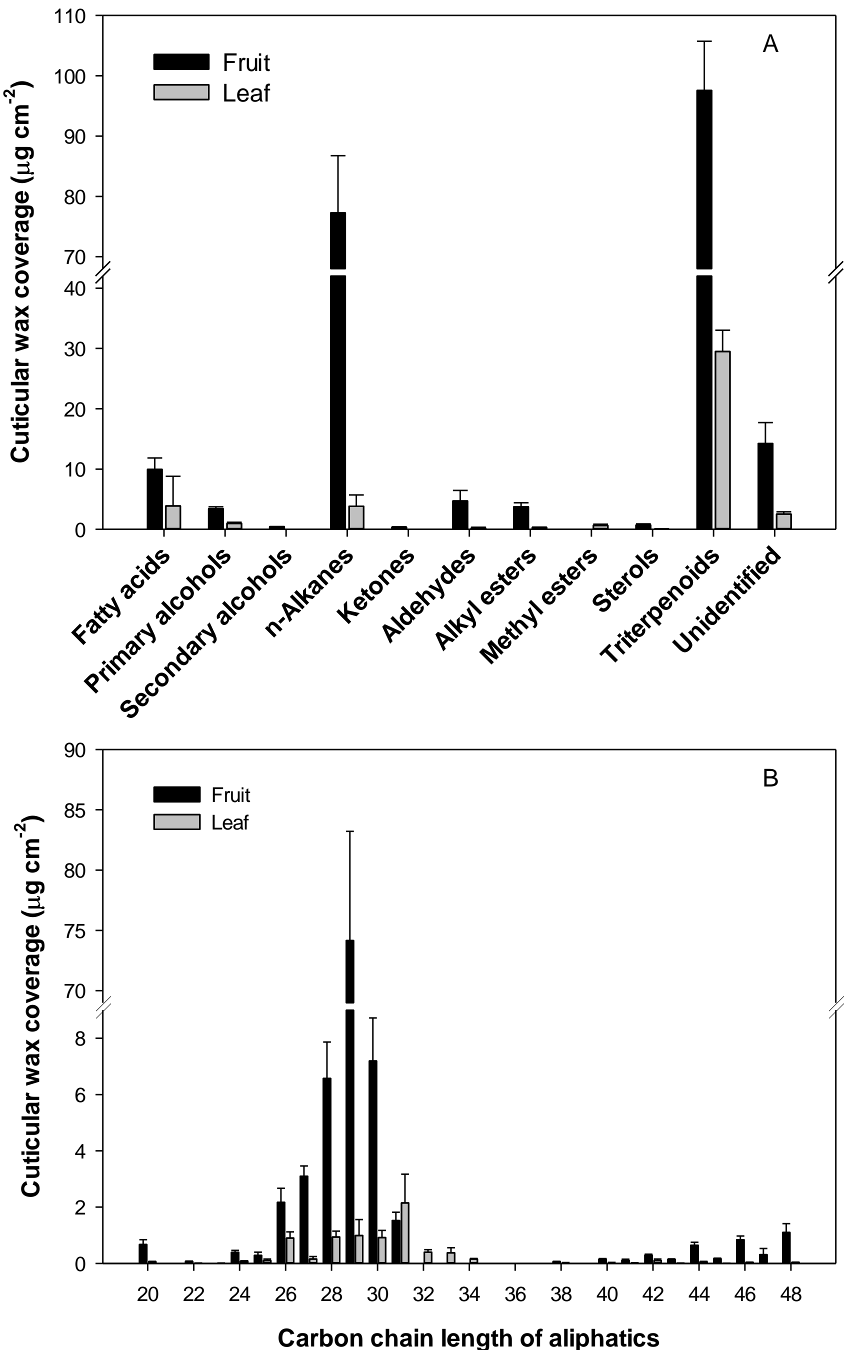

Figure 17. Cuticular wax compositions from *Prunus domestica* L. subsp. *syriaca* Janich. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 17. The cuticular wax coverage and compositions of *Prunus domestica* L. subsp. *syriaca* Janich. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	0.67 ± 0.17	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.02
22	0.07 ± 0.02	0.01 ± 0.00	0.03 ± 0.00	
23		0.01 ± 0.00	0.02 ± 0.01	
24	0.30 ± 0.08	0.05 ± 0.02	0.10 ± 0.03	
25	0.20 ± 0.10	0.03 ± 0.01	0.05 ± 0.02	
26	1.37 ± 0.32	0.26 ± 0.11	0.52 ± 0.22	
27	0.34 ± 0.07	0.03 ± 0.01	0.05 ± 0.01	
28	3.22 ± 0.63	0.29 ± 0.02	0.52 ± 0.06	0.05 ± 0.03
29	0.41 ± 0.16	0.04 ± 0.03	0.09 ± 0.07	
30	3.37 ± 0.99	0.54 ± 0.21	0.75 ± 0.33	0.33 ± 0.20
32	0.00 ± 0.00	0.27 ± 0.06	0.37 ± 0.11	0.18 ± 0.07
Primary alcohols				
24	0.09 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	
25	0.08 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	
26	0.39 ± 0.07	0.37 ± 0.15	0.74 ± 0.30	
27	0.32 ± 0.05	0.02 ± 0.01	0.04 ± 0.01	
28	1.14 ± 0.11	0.22 ± 0.03	0.37 ± 0.06	0.07 ± 0.01
29	0.38 ± 0.10	0.02 ± 0.00	0.03 ± 0.01	
30	1.00 ± 0.13	0.15 ± 0.02	0.19 ± 0.05	0.10 ± 0.02
32		0.03 ± 0.01	0.06 ± 0.02	
34		0.15 ± 0.02	0.10 ± 0.05	0.21 ± 0.03
Secondary alcohols				
29 (pos.2)	0.21 ± 0.04			
29 (pos.3)	0.21 ± 0.02			
n-Alkane				
25		0.06 ± 0.04	0.12 ± 0.09	
27	2.43 ± 0.29	0.11 ± 0.08	0.21 ± 0.17	
28	0.24 ± 0.00			
29	72.59 ± 8.98	0.93 ± 0.57	1.53 ± 1.12	0.33 ± 0.09
30	0.68 ± 0.09	0.13 ± 0.05	0.21 ± 0.11	0.05 ± 0.02
31	1.52 ± 0.30	2.12 ± 1.02	3.70 ± 2.18	0.54 ± 0.18
32		0.13 ± 0.04	0.25 ± 0.08	
33		0.38 ± 0.18	0.75 ± 0.36	
Ketones				
29 (pos.10)	0.35 ± 0.07			
Aldehydes				
26	0.40 ± 0.17			
28	2.15 ± 0.88	0.15 ± 0.17	0.30 ± 0.33	
30	2.14 ± 0.87	0.02 ± 0.01	0.04 ± 0.02	
Methyl esters				
26		0.27 ± 0.14	0.55 ± 0.28	
28		0.28 ± 0.08	0.57 ± 0.16	
30		0.08 ± 0.02	0.16 ± 0.04	
Alkyl esters				
38	0.06 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	
40	0.15 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	

Table 17. continued

41	0.11 ± 0.04	0.01 ± 0.01	0.03 ± 0.01	
42	0.29 ± 0.03	0.10 ± 0.05	0.20 ± 0.11	
43	0.14 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	
44	0.64 ± 0.11	0.05 ± 0.02	0.10 ± 0.04	
45	0.16 ± 0.02	traces	0.02 ± 0.00	
46	0.84 ± 0.13	0.03 ± 0.01	0.06 ± 0.02	
47	0.31 ± 0.22			
48	1.10 ± 0.31	0.03 ± 0.01	0.07 ± 0.02	
Sum aliphatic components				
	99.52 ± 10.31	7.52 ± 2.34	13.13 ± 4.65	1.91 ± 0.36
	46.9%	**19.7%**	**34.9%**	**4.5%**
Sterols				
ß-sitosterol	0.73 ± 0.18	0.06 ± 0.04		0.13 ± 0.08
Triterpenoids				
ß-amyrin		0.02 ± 0.00		0.04 ± 0.01
α-amyrin	0.29 ± 0.08	0.66 ± 0.14	0.59 ± 0.06	0.73 ± 0.25
erythrodiol	1.03 ± 1.00			
uvaol	1.50 ± 0.12	0.20 ± 0.16		0.51 ± 0.25
oleanolic acid	25.03 ± 0.92	4.15 ± 0.64	3.33 ± 1.57	4.97 ± 0.64
betulinic acid	1.58 ± 0.41			
ursolic acid	50.75 ± 5.38	21.13 ± 3.51	14.01 ± 2.71	28.25 ± 4.81
maslinic acid	4.13 ± 2.84	0.24 ± 0.05	0.33 ± 0.08	0.15 ± 0.03
oleanolic acid methyl ester		0.33 ± 0.30	0.58 ± 0.49	0.72 ± 0.72
oleanolic acid aldehyde		0.10 ± 0.23	1.05 ± 0.00	
other triterpenoids	13.27 ± 1.36	2.66 ± 0.69	1.80 ± 0.32	3.52 ± 1.62
Sum cyclic components				
	98.31 ± 8.04	29.57 ± 3.55	20.49 ± 2.64	38.64 ± 5.27
	46.4%	**73.7%**	**56.2%**	**91.3%**
Unidentified				
	14.24 ± 3.46	2.54 ± 0.38	3.27 ± 0.45	1.81 ± 0.65
Total wax	212.07 ± 12.27	39.62 ± 2.41	36.89 ± 3.66	42.35 ± 5.91

2.11 Cuticular waxes of *Prunus domestica* subsp. *insititia* (L.)

The total wax coverage of *Prunus domestica* subsp. *insititia* (L.) fruit was 246.75 ± 21.19 µg cm^{-2}. The overall wax load on leaf surfaces was 23.70 ± 1.68 µg cm^{-2}. The leaf waxes were distributed by 20.25 ± 1.24 µg cm^{-2} and 27.15 ± 2.90 µg cm^{-2} on the ad- and abaxial surfaces, respectively (Table 18). The fruit wax composed a major portion of aliphatic components (82.20%, 202.95 ± 19.41 µg cm^{-2}) and a minor pattern of cyclic components (2.2%, 5.35 ± 0.66 µg cm^{-2}). The leaf wax composed a major portion of cyclic components (51.3%, 12.38 ± 1.11 µg cm^{-2}) and a minor portion of aliphatic compounds (32.2%, 7.38 ± 0.50 µg cm^{-2}).

The main aliphatic components of fruit wax were secondary alcohols (36.1%, 88.04 ± 9.94 µg cm^{-2}) followed by *n*-alkanes (14.7%, 35.69 ± 3.81 µg cm^{-2}), alkyl esters (11.9%, 28.89 ± 1.16 µg cm^{-2}), and primary alcohols (11.9%, 29.01 ± 5.10 µg cm^{-2}), and small amount of fatty acids, aldehydes, and ketones. The leaf aliphatic components were dominated by primary alcohols (10.7%, 2.52 ± 0.18 µg cm^{-2}), followed by *n*-alkanes (7.9%, 1.87 ± 0.15 µg cm^{-2}), alkyl esters (7.8%, 1.84 ± 0.24 µg cm^{-2}) fatty acids (4.0%), and small amount of secondary alcohols and hydroxyl fatty acids (Figure 18 A). Carbon chain lengths ranged from C_{20} to C_{52}, and the most abundant chain lengths were C_{28}, C_{29} and C_{30} for both fruit and leaf wax (Figure 18 B). Nonacosan-10-ol (33.8%, 82.18 ± 9.55 µg cm^{-2}) and *n*-nonacosane (17.1%, 31.86 ± 3.36 µg cm^{-2}) dominated the aliphatics for fruit. *N*-nonacosane (2.5%) and *n*-hentriacontane (1.9%) were the main alipahtics of leaf wax. The ACL value of aliphatics was 29.68 for fruit, and was 30.87 for leaf (Table 6).

The cyclics were dominated by ursolic acid (39.8%, 9.42 ± 0.86 µg cm^{-2}) and oleanolic acid (9.4%, 2.23 ± 0.25 µg cm^{-2}) in leaf wax. Small amount of oleanolic acid (1.3%, 3.18 ± 0. 62 µg cm^{-2}), and ursolic acid were found in fruit wax (Table 18).

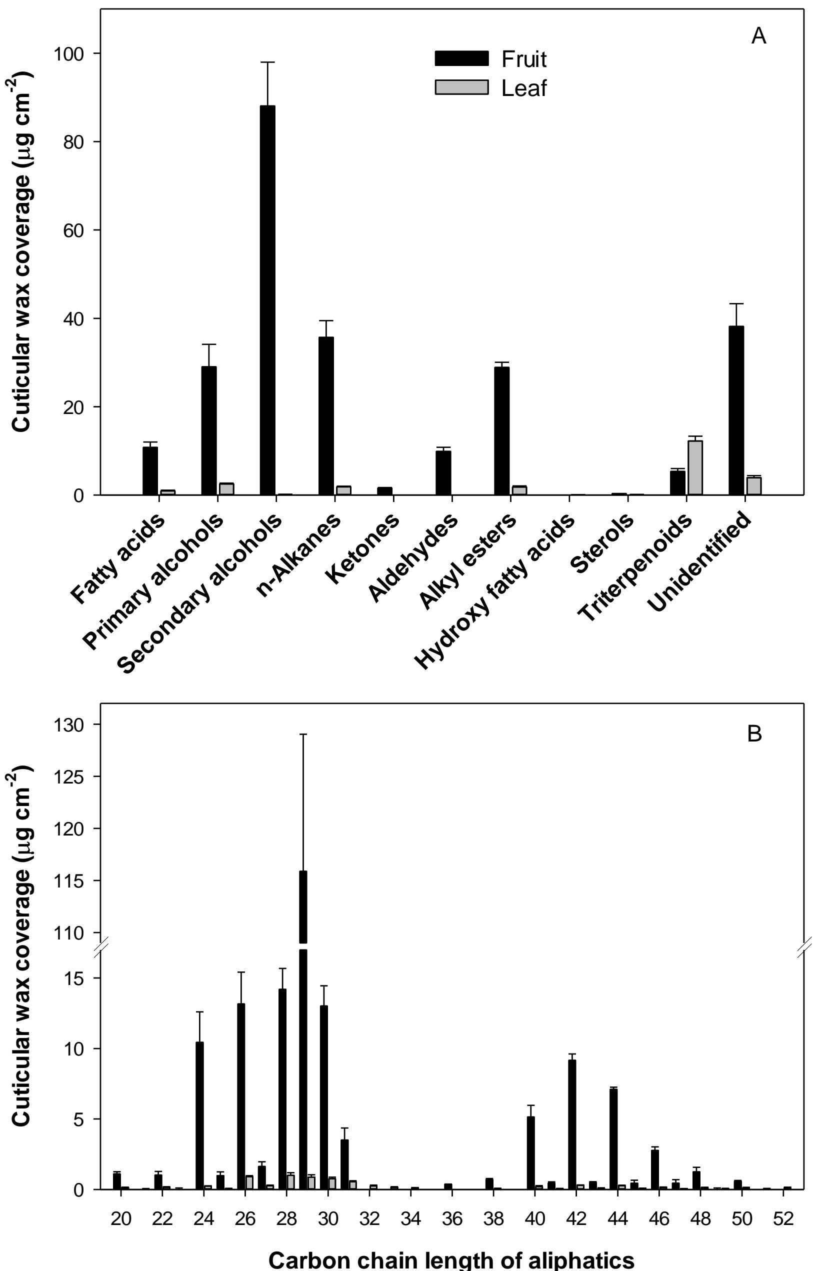

Figure 18. Cuticular wax compositions from *Prunus domestica* subsp. *insititia* (L.) fruits and leaves. (A) Cuticular wax compound classes of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 18. The cuticular wax coverage and compositions of *Prunus domestica* subsp. *insititia* (L.). Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	1.09 ± 0.16	0.14 ± 0.02	0.11 ± 0.03	0.17 ± 0.04
21		0.05 ± 0.02		0.09 ± 0.04
22	0.12 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
24	0.80 ± 0.16	0.04 ± 0.00	0.09 ± 0.01	
25	0.18 ± 0.04	0.04 ± 0.01	0.08 ± 0.01	
26	1.40 ± 0.21	0.14 ± 0.02	0.21 ± 0.03	0.06 ± 0.01
27	0.61 ± 0.39			
28	3.04 ± 0.34	0.25 ± 0.11	0.36 ± 0.15	0.14 ± 0.07
29	0.31 ± 0.23	0.06 ± 0.02	0.07 ± 0.03	0.05 ± 0.01
30	2.89 ± 0.45	0.15 ± 0.05	0.23 ± 0.12	0.07 ± 0.04
32	0.33 ± 0.14	0.05 ± 0.01	0.10 ± 0.03	
Primary alcohols				
22	0.90 ± 0.26	0.13 ± 0.03	0.13 ± 0.03	0.12 ± 0.04
24	9.63 ± 2.02	0.20 ± 0.01	0.31 ± 0.02	0.09 ± 0.01
25	0.56 ± 0.27	0.03 ± 0.00	0.06 ± 0.01	
26	10.96 ± 1.91	0.52 ± 0.05	0.83 ± 0.11	0.20 ± 0.05
27	0.18 ± 0.04	0.06 ± 0.01	0.12 ± 0.02	
28	3.83 ± 0.66	0.68 ± 0.08	1.07 ± 0.16	0.28 ± 0.04
29		0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.05
30	2.95 ± 0.42	0.46 ± 0.04	0.52 ± 0.04	0.40 ± 0.09
31		0.11 ± 0.03	0.14 ± 0.03	0.09 ± 0.04
32		0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.04
33		0.03 ± 0.01	0.06 ± 0.02	
34		0.11 ± 0.02	0.12 ± 0.03	0.09 ± 0.01
Secondary alcohols				
28 (pos. 9/10)	4.04 ± 0.59			
29 (pos. 9/10)	82.18 ± 9.55	0.14 ± 0.08	0.05 ± 0.01	0.24 ± 0.15
30 (pos.10/11)	0.61 ± 0.12			
31 (pos.10/11)	1.22 ± 0.14			
Hydroxy fatty acids				
26		0.05 ± 0.07	0.11 ± 0.13	
Ketones				
29 (pos.10)	1.54 ± 0.17			
n-Alkanes				
23	0.17 ± 0.00			
25	0.24 ± 0.03			
26		0.20 ± 0.12		0.40 ± 0.24
27	0.84 ± 0.20	0.20 ± 0.05	0.10 ± 0.03	0.30 ± 0.11
28	0.32 ± 0.26	0.07 ± 0.04		0.15 ± 0.09
29	31.86 ± 3.36	0.59 ± 0.08	0.22 ± 0.03	0.96 ± 0.19
30	0.44 ± 0.15	0.15 ± 0.03	0.05 ± 0.02	0.26 ± 0.05
31	1.96 ± 0.91	0.44 ± 0.04	0.29 ± 0.03	0.60 ± 0.08
32		0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.04
33		0.13 ± 0.02	0.06 ± 0.01	0.21 ± 0.03

Table 18. continued

Aldehydes				
26	0.80 ± 0.21			
28	2.96 ± 0.37			
30	6.12 ± 0.75			
Alkyl esters				
36	0.36 ± 0.02			
38	0.70 ± 0.08	0.07 ± 0.02	0.09 ± 0.03	0.05 ± 0.02
40	5.14 ± 0.83	0.21 ± 0.07	0.19 ± 0.03	0.22 ± 0.14
41	0.44 ± 0.10	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.03
42	9.16 ± 0.45	0.29 ± 0.03	0.32 ± 0.05	0.26 ± 0.07
43	0.48 ± 0.07	0.08 ± 0.03	0.07 ± 0.01	0.10 ± 0.05
44	7.10 ± 0.16	0.27 ± 0.03	0.27 ± 0.04	0.27 ± 0.06
45	0.44 ± 0.21	0.07 ± 0.02	0.10 ± 0.04	0.04 ± 0.02
46	2.77 ± 0.25	0.16 ± 0.00	0.15 ± 0.02	0.18 ± 0.03
47	0.45 ± 0.25	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.02
48	1.25 ± 0.32	0.13 ± 0.02	0.14 ± 0.03	0.12 ± 0.02
49	0.17 ± 0.00	0.06 ± 0.01	0.08 ± 0.02	0.04 ± 0.02
50	0.58 ± 0.05	0.12 ± 0.03	0.12 ± 0.03	0.12 ± 0.04
51		0.05 ± 0.02	0.10 ± 0.04	
52		0.12 ± 0.05	0.12 ± 0.06	0.11 ± 0.04
54		0.10 ± 0.02	0.11 ± 0.04	0.08 ± 0.04
Sum aliphatic components				
	202.95 ± 19.41	7.38 ± 0.50	7.82 ± 0.42	6.95 ± 0.65
	82.2%	**32.2%**	**38.7%**	**25.7%**
Sterols				
ß-sitosterol	0.31 ± 0.05	0.15 ± 0.02	0.10 ± 0.02	0.21 ± 0.04
Triterpenoids				
erythrodiol		0.10 ± 0.03		0.19 ± 0.06
uvaol		0.17 ± 0.03	0.15 ± 0.07	0.19 ± 0.01
oleanolic acid	3.18 ± 0.62	2.23 ± 0.25	1.40 ± 0.15	3.06 ± 0.48
ursolic acid	0.90 ± 0.11	9.42 ± 0.86	7.24 ± 0.98	11.61 ± 1.27
maslinic acid	1.26 ± 0.22	0.31 ± 0.10	0.20 ± 0.08	0.42 ± 0.20
Sum cyclic components				
	5.35 ± 0.66	12.38 ± 1.11	9.10 ± 1.18	15.67 ± 1.70
	2.2%	**51.3%**	**44.8%**	**57.7%**
Unidentified				
	38.18 ± 5.16	3.93 ± 0.47	3.33 ± 0.26	4.53 ± 0.86
Total wax	246.75 ± 21.19	23.70 ± 1.68	20.25 ± 1.24	27.15 ± 2.90

2.12 Cuticular waxes of *Prunus persica* L.

The total wax load of *Prunus persica* L. (nectarine) fruit was 288.04 ± 32.59 μg cm^{-2}. The overall coverage of waxes on leaf surfaces was 47.29 ± 12.44 μg cm^{-2}. The leaf waxes were distributed by a lower coverage of 31.18 ± 5.84 μg cm^{-2} on the adaxial surfaces, in coamparison to the deposition of 63.41 ± 28.19 μg cm^{-2} on the abaxial surfaces (Table 19). The fruit wax composed mainly cyclic components (81.5%, 234.53 ± 25.71 μg cm^{-2}) and a minor portion of aliphatics (10.9%, 31.49 ± 5.84 μg cm^{-2}). The leaf wax composed a similar portion of cyclic components (40.3%, 20.21 ± 5.88 μg cm^{-2}) and aliphatic compounds (48.1%, 22.24 ± 6.04 μg cm^{-2}).

The main aliphatic components of fruit wax were alkyl esters (3.5%, 20.21 ± 5.88 μg cm^{-2}), *n*-alkanes (3.1%, 9.11 ± 2.77 μg cm^{-2}), primary alcohols (2.5%), and fatty acids (1.1%). The leaf aliphatic components were dominated by primary alcohols (15.8%) and *n*-alkanes (15.6%), followed by alkyl esters (11.8%), fatty acids (4.7%), and small amount of aldehydes (1.8%) (Figure 19 A). Carbon chain lengths ranged from C_{19} to C_{52} for fruit and leaf wax. The most abundant chain lengths were C_{26}, C_{28} and C_{29} for fruit wax, and were C_{31} and C_{32} in leaf wax (Figure 19 B). *N*-nonacosane (1.6%) dominated the aliphatic pattern of fruit wax. *N*-hentriscontane (8.8%) was the main alipahtics for leaf wax. The ACL value of aliphatics was 29.92 for fruit, and was 33.85 for leaf (Table 6).

Triterpenoids were the prominent cyclic components in both of fruit (80.8%, 232.72 ± 25.6 μg cm^{-2}) and leaf (40.0%, 20.08 ± 5.93 μg cm^{-2}) wax. The triterpenoids were dominated by ursolic acid (51.5%, 148.48 ± 16.60 μg cm^{-2} for fruit; 29.2%, 13.80 ± 4.92 μg cm^{-2} for leaf) and oleanolic acid (15.0%, 43.35 ± 2.71 μg cm^{-2} for fruit; 5.5%, 2.62 ± 1.03 μg cm^{-2} for leaf). Small amount of maslinic acid, uvaol and β-sitosterol were also found in fruit and leaf wax (Table 19).

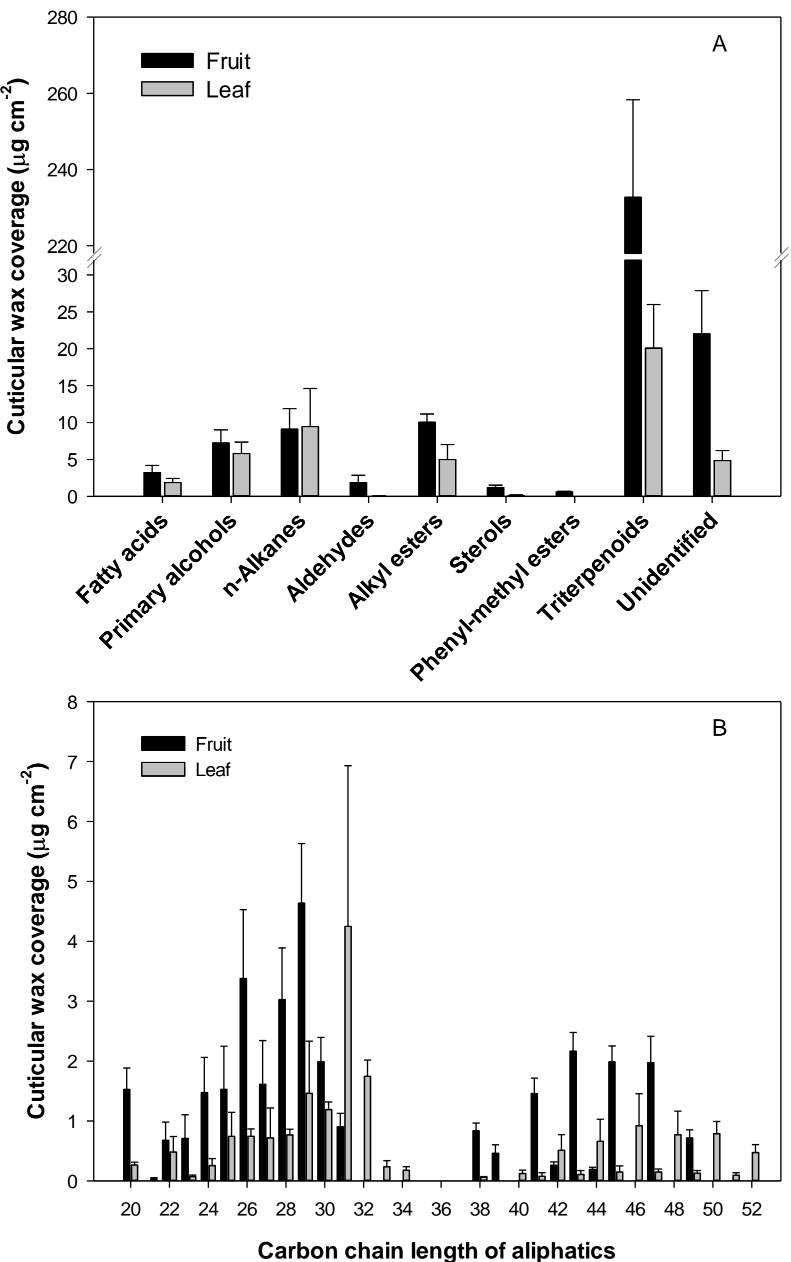

Figure 19. Cuticular wax compositions from *Prunus persica* L. (nectarine) fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit ad- and abaxial leaf (mean values ± SD, n = 5).

Table 19. The cuticular wax coverage and compositions of *Prunus persica* L. (nectarine) fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
19		0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
20	1.36 ± 0.30	0.21 ± 0.05	0.26 ± 0.11	0.23 ± 0.05
21	0.00 ± 0.00	0.03 ± 0.02	0.04 ± 0.03	0.03 ± 0.02
22	0.19 ± 0.09	0.30 ± 0.16	0.07 ± 0.05	0.52 ± 0.30
23	0.07 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
24	0.56 ± 0.29	0.10 ± 0.08	0.16 ± 0.14	0.04 ± 0.02
25	0.00 ± 0.00	0.03 ± 0.03	0.06 ± 0.05	
26	0.51 ± 0.20	0.23 ± 0.11	0.33 ± 0.23	0.13 ± 0.08
27		0.03 ± 0.02	0.07 ± 0.05	
28	0.40 ± 0.12	0.18 ± 0.11	0.28 ± 0.21	0.08 ± 0.05
29		0.05 ± 0.02	0.09 ± 0.05	
30	0.13 ± 0.03	0.31 ± 0.19	0.41 ± 0.26	0.22 ± 0.15
31		0.04 ± 0.08	0.08 ± 0.15	
32		0.26 ± 0.13	0.21 ± 0.07	0.31 ± 0.23
34		0.02 ± 0.02		0.07 ± 0.03
Primary alcohols				
20	0.16 ± 0.06	0.05 ± 0.02	0.02 ± 0.01	0.08 ± 0.05
22	0.23 ± 0.13	0.19 ± 0.12	0.12 ± 0.08	0.35 ± 0.23
23	0.05 ± 0.02			
24	0.80 ± 0.28	0.15 ± 0.08	0.36 ± 0.19	0.26 ± 0.16
25	0.15 ± 0.04	0.01 ± 0.01	0.03 ± 0.01	
26	1.96 ± 0.70	0.47 ± 0.08	1.29 ± 0.42	0.42 ± 0.11
27		0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
28	2.04 ± 0.55	0.58 ± 0.08	0.98 ± 0.33	0.64 ± 0.14
29		0.06 ± 0.04	0.07 ± 0.06	0.05 ± 0.02
30	1.85 ± 0.42	0.74 ± 0.17	0.99 ± 0.59	0.93 ± 0.14
31		0.04 ± 0.01	0.07 ± 0.03	
32		1.23 ± 0.22	2.38 ± 1.13	1.17 ± 0.33
33		0.05 ± 0.01	0.09 ± 0.02	
34		0.46 ± 0.09	0.71 ± 0.31	0.56 ± 0.13
n-Alkanes				
23	0.58 ± 0.39	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
25	1.37 ± 0.71	0.70 ± 0.40	0.16 ± 0.05	1.23 ± 0.82
26		0.03 ± 0.02		0.06 ± 0.04
27	1.61 ± 0.73	0.64 ± 0.49	0.98 ± 0.50	1.20 ± 1.00
29	4.64 ± 1.00	1.34 ± 0.86	0.08 ± 0.02	2.61 ± 1.71
30		0.14 ± 0.10	0.07 ± 0.02	0.21 ± 0.19
31	0.90 ± 0.23	4.17 ± 2.65	0.12 ± 0.04	8.22 ± 5.30
32		0.25 ± 0.15	0.07 ± 0.03	0.43 ± 0.29
33		1.54 ± 0.77		3.07 ± 1.53
34		0.19 ± 0.10		0.37 ± 0.20
Aldehydes				
22	0.26 ± 0.15			
24	0.12 ± 0.09			
26	0.91 ± 0.59	0.01 ± 0.00	0.02 ± 0.01	
27		0.02 ± 0.01	0.03 ± 0.02	
28	0.59 ± 0.25	0.01 ± 0.00	0.02 ± 0.01	

Table 19. continued

30		0.01 ± 0.01	0.03 ± 0.03	
Alkyl esters				
36	0.84 ± 0.13			
38	0.46 ± 0.14	0.06 ± 0.01	0.09 ± 0.02	0.03 ± 0.03
40	1.46 ± 0.26	0.12 ± 0.06	0.13 ± 0.05	0.11 ± 0.10
41	0.26 ± 0.05	0.08 ± 0.06	0.11 ± 0.14	0.05 ± 0.03
42	2.16 ± 0.31	0.51 ± 0.26	0.46 ± 0.22	0.56 ± 0.42
43	0.19 ± 0.04	0.11 ± 0.07	0.13 ± 0.14	0.08 ± 0.03
44	1.99 ± 0.27	0.66 ± 0.37	0.56 ± 0.28	0.76 ± 0.58
45		0.15 ± 0.10	0.19 ± 0.21	0.11 ± 0.06
46	1.97 ± 0.44	0.92 ± 0.53	0.79 ± 0.38	1.05 ± 0.88
47		0.15 ± 0.05	0.15 ± 0.06	0.14 ± 0.11
48	0.72 ± 0.14	0.77 ± 0.39	0.62 ± 0.17	0.92 ± 0.70
49		0.13 ± 0.04	0.14 ± 0.02	0.12 ± 0.09
50		0.79 ± 0.20	0.94 ± 0.26	0.64 ± 0.49
51		0.10 ± 0.04	0.10 ± 0.02	0.11 ± 0.08
52		0.47 ± 0.13	0.54 ± 0.08	0.40 ± 0.27
Sum aliphatic components				
	31.49 ± 5.84	22.24 ± 6.04	15.84 ± 2.54	28.63 ± 14.32
	10.9%	**48.1%**	**44.8%**	**51.4%**
Sterols				
ß-sitosterol	1.22 ± 0.31	0.13 ± 0.06	0.16 ± 0.06	0.15 ± 0.07
Triterpenoids				
ß-amyrin	0.27 ± 0.08			
erythrodiol	1.18 ± 0.59			
uvaol	2.18 ± 0.16	0.36 ± 0.16	0.38 ± 0.23	0.33 ± 0.20
oleanolic acid methyl ester	1.96 ± 1.03	0.73 ± 0.72		1.46 ± 1.43
oleanolic aldehyde	20.09 ± 8.83			
oleanolic acid	43.35 ± 2.71	2.62 ± 1.03	0.76 ± 0.28	4.49 ± 2.08
betulinic acid		0.17 ± 0.07		0.35 ± 0.15
ursolic acid	148.48 ± 16.60	13.80 ± 4.92	5.66 ± 2.17	22.17 ± 9.33
maslinic acid	2.09 ± 1.12	0.43 ± 0.11	0.48 ± 0.18	0.38 ± 0.20
corosolic acid	3.71 ± 1.86			
other triterpenoids	9.42 ± 3.47	0.69 ± 0.34	3.09 ± 1.94	0.62 ± 0.43
Phenyl-methyl ester				
30	0.58 ± 0.12			
Sum cyclic components				
	234.53 ± 25.71	20.21 ± 5.88	10.50 ± 3.43	29.92 ± 12.51
	81.5%	**40.3%**	**47.7%**	**33.0%**
Unidentified				
	22.02 ± 5.86	4.85 ± 1.37	4.84 ± 1.19	4.86 ± 2.53
Total wax	288.04 ± 32.60	47.29 ± 12.44	31.18 ± 5.84	63.41 ± 28.19

2.13 Cuticular waxes of *Vitis vinifera* L. cv. 'Nelly'

The total wax coverage of *Vitis vinifera* L. cv. 'Nelly' fruit was 257.90 ± 22.08 μg cm^{-2}. The overall coverage of waxes on leaf surfaces was 11.04 ± 2.32 μg cm^{-2}. The leaf waxes were distributed by a higher coverage of 16.81 ± 2.61 μg cm^{-2} on the adaxial surfaces, in comparison to the amount of 5.26 ± 4.44 μg cm^{-2} on the abaxial surfaces (Table 20). The fruit wax composed a similar portion of cyclic components (48.9%, 127.37 ± 30.81 μg cm^{-2}) and aliphatic compounds (43.6%, 111.55 ± 4.91 μg cm^{-2}). The leaf wax composed major aliphatic components (80.5%, 9.16 ± 2.00 μg cm^{-2}) and only a small amount of cyclic components (1.1%).

The main aliphatic components of fruit wax were fatty acids (18.2%, 46.44 ± 6.28 μg cm^{-2}) followed by primary alcohols (9.1%, 23.56 ± 3.62 μg cm^{-2}), alkyl esters (8.8%, 21.90 ± 9.50 μg cm^{-2}), aldehydes (7.0%), and very small amount of *n*-alkanes. The leaf aliphatic components were dominated by primary alcohols (31.5%, 3.67± 1.01 μg cm^{-2}) followed by alkyl esters (22.9%, 2.85 ± 0.36 μg cm^{-2}), aldehydes (13.3%), fatty acids (7.5%), and *n*-alkanes (5.4%) (Figure 20 A). Carbon chain lengths ranged from C_{20} to C_{52} for fruit and leaf wax. The most abundant chain lengths were C_{26}, C_{28} and C_{30} for fruit wax, and were C_{28} and C_{30} for leaf wax (Figure 20 B). The ACL value of aliphatics was 30.12 for fruit, and was 31.18 for leaf (Table 6).

Triterpenoids were the prominent cyclic compounds in fruit wax (48.6%, 126.80 ± 30.93 μg cm^{-2}). The triterpenoids were dominated by oleanolic acid (40.6%, 104.74 ± 34.56 μg cm^{-2}). Small amount of erythrodiol, uvaol, β-amyrin, α-amyrin, ursolic acid, taraxerol and β-sitosterol were also detected. Only traces of β-sitosterol, taraxerol and β-amyrin were detected in leaf wax (Table 20).

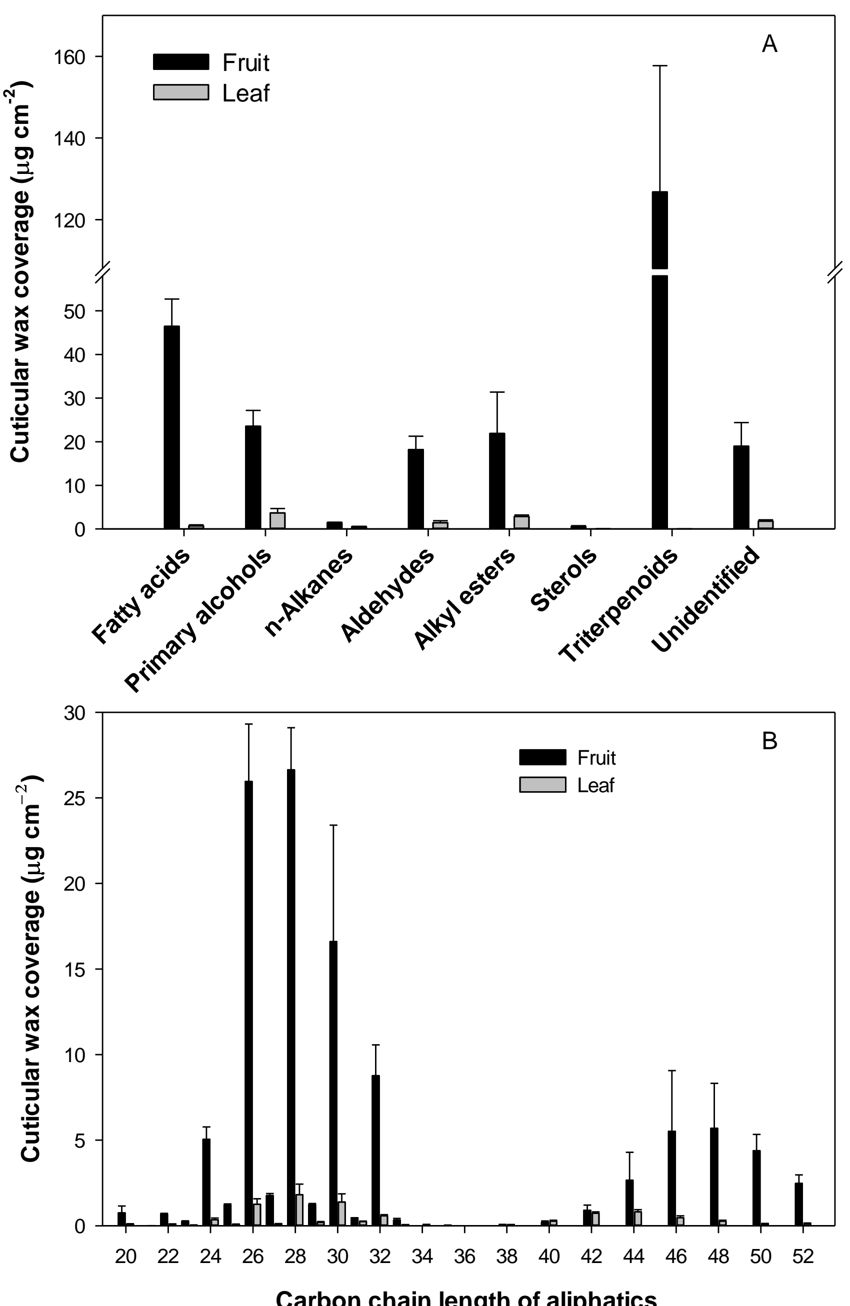

Figure 20. Cuticular wax compositions from *Vitis vinifera* L. cv. 'Nelly' fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 20. The cuticular wax coverage and compositions of *Vitis vinifera* L. cv. 'Nelly' fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit			Whole leaf			Leaf adaxial			Leaf abaxial		
Fatty acids												
20	0.75	±	0.40	0.08	±	0.04	0.08	±	0.01	0.08	±	0.08
21				traces	±	0.00	0.01	±	0.00			
22	0.42	±	0.06	0.07	±	0.02	0.10	±	0.01	0.04	±	0.04
23	0.09	±	0.04	0.02	±	0.01	0.02	±	0.00	0.02	±	0.02
24	2.18	±	0.32	0.11	±	0.05	0.14	±	0.03	0.07	±	0.09
25	0.35	±	0.03	0.01	±	0.00	0.02	±	0.00	0.01	±	0.01
26	11.83	±	0.71	0.10	±	0.02	0.16	±	0.02	0.04	±	0.04
27	0.74	±	0.10	0.02	±	0.01	0.03	±	0.01	0.01	±	0.01
28	14.95	±	1.31	0.13	±	0.05	0.14	±	0.01	0.11	±	0.09
29	0.46	±	0.08	0.03	±	0.02	0.04	±	0.02	0.02	±	0.02
30	11.84	±	7.48	0.13	±	0.04	0.13	±	0.01	0.12	±	0.09
32	2.70	±	1.31	0.02	±	0.01	0.03	±	0.03	0.01	±	0.01
34	0.14	±	0.09									
Primary alcohols												
22	0.26	±	0.02	0.02	±	0.00	0.03	±	0.01	0.01	±	0.00
23	0.07	±	0.01	traces	±	0.00	0.01	±	0.00			
24	2.56	±	0.37	0.19	±	0.06	0.36	±	0.11	0.02	±	0.00
25	0.36	±	0.05	0.03	±	0.01	0.03	±	0.01	0.03	±	0.03
26	8.47	±	1.45	0.87	±	0.25	1.59	±	0.45	0.15	±	0.18
27	0.45	±	0.04	0.03	±	0.01	0.05	±	0.01	0.01	±	0.01
28	6.76	±	1.19	1.29	±	0.42	2.03	±	0.55	0.56	±	0.57
29	0.31	±	0.05	0.04	±	0.02	0.04	±	0.01	0.04	±	0.03
30	2.03	±	0.25	0.78	±	0.27	1.03	±	0.25	0.52	±	0.44
31				0.03	±	0.01	0.04	±	0.01	0.02	±	0.02
32	2.10	±	0.29	0.35	±	0.02	0.57	±	0.13	0.14	±	0.12
34	0.18	±	0.02	0.03	±	0.01	0.04	±	0.01	0.03	±	0.03
n-Alkanes												
23	0.08	±	0.01	0.01	±	0.00	0.01	±	0.00	0.01	±	0.00
25	0.15	±	0.03	0.02	±	0.01	0.02	±	0.02	0.01	±	0.01
26				0.01	±	0.00	0.01	±	0.00	traces		
27	0.24	±	0.05	0.03	±	0.01	0.05	±	0.02	0.02	±	0.01
29	0.23	±	0.03	0.10	±	0.01	0.14	±	0.04	0.05	±	0.03
30	0.14	±	0.04	0.03	±	0.02	0.04	±	0.04	0.02	±	0.01
31	0.44	±	0.03	0.19	±	0.02	0.31	±	0.07	0.07	±	0.05
32	0.17	±	0.03	0.03	±	0.02	0.04	±	0.04	0.03	±	0.01
33				0.05	±	0.02	0.06	±	0.04	0.04	±	0.02
34				0.03	±	0.01	0.02	±	0.03	0.03	±	0.01
35				0.02	±	0.01	0.03	±	0.02	0.02	±	0.01
Aldehydes												
24	0.32	±	0.24	0.05	±	0.01	0.10	±	0.03			
25	0.35	±	0.08	0.01	±	0.00	0.03	±	0.01			

Table 20. continued

26	5.65 ± 1.83	0.27 ± 0.06	0.49 ± 0.12	0.06 ± 0.03
27	0.34 ± 0.06	0.01 ± 0.00	0.03 ± 0.01	
28	4.93 ± 0.68	0.39 ± 0.17	0.52 ± 0.14	0.26 ± 0.28
29	0.23 ± 0.04	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.02
30	2.58 ± 0.63	0.45 ± 0.17	0.57 ± 0.16	0.33 ± 0.29
31		0.02 ± 0.01	0.04 ± 0.01	
32	3.80 ± 0.46	0.17 ± 0.06	0.26 ± 0.08	0.08 ± 0.07
Alkyl esters				
38	0.06 ± 0.01	0.05 ± 0.02	0.06 ± 0.01	0.04 ± 0.05
40	0.21 ± 0.07	0.27 ± 0.05	0.50 ± 0.11	0.04 ± 0.04
42	0.90 ± 0.30	0.74 ± 0.08	1.39 ± 0.17	0.08 ± 0.11
44	2.66 ± 1.63	0.81 ± 0.12	1.34 ± 0.13	0.29 ± 0.30
46	5.51 ± 3.55	0.47 ± 0.11	0.66 ± 0.11	0.27 ± 0.24
48	5.69 ± 2.63	0.27 ± 0.05	0.39 ± 0.06	0.15 ± 0.13
50	4.39 ± 0.95	0.11 ± 0.02	0.14 ± 0.02	0.08 ± 0.06
52	2.48 ± 0.50	0.13 ± 0.03	0.22 ± 0.04	0.05 ± 0.04
Sum aliphatic components				
	111.55 ± 4.91	9.16 ± 2.00	14.21 ± 2.41	4.12 ± 3.58
	43.6%	**80.5%**	**84.3%**	**76.7%**
Sterols				
ß-sitosterol	0.57 ± 0.17	0.04 ± 0.03	0.01 ± 0.00	0.07 ± 0.07
Triterpenoids				
taraxerol	traces	traces	traces	traces
ß-amyrin	0.36 ± 0.07	0.04 ± 0.01	0.06 ± 0.01	0.02 ± 0.01
α-amyrin	0.24 ± 0.08			
erythrodiol	11.46 ± 8.28			
uvaol	0.94 ± 0.18			
gypsognein	0.25 ± 0.10			
oleanolic acid	104.74 ± 34.56			
ursolic acid	5.34 ± 1.89			
oleanolic aldehyde	3.46 ± 0.67			
Sum cyclic components				
	127.37 ± 30.81	0.08 ± 0.04	0.07 ± 0.01	0.09 ± 0.08
	48.9%	**1.1%**	**0.4%**	**1.7%**
Unidentified				
	18.98 ± 5.40	1.79 ± 0.31	2.53 ± 0.33	1.05 ± 0.79
Total wax	257.90 ± 22.08	11.04 ± 2.32	16.81 ± 2.61	5.26 ± 4.44

2.14 Cuticular waxes of *Vitis vinifera* L. cv. 'Silvana'

The total wax coverage of *Vitis vinifera* L. cv. 'Silvana' fruit was 168.20 ± 27.16 μg cm^{-2}. The overall coverage of wax on leaf surfaces was 16.36 ± 1.80 μg cm^{-2}. The leaf waxes were distributed by a higher coverage of 25.30 ± 2.60 μg cm^{-2} on the adaxial surfaces, in comparison to the deposition of 7.43 ± 2.72 μg cm^{-2} on the abaxial surfaces (Table 21). The fruit wax composed a major portion of cyclic components (53.5%, 89.45 ± 13.99 μg cm^{-2}) and a minor portion of aliphatic compounds (36.6%, 62.37 ± 16.76 μg cm^{-2}). The leaf wax composed major aliphatic components (73.0%, 12.31 ± 0.93 μg cm^{-2}) and a small amount of cyclic components (5.0%).

The main aliphatic components of fruit wax were primary alcohols (13.9%, 23.63 ± 6.05 μg cm^{-2}) followed by aldehydes (9.7%, 16.46 ± 4.31 μg cm^{-2}), fatty acids (8.2%, 14.91 ± 3.67 μg cm^{-2}), alkyl esters (3.4%), and very small amount of *n*-alkanes. The leaf aliphatic components were dominated by primary alcohols (34.7%, 6.28 ± 0.68 μg cm^{-2}) followed by aldehydes (13.2%), alkyl esters (11.4%), fatty acids (9.3%), and *n*-alkanes (4.4%) (Figure 21 A). Carbon chain lengths ranged from C_{20} to C_{54} for both fruit and leaf wax. The most abundant chain lengths were C_{26} and C_{28} for fruit wax, and were C_{28} and C_{30} for leaf wax (Figure 21 B). The ACL value of aliphatics was 28.20 for fruit, and was 30.49 for leaf (Table 6).

Oleanolic acid (46.1%, 77.48 ± 13.55 μg cm^{-2}) was the predominant compound of triterpenoids in fruit wax. Small amount of β-sitosterol, erythrodiol, β-amyrin, and taraxerol were found for both fruit and leaf wax (Table 21).

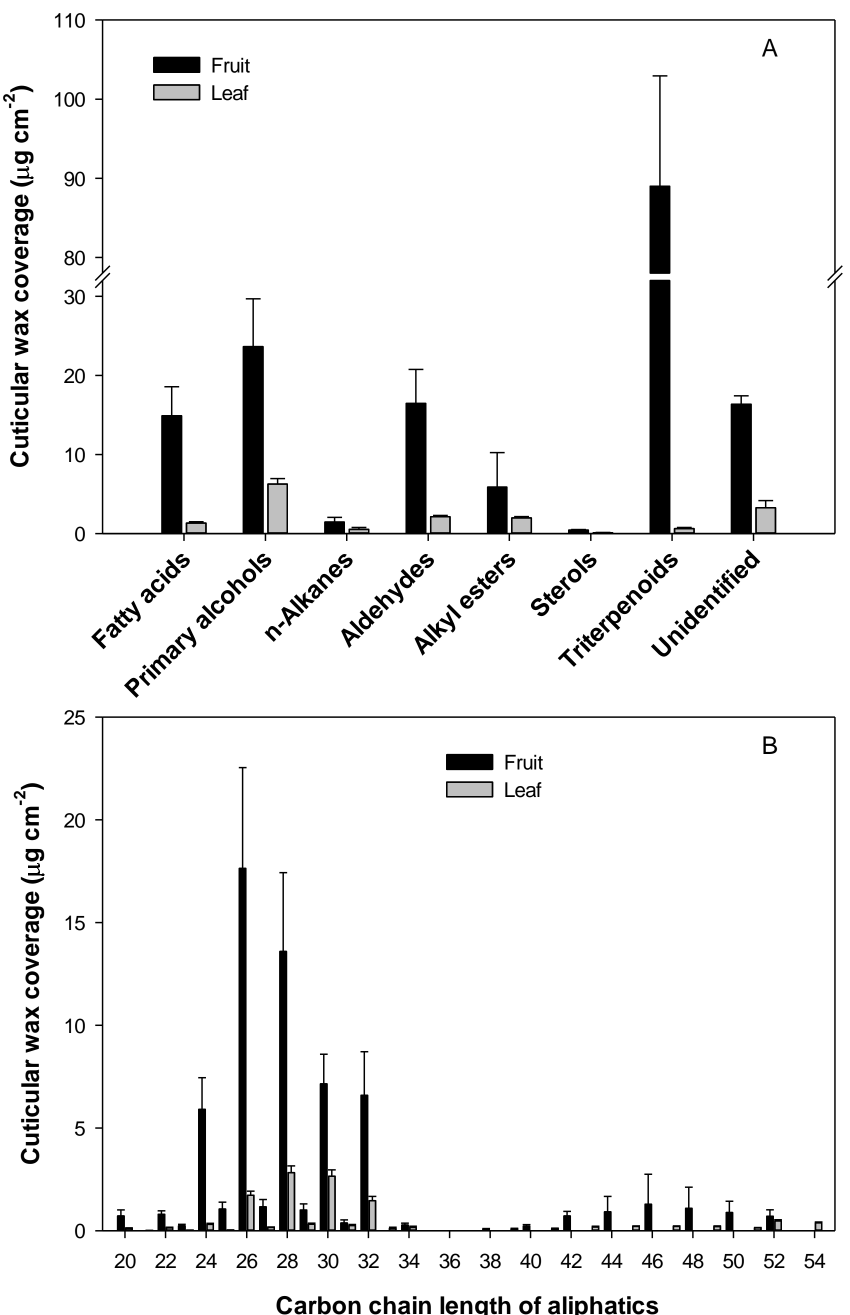

Figure 21. Cuticular wax compositions from *Vitis vinifera* L. cv. 'Silvana' fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaixial leaf (mean values ± SD, n = 5).

Table 21. The cuticular wax coverage and compositions of *Vitis vinifera* L. cv. 'Silvana' fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	0.61 ± 0.33	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.04
21		0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
22	0.17 ± 0.08	0.09 ± 0.02	0.14 ± 0.04	0.03 ± 0.01
23	0.06 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	traces
24	1.08 ± 0.41	0.10 ± 0.02	0.17 ± 0.04	0.02 ± 0.01
25	0.22 ± 0.10	0.02 ± 0.00	0.03 ± 0.00	
26	3.93 ± 1.31	0.16 ± 0.02	0.28 ± 0.04	0.04 ± 0.01
27	0.30 ± 0.09	0.04 ± 0.01	0.06 ± 0.02	0.01 ± 0.01
28	3.87 ± 1.47	0.19 ± 0.03	0.24 ± 0.02	0.15 ± 0.04
29	0.22 ± 0.08	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.03
30	3.00 ± 1.57	0.39 ± 0.07	0.44 ± 0.03	0.33 ± 0.11
32	1.46 ± 0.76	0.16 ± 0.05	0.27 ± 0.08	0.06 ± 0.05
Primary alcohols				
20	0.11 ± 0.06	0.01 ± 0.00	0.02 ± 0.01	
22	0.63 ± 0.11	0.05 ± 0.01	0.09 ± 0.02	0.01 ± 0.01
23	0.11 ± 0.04			
24	3.11 ± 0.68	0.16 ± 0.03	0.30 ± 0.06	0.01 ± 0.00
25	0.41 ± 0.13	0.02 ± 0.01	0.03 ± 0.01	traces
26	8.78 ± 2.43	1.30 ± 0.19	2.44 ± 0.41	0.17 ± 0.09
27	0.50 ± 0.12	0.06 ± 0.01	0.09 ± 0.02	0.02 ± 0.01
28	6.13 ± 1.62	2.14 ± 0.32	3.48 ± 0.78	0.80 ± 0.39
29	0.36 ± 0.06	0.11 ± 0.02	0.19 ± 0.04	0.03 ± 0.01
30	1.87 ± 0.48	1.46 ± 0.20	2.10 ± 0.46	0.82 ± 0.32
31		0.09 ± 0.01	0.14 ± 0.01	0.03 ± 0.01
32	1.34 ± 0.83	0.78 ± 0.05	1.40 ± 0.10	0.16 ± 0.06
33		0.03 ± 0.01	0.05 ± 0.01	0.01 ± 0.00
34	0.28 ± 0.09	0.09 ± 0.02	0.12 ± 0.03	0.05 ± 0.01
n-Alkanes				
23	0.07 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
25	0.18 ± 0.03			
27	0.14 ± 0.05	0.05 ± 0.01	0.08 ± 0.01	0.02 ± 0.01
29	0.25 ± 0.12	0.09 ± 0.03	0.12 ± 0.03	0.06 ± 0.03
30	0.31 ± 0.27	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.02
31	0.38 ± 0.15	0.12 ± 0.05	0.16 ± 0.06	0.08 ± 0.06
32	0.16 ± 0.07	0.07 ± 0.05	0.07 ± 0.06	0.07 ± 0.07
33		0.09 ± 0.05	0.09 ± 0.06	0.09 ± 0.07
34		0.07 ± 0.05	0.06 ± 0.06	0.09 ± 0.04
Aldehydes				
24	1.71 ± 0.53	0.06 ± 0.01	0.11 ± 0.03	
25	0.24 ± 0.13			
26	4.93 ± 1.31	0.26 ± 0.02	0.46 ± 0.05	0.06 ± 0.03
27	0.23 ± 0.12	0.02 ± 0.00	0.03 ± 0.01	0.01 ± 0.00
28	3.58 ± 0.84	0.49 ± 0.03	0.72 ± 0.11	0.27 ± 0.16
29	0.17 ± 0.08	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.03
30	1.97 ± 0.59	0.76 ± 0.11	0.98 ± 0.10	0.54 ± 0.31
31		0.05 ± 0.01	0.07 ± 0.01	0.02 ± 0.01
32	3.63 ± 0.83	0.44 ± 0.13	0.78 ± 0.25	0.11 ± 0.06

Table 21. continued

Alkyl esters												
38	0.08	±	0.03	0.09	±	0.03	0.07	±	0.01	0.11	±	0.05
40	0.22	±	0.08	0.10	±	0.03	0.12	±	0.02	0.08	±	0.04
42	0.72	±	0.22	0.19	±	0.03	0.33	±	0.06	0.04	±	0.01
44	0.92	±	0.75	0.21	±	0.02	0.36	±	0.04	0.07	±	0.02
46	1.29	±	1.46	0.21	±	0.03	0.34	±	0.05	0.08	±	0.02
48	1.09	±	1.02	0.20	±	0.03	0.33	±	0.04	0.07	±	0.02
50	0.88	±	0.55	0.14	±	0.02	0.23	±	0.01	0.05	±	0.03
52	0.70	±	0.31	0.48	±	0.06	0.91	±	0.13	0.04	±	0.02
54				0.38	±	0.05	0.67	±	0.07	0.09	±	0.05
Sum aliphatic components												
	62.37	±	16.76	12.31	±	0.93	19.58	±	1.79	5.04	±	1.77
		36.6%			**73.0%**			**77.5%**			**68.5%**	
Sterols												
β-sitosterol	0.45	±	0.09	0.12	±	0.04	0.10	±	0.02	0.15	±	0.07
Triterpenoids												
taraxerol	0.21	±	0.09	0.43	±	0.13	0.73	±	0.24	0.14	±	0.08
β-amyrin	0.44	±	0.09	0.14	±	0.03	0.18	±	0.05	0.09	±	0.03
erythrodiol	5.58	±	4.41									
oleanolic acid	77.48	±	13.55	0.08	±	0.02	0.12	±	0.03	0.04	±	0.02
oleanolic acid methyl ester	3.00	±	2.35									
oleanolic aldehyde	2.28	±	2.20									
Sum cyclic components												
	89.45	±	13.99	0.77	±	0.16	1.13	±	0.27	0.42	±	0.16
		53.5%			**5.0%**			**4.4%**			**5.6%**	
Unidentified												
	16.37	±	1.06	3.28	±	0.90	4.59	±	1.02	1.97	±	1.17
Total wax	168.20	±	27.16	16.36	±	1.80	25.30	±	2.60	7.43	±	2.72

2.15 Cuticular waxes of *Cornus officinalis* Siebold & Zucc.

The total wax coverage of *Cornus officinalis* fruit was 82.11 ± 14.35 μg cm^{-2}. The overall coverage of wax on leaf surfaces was 18.42 ± 3.91 μg cm^{-2}. The leaf waxes were distributed by 20.26 ± 3.07 μg cm^{-2} and 16.58 ± 5.75 μg cm^{-2} on the ad- and abaxial surfaces, repsectively (Table 22). The cyclic compounds dominated both the fruit (66.5%, 54.72 ± 10.40 μg cm^{-2}) and leaf (59.3%, 10.84 ± 3.25 μg cm^{-2}) wax. The minor portion of aliphatics was 31.3% (25.19 ± 3.44 μg cm^{-2}) for fruit, and was 25.9% (4.83 ± 0.44 μg cm^{-2}) for leaf wax.

The main aliphatic components of fruit wax were *n*-alkanes (19.8%, 15.94 ± 3.33 μg cm^{-2}), followed by primary alcohols (4.9%), fatty acids (4.9%), and alkyl esters (1.7%). The predominant leaf aliphatic components were primary alcohols (10.8%, 1.99 ± 0.25 μg cm^{-2}) followed by *n*-alkanes (5.8%), fatty acids (5.4%), alkyl esters (3.0%), and traces of alcohol acetates (Figure 22 A). Carbon chain lengths ranged from C_{20} to C_{48} in fruit and to C_{50} for leaf wax. The most abundant chain lengths were C_{29} and C_{30} for fruit wax, and was C_{32} for leaf wax (Figure 22 B). The *n*-nonacosane (15.1%, 12.40 ± 3.24 μg cm^{-2}) was the main aliphatics of fruit wax. The ACL value of aliphatics was 28.86 for fruit, and was 29.63 for leaf (Table 6).

Triterpenoids were the predominant cyclic compounds in both of fruit (66.2%, 54.47 ± 10.43 μg cm^{-2}) and leaf (58.2%, 10.64 ± 3.18 μg cm^{-2}) waxes. They were dominated by ursolic acid (37.1%, 30.46 ± 6.53 μg cm^{-2} for fruit wax; 22.3%, 4.12 ± 1.61 μg cm^{-2} in leaf wax) and oleanolic acid (12.2%, 10.04 ± 2.14 μg cm^{-2} for fruit wax; 11.1%, 2.04 ± 0.43 μg cm^{-2} for leaf wax). Additional small amount of β-sitosterol, amyrins, erythrodiol, and lupeol were also detected for both fruit and leaf wax (Table 22).

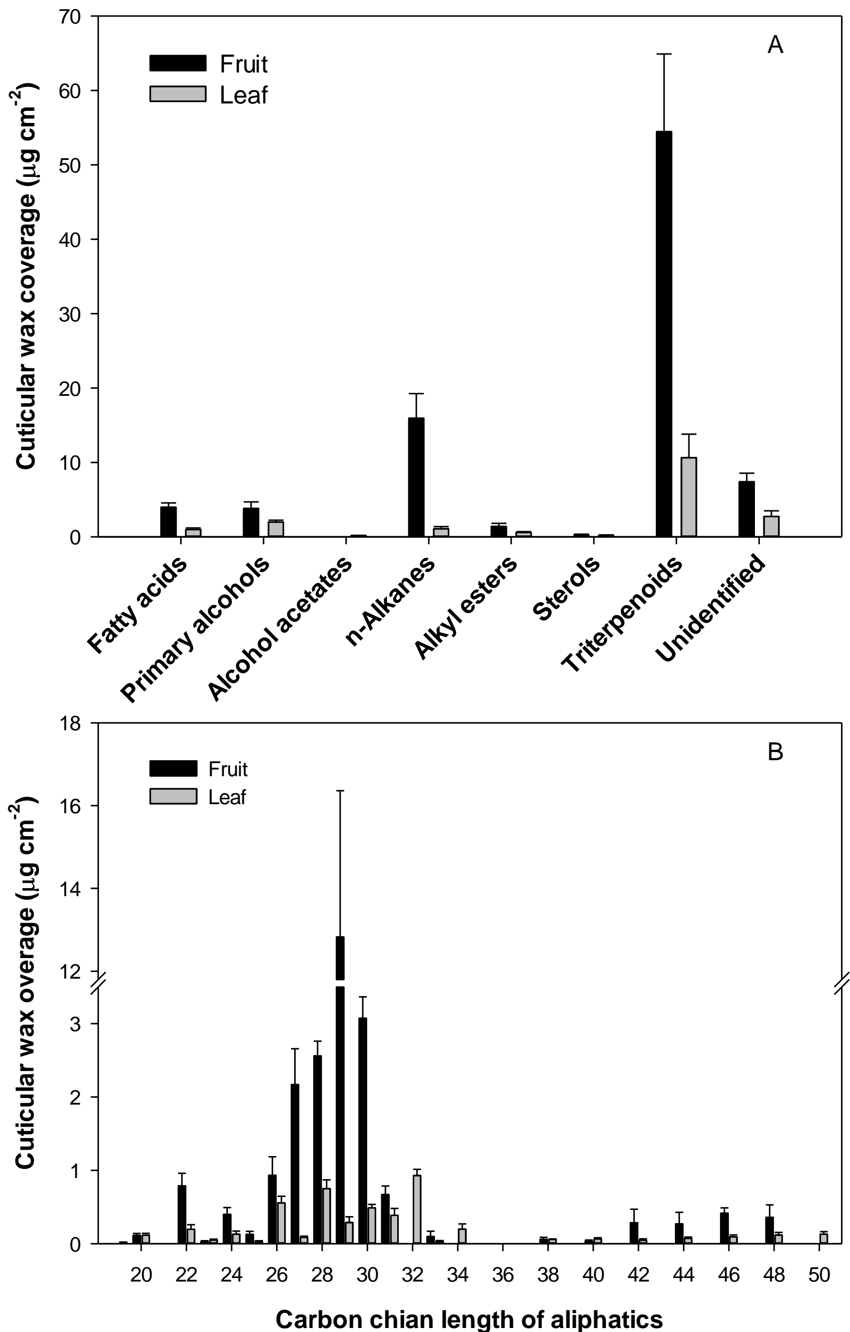

Figure 22. Cuticular wax compositions from *Cornus officinalis* Siebold & Zucc. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 22. The cuticular wax coverage and compositions of fruit and leaf of *Cornus officinalis*. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
20	0.11 ± 0.03	0.10 ± 0.02	0.10 ± 0.04	0.10 ± 0.03
22	0.15 ± 0.04	0.06 ± 0.02	0.07 ± 0.04	0.04 ± 0.01
23		0.01 ± 0.00	0.02 ± 0.01	
24	0.37 ± 0.08	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.01
25	0.10 ± 0.04	0.02 ± 0.00	0.05 ± 0.01	
26	0.38 ± 0.12	0.08 ± 0.02	0.14 ± 0.04	0.03 ± 0.01
27	0.07 ± 0.07	0.01 ± 0.00	0.02 ± 0.01	
28	0.91 ± 0.20	0.30 ± 0.16	0.36 ± 0.25	0.24 ± 0.12
29	0.11 ± 0.03			
30	1.79 ± 0.17	0.11 ± 0.04	0.10 ± 0.07	0.12 ± 0.04
32		0.24 ± 0.06	0.28 ± 0.06	0.19 ± 0.09
Primary alcohols				
20		0.01 ± 0.01	0.03 ± 0.01	
22	0.64 ± 0.18	0.15 ± 0.06	0.20 ± 0.12	0.10 ± 0.03
24	0.03 ± 0.01	0.08 ± 0.04	0.16 ± 0.07	
25	0.03 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	
26	0.55 ± 0.20	0.34 ± 0.06	0.60 ± 0.10	0.08 ± 0.02
27	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	
28	1.45 ± 0.26	0.42 ± 0.07	0.50 ± 0.06	0.35 ± 0.12
29	0.31 ± 0.18	0.03 ± 0.01	0.06 ± 0.01	
30	0.81 ± 0.37	0.30 ± 0.04	0.32 ± 0.03	0.29 ± 0.06
31		0.05 ± 0.02	0.09 ± 0.03	
32		0.45 ± 0.07	0.59 ± 0.13	0.31 ± 0.13
33		0.03 ± 0.01	0.06 ± 0.02	
34		0.11 ± 0.04	0.14 ± 0.04	0.08 ± 0.07
n-Alkanes				
23	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
26		0.02 ± 0.01	0.03 ± 0.01	
27	2.07 ± 0.52	0.07 ± 0.01	0.10 ± 0.03	0.03 ± 0.01
28	0.20 ± 0.06			
29	12.40 ± 3.24	0.26 ± 0.08	0.41 ± 0.18	0.10 ± 0.04
30	0.47 ± 0.10	0.05 ± 0.03	0.09 ± 0.06	
31	0.67 ± 0.12	0.34 ± 0.10	0.61 ± 0.21	0.07 ± 0.03
32		0.24 ± 0.07	0.39 ± 0.14	0.10 ± 0.06
33	0.10 ± 0.07			
34		0.09 ± 0.05	0.17 ± 0.11	
Alcohol acetates				
26		0.12 ± 0.03	0.24 ± 0.06	
28		0.03 ± 0.01	0.05 ± 0.03	
30		0.03 ± 0.02	0.06 ± 0.03	
Alkyl esters				
38	0.06 ± 0.03	0.06 ± 0.01	0.04 ± 0.00	0.08 ± 0.02
40	0.03 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.02
42	0.29 ± 0.18	0.05 ± 0.02	0.04 ± 0.02	0.06 ± 0.02
44	0.27 ± 0.16	0.07 ± 0.02	0.11 ± 0.03	0.04 ± 0.02
46	0.42 ± 0.07	0.09 ± 0.03	0.15 ± 0.04	0.04 ± 0.02

Table 22. continued

48	0.36 ± 0.17	0.12 ± 0.03	0.23 ± 0.07	
50		0.13 ± 0.03	0.26 ± 0.07	
Sum aliphatic components				
	25.19 ± 3.44	4.83 ± 0.44	7.12 ± 0.93	2.55 ± 0.42
	31.3%	**25.9%**	**35.5%**	**16.4%**
Sterols				
cholesterol		0.01 ± 0.01	0.03 ± 0.02	
ß-sitosterol	0.25 ± 0.12	0.19 ± 0.07	0.13 ± 0.08	0.24 ± 0.12
Triterpenoids				
δ-amyrin	1.12 ± 0.24	0.26 ± 0.05	0.26 ± 0.10	0.26 ± 0.12
ß-amyrin	0.99 ± 0.15	0.36 ± 0.17	0.32 ± 0.11	0.41 ± 0.26
α-amyrin	2.25 ± 0.19	1.10 ± 0.55	0.99 ± 0.43	1.20 ± 0.76
lupeol	2.92 ± 0.56	0.34 ± 0.09	0.49 ± 0.13	0.19 ± 0.10
friedelin		0.09 ± 0.03		0.18 ± 0.07
erythrodiol	0.28 ± 0.08	0.30 ± 0.19	0.29 ± 0.21	0.30 ± 0.18
uvaol	1.91 ± 0.45	0.48 ± 0.19	0.41 ± 0.10	0.55 ± 0.29
oleanolic acid	10.04 ± 2.14	2.04 ± 0.43	1.66 ± 0.45	2.42 ± 0.69
betulinic acid	2.94 ± 0.67	0.36 ± 0.13	0.45 ± 0.13	0.26 ± 0.13
ursolic acid	30.46 ± 6.53	4.12 ± 1.61	3.03 ± 0.68	5.21 ± 2.54
maslinic acid	1.54 ± 0.48	0.52 ± 0.49	0.71 ± 0.70	0.32 ± 0.29
other triterpenoids		0.68 ± 0.46	0.90 ± 0.73	0.46 ± 0.22
Sum cyclic components				
	54.72 ± 10.40	10.84 ± 3.25	9.67 ± 1.96	12.02 ± 5.00
	66.5%	**59.3%**	**47.5%**	**71.1%**
Unidentified				
	7.40 ± 1.18	2.74 ± 0.77	3.47 ± 1.09	2.01 ± 0.51
Total wax	82.11 ± 14.35	18.42 ± 3.91	20.26 ± 3.07	16.58 ± 5.75

2.16 Cuticular waxes of *Ficus carica* L.

The total wax coverage of *Ficus carica* L. fruit was 41.32 ± 9.90 μg cm^{-2}. The coverage of wax on adaxial leaf surfaces was 8.60 ± 1.69 μg cm^{-2} (Table 23). The aliphatic components dominated the fruit (84.1%, 33.81 ± 6.24 μg cm^{-2}) and adaxial leaf (57.3%, 4.87 ± 0.65 μg cm^{-2}) wax. Only minor portion of cyclic compounds of fruit (4.9%) and leaf (8.3%) wax was detected.

The main aliphatic components of fruit wax were fatty acids (51.1%, 20.29 ± 2.43 μg cm^{-2}) followed by aldehydes (21.2%, 8.52 ± 2.24 μg cm^{-2}), primary alcohols (8.8%), and *n*-alkanes (3.0%). The predominant leaf aliphatic components were *n*-alkanes (34.7%, 2.26 ± 0.33 μg cm^{-2}) followed by primary alcohols (12.7%, 1.04 ± 0.16 μg cm^{-2}), alkyl esters (10.1%), fatty acids (5.7%), and very small amount of aldehydes (Figure 23 A). Carbon chain lengths ranged from C_{20} to C_{32} for fruit and to C_{50} for leaf wax. The most abundant chain lengths were C_{24}, C_{26}, C_{28} and C_{30} for fruit wax, and were C_{29} and C_{31} in leaf wax (Figure 23 B). The ACL value of aliphatics was 26.28 for fruit, and was 30.90 for leaf (Table 6).

The cyclic compounds were distributed by small amount of β-amyrin, α-amyrin, lupeol, and sterols for both fruit and leaf wax. Small amount of lanosterol, β-amyrin acetate, and phenyl-methyl esters were only detected in fruit wax, while tocopherols were only detected in leaf wax (Table 23).

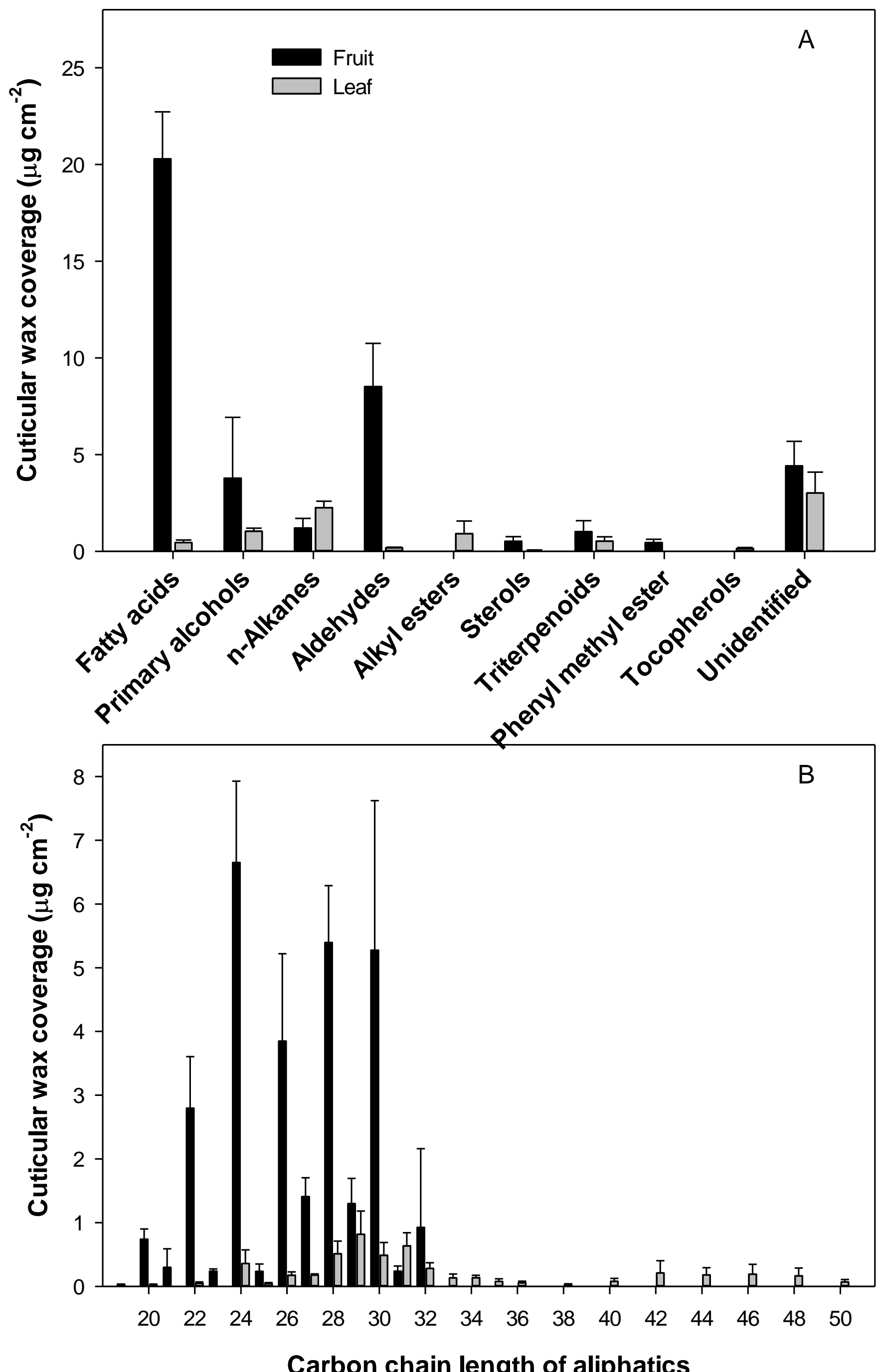

Figure 23. Cuticular wax compositions from *Ficus carica* L. fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Fruit wax was extracted from isolated cuticular membranes, the leaf waxes were extracted from the fresh adaxial leaf surfaces (mean values ± SD, n = 5).

Table 23. The cuticular wax coverage and compositions of *Ficus carica* L. fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Leaf adaxial
Fatty acids		
20	0.71 ± 0.16	0.02 ± 0.01
21	0.30 ± 0.29	
22	2.46 ± 0.57	0.02 ± 0.01
23	0.18 ± 0.03	
24	6.06 ± 1.13	0.17 ± 0.09
25	0.16 ± 0.10	0.03 ± 0.01
26	2.81 ± 0.64	0.05 ± 0.02
27	0.26 ± 0.05	0.03 ± 0.01
28	4.34 ± 0.70	0.09 ± 0.04
29	0.32 ± 0.15	
30	2.49 ± 1.87	0.05 ± 0.02
31	0.09 ± 0.03	
32	0.09 ± 0.03	
Primary alcohols		
20	0.03 ± 0.01	
22	0.34 ± 0.29	0.03 ± 0.02
23	0.02 ± 0.01	
24	0.59 ± 0.52	0.19 ± 0.13
25	0.08 ± 0.03	0.02 ± 0.01
26	0.48 ± 0.34	0.10 ± 0.05
27	0.18 ± 0.11	
28	1.05 ± 0.24	0.27 ± 0.09
29	0.27 ± 0.04	0.02 ± 0.01
30	0.66 ± 0.33	0.20 ± 0.17
31	0.09 ± 0.07	
32		0.13 ± 0.06
34		0.03 ± 0.01
n-Alkanes		
23	0.04 ± 0.01	
26		0.03 ± 0.01
27	0.66 ± 0.24	0.13 ± 0.02
28		0.07 ± 0.06
29	0.43 ± 0.30	0.79 ± 0.36
30	0.03 ± 0.00	0.13 ± 0.03
31	0.06 ± 0.02	0.60 ± 0.22
32		0.15 ± 0.04
33		0.13 ± 0.06
34		0.10 ± 0.03
35		0.08 ± 0.04
36		0.06 ± 0.02
Aldehydes		
26	0.86 ± 0.33	
27	0.28 ± 0.15	
28	4.61 ± 1.68	0.08 ± 0.04
29	0.33 ± 0.07	
30	2.20 ± 0.58	0.10 ± 0.02
32	0.25 ± 0.43	

Table 23. continued

Alkyl esters						
38				0.03	±	0.02
40				0.08	±	0.05
42				0.21	±	0.19
44				0.18	±	0.11
46				0.19	±	0.16
48				0.16	±	0.13
50				0.07	±	0.04
Sum aliphatic components						
	33.81	±	6.24	4.87	±	0.65
		84.1%			**57.3%**	
Sterols						
ß-sitosterol	0.13	±	0.06	0.04	±	0.04
Lanosterol (drivatives)	0.45	±	0.15			
Triterpenoids						
ß-amyrin	0.17	±	0.05	0.05	±	0.03
α-amyrin	0.35	±	0.32	0.04	±	0.03
ß-amyrin acetate	0.32	±	0.14			
lupeol	0.19	±	0.08	0.26	±	0.19
obtusifoliol				0.19	±	0.05
Phenyl-methyl esters						
26	0.15	±	0.06			
28	0.31	±	0.11			
Tocopherols						
δ-tocopherol				0.04	±	0.04
ß-tocopherol				0.03	±	0.02
γ-tocopherol				0.03	±	0.01
α-tocopherol				0.04	±	0.02
Sum cyclic components						
	2.06	±	0.76	0.71	±	0.28
		5.0%			**8.3%**	
Unidentified						
	4.42	±	1.27	3.02	±	1.08
Total wax	40.29	±	7.98	8.60	±	1.69

2.17 Cuticular waxes of *Capsicum annuum* L. cv. 'Kalocsai'

The total wax of *Capsicum annuum* L. cv. 'Kalocsai' fruit was 28.40 ± 4.63 µg cm^{-2}. The overall coverage of waxes on leaf surfaces was 5.17 ± 1.66 µg cm^{-2}. The leaf waxes were distributed by 5.10 ± 1.38 µg cm^{-2} and 5.33 ± 3.06 µg cm^{-2} on the ad- and abaxial surfaces, respectively (Table 24). The fruit wax composed a higher portion of cyclic compounds (30.5%, 8.58 ± 0.78 µg cm^{-2}), in comparison to aliphatic compounds (12.2%, 3.49 ± 0.77 µg cm^{-2}). The leaf wax was dominated by aliphatics (70.7% 4.69 ± 2.58 µg cm^{-2}) with very small amount of cyclic component (3.7%, 0.25 µg cm^{-2}).

The main aliphatic components of fruit wax were *n*-alkanes (6.1%, 1.87 ± 0.39 µg cm^{-2}), followed by fatty acids (5.2%, 1.62 ± 0.44 µg cm^{-2}), and very small amount of alcohols. The predominant leaf aliphatic components were *n*-alkanes (35.7%, 2.44 ± 1.58 µg cm^{-2}) followed by primary alcohols (21.9%, 1.27 ± 0.48 µg cm^{-2}), fatty acids (6.3%), aldehydes (1.9%), and small amount of ketones (Figure 24 A). Carbon chain lengths ranged from C_{19} to C_{35}. The most abundant chain lengths were C_{20} and C_{29} for fruit wax, and were C_{28} and C_{33} for leaf wax (Figure 24 B). The ACL value of aliphatics was 25.99 for fruit, and was 30.38 for leaf (Table 6).

The cyclic compounds were dominated by β-amyrin (5.2%), α-amyrin (4.7%), lupeol (6.2%) and α-tocopherol (6.7%) in fruit wax. Additional small amount of β-sitosterol, campesterol, epi-friedelenol, and tocopherols were also detected in fruit wax. Only very small amount of β-sitosterol, β-amyrin, α-amyrin, lupenon, friedelin as the cyclic components were detected from adaxial leaf surfaces (Table 24).

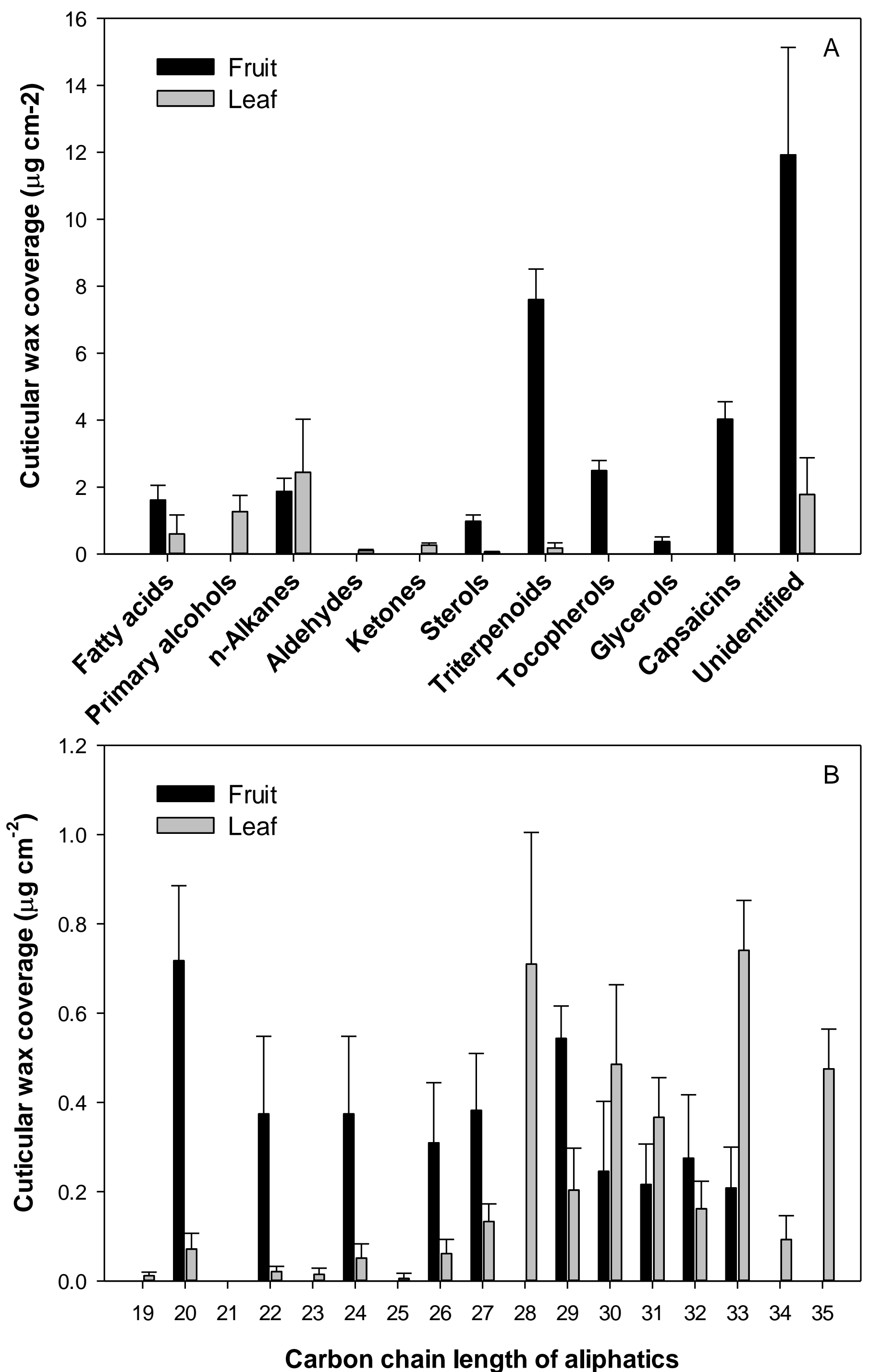

Figure 24. Cuticular wax compositions from *Capsicum annuum* L. cv. 'Kalocsai' fruits and leaves. (A) Cuticular wax compositions of fruits and leaves; (B) the carbon chain length distribution of aliphatics. Waxes were extracted from isolated cuticular membranes of fruit, ad- and abaxial leaf (mean values ± SD, n = 5).

Table 24. The cuticular wax coverage and compositions of *Capsicum annuum* L. cv. 'Kalocsai' fruits and leaves. Data were given as mean values ± SD (in µg cm^{-2}, n = 5).

Compound	Fruit	Whole leaf	Leaf adaxial	Leaf abaxial
Fatty acids				
19		0.01 ± 0.01		0.02 ± 0.02
20	0.72 ± 0.17	0.07 ± 0.04	0.07 ± 0.07	0.07 ± 0.08
22	0.37 ± 0.17	0.02 ± 0.01		0.03 ± 0.02
23		0.01 ± 0.01		0.02 ± 0.01
24	0.31 ± 0.13	0.05 ± 0.03	0.02 ± 0.02	0.07 ± 0.05
25		0.00 ± 0.01		0.03 ± 0.00
26	0.22 ± 0.12	0.04 ± 0.03	0.02 ± 0.01	0.06 ± 0.06
27		0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.00
28		0.04 ± 0.04	0.03 ± 0.01	0.26 ± 0.09
30		0.05 ± 0.07	0.03 ± 0.02	0.22 ± 0.13
31		0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02
32		0.03 ± 0.04		0.03 ± 0.02
34		0.03 ± 0.03		0.08 ± 0.08
Primary alcohols				
22		0.01 ± 0.00		0.01 ± 0.00
23		traces		0.02 ± 0.00
24		traces		0.01 ± 0.00
25		traces		0.01 ± 0.00
26	traces	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
27		0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
28	traces	0.63 ± 0.28	1.22 ± 0.47	0.03 ± 0.01
29		0.05 ± 0.02	0.07 ± 0.02	0.02 ± 0.02
30		0.34 ± 0.13	0.63 ± 0.23	0.03 ± 0.02
31		0.02 ± 0.01		0.03 ± 0.03
32		0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
33		0.01 ± 0.01		0.03 ± 0.03
34		0.02 ± 0.01		0.03 ± 0.02
n-Alkanes				
23		0.01 ± 0.01		0.01 ± 0.01
27	0.38 ± 0.13	0.10 ± 0.03	0.15 ± 0.03	0.06 ± 0.04
29	0.54 ± 0.07	0.16 ± 0.08	0.18 ± 0.03	0.14 ± 0.14
30	0.25 ± 0.16	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
31	0.22 ± 0.09	0.33 ± 0.07	0.35 ± 0.10	0.33 ± 0.10
32	0.28 ± 0.14	0.10 ± 0.02	0.06 ± 0.02	0.14 ± 0.05
33	0.21 ± 0.09	0.73 ± 0.10	0.37 ± 0.13	1.13 ± 0.19
34		0.05 ± 0.02		0.09 ± 0.03
35		0.23 ± 0.04	0.27 ± 0.08	0.21 ± 0.04
Aldehydes				
28		0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.02
30		0.06 ± 0.02	0.10 ± 0.03	0.02 ± 0.02
Ketones				
35 (pos.2)		0.24 ± 0.05	0.16 ± 0.06	0.34 ± 0.08
Sum aliphatic components				
	3.49 ± 0.77	4.69 ± 2.58	3.93 ± 1.24	3.34 ± 1.44
	12.2%	**70.7%**	**76.1%**	**65.5%**

Table 24. continued

Sterols				
ß-sitosterol	0.66 ± 0.17	0.06 0.01	0.04 ± 0.02	0.07 ± 0.02
campsterol	0.32 ± 0.04			
Triterpenoids				
ß-amyrin	1.49 ± 0.31	0.03 ± 0.02		0.06 ± 0.03
α-amyrin	1.33 ± 0.29	0.01 ± 0.00		0.02 ± 0.01
lupeol	1.77 ± 0.20			
lupenon		0.03 ± 0.02		0.06 ± 0.04
friedelin	0.22 ± 0.07	0.02 ± 0.01		0.04 ± 0.01
epi-friedelenol	0.30 ± 0.12			
Tocopherols				
δ-tocopherol	0.10 ± 0.02			
ß-tocopherol	0.10 ± 0.01			
γ-tocopherol	0.38 ± 0.03			
α-tocopherol	1.91 ± 0.31			
Sum cyclic components				
	8.58 ± 0.78	0.25 ± 0.16	0.04 ± 0.02	0.38 ± 0.32
	30.5%	**3.7%**	**0.9%**	**6.4%**
Capsaicins				
noniamide	0.37 ± 0.02			
capsaicin	2.53 ± 0.40			
dihydrocapsaicin	1.13 ± 0.13			
Glycerols				
2-linoleoyglycerol	0.37 ± 0.14			
Unidentified				
	11.92 ± 3.21	1.36 ± 0.64	1.12 ± 0.16	1.62 ± 1.30
Total wax	28.40 ± 4.63	5.17 ± 1.66	5.10 ± 1.38	5.33 ± 3.06

3 Morphological characteristics of fruit and leaf surfaces

Fruit and leaf surface characteristics of the studied species were observed. The surfaces of olive fruit and leaf (*Olea europaea* L. cv. 'Arbequina' and *Olea europaea* subsp. *europaea* var. *sylvestris*) were covered by scarce incompletely degradation of scale-like peltate (non-glandular) trichomes with stalks (Figure 36 and 37). The epicuticular wax crystals were shown as granules on fruits surfaces (Barthlott et al., 1998), and no obvious epicuticular wax crystals were observed on adaxial leaf surfaces. The abaxial leaf surfaces were covered by dense of scale-like peltate trichomes (Levizou et al., 2005). Stomata occurred on the adaxial leaf surfaces below the trichomes (Figure 37).

The fruit surface of *Averrhoa carambola* L. was covered by a wax film (Figure 38). The adaxial and abaxial leaf surfaces were covered by plates and platelets type epicuticular wax crystals. Stomata occurred on abaxial leaf surface. The guard cell of stomata was around wax film.

The fruit surface of *Coffea arabica* L. was covered by a wax film (Figure 39). The adaxial and abaxial leaf epicuticular wax crystals were observed as plates, platelets, and granules types. Stomata occurred on both fruit and abaxial leaf surfaces.

The fruit surface of *Crataegus pedicellata* Sarg. was covered by a variety of epicuticular wax crystals, e.g. transversely ridged ribbons, plates and platelets, and clusters of hollow tubules, which were constituted by nonacosan-10-ol (Figure 40, Jeffree, 2006). Scarce foliar acicular trichomes occurred on the adaxial leaf surfaces (Tschan and Denk, 2012). A wax film covered on adaxial leaf surface. Stomata was found on abaxial leaf surface and the epicuticular wax exhibited as irregularly granulated features.

The epicuticular wax cystals of *Malus domestica* L. cv. 'Topaz' fruit was constituted by a syntopism of plates and platelets (Figure 41, Al Bitar et al., 2014). No obvious epicuticular wax crystals but irregularly granulated structures were observed on adaxial leaf surface. The foliar fasciculate (thread- and spiral-shaped) trichomes that irregularly dispersed throughout the lamina on abaxial leaf surface were observed (Al Bitar et al., 2012; Tschan and Denk, 2012). Stomata occurred on abaxial leaf surface and the epicuticular wax crystals were granules (Barthlott et al., 1998).

The fruit epicuticular wax crystals of *Prunus cerasifera* Ehrh. and *Prunus domestica* L. subsp. s*yriaca* Janich., were non-entire or entire platelets and membranous

platelets (Figure 42 and 43, Jeffree, 2006). Both the ad- and abaxial leaf surface were covered by a smooth wax film. Stomata occurred on the abaxial leaf surface.

Fruit epicuticular wax crystals of *Prunus persica* L. were constituted by simple plate-type wax (Figure 44). The ad- and abaxial leaf surfaces were covered by a smooth wax film. Stomata occurred on both fruit and abaxial leaf surfaces.

For the fruit of *Prunus avium* L. and *Prunus domestica* subsp. *Insititia* (L.), the surface characteristics have been reported as relative smooth on sweet cherry fruit surface, while dense of platelets crystals occurred on European plum fruit surface. Stomata and microcracks occurred on both these two fruit surfaces (Knoche and Peschel, 2007, Mukhtar et al., 2014).

The fruit surfaces of *Vitis vinifera* L. cv. ‘Nelly’ and cv. ‘Silvana’ were covered by dense of non-entire platelets, and simple plate-type wax (Figure 45 and 46, Barthlott et al., 1998). The adaxial leaf surface of cv. ‘Nelly’ showed irregularly granulated features with granules, small simple plate-type wax crystals, whereas the adaxial leaf epicuticular wax crystals of cv. ’Silvana’ were rosettes of platelets. The abaxial leaf surfaces of cv. ‘Nelly’ and cv. ‘Silvana’ were covered by small parallel stacked platelets wax (Barthlott et al., 1998). For these two grape vines, stomata occurred both on abaxial leaf surfaces.

The fruit surface of *Cornus officinalis* Siebold & Zucc. was covered by plates and platelets (Figure 47). The adaxial leaf surface showed irregularly granulated structure. A smooth wax film covered on the adaxial and abaxial leaf surfaces. Stomata occurred on fruit and abaxial leaf surfaces. Most of the stomata on fruit surfaces were completely covered by wax.

The crust wax with fissured layers represented the epicuticular wax features for fruit surface of *Ficus carica* L. (Figure 48, Barthlott, 1998). Acicular trichomes occurred on fruit, adaxial, and abaxial leaf surfaces (Tschan and Denk, 2012). Smooth wax film covered on both ad- and abaxial leaf surfaces. Stomata occurred on fruit and abaxial leaf surfaces.

Discussion

The principal goal of the present study was to characterize the water permeability of fruits and leaves to quantify the possible contribution of cuticular wax components to the transpiration barrier. Comparative analyses of the deposition of total wax, cyclics, and aliphatics, as well as the chain length distribution of aliphatic compounds between fruits and leaves were conducted. The correlation of the cuticular chemical compositions with the transpiration barrier properties are discussed.

1 The transpiration barrier properties of fruits and leaves

The transpiration of fruits and leaves varies between different species and organs. The water permeability of fruit varied between 3.7 x 10^{-5} m s^{-1} (*Prunus domestica* L. subsp. *Syriaca* (L.)) and 37.4 x 10^{-5} m s^{-1} (*Coffea Arabica*), whereas an overall lower permeability or minimum conductance for leaves ranging between 1.6 x 10^{-5} m s^{-1} (*Cornus officinalis*) and 4.5 x 10^{-5} m s^{-1} (*Prunus domestica* L. subsp. *syriaca* (L.)) was detected. The permeability values determined in the present study are well within the previously reported range of 0.9 x 10^{-5} m s^{-1} to 20.0 x 10^{-5} m s^{-1} for fruits (Knoche et al., 2000; Riederer and Schreiber, 2001; Leide et al., 2007), and of 0.4 x 10^{-6} m s^{-1} to 14.4 x 10^{-5} m s^{-1} for leaves (Riederer and Schreiber, 2001; Jetter and Riederer, 2016; Schuster et al., 2016). Most of the studied species are shown as intraspecies higher water permeability for fruit than the leaf (Table 3). Consequently, the interspecies comparison of water permeability in fruits was significantly higher than that of leaves (Figure 25A). This further supports the trend that transpiration levels are higher in fruits than leaves; a result that has been observed in tomatoes, apples and peppers (Schreiber and Riederer, 1996; Riederer and Schreiber, 2001).

The residual stomatal or lenticular transpiration may affect the total transpiration, where they occurred on the fruit or leaf surfaces. The residual stomatal transpiration of the leaves of several species (*Ficaria verna*, *Plantago lanceolate*, *Teucrium chamaedrys*, *Alnus glutinosa*, and *Quercus robur*) has been observed to compose less than 6% of the total minimum conductance (Thibaud et al., unpublished, data not shown). Only one species of *Hedera helix* has so far been detected to have a comparable ratio of the residual stomatal transpiration to the leaf minimum conductance (Burghardt and Riederer, 2003). In the present study, there were no

significant differences between the cuticular permeances of adaxial astomatous leaf after sealing the hypostomatic surfaces, and the minimum conductance of intact leaves for the species of *Olea europaea*, *Averrhoa carambola*, and *Prunus domestica* (Table 28). The result indicates that, therefore, both astomatous adaxial water permeability and the minimum conductance of intact leaf provides the references as the cuticular transpiration.

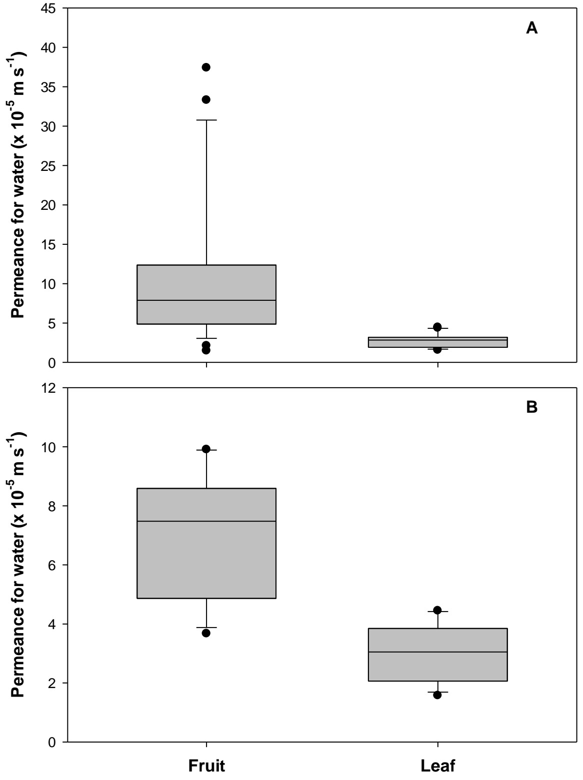

Figure 25. The cuticular permeability of fruits and leaves of different species. (A) The interspecies comparison of water permeabilities for fruits was significantly higher than that of leaves (P < 0.01). (B) The cuticular water permeabilities of leaves and fruits of *Ligustrum valgure* L., *Prunus avium* L., *Prunus domestica* subsp. *insititia* (L.), *Prunus persica L., Coffea Arabica L.*, *Ficus carica* L., and *Cornus officinalis* with observed stomata on fruit surfaces were excluded. The interspecies comparison of water permeabilities for leaves was significantly lower than that of fruits (P < 0.01).

The water permeability of fruit has been studied in only a limited number of species and values ranged between 0.9 x 10^{-5} m s^{-1} (tomato) and 20.0 x 10^{-5} m s^{-1} (*Capsicum annuum*) (Knoche et al., 2000; Riederer and Schreiber, 2001; Leide et al., 2007). Water permeability of olive fruits from different cultivars or subspecies ranged from 7.2 x 10^{-5} m s^{-1} to 9.9 x 10^{-5} m s^{-1} in different growing years. Rosaceae fruits demonstrated

a similar transpiration (around 3.0 - 4.0 x 10^{-5} m s^{-1}). The transpiration of grape berries was around 7.5 x 10^{-5} m s^{-1}, which was higher than that of previous studies (Rogiers et al., 2004). However, fruit of *Ligustrum valgure* L., *Prunus avium* L.*, Prunus domestica* subsp. *insititia* (L.)*, Prunus persica L., Coffea Arabica L.*, *Ficus carica* L., and *Cornus officinalis* occurred stomata on the fruit surfaces and exhibited therefore a higher transpiration of over 10.0 x 10^{-5} m s^{-1}. These values are comparable to those found in literature of the sweet cherry (Knoche et al., 2000, 2001; Riederer and Schreiber, 2001) and young grape berry (Rogiers et al., 2004). The occurrence of lenticels on fruit surfaces, such as apple, hawthorn and plum, may affect the total transpiration for the fruits (Pieniazek, 1944; Veraverbeke et al., 2003). Nevertheless, the values for water transpiration of fruits, even the values of fruits where the stomata were excluded, exhibited significantly higher values than those of leaves (Figure 25B).

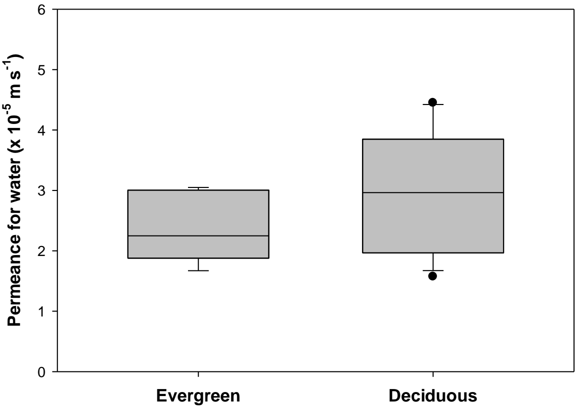

Figure 26. The water permeances of evergreen and deciduous leaves. The transpiration displayed a trend increased from evergreen to deciduous type leaves on a median level obtained in the present study.

Previous studies indicate a general tendency that evergreen leaves display the lowest cuticular permeabilities, followed by Mediterranean evergreen or deciduous leaves, then temperate deciduous leaves and desert leaves (Riederer and Schreiber, 2001; Schuster et al., 2016). The cuticular permeability ranged from 2.6 x 10^{-5} m s^{-1} to 3.1 x 10^{-5} m s^{-1} for *Olea europaea* L. (cv. ‘Arbequina’ and subsp. *europaea* var. *sylvestris*); these values are remarkably lower than the reported values of different olive cultivars (2.8 x 10^{-4} m s^{-1} and 5.1 x 10^{-4} m s^{-1}) (Bacelar et al., 2004). While as the drought tolerance leaves were found to be a relative high leavel of transpiration. Thus, the transpiration displayed a trend increased from evergreen to deciduous type leaves on a median level obtained in the present study (Figure 26). Whereas the measured

values for transpiration of *Olea europaea* L. and *Coffea Arabica* L. leaf permeabilities were approximately 10-fold and 3.8-fold higher than the data from literatures, respectively (Riederer and Schreiber, 2001). Permeabilties of *Ligustrum vulgare* L. in the present study are very close to the literature data (Riederer and Schreiber, 2001). The other deciduous leaves of Rosaceae, Vitaceae and other species, ranging from 1.8×10^{-5} m s^{-1} to 4.5×10^{-5} m s^{-1}, have not been previously reported.

The following factors may help explain the observed differences in water permeabilities between fruit and leaf. Firstly, fruit with stomata or lenticel may induce higher transpiration. Thus, the fruit transpiration includes not only cuticular transpiration, but may also include measurable lenticular or stomatal transpiration (Pieniazek, 1944). The stomata density of fruit may be lower than that of leaves. It has been shown in leaves that an increased density of stomata results in a better control over transpiration (Bosabalidis and Kofidis, 2002). Secondly, an efficient transpiration or gas exchange is necessary for the transportation of water and nutrients through the xylem into fruit. Thirdly, the lifespan of fruits is commonly shorter than that of leaves. For instance, the transpiration of evergreen leaves showed the lowest level increased by the deciduous leaves, which may be related to their longer lifetime period than that of the latter ones.

With the development of fruit and leaf, the leaf conductance increases, and exhibits more sensitive to the environmental stresses, especially to dehydration stress. While the xylem flow is reduced following irrigation, which leads to an increased phloem flow in the ripe stage for fruit (Greenspan et al., 1996; Matthews and Shackel, 2005). Following dehydration, the efficiency of water consumption and flow shifts, the turgor pressure decreases and the stomata closes to reduce leaf transpiration. Xylem water flow thereby increases into the fruit to recover turgor pressure for osmotic adjustment (Kaufmann, 1970; Dell'Amico et al., 2012). Variations in leaf–fruit water status may shift the water flow between xylem and phloem, consequently stabilizing the fruit water status and reducing the effect of stress on fruit growth and yield. Finally, it also should be noted, as hypothesized that fruit demonstrated a higher drought tolerance than leaves (Dell'Amico et al., 2012) meaning that a more efficient cuticular transpiration barrier is necessary to adapt the fast leaf hydration in leaves.

2 Wax compositions of fruits and leaves

The compositions and coverage of the wax mixtures in fruits and leaves in this study were consistent with those reported previously. Thorough study of the surface wax composition profiles for fruits of olive (Bianchi et al., 1992; Vichi et al., 2015), sweet cherry (Peschel et al., 2007), apple (Verardo et al., 2003), peach (Belge et al., 2014), and pepper (Parsons et al., 2012; Parsons et al., 2013), and leaves of olive (Bianchi et al., 1992) and peach (Baker et al., 1979) have been conducted. Genrally, fruits and leaves of different speceis were characterized by an organ-specific pattern of aliphatic and cyclic compositions for the cuticular waxes (Figure 27).

Cuticular waxes of olive contained mainly pentacyclic triterpenoids from the oleanane type: predominantly oleanolic acid and maslinic acid in fruits, and oleanolic acid and erythrodiol in leaves. As for olive fruits, oleanolic acid was also found as the main triterpenoid in other fruit crops, e.g. *Prunus domestica* L. (Ismail et al., 1977), *Vaccinium myrtillus* L. (Szakiel et al. 2012) and *Vitis vinifera* L. (Possingham et al., 1967; Zhang et al., 2004; Pensec et al., 2014). Except for the deposition on the olive fruit surface, maslinic acid has been very rarely detected in fruits, e.g. in *Ziziphus jujuba* Mill. (Guo et al., 2010), *Rubus chingii* Hu (Guo et al., 2005) and *Malus pumila* Mill. (He and Liu, 2007). Like in fruits, oleanolic acid was the major cuticular wax compound in leaves, which is not common for leaf cuticular waxes (Kolattukudy, 1970). Additionally, cuticular waxes of olive leaves exhibited a high amount of ursolic acid and uvaol that belong to the ursane type of triterpenoids, whereas only fruit cuticular waxes had betulinic acid of the lupane type (Jäger et al., 2009). Fruits and leaves of olive were characterized by an organ-specific pattern of triterpenoid composition which might indicate differences in the triterpenoid biosynthesis in both organs.

The aliphatic components, mainly fatty acids and primary alcohols, constituted the smaller portion of cuticular waxes. An increase of the aliphatic fraction during fruit development was primarily due to a higher accumulation of alkyl esters and primary alcohols. Bianchi et al. (1992) compared the green and black developmental stages of fruits of the 'Coratina' cultivar. Similarly, as found for the cultivar 'Arbequina', the black-stage fruits exhibit a higher percentage of aliphatic components compared to the green-stage fruit. Also in the case of the 'Coratina' cultivar, the fraction of pentacyclic triterpenoids decreased from the green to the black stage.

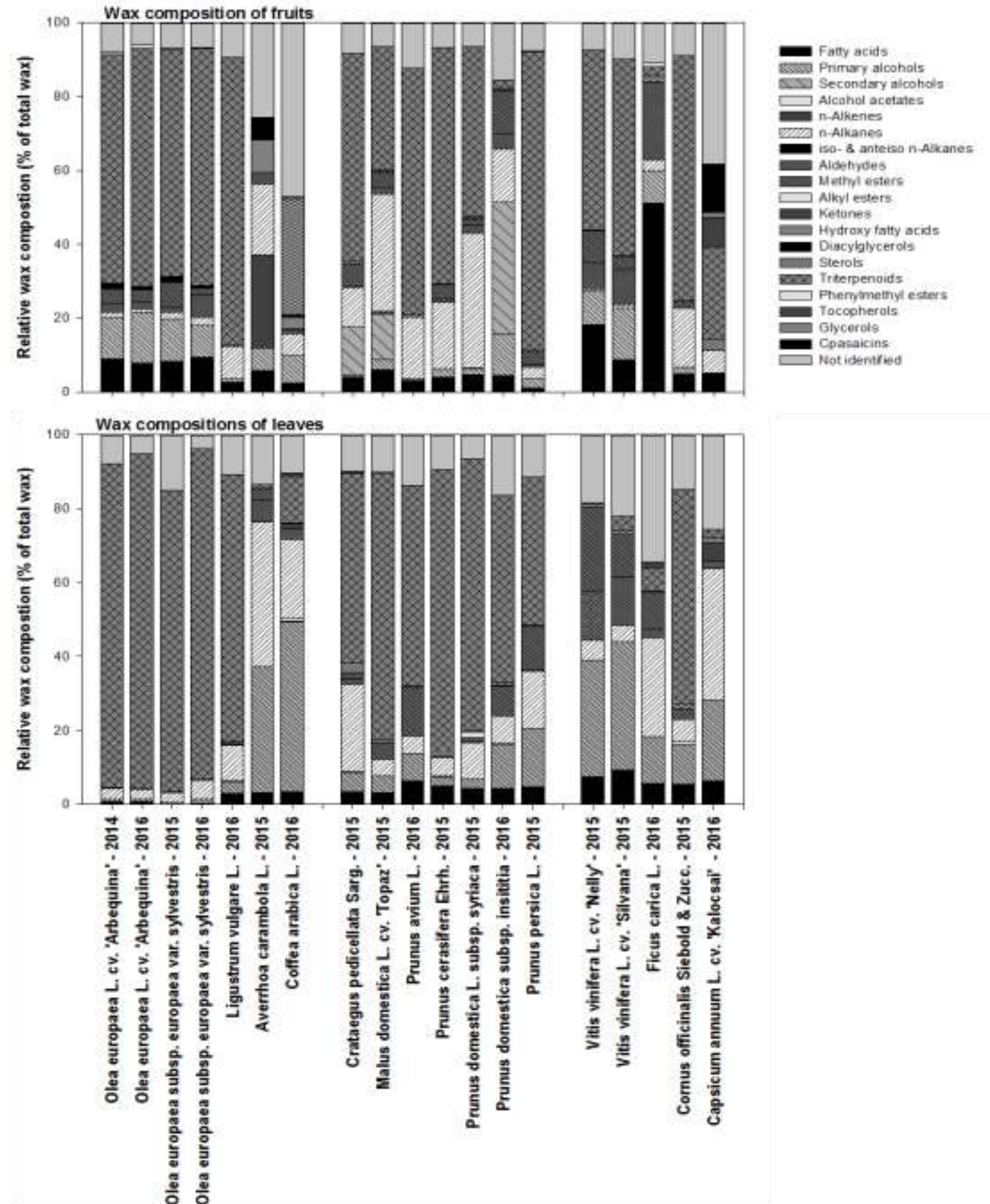

Figure 27. Relative wax compositions of fruits and leaves from different species. The cuticular wax components were etracted from fresh or CM samples. Data were given as the mean value of relative content in percentage (%, n = 4-5).

The aldehydes and methyl esters, and 2-phenyl-ethanol-1-esters detected in adaxial leaf surfaces of various cultivars of olives (Bianchi et al., 1992), and mono-, triacylglycerols and geranylgeranyl esters in the fruits (Bianchi et al., 1992; Vichi et al., 2015) have not yet been found in *Olea europaea* L. cv. 'Arbequina' or var. *sylvestris*. The coverage of waxes on the black stage fruit of cv. 'Arbequina' surfaces were very

similar in 2014 and 2015, while a decline in 2016 was noted. Similar changes in total wax coverages on green and turning fruit surfaces were found whilst analyzing the waxes in 2014 and 2016. The wax compositions in the fruits and leaves of var. *sylvestris* were very similar to waxes of cv. 'Arbequina'. The alteration of wax load, but not composition, by the environment conditions has also been reported on citrus leaves grown under different temperature and humidity environments (Riederer and Schneider, 1990).

The wax coverage and composition of the leaf of *Ligustrum vulgare* L. (privet), also a member of the Oleaceae plant family in the present study were very close to the results that obtained by extracted directly intact leaves or by extracted separately of the epi- and intracuticular wax (Buschhaus et al., 2007). Different from the olive fruit and leaf, the dominating cyclic compounds were ursolic acid and small amount of oleanolic acid and, while *n*-alkanes were the most abundant aliphatic compounds in privet fruit and leaf. Phenylmethyl esters, which have been identified in previous studies, were not detected in the present study. Whereas traces of secondary alcohols with odd-numbered chain-length ranging from C_{27} to C_{33} have been identified in the present study, but not in previous report (Buschhaus et al., 2007).

Similarly to previous reports, *n*-alkanes, predominantly *n*-Nonacosane (C_{29}, 70% of total *n*-alkanes), were the main aliphatic component (16% of total wax) and triterpenoids, such as ursolic acid and oleanolic acid for the sweet cherry fruit (Peschel et al., 2007), were the prominent wax component. Compared to the previous study, a lower proportion of triterpenoids (67% vs 93%) and a higher level of aliphatics (20% vs 6%) were detected in sweet cherry fruit wax (Peschel et al., 2007).

The total cuticular wax of apple fruit varied between 366.0 μg cm^{-2} and 1038.7 μg cm^{-2} for a number of different cultivars (Belding et al., 1998; Belding et al., 2000). Compared to the literature data, a remarkably lower wax load, approximately 230.2 μg cm^{-2} for *Malus domestica* L. cv. 'Topaz' was found. The coverage of aliphatics (60% of total wax) was higher than that of cyclics (34% of total wax). Nonacoan-10-ol (C_{29}, 11% of total wax) and *n*-Nonacosane (C_{29}, 28% of total wax) were the main aliphatic compounds and ursolic acid and oleanolic acid were the prominent triterpenoids (Belding et al., 1998; Verardo et al., 2003).

Recently, the cuticular wax of melting and non-meting peaches (*Prunus persica* L.) was investigated (Belge et al., 2014). The total wax yield of these two peaches were 518.3 μg cm^{-2} and 425.6 μg cm^{-2}, respectively, which is approximately 2-fold higher

than nectarine fruit (288.0 μg cm^{-2}). The accumulation of aliphatics in nectarines was similar to that of peaches, while the predominant triterpenoids were remarkably higher in nectarine fruit (52% vs 82%). In addition, a relatively high concentration of alkyl esters (32% of aliphatics) and a minor portion of aldehydes (6% of aliphatics) were found in nectarine fruit, while these compounds were not reported in melting or non-melting peaches (Belge et al., 2014).

Similar to previous reports, the total wax load of plum fruits varied between 200 μg cm^{-2} and 300 μg cm^{-2} (Bain and Mcbean, 1969; Knoche and Peschel, 2007; Mukhtar et al., 2014). The main wax compositions of plum fruits were consistent with those reported for egg plums, whereas trace amount of sterols and triterpenols have not been reported (Ismail et al., 1977). Nonacosan-10-ol was detected in relatively high levels in wax 'bloom' surfaces of *Crataegus pedicellata* L. (18% total aliphatics), apple (cv. 'Topaz', 40% total aliphatics), and European plum (40% total aliphatics) fruits. The deposition of *n*-Nonacosane (C_{29}) varied between 15% and 73% (total aliphatics) and was the main aliphatic component of all the Rosaceae fruits. Similar results on fruit and leaf surfaces of *Prunus domestica* L. (Holloway et al., 1976; Ismail et al., 1977), *Crataegus oxyacantha* L. (hawthorn) (Wollrab, 1969), sweet cherry (Peschel et al., 2007) and several cultivars of apple (Verardo et al., 2003) have been reported.

The wax 'bloom' appearance of grape berries covered with a relative high amount of wax ranging between 100 μg cm^{-2} and 220 μg cm^{-2} (Grncarevic and Radler, 1971; Yamamura and Naito, 1983; Commenil et al., 1997). The total wax load of *Vitis vinifera* L. v. 'Nelly' and cv. 'Silvana' berries were detected to be in the reported relative wide range. Fatty acids, primary alcohols and aldehydes with even numbered chain-lengths varying between C_{26} and C_{30} were the predominant aliphatic compound profiles (Radler, 1965). The main pattern of wax, cyclic compounds was distributed by the portion of 60% to 80% in those previous reports, while a lower portion of 49% and 54% were found for cv. 'Nelly' and cv. 'Silvana', respectively. Similarly, ursolic acid, as well as a small amount of ß-sitosterol, ß-amyrin, erythrodiol and oleanolic acid derivatives were detected for the two cultivars of grape berries (Neto, 2011; Pensec et al., 2014). The presented results provided detailed quantitative and qualitative wax compositions, which have increased the understanding of cuticular waxes of plum fruits and leaves of the Rosaceae family.

Like previous reports, fatty acids and *n*-alkanes were the main aliphatic compounds in the cuticular wax of a various collection pepper fruits. And ß-amyrin, α-amyrin and

lupeol dominated the cyclics in the cv. Kalocsai (Bauer et al., 2005; Parsons et al., 2012; Parsons et al., 2013).

The deposition of leaf cuticular wax of the studied species varied in a relatively narrow range between 7.0 and 46.4 µg cm^{-2}, except for the high amount of wax (over 144.4 µg cm^{-2}) in olive leaves. In many cases, the most pronounced aliphatic compounds were *n*-alkanes with odd numbered chain-lengths ranging between C_{27} and C_{33}. Trace amounts of cyclics accumulated in leaf wax mixtures of *Vitis vinifera* L. cv. 'Nelly' and cv. 'Silvana', *Averrhoa carambola* L., *Coffea Arabica* L., *Capsicum annuum* L. cv. 'Kalocsai', and *Ficus carica* L. The cuticular wax of both the fruit and the leaf of *Averrhoa carambola* L., *Coffea Arabica* L., *Cornnus officinalis* and *Ficus carica* L., were reported for the first time in detail in the present study.

Overall, the level of conformity of literature data sets and the present study regarding wax load and chemical composition varied for the different species of leaves (Jetter et al., 2008; Jetter and Riederer, 2016; Schuster, 2016) and fruits (Belge et al., 2014; Lara et al., 2015). Both the present data and the published information highlighted that the fatty acids, primary alcohols, *n*-alkanes, aldehydes and alkyl esters were the main very-long-chain aliphatic compound classes; and that ursolic acid and oleanolic acid dominated the pentacyclic triterpenoids (main cyclic constituents). Consequently, comprehensive correlation analyses could be conducted to investigate the permeances and chemical data variances between fruit and leaf.

3 Cuticular waxes and the transpiration barrier properties

It is widely accepted that the barrier properties of plant cuticles are determined by the chemical compositions of cutiuclar wax (Schönherr, 1976; Schönherr and Riederer, 1989; Riederer and Schreiber, 1995; Riederer and Schreiber, 2001). It can be assumed that (1) the cuticular barrier properties are directly dependent upon the aliphatic compounds, especially chain-length distribution of aliphatics, and (2) different concentrations of cyclic and aliphatic components accumulate to provide the mechanical support for the cuticle.

The first assumption is based upon the proposed molecular wax structure barrier model, which is composed of crystalline zones and amorphous zones (Riederer and Schneider, 1990; Riederer and Schreiber, 1995). The water molecules or other solutes pass through the less ordered and hydrophilic aqueous zones, while the tightly-packed

hydrophobic aliphatic zones remain impermeable (Riederer and Schreiber, 2001; Arand et al., 2010; Niemann et al., 2013). The second assumption is based upon previous studies that have demonstrated that cuticular waxes may serve as a filler to provide mechanical support for the cuticle (Petracek and Bukovac, 1995; Khanal et al., 2013). The cuticular wax, especially the intracuticular wax, has been reported to be associated molecularly with the cutin matrix, which showed a small resistance for transpiration (Nawrath, 2006). The wax mixtures may also have an indirect effect on the rheological properties of cuticle. However, this indirect effect cannot be concluded at present. It is proposed here that the various coverage of aliphatic and cyclic compounds may result in different biomechanical properties of cuticle, thereby inducing a variety of barrier properties.

3.1 Correlations between wax load and transpiration barrier properties

In order to link the cuticular chemical composition and the transpiration barrier properties, comprehensive correlation analyses were carried out between the chemical parameters of total wax, aliphatics, cyclics as well as their main components, and the natural logarithm water permeabilities for fruit and leaf of different species. The most important correlation pairs were summarized in Table 27.

The total wax load of various species and organs varied between 15.5 μg cm^{-2} (*Coffea arabica*) and 451.1 μg cm^{-2} (*Crataegus pedicellata*) for fruits, and between 5.2 μg cm^{-2} (*Capsicum annuum*) and 277.1 μg cm^{-2} (*Olea europaea*) for leaves (Table 4 and 5). For the various deposition of wax amount for a wide species and organ-specific manner, no significant correlations were found between the total wax load and the water permeabilities for fruits and leaves independently or in combination together (Figure 28). Similar results have been reported for the total wax obtained either by weighted gravimetrically (weight difference between CMs before and after extraction) (Schreiber and Riederer, 1996) or by GC analyses (Jetter and Riederer, 2016; Schuster, 2016). In addition, the various coverage of cuticular wax per unit area of the intrinsic size of different fruits and leaves may induce a different thickness of cuticles. However, similar as has been previously tested (Riederer and Schreiber, 2001; Jetter and Riederer, 2016), the thickness of cuticle and cuticular wax layer that were carried out based on the weight of CMs and total cuticular wax load, were not detected to be correlated with the transpiration properties of fruits and leaves (Table 27). Thus, the

accumulation of various total wax coverage seemly does not provide a more efficient barrier for transpiration.

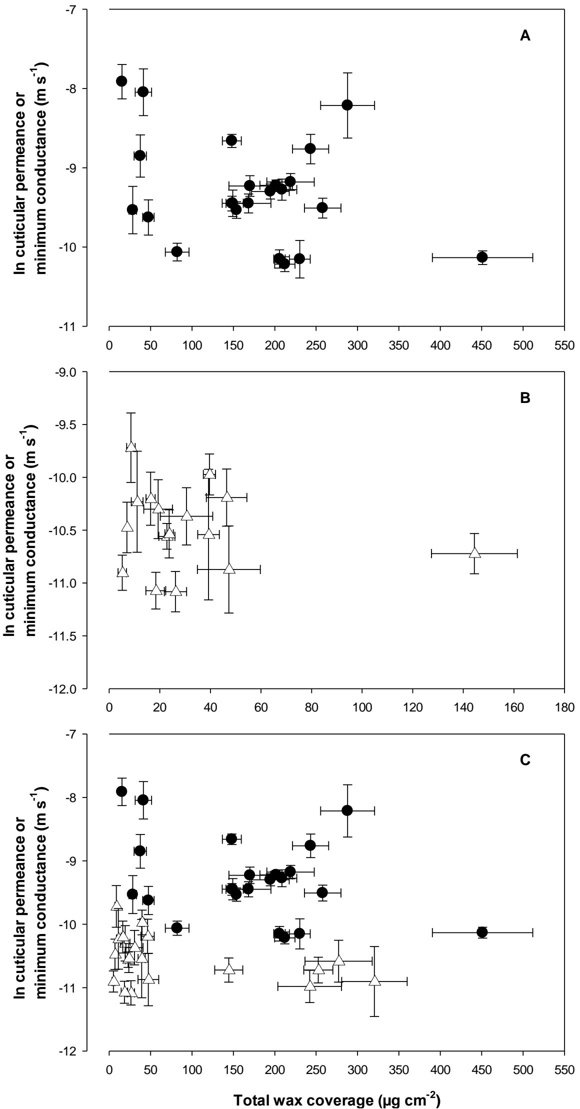

Figure 28. The natural logarithm of the minimum conductance or cuticular permeance as a function of total wax coverage of (A) fruits, (B) leaves, (C) fruits and leaves together (●, fruits; Δ, leaves).

Like the total wax load, the deposition of cyclic and aliphatic compounds varied based upon the different species and organs (Figure 27, Table 4 and 5). Over 60% of the total wax was dominated by cyclic compounds in mature Oleaceae fruits and leaves. Besides European plum fruit (only 2%), cyclics in the other Rosaceae fruits varied from 35% up to 80% of the total wax. Grape berries contained a similar proportion of aliphatics and cyclics (49%-54%). Between 50% and 90% of total wax were dominated by cyclic compounds in the wax of Oleaceae and Rosaceae leaves.

While very small portions, not more than 15%, were detected in the leaf waxes of *Vitis vinifera* L. cv. 'Nelly', and cv. 'Silvana', *Averrhoa carambola* L., *Coffea arabica* L., *Ficus carica* L. and *Capsicum annuum* L. cv. 'Kalocsai'. The various deposition of cyclic compounds as well as the main composition of oleanolic acid and ursolic acid, were not directly related to the transpiration barrier (Figure 29 and Table 27).

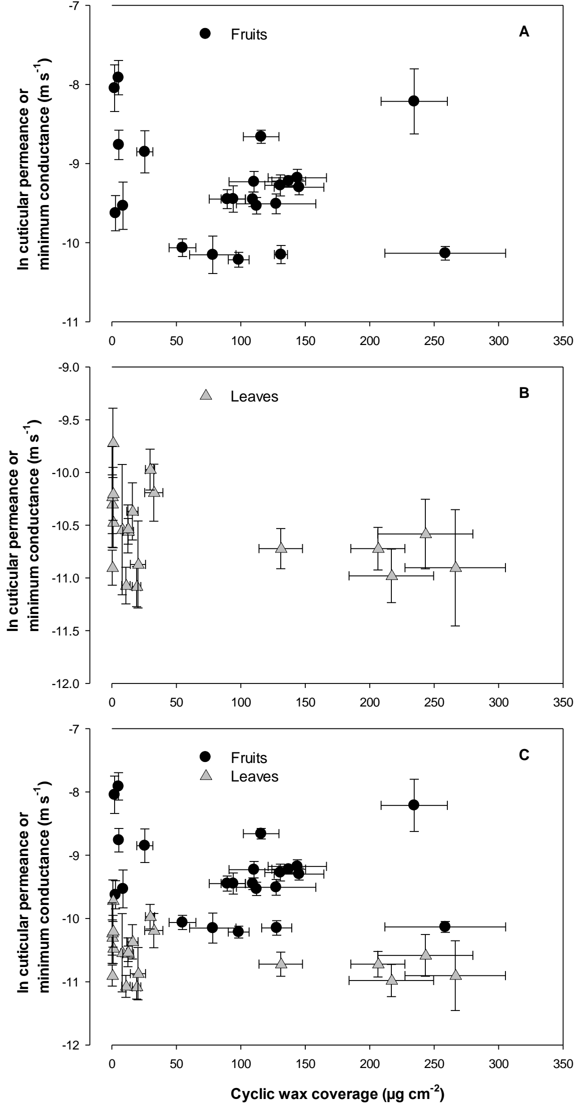

Figure 29. The natural logarithm of the minimum conductance or cuticular permeance as a function of cyclic wax coverage of (A) fruits, (B) leaves, (C) fruits and leaves together (●, fruits; Δ, leaves).

The deposition of alipahtics ranged from 20% to 35% of total wax in olive fruits, while this value was only 5% for leaves (Figure 27). The concentration of aliphatics varied from 12% (nectarine) to 90% (European plum) fruits, whereas it composed from 14% (cherry plum) to 50% (nectarine) of leaf wax. In comparison to the higher

accumulation of aliphatics, ranging from 50% to 90% for leaf waxes of *Vitis vinifera* L. cv. 'Nelly', and cv. 'Silvana', *Averrhoa carambola* L., *Coffea arabica* L., *Ficus carica* L. and *Capsicum annuum* L. cv. 'Kalocsai'., lower concentrations of aliphatics were detected in their fruits (35 % for *Vitis vinifera* L. cv. 'Silvana', 45% for cv. 'Nelly' berry fruit, 45% for star fruit and over 70% for figs).

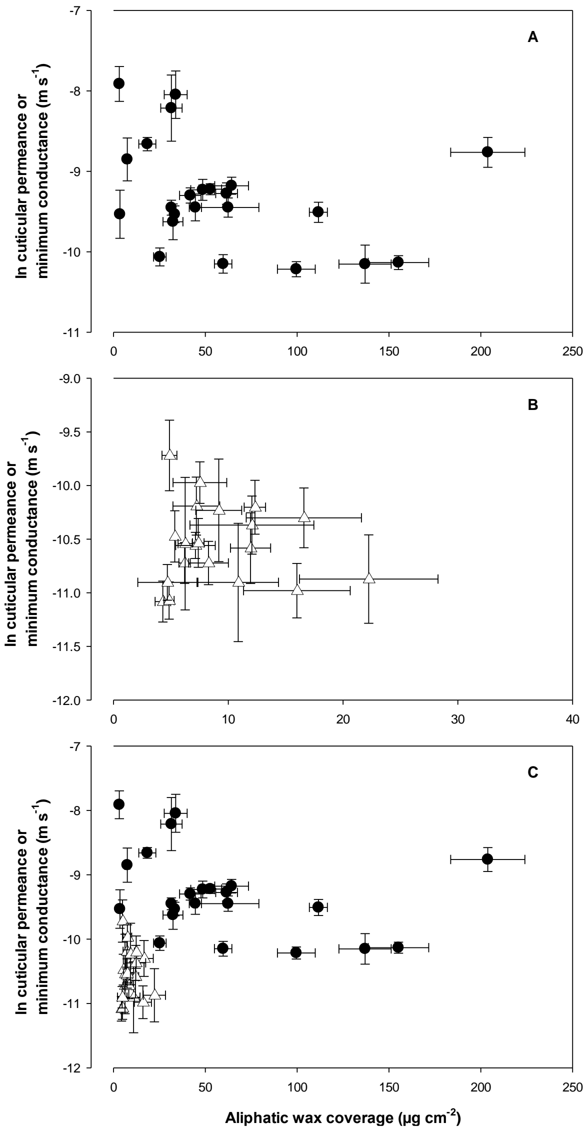

Figure 30. The natural logarithm of the minimum conductance or cuticular permeance as a function of aliphatic wax coverage of (A) fruits, (B) leaves, (C) fruits and leaves together (●, fruits; Δ, leaves).

Subsequently, the ratio of aliphatics to cyclics varied from 0.13 (nectarine) to 38.59 (European plum) for fruits (Table 4), while a larger range was found for leaves (Table 5), namely between 0.04 (*Olea europaea* L.) and 123.02 (*Vitis vinifera* L. cv. 'Nelly'). The deposition of total amount of aliphatics and the various aliphatic components,

mainly primary alcohols, *n*-alkanes, alkyl esters as well as the most abundant *n*-alkanes of *n*-Nonacosane (C_{29}), were not detected to be related to the transpiration barrier properties (Figure 30 and Table 27).

Overall, the deposition of cuticular waxes appears to vary in a species-specific manner. The total wax load and accumulation of cyclics could not contribute to more efficient water transpiration (Riederer and Schreiber, 2001; Oliveira et al., 2003; Jetter and Riederer, 2016; Schuster, 2016). Recently, the transpiration barrier was putatively associated with the coverage of aliphatics (55%) and this theory was tested using different xeric plant leaves (Schuster, 2016). However, significant correlations between the deposition of aliphatics and the permeabilities of fruits and leaves independently or together were not confirmed. The absence of accumulation of *n*-alkanes and aldehydes and the increased deposition of triterpenoids induced increase water loss in the tomato fruit (Leide et al., 2011). An additional two studies on the post-harvest wax change of sweet cherry and peach fruits indicated that higher ratios of *n*-alkanes to triterpenoids were associated with a decreased weight loss for fruits (Belge et al., 2014; Belge et al., 2014). Therefore, the contribution of aliphatic or cyclic compounds for the transpiration barrier remains uncertain.

3.2 Effect of cyclic waxes on the transpiration barrier properties

The proposed wax structure model suggests a possible link between the wax compositions and the barrier properties. The amorphous zone of cuticular wax layer was predominantly filled by cyclic compounds and short carbon chain aliphatics (Reynhardt and Riederer, 1991, 1994). Water was thought to diffuse solely through the amorphous zones. The potential effect of triterpenoids, the predominant cyclic components, on transpiration properties has been implied following genetic modifications of tomato fruit and *Arabidopsis* leaves. The *lecer6* and *ps* mutant tomato fruits showed an absence of *n*-alkanes and aldehydes, an increased deposition of triterpenoids and sterol derivatives and an significant induced increase of water transpiration when compared to wild type fruit (Leide et al., 2007; Leide et al., 2011). In comparison to the control plant, the accumulation of β-amyrin in the intracuticular wax layer, which was induced by overexpression of *AtLUP4* in the *Arabidopsis* leaf, exhibited significantly higher water transpiration (Buschhaus and Jetter, 2012).

Recent studies on desert plant leaves of woods, shrubs and grasses found that these desert leaves showed a relatively high transpiration similar to deciduous plants.

The cuticular waxes were dominated by triterpenoids (85% total wax) (Schuster et al., 2016). The pronounced accumulation of ursolic acid as the main cyclic compound was positively correlated to weight loss and softening change of highbush blueberries during post-harvest shelf life (Moggia et al., 2016). In addition, detection of the wax change of sweet cherry and peach fruits during post-harvest storage found that a higher weight loss of fruits was associated with lower ratios of *n*-alkanes to triterpenoids (Belge et al., 2014; Belge et al., 2014). This suggests, as proposed for the structure model, that the accumulation of pentacyclic triterpenoids in the cuticle may broaden the amorphous fractions; thus, leading to an increase in transpiration.

The distinguishable analysis of epicuticular and intracuticular waxes of selected leaves and Micro-Tom fruit provided a further possibility to examine the relationships between transpiration barrier properties and the chemical characteristics (Vogg et al., 2004; Buschhaus and Jetter, 2012; Jetter and Riederer, 2016; Zeisler and Schreiber, 2016). In species where a high amount of intracuticular triterpenoids or other alicyclic compounds were detected, the cuticular transpiration barrier may be distributed in series (up to 1:1) between the epi- and intracuticular wax layers. On the other hand, the transpiration barrier was largely constructed by intracuticular waxes when triterpenoids and other cyclic compounds were lacking (Jetter and Riederer, 2016). Another study on flower petals demonstrated that two-thirds of the water barrier was located in the epicuticular waxes, where aliphatic compounds solely occur. The residual one-third of the barrier was contributed by the intracuticular wax layer with a large amount of triterpenoids (Buschhaus et al., 2015).

In the present study, various comparable coverages of triterpenoids, mainly oleanolic acid and ursolic acid, were detected in waxes of the fruits and leaves of Oleaceae and in most Rosaceae species (Table 26), while they were not detected to be related to the transpiration barrier properties (Table 27). When previous literature was combined with the present data, it was found that the accumulation of triterpenoids together with other cyclic components could not provide a more efficient barrier for water transpiration.

3.3 Effect of aliphatic waxes on the transpiration barrier properties

It has been shown that when the heterogeneous cuticular wax mixture was entirely dominated by VLC aliphatic compounds the transpiration barrier was largely located in the intracuticular wax layer. Whereas the barrier was substantially formed by

epicuticular and intracuticular wax layers, when VLC aliphatic compounds located either only in epicuticular or together with high amount of cyclics in intracuticular wax layer. This was confirmed by studying selective evergreen leaves, tomato and sweet cherry fruits and *Cosmos* petals (Knoche et al., 2000; Vogg et al., 2004; Buschhaus et al., 2015; Jetter and Riederer, 2016). Thus, the transpiration barrier properties were putatively associated to the aliphatic compositions rather than to cyclic compounds.

The aliphatic components, especially the *n*-alkanes, can be stimulated to accumulate in leaf waxes for improved drought tolerance (Cameron et al., 2006; Kosma et al., 2009; Al-Abdallat et al., 2014). The deposition of *n*-alkanes also showed a significant negative correlation with the minimum leaf water permeability of selective xeric plants. It implied that the aliphatic components contributed nearly 55% of the transpiration barrier (Schuster, 2016). These studies further confirmed the crucial role of aliphatic components in transpiration barrier properties. However, in the present study, no significant correlations between the total aliphatics and individual aliphatic compositions of different fruits and leaves independently or together were found. However, the importance of the absolute amount of aliphatic compounds, and deposition of individual aliphatic compositions for the transpiration barrier remains uncertain (Riederer and Schneider, 1990; Schreiber and Riederer, 1996; Jetter and Riederer, 2016).

As in the proposed wax structural model, the crystalline zone is solely packed by very-long hydrocarbon chains, which are rigidly arranged and tightly aligned to form impermeable flake obstacles (Riederer and Schreiber, 1995). These molecules are forced by the randomly-distributed impermeable flakes to follow a tortuous pathway through the amorphous zones (Cussler et al., 1988; Riederer and Schreiber, 1995). Consequently, the transpiration barrier properties vary with the volume fractions of the flakes, which are largely dependent upon the chain-length distribution of the aliphatics (Riederer and Schreiber, 1995). However, for this proposed model, only a limited number of realistic studies have been elucidated. Studies on the tomato fruit with the *lecer6* wax mutant, which is defective in very-long-chain fatty acid elongation, induced an eight-fold increase in water permeability for fruit when compared to the wild type. Another positional sterile (*ps*) mutant line, characterized by a strikingly similar phenotype to *lecer6* for the floral organ, showed a five- to eight-fold increase in water transpiration in comparison to the wild type. Both mutant lines could not synthesize VLC *n*-alkanes and aldehydes, especially with chain-lengths larger than C_{28} (Leide,

2008; Leide et al., 2011). Recently, the deposition of *n*-alkanes (dominated by chain-lengths ranging between C_{29} and C_{33}) demonstrated a significant negative correlation with the minimum leaf water permeability of selective xeric plants (Schuster, 2016). These reported results provide somewhat evidences that the accumulation of aliphatic compositions, especially the chain-length distribution, can affect the transpiration barrier properties.

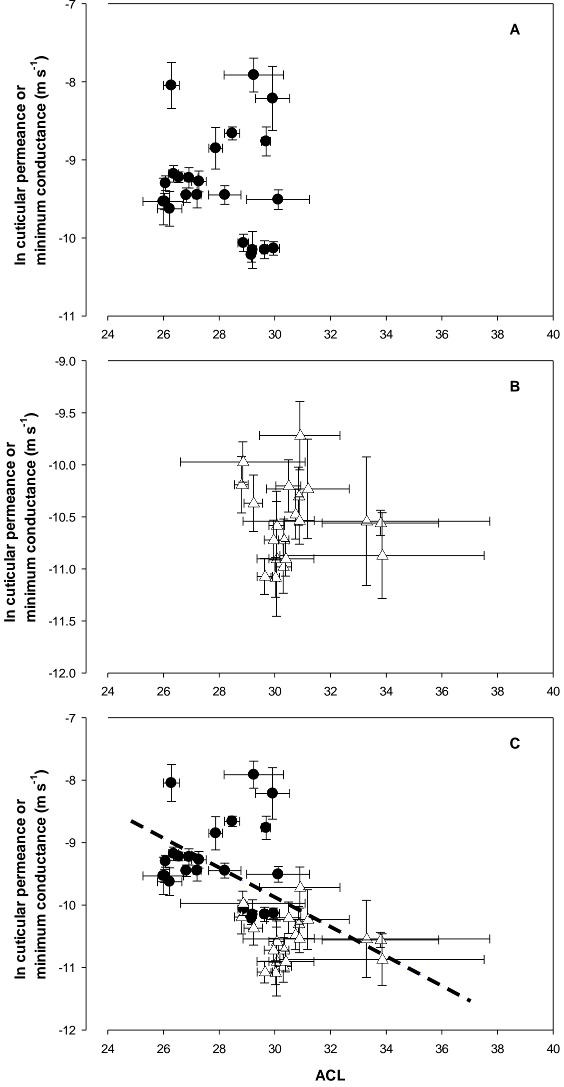

Figure 31. The natural logarithm of the minimum conductance or cuticular permeance as a function of ACL of (A) fruits, (B) leaves, (C) fruits and leaves together (r^2 = 0.42, Spearman Rank Order Correlation Coefficient = - 0.65, $P < 0.001$; ●, fruits; Δ, leaves).

In the present study, the aliphatic waxes of leaves from several different species were predominantly composed of *n*-alkanes with odd numbered chain-lengths ranging between C_{29} and C_{33}. The aliphatic wax fraction of leaves showed a narrow range between C_{29} and C_{34} in ACL, which describes the average number of carbon atoms of

for the chain-length for aliphatic components (Poynter et al. 1989). Consequently, no significantly correlations between ACLs and the permeabilities of leaves was observed (Figure 31A). The ACL value is widely accepted as a proxy indicator for the cuticular wax quality in plants (Poynter et al., 1989; Wang et al., 2015). From this point of view, a similar chain length distribution of aliphatic fractions for the same organ of leaves in different xeric species, therefore, which may result no significant correlations between the ACL and the water transpiration (Schuster, 2016).

The aliphatic fractions of fruit from different species exhibited various chain-lengths and composition distributions. The aliphatics of olive, figs, grape berries of *Vitis vinifera* L. cv. 'Nelly' and cv. 'Silvana' were dominated by fatty acids and primary alcohols with even numbered chain-lengths ranging from C_{24} and C_{28} or C_{30} (Table 26). The fruits of the Rosaceae family and *Cornus officinalis* were dominated by *n*-Nonacosane (C_{29}). In addition, secondary alcohols with a chain-length of C_{29} were distributed at a higher level in fruits of *Crataegus pedicellata*, *Malus domestica* L. cv. 'Topaz' and European plum. The major aliphatics of the star fruit were *n*-alkenes and *n*-alkanes with a chain-length of C_{23} and C_{25} (Table 26). The ACLs for olive, pepper, figs, star fruit were C_{26} or C_{27}, and C_{28} or C_{29} for the Rosaceae fruits. The overall ACL ranged between C_{26} and C_{30} and were detected for the various studied fruits. Similar as in leaves, the water transpiration did not relate to ACL for fruits (Figure 31B).

When the fruit and leaf data sets were combined, the interspecies comparison of ACL for leaves was significantly greater than that of fruits (Figure 32A). Meanwhile, a significant negative correlation between ACLs and the natural logarithm of permeabilities was detected (Fig. 31C, $r^2 = 0.42$, $P < 0.001$). Hence, in comparison to fruits, the longer average chain length of aliphatic fractions and the lower water permeability was found for leaves.

It has been indicated that the non-functional stomata occur on the sweet cherry fruit (Knoche et al., 2000). The occurrence of stomata may affect the cuticular transpiration. However, the interspecies comparison of ACL for leaves was also found significantly greater than that of fruits, when the data sets of the fruit of *Ligustrum valgure* L., *Prunus avium* L., *Prunus domestica* subsp. *insititia* (L.), *Prunus persica L., Coffea Arabica L.*, *Ficus carica* L., and *Cornus officinalis* with stomata on their surfaces were excluded (Figure 32B). As the organ-specific for fruit and leaf, no significant correlations between the ACLs and the nature logarithm of permeabilities for fruits or leaves independently (Figure 33A and B). While a significant negative correlation

between the ACL and nature logarithm of permeabilities in combination of fruits and leaves was also detected (Figure 33C, $r^2 = 0.56$, $P < 0.001$). The absolute correlation coefficient was even higher than that of ACL including fruits with stomata on their surfaces. These results further indicate that the lower permeabilities are related to the longer chain-length distributions.

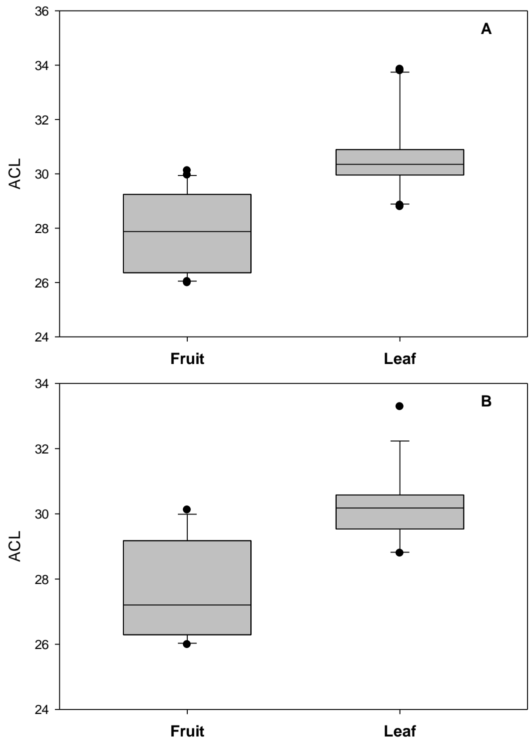

Figure 32. The ACL of fruits and leaves of different species. (A) The interspecies comparison of ACL for leaves was significantly longer than that of fruits ($P < 0.001$). (B) The ACL of leaves and fruits of *Ligustrum valgure* L., *Prunus avium* L., *Prunus domestica* subsp. *institia* (L.), *Prunus persica L., Coffea Arabica L.*, *Ficus carica* L., and *Cornus officinalis* with observed stomata on fruit surfaces were excluded. The interspecies comparison of ACL for leaves was significantly longer than that of fruits ($P < 0.001$).

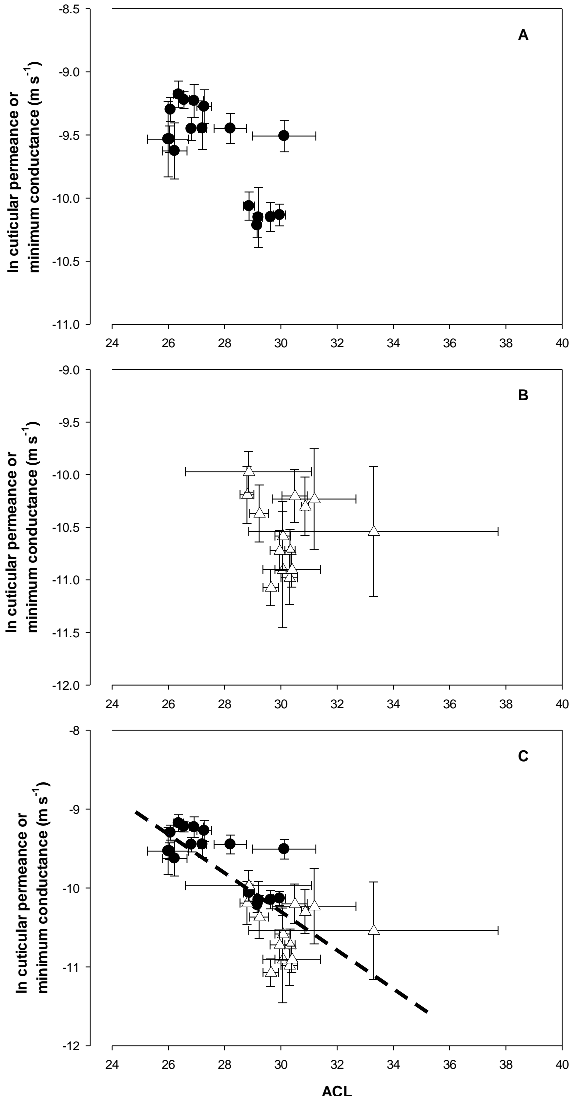

Figure 33. The natural logarithm of the minimum conductance or cuticular permeance as a function of ACL of (A) fruits, (B) leaves, (C) fruits and leaves together (r^2 = 0.56, Spearman Rank Order Correlation Coefficient = - 0.75, $P < 0.001$; ●, fruits; Δ, leaves).

Additionally, the chain ends of VLC hydrocarbon chains formed solid amorphous zones between two flakes (Reynhardt and Riederer, 1991; Riederer and Schreiber, 1995). The fraction of adjacent amorphous zones in the wax barrier functioned as the reverse of ΔACL (root mean square deviation) over ACL. As a measurement of dispersion of the functional-group contents, the hydrocarbon and polar groups, thus, the greater ΔACL ACL^{-1}, the smaller is for the volume fraction of crystallines (Riederer and Schneider, 1990). The impact of ΔACL ACL^{-1} on the transpiration barrier was not detected in this study (Figure 34).

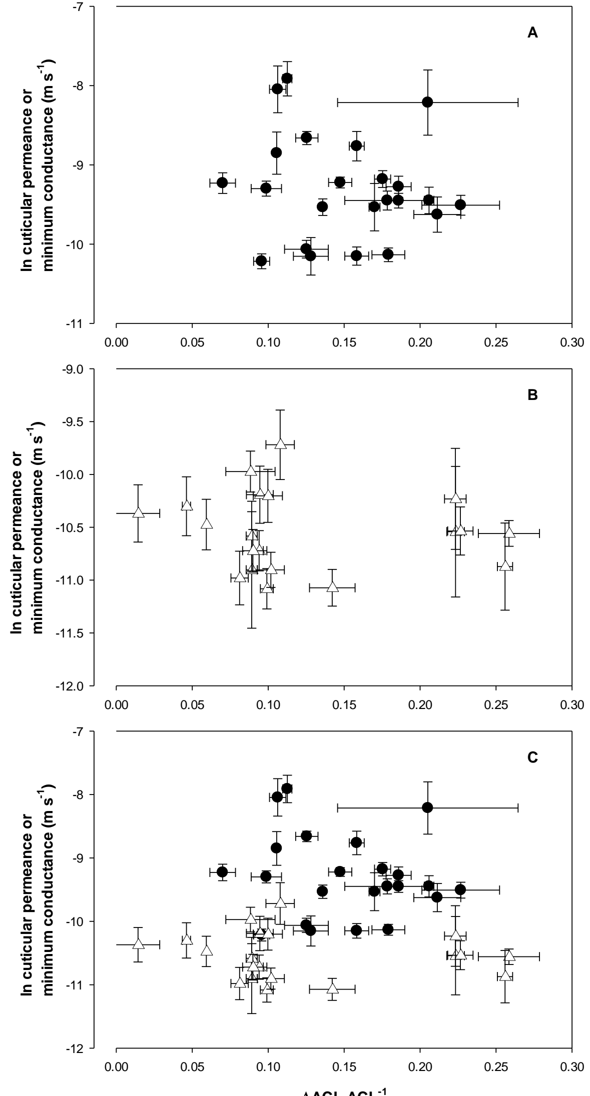

Figure 34. The natural logarithm of the minimum conductance or cuticular permeance as a function of ΔACL ACL-1 of (A) fruits, (B) leaves, (C) and in combination of fruits and leaves (●, fruits; Δ, leaves).

Within the plant cuticle, the cuticular waxes establish the main transport-limiting barrier. Water diffusion is assumed to occur only in the amorphous zone following a very tortuous pathway around the crystalline flakes (Riederer and Schreiber 1995, Riederer and Schreiber 2001). In analogy to polyethylene which is a semi-crystalline aliphatic material comparable to cuticular waxes, the degree of crystallinity should determine the barrier properties of cuticular waxes. The higher the crystallinity is, the longer is the effective pathway, and consequently, the lower is the effective diffusion coefficient of water molecules across this barrier (Lasoski and Cobbs, 1959). The ACL of the aliphatic wax components is one parameter influencing the crystallinity. The

greater of ACL was proposed to enhance the volume of crystalline fractions; thereby enhancing the number of impermeable flakes and resulting in lower permeability.

Conversely, the high concentration of relatively short chain-length aliphatics with polar groups of hydroxyl, carbonhydroxyl, such as fatty acids, primary alcohols, and aldehydes *etc.* (Table 26), may result a smaller ACL. In the presentstudy, the absolute amount of fatty acids and aldehydes were even detected to be slightly positively related to the transpiration barrier (Table 27). These relatively chain-length aliphatics may increase hydrophilicity and broaden the amorphous zones, therefore, inducing a higher water permeability was found in fruits rather than leaves. Therefore, the greater ACL in leaves as compared to fruits may be a factor leading to the considerably lower permeability of the leaf cuticles. The results obtained in the present study demonstrated an organ-specific differences of cuticular permeability basis of the ACL of cuticular aliphatic waxes between fruit and leaf from different species. The correlations between ACL and the permeabilities in combination of fruits and leaves, therefore, the proposed importance of chain-length-dependent contributions of aliphatics for the cuticular wax barrier was further supported by the presented results.

4 Cuticular waxes and the mechanical properties of cuticle

Rheological studies demonstrated that the cuticular waxes served as fillers to strengthen the mechanical properties of cuticular membranes (Petracek and Bukovac, 1995). It has been reported that under environmental stresses, such as hydration and temperature, the dense molecular structure of aliphatics or triterpenoids may affect the packing of the reconstituted cuticular waxes and maintenance of the cuticle integrity (Casado and Heredia, 1999; Stark et al., 2008). There have also been reports which demonstrated that dynamic changes of chemical composition of the tomato fruit cuticle are likely to account for the various mechanical properties (Bargel et al., 2006). Therefore, the importance of different cuticular wax components for the mechanical properties cannot be ignored.

4.1 Attribution of wax load to the integrity of cuticle

Following drought or high temperature stresses, one of the main strategies to enhance the efficiency of water-use and to limit water loss is to decrease leaf area (Blum, 1996; Bacelar et al., 2007; Farooq et al., 2009). As a result, plants that grow under high

temperatures or under water-deficit environments accumulate a thick cuticle on both the fruit and leaf surfaces (Premachandra et al., 1991; Patumi et al., 2002; Ristic and Jenks, 2002; Bacelar et al., 2004; Hammami and Rapoport, 2012; Gómez-del-Campo et al., 2014). Though no correlations have been found between wax accumulation or cuticle thickness and the permeability characteristics (Riederer and Schreiber, 2001; Jetter and Riederer, 2016), the cuticle thickness has been putatively related to the mechanical properties of the cuticle (Matas et al., 2004). For instance, the cuticle thickness of olive fruit increased following the decrease of irrigation regimes (Patumi et al., 2002). However, leaves with thicker cuticles do not provide more efficient transpiration barriers (Bacelar et al., 2004).

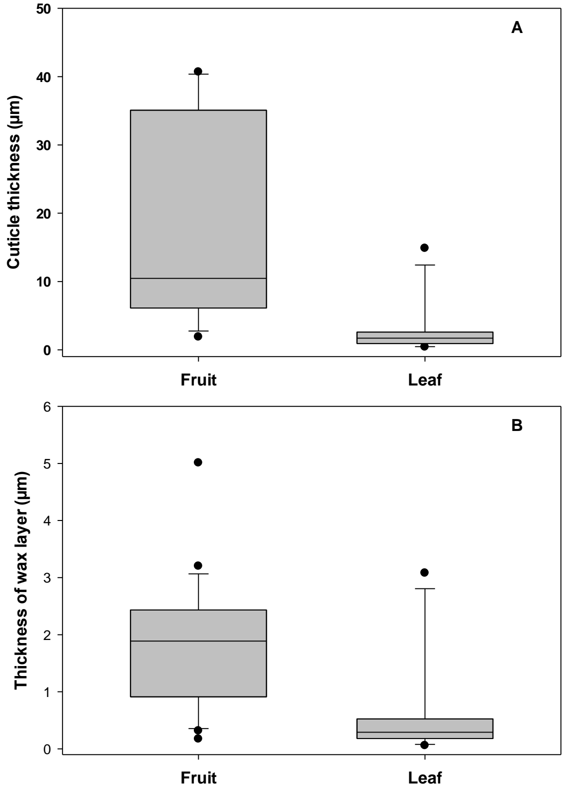

Figure 35. The thickness of intact cuticle and cuticular wax layer of fruits and leaves. (A) The thickness of cuticles were carried out according to the reported density of cuticles and the dry weigt of CMs. The thickness of fruit cuticles was significantly thicker than that of leaf cuticles (P < 0.01). (B) The thickness of cuticular wax layers were calculated according to the wax density and total amount of wax. The thickness of cuticular wax layer of fruit was significantly thickerr than that of leaves (P < 0.01).

In the present study, the thickness of cuticle or the cuticular wax layer of different fruits and leaves could be calculated by dividing the weight of CMs by the density of

cuticle, which is between 1.0-1.1 g cm^{-3} (Schreiber and Schönherr, 1990) or of wax of around 0.9 g cm^{-3}. Consequently, the interspecies comparison of thickness of cuticle or cuticular wax layer of fruits was significantly greater than that of leaves (Figure 35). The cuticles of olive fruits, *Ligustrum vulgare*, Rosaceae species and grape berries were thicker than other fruit cuticles. These results are in accordance with the reported ones, such as the cuticle thickness of sweet cherry, plum and olive fruit (Demirsoy and Demirsoy, 2004; Gómez-del-Campo et al., 2014; Konarska, 2015) and olive leaf (Bacelar et al., 2004). Meanwhile, the olive leaf, which grows for an extended period under drought stress, accumulates a much thicker cuticle than the other studied leaves.

The change of cuticle thickness could not only be stimulated by water deficit stress but also by other abiotic stresses, such as UV light irradiation (Grammatikopoulos et al., 1998; Liakoura et al., 1999; Semerdjieva et al., 2003) and pathogen infection (Biles et al., 1993; Gabler et al., 2003; Gomes et al., 2012). The cuticle thickness was thought to provide a mechanical protection against pathogens and UV-light radiation (Solovchenko and Merzlyak, 2003; Gomes et al., 2012). Moreover, the crack susceptibility of different cultivars of cherry tomato and sweet cherry fruits has been suggested to be related to their cuticle thickness (Demirsoy and Demirsoy, 2004; Matas et al., 2004). During the development of the sweet cherry fruit, the fruit surface expansion, strain and formation of micro-cracks in the CMs is implicated to be related to the lack of deposition of waxes in the cuticle membrane (Alkio et al., 2012). It has also been suggested that the thickness of cuticle in insects provides the insect with ‘hardness’ and ‘intractability’ (Evans and Sanson, 2005). Therefore, it might be indicated that the turgor-driven growth of fruits and leaves induces the various thickness for cuticles to stabilize the integrity of plant organs, which help them to adapt following abiotic environmental stresses, such as water deficit, pathogen infection and UV light radiation *etc* (Bargel et al., 2006).

4.2 Attribution of cyclic waxes to the mechanical properties of cuticle

The presence of large amounts of cyclic components has been suggested to play a role in the high degree of molecular order in cuticular wax layer (Casado and Heredia, 1999). Moreover, thermodynamic analyses revealed that cyclic compounds mixed with other wax components in the amorphous zone may shift the melting point of the wax (Reynhardt and Riederer, 1994; Reynhardt, 1997). A single study has thus far analyzed the effect of temperature on the cuticular transpiration of the hot-desert plant

leaf of *Rhazya stricta* and found that an increase in temperature (15 °C to 50 °C) induced a limited increase in the minimum leaf conductance in comparison to other evergreen or deciduous leaves. The effectiveness of the cuticular transpiration barrier was thought to be related to the high amount of waxes (about 251.4 µg cm^{-2}), which were dominated by triterpenoids (85%) (Schuster et al., 2016). Similar to the *Rhazya stricta*, the permeability of olive fruit showed no significant differences between 25 °C and 50 °C (Table 29). The permeability increased 1.4-fold from 25 °C to 50 °C for the adaxial leaf, which showed a similar increase as reported by *Rhazya stricta* (Schuster et al., 2016). The triterpenoids dominated in both the olive fruit (about 63%) and leaf (88%-91%) waxes (Figure 27). As suggested by Schuster et al. (2016), high amounts of waxes, such as triterpenoids, may help to protect the thermal expansion of the cuticular membrane of the olive fruit and leaf for environment adaptation.

The cyclic compounds were predominantly embeded in the intracuticular wax layer which penetrates directly into the cutin matrix (Jetter et al., 2000; Vogg et al., 2004). The intracuticular waxes have been described as fillers and have been proven to be important for the enhancement of the mechanical properties of cuticle, i.e. the reduction of free spaces and segmental mobility within the cutin matrix (Bargel et al., 2006; Khanal et al., 2013). Quantitative changes in cuticle components influence the elastic/viscoelastic behavior of the cuticle (España et al., 2013). Triterpenoid accumulation caused a reduction in the water barrier effectiveness of the intracuticular wax (Bushchaus, and Jetter, 2012) and the increased accumulation of triterpenoids in *Rhazya stricta* also induced a relatively high permeability compared to deciduous leaves. Nevertheless, high amounts of triterpenoids or other cyclic compounds have been suggested to act as fillers to enhance the tolerance to drought stress and to strengthen and uphold the integrity of the cuticle (Schuster et al., 2016). Additionally, most fruits and leaves from different species were detected to contain higher amount of cyclics than aliphatics, with the ratio of aliphaitcs over cyclics being less than the value of unit (Table 4 and 5). From an ecological point of view, in order to adapt to the various environmental impact factors, the relatively high concentration of triterpenoids seems a more likely link to the mechanical properties of cuticles (Bargel et al., 2006).

4.3 Attribution of aliphatic waxes to the mechanical properties of cuticle

The cuticle mechanical properties have been proven to be important for the integrity of plant organs, for example to avoid fruit cracking (Matas et al., 2004; Hetzroni et al.,

2011), invagination of fruit texture and shelf life (Matas et al., 2004; Saladié et al., 2007). These properties are especially important for the adjustment of fruit with a stable and high turgor pressure, especially for turgor-driven growth of berries. For instance, the thickness of the cuticle following fruit surface expansion is found to be positively related to protect sweet cherry and tomato from micro-crack formation in the CMs and fruit cracking (Demirsoy and Demirsoy, 2004; Matas et al., 2004).

Studies on different cultivars of sweet cherry fruits indicated that a higher level of VLC *n*-alkanes, especially with a chain-length of C_{29}, exhibits a better cracking tolerance for the fruit (Balbontín et al., 2013; Ríos et al., 2015). The cherry and tomato cracking was reported to be induced by fast water uptake through the surface water droplets by increasing the turgor pressure (Cline et al., 1995; Balbontín et al., 2013). Genetic modifications of tomato fruit, in which the synthesis of VLC *n*-alkanes (> C_{28}) in the tomato cuticle were altered, produced not only an increased water transpiration, but also a faster shrinkage of ripe fruit (Vogg et al., 2004). In addition, under dehydration conditions, the VLC *n*-alkanes (> C28) were stimulated to accumulate and increase the thickness of cuticle for leaves, which subsequently exhibited better dehydration stress tolerance (Cameron et al., 2006; Kosma et al., 2009; Al-Abdallat et al., 2014). Therefore, the accumulation of aliphatic components, especially VLC *n*-alkanes, may also play a crucial role in protecting the integrity and mechanical properties of the cuticle for plant organs.

Interestingly, as summarized by Lara et al. (2015), predominant aliphatic wax components of C_{29} or C_{31} *n*-alkane were found in some fruits of the Solananceae, Rosaceae and Rutaceae families. Similar results with prounounced abound of C_{29} *n*-alkanes, as seen in the dominant aliphatic composition in the drupe fruits, such as fruit of Rosaceae, *Ligustrum vulgare* L., and *Cornus officinalis,* were also detected in the present study (Table 26). Furthermore, both the present study and previous reports showed a common distribution of *n*-alkanes with odd numbered chain-lengths ranging from C_{29} to C_{33} as the prominent aliphatic waxes for leaves (Riederer and Schneider, 1990; Jetter et al., 2000; Szafranek and Synak, 2006; Buschhaus et al., 2007; Jetter and Riederer, 2016; Schuster, 2016). Meanwhile, the VLC *n*-alkanes were deposited both in the epicuticular and the intracuticular wax layers of leaves (Jetter et al., 2000; Buschhaus et al., 2007; Jetter and Riederer, 2016) and tomato fruit (Vogg et al., 2004). The aliphatics, especially, the VLC aliphatic components are proposed to form a more

efficient cuticular transpiration barrier, as evidenced by the genetic modification and physical treatments which induced drought stresses.

The structure-function relationship suggests that the aliphatic waxes, especially the VLC *n*-alkanes, are the pivotal components which enhance the tightly-packed orthorhombic impermeable crystalline fractions (Riederer and Schreiber, 1995; Leide et al., 2007). When previous data was combined with the present results, it could be proposed that the aliphtics, especially, the VLC *n*-alkanes are essential for both the transpiration barrier and the mechanical properties of cuticle of fruits and leaves. On the one hand, the VLC *n*-alkanes (especially > C_{28}) may help to establish a more efficient barrier according to extend the ACL value of aliphatics to enhance the volume fraction of crystalline fractions, therefore, inducing relative low permeability to prevent plant organs from fast hydration. On the other hand, the accumulation of these VLC *n*-alkanes together with other aliphatic or cyclic constituents may also act as fillers to strengthen the cuticle mechanical properties, thereby protecting the integrity of organs against shrinkage or cracking under different environmental conditions.

5 Conclusions and Outlook

In the present study, a wide range of plant species was evaluated with the goal of comparing the cuticular water permeability and the chemical composition of the cuticle of fruits against leaves. The results obtained here allowed for integrative correlations between the qualitative and quantitative deposition of cuticular waxes and transpiration barrier properties. For the 17-investigated species, the water permeability of fruits was significantly higher than that of leaves. Chemical analyses showed that the amounts and compositions of the cuticular wax mixtures of fruits and leaves of all studied species were very similar to previously described patterns of plant waxes. The accumulation of total cuticular waxes, aliphatic wax fractions and cyclic wax fractions varied between fruits and leaves of different species.

These results demonstrate that transpiration is not directly related to the deposition of total wax, aliphatic fractions or cyclic fractions. It is more likely that the water transpiration is related to the average chain length of very-long-chain aliphatic wax components. Therefore, the results obtained in this study corroborate with the cuticular wax structure model, which proposes that the high average chain length of aliphatic

compounds might enhance the wax crystalline fractions; thereby, reducing the cuticular water permeability.

A pronounced accumulation of C_{29} *n*-alkanes was found in the waxes of drupe fruits, especially in the Rosaceae family, and of the homologous C_{29} to C_{33} in most of investigated leaves. Taking the present results together with the reported ones, it may be proposed that the accumulation of very-long-chain *n*-alkanes in both fruits and leaves is important for the physiological barrier properties of the plant cuticle. The research performed here enhances the understanding of the potential contributions of cuticular wax composition for the transpiration barrier properties.

From the present results, several future studies are possible. Water loss through possible surface structures, such as stomata or lenticels, may affect the cuticular transpiration. Non-functional stomata were found in the mature sweet cherry fruit (Peschel et al., 2003), while no study regarding functionality analyses of stomata on the fruit surface of *Ligustrum valgure* L., *Prunus domestica* subsp. *institia* (L.)*, Prunus persica L., Coffea Arabica L.*, *Ficus carica* L., and *Cornus officinalis* have been reported. Functional stomata occurred on young grape berries and olive fruits, while the mature fruit was wax-covered (Proietti et al., 1999; Rogiers et al., 2004). Analyses of the residual stomata transpiration of leaves after the transition point is reached, which indicates a maximum closure of stomata was available. Thus, the stomatal or lenticular transpiration effect can be elucidated.

It has been previously suggested that fruit-cracking tolerance is associated with the deposition of C_{29} *n*-alkanes (Balbontín et al., 2013; Ríos et al., 2015); therefore, further comparative studies on the mechanical properties of fruit and leaf cuticles, especially the abundance of odd-numbered carbon chain length longer than C_{28}, are necessary to shed light on their contributions to mechanical properties for the cuticle.

The alterations in cuticle composition are commonly proposed to affect the cuticular transport barrier. Also, differences in the polymer composition and the corresponding primary and secondary ester linkages are proposed to influence the barrier properties (Goodwin and Jenks, 2005; Kosma and Jenks, 2007; Fich et al., 2016). Other factors like cutin polymer structure and cutin/wax interactions may additionally be responsible for the organ-specific differences in cuticular permeability should be further cunducted.

References

Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. The Plant Cell 16: 2463-2480

Al-Abdallat AM, Al-Debei HS, Ayad JY, Hasan S (2014) Over-expression of SlSHN1 gene improves drought tolerance by increasing cuticular wax accumulation in tomato. International Journal of Molecular Sciences 15: 19499-19515

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell & Environment 24: 1337-1344

Alkio M, Jonas U, Sprink T, van Nocker S, Knoche M (2012) Identification of putative candidate genes involved in cuticle formation in Prunus avium (sweet cherry) fruit. Annals of Botany 110: 101-112

Aloni B, Karni L, Rylski I, Cohen Y, Lee Y, Fuchs M, Moreshet S, Yao C (1998) Cuticular cracking in pepper fruit. I. Effects of night temperature and humidity. The Journal of Horticultural Science and Biotechnology 73: 743-749

Arand K, Stock D, Burghardt M, Riederer M (2010) pH-dependent permeation of amino acids through isolated ivy cuticles is affected by cuticular water sorption and hydration shell size of the solute. Journal of Experimental Botany 61: 3865-3873

Asmus E, Popp C, Friedmann AA, Arand K, Riederer M (2016) Water Sorption Isotherms of Surfactants: A Tool To Evaluate Humectancy. Journal of Agricultural and Food Chemistry 64: 5310-5316

Augustin JM, Kuzina V, Andersen SB, Bak S (2011) Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry 72: 435-457

Bacelar EA, Correia CM, Moutinho-Pereira JM, Gonçalves BC, Lopes JI, Torres-Pereira JM (2004) Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. Tree Physiology 24: 233-239

Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Ferreira HF, Correia CM (2007) Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environmental and Experimental Botany 60: 183-192

Bacelar EA, Moutinho-Pereira JM, Gonçalves BC, Lopes JI, Correia CM (2009) Physiological responses of different olive genotypes to drought conditions. Acta Physiologiae Plantarum 31: 611-621

Bain JM, Mcbean DM (1969) The development of the cuticular wax layer in prune plums and the changes occurring in it during drying. Australian Journal of Biological Sciences 22: 101-110

Baker E, Procopiou J (1997) The leaf and fruit cuticles of selected drought tolerant plants. *In* International Symposium on Growth and Development of Fruit Crops 527: 85-94

Baker EA, Bukovac MJ, Flore JA (1979) Ontogenetic variations in the composition of peach leaf wax. Phytochemistry 18: 781-784

Balbontín C, Ayala H, M Bastías R, Tapia G, Ellena M, Torres C, Yuri JA, Quero-García J, Ríos JC, Silva H (2013) Cracking in sweet cherries: A comprehensive review from a physiological, molecular, and genomic perspective. Chilean Journal of Agricultural Research 73: 66-72

Bargel H, Koch K, Cerman Z, Neinhuis C (2006) Structure–function relationships of the plant cuticle and cuticular waxes—a smart material? Functional Plant Biology 33: 893-910

Bargel H, Neinhuis C (2005) Tomato (Lycopersicon esculentum Mill.) fruit growth and ripening as related to the biomechanical properties of fruit skin and isolated cuticle. Journal of Experimental Botany 56: 1049-1060

Barthlott W, Mail M, Bhushan B, Koch K (2017) Plant Surfaces: Structures and Functions for Biomimetic Innovations. Nano-Micro Letters 9: 23

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202: 1-8

Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wilhelmi H (1998) Classification and terminology of plant epicuticular waxes. Botanical Journal of the Linnean Society 126: 237-260

Basson I, Reynhardt E (1988) An investigation of the structures and molecular dynamics of natural waxes. I. Beeswax. Journal of Physics D: Applied Physics 21: 1421

Basson I, Reynhardt E (1992) The structure and melting of paraffinic Fischer—Tropsch waxes. Chemical Physics Letters 198: 367-372

Bateman RM, Crane PR, DiMichele WA, Kenrick PR, Rowe NP, Speck T, Stein WE (1998) Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. Annual Review of Ecology and Systematics 29: 263-292

Bauer S, Schulte E, Thier H-P (2005) Composition of the surface waxes from bell pepper and eggplant. European Food Research and Technology 220: 5-10

Baur P, Marzouk H, Schönherr J, Bauer H (1996) Mobilities of organic compounds in plant cuticles as affected by structure and molar volumes of chemicals and plant species. Planta 199: 404-412

Beisson F, Li-Beisson Y, Pollard M (2012) Solving the puzzles of cutin and suberin polymer biosynthesis. Current Opinion in Plant Biology 15: 329-337

Belding RD, Blankenship SM, Young E, Leidy RB (1998) Composition and variability of epicuticular waxes in apple cultivars. Journal of the American Society for Horticultural Science 123: 348-356

Belding RD, Sutton TB, Blankenship SM, Young E (2000) Relationship between apple fruit epicuticular wax and growth of Peltaster fructicola and Leptodontidium elatius, two fungi that cause sooty blotch disease. Plant Disease 84: 767-772

Belge B, Llovera M, Comabella E, Gatius F, Guillen P, Graell J, Lara I (2014) Characterization of cuticle composition after cold storage of "Celeste" and

"Somerset" sweet cherry fruit. Journal of Agricultural and Food Chemistry 62: 8722-8729

Belge B, Llovera M, Comabella E, Graell J, Lara I (2014) Fruit cuticle composition of a melting and a nonmelting peach cultivar. Journal of Agricultural and Food Chemistry 62: 3488-3495

Bernard A, Domergue F, Pascal S, Jetter R, Renne C, Faure J-D, Haslam RP, Napier JA, Lessire R, Joubès J (2012) Reconstitution of plant alkane biosynthesis in yeast demonstrates that Arabidopsis ECERIFERUM1 and ECERIFERUM3 are core components of a very-long-chain alkane synthesis complex. The Plant Cell 24: 3106-3118

Bernard A, Joubès J (2013) Arabidopsis cuticular waxes: advances in synthesis, export and regulation. Progress in Lipid Research 52: 110-129

Bernards MA (2002) Demystifying suberin. Canadian Journal of Botany 80: 227-240

Bernards MA, Lopez ML, Zajicek J, Lewis NG (1995) Hydroxycinnamic acid-derived polymers constitute the polyaromatic domain of suberin. Journal of Biological Chemistry 270: 7382-7386

Bianchi G, Murelli C, Vlahov G (1992) Surface waxes from olive fruits. Phytochemistry 31: 3503-3506

Bianchi G, Vlahov G, Anglani C, Murelli C (1992) Epicuticular wax of olive leaves. Phytochemistry 32: 49-52

Biles CL, Wall MM, Waugh M, Palmer H (1993) Relationship of Phytophthora fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. Phytopathology 83: 607-611

Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, Kunst L, Wu X, Yephremov A, Samuels L (2007) Characterization of Arabidopsis ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. The Plant Journal 52: 485-498

Blum A (1996) Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20: 135-148

Boom A, Damsté JS, De Leeuw J (2005) Cutan, a common aliphatic biopolymer in cuticles of drought-adapted plants. Organic Geochemistry 36: 595-601

Bosabalidis AM, Kofidis G (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Science 163: 375-379

Boughalleb F, Hajlaoui H (2010) Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiologiae Plantarum 33: 53-65

Bourdenx B, Bernard A, Domergue F, Pascal S, Léger A, Roby D, Pervent M, Vile D, Haslam RP, Napier JA (2011) Overexpression of Arabidopsis ECERIFERUM1 promotes wax very-long-chain alkane biosynthesis and influences plant response to biotic and abiotic stresses. Plant Physiology 156: 29-45

Buchholz A (2006) Characterization of the diffusion of non-electrolytes across plant cuticles: properties of the lipophilic pathway. Journal of Experimental Botany 57: 2501-2513

Burghardt M, Riederer M (2003) Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. Journal of Experimental Botany 54: 1941-1949

Buschhaus C, Hager D, Jetter R (2015) Wax layers on Cosmos bipinnatus petals contribute unequally to total petal water resistance. Plant Physiology 167: 80-88

Buschhaus C, Herz H, Jetter R (2007) Chemical composition of the epicuticular and intracuticular wax layers on the adaxial side of Ligustrum vulgare leaves. New Phytologist 176: 311-316

Buschhaus C, Jetter R (2011) Composition differences between epicuticular and intracuticular wax substructures: how do plants seal their epidermal surfaces? Journal of Experimental Botany 62: 841-853

Buschhaus C, Jetter R (2012) Composition and physiological function of the wax layers coating Arabidopsis leaves: β-amyrin negatively affects the intracuticular water barrier. Plant Physiology 160: 1120-1129

Cameron KD, Teece MA, Smart LB (2006) Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiology 140: 176-183

Casado CG, Heredia A (1999) Structure and dynamics of reconstituted cuticular waxes of grape berry cuticle (Vitis vinifera L.). Journal of Experimental Botany 50: 175-182

Chamel A, Pineri M, Escoubes M (1991) Quantitative determination of water sorption by plant cuticles. Plant, Cell & Environment 14: 87-95

Clayton M, Amos ND, Banks NH, Morton RH (1995) Estimation of apple fruit surface area. New Zealand Journal of Crop and Horticultural Science, 23:3, 345-349

Cline JA, Sekse L, Meland M, Webster AD (1995) Rain-induced fruit cracking of sweet cherries: I. Influence of cultivar and rootstock on fruit water absorption, cracking and quality. Acta Agriculturae Scandinavica B-Plant Soil Sciences 45: 213-223

Commenil P, Brunet L, Audran J-C (1997) The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. Journal of Experimental Botany 48: 1599-1607

Cussler E, Hughes SE, Ward WJ, Aris R (1988) Barrier membranes. Journal of Membrane Science 38: 161-174

DeBono A, Yeats TH, Rose JK, Bird D, Jetter R, Kunst L, Samuels L (2009) Arabidopsis LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. The Plant Cell 21: 1230-1238

Dell'Amico J, Moriana A, Corell M, Girón IF, Morales D, Torrecillas A, Moreno F (2012) Low water stress conditions in table olive trees (Olea europaea L.) during pit hardening produced a different response of fruit and leaf water relations. Agricultural Water Management 114: 11-17

Delude C, Moussu S, Joubès J, Ingram G, Domergue F (2016) Plant Surface Lipids and Epidermis Development. *In* Lipids in Plant and Algae Development. Springer, 287-313

Demirsoy L, Demirsoy H (2004) The epidermal characteristics of fruit skin of some sweet cherry cultivars in relation to fruit cracking. Pakistan Journal of Botany 36: 725-731

Deshmukh AP, Simpson AJ, Hadad CM, Hatcher PG (2005) Insights into the structure of cutin and cutan from Agave americana leaf cuticle using HRMAS NMR spectroscopy. Organic Geochemistry 36: 1072-1085

Deshmukh AP, Simpson AJ, Hatcher PG (2003) Evidence for cross-linking in tomato cutin using HR-MAS NMR spectroscopy. Phytochemistry 64: 1163-1170

Dichio B, Xiloyannis C, Angelopoulos K, Nuzzo V, Bufo SA, Celano G (2003) Drought-induced variations of water relations parameters in Olea europaea. Plant and Soil 257: 381-389

Domínguez E, Heredia A (1999) Water hydration in cutinized cell walls: a physico-chemical analysis. Biochimica et Biophysica Acta (BBA)-General Subjects 1426: 168-176

Doupis G, Bosabalidis A, Patakas A (2016) Comparative effects of water deficit and enhanced UV-B radiation on photosynthetic capacity and leaf anatomy traits of two grapevine (Vitis vinifera L.) cultivars. Theoretical and Experimental Plant Physiology 28: 131-141

Edelmann HG, Neinhuis C, Bargel H (2005) Influence of hydration and temperature on the rheological properties of plant cuticles and their impact on plant organ integrity. Journal of Plant Growth Regulation 24: 116-126

Emmons CLW, Scott JW (1997) Environmental and physiological effects on cuticle cracking in tomato. Journal of the American Society for Horticultural Science 122: 797-801

Ennajeh M, Vadel AM, Khemira H, Ben Mimoun M, Hellali R (2006) Defense mechanisms against water deficit in two olive (Olea europaeaL.) cultivars ‘Meski’ and ‘Chemlali’. The Journal of Horticultural Science and Biotechnology 81: 99-104

Espana L, Heredia-Guerrero JA, Segado P, Benitez JJ, Heredia A, Dominguez E (2014) Biomechanical properties of the tomato (Solanum lycopersicum) fruit cuticle during development are modulated by changes in the relative amounts of its components. New Phytologist 202: 790-802

Evans AR, Sanson GD (2005) Biomechanical properties of insects in relation to insectivory: cuticle thickness as an indicator of insect ‘hardness’ and ‘intractability’. Australian Journal of Zoology 53: 9-19

Fabre G, Garroum I, Mazurek S, Daraspe J, Mucciolo A, Sankar M, Humbel BM, Nawrath C (2016) The ABCG transporter PEC1/ABCG32 is required for the formation of the developing leaf cuticle in Arabidopsis. New Phytologist 209: 192-201

Fahn A (1986) Structural and functional properties of trichomes of xeromorphic leaves. Annals of Botany 57: 631-637

Fang X, Qiu F, Yan B, Wang H, Mort AJ, Stark RE (2001) NMR studies of molecular structure in fruit cuticle polyesters. Phytochemistry 57: 1035-1042

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S (2009) Plant drought stress: effects, mechanisms and management. *In* Sustainable Agriculture. Springer, 153-188

Fernández V, Khayet M (2015) Evaluation of the surface free energy of plant surfaces: toward standardizing the procedure. Frontiers in Plant Science 6: 510

Fich EA, Segerson NA, Rose JK (2016) The Plant Polyester Cutin: Biosynthesis, Structure, and Biological Roles. Annual Review of Plant Biology 67: 207-233

Ficke A, Gadoury DM, Seem RC (2002) Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytopathology 92: 671-675

Franke R, Briesen I, Wojciechowski T, Faust A, Yephremov A, Nawrath C, Schreiber L (2005) Apoplastic polyesters in Arabidopsis surface tissues–a typical suberin and a particular cutin. Phytochemistry 66: 2643-2658

Franke R, Schreiber L (2007) Suberin—a biopolyester forming apoplastic plant interfaces. Current Opinion in Plant Biology 10: 252-259

Gabler FM, Smilanick JL, Mansour M, Ramming DW, Mackey BE (2003) Correlations of morphological, anatomical, and chemical features of grape berries with resistance to Botrytis cinerea. Phytopathology 93: 1263-1273

Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15: 688-695

Girard A-L, Mounet F, Lemaire-Chamley M, Gaillard C, Elmorjani K, Vivancos J, Runavot J-L, Quemener B, Petit J, Germain V (2012) Tomato GDSL1 is required for cutin deposition in the fruit cuticle. The Plant Cell 24: 3119-3134

Gomes S, Bacelar E, Martins-Lopes P, Carvalho T, Guedes-Pinto H (2012) Infection process of olive fruits by Colletotrichum acutatum and the protective role of the cuticle and epidermis. Journal of Agricultural Science 4: 101

Gómez-del-Campo M, Pérez-Expósito MÁ, Hammami SBM, Centeno A, Rapoport HF (2014) Effect of varied summer deficit irrigation on components of olive fruit growth and development. Agricultural Water Management 137: 84-91

Goodwin SM, Jenks MA (2005) Plant cuticle function as a barrier to water loss. *In* Plant Abiotic Stress pp 14-36

Gorb E, Haas K, Henrich A, Enders S, Barbakadze N, Gorb S (2005) Composite structure of the crystalline epicuticular wax layer of the slippery zone in the pitchers of the carnivorous plant Nepenthes alata and its effect on insect attachment. Journal of Experimental Biology 208: 4651-4662

Graça J, Pereira H (2000) Suberin structure in potato periderm: glycerol, long-chain monomers, and glyceryl and feruloyl dimers. Journal of Agricultural and Food Chemistry 48: 5476-5483

Grammatikopoulos G, Kyparissis A, Drilias P, Petropoulou Y, Manetas Y (1998) Effects of UV-B radiation on cuticle thickness and nutritional value of leaves in two Mediterranean evergreen sclerophylls. Journal of Plant Physiology 153: 506-512

Greenspan MD, Schultz HR, Matthews MA (1996) Field evaluation of water transport in grape berries during water deficits. Physiologia Plantarum 97: 55-62

Greer S, Wen M, Bird D, Wu X, Samuels L, Kunst L, Jetter R (2007) The cytochrome P450 enzyme CYP96A15 is the midchain alkane hydroxylase responsible for formation of secondary alcohols and ketones in stem cuticular wax of Arabidopsis. Plant Physiology 145: 653-667

Grncarevic M, Radler F (1971) A review of the surface lipids of grapes and their importance in the drying process. American Journal of Enology and Viticulture 22: 80-86

Gucci R, Goldhamer D, Fereres E (2012) Olive. Crop yield response to water. Irrigation and Drainage 298-313

Guerfel M, Baccouri O, Boujnah D, Chaïbi W, Zarrouk M (2009) Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (Olea europaea L.) cultivars. Scientia Horticulturae 119: 257-263

Guo Q, Gao J, Yang J (2005) Analysis of bioactive triterpenes from Rubus chingii by cyclodextrin-modified capillary electrophoresis. Chromatographia 62: 145-150

Guo S, Duan J-A, Tang Y-P, Yang N-Y, Qian D-W, Su S-L, Shang E-X (2010) Characterization of triterpenic acids in fruits of Ziziphus species by HPLC-ELSD-MS. Journal of Agricultural and Food Chemistry 58: 6285-6289

Gupta NS, Collinson ME, Briggs DE, Evershed RP, Pancost RD (2006) Reinvestigation of the occurrence of cutan in plants: implications for the leaf fossil record. Paleobiology 32: 432-449

Hammami SBM, Rapoport HF (2012) Quantitative Analysis of Cell Organization in the External Region of the Olive Fruit. International Journal of Plant Sciences 173: 993-1004

Hansjakob A, Bischof S, Bringmann G, Riederer M, Hildebrandt U (2010) Very-long-chain aldehydes promote in vitro prepenetration processes of Blumeria graminis in a dose-and chain length-dependent manner. New Phytologist 188: 1039-1054

Haralampidis K, Trojanowska M, Osbourn AE (2002) Biosynthesis of triterpenoid saponins in plants. *In* History and Trends in Bioprocessing and Biotransformation. Springer, 31-49

Haslam TM, Kunst L (2013) Extending the story of very-long-chain fatty acid elongation. Plant Science 210: 93-107

He X, Liu RH (2007) Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. Journal of Agricultural and Food Chemistry 55: 4366-4370

Heredia-Guerrero JA, Benítez JJ, Domínguez E, Bayer IS, Cingolani R, Athanassiou A, Heredia A (2014) Infrared and Raman spectroscopic features of plant cuticles: a review. Frontiers in Plant Science 5: 305

Hetzroni A, Vana A, Mizrach A (2011) Biomechanical characteristics of tomato fruit peels. Postharvest biology and technology 59: 80-84

Holloway P (1982) The chemical constitution of plant cutins. Cutler, D, F,, Alvin, K, L,, Price, C, E ed (s). The Plant Cuticle. Academic Pres: London etc: 45-85

Holloway PJ, Jeffree CE, Baker EA (1976) Structural determination of secondary alcohols from plant epicuticular waxes. Phytochemistry 15: 1768-1770

Holmes MG, Keiller DR (2002) Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant, Cell & Environment 25: 85-93

Hunt L, Amsbury S, Baillie AL, Movahedi M, Mitchell A, Afsharinafar M, Swarup K, Denyer T, Hobbs J, Swarup R (2017) Formation of the Stomatal Outer Cuticular Ledge Requires a Guard Cell Wall Proline-Rich Protein. Plant Physiology 174: 689-699.

Isaacson T, Kosma DK, Matas AJ, Buda GJ, He Y, Yu B, Pravitasari A, Batteas JD, Stark RE, Jenks MA (2009) Cutin deficiency in the tomato fruit cuticle consistently affects resistance to microbial infection and biomechanical properties, but not transpirational water loss. The Plant Journal 60: 363-377

Ismail HM, Brown GA, Tucknott OG, Holloway PJ, Williams AA (1977) Nonanal in epicuticular wax of golden egg plums (Prunus domestica). Phytochemistry 16: 769-770

Jäger S, Trojan H, Kopp T, Laszczyk MN, Scheffler A (2009) Pentacyclic triterpene distribution in various plants–rich sources for a new group of multi-potent plant extracts. Molecules 14: 2016-2031

Jeffree CE (1996) Structure and ontogeny of plant cuticles. Plant cuticles: an integrated functional approach. BIOS Scientific Publishers Ltd.: Oxford, UK: 33-82

Jeffree CE (2006) The fine structure of the plant cuticle. Biology of the Plant Cuticle 23: 11-125

Jenks MA, Hasegawa PM (2008) Plant abiotic stress. John Wiley & Sons

Jenks MA, Tuttle HA, Eigenbrode SD, Feldmann KA (1995) Leaf epicuticular waxes of the eceriferum mutants in Arabidopsis. Plant Physiology 108: 369-377

Jessen D, Olbrich A, Knüfer J, Krüger A, Hoppert M, Polle A, Fulda M (2011) Combined activity of LACS1 and LACS4 is required for proper pollen coat formation in Arabidopsis. The Plant Journal 68: 715-726

Jetter R, Kunst L, Samuels AL (2008) Composition of plant cuticular waxes. Biology of the Plant Cuticle 23: 145-181

Jetter R, Riederer M (2016) Localization of the Transpiration Barrier in the Epi- and Intracuticular Waxes of Eight Plant Species: Water Transport Resistances Are Associated with Fatty Acyl Rather Than Alicyclic Components. Plant Physiology 170: 921-934

Jetter R, Riederer M (2016) Localization of the transpiration barrier in the epi-and intracuticular waxes of eight plant species: water transport resistances are associated with fatty acyl rather than alicyclic components. Plant Physiology 170: 921-934

Jetter R, Schäffer S (2001) Chemical composition of the Prunus laurocerasus leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant physiology 126: 1725-1737

Jetter R, Schäffer S, Riederer M (2000) Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from Prunus laurocerasus L. Plant, Cell & Environment 23: 619-628

Joubes J, Raffaele S, Bourdenx B, Garcia C, Laroche-Traineau J, Moreau P, Domergue F, Lessire R (2008) The VLCFA elongase gene family in Arabidopsis thaliana: phylogenetic analysis, 3D modelling and expression profiling. Plant Molecular Biology 67: 547

Kandel S, Sauveplane V, Olry A, Diss L, Benveniste I, Pinot F (2006) Cytochrome P450-dependent fatty acid hydroxylases in plants. Phytochemistry Reviews 5: 359-372

Kaufmann MR (1970) Water potential components in growing citrus fruits. Plant Physiology 46: 145-149

Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389: 33-39

Khanal BP, Grimm E, Finger S, Blume A, Knoche M (2013) Intracuticular wax fixes and restricts strain in leaf and fruit cuticles. New Phytologist 200: 134-143

Kim H, Lee SB, Kim HJ, Min MK, Hwang I, Suh MC (2012) Characterization of glycosylphosphatidylinositol-anchored lipid transfer protein 2 (LTPG2) and overlapping function between LTPG/LTPG1 and LTPG2 in cuticular wax export or accumulation in Arabidopsis thaliana. Plant and Cell Physiology 53: 1391-1403

Kim KS, Park SH, Jenks MA (2007) Changes in leaf cuticular waxes of sesame (Sesamum indicum L.) plants exposed to water deficit. Journal of Plant Physiology 164: 1134-1143

King GA, Henderson KG, Lill RE (1987) Growth and anatomical and ultrastructural studies of nectarine fruit wall development. Botanical Gazette 148: 443-455

Knoche M, Peschel S (2007) Deposition and strain of the cuticle of developing European plum fruit. Journal of the American Society for Horticultural Science 132: 597-602

Knoche M, Peschel S, Hinz M, Bukovac MJ (2000) Studies on water transport through the sweet cherry fruit surface: characterizing conductance of the cuticular membrane using pericarp segments. Planta 212: 127-135

Knoche M, Peschel S, Hinz M, Bukovac MJ (2001) Studies on water transport through the sweet cherry fruit surface: II. Conductance of the cuticle in relation to fruit development. Planta 213: 927-936

Koch K, Hartmann KD, Schreiber L, Barthlott W, Neinhuis C (2006) Influences of air humidity during the cultivation of plants on wax chemical composition, morphology and leaf surface wettability. Environmental and Experimental Botany 56: 1-9

Koch K, Neinhuis C, Ensikat HJ, Barthlott W (2004) Self assembly of epicuticular waxes on living plant surfaces imaged by atomic force microscopy (AFM). Journal of Experimental Botany 55: 711-718

Kolattukudy P (1970) Plant waxes. Lipids 5: 259-275

Kolattukudy P (1980) Biopolyester membranes of plants: cutin and suberin. Science 208: 990-1000

Kolattukudy Pt (1981) Structure, biosynthesis, and biodegradation of cutin and suberin. Annual Review of Plant Physiology 32: 539-567

Konarska A (2015) Characteristics of Fruit (Prunus domesticaL.) Skin: Structure and Antioxidant Content. International Journal of Food Properties 18: 2487-2499

Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lü S, Joubès J, Jenks MA (2009) The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiology 151: 1918-1929

Kosma DK, Jenks MA (2007) Eco-physiological and molecular-genetic determinants of plant cuticle function in drought and salt stress tolerance. *In* Advances in Molecular breeding toward drought and salt tolerant crops. Springer, pp 91-120

Kosma DK, Parsons EP, Isaacson T, Lü S, Rose JK, Jenks MA (2010) Fruit cuticle lipid composition during development in tomato ripening mutants. Physiologia Plantarum 139: 107-117

Kurdyukov S, Faust A, Trenkamp S, Bär S, Franke R, Efremova N, Tietjen K, Schreiber L, Saedler H, Yephremov A (2006) Genetic and biochemical evidence for involvement of HOTHEAD in the biosynthesis of long-chain α-, ω-dicarboxylic fatty acids and formation of extracellular matrix. Planta 224: 315-329

Lara I, Belge B, Goulao LF (2015) A focus on the biosynthesis and composition of cuticle in fruits. Journal of Agricultral and Food Chemistry 63: 4005-4019

Lardizabal KD, Metz JG, Sakamoto T, Hutton WC, Pollard MR, Lassner MW (2000) Purification of a jojoba embryo wax synthase, cloning of its cDNA, and production of high levels of wax in seeds of transgenic Arabidopsis. Plant Physiology 122: 645-656

Lasoski S, Cobbs W (1959) Moisture permeability of polymers. I. Role of crystallinity and orientation. Journal of Polymer Science 36: 21-33

Le Roux N (1980) Nuclear magnetic resonance investigation of the mobile phase in paraffinic Fischer-Tropsch waxes. South African Journal of Science 76: 157-161

Lee SB, Suh MC (2014) Cuticular wax biosynthesis is up-regulated by the MYB94 transcription factor in Arabidopsis. Plant and Cell Physiology 56: 48-60

Lee SC, Luan S (2012) ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant, Cell & Environment 35: 53-60

Leide J (2008) Cuticular wax biosynthesis of Lycopersicon esculentum and its impact on transpiration barrier properties during fruit development. Graduate thesis, Würzburg, Germany

Leide J, Hildebrandt U, Hartung W, Riederer M, Vogg G (2012) Abscisic acid mediates the formation of a suberized stem scar tissue in tomato fruits. New Phytologist 194: 402-415

Leide J, Hildebrandt U, Reussing K, Riederer M, Vogg G (2007) The developmental pattern of tomato fruit wax accumulation and its impact on cuticular transpiration barrier properties: effects of a deficiency in a β-ketoacyl-coenzyme A synthase (LeCER6). Plant Physiology 144: 1667-1679

Leide J, Hildebrandt U, Vogg G, Riederer M (2011) The positional sterile (ps) mutation affects cuticular transpiration and wax biosynthesis of tomato fruits. Journal of Plant Physiology 168: 871-877

Levizou E, Drilias P, Psaras GK, Manetas Y (2005) Nondestructive assessment of leaf chemistry and physiology through spectral reflectance measurements may be misleading when changes in trichome density co-occur. New Phytologist 165: 463-472

Li-Beisson Y, Pollard M, Sauveplane V, Pinot F, Ohlrogge J, Beisson F (2009) Nanoridges that characterize the surface morphology of flowers require the synthesis of cutin polyester. Proceedings of the National Academy of Sciences 106: 22008-22013

Li F, Wu X, Lam P, Bird D, Zheng H, Samuels L, Jetter R, Kunst L (2008) Identification of the wax ester synthase/acyl-coenzyme A: diacylglycerol acyltransferase WSD1 required for stem wax ester biosynthesis in Arabidopsis. Plant Physiology 148: 97-107

Li Y, Beisson F, Koo AJ, Molina I, Pollard M, Ohlrogge J (2007) Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers. Proceedings of the National Academy of Sciences 104: 18339-18344

Liakoura V, Stavrianakou S, Liakopoulos G, Karabourniotis G, Manetas Y (1999) Effects of UV-B radiation on Olea europaea: comparisons between a greenhouse and a field experiment. Tree Physiology 19: 905-908

López-Casado G, Matas AJ, Domínguez E, Cuartero J, Heredia A (2007) Biomechanics of isolated tomato (Solanum lycopersicum L.) fruit cuticles: the role of the cutin matrix and polysaccharides. Journal of Experimental Botany 58: 3875-3883

Loza-Cornejo S, Terrazas T (2003) Epidermal and hypodermal characteristics in North American Cactoideae (Cactaceae). Journal of Plant Research 116: 27-35

Lü S, Song T, Kosma DK, Parsons EP, Rowland O, Jenks MA (2009) Arabidopsis CER8 encodes LONG-CHAIN ACYL-COA SYNTHETASE 1 (LACS1) that has overlapping functions with LACS2 in plant wax and cutin synthesis. The Plant Journal 59: 553-564

Lü S, Zhao H, Des Marais DL, Parsons EP, Wen X, Xu X, Bangarusamy DK, Wang G, Rowland O, Juenger T (2012) Arabidopsis ECERIFERUM9 involvement in cuticle formation and maintenance of plant water status. Plant Physiology 159: 930-944

Lulai E, Corsini D (1998) Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (Solanum

tuberosumL.) wound-healing. Physiological and Molecular Plant Pathology 53: 209-222

Luque P, Gavara R, Heredia A (1995) A study of the hydration process of isolated cuticular membranes. New Phytologist 129: 283-288

Mamrutha H, Mogili T, Lakshmi KJ, Rama N, Kosma D, Kumar MU, Jenks MA, Nataraja KN (2010) Leaf cuticular wax amount and crystal morphology regulate post-harvest water loss in mulberry (Morus species). Plant Physiology and Biochemistry 48: 690-696

Maréchal Y, Chamel A (1996) Water in a biomembrane by infrared spectrometry. The Journal of Physical Chemistry 100: 8551-8555

Maréchal Y, Chamel A (1997) Interaction configurations of H2O molecules absorbed in isolated plant cuticles by infrared spectrometry. Biospectroscopy 3: 143-153

Martin LB, Romero P, Fich EA, Domozych D, Rose JK (2017) Cuticle Biosynthesis is Developmentally Regulated by Abscisic Acid. Plant Physiology 00387.02017

Matas AJ, Cobb ED, Bartsch JA, Paolillo DJ, Niklas KJ (2004) Biomechanics and anatomy of Lycopersicon esculentum fruit peels and enzyme-treated samples. American Journal of Botany 91: 352-360

Matas AJ, Cobb ED, Paolillo DJ, Niklas KJ (2004) Crack resistance in cherry tomato fruit correlates with cuticular membrane thickness. HortScience 39: 1354-1358

Matas AJ, Cuartero J, Heredia A (2004) Phase transitions in the biopolyester cutin isolated from tomato fruit cuticles. Thermochimica Acta 409: 165-168

Matas AJ, López-Casado G, Cuartero J, Heredia A (2005) Relative humidity and temperature modify the mechanical properties of isolated tomato fruit cuticles. American Journal of Botany 92: 462-468

Matthews MA, Shackel KA (2005) Growth and water transport in fleshy fruit. Vascular Transport in Plants 181-197

Mazurek S, Garroum I, Daraspe J, De Bellis D, Olsson V, Mucciolo A, Butenko MA, Humbel BM, Nawrath C (2017) Connecting the Molecular Structure of Cutin to Ultrastructure and Physical Properties of the Cuticle in Petals of Arabidopsis. Plant Physiology 173: 1146-1163

McFarlane HE, Shin JJ, Bird DA, Samuels AL (2010) Arabidopsis ABCG transporters, which are required for export of diverse cuticular lipids, dimerize in different combinations. The Plant Cell 22: 3066-3075

McFarlane HE, Watanabe Y, Yang W, Huang Y, Ohlrogge J, Samuels AL (2014) Golgi- and trans-Golgi network-mediated vesicle trafficking is required for wax secretion from epidermal cells. Plant Physiology 164: 1250-1260

McKinney D, Bortiatynski J, Carson D, Clifford D, De Leeuw J, Hatcher P (1996) Tetramethylammonium hydroxide (TMAH) thermochemolysis of the aliphatic biopolymer cutan: insights into the chemical structure. Organic Geochemistry 24: 641-650

Moggia C, Graell J, Lara I, Schmeda-Hirschmann G, Thomas-Valdés S, Lobos GA (2016) Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. Scientia Horticulturae 211: 449-457

Mukhtar A, Damerow L, Blanke M (2014) Non-invasive assessment of glossiness and polishing of the wax bloom of European plum. Postharvest Biology and Technology 87: 144-151

Müller C, Riederer M (2005) Plant surface properties in chemical ecology. Journal of Chemical Ecology 31: 2621-2651

Nawrath C (2006) Unraveling the complex network of cuticular structure and function. Current Opinion in Plant Biology 9: 281-287

Neinhuis C, Barthlott W (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. Annals of Botany 79: 667-677

Neto CC (2011) Ursolic acid and other pentacyclic triterpenoids: anticancer activities and occurrence in berries. *In* Berries and Cancer Prevention. Springer, 41-49

Ni Y, Guo Y, Han L, Tang H, Conyers M (2012) Leaf cuticular waxes and physiological parameters in alfalfa leaves as influenced by drought. Photosynthetica 50: 458-466

Niemann S, Burghardt M, Popp C, Riederer M (2013) Aqueous pathways dominate permeation of solutes across Pisum sativum seed coats and mediate solute transport via diffusion and bulk flow of water. Plant, Cell & Environment 36: 1027-1036

Nip M, Tegelaar E, Brinkhuis H, De Leeuw J, Schenck P, Holloway P (1986) Analysis of modern and fossil plant cuticles by Curie point Py-GC and Curie point Py-GC-MS: recognition of a new, highly aliphatic and resistant biopolymer. Organic Geochemistry 10: 769-778

Nobel P (2009) Physicochemical and Environmental Plant Physiology–Academic Press. *In*. Oxford

Oliveira AF, Meirelles ST, Salatino A (2003) Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. Anais da Academia Brasileira de Ciências 75: 431-439

Panikashvili D, Shi JX, Schreiber L, Aharoni A (2011) The Arabidopsis ABCG13 transporter is required for flower cuticle secretion and patterning of the petal epidermis. New Phytologist 190: 113-124

Parsons EP, Popopvsky S, Lohrey GT, Alkalai-Tuvia S, Perzelan Y, Bosland P, Bebeli PJ, Paran I, Fallik E, Jenks MA (2013) Fruit cuticle lipid composition and water loss in a diverse collection of pepper (Capsicum). Physiologia Plantarum 149: 160-174

Parsons EP, Popopvsky S, Lohrey GT, Lü S, Alkalai-Tuvia S, Perzelan Y, Paran I, Fallik E, Jenks MA (2012) Fruit cuticle lipid composition and fruit post-harvest water loss in an advanced backcross generation of pepper (Capsicum sp.). Physiologia Plantarum 146: 15-25

Patumi M, d'Andria R, Marsilio V, Fontanazza G, Morelli G, Lanza B (2002) Olive and olive oil quality after intensive monocone olive growing (Olea europaea L., cv. Kalamata) in different irrigation regimes. Food Chemistry 77: 27-34

Pensec F, Pączkowski C, Grabarczyk M, Woźniak A, Bénard-Gellon MI, Bertsch C, Chong J, Szakiel A (2014) Changes in the triterpenoid content of cuticular

waxes during fruit ripening of eight grape (Vitis vinifera) cultivars grown in the Upper Rhine Valley. Journal of Agricultural and Food Chemistry 62: 7998-8007

Peschel S, Beyer M, Knoche M (2003) Surface characteristics of sweet cherry fruit: stomata-number, distribution, functionality and surface wetting. Scientia Horticulturae 97: 265-278

Peschel S, Franke R, Schreiber L, Knoche M (2007) Composition of the cuticle of developing sweet cherry fruit. Phytochemistry 68: 1017-1025

Petracek PD, Bukovac MJ (1995) Rheological properties of enzymatically isolated tomato fruit cuticle. Plant Physiology 109: 675-679

Pfündel EE, Agati G, Cerovic GZ (2008) Optical properties of plant surfaces. Biology of the plant cuticle 3: 216-239

Phillips DR, Rasbery JM, Bartel B, Matsuda SP (2006) Biosynthetic diversity in plant triterpene cyclization. Current Opinion in Plant Biology 9: 305-314

Pieniazek S (1944) Physical characters of the skin in relation to apple fruit transpiration. Plant Physiology 19: 529

Pighin JA, Zheng H, Balakshin LJ, Goodman IP, Western TL, Jetter R, Kunst L, Samuels AL (2004) Plant cuticular lipid export requires an ABC transporter. Science 306: 702-704

Pollard M, Beisson F, Li Y, Ohlrogge JB (2008) Building lipid barriers: biosynthesis of cutin and suberin. Trends in Plant Science 13: 236-246

Possingham J, Chambers T, Radler F, Grncarevic M (1967) Cuticular transpiration and wax structure and composition of leaves and fruit of Vitis vinifera. Australian Journal of Biological Sciences 20: 1149-1154

Poynter J, Farrimond P, Robinson N, Eglinton G (1989) Aeolian-derived higher plant lipids in the marine sedimentary record: Links with palaeoclimate. *In* Paleoclimatology and paleometeorology: modern and past patterns of global atmospheric transport. Springer, pp 435-462

Premachandra G, Saneoka H, Kanaya M, Ogata S (1991) Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. Journal of Experimental Botany 42: 167-171

Proietti P, Famiani F, Tombesi A (1999) Gas exchange in olive fruit. Photosynthetica 36: 423-432

Radler F (1965) The main constituents of the surface waxes of varieties and species of the genus Vitis. American Journal of Enology and Viticulture 16: 159-167

Rautengarten C, Ebert B, Ouellet M, Nafisi M, Baidoo EE, Benke P, Stranne M, Mukhopadhyay A, Keasling JD, Sakuragi Y (2012) Arabidopsis deficient in cutin ferulate encodes a transferase required for feruloylation of ω-hydroxy fatty acids in cutin polyester. Plant Physiology 158: 654-665

Reynhardt E (1997) The role of hydrogen bonding in the cuticular wax of Hordeum vulgare L. European biophysics journal 26: 195-201

Reynhardt E, Riederer M (1991) Structure and molecular dynamics of the cuticular wax from leaves of Citrus aurantium L. Journal of Physics D: Applied Physics 24: 478

Reynhardt E, Riederer M (1994) Structures and molecular dynamics of plant waxes. European Biophysics Journal 23: 59-70

Riederer M, Müller C (2008) Annual Plant Reviews, Biology of the Plant Cuticle, Vol 23. John Wiley & Sons

Riederer M, Schneider G (1990) The effect of the environment on the permeability and composition of Citrus leaf cuticles. Planta 180: 154-165

Riederer M, Schreiber L (1995) Waxes: the transport barriers of plant cuticles. *In*, Vol 6. The Oily Press: Dundee, Scotland, 131-156

Riederer M, Schreiber L (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. Journal of Experimental Botany 52: 2023-2032

Ríos JC, Robledo F, Schreiber L, Zeisler V, Lang E, Carrasco B, Silva H (2015) Association between the concentration of n-alkanes and tolerance to cracking in commercial varieties of sweet cherry fruits. Scientia Horticulturae 197: 57-65

Ristic Z, Jenks MA (2002) Leaf cuticle and water loss in maize lines differing in dehydration avoidance. Journal of Plant Physiology 159: 645-651

Rogiers SY, Hatfield JM, Jaudzems VG, White RG, Keller M (2004) Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. American Journal of Enology and Viticulture 55: 121-127

Rowland O, Domergue F (2012) Plant fatty acyl reductases: enzymes generating fatty alcohols for protective layers with potential for industrial applications. Plant Science 193: 28-38

Saladié M, Matas AJ, Isaacson T, Jenks MA, Goodwin SM, Niklas KJ, Xiaolin R, Labavitch JM, Shackel KA, Fernie AR (2007) A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiology 144: 1012-1028

Scholz I, Bückins M, Dolge L, Erlinghagen T, Weth A, Hischen F, Mayer J, Hoffmann S, Riederer M, Riedel M (2010) Slippery surfaces of pitcher plants: Nepenthes wax crystals minimize insect attachment via microscopic surface roughness. Journal of Experimental Biology 213: 1115-1125

Schönherr J (1976) Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. Planta 131: 159-164

Schönherr J, Riederer M (1986) Plant cuticles sorb lipophilic compounds during enzymatic isolation. Plant, Cell & Environment 9: 459-466

Schönherr J, Riederer M (1989) Foliar penetration and accumulation of organic chemicals in plant cuticles. *In* Reviews of Environmental Contamination and Toxicology. Springer, 1-70

Schouten S, Moerkerken P, Gelin F, Baas M, de Leeuw JW, Damsté JSS (1998) Structural characterization of aliphatic, non-hydrolyzable biopolymers in freshwater algae and a leaf cuticle using ruthenium tetroxide degradation. Phytochemistry 49: 987-993

Schreiber L (2001) Effect of temperature on cuticular transpiration of isolated cuticular membranes and leaf discs. Journal of Experimental Botany 52: 1893-1900

Schreiber L, Riederer M (1996) Ecophysiology of cuticular transpiration: comparative investigation of cuticular water permeability of plant species from different habitats. Oecologia 107: 426-432

Schreiber L, Schönherr J (1990) Phase transitions and thermal expansion coefficients of plant cuticles. Planta 182: 186-193

Schuster A-C (2016) Chemical and functional analyses of the plant cuticle as leaf transpiration barrier. Dissertation, Würzburg, Universität Würzburg, 2016

Schuster A-C, Burghardt M, Alfarhan A, Bueno A, Hedrich R, Leide J, Thomas J, Riederer M (2016) Effectiveness of cuticular transpiration barriers in a desert plant at controlling water loss at high temperatures. AoB Plants: plw027

Semerdjieva S, Phoenix G, Hares D, Gwynn-Jones D, Callaghan T, Sheffield E (2003) Surface morphology, leaf and cuticle thickness of four dwarf shrubs from a sub-Arctic heath following long-term exposure to enhanced levels of UV-B. Physiologia Plantarum 117: 289-294

Seo PJ, Lee SB, Suh MC, Park M-J, Go YS, Park C-M (2011) The MYB96 transcription factor regulates cuticular wax biosynthesis under drought conditions in Arabidopsis. The Plant Cell 23: 1138-1152

Seo PJ, Park C-M (2011) Cuticular wax biosynthesis as a way of inducing drought resistance. Plant Signaling & Behavior 6: 1043-1045

Serrano M, Coluccia F, Martha T, LHardion F, Métraux J (2015) The cuticle and plant defense to pathogens. Plant Cell Wall in Pathogenesis, Parasitism and Symbiosis 5: 6-13

Shepherd T, Wynne Griffiths D (2006) The effects of stress on plant cuticular waxes. New Phytologist 171: 469-499

Simpson JP, Ohlrogge JB (2016) A novel pathway for triacylglycerol biosynthesis is responsible for the accumulation of massive quantities of glycerolipids in the surface wax of bayberry (Myrica pensylvanica) fruit. The Plant Cell 28: 248-264

Smirnova A, Leide J, Riederer M (2013) Deficiency in a very-long-chain fatty acid β-ketoacyl-coenzyme A synthase of tomato impairs microgametogenesis and causes floral organ fusion. Plant Physiology 161: 196-209

Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochemical & Photobiological Sciences 2: 861-866

Stark RE, Yan B, Stanley-Fernandez SM, Chen ZJ, Garbow JR (2008) NMR characterization of hydration and thermal stress in tomato fruit cuticles. Phytochemistry 69: 2689-2695

Stenglein SA, Arambarri AM, Sevillano MdCM, Balatti PA (2005) Leaf epidermal characters related with plant's passive resistance to pathogens vary among accessions of wild beans Phaseolus vulgaris var. aborigineus (Leguminosae–Phaseoleae). Flora-Morphology, Distribution, Functional Ecology of Plants 200: 285-295

Sterling C (1953) Developmental anatomy of the fruit of Prunus domestica L. Bulletin of the Torrey Botanical Club: 457-477

Szafranek BM, Synak EE (2006) Cuticular waxes from potato (Solanum tuberosum) leaves. Phytochemistry 67: 80-90

Szakiel A, Pączkowski C, Huttunen S (2012) Triterpenoid content of berries and leaves of bilberry Vaccinium myrtillus from Finland and Poland. Journal of Agricultural and Food Chemistry 60: 11839-11849

Szakiel A, Pączkowski C, Koivuniemi H, Huttunen S (2012) Comparison of the triterpenoid content of berries and leaves of lingonberry Vaccinium vitis-idaea from Finland and Poland. Journal of Agricultural and Food Chemistry 60: 4994-5002

Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn A (2014) Triterpene biosynthesis in plants. Annual Review of Plant Biology 65: 225-257

Tschan GF, Denk T (2012) Trichome types, foliar indumentum and epicuticular wax in the Mediterranean gall oaks, Quercus subsection Galliferae (Fagaceae): implications for taxonomy, ecology and evolution. Botanical Journal of the Linnean Society 169: 611-644

Verardo G, Pagani E, Geatti P, Martinuzzi P (2003) A thorough study of the surface wax of apple fruits. Analytical and Bioanalytical Chemistry 376: 659-667

Veraverbeke EA, Verboven P, Van Oostveldt P, Nicolaï BM (2003) Prediction of moisture loss across the cuticle of apple (Malus sylvestris subsp. Mitis (Wallr.)) during storage: part 1. Model development and determination of diffusion coefficients. Postharvest Biology and Technology 30: 75-88

Vichi S, Cortés-Francisco N, Caixach J, Barrios G, Mateu J, Ninot A, Romero A (2016) Epicuticular wax in developing olives (Olea europaea) is highly dependent on cultivar and fruit ripeness. Journal of Agricultural and Food Chemistry 64: 5985-5994.

Vichi S, Cortés-Francisco N, Romero A, Caixach J (2015) Direct chemical profiling of olive (Olea europaea) fruit epicuticular waxes by direct electrospray-ultrahigh resolution mass spectrometry. Journal of Mass Spectrometry 50: 558-566

Villena JF, Domínguez E, Stewart D, Heredia A (1999) Characterization and biosynthesis of non-degradable polymers in plant cuticles. Planta 208: 181-187

Vogg G, Fischer S, Leide J, Emmanuel E, Jetter R, Levy AA, Riederer M (2004) Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid β-ketoacyl-CoA synthase. Journal of Experimental Botany 55: 1401-1410

Wang J, Hao H, Liu R, Ma Q, Xu J, Chen F, Cheng Y, Deng X (2014) Comparative analysis of surface wax in mature fruits between Satsuma mandarin (Citrus unshiu) and ‘Newhall’navel orange (Citrus sinensis) from the perspective of crystal morphology, chemical composition and key gene expression. Food Chemistry 153: 177-185

Wang M, Zhang W, Hou J (2015) Is average chain length of plant lipids a potential proxy for vegetation, environment and climate changes. Biogeosciences Discuss 12: 5477-5501

Wattendorff J, Holloway P (1982) Studies on the ultrastructure and histochemistry of plant cuticles: isolated cuticular membrane preparations of Agave americana L. and the effects of various extraction procedures. Annals of Botany 49: 769-804

Wellesen K, Durst F, Pinot F, Benveniste I, Nettesheim K, Wisman E, Steiner-Lange S, Saedler H, Yephremov A (2001) Functional analysis of the LACERATA gene of Arabidopsis provides evidence for different roles of fatty acid ω-hydroxylation in development. Proceedings of the National Academy of Sciences 98: 9694-9699

Wellman CH, Osterloff PL, Mohiuddin U (2003) Fragments of the earliest land plants. Nature 425: 282-285

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology 5: 218-223

Wollrab V (1969) Secondary alcohols and paraffins in the plant waxes of the family Rosaceae. Phytochemistry 8: 623-627

Xiao F, Goodwin SM, Xiao Y, Sun Z, Baker D, Tang X, Jenks MA, Zhou JM (2004) Arabidopsis CYP86A2 represses Pseudomonas syringae type III genes and is required for cuticle development. The EMBO Journal 23: 2903-2913

Yamamura H, Naito R (1983) The surface wax of several grapes in Japan. Journal of the Japanese Society for Horticultural Science 52: 266-272

Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB (2012) A land-plant-specific glycerol-3-phosphate acyltransferase family in Arabidopsis: substrate specificity, sn-2 preference, and evolution. Plant Physiology 160: 638-652

Yeats TH, Huang W, Chatterjee S, Viart HMF, Clausen MH, Stark RE, Rose JK (2014) Tomato Cutin Deficient 1 (CD1) and putative orthologs comprise an ancient family of cutin synthase-like (CUS) proteins that are conserved among land plants. The Plant Journal 77: 667-675

Yeats TH, Martin LB, Viart HM, Isaacson T, He Y, Zhao L, Matas AJ, Buda GJ, Domozych DS, Clausen MH (2012) The identification of cutin synthase: formation of the plant polyester cutin. Nature Chemical Biology 8: 609-611

Yeats TH, Rose JK (2013) The formation and function of plant cuticles. Plant Physiology 163: 5-20

Zeisler V, Schreiber L (2016) Epicuticular wax on cherry laurel (Prunus laurocerasus) leaves does not constitute the cuticular transpiration barrier. Planta 243: 65-81

Zhang Y, Jayaprakasam B, Seeram NP, Olson LK, DeWitt D, Nair MG (2004) Insulin secretion and cyclooxygenase enzyme inhibition by cabernet sauvignon grape skin compounds. Journal of Agricultural and Food Chemistry 52: 228-233

Zhu X, Xiong L (2013) Putative megaenzyme DWA1 plays essential roles in drought resistance by regulating stress-induced wax deposition in rice. Proceedings of the National Academy of Sciences 110: 17790-17795

Annex

Table 25. The plant species, growing places, the sample types, and fruit and leaf sample types used for cuticular chemical analysis.

Family	Species	Year	Fruit type	Leaf type	Sampling place	Fruit	Leaf
Oleaceae							
	Olea europaea L. cv. ‘Arbequina’	November 2014	drupe	Evergreen	Field, Lleida, Spain	fresh	CMs
	Olea europaea L. cv. ‘Arbequina’	November 2015	drupe	Evergreen	Field, Lleida, Spain	fresh	fresh
	Olea europaea L. cv. ‘Arbequina’	December 2016	drupe	Evergreen	Field, Constanti, Spain	fresh	fresh
	Olea europaea subsp. *europaea* var. *sylvestris*	December 2015	drupe	Evergreen	Botanical Garden, Würzburg	fresh	fresh
	Olea europaea subsp. *europaea* var. *sylvestris*	December 2016	drupe	Evergreen	Botanical Garden, Würzburg	fresh	fresh
	Ligustrum vulgare L.	March 2016	drupe	Semi-evergreen	Botanical Garden, Würzburg	CMs	fresh
Oxalidaceae							
	Averrhoa carambola L.	July 2015	berry	Evergreen	Botanical Garden, Würzburg	CMs	fresh
Rubiaceae							
	Coffea arabica L.	April 2016	drupe	Evergreen	Botanical Garden, Würzburg	fresh	fresh
Rosaceae							
	Crataegus pedicellata Sarg.	September 2015	pome	Deciduous	Botanical Garden, Würzburg	CMs	CMs
	Malus domestica L. cv.'Topaz'	September 2015	pome	Deciduous	Botanical Garden, Würzburg	CMs	CMs
	Prunus avium L.	June 2015	drupe	Deciduous	Field, Würzburg	CMs	CMs
	Prunus cerasifera Ehrh.	August 2015	drupe	Deciduous	Field, Würzburg	CMs	CMs
	Prunus domestica L. subsp. *syriaca* Janich.	August 2015	drupe	Deciduous	Field, Würzburg	CMs	CMs
	Prunus domestica subsp. insititia (L.)	August 2016	drupe	Deciduous	Field, Würzburg	CMs	CMs
	Prunus persica L.	July 2015	drupe	Deciduous	Botanical Garden, Würzburg	CMs	CMs
Vitaceae							
	Vitis vinifera L. cv. 'Nelly'	September 2015	berry	Deciduous	Botanical Garden, Würzburg	CMs	CMs
	Vitis vinifera L. cv. 'Silvana'	September 2015	berry	Deciduous	Botanical Garden, Würzburg	CMs	CMs
Cornaceae							
	Cornus officinalis Siebold & Zucc.	August 2015	berry	Deciduous	Botanical Garden, Würzburg	CMs	CMs
Moraceae							
	Ficus carica L.	August 2015	drupe	Deciduous	Botanical Garden, Würzburg	CMs	CMs
Solanaceae							
	Capsicum annuum L. cv. 'Kalocsai'	September 2016	berry	Annual/deciduous	Botanical Garden, Würzburg	CMs	CMs

Table 26. Main aliphatic and cyclic components of cuticular waxes detected in fruit and leaf of different species in the present study.

Family	Species	Aliphatics		Cyclics	
		Fruit	Leaf	Fruit	Leaf
Oleaceae					
	Olea europaea L. cv. 'Arbequina'	Hexacosanoic acid (C_{26}) Octacosanoic acid (C_{28}) Hexacosanol (C_{26})	*n*-Hentriacotane (C_{31}) *n*-Tritriacotane (C_{33})	Oleanolic acid	Oleanolic acid
	Olea europaea subsp. *europaea* var. *sylvestris*	Hexacosanoic acid (C_{26}) Octacosanoic acid (C_{28}) Hexacosanol (C_{26})	*n*-Hentriacotane (C_{31}) *n*-Tritriacotane (C_{33})	Oleanolic acid	Oleanolic acid
	Ligustrum vulgare L.	*n*-Hentriacotane (C_{31})	*n*-Hentriacotane (C_{31}) *n*-Tritriacotane (C_{33})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
Oxalidaceae					
	Averrhoa carambola L.	*n*-Tricosene (C_{23}) *n*-Tricosane (C_{23}) *n*-Pentacosene (C_{25}) *n*-Pentacosane (C_{25})	*n*-Hentriacotane (C_{31})	ß-sitosterol	ß-sitosterol
Rubiaceae					
	Coffea arabica L.	Triacotanol (C_{30}) Dotriacotanol (C_{32})	Triacotanol (C_{30}) Dotriacotanol (C_{33})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
Rosaceae					
	Crataegus pedicellata Sarg.	*n*-Nonacosane (C_{29}) Nonacosan-10-ol (C_{29})	*n*-Nonacosane (C_{29}) *n*-Hentriacotane (C_{31})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
	Malus domestica L. cv.'Topaz'	*n*-Nonacosane (C_{29}) Nonacosan-10-ol (C_{29})	*n*-Hentriacotane (C_{31})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
	Prunus avium L.	*n*-Nonacosane (C_{29})	*n*-Nonacosane (C_{29}) Triacosanoic acid (C_{30})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
	Prunus cerasifera Ehrh.	*n*-Nonacosane (C_{29})	Hentriacotane (C_{31})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
	Prunus domestica L. subsp. *Syriaca* Janich.	*n*-Nonacosane (C_{29})	Hentriacotane (C_{31})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
	Prunus domestica subsp. *Insititia* (L.)	*n*-Nonacosane (C_{29}) Nonacosan-10-ol (C_{29}) Hexacosanol (C_{26})	Octacosanol (C_{28}) *n*-Nonacosane (C_{29})	Oleanolic acid	Ursolic acid Oleanolic acid
	Prunus persica L.	*n*-Nonacosane (C_{29})	*n*-Hentriacotane (C_{31})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid

Table 26. continued

Vitaceae					
	Vitis vinifera L. cv. 'Nelly'	Hexacosanoic acid (C_{26}) Octacosanoic acid (C_{28}) Triacotanoic acid (C_{30})	Octacosanol (C_{28})	Oleanolic acid	
	Vitis vinifera L. cv. 'Silvana'	Hexacosanol (C_{26}) Octacosanol (C_{28})	Octacosanol (C_{28})	Oleanolic acid	
Solanaceae					
	Capsicum annuum L. cv. 'Kalocsai'	*n*-nonacosane (C_{29})	n-Hentriacotane (C_{31}) n-Tritriacotane (C_{33})	β/α-amyrin, lupeol	β-amyrin
Cornaceae					
	Cornus officinalis Siebold & Zucc.	*n*-Nonacosane (C_{29})	Octacosanol (C_{28}) Dotriacotanol (C_{32})	Ursolic acid Oleanolic acid	Ursolic acid Oleanolic acid
Moraceae					
	Ficus carica L.	Teracosanoic acid (C_{24}) Octacosanoic acid (C_{28})	*n*-Nonacosane (C_{29}) *n*-Hentriacotane (C_{31})	β/α-amyrin, Lupeol	Lupeol

Table 27. Correlation coefficients between amount of total wax, aliphatics, cyclics as well as their main compositions and the nature logarithm water permeabilities for fruit and leaf of different species.

	lnP (Fruit & Leaf)			*lnP* (Fruit)			*lnP* (Leaf)		
	r	r^2	*P*	*r*	r^2	*P*	*r*	r^2	*P*
Total wax	0.206	0.042	0.184	- 0.342	0.117	0.111	- 0.328	0.108	0.155
Total aliphatics	0.472	0.223	< 0.01	- 0.331	0.110	0.121	0.167	0.028	0.476
Total cyclics	0.084	0.007	0.589	- 0.074	0.005	0.733	- 0.402	0.162	0.077
Aliphatics $Cyclics^{-1}$	0.077	0.006	0.622	- 0.232	0.054	0.282	- 0.182	0.033	0.437
ACL	**- 0.651**	**0.424**	**< 0.001**	- 0.061	0.004	0.778	0.093	0.009	0.691
ΔACL ACL^{-1}	0.232	0.054	0.134	- 0.334	0.112	0.117	- 0.090	0.008	0.700
ACL[a]	**- 0.647**	**0.419**	**< 0.001**	- 0.130	0.017	0.555	0.245	0.060	0.450
ACL[b]	**- 0.751**	**0.564**	**< 0.001**	- 0.461	0.213	0.061	- 0.046	0.002	0.868
Thickness of wax layer [c]	0.279	0.078	0.073	- 0.342	0.117	0.111	- 0.263	0.069	0.271
Thickness of cuticle [d]	0.493	0.243	0.008	- 0.270	0.073	0.288	0.245	0.060	0.450
Main components									
Fatty acids	**0.582**	**0.339**	**< 0.001**	0.005	0.000	0.980	0.059	0.003	0.802
Primary alcohols	0.430	0.185	< 0.01	0.021	0.000	0.924	0.098	0.010	0.676
n-Alkanes	- 0.082	0.007	0.589	- 0.431	0.186	< 0.05	- 0.290	0.084	0.210
Aldehydes	**0.607**	**0.368**	**< 0.001**	0.093	0.009	0.699	**0.600**	**0.360**	**< 0.05**
Alkyl esters	0.203	0.041	0.271	0.140	0.020	0.560	- 0.441	0.194	0.143
n-Nonacosane (C_{29})	0.022	0.000	0.886	- 0.128	0.016	0.560	- 0.016	0.000	0.942
Oleanolic acid	- 0.013	0.000	0.939	- 0.156	0.024	0.510	- 0.324	0.105	0.215
Ursolic acid	0.039	0.002	0.850	- 0.345	0.119	0.283	0.213	0.045	0.355

[a] ACL calculated excluded Alkyl esters;

[b] ACL calculated excluded species that sotama were observed on fruit surfaces;

[c] the thickness of wax layer of fruits and leaves were calculated according to the total amount of wax;

[d] the thickness of cuticle was calculated according to the reported density of cuticles and the dry weight of CMs.

Table 28. The cuticular transpiration via adaxial leaf surfaces or minimum conductance of intact leaves obtained by leaf drying curve. Samples from three soecies were performed. Data were given as mean values ± SD (x 10^{-5} m s^{-1}, n = 9-17).

Species	Adaxial leaf	Intact leaf
Olea europaea L. cv. ‘Arbequina’	1.88 ± 1.24	1.75 ± 0.46
Averrhoa carambola L.	2.73 ± 0.57	3.00 ± 0.65
Prunus domestica subsp. *insititia* (L.)	2.42 ± 1.14	2.73 ± 0.64

Table 29. Permeances for via olive fruit (black stage) and leaf adaxial surface (*Olea europaea* L. sub. *europaea* var. *sylvestris*) under 25 °C and 50 °C, respectively. Data were given as mean values ± standard deviation (x 10^{-5} m s^{-1}, n = 12 biological replicates).

T (°C)	Black fruit	Adaxial leaf
25	9.89 ± 1.26	2.25 ± 0.47
50	9.28 ± 0.98	3.23 ± 0.78

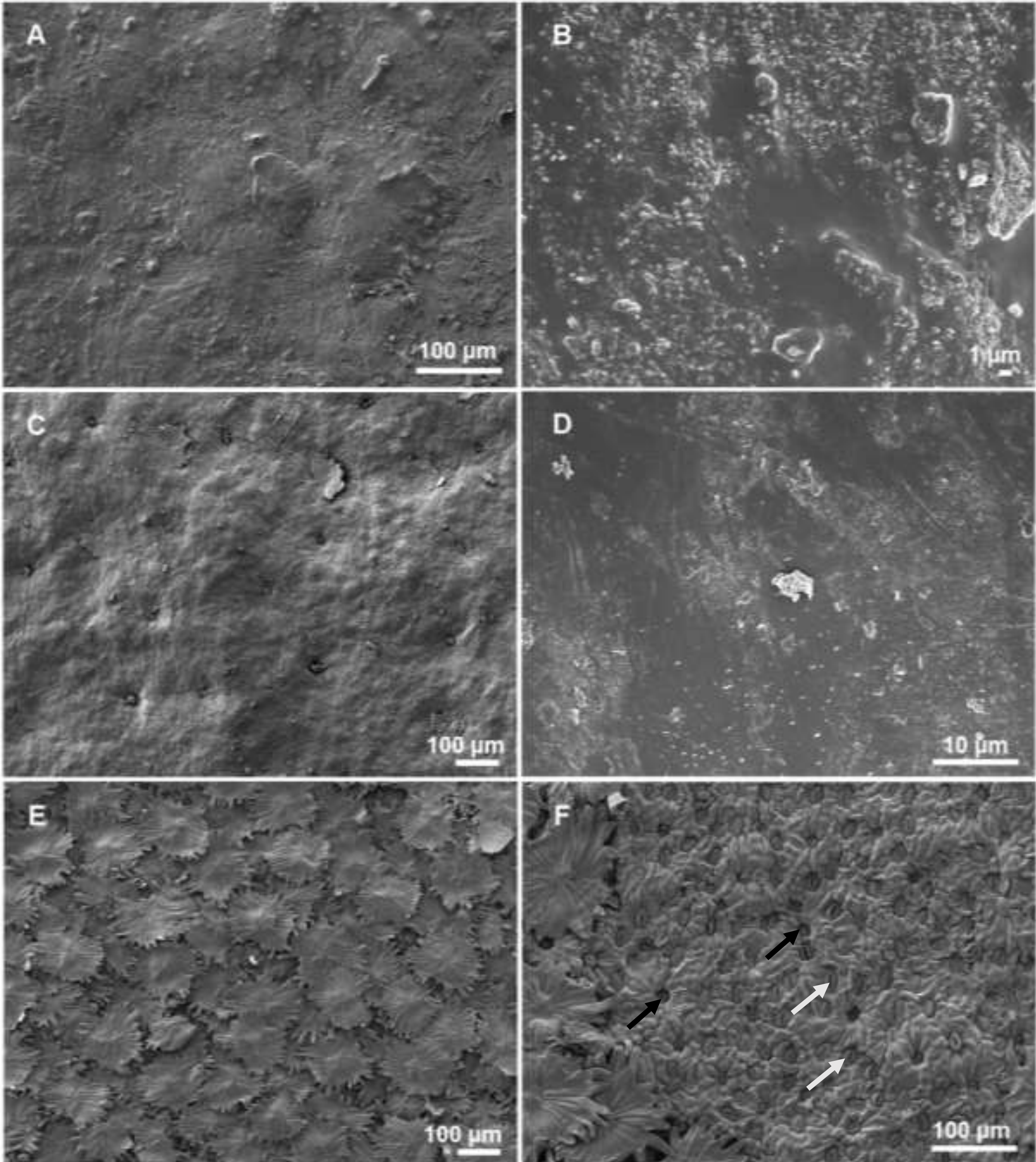

Figure 36. The native fruit, ad- and abaxial leaf surfaces of *Olea europaea* L. cv. 'Arbequina'. The fruit (A) and adaxial leaf (C) surfaces were covered by scarce incompletely degradation of scale-like peltate (non-glandular) trichomes with stalks (black arrows). (B) The epicuticular wax crystals were shown as granules (Barthlott et al., 1998). (D) No obvious epicuticular wax crystals were observed on adaxial leaf surfaces. (E) The abaxial leaf surfaces were covered by dense of scale-like peltate trichomes (Levizou et al., 2005). (F) Stomata (gray arrows) occurred below the trichomes.

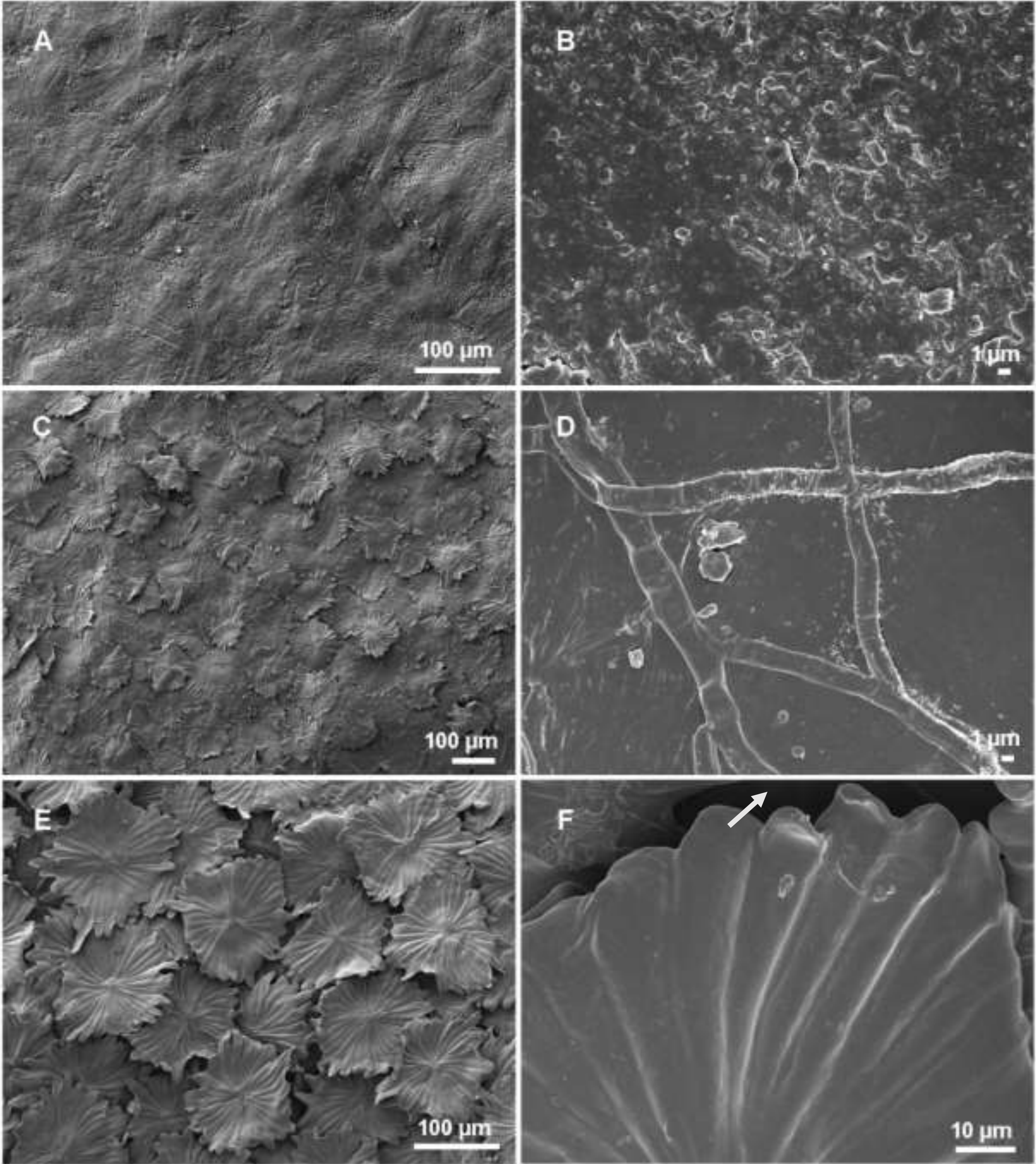

Figure 37. The native fruit, ad- and abaxial leaf surfaces of *Olea europaea* subsp. *europaea* var. *sylvestris*. (A, B) The epicuticular wax crystals were shown as granules on the fruit surfaces. (C) The adaxial leaf surfaces were covered by scarce incompletely degradation of scale-like peltate trichomes. (D) No obvious epicuticular wax crystals was observed. (E) The abaxial leaf surface was covered by dense of scale-like peltate trichomes (Levizou et al., 2005). (F) Stomata (gray arrows) occurred below the trichomes of abaxial leaf surfaces.

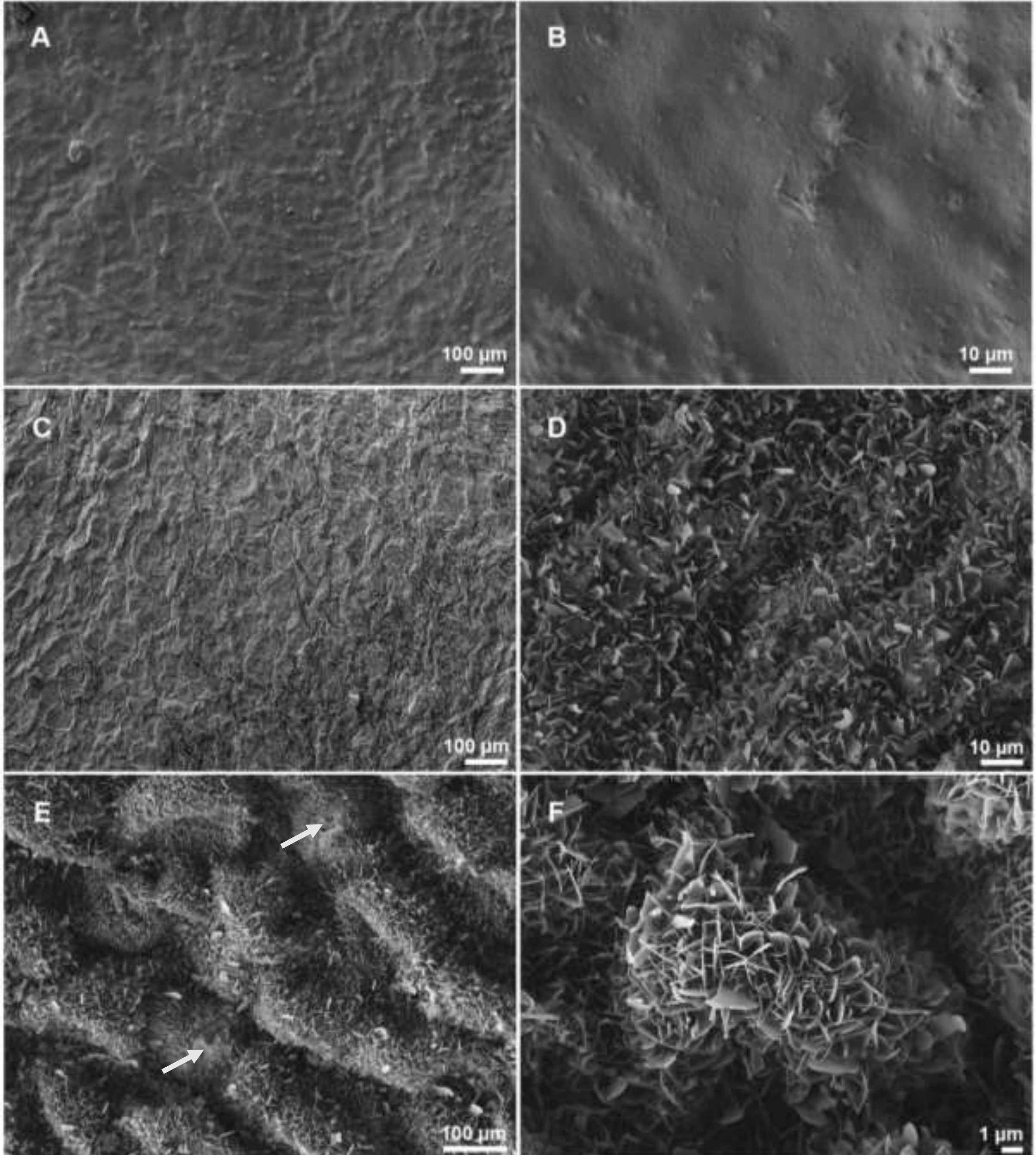

Figure 38. The native fruit, ad- and abaxial leaf surfaces of *Averrhoa carambola* L. (A, B) Fruit surface was covered by wax film. The adaxial (C, D) and abaxial leaf (F) surfaces were covered by plates and platelets type epicuticular wax crystals. (E) Stomata (gray arrows) occurred on abaxial leaf surface. The guard cell was around wax film.

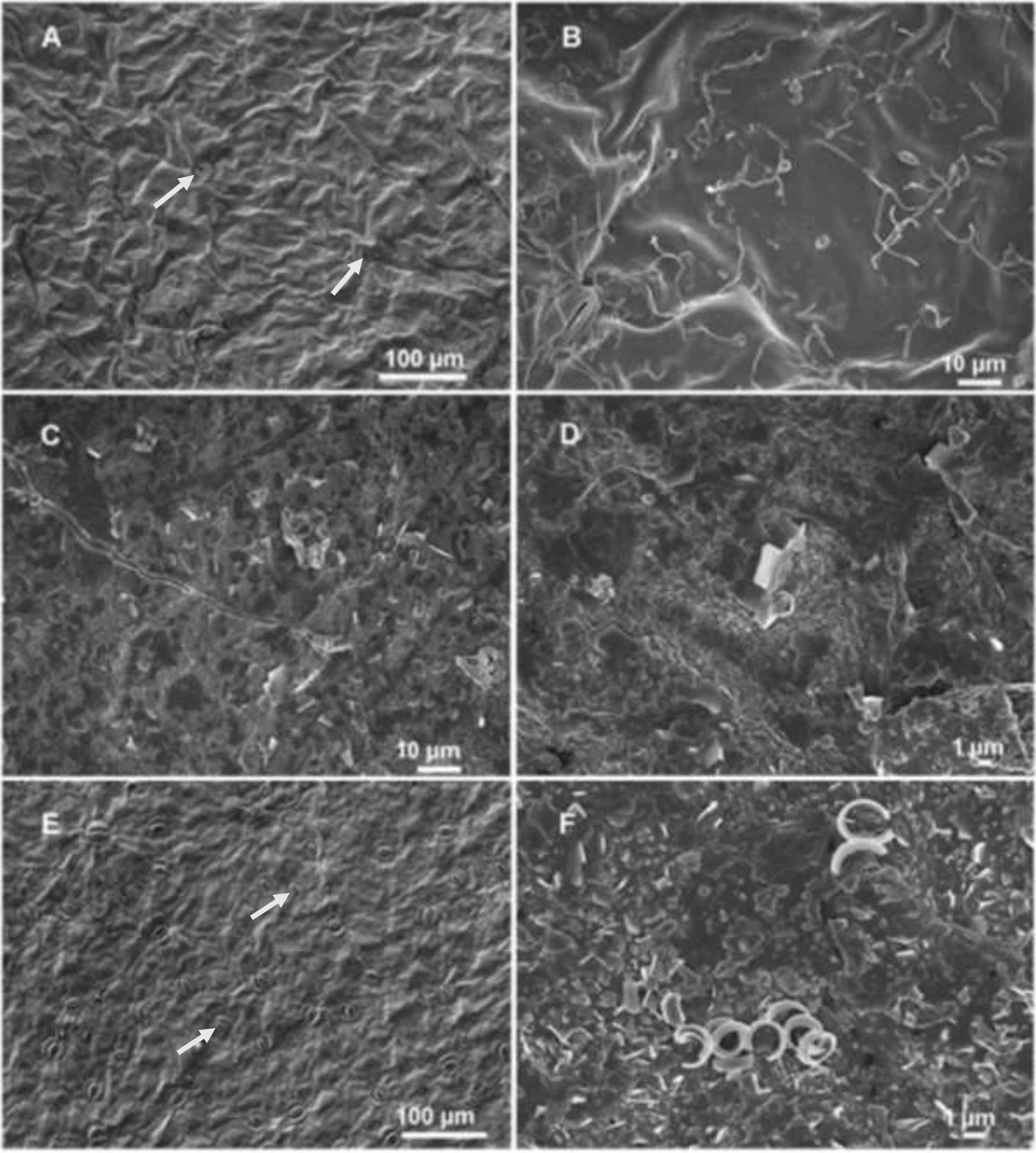

Figure 39. The native fruit, ad- and abaxial leaf surfaces of *Coffea arabica* L. (A, B) Fruit surface was covered by wax film. The adaxial (C, D) and abaxial leaf (F) epicuticular wax crystals were shown as plates, platelets, and granules types. Stomata (gray arrows) occurred on fruit (A) and abaxial leaf (E) surfaces.

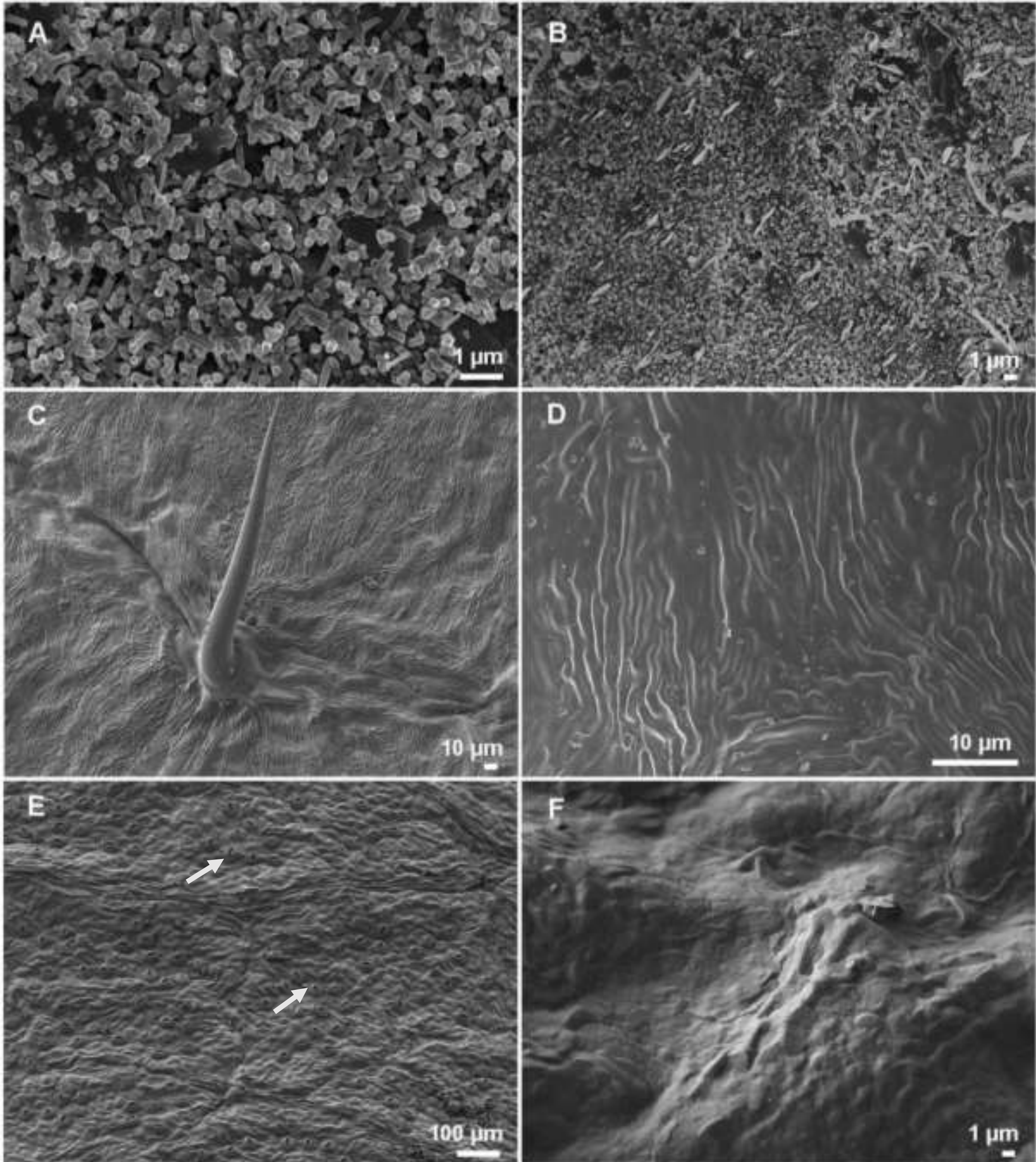

Figure 40. The native fruit, ad- and abaxial leaf surfaces of *Crataegus pedicellata* Sarg. (A, B) The hawthorn fruit surfaces were covered by variety of epicuticular wax crystals, e.g. transversely ridged ribbons, plates and platelets, and clusters of hollow tubules, which were constituted by 10-nonacoanol (Jeffree, 2006). (C) Scarce foliar acicular trichomes occurred on the adaxial leaf surfaces (Tschan and Denk, 2012). (D) A wax film covered on adaxial leaf surface. (E) Stomata (gray arrows) occurred on abaxial leaf surfaces. (F) The epicuticular wax exhibited as irregularly granulated features.

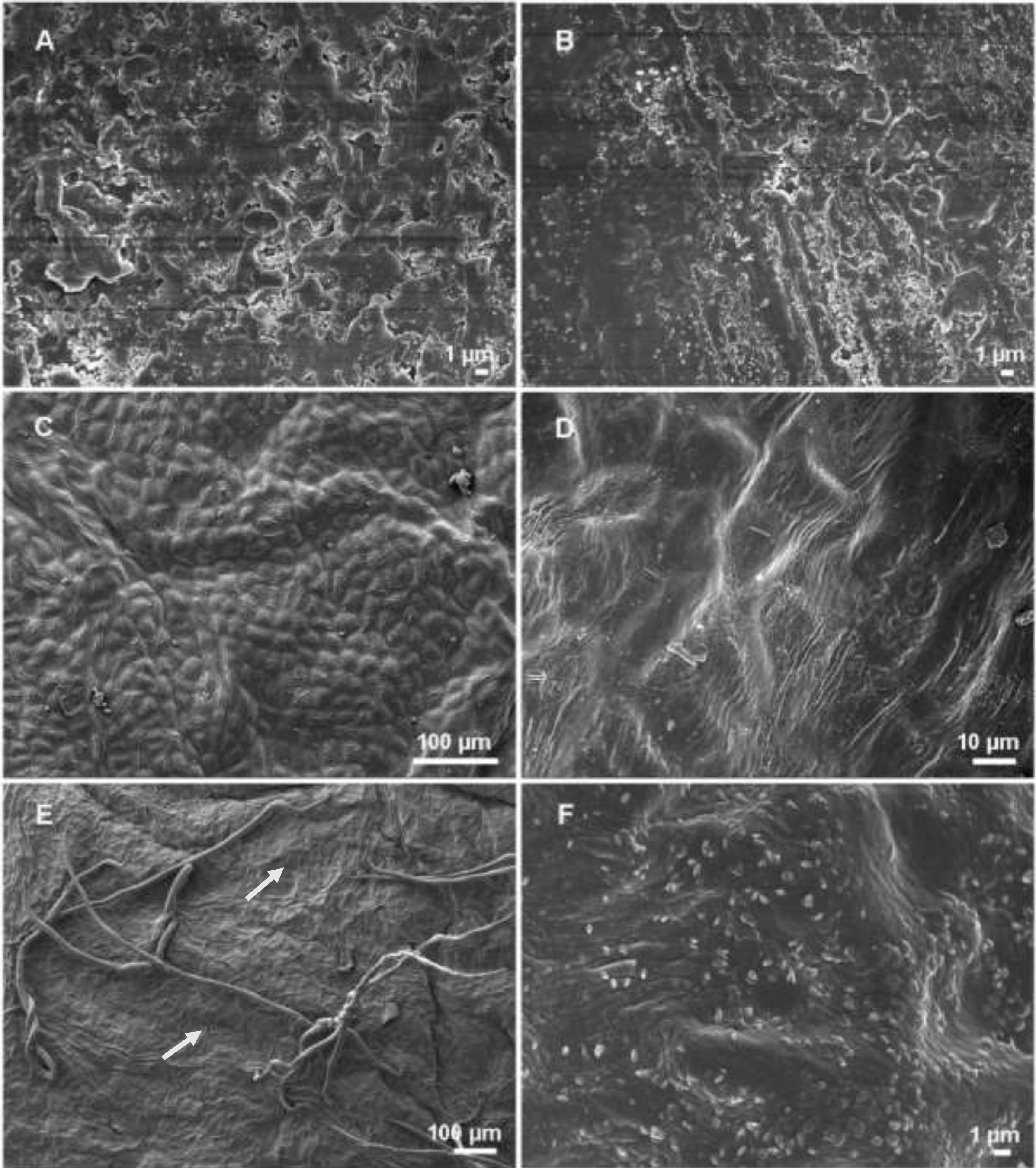

Figure 41. The native fruit, ad- and abaxial leaf surfaces of *Malus domestica* L. cv. 'Topaz'. (A, B) The topaz fruit epicuticular wax was constituted by a syntopism plates and platelets (Al Bitar et al., 2014). (C, D) No obvious epicuticular wax crystals but irregularly granulated structure was observed on adaxial leaf surfaces. (E) The foliar fasciculate (thread- and spiral-shaped) trichomes on abaxial leaf surfaces was observed (Al Bitar et al., 2012; Tschan and Denk, 2012). Stomata (gray arrows) occurred on abaxial leaf surfaces. (F) The epicuticular wax crystals were granules on abaxial leaf surfaces (Barthlott et al., 1998).

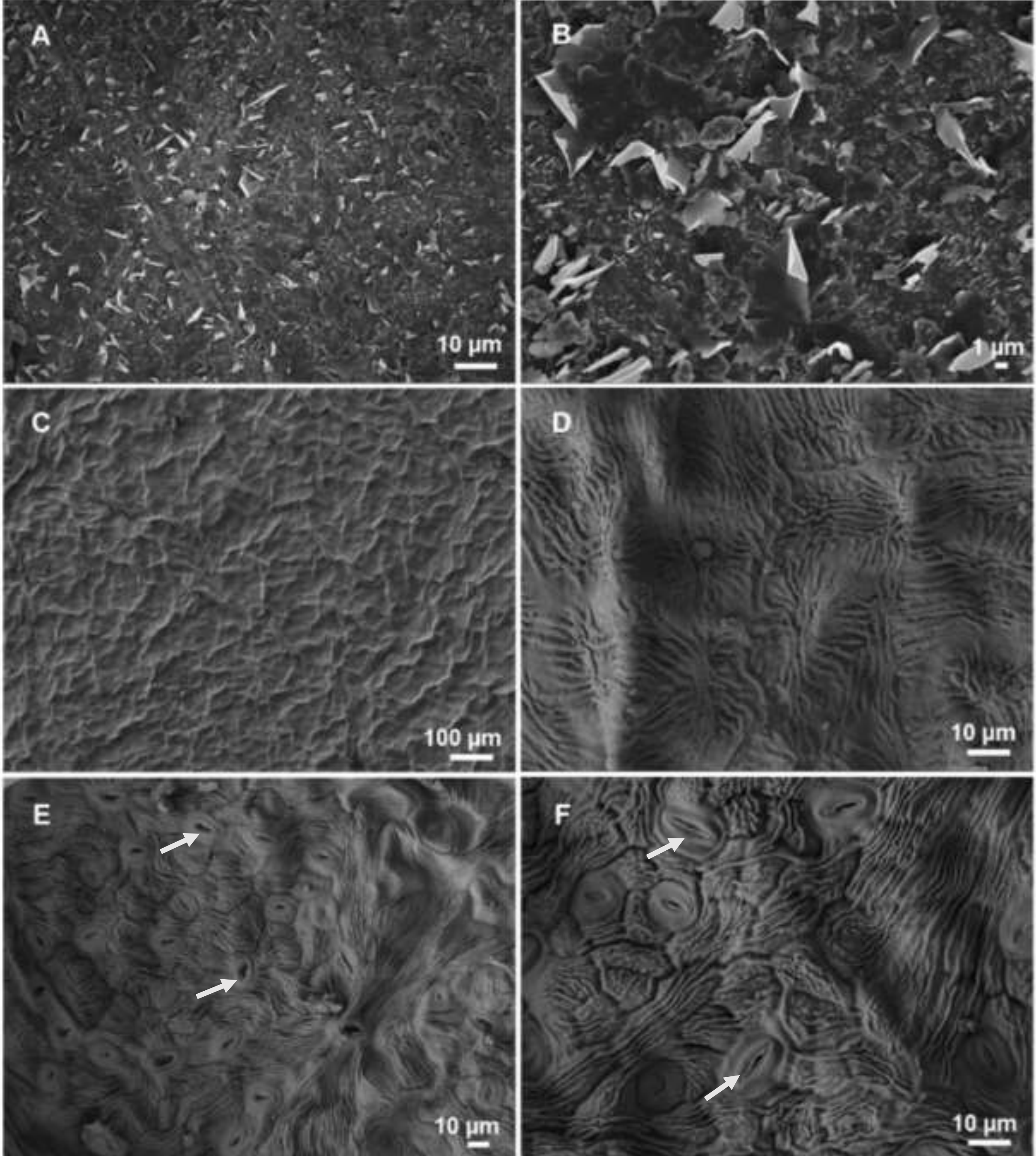

Figure 42. The native fruit, adaxial, and abaxial leaf surfaces of *Prunus cerasifera* Ehrh. (A, B) Cherry plum fruit epicuticular wax crystals were non-entire or entire platelets and membranous platelets (Jeffree, 2006). (C, D) The adaxial leaf surface was covered by smooth wax film. (E) Stomata (gray arrows) occurred on the leaf abaxial surfaces. (F) A wax film covered on abaxial leaf surface.

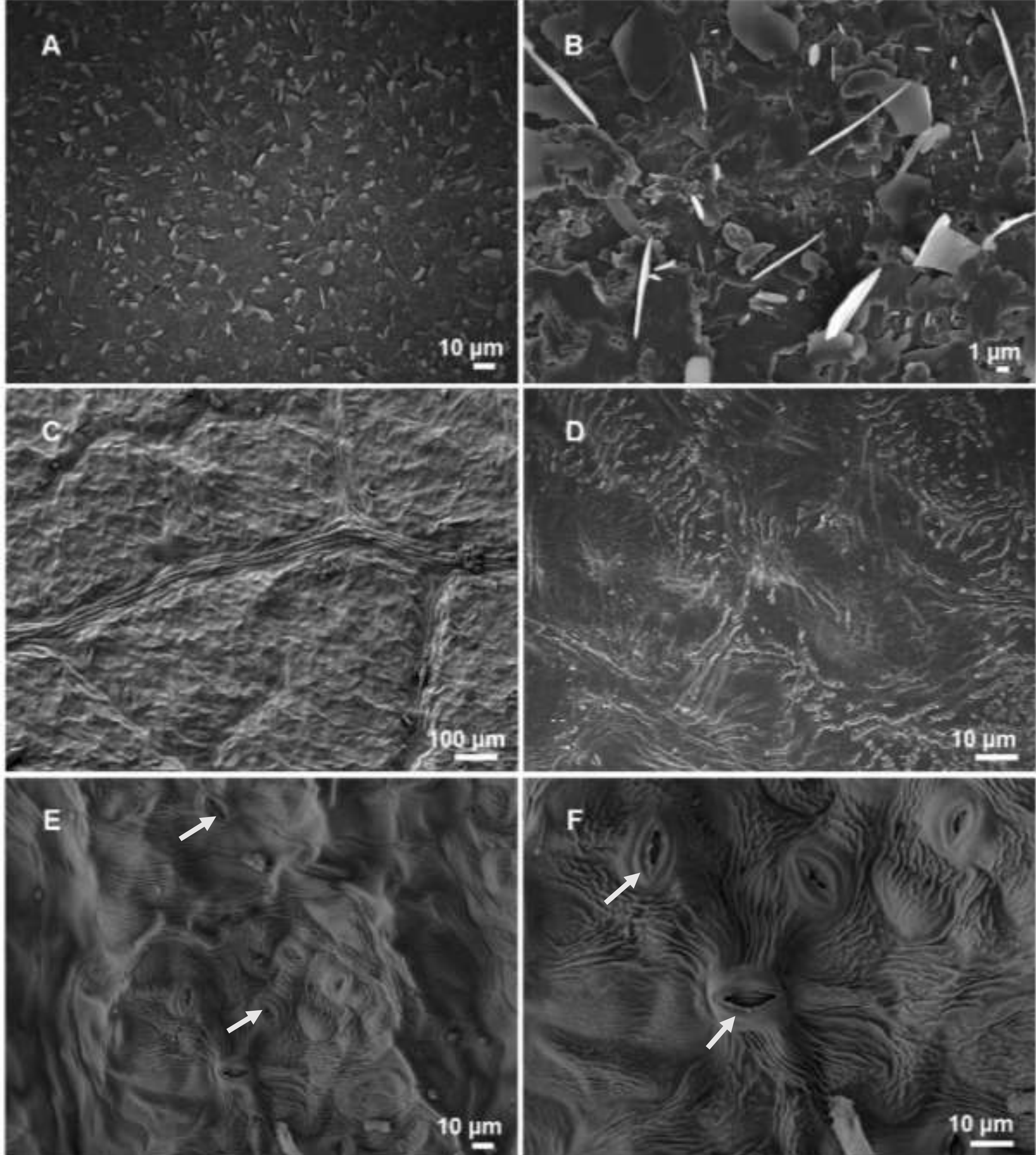

Figure 43. The native fruit, ad- and abaxial leaf surfaces of *Prunus domestica* L. subsp. S*yriaca* Janich. (A, B) similar as on cherry plum fruit surface, mirabelle plum fruit epicuticular wax crystals were plates, non-entire or entire platelets, and membranous platelets (Jeffree, 2006). (C, D) Very small granule-type epicuticular wax crystals occurred on adaxial leaf surfaces. (E) Stomata (gray arrows) occurred on the leaf abaxial leaf surfaces. (F) A wax film covered on abaxial leaf surface.

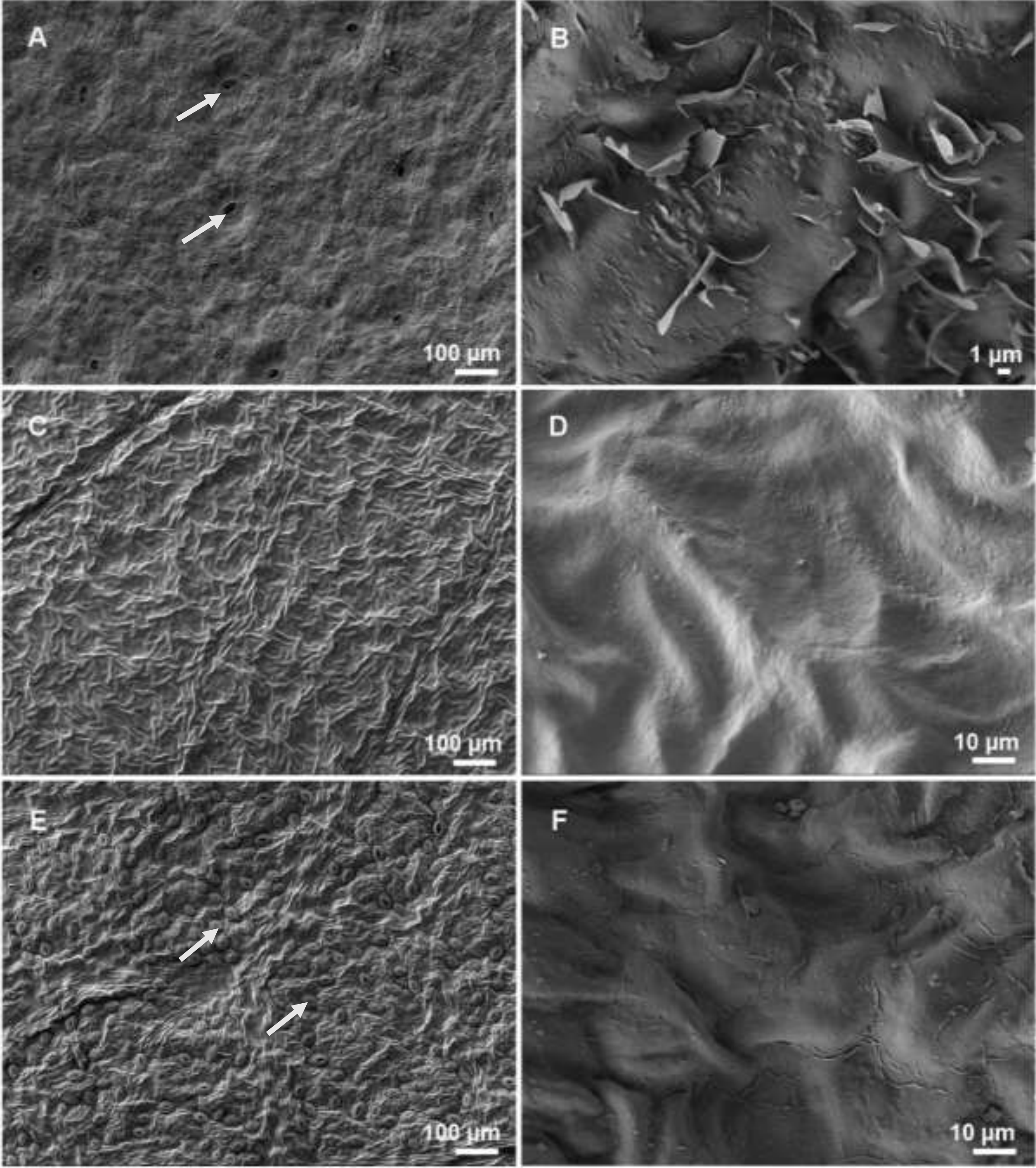

Figure 44. The native fruit, ad- and abaxial leaf surfaces of *Prunus persica* L. (A, B) Fruit epicuticular wax crystals were constituted by simple plate-type wax. The adaxial (C, D) and abaxial leaf (F) surfaces were covered by smooth wax film. Stomata (gray arrows) occurred on both fruit (A) and abaxial leaf (E) surfaces.

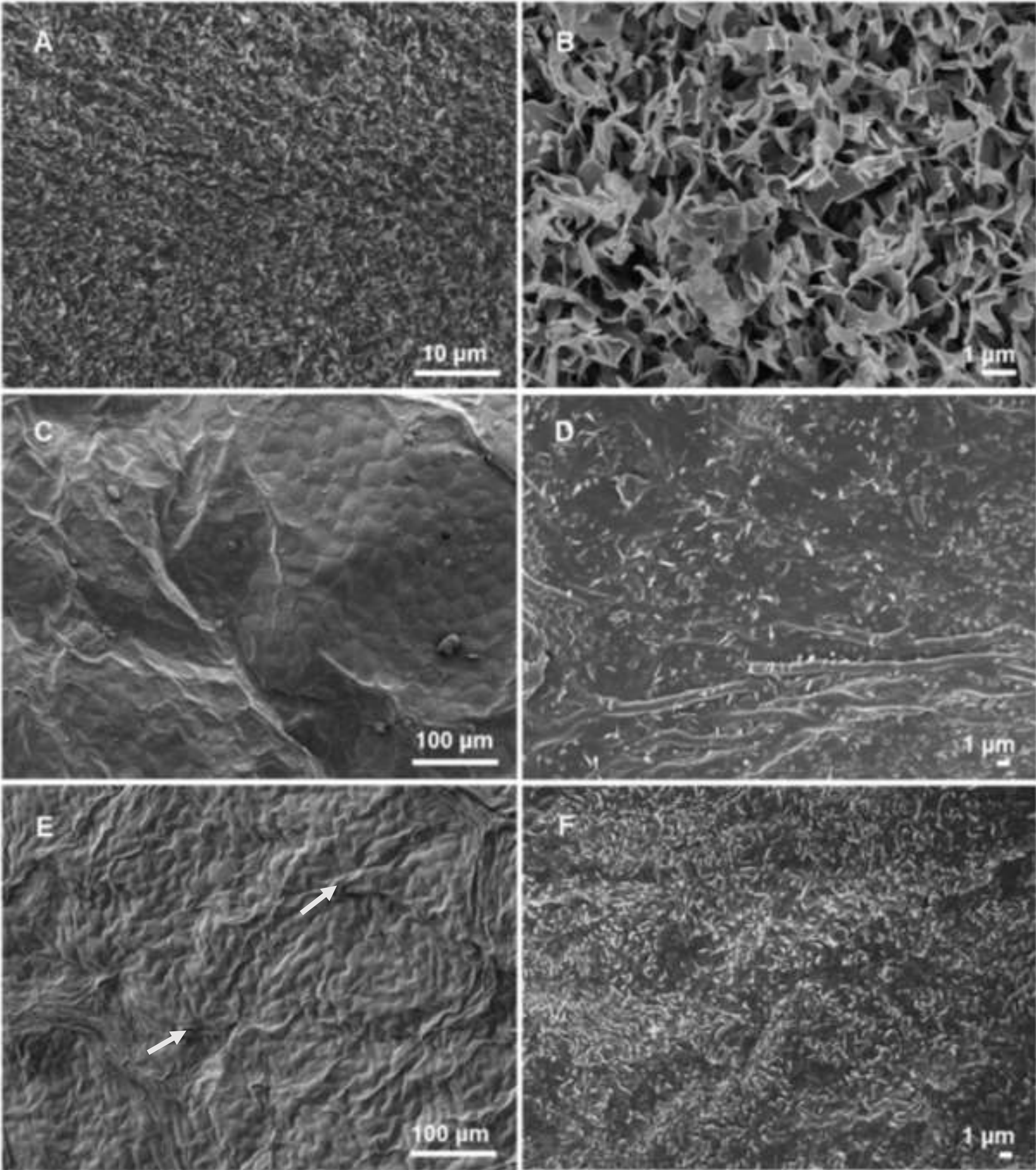

Figure 45. The native fruit, ad- and abaxial leaf surfaces of *Vitis vinifera* L. cv. 'Nelly'. (A, B) The surface of cv. 'Nelly' berry was covered by dense of non-entire platelets, and simple plate-type wax (Barthlott et al., 1998). (C) The adaxial leaf surfaces showed irregularly granulated features. (D) The epicuticular wax crystals were granules, small simple plate-type wax. (E) Stomata occurred (gray arrows) on abaxial leaf surfaces. (F) The abaxial leaf surface was covered by small parallel stacked platelets wax (Barthlott et al., 1998).

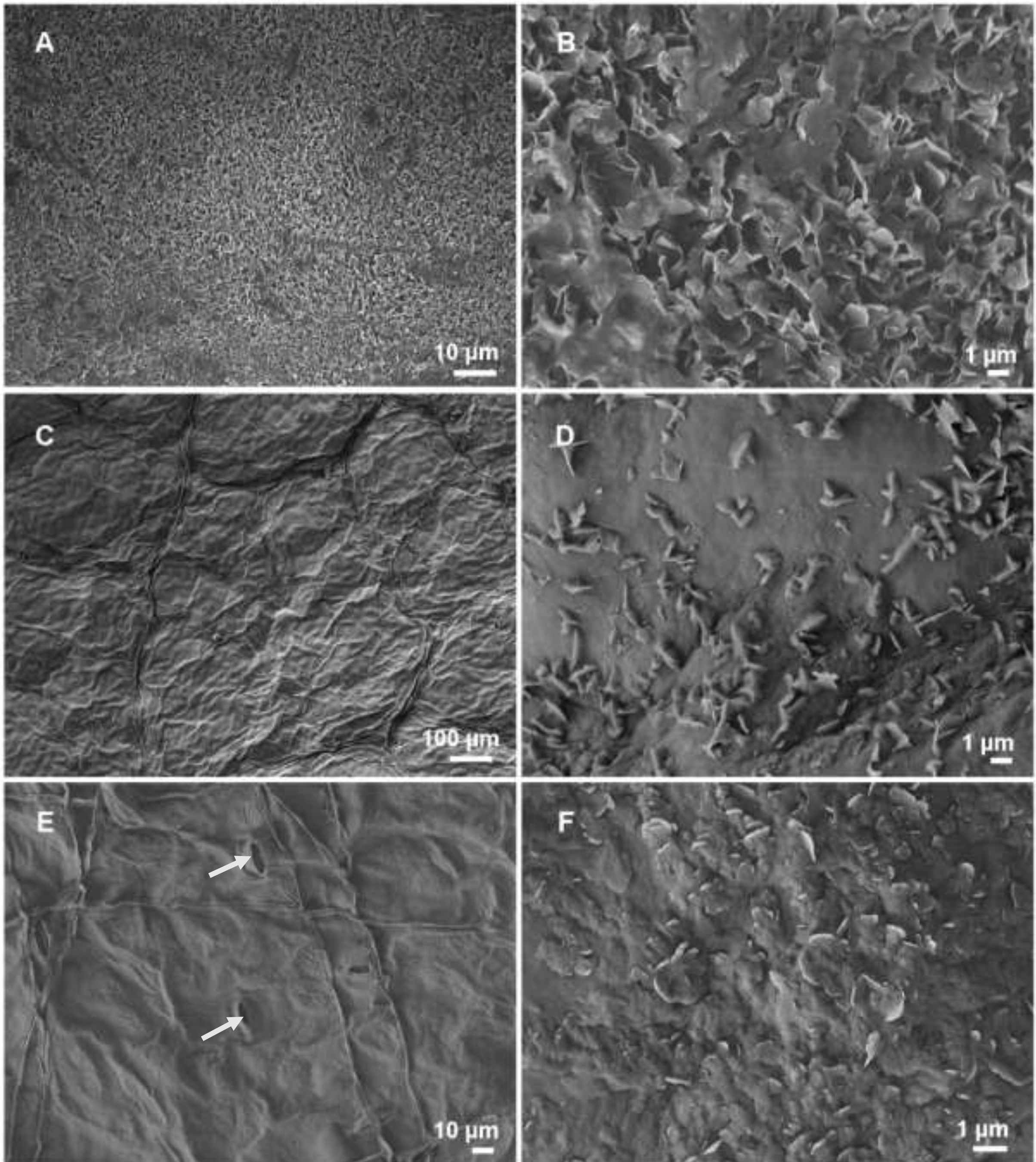

Figure 46. The native fruit, ad- and abaxial leaf surfaces of *Vitis vinifera* L. cv. 'Silvana'. (A, B) The surface of cv. 'Silvana' berry was covered by dense of platelets, and membranous platelets wax (Barthlott et al., 1998). (C, D) The adaxial leaf epicuticular wax crystals were rosettes of platelets (Barthlott et al., 1998). (E) Stomata occurred (gray arrows) on abaxial leaf surfaces. A filament network on the abaxial leaf surface was observed. (F) Small parallel stacked platelets wax covered on abaxial leaf surfaces (Barthlott et al., 1998).

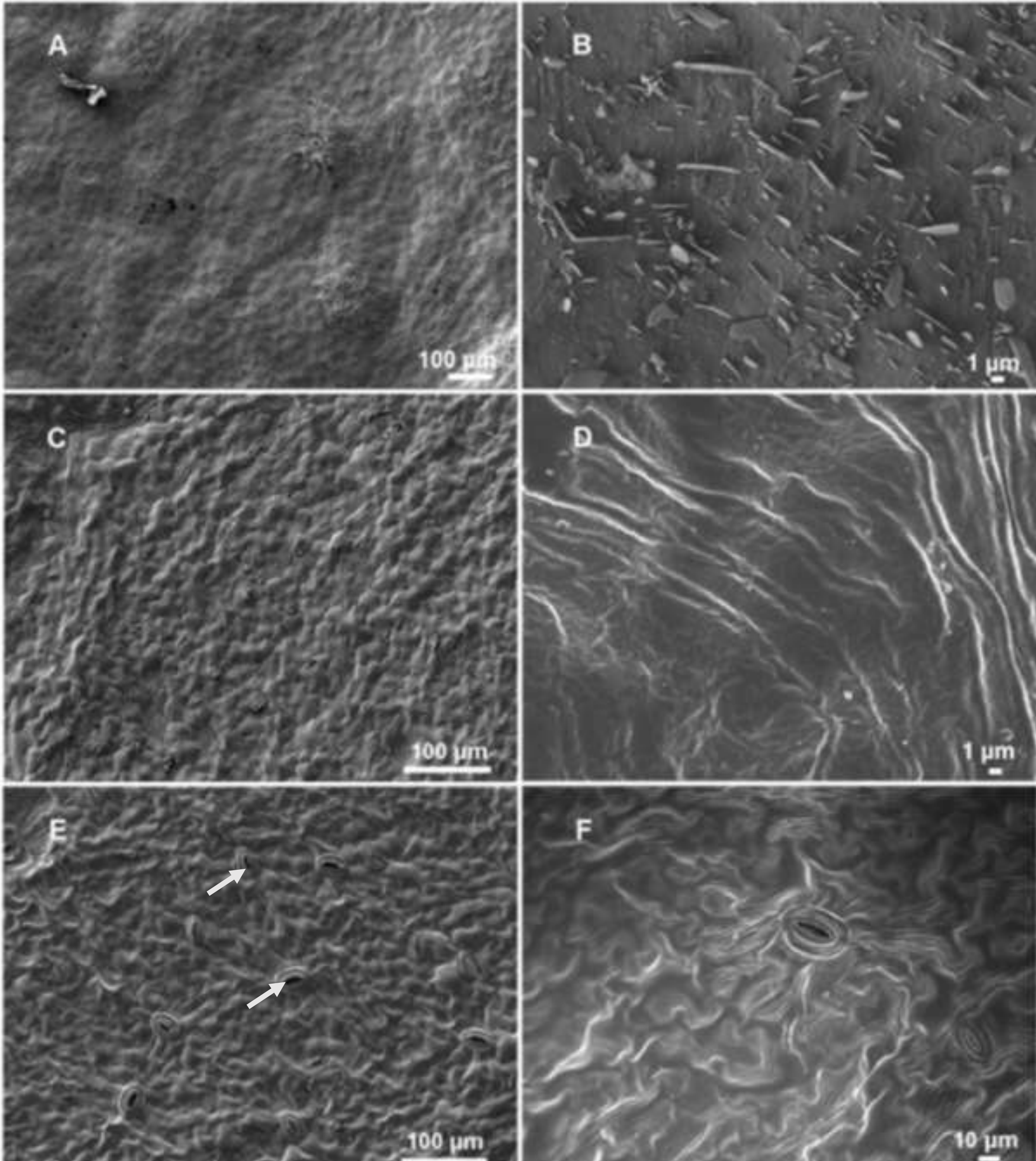

Figure 47. The native fruit, ad- and abaxial leaf surfaces of *Cornus officinalis* Siebold & Zucc. (A, B) Fruit epicuticular wax crystals were plates and platelets. Stomata (gray arrows) occurred on fruit (A) and abaxial (E) leaf surfaces. Most of the stomata on fruit surfaces were completely covered by wax. (C) The adaxial leaf surface showed irregularly granulated structure. Smooth wax film covered on the adaxial (D) and abaxial (F) leaf surfaces.

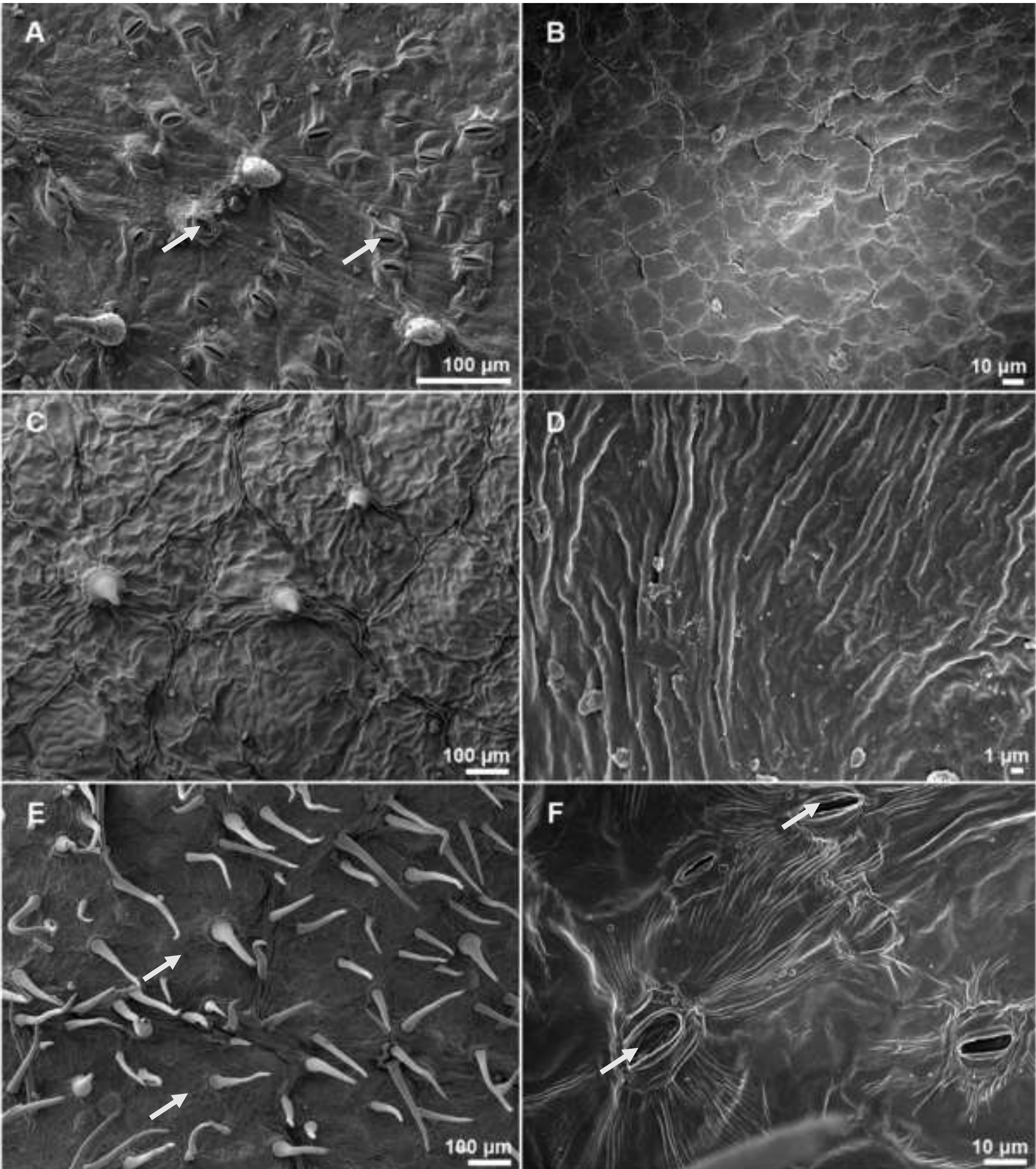

Figure 48. The native fruit, ad- and abaxial leaf surfaces of *Ficus carica* L. Crust wax with fissured layers represented the epicuticular wax features for fruit (Barthlott, 1998). Acicular trichomes occurred on fruit (A), adaxial (C), and abaxial (E) leaf surfaces (Tschan and Denk, 2012). Stomata (gray arrows) occurred on fruit (A) and abaxial leaf (E) surfaces. Smooth wax film covered on both adaxial (D) and abaxial (F) leaf surfaces.

Acknowledgments

Publications and Presentations

Publications

Huang H, Burghardt M, Schuster A, Leide J, Lara I and Riederer M (2017) Chemical compositions and water permeabilities of fruit and leaf cuticles of *Olea europaea* L. *Journal of Agricultural and Food Chemistry,* ***Accepted***

Riederer M, Arand K, Burghardt M, **Huang H**, Riedel M, Schuster A-C, Smirnova A, Jiang Y (2015) Water loss from litchi (Litchi chinensis) and longan (Dimocarpus longan) fruits is biphasic and controlled by a complex pericarpal transpiration barrier. *Planta* 242 (5):1207-1219

Presentations

10/2016 Eureka! 2016 – The 11th International Student Symposium, Würzburg (GER)
Poster Presentation: Comparative investigation of the chemical composition and the water permeability of fruit and leaf cuticles of *Olea europaea* L.

10/2015 Retreat of GK1342: Lipid Signalling, Rhön (GER)
Oral Presentation: Comparative investigation of the chemical composition and the water permeability of fruit and leaf cuticles of Olea europaea L.

10/2015 Eureka! 2015 – The 10th International Student Symposium, Würzburg (GER)
Poster presentation: Water movement through the surface of tomato fruit: effect of humidity and the cuticular wax barrier

06/2015 Conference of ‘Plant Waxes: From Biosynthesis to Burial’, Ascona (CH)
Poster presentation: Comparative investigation of the chemical composition and the water permeability of fruit and leaf cuticles of *Olea europaea* L.

10/2014 Retreat of GK 1342: Lipid Signalling, Luisenthal (GER)
Poster presentation: Effect of humidity on water transpiration of tomato fruit

Curriculum Vitae

Affidavit

I hereby confirm that my thesis entitled ‘Comparative investigation of the chemical composition and the water permeability of fruit and leaf cuticles’ is the result of my own work. I did not receive any help or support from commercial consultants. All sources and/or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Würzburg,
Place, Date Signature

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „Vergleichende Untersuchung zur chemischen Zusammensetzung und zur Wasserpermeabilität der Kutikula von Früchten und Blättern“ eigenständig, d.h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg,
Ort, Datum Unterschrift