Ecophysiological adaptations of the cuticular water permeability within the Solanaceae family

Ökophysiologische Anpassungen der kutikulären Wasserpermeabilität innerhalb der Solanaceae Familie

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I dedicate this thesis to my grandparents that inspired me in many ways

and taught me to admire and love plants.

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“Working hard is important. But there’s something that matters even more.

Believing in yourself.”

— J.K. Rowling, Harry Potter and the Order of the Phoenix

Summary

The cuticle, a complex lipidic layer synthesized by epidermal cells, covers and protects primary organs of all land plants. Its main function is to avoid plant desiccation by limiting non-stomatal water loss. The cuticular properties vary widely among plant species. So far, most of the cuticle-related studies have focused on a limited number of species, and studies addressing phylogenetically related plant species are rare. Moreover, comparative studies among organs from the same plant species are still scarce.

Thus, this study focus on organ-specificities of the cuticle within and between plant species of the Solanaceae family. Twenty-seven plant species of ten genera, including cultivated and non-cultivated species, were investigated to identify potential cuticular similarities. Structural, chemical and functional traits of fully expanded leaves, inflated fruiting calyces, and ripe fruits were analyzed.

The surface morphology was investigated by scanning electron microscopy. Leaves were mainly amphistomatic and covered by an epicuticular wax film. The diversity and distribution of trichomes varied among species. Only the leaves of *S. grandiflora* were glabrous. Plant species of the *Leptostemonum* subgenus had numerous prickles and non-glandular stellate trichomes. Fruits were stomata-free, except for *S. muricatum,* and a wax film covered their surface. Last, lenticel-like structures and remaining scars of broken trichomes were found on the surface of some *Solanum* fruits.

Cuticular water permeability was used as indicators of the cuticular transpiration barrier efficiency. The water permeability differed among plant species, organs and fruit types with values ranging up to one hundred-fold. The minimum leaf conductance ranged from 0.35×10^{-5} m s^{-1} in *S. grandiflora* to 31.54×10^{-5} m s^{-1} in *S. muricatum*. Cuticular permeability of fruits ranged from 0.64×10^{-5} m s^{-1} in *S. dulcamara* (fleshy berry) to 34.98×10^{-5} m s^{-1} in *N. tabacum* (capsule). Generally, the cuticular water loss of dry fruits was about to 5-fold higher than that of fleshy fruits.

Interestingly, comparisons between cultivated and non-cultivated species showed that wild species have the most efficient cuticular transpiration barrier in leaves and fruits. The average permeability of leaves and fruits of wild plant species was up to three-fold lower in comparison to the cultivated ones. Moreover, ripe fruits of *P. ixocarpa* and *P. peruviana* showed two-times lower cuticular transpiration when enclosed by the inflated fruiting calyx.

The cuticular chemical composition was examined using gas chromatography. Very-long-chain aliphatic compounds primarily composed the cuticular waxes, being mostly dominated by *n*-alkanes (up to 80% of the total wax load). Primary alkanols, alkanoic acids, alkyl esters and branched *iso*- and *anteiso*-alkanes were also frequently found. Although in minor amounts, sterols, pentacyclic triterpenoids, phenylmethyl esters, coumaric acid esters, and tocopherols were identified in the cuticular waxes. Cuticular wax coverages highly varied in solanaceous (62-fold variation). The cuticular wax load of fruits ranged from 0.55 µg cm^{-2} (*Nicandra physalodes*) to 33.99 µg cm^{-2} (*S. pennellii*), whereas the wax amount of leaves varied from 0.90 µg cm^{-2} (*N. physalodes*) to 28.42 µg cm^{-2} (*S. burchellii*). Finally, the wax load of inflated fruiting calyces ranged from 0.56 µg cm^{-2} in *P. peruviana* to 2.00 µg cm^{-2} in *N. physalodes*.

For the first time, a comparative study on the efficiency of the cuticular transpiration barrier in different plant organs of closely related plant species was conducted. Altogether, the cuticular chemical variability found in solanaceous species highlight species-, and organ-specific wax biosynthesis. These chemical variabilities might relate to the waterproofing properties of the plant cuticle, thereby influencing leaf and fruit performances. Additionally, the high cuticular water permeabilities of cultivated plant species suggest a potential existence of a trade-off between fruit organoleptic properties and the efficiency of the cuticular transpiration barrier. Last, the high cuticular water loss of the solanaceous dry fruits might be a physiological adaptation favouring seed dispersion.

Zusammenfassung

Die Kutikula, eine von Epidermiszellen gebildete, komplexe Lipidschicht, bedeckt und schützt die primären Organe niederer und höherer Landpflanzen. Ihre Hauptfunktion besteht darin, die Austrocknung der Pflanzen zu vermeiden, indem der nicht-stomatäre Wasserverlust an die Atmosphäre begrenzt wird. Ihre Eigenschaften können je nach Pflanzenart stark variieren, dennoch wurden kutikuläre Charakteristika unter phylogenetisch nah verwandten Pflanzenarten bisher wenig diskutiert. Die meisten Studien im Zusammenhang mit der Kutikula fokussierten sich auf eine begrenzte Anzahl von Pflanzenarten. Vergleichsstudien zwischen Organen derselben Pflanzenart sind kaum vorhanden.

Die vorliegende Studie konzentriert sich daher auf die Kutikula von Pflanzenarten aus der Familie der Solanaceae und deren Organe. Die kutikulären Eigenschaften von 27 Pflanzenarten aus zehn verschiedenen Pflanzengattungen wurden untersucht, einschließlich Wild- und Kulturarten. Strukturelle, chemische und funktionelle Merkmale wurden für vollständig entwickelte Blätter, vergrößerte Blütenkelche und reife Früchte vergleichend analysiert.

Die Oberflächenmorphologie wurde mit Hilfe der Rasterelektronenmikroskopie untersucht. Strukturell wiesen die meisten Blätter eine amphistomatische Oberfläche auf, die mit einem epikutikulären Wachsfilm bedeckt war, wobei einfache Trichome und Trichome mit Drüsen eine artspezifische Verteilung und Vielfalt aufzeigten. Bei den meisten Blättern der Untergattung *Leptostemonum* wurden Stacheln und zahlreiche sternenförmige Trichome beobachtet. Allein *Solandra grandiflora* hatte eine Blattoberfläche ohne Trichome. Früchte zeichneten sich hauptsächlich durch einen epikutikulären Wachsfilm aus, der ihre Oberfläche bedeckte. Als einzige Pflanzenart besaß *Solanum muricatum* auf der Fruchtoberfläche Stomata, dennoch wurden Lentizellen und Fragmente von Trichomen auf der Fruchtoberfläche von *Solanum tuberosum*, *Solanum quitoense* und *Solanum lycopersicum* gefunden.

Für die Effizienzbestimmung der kutikulären Transpirationsbarriere von Oberflächen mit und ohne Stomata wurden die minimale Wasserleitfähigkeit unter Bedingungen des maximalen Stomaschlusses beziehungsweise die kutikuläre Wasserpermeabilität untersucht. Dieses ergab ein art-, organ- und fruchttypspezifisches Muster. Die Werte variierten zwischen den

Pflanzenarten bis zu hundertfach und lagen zwischen 10^{-6} m s^{-1} und 10^{-4} m s^{-1}. Im Gegensatz zu den Ergebnissen früherer Studien zeigte der Vergleich der Wasserpermeabilität von verschiedenen Organen derselben Pflanzenarten, dass eine höhere Wasserpermeabilität für Blätter oder für Früchte gefunden werden kann oder dass sie für beide Organe nahezu gleich sein kann. Die minimale Wasserleitfähigkeit der Blätter lag im Bereich von 0.35×10^{-5} m s^{-1} für *S. grandiflora* bis 31.54×10^{-5} m s^{-1} für *S. muricatum*. Die kutikuläre Wasserpermeabilität lag im Bereich von 0.64×10^{-5} m s^{-1} für fleischige Früchte von *Solanum dulcamara* bis 34.98×10^{-5} m s^{-1} für Kapselfrüchte von *Nicotiana tabacum*. Allgemein zeigte sich, dass trockene Früchte eine etwa fünffach höhere kutikuläre Wasserpermeabilität als fleischige Früchte besaßen. Interessanterweise zeigten Vergleiche zwischen Wild- und Kulturarten, dass Wildarten eine wirksamere kutikuläre Transpirationsbarriere der Blätter und Früchte aufwiesen, da ihre Wasserpermeabilität etwa zwei- bis dreifach niedriger war als die der kultivierten Pflanzenarten. Des Weiteren zeigten *Physalis ixocarpa* und *Physalis peruviana*, deren Früchte von einem vergrößerten Blütenkelch umschlossen waren, einen schützenden Einfluss dieses Blütenkelches auf die reife Frucht. Eine Reduktion der kutikulären Wasserpermeabilität um den Faktor zwei wurde nachgewiesen.

Die chemische Zusammensetzung der kutikulären Transpirationsbarriere wurde mit Hilfe der Gaschromatographie detektiert. Die Analysen ergaben art- und organspezifische Mengen und Zusammensetzungen der kutikulären Wachse, die vor allem aus sehr langkettigen aliphatischen Verbindungen bestanden. Bis zu 80% der kutikulären Wachszusammensetzung bildete die Stoffklasse der *n*-Alkane. Andere häufig identifizierte Stoffklassen waren primäre Alkanole, Alkansäuren, Alkylester sowie *iso*- und *anteiso*-Alkane. Obwohl in geringen Mengen, wurden in den meisten kutikulären Wachsen auch alicyclische und aromatische Stoffklassen gefunden. Hauptsächlich handelte es sich um Phytosterole, pentacyclische Triterpenoide, Phenylmethylester, Cumarsäureester, Tocopherole und Flavonoide. Die kutikuläre Wachsschicht variierte zwischen den Pflanzenarten bis zu 62-fach und betrug zwischen 0.55 µg cm^{-2} für *Nicandra physalodes* und 33.99 µg cm^{-2} für *Solanum pennellii*, wobei sowohl niedrigste als auch höchste kutikuläre Wachsmenge für Früchte gefunden wurde. Die kutikulären Wachse der Blätter

reichten von 0.90 µg cm^{-2} für *N. physalodes* bis 28.42 µg cm^{-2} für *Solanum burchellii*. Die kutikuläre Wachsmenge der vergrößerten Blütenkelche lag zwischen 0.56 µg cm^{-2} für *P. peruviana* und 2.00 µg cm^{-2} für *N. physalodes*.

Zum ersten Mal wurde eine umfangreiche Studie zur Effizienz der kutikulären Transpirationsbarriere verschiedener Pflanzenorgane von phylogenetisch nah verwandten Pflanzenarten durchgeführt. Insgesamt zeigt die vergleichende Untersuchung innerhalb der Familie der Solanaceae die funktionelle und chemische Variabilität der kutikulären Wasserpermeabilität und der kutikulären Wachsbiosynthese. Die art- und organspezifische Divergenz kann dabei einen Einfluss auf die hydrophoben Eigenschaften der Kutikula haben und wichtige Konsequenzen für die Blatt- und Fruchtleistung mit sich führen. Darüber hinaus deuten diese Ergebnisse auf einen Kompromiss zwischen Fruchteigenschaften und Oberflächenschutz bei den Kulturarten hin. Es wird auch vermutet, dass die verringerte kutikuläre Barriereleistung der trockenen Früchte eine physiologische Anpassung an die Samenausbreitung dieser Pflanzenarten ist.

General introduction

Stay hydrated: the evolution of the plant cuticle

The origin and early diversification of terrestrial plants, in the mid-Palaeozoic era between about 480 and 360 million years ago, was a significant event in the history of life. The greening of the continents increased the atmospheric oxygen enabling the rise of other aerobic organisms and provided a diverse source of nutrition and habitats to animals and microorganism that evolved in parallel (Kenrick & Crane, 1997; Davis & Matthews, 2019). However, surviving in a gaseous environment would require the overcoming of countless challenges such as support against gravity, exposure to large temperature fluctuations, intense solar radiation and atmospheric dryness (Riederer & Schreiber, 2001; Yeats & Rose, 2013).

The transition from an exclusively aqueous to a gaseous environment resulted in crucial physiological and structural innovations in plants. From a simple plant body composed of a small number of cells, land plants evolved to elaborate organisms formed by a remarkable display of complex tissues and organs (Kenrick & Crane, 1997). One of the most important novelties in the history of plant evolution was the development of essential components that regulate transpirational water loss, such as hydrophobic coatings to reduce desiccation, pores to facilitate gas exchange and specialized cells for the transport of water and nutrients (Arteaga-Vasquez, 2016; Kenrick, 2018).

Since water is a universal requirement for plant survival and growth, the ability to conserving water was a critical evolutionary step for the successful terrestrialization of plants (Oliver et al., 2000; Yeats & Rose, 2013). The earliest land plants already displayed adaptations that facilitated regulation of the plant water status (Edwards, 1993). Significant innovations, such as cuticular fragments, stomatal pores, tracheid cells and rhizoids, were found in early terrestrial plant micro- and macrofossil records, integrating an anatomical, morphological and physiological platform of

fundamental importance to the diversification of vascular plants (Edwards, 1996; Edwards et al., 1998; Kerp et al., 2003; Kenrick, 2018; Davis & Matthews, 2019).

The plant cuticle and the stomata act as ubiquitous features to avoid transpirational water loss among land plants (Croxdale, 2001; Reina-Pinto & Yephremov, 2009; Budke et al., 2012). At the present evolutionary time, the majority of plants are desiccation-sensitive and evolve solutions to keep them from drying-out by maintaining a chronic imbalance between water-filled cells and low-moisture environments (Alpert & Oliver, 2002). The ability to synthesize, accumulation, and maintain a hydrophobic deposit on aerial plant surfaces and to dynamical control gas exchange and transpiration through the stomatal aperture enabled plants to conserve water even during severe dry seasons (Kerstiens, 1996a; Yeats & Rose, 2013).

The hydrophobic plant cuticle was a key innovation that allowed plants to tolerate the drought atmosphere and cope with the unfavourable conditions on land. This continuous extracellular cuticular layer is synthetized by the epidermal cells and covers primary plant organs of lower and higher land plants (Jeffree, 2006; Koch et al., 2008; Samuels et al., 2008; Buschhaus & Jetter, 2011). This protective layer that enabled plants to thrive in a wide range of desiccating environments coats the outer surfaces of leaves, flowers, fruits, stems and seeds (Koch et al., 2008; Yeats & Rose, 2013).

Functional importance of the plant cuticle

Limiting transpirational water loss to a minimum is of significant importance for plant survival during extreme drought events (Schönherr, 1982; Burghardt & Riederer, 2003). However, during periods of water deficit, when stomata are closed, the only route for water diffusion from inner plant tissue to the atmosphere is across the plant cuticle. Due to its extracellular deposition, the cuticle acts as a natural interface between plant organs and their surrounding environment (Kerstiens, 1996c; Heredia & Dominguez, 2009). The restriction of non-stomatal water loss is provided by the low permeability of the cuticle, and its study deserves for the comprehension of

many cuticular functions (Kerstiens, 1996a; Burghardt & Riederer, 2006; Riederer et al., 2015; Schuster et al., 2017).

According to previous data available, cuticle permeability of leaves range from 2.5×10^{-7} in *Zamioculcas zamiifolia* to 4.0×10^{-3} m s^{-1} in *Ipomoea batatas* (Karbulková et al., 2008; Zobayed et al., 2000; Schuster et al., 2017). On the other hand, water permeability of fruits was investigated in fewer cases having a lower variability than that of leaves, ranging from 0.9×10^{-5} to 2.0×10^{-4} m s^{-1} for *S. lycopersicum* cv. 'Micro-Tom' and *Capsicum annum*, respectively (Riederer & Schreiber, 2001; Leide et al., 2007).

In addition to its major role as the foremost barrier to transpirational water loss, the cuticle has multiple functions in plant development and physiology besides conferring resistance to a wide range of biotic and abiotic stresses (Buschhaus & Jetter, 2011; Domínguez et al., 2011; Yeats & Rose, 2013). These include the protection against ultraviolet radiation, heavy wind and rain, prevention of plant organ fusion, promotion of a self-cleaning surface, and mechanical support to maintain plant organ integrity. It also plays a vital role in cuticular water uptake and diffusion of solutes into the cuticle, protection against herbivores and defence against pathogenic infection (Kolattukudy, 1980; Kerstiens, 1996b; Reina-Pinto & Yephremov, 2009; Domínguez et al., 2011; Lara et al., 2014; Martin & Rose, 2014).

The numerous roles of the plant cuticle have been studied in a broad range of plant species, and the increasing interest seeing especially during the past twenty years has fostered our understanding of the plant cuticle biology. Several contributions in this field of research, including studies on characterization, biochemistry and molecular biology of the biosynthesis of the cuticle, has led to connections to other aspects of plant biology. Numerous findings revealed that the cuticle is much more than a mere passive barrier to transpirational water loss (Yeats & Rose, 2013; Domínguez et al., 2017; Ziv et al., 2018).

Compositional and structural properties of the plant cuticle

The plant cuticle is a multi-functional structure with a complex and heterogeneous nature ranging from < 1 µm to >20 µm in thickness (Jeffree, 2006; Khayet & Fernández, 2012; Fernández et al., 2016; Schuster et al., 2016; Segado et al., 2016). Chemically, the cuticle is composed of an insoluble cutin polymer associated with solvent-soluble lipids, the cuticular waxes, but polysaccharides, phenolic compounds, as flavonoids and tocopherols, and cutan can also be present (Guzmán-Delgado et al., 2016). Two spatially distinct layers are identified according to the cuticular wax deposition: the intracuticular wax, embedded within the cutin matrix, and the epicuticular wax, coating the outer surface as either a cuticular wax film or crystals (Baker, 1982; Holloway, 1982; Walton, 1990).

The cutin matrix, as the structure-providing component of the cuticle, comprises up to 80% of the cuticular weight. This biopolymer is mainly composed of ester-linked long-chain alkanoic acid monomers with chain-lengths of 16 and 18 carbon atoms (Heredia, 2003; Niklas et al., 2017). Common cutin monomers include ω-hydroxy alkanoic acids with mid-chain oxygenated substitutions, usually epoxy, oxo and hydroxy groups, and occasionally α,ω-dicarboxylic acids. In addition, minor proportions of aromatic acids, such as hydroxy cinnamic acids, are linked within the cutin matrix (Graça et al., 2002; Isaacson et al., 2009; Domínguez et al., 2011; Khanal & Knoche, 2017).

As well as cuticle thickness, cuticular wax coverage varies strongly between plant species, ranging from coverages as low as 0.4 µg cm^{-2}, as reported to *Morus alba* L. leaves (Mamrutha et al., 2010), to major coverages up to 160 µg cm^{-2}, as found for *Argania spinosa* (L.) Skeels leaves (Bouzoubaâ et al., 2006). However, water permeability is not correlated to the thickness or to the wax amount of the cuticle (Riederer & Schreiber, 2001).

The cuticular waxes consist of a complex mixture of homologues series of very-long-chain aliphatic compounds with more than 20 carbon atoms. Common components are *n*-alkanes,

primary alkanols, alkanoic acids, alkanals and alkyl esters, and highly variable amounts of cyclic compounds, like pentacyclic triterpenoids and hydroxy cinnamic acids (Kolattukudy, 1970; Walton, 1990; Jetter et al., 2006; Heredia & Dominguez, 2009). The cuticular waxes comprise the true barrier of the plant cuticle against passive water loss and diffusion of solutes, and its compositional characteristics and spatial arrangement affect the properties of the cuticular transport-limiting barrier (Schönherr & Riederer, 1989; Wijmans & Baker, 1995; Veraverbeke et al., 2003a; Vogg et al., 2004).

The fruit cuticle

Cuticle function, ultrastructure and composition can vary across plant species, ecotypes, and even within individual plants in a tissue- and organ-specific manner. Addressing these variables is fundamental to broaden our knowledge of different strategies and adaptations of plants to minimize transpirational water loss (Matzke & Riederer, 1991; Kerstiens, 1996c; Bonaventure et al., 2004; Xu et al., 2014; Fernandéz et al., 2016; Leide et al., 2018).

Vegetative plant organs, particularly leaves, have been in the focus of cuticle-related studies in the past decades. Although sharing many features with those of vegetative plant organs, only recently generative fruits have stood out as a valuable model of research. In comparison to other standard models, such as *Arabidopsis thaliana* (L.) Heynh. stems and leaves, the fruit cuticle has unique features that make it experimentally attractive for functional, structural and compositional analyses: a continuous, often astomatous plant material, which is thicker and more robust than those of most leaves, relatively readily to isolate. Therefore, the fruit cuticle offers a useful experimental system that can contribute to deepen our understanding of the cuticle properties and uncover possible relationships among them (Lara et al., 2014, 2019; Martin & Rose, 2014; Ziv et al., 2018).

In spite of specific fruit cuticle attributes and their importance as determinants in fruit quality and post-harvest shelf life, studies addressing fruit cuticle biosynthesis and composition are still

scarce (Lara et al., 2014; Martin & Rose, 2014; Zarrouk et al., 2018). Information is available especially for tomato (*Solanum lycopersicum* L.; Leide et al., 2007, 2011; Lopez-Casado, 2007; Martin & Rose, 2014). However, other fruits including eggplant (*Solanum melongena* L.; Schönherr & Schmidt, 1979; Becker et al., 1986), bell pepper (*Capsicum annuum* L.; Elshatshat et al., 2007), apple (*Malus domestica* Borkh.; Veraverbeke et al., 2003a,b; Leide et al., 2018), sweet cherry (*Prunus avium* (L.) L.; Peschel et al., 2007; Belge et al., 2014) and olive (*Olea europaea* L., Huang et al., 2017) have been investigated although not to the same degree. Hence, many knowledge gaps concerning the composition and physiological roles of the fruit cuticle and specificities in fruits of different plant species and varieties remain to be elucidated (Lara et al., 2014, 2015).

Agricultural importance of the plant cuticle

The cuticle is a crucial determinant in fruit quality and post-harvest performance of horticultural crops. Fruit disorders, such as russeting and fruit cracking, sensory and nutritional qualities, are strongly affected by cuticular properties (Lara et al., 2014; Moggia et al., 2017; Petit et al., 2017). Water and weight loss during storage lead to fruit over softening, tissue collapse and higher incidence of pathogenic infection in fruit crops, impairing their quality and availability in the markets (Martin & Rose, 2014; Tafolla-Arellano et al., 2018). Among fruit traits, dehydration and softening are the typical reasons of shipment rejections and causes direct economic loss due to the decrease in saleable weight (Veraverbeke et al., 2003b; Moggia et al., 2016, 2017).

Studies of post-harvest behaviour of horticultural commodities have shown that the cuticle is the most important determinant in water loss. Cuticular composition and its physical properties might have a key role in controlling water status and maintaining fruit surface integrity, thus, promoting post-harvest firmness and shelf life. In some cases, the fruit cuticle is the only transpiration barrier regulating the water status, due to the absence of stomata at maturity (Veraverbeke et al., 2003b,b; Leide et al., 2007; Saladié et al., 2007; Riederer et al., 2015; Tafolla-Arellano et al., 2018).

It is clear that plant cuticle relevance extends far beyond the basic research having a great influence on desirable horticultural traits and crop production. By conferring adaptation to multiple biotic and abiotic stresses, including desiccation control, limiting pathogen and herbivore attack, plant cuticle acts as an important trait to be considered in crop protection and quality improvement strategies (Kerstiens, 2006; Martin & Rose, 2014; Domínguez et al., 2017; Dhanyalakshmi et al., 2019).

Drought stress is one the main limiting factors in agricultural production and future changes in water regime patterns can further worsen this scenario leading to reductions in the yield and quality of horticultural crops (Tafolla-Arellano et al., 2018). Improving cuticular traits, such as manipulating cuticular waxes for breeding new crop types, could enhance crop adaptability to adverse conditions, especially under the current threat of the global climate changes and its consequences for agriculture (Dhanyalakshmi et al., 2019).

Solanaceous species as model organisms

The Solanaceae family, or nightshades, is one of the largest and most complex plant families of angiosperms. It has a worldwide distribution with the main centre of diversity and endemism in South America (D'Arcy, 1991; Hunziker, 2001). This cosmopolitan plant family comprises between 3000 and 4000 plant species and 90 genera, including important crops of historical and economic value to human beings, such as potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), eggplant (*S. melongena* L.) and peppers (*Capsicum* spp.). Besides its fundamental importance as a source of nutrient, several solanaceous species are used for their pharmacological compounds. Many plant species contain potent alkaloids, and some are highly toxic, e.g. deadly nightshade (*Atropa bella-donna* L.), downy thorn apple (*Datura innoxia* Mill.) and mandrake (*Mandragora officinarum* L.). Several others are used as ornamentals, e.g. petunia (*Petunia hybrida* Vilm.) and tobacco (*Nicotiana tabacum* L.), and even as models in biological studies (Knapp et al., 2004; Wilf et al., 2017).

Members of the Solanaceae family display a remarkable diversity of life forms, ranging from annual to perennial herbs, shrubs or rarely trees, habitats, morphological traits, especially floral parts and fruits, and chemical composition (Knapp, 2002; Barboza et al., 2016). Therefore, solanaceous species offer great potential to investigate questions on cuticle-related research by providing a wide range of working resources. The function and composition of the cuticle can be studied at various levels, including different developmental stages, plant organs, fruit types, cultivars and mutant species of the same genetic background. Additionally, comparative analyses between domesticated plant species and their wild relatives and among plant species from different habitats are also possible.

Indeed, studies on leaf and fruit surfaces of solanaceous species, including *S. lycopersicum*, *S. melongena*, *C. annuum* and *Solanum pennellii* (Correll), had already shown that solanaceous species are very suitable experimental systems for analyses of the cuticle composition. Even more, distinct compositional phenotypes can be correlated with functional aspects and gene expression patterns (Alba et al., 2004, 2005; Fei et al., 2004; Rose et al., 2004; Moore et al., 2005; Leide et al., 2007; 2011; Bolger et al., 2014; Haliński et al., 2015; Romero & Rose, 2019). Thus, the Solanaceae family comprise a valuable source of model organisms to investigate ecophysiological functions and mechanisms that determine the different degrees of cuticular water permeability in plants.

Aim of the present study

As the primary determinant of the water loss rate during cuticular transpiration, the permeability of the cuticle and is its variation can have significant physiological and ecological consequences for plant survival and competitiveness (Goodwin and Jenks, 2005). Studying the processes that influence the cuticular transpiration barrier efficiency not only contributes to the basic understanding of the performance and adaptation of plants in the field but can also promote the development of strategies to cope with the increasing water demand by crops (Drastig et al., 2016). However, cuticular biology studies on composition, structure and function of fruit cuticles

and investigations of cuticular traits in different organs of the same species are still needed (Lara et al., 2014; 2015; Fernandéz, 2016; Huang et al., 2017; Zarrouk et al., 2018; Leide et al., 2018).

Thus, the general objective of this project was to investigate cuticular properties among taxonomically related species. For that, the compositional, structural and functional cuticle attributes were analysed in a wide range of plant species. Plant species from the Solanaceae family were chosen as experimental models, resulting in thirty solanaceous plants belonging to ten different genera: *Atropa*, *Capsicum*, *Cestrum*, *Datura*, *Hyoscyamus*, *Nicandra*, *Nicotiana*, *Physalis*, *Solandra* and *Solanum*. To reveal possible cuticle-related specificities within and between species, the cuticular transpiration barrier properties were analysed in leaf, inflated calyx and fruit, resulting in three chapters:

Chapter I evaluated the efficiency of the cuticular transpiration barrier at a genus level in fruit and leaf cuticles of fourteen *Solanum* representatives. Wild and cultivated plant species were analysed organ- and species-specifically and among different cultivars of the same species;

Chapter II investigated the cuticle properties of solanaceous species presenting an inflated papery calyx enclosing the fruit as a special morphological feature. Compositional, structural and functional cuticle attributes were analyzed in leaves, inflated fruiting calyces and fruits. Additionally, the inflated fruiting calyx was put into perspective of its functional significance for the cuticular water loss in ripe fruits;

By last, chapter III focused on the primary function of the plant cuticle as a barrier to transpirational water loss within the Solanaceae family. Cuticular water permeability of leaves and fruits were compared species and organ-specifically. Furthermore, to investigate whether the life strategies influence on the cuticular permeabilities of the solanaceous investigated, the cuticular function was put into perspective of the respective plant life forms, fruit types, and climatic origin of the plant species.

Materials and methods

Plant material and growing conditions

Twenty-eight plant species of the family Solanaceae belonging to ten different genera were selected for this study (Table 1). The most recent taxonomic revisions of the Solanaceae species were used in this work (see Solanaceae Source, http://solanaceaesource.org and http://www.theplantlist.org). The solanaceous plants were cultivated in pots using ED73 substrate (Einheitserde Werkverband eV) inside a greenhouse or in garden beds located at the Botanical Garden (49° 45' N, 9° 55' E) of the University of Würzburg, Germany. Würzburg lies in Central Europe at an elevation of 177 meters above mean sea level. Low annual precipitation (601 mm) and high summer temperatures characterize the climate in this area. Mean annual temperature and the average temperature of the warmest month (July) are 9.6°C and 18.3°C, respectively. Rainfall concentrates mainly during the vegetation period, which has an average temperature above 10°C and lasts around 150 days (1981 to 2010, Germany's National Meteorological Service).

Plants were watered and supplemented with NPK fertilizer (nitrogen, phosphorus, and potassium; Osmocote® Plus, ICL Specialty Fertilizers) as needed. Annual plant species were grown yearly from 2016 to 2019, while perennial plant species were reused during the whole period of study. During the summer season, plants were moved to an open greenhouse, thus being subjected to the external climate conditions. In winter, perennial plants stayed inside a greenhouse with a temperature of 24°C/18°C day/night and 50% ± 5% relative humidity under a 14 h light regime at 250 µmol m^{-2} s^{-1} white light (400 W, Philips Master Agro). Intact, fully expanded leaves and fruits at the full ripening stage were collected by detaching at the petiole or peduncle base, put into plastic bags and transported to the laboratory for experimental analyses. Plant material was obtained mainly during the summer season (June to August) during their reproductive phase. For *Physalis* and *Nicandra* species, immature green inflated fruiting calyces, characterized by a purple colouring on their base, were also investigated.

Table 1. Solanaceous plant species selected for this study organized by plant tribes. Plant origin was classified according to the seven-continent model (Encyclopaedia Britannica, 2008).

plant tribe	scientific name	common name	origin	life-form
Hyoscyameae	*Atropa bella-donna* L.	deadly nightshade	Asia and Europe	herb
Hyoscyameae	*Atropa bella-donna var. lutea* Döll	yellow deadly nightshade	Asia and Europe	herb
Capsiceae	*Capsicum annuum* L. cv. 'Kapia'	bell pepper	North America	herb
Cestreae	*Cestrum parqui* (Lam.) L'Hér	green cestrum	South America	shrub
Cestreae	*Cestrum elegans* (Brongn. ex Neumann) Schltdl.	red cestrum	North America	shrub
Datureae	*Datura inoxia* Mill.	devil's trumpet	Americas	shrub
Hyoscyameae	*Hyoscyamus albus* L.	white henbane	Asia and Europe	herb
Hyoscyameae	*Hyoscyamus niger* L.	black henbane	Asia and Europe	herb
Nicandreae	*Nicandra physalodes* (L.) Gaertn.	apple of Peru	South America	herb
Nicotianeae	*Nicotiana tabacum* L. cv. 'Izmir'	cultivated tobacco	South America	herb
Physalideae	*Physalis alkekengi* var. *franchetii* (Mast.) Makino	chinese lantern	Asia and Europe	herb
Physalideae	*Physalis ixocarpa* Brot. ex. Hornem	tomatillo	North America	herb
Physalideae	*Physalis peruviana* L.	cape gooseberry	South America	shrub
Solandreae	*Solandra grandiflora* Sw.	chalice vine	Americas	vine
Solaneae	*Solanum burchellii* Dunal	lemoenbossie	Africa	shrub
Solaneae	*Solanum dulcamara* L.	bittersweet	Asia and Europe	vine
Solaneae	*Solanum linnaeanum* Hepper & P.-M.L. Jaeger & Jaeger	devil's apple	Africa	shrub
Solaneae	*Solanum lycocarpum* A.St.-Hil.	wolf apple	South America	shrub
Solaneae	*Solanum lycopersicum* L. cv. 'Benarys Gartenfreude'	tomato	South America	herb
Solaneae	*Solanum lycopersicum* L. cv. 'John Baer'	tomato	South America	herb
Solaneae	*Solanum lycopersicum* L. cv. 'Pearson'	tomato	South America	herb
Solaneae	*Solanum melongena* L. cv. 'Slim Jim'	eggplant	Asia	shrub
Solaneae	*Solanum muricatum* Aiton	sweet cucumber	South America	shrub
Solaneae	*Solanum pennellii* Correll	wild tomato	South America	shrub
Solaneae	*Solanum pseudocapsicum* L.	jerusalem cherry	Americas	shrub
Solaneae	*Solanum quitoense* Lam.	lulo	South America	shrub
Solaneae	*Solanum retroflexum* Dunal	sunberry	Africa	herb
Solaneae	*Solanum sisymbriifolium* Lam.	litchi tomato	South America	shrub
Solaneae	*Solanum virginianum* L.	yellow-fruit nightshade	Asia	shrub
Solaneae	*Solanum tuberosum* L. ssp. andigena Hawkes	potato	South America	herb

Determination of leaf, inflated fruiting calyx and fruit traits

Prior to measurement, leaves and green inflated calyces were rehydrated overnight in humid chambers for the saturated fresh weight ($FW_{saturated}$) determination (MC-1 AC210S, Sartorius). The actual fresh weight (FW_{actual}) during cuticular transpiration experiments was obtained to calculate the relative water deficit (*RWD*) according to:

$$RWD = 1 - \frac{FWactual - DW}{FWsaturated - DW}$$

The dry weight (*DW*) was detected after oven-drying the plant material at 60°C (Memmert UF55) for seven days. For calculation of the relative water content (*RWC*) of fruits, the fresh weight (*FW*) was related to the *DW* using the formula:

$$RWC = 100 - \frac{DW \times 100}{FW}$$

The surface area (*A*) of leaves, inflated fruiting calyces and fruits with irregular shapes was determined according to the pixel values of the planar surface scanned at high resolution (600 dpi) in comparison to a reference area (Corel® Photopaint® 2018). For total leaf area calculation, the dual projected leaf area was considered. In species of the 'spiny solanums' clade (*Solanum* subgenus *Leptostemonum*), the area of prickles were calculated as a dual projected prickle area after scanning of their surfaces, and summed to the total leaf area. The surface area of fruits with spherical shapes was calculated from the average of a vertical and a horizontal diameter. The specific area was calculated by dividing the leaf surface area or fruit surface area by the dry weight.

Isolation of cuticular membranes

Circular sections of the fruit surface were obtained using metal cork borers. Fruit cuticular membranes were enzymatically isolated with pectinase (Trenolin Super DF, Erbslöh) and cellulase (Celluclast, Novo Nordisk AIS) in 20 mM citric acid (AppliChem), pH 3.0, containing 1 mM sodium azide (Sigma-Aldrich) at room temperature. The enzyme solution was exchanged weekly. Isolated cuticular membranes were extensively washed in deionized water, air-dried and stored in Petri dishes at room temperature until use. Leaf cuticle and inflated fruiting calyx cuticle of the selected solanaceous plant species were extremely fragile, and thereby isolation was not feasible.

Scanning electron microscopy

Air-dried plant material was mounted on aluminium holders using a conductive double-sided adhesive tape (Plannet Plano), 5 min coated with gold/palladium (60/40) at 25 mA using a Bal-Tec SCD 005 sputter coater (300 s; Balzers) and examined in a field emission scanning electron microscope (JEOL JSM-7500F) at 10 kV. The sputter conditions, depositing an alloy coat thickness of approximately 20 nm, were optimized for the acceleration voltage in the scanning electron microscope. Images from plant surface were taken from both the adaxial and abaxial leaf sides and the inflated fruiting calyx, and from the outer and inner side of isolated fruit cuticles.

Minimum conductance for water

Water permeability obtained from stomatous cuticles is named minimum water conductance (g_{min}) and corresponds to the lowest cuticular water loss when stomata are maximally closed in response to plant dehydration (Kerstiens, 1996a). Minimum conductance was determined from the consecutive weight loss of water-saturated leaves and green-inflated calyces. Cut petioles and the opened-tip of the inflated fruiting calyces were sealed with bee wax (Roth). Then, the sealed plant material was exposed to the desiccating atmosphere inside plastic boxes over silica gel (Applichem) for maintaining the low air humidity. Boxes were kept in a climate incubator at

25°C and dark conditions (IPP 110, Memmert). The weight loss of the desiccating plant material was recorded using a high precision balance (MC-1 AC210S, Sartorius). The relative humidity and temperature inside the climate incubator were monitored using a digital thermo-hygrometer (Testoterm 6010, Testo).

Minimum conductance was calculated by dividing the transpiration rate (*J*) by the driving force (Δ*c*) for water loss. The transpiration rate (*J*) was determined from the decline in fresh weight (Δ*FW*) with time (Δ*t*) divided by the surface area (*A*) according to the formula:

$$J = \frac{\Delta FW}{\Delta t \times A}$$

The driving force (Δ*c*) for water loss corresponded to the difference between the concentration of water vapour in the leaf or inflated fruiting calyx epidermis ($C_{wv\ epidermis}$) and the surrounding atmosphere ($C_{wv\ air}$):

$$\Delta c = C_{wv\ epidermis} - C_{wv\ air}$$

The water vapour concentration in the epidermis ($C_{wv\ epidermis}$) is the product of the water activity in the epidermis ($\alpha_{epidermis}$), and the concentration of water vapour saturation at the epidermal surface ($C_{wv\ sat\ epidermis}$). The water activity in the epidermal apoplast ($\alpha_{epidermis}$) is assumed to be close to one. The concentration of water vapour in the atmosphere ($C_{wv\ air}$) is given by multiplying the water activity of the air (α_{air}) by the concentration of water vapour saturation ($C_{wv\ sat\ air}$), which is nearly zero over silica gel (Slavík, 1974). Thus, the active driving force was the saturation concentration of water vapour at the actual leaf or inflated fruiting calyx temperature:

$$gmin = \frac{J}{\Delta c} = \frac{J}{\alpha_{epidermis} \times C_{wv\ sat\ epidermis} - \alpha_{air} \times C_{wv\ sat\ air}}$$

Leaf and inflated fruiting calyx temperatures were measured using an infrared laser thermometer (one point measurements, Harbor Freight Tools). The corresponding water vapour saturation

concentrations at leaf and inflated fruiting calyx temperature were derived from tabulated values (Nobel, 2009).

The water permeability at a given dehydration point was plotted versus the respective *RWD* (Fig. 1). Initially, high conductance resulted from the stomatal transpiration. At a certain *RWD*, the continuous dehydration and maximum stomatal closure culminate in a plateau, where conductance is no longer altered by the subsequent decline of the *RWD* corresponding to g_{min} (Burghardt and Riederer, 2003).

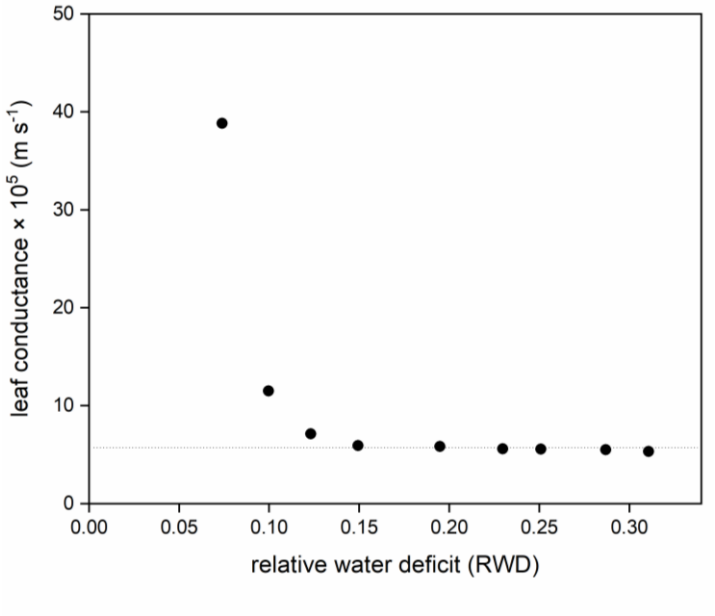

Fig. 1. Representative leaf drying curve of *Physalis alkekengi* at 25°C showing the leaf conductance as a function of the relative water deficit (*RWD*). The maximal stomatal closure occurs in the transition between the decreasing conductance to the constant values (plateau, dotted line).

Cuticular permeance for water

Values obtained from stomata-free systems, such as astomatous fruits, is termed cuticular water permeance. Water permeability will be used to refer to both minimum conductance and cuticular permeance for water (Kerstiens, 1996a; Schuster et al., 2017).

Cut peduncles were sealed with bee wax (Roth), and cuticular permeance was determined from the consecutive weight loss of fruits, similarly to the calculation of the minimum conductance described above. Additionally, the post-floral contribution of the inflated calyx to the water loss in mature fruits was investigated for *Physalis* and *Nicandra* species. To assure that the water loss determined was solely the fruit water permeability, inflated fruiting calyces were dried overnight at 24°C according to Novoa et al. (2006), with adaptations. Then, fruits with inflated calyx and after its removal were analysed for cuticular permeability.

Cuticular permeability was calculated by dividing the transpiration rate (J) by the driving force (Δc) for water loss. Cuticular conductance from the astomatous fruits resulted in linear plots of cumulative water loss (ΔFW) per the time (Δt; Fig. 2).

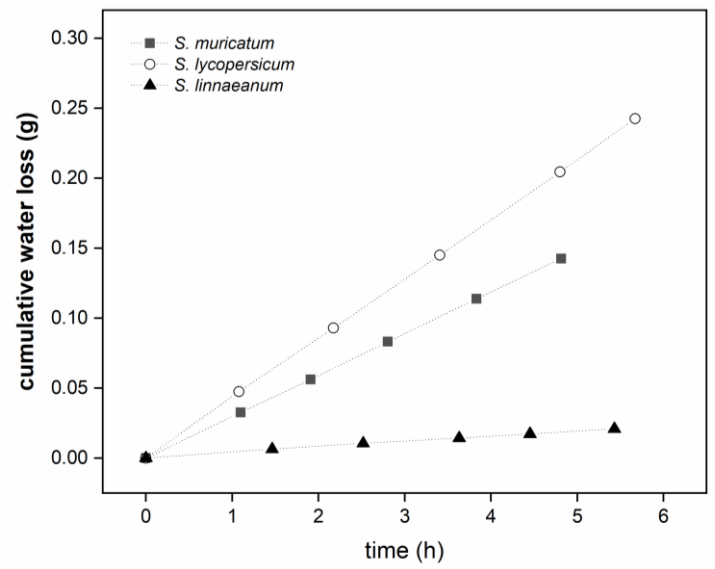

Fig. 2. Slopes of the cumulative water loss per time of mature fruits of representative solanaceous plant species *Solanum muricatum* (r^2 = 0.999), *Solanum lycopersicum* cv. 'Pearson' (r^2 = 0.999) and *Solanum linnaeanum* (r^2 =0.998).

Cuticular wax analysis and calculation of the average chain-length distribution

For cuticular wax analysis, leaves or leaflets were pre-washed with deionized water to assure the removal of any contaminating particles from the surface and gently dried before the extraction. Prior to the wax extraction experiment, inflated calyces were cut on the top and fruits were carefully removed. Cuticular waxes of leaves and inflated calyces were extracted by their dipping in trichloromethane (≥ 99.8%, Roth) for 1 min at room temperature avoiding contact with cut parts (cut petioles or inflated fruiting calyx top). In species-rich in acyl sugars on its leaf surfaces, the wax extraction was made according to Bolger et al. (2014) with adaptations. Waxes from enzyme-isolated fruit cuticles were extracted by their full immersion in trichloromethane for 5 min at room temperature. As an internal standard, *n*-tetracosane (> 99.5%, Sigma-Aldrich) was added. The organic solvent was evaporated under a continuous flow of nitrogen.

Before gas chromatographic analysis, dry cuticular wax samples were derivatized with *N*,*O*-bis-trimethylsilyl-trifluoroacetamide (BSTFA, Macherey-Nagel) in pyridine (≥ 99.5%, Merck) at 70°C for 60 min to transform functional group-containing cuticular wax compounds into their corresponding trimethylsilyl derivatives.

The qualitative composition of cuticular waxes was determined by temperature-controlled capillary gas chromatography (6890N GC, Agilent Technologies) and on-column injection (DB-1, length of 30 m, inner diameter of 320 µm, film thickness of 0.1 µm; Agilent J&W GC column) with helium carrier gas inlet pressure programmed at 50 kPa for 5 min, 3 kPa min^{-1} to 150 kPa, and at 150 kPa for 40 min using a mass spectrometric detector (ionization energy 70 eV, mass range *m/z* 10 to 800; 5975 MSD, Agilent Technologies). Separation of the cuticular compounds was achieved using an initial temperature of 50°C for 2 min, raised by 40°C min^{-1} to 200°C, held at 200°C for 2 min, and, subsequently, raised by 3°C min^{-1} to 320°C and maintained at 320°C for 30 min. Quantitative composition of cuticular waxes was studied using capillary gas chromatography (7890A GC, Agilent Technologies) and flame ionization detection under the same gas chromatographic conditions but with hydrogen as the carrier gas.

The average chain length (*ACL*) distribution of the aliphatic compounds was calculated based on the cuticular wax amount using the equation:

$$ACL = \frac{\Sigma(C_n \times n)}{\Sigma(C_n)}$$

where C_n is the abundance of aliphatic moieties with n carbon units (Poynter and Eglinton, 1990).

Cutin matrix analysis

To depolymerize the non-extractable cutin matrix, enzyme-isolated and dewaxed fruit cuticles were transesterified with boron trifluoride in methanol (Roth) at 70°C overnight to release methyl esters of cutin acids. Sodium chloride-saturated aqueous solution, trichloromethane, and *n*-dotriacontane (Sigma-Aldrich) as an internal standard were added to all reaction mixtures. From this two-phase system, the transesterified cutin monomers were extracted three times with trichloromethane. The combined extracts were dried over anhydrous sodium sulphate. All extracts were filtered, and the organic solvent was evaporated under a continuous flow of nitrogen. Derivatisation with *N*,*O*-bis-trimethylsilyl-trifluoroacetamide in pyridine was performed at 60°C for 60 min.

Analysis of cutin monomers was performed similarly to the gas chromatographic analysis of cuticular waxes. Separation of cutin monomers was carried out at 50 kPa for 60 min, 10 kPa min^{-1} to 150 kPa, and at 150 kPa for 30 min using a temperature program of 50°C for 1 min, raised by 10°C min^{-1} to 150°C, held at 150°C for 2 min, and, subsequently, raised by 3°C min^{-1} to 320°C and maintained at 320°C for 30 min. Qualitative and quantitative composition was studied using capillary gas chromatography with mass spectrometric and flame ionization detection under the same chromatographic conditions. Single compounds were identified based on the electron ionization mass spectra using authentic standards, the Wiley 10th/NIST 2014 mass spectral library (W10N14, John Wiley & Sons) or by interpretation of the spectra, by the retention times and/or by comparison with literature data and quantitated by the internal standard.

Statistical and graphical analysis

Graphical data was created with Origin(Pro) (Version 2019, OriginLab Corporation), and statistical analyses were performed with SPSS Statistics software version 23.0 (IBM Corporation). Data were tested for normality by Shapiro-Wilk test. Two independent groups were compared using Student's *t*-test and Mann-Whitney *U*-test for normal and non-normal distributed data, respectively. More than two independent groups were compared using one-way analysis of variance (ANOVA) for normal distributed data followed by HSD posthoc test for unequal sample number. Kruskal-Wallis ANOVA compared non-normal distributed groups followed by posthoc Dunn's test. Correlation analyses between two normal distributed variables were carried out using Pearson correlation and non-normal distributed variables using Spearman's Rank correlation. For all tests, the considered level of significance was $p < 0.05$.

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Chapter I. Comparative analysis of the cuticular transpiration barrier of wild and cultivated *Solanum* species

1 Introduction

Amongst the abiotic stresses, drought is the leading cause of productivity losses in crop and other plant species, seriously impairing plant growth and development besides inducing a higher vegetation mortality rate (da Silva et al., 2013; Bodner et al., 2015). Thus, the understanding of physiological and biochemical responses to drought stress is crucial for efficient management of the plant water status (Fahad et al., 2017). The development of a protective lipidic extracellular layer, named the cuticle, was an evolutionary response of land plants to excessive water loss. The cuticle is composed of a cutin matrix and associated cuticular waxes, but polysaccharides and phenolic compounds such as tocopherols can also be present (Guzmán-Delgado et al., 2016). As the interface between the plant tissue and its environment, one of its most important functions is the barrier against uncontrolled water loss from primary organs from primary plant parts to the atmosphere (Riederer & Schreiber, 2001).

Cuticular waxes provide the main barrier properties of the plant cuticle and largely influence plant interaction with biotic and abiotic factors, being a critical determinant in agricultural crop quality. Wild plant species also rely on cuticle properties to enhance their responsiveness to naturally occurring abiotic stresses, such as temperature extremes, drought and salinity, as well as its defences against herbivore and pathogen attacks (Jetter & Riederer, 2016; Klavins & Klavins, 2020).

The *Solanum* genus comprises some of the world's most agriculturally important crop species, including *S. lycopersicum*, *S. tuberosum* and *S. melongena*. Minor or underutilized species of regional or local significance include *S. quitoense*, *S. muricatum* and *S. retroflexum* (Edmonds & Chweya, 1997; Poczai et al., 2010; Rodríguez-Burruezo et al., 2011; Vorontsova et al., 2013; Herraiz et al., 2015). Besides its agricultural importance, species of this genus are extensively

used as medicinal plants, being a rich source of steroidal sapogenins and alkaloids, or as ornamental plants due to their attractive fruits and flowers, such as *S. pseudocapsicum* (Roia & Smith, 1977; Pereira et al., 2014; Modilal et al., 2015). Moreover, numerous *Solanum* species grow as wild and are less known or used plant species, such as *S. burchellii, S. dulcamara*, *S. linnaeanum*, *S. virginianum* and *S. pennellii* (Table 1; Gbile & Adesina, 1988; National Research Council, 1989; Eijlander & Stiekema, 1994; Fawzi & Habeeb, 2016; Syfert et al., 2016).

Up to now, apart from studies based on plant morphology and taxonomy, most of the information available about cuticle biosynthesis, composition and function of *Solanum* species are limited to few plant species, mostly cultivated plant species from *Solanum* subgenera *Leptostemonum* and *Potatoe* (Barnes et al., 1996; Szafranek & Synak, 2006; Haliński et al., 2011; Leide et al., 2011; da Silva et al., 2012; Haliński & Stepnowski, 2016). However, the long domestication process has been selecting a limited set of desirable traits, such as attractive fruit colour, flavour and size. This intensive selection might have affected plant surface properties, but the existence of unique cuticle features between wild and cultivated plant species is still unknown. Furthermore, despite fruit cuticle importance as a determinant in quality and shelf-life, such as firmness and susceptibility to infections, cuticle-related plant properties have not been investigated extensively (Lara et al., 2014, 2015; Martin & Rose, 2014; Zarrouk et al., 2018).

Cuticle function, ultrastructure and chemical composition can vary across plant species, cultivars, and even within individual plants. Hence, addressing these variables is fundamental to broaden our knowledge of different strategies and adaptations of plants to minimize the transpirational water loss (Matzke & Riederer, 1991; Kerstiens, 1996c; Bonaventure et al. 2004; Khayet & Fernández, 2012; Xu et al., 2014; Fernandéz et al., 2016; Leide et al., 2018). Thus, this study aims to investigate the cuticle composition, function and structure on leaves and fruits of plant species belonging to the *Solanum* genus to identify possible similarities among taxonomically related plant species. The properties of the cuticular transpiration barrier of wild and cultivated plant species were analysed organ- and species-specifically and among different cultivars to determine potential cuticle specificities (Fig. 1, Table 1).

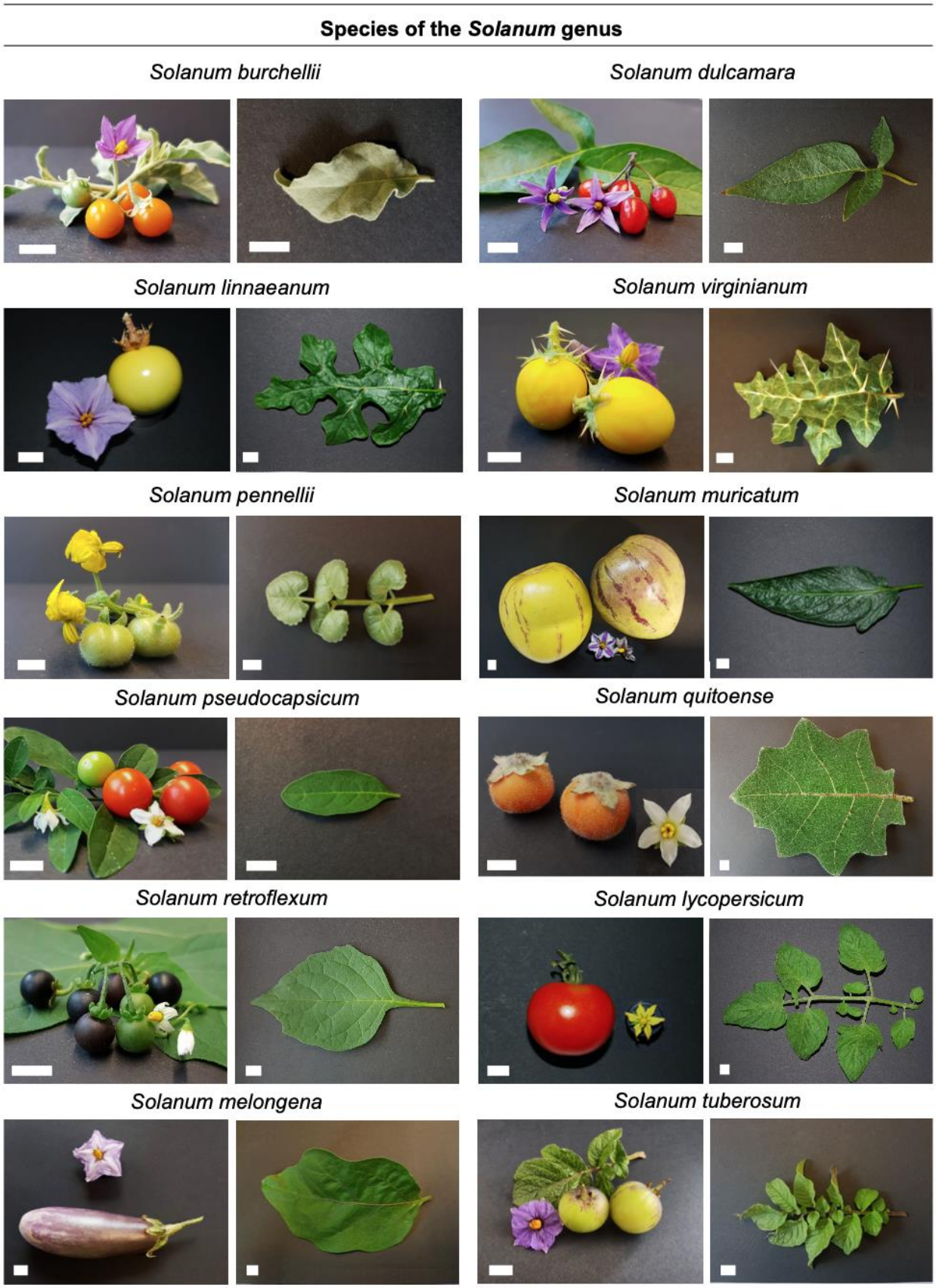


Fig. 1. Fruits, flowers and leaves of *Solanum* species showing the morphological diversity of this genus. *Solanum linnaeanum, S. virginianum, S. melongena, and S. quitoense* had prickles on their leaf surfaces. Scale bar = 1 cm.

Table 1. Plant species of the *Solanum* genus selected for this study. Plant species are classified according to their cultivations status, following the classification summarized by Messinger (2019) with adaptations, in wild, minor and major cultivated plant species.

***Solanum* species**	**subgenus**	**common name**	**origin**	**cultivation status**
Solanum burchellii Dunal	*Leptostemonum*	lemoenbossie	South Africa	wild
Solanum dulcamara L	*Solanum*	bittersweet	Eurasia	wild
Solanum linnaeanum Hepper & P.-M.L. Jaeger	*Leptostemonum*	devil's apple	South Africa	wild
Solanum pennellii Correll	*Potatoe*	wild tomato	South America	wild
Solanum virginianum L.	*Leptostemonum*	yellow-fruit nightshade	Asia	wild
Solanum muricatum Aiton	*Potatoe*	sweet cucumber	South America	minor cultivated
Solanum pseudocapsicum L.	*Solanum*	jerusalem cherry	Americas	minor cultivated
Solanum quitoense Lam.	*Leptostemonum*	lulo	South America	minor cultivated
Solanum retroflexum Dunal	*Solanum*	sunberry	South Africa	minor cultivated
Solanum lycopersicum L. cv. 'Benarys Gartenfreude'	*Potatoe*	tomato	South America	major cultivated
Solanum lycopersicum L. cv. 'John Baer'	*Potatoe*	tomato	South America	major cultivated
Solanum lycopersicum L. cv. 'Pearson'	*Potatoe*	tomato	South America	major cultivated
Solanum melongena L. cv. 'Slim Jim'	*Leptostemonum*	eggplant	Asia	major cultivated
Solanum tuberosum L. ssp. andigena Hawkes	*Potatoe*	potato	South America	major cultivated

2 Results

2.1 Epicuticular structure of the leaf and fruit surface of Solanum species

Samples of fully expanded leaves and ripe fruits were analysed by scanning electron microscopy to assess the tissue-, organ- and species-specific surface morphology of *Solanum* species (Fig. 2). Principal features investigated on the leaf and fruit surfaces were the presence of stomata or lenticels, trichomes and the epicuticular wax structure. Additional scanning electron micrographs in higher magnifications are presented in the appendix section.

Amphistomatic leaves with stomata on both the adaxial and the abaxial leaf surface were detected in the *Solanum* species. Stomata could not be observed due to the stellate indumentum completely covering the abaxial leaf surface of *S. quitoense* and both leaf surfaces of *S. burchellii*.

Trichomes were present on the adaxial and abaxial surfaces of all *Solanum* species studied. Most of the *Solanum* species bore simple, non-glandular and glandular trichomes. Non-glandular, stellate trichomes were observed exclusively in the plant species of the Leptostemonum subgenus *S. burchellii*, *S. melongena*, *S. quitoense*, *S. linnaeanum* and *S. virginianum*. Thick and short four-lobed, glandular trichomes were observed in the *S. lycopersicum* cultivars 'Benarys Gartenfreude', 'John Baer' and 'Pearson'. *S. pennellii* and *S. retroflexum* leaves bore solely glandular trichomes on their leaf surfaces. Contrary, only simple or branched non-glandular trichomes were observed on *S. pseudocapsicum* leaves.

The scanning electron micrographs from leaves revealed surfaces covered by an epicuticular wax film with irregular epicuticular wax granules intermittently covering both leaf surfaces in most of these *Solanum* species. Platelets were also found in *S. melongena* and *S. linnaeanum* leaves. Clusters of rodletes were observed on the surface of *S. retroflexum*, *S. dulcamara, S. melongena* and *S. tuberosum*.

The fruit surface was free of trichomes or stomata for most of these *Solanum* species. Exceptionally, trichome or scars of broken-off trichomes were found on the fruit surface of *S. lycopersicum* (all three cultivars), *S. pennellii*, *S. quitoense*, and *S. burchellii*. Stomata were observed solely on *S. muricatum*. *S. tuberosum* had lenticels, mostly covered by epicuticular waxes, on its fruit surface. Several microcracks filled with cuticular waxes were present on *S. melongena* fruit surface. A smooth layer of an epicuticular wax film covered the fruit cuticles, and epicuticular granules were irregularly distributed in most of the *Solanum* species. Vertical platelets were found mainly on *S. burchellii* and *S. tuberosum*, whereas, *S. pennellii* had horizontal platelets covering its fruit surface.

The shape of pavement cells was revealed by the imprints of their periclinal and anticlinal walls on the fruit cuticle. The inner cuticular side was characterized by straight cell boundaries giving to the cuticle interior a honeycomb-like pattern for *S. dulcamara*, *S. muricatum*, *S. pennellii*, *S. retroflexum* and *S. tuberosum*. All other *Solanum* species investigated had a sponge-like porous structure on its inner cuticular side.

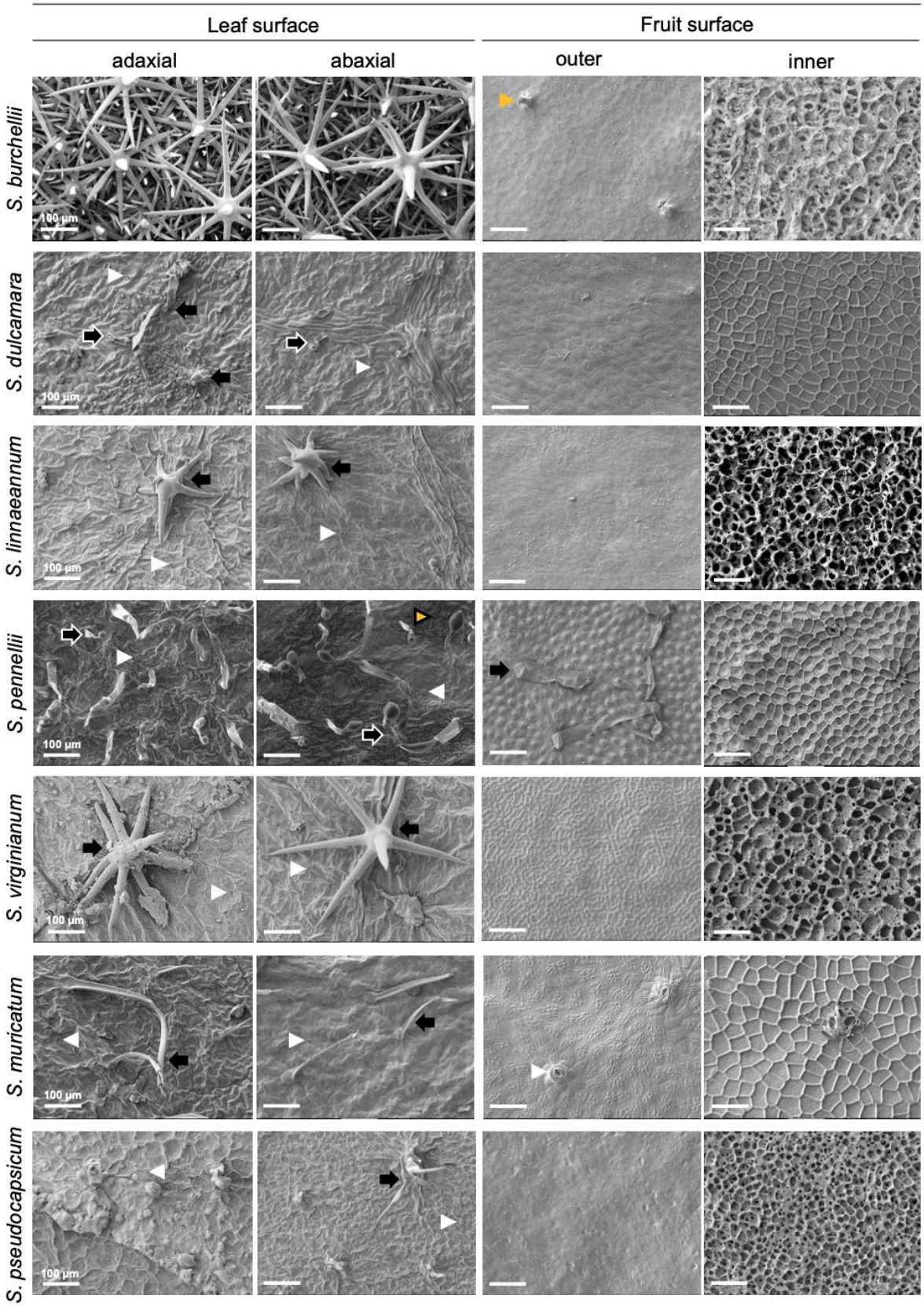


Fig. 2. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and isolated fruit cuticles (outer and inner sides) of *Solanum* species: wild plant species (A-E), minor cultivated (F-I) and major cultivated plant species (J-N). Arrowheads indicate stomata (white), lenticels (black contour and white filling), protuberances or trichome attachment scars (light orange), glandular trichome exudates (black contour and orange filling). Arrows indicate non-glandular trichomes (black) and glandular trichomes (white contour and black filling).

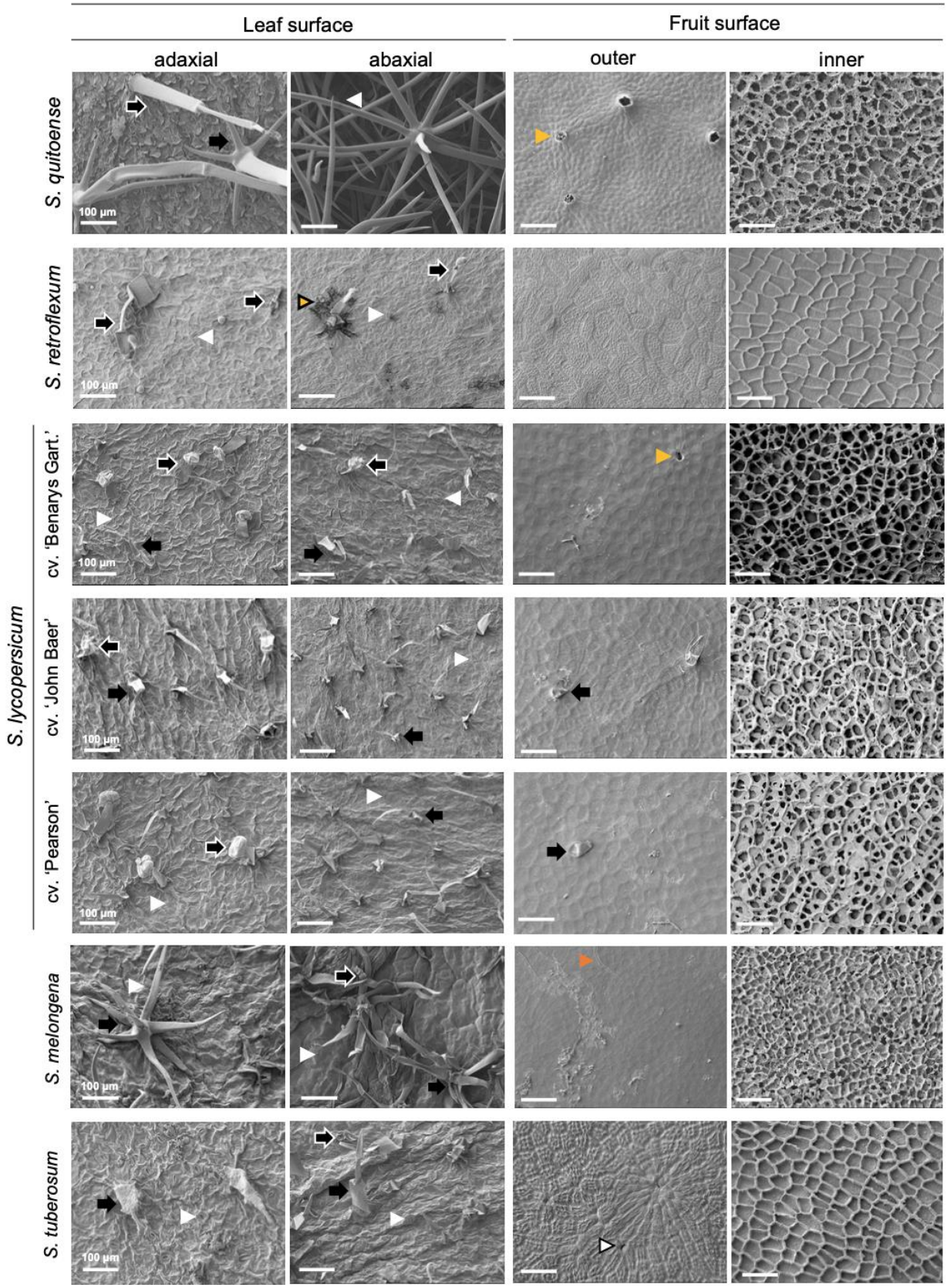


Fig. 2 (continued). Scanning electron micrographs from leaf (adaxial and abaxial surface) and isolated fruit cuticles (outer and inner side) of *Solanum* species selected for this study: wild plant species (A-E), minor cultivated (F-I) and major cultivated plant species (J-N). Arrowheads indicate stomata (white), lenticels (black contour and white filling), protuberances or trichome attachment scars (light orange), microcracks (dark orange), glandular trichome exudates (black contour and

orange filling). Arrows indicate non-glandular trichomes (black) and glandular trichomes (white contour and black filling).

2.2 Water permeability in the leaf and the fruit of Solanum species

The cuticular water conductance for astomatous fruits and the minimum water conductance for stomatous fruits and leaves with maximal stomatal closure was determined in *Solanum* species by relating the water loss rate to the surface area (Table 2).

At an organ-specific level, most of the *Solanum* species showed significant differences for the water permeability between fully expanded leaves and ripe fruits (Fig. 3). Within the same *Solanum* species, the leaf had a significantly higher water permeability in comparison to fruit for the majority of the *Solanum* species studied, being a difference up to nine-fold in *S. muricatum*. Exceptionally, *S. lycopersicum* cultivar 'Benarys Gartenfreude' and *S. quitoense* had two-fold higher water permeability for fruits than for leaves. Differences in water permeability between leaves and fruits of *S. lycopersicum* cultivars 'John Baer' and 'Pearson', *S. retroflexum* and *S. tuberosum* were not detected.

Among the *S. lycopersicum* cultivars, a difference was found only for leaves of 'Benarys Gartenfreude', which had a significantly lower minimum water conductance in comparison to the leaves of *S. lycopersicum* cultivars 'John Baer' and 'Pearson' (F (2.29) = 43.439, $p < 0.001$). Cultivar-specific differences in the cuticular water conductance of *S. lycopersicum* fruits were not detected ($p = 0.63$).

2.2.1 Species-specific differences among the Solanum species

Species-specifically, water permeability for fruits ranged from 0.64×10^{-5} m s^{-1} for the wild *Solanum* species *S. dulcamara* to 9.64×10^{-5} m s^{-1} in the major cultivated *Solanum* species *S. lycopersicum* cultivar 'John Baer'. Leaves had a broader range of minimum water conductance. The leaf minimum water conductance varied up to twelve-fold among the *Solanum* species, with the lowest minimum water conductance found for the wild *Solanum* species *S. linnaeanum* with

2.64×10^{-5} m s^{-1} and the highest for leaves of the minor cultivated *Solanum* species *S. muricatum* with 31.54×10^{-5} m s^{-1}.

Table 2. Water permeability of fully expanded leaves and ripe fruits of *Solanum* species grouped by cultivation status. Each value represents the mean ± standard deviation ($n \geq 8$). leaves ($n \geq 8$) and fruits ($n \geq 10$); Statistics (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Statistical differences were analysed by Mann-Whitney-*U*-Test or *t*-test.

***Solanum* species**	**Water permeability × 10^5 (m s^{-1})**		
	leaf	**fruit**	**ratio$_{leaf:fruit}$**
S. burchellii	5.16 ± 1.24	3.82 ± 1.61	1.4*
S. dulcamara	3.85 ± 1.04	0.64 ± 0.19	6.0***
S. linnaeanum	2.64 ± 0.88	1.10 ± 0.59	2.4***
S. pennellii	4.17 ± 0.59	2.09 ± 0.64	1.9***
S. virginianum	5.22 ± 1.08	1.73 ± 0.77	3.0***
S. muricatum	31.54 ± 7.27	3.48 ± 1.11	9.1***
S. pseudocapsicum	6.43 ± 1.11	2.02 ± 0.44	3.2***
S. quitoense	3.62 ± 0.96	6.68 ± 1.13	1.8***
S. retroflexum	3.42 ± 0.74	4.33 ± 1.39	0.8 n.s.
S. lycopersicum cv. 'Benarys Gartenfreude'	3.05 ± 0.56	6.01 ± 2.29	2.0***
S. lycopersicum cv. 'John Baer'	10.07 ± 3.33	9.64 ± 3.88	1.0 n.s.
S. lycopersicum cv. 'Pearson'	8.99 ± 3.87	6.63 ± 2.45	1.4 n.s.
S. melongena	12.87 ± 3.75	2.64 ± 0.66	4.9***
S. tuberosum	7.39 ± 1.16	6.72 ± 1.19	1.1 n.s.

Considering the degree of domestication of the *Solanum* species (Fig. 4), wild *Solanum* species possessed a lower leaf minimum water conductance among the groups with an average of 4.21×10^{-5} m s^{-1} (X^2 (2) = 26.357, $p < 0.001$). Leaves of minor and major cultivated *Solanum* species did not differ significantly (average of 9.38×10^{-5} m s^{-1}, $p > 0.05$).

The water permeability of fruits was significantly different among the three clusters (X^2 (2) = 92.938, $p < 0.001$). Lower water permeability was found for fruits of wild *Solanum* species (average of 2.21×10^{-5} m s^{-1}) followed by minor cultivated *Solanum* species (average of 3.92×10^{-5} m s^{-1}), while fruits of major cultivated *Solanum* species had the highest water permeability among the clusters (average of 6.45×10^{-5} m s^{-1}).

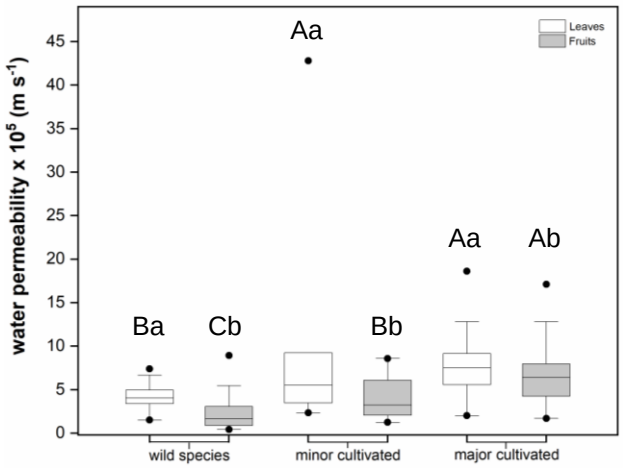


Fig. 3. Water permeability of fully expanded leaves and ripe fruits among wild, minor cultivated and major cultivated *Solanum* species. Different capital letters indicate a significant difference of water permeability (leaf or fruit) among the cultivation status and minor letters between leaves and fruits of the same group ($p < 0.05$). Statistical differences were analysed by Kruskal-Wallis or t-test.

2.3 Chemical analyses of the leaf and fruit cuticular waxes of Solanum species

Cuticular waxes were quantitatively and qualitatively analysed by gas chromatography. Detailed results of leaf and fruit cuticular wax composition of the twelve *Solanum* species studied are presented in the appendix section.

Leaves and fruits of the *Solanum* species were characterized by a wide range of total cuticular wax coverage and varied cuticular chemical composition, primarily composing of very-long-chain aliphatic compounds. The cuticular wax coverage of the *Solanum* leaves ranged up to 23-fold from 1.24 µg cm^{-2} for *S. tuberosum* to 28.42 µg cm^{-2} for *S. burchellii*, and almost ten-fold in *Solanum* fruits from 3.40 µg cm^{-2} for *S. melongena* to 33.99 µg cm^{-2} for *S. pennellii*. The average chain length of the aliphatic wax fraction varied from 29.91 to 33.17 carbon atoms in leaves and from 28.11 to 35.34 carbon atoms in fruits (Table 3).

Aliphatic wax compounds comprised more than 66% of the total cuticular waxes of fully expanded leaves (Fig. 5) and ripe fruits (Fig. 6). The main compound classes identified were alkanals (C_{23} to C_{37}), alkanoic acids (C_{20} to C_{35}), alkyl esters (C_{31} to C_{52}), *n*-alkanes (C_{25} to C_{37}), branched-

alkanes (C_{27} to C_{37}) and primary alkanols (C_{22} to C_{34}). Additionally, *n*-alkenes (C_{31} to C_{37}) were present exclusively in *S. quitoense* leaves (4%, 0.53 ± 0.33 µg cm^{-2}) and traces of alkanones (C_{33}) were found on fruits of *S. lycopersicum* cultivar 'Benarys Gartenfreude' (<1%). Leaves of all *Solanum* species had *n*-alkanes as the major aliphatic compound class identified, comprising from 42% to 73% of the total cuticular waxes. A comparable result was obtained for fruits with *n*-alkanes corresponding from 36% to 80% of the total cuticular waxes, except for *S. tuberosum* fruit, in which alkyl esters was the predominant compound class (52%, 7.34 µg cm^{-2}).

Branched-alkanes (*iso*- and *anteiso*) were also found in the leaves of all *Solanum* species. These aliphatic wax components were identified in larger proportions in the majority of the *Solanum* species, ranging from 12% of the total cuticular waxes in *S. quitoense* (1.60 µg cm^{-2}) to 41% in *S. pennellii* (2.83 µg cm^{-2}). An exception was found for *S. linnaeanum* and *S. muricatum* leaves, which had primary alkanols as the second most abundant wax compound class, and alkanoic acids in *S. dulcamara*, *S. pseudocapsicum* and *S. virginianum* leaves.

Except for *S. dulcamara*, branched-alkanes were also present on *Solanum* fruits but in lower proportions than found for leaves (≤ 10%). Following the very-long-chain *n*-alkanes, alkanoic acids were the second prominent aliphatic wax compound class on fruits and comprised up to 38% of the total cuticular waxes. In contrast, the second most abundant aliphatic wax compound class was primary alkanols in *S. burchellii* (30%, 7.93 µg cm^{-2}), alkyl esters in *S. lycopersicum* cultivar 'Benarys Gartenfreude' (12%, 2.41 µg cm^{-2}), and branched-alkanes in *S. pennellii* (7%, 2.46 µg cm^{-2}) and *S. retroflexum* fruits (10%, 0.53 µg cm^{-2}).

Table 3. Cuticular wax coverage and average chain length (ACL) of fully expanded leaves and ripe fruits of *Solanum* species. Values represent the mean ± standard deviation (n ≥ 4).

		Leaf		Fruit	
		wax coverage	ACL	wax coverage	ACL
Cultivation status	***Solanum* species**	(µg cm^{-2})		(µg cm^{-2})	
wild species	*S. burchellii*	28.42 ± 5.74	32.84	26.76 ± 3.24	30.89
	S. dulcamara	2.21 ± 0.31	30.58	4.70 ± 0.51	28.26
	S. linnaeanum	7.38 ± 1.34	32.02	13.60 ± 3.26	29.83
	S. pennellii	6.97 ± 1.38	31.29	33.99 ± 5.70	30.84
	S. virginianum	5.23 ± 1.06	33.17	3.51 ± 0.78	30.12
minor cultivated	*S. muricatum*	2.14 ± 0.13	32.14	7.87 ± 1.16	31.20
	S. pseudocapsicum	3.08 ± 0.32	29.91	5.14 ± 1.03	30.21
	S. quitoense	1.91 ± 0.21	32.65	14.92 ± 1.27	26.50
	S. retroflexum	13.60 ± 3.25	31.88	5.12 ± 0.70	29.09
major cultivated	*S. lycopersicum* cv. ‘Benarys Gartenfreude.’	2.27 ± 0.46	31.00	20.42 ± 7.93	32.21
	S. lycopersicum cv. ‘John Baer’	1.94 ± 0.11	31.05	12.61 ± 2.90	29.98
	S. lycopersicum cv. ‘Pearson’	2.66 ± 0.10	31.09	10.37 ± 1.95	30.32
	S. melongena	6.32 ± 0.68	33.05	3.40 ± 0.87	30.79
	S. tuberosum	1.24 ± 0.08	30.09	14.11 ± 3.05	35.34

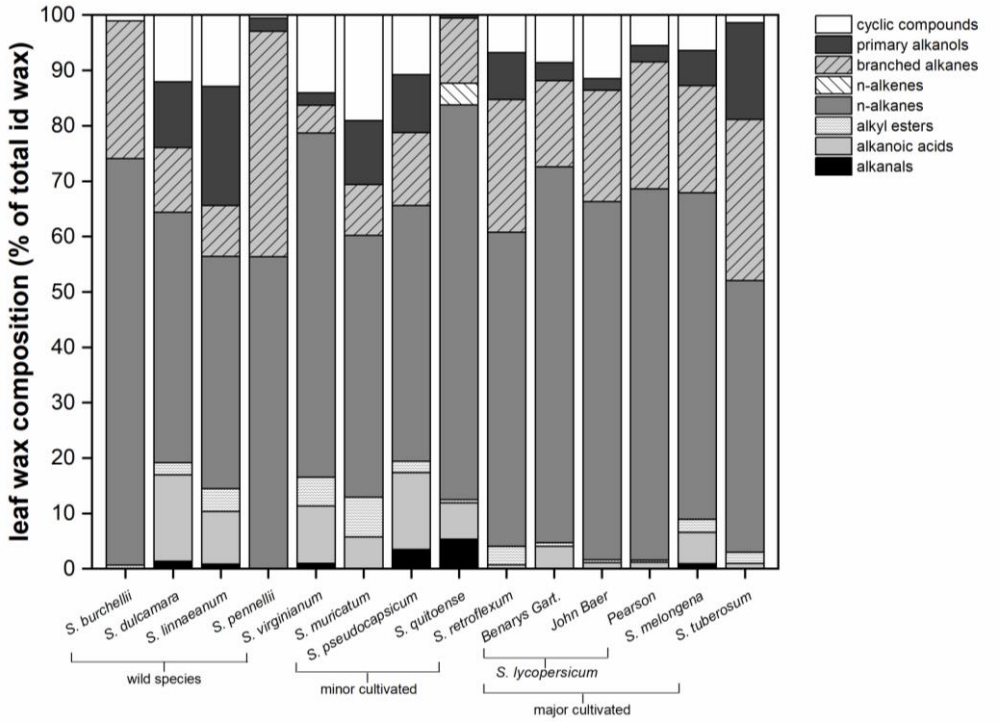


Fig.5. Relative cuticular wax composition of fully expanded leaves of *Solanum* species (n ≥ 4) according to their cultivation status (wild, minor cultivated and major cultivated).

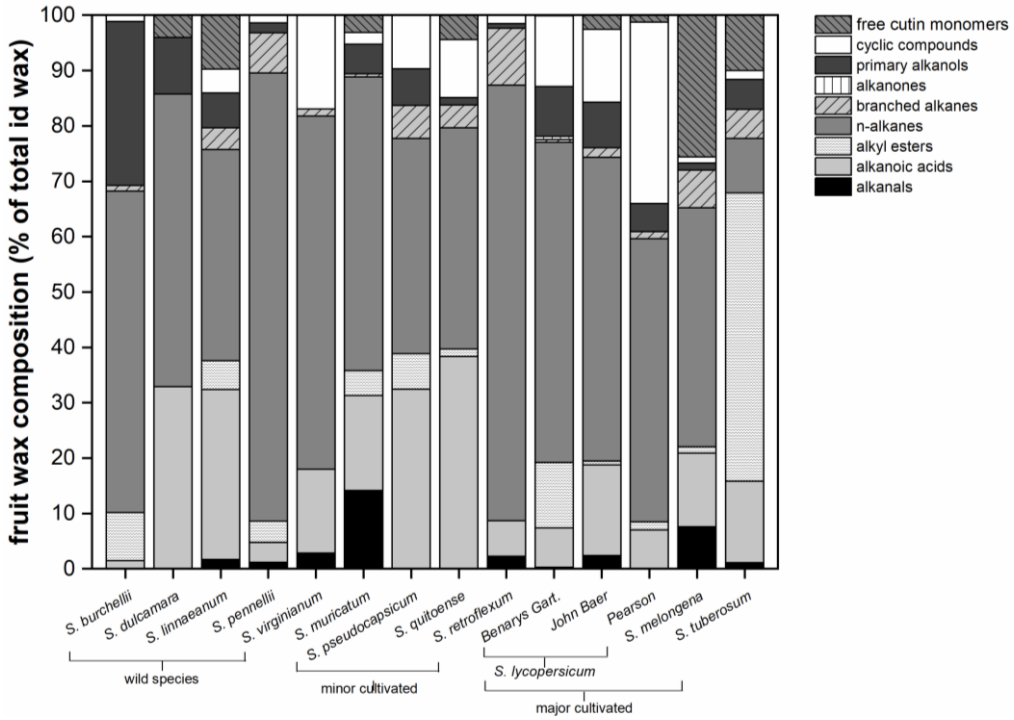


Fig. 6. Relative cuticular wax composition of ripe fruits of *Solanum* species (n ≥ 4) according to their cultivation status (wild, minor cultivated and major cultivated).

Although in minor amounts, alicyclic and aromatic components were also identified in most of the cuticular waxes, except for *S. quitoense* leaf and *S. dulcamara* fruit. The alicyclic compounds identified were sterols (campesterol, cholesterol, stigmasterol and β-sitosterol) and pentacyclic triterpenoids (α, β, δ-amyrins, ursolic acid and lupeol). The aromatic wax fraction was composed of phenylmethyl esters (C_{26} to C_{36}), coumaric acid esters (C_{26} to C_{32}), tocopherols (α, β, δ, γ-tocopherol) and flavonoids (naringenin).

Among *Solanum* leaves, *S. muricatum* had the highest cyclic wax fraction corresponding to 19% (0.59 µg cm^{-2}), followed by *S. virginianum* (14%, 0.73 µg cm^{-2}) and *S. linnaeanum* (13%, 0.95 µg cm^{-2}), while *S. pennellii* and *S. burchellii* had less than 1% of cyclic compounds in their cuticular waxes. Among the *Solanum* fruits, *S. lycopersicum* cultivar 'Pearson' had the highest cyclic wax fraction (33%, 3.40 µg cm^{-2}), which was more than 2.5-fold higher than the proportion found for the *S. lycopersicum* cultivars (13%) 'Benarys Gartenfreude' (2.61 µg cm^{-2}) and 'John Baer' (1.67 µg cm^{-2}). On the other hand, only traces of cyclic components (≤ 1%) were found in cuticular waxes of *S. burchellii, S. melongena, S. pennellii and S. retroflexum*.

Dihydroxy hexadecanoic acid and trihydroxy octadecenoic acid as non-esterified cutin monomers were identified exclusively on fruits of seven out of twelve *Solanum* species: *S. dulcamara*, *S. linnaeanum*, *S. lycopersicum*, *S. melongena*, *S. muricatum*, *S. quitoense* and *S. tuberosum*.

2.4 Correlation analysis

The water permeability was positively correlated with the cyclic fraction of cuticular wax coverage but only for fruits (Fig. 7). However, no correlation was found between water permeability and the total cuticular waxes, very-long-chain aliphatic wax fraction, *n*-alkanes as the predominant compound class or the average chain length of the aliphatic wax fraction neither on leaves nor on fruits of the *Solanum* species.

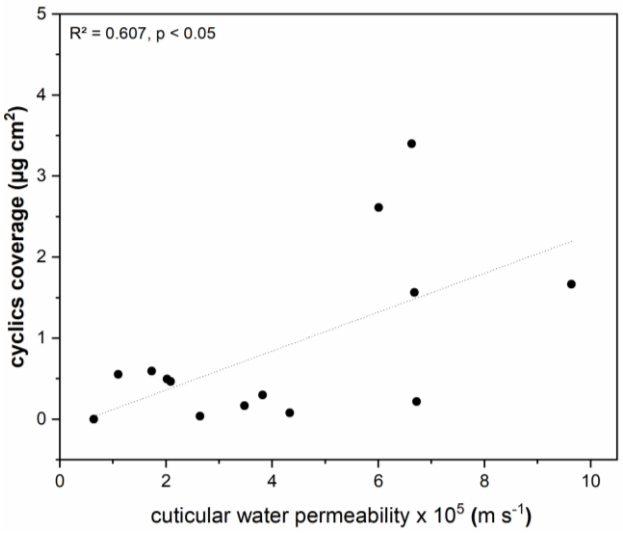


Fig. 7. Water permeability of fruits of *Solanum* species as a function of the cyclic fraction of the cuticular wax coverage (R^2 = 0.607, p = 0.02).

3 Discussion

In this comparative study, the leaf and fruit cuticle were analysed in twelve plant species belonging to the *Solanum* genus to identify possible similarities among taxonomically related plant species. Although being related plant species, it is still unclear whether wild and cultivated *Solanum* species, as well as different *Solanum* cultivars of the same plant species, differ in terms of chemical, structural and functional cuticle traits. Since the domestication process may additionally have been altered plant cuticle properties, wild plant species might present particular cuticle attributes that are less prevalent in the cultivated ones (Martin & Rose, 2014).

3.1 Structural diversity of leaves and fruits of Solanum species

The most distinguishable structural feature among the surfaces of the *Solanum* species investigated was their indumentum. Characterization of the plant surface revealed the presence of diverse types of trichomes, especially on leaves. Most of these trichomes are epidermal extensions and, therefore, are not connected to the vascular system, and composed of a single-cell or multicellular structures. Generally, they are divided into glandular or non-glandular trichomes (Wagner et al., 2004; Atito et al., 2018). Trichomes are important morphological features commonly used as taxonomic markers, including the *Solanum* genus (Ali & Al-Hemaid, 2011; McDowell et al., 2011; Bello et al., 2017). In accordance, non-glandular, stellate trichomes were only identified on leaf surfaces of the *Solanum* species belonging to the *Leptostemonum* subgenus *S. burchellii*, *S. linnaeanum*, *S. melongena*, *S. quitoense* and *S. virginianum*. Bifurcate, non-glandular trichomes were only present *in S. pseudocapsicum,* while four-lobed glandular trichomes were solely observed on *S. lycopersicum* leaf surface. The majority of the *Solanum* species had amphistomatic leaves with stomata mainly distributed on the abaxial side, which is a common feature among solanaceous plant species (Ahmad, 1964).

Glandular trichomes of type IV of *S. retroflexum* and *S. pennellii* leaves are characterized by having a single flat basal cell and a short stalk with a secretory cell on the tip. Acyl sugars, glucose or sucrose esters containing acyl groups are present inside of this glandular trichomes. These compounds are commonly produced by glandular trichomes of the solanaceous plant species (Vendemiatti et al., 2017; Nyaku & Danquah, 2019; Mihaylova-Kroumova et al., 2020). Previous studies on crop species and their wild relatives have shown that the domestication process can increase the susceptibility of plants to herbivore and pathogen attacks through the loss of important glandular trichome-derived metabolites. For instance, cultivated *Solanum* species *S. lycopersicum* produces only minor amounts of acyl sugars, and type-IV-trichomes are considered absent on their leaves (Oghiakhe, 1997; Medeiros and Tingey, 2006; Zhang et al., 2008; Besser et al., 2009; McDowell et al., 2011; Vendemiatti et al., 2017). Accordingly,

none of the *S. lycopersicum* cultivars here investigated, cv. 'Benarys Gartenfreude', cv. 'John Baer' and cv. 'Pearson', presented these type of glandular trichomes on their leaf surfaces, whereas type-IV-trichomes densely covered wild *Solanum* species *S. pennellii*.

The majority of fruits studied had stomata- and trichome-free surfaces. Exceptionally, stomata were found on the *S. muricatum* fruit surface. Although much less common than on leaves, the presence of stomata on the fruit surface suggests that *S. muricatum* fruits were photosynthetic active in earlier fruit developmental stages as observed for other solanaceous plant species (Sui et al., 2017; Yahia & Carrillo-Lopez, 2018). Nevertheless, the minimum water conductance found for stomatous *S. muricatum* fruits was in the same range than the cuticular water permeance measured for astomatous *Solanum* fruits. Other *Solanum* species possessing lenticels or holes impregnated with cuticular waxes on the fruit surface were *S. tuberosum*, *S. quitoense* and *S. lycopersicum* cv. 'Benarys Gartenfreude'. Epicuticular waxes also filled the micro-cracks observed on *S. melongena,* but its fruit had similar permeability to the measured for the wild species.

Interestingly, cuticle imperfections were only observed on minor and major cultivated *Solanum* species. It is known that changes in surface structure to achieve desirable agricultural traits, such as fruit colour or glossiness, can also favour the occurrence of physiological disorders. Remaining scars of trichomes are preferential sites of water loss and pathogenic infection that can lead to dehydration and fruit rot (Glenn & Poovaiah, 1989; Børve et al., 2000; Fernandéz et al., 2014; Lara et al., 2019). Hence, more studies should be conducted with these *Solanum* species in order to clarify the functionalities of stomata and lenticels of ripe fruits and their contribution to the water permeability.

3.2 Water permeability profiles in leaf and fruit of Solanum species

Analyses of the leaf and fruit revealed species- and organ-specific patterns for water permeability in most of the *Solanum* species studied. Surprisingly, leaves had a higher water cuticular transpiration, especially among the wild *Solanum* species, with fruits having values up to nine-fold lower or at least comparable to the leaves. These results are different from previous reports showing lower water permeability for leaves in comparison to fruits (Riederer & Schreiber, 2001; Huang, 2017). In this study, the cultivated species *S. quitoense* and *S. lycopersicum* cultivar 'Benarys Gartenfreude' were the only species having higher cuticular water losses in fruits in comparison to leaves. Most of the studies on water permeability of plant cuticle published so far have been conducted with a limited number of plant species, and mainly with cultivated ones (Martin & Rose, 2014; Lara et al., 2015; Domínguez et al., 2017). Therefore, the assumption that fruits have a higher cuticular water loss in relation to leaves cannot be generally applied, especially when considering wild and minor cultivated plant species or comparing leaves and fruits of different plant species.

Leaves of *S. muricatum*, an evergreen shrub, whose origin is the Andean region of South America, had the highest water permeability among the *Solanum* species (31.5×10^{-5} m s^{-1}), being up to twelve-fold higher in comparison to the other *Solanum* species. Moreover, a relatively low cuticular wax coverage with a proportionally very high cyclic wax fraction was identified in this same *Solanum* species, comprising 19% of the total cuticular waxes of leaves. Tocopherols, mainly β-tocopherol, were the most prominent aromatic components identified in *S. muricatum*. These lipophilic compounds were previously reported as leaf cuticular wax constituents of numerous plant species, which are associated with the response of photosynthetic tissues to abiotic stresses, including low temperature, high-intensity light and drought (Gülz et al., 1992; Maeda et al., 2006; Riederer & Muller, 2008; Schuster, 2016). However, while the aliphatic fraction of cuticular waxes primarily forms the cuticular transpiration barrier, cyclic compounds have a minor contribution to the avoidance of the water loss of plant cuticles (Jetter and Riederer; 2016; Schuster et al., 2016).

3.3 Differences of the cuticular transpiration barrier between wild and cultivated species

To further examine whether the degrees of plant domestication has an impact on water permeability, wild, minor cultivated and major cultivated *Solanum* species were compared. Considering the cultivation status of the *Solanum* species studied, wild plant species showed significantly lower water permeability for leaves and fruits, and, thus, a better cuticular transpiration barrier in relation to the cultivated ones. Leaves showed two-fold lower water permeability when compared to the cultivated ones, and fruits had two- and three-fold lower water permeability than that of minor and major cultivated plant species, respectively. Thus, especially for fruits of *Solanum* species, the most target organs in the selection of organoleptic properties and breeding programs (Prohens et al., 2003; Rodríguez-Burruezo, 2011; Sim et al., 2011), a gradient of water permeability was identified according to their cultivation status: the higher is the degree of domestication, the higher is the water permeability. These results indicate that the domestication process likely impacts cuticular properties leading to increased water permeability in cultivated leaves and fruits.

3.4 Cuticular wax composition of leaves and fruits of Solanum species

Considering the cuticular wax composition, fruits and leaves of the *Solanum* species were predominantly composed of very-long-chain *n*-alkanes, which is in accordance with other studies on solanaceous plant species, being this compound class even considered a taxonomic marker within the Solanaceae family (da Silva et al., 2012; Yeats et al., 2012; Lara et al., 2015). However, the high variability in cuticular wax coverage was the most remarkable outcome concerning the cuticular waxes of the *Solanum* species. Leaves of *S. burchellii*, an evergreen shrub, showed an impressive cuticular wax accumulation of 28.42 µg cm^{-2}, while fruits of *S. pennellii*, a perennial herb, had a cuticular wax accumulation of 33.99 µg cm^{-2}. Interestingly, both species are endemic to arid regions, with *S. burchellii* found in shrublands of southern Namibia and north-western Cape Province, while *S. pennellii* is confined to coastal

areas of the Atacama Desert of Peru (Jaeger, 1985; Coneva et al., 2017). Thus, this very high cuticular wax accumulation could represent an adaption of these *Solanum* species to their habitats characterized by arid conditions. Indeed, it was previously described that plants under water-limiting conditions had increased deposition of cuticular waxes, including other solanaceous plant species such as *Nicotiana glauca* (Cameron et al., 2006; Kosma et al., 2009; Bueno et al., 2019). Nevertheless, the relationship between cuticular wax amount and composition and the cuticular water permeability is still unclear, and further efforts to promote a better understanding of how cuticle properties contribute to avoiding uncontrolled water loss should be made.

4 Conclusion

The efficiency of the cuticular transpiration barrier of leaves and fruits was investigated in twelve *Solanum* species with different cultivation status. A species- and organ-specific pattern for water permeability, cuticular wax amount and composition was found for most of the *Solanum* species. To the authors' best knowledge, this is the first time it was shown that wild *Solanum* species have a more efficient cuticular transpiration barrier in vegetative and reproductive organs in comparison to minor and major cultivated species. This finding suggests that the domestication process and selection for desirable traits affect the plant cuticle functionality. Additionally, cuticle properties are most likely affected systemically, since higher water permeability was not limited to target organs by breeding. A moderate positive correlation between water permeability and the cyclic fraction of the cuticular waxes implies that the cuticular wax biosynthesis of fruit cuticles are modified in the cultivated plant species to favour their organoleptic properties. Thus, this results with the *Solanum* species serve as a base for future studies on plant cuticle-associated traits and improvement of transpiration efficiency in plant species of agricultural relevance. Furthermore, efforts should be made, especially investigating wild and minor cultivated plant species, in order to clarify the mechanisms behind water permeability and how cuticular traits are affected by artificial selection leading to an increased water loss in plants.

Chapter II. Chemical, functional and structural analyses of the plant cuticle in solanaceous species bearing inflated fruiting calyx

1 Introduction

Land plants have evolved and diversified to reduce the detrimental factors imposed by living on an atmospheric environment and developed innumerable adaptations to increase their performance and fitness (Huot et al., 2013), such as the plant cuticle. This continuous extracellular hydrophobic layer acts as a natural interface between aerial plant organs and its surrounding environment, allowing plants to tolerate the drier atmosphere and cope with the unfavourable conditions on land (Riederer & Schreiber, 2001). It plays a significant role in preventing plant desiccation by limiting the non-stomatal water loss from primary plant surfaces (Kerstiens,1996c; Burghardt & Riederer, 2006).

The cuticle is formed by a cutin polymer matrix, in which the cuticular waxes are deposited. Cuticular waxes predominantly composed of very-long-chain aliphatic compounds (*n*-alkanes, primary alkanols, alkanoic acids, alkanals and alkyl esters) and variable amounts of pentacyclic triterpenoids. By impregnating as well as covering the cutin matrix, this complex mixture of cuticular waxes provides the main barrier properties of the cuticle (Riederer & Schreiber, 2001; Schreiber, 2010). Cuticular biology studies have focused on vegetative plant tissues, principally leaves, and studies on composition, structure and function of fruit cuticles but comparative studies of the cuticular properties in different plant organs of the same plant species are still scarce (Lara et al., 2014; Fernandéz et al., 2016; Huang et al., 2017).

The Solanaceae family is one of the largest and most complex groups of angiosperms with many plant species having considerable economic importance as crop plants, drug plants and ornamental plants (Knapp et al., 2004). A remarkable diversity of habitats, life-forms, morphological traits, especially floral parts and fruits, and chemical compositions characterise the members of this family (Knapp, 2002; Barboza et al., 2016). The presence of an inflated

papery calyx is a prominent feature that makes *Physalis* and *Nicandra* highly recognisable solanaceous genera (Wilf et al., 2017). However, the inflated fruiting calyx is found at least in seven more Solanaceae genera, including *Cuatreasia*, *Exodeconus*, *Margaranthus*, *Physaliastrum*, *Physochlaina*, *Przewalskia*, and *Withania* (D'Arcy, 1991; Hu & Saedler, 2007). The inflated calyx syndrome (ICS) is a post-floral morphological novelty within Solanaceae, which causes the sepals to increase and inflate after flower fertilisation, forming a charismatic structure popularly known as the Chinese lantern (He & Saedler, 2007; Wilf et al., 2017; Li et al., 2019). Interestingly, the ICS probably represent a plesiomorphic character that was lost in some lineages during the evolution (Hu & Saedler, 2007; Zhang et al., 2012), being lantern fruits recorded in fossils (*Physalis infinemundi* sp. nov.) from the Eocene (52.2 million years ago) suggesting an ancient origin of the inflated fruiting calyx structure in Solanaceae (Wilf et al., 2017).

The Chinese lantern comprises an accrescent, highly inflated, five-angled, gamosepalous, and pedicellate calyx encapsulating a fleshy berry (Wilf et al., 2017). *Nicandra* is unique for having auriculate calyx segments, with a deeply cordate or sagittate base (Horton, 1979). This evolutionary adaptive trait protects the fruit against herbivory and adverse climatic conditions, favours the dispersion of the fruit by water, and it is a source of carbohydrates during the first days of fruit development, thus improving plant fitness (Fischer,1995; Flórez et al., 2000; Herrera, 2005; Li et al., 2019). Besides similarly displaying the ICS, *Physalis* and *Nicandra* genera belong to the subfamily Solanoideae. The fifth-largest genus of the family, *Physalis* comprises about 100 plant species belonging to the tribe Physalideae, whereas Nicandreae is a monotypic and basal tribe (Knapp, 2002; Olmstead et al., 2008). *Physalis* and *Nicandra* are originated from tropical America, while *P. alkekengi var. franchetii* (Mast.) Makino (synonym: *Alkekengi officinarum* Moench) is from the old world most probably originated from Asia (te Beest et al., 1999).

The ICS in solanaceous species is well discussed in the literature. Yet, its post-floral functionality has been little investigated, and there is still a lack of information on the potential

adaptive and ecological functions of the inflated fruiting calyx (Herrera 2005; Wilf et al., 2017). Thus, this study aims to investigate the cuticular properties of *Physalis alkekengi* L.*, Physalis peruviana*, *Physalis* ixocarpa and *Nicandra physalodes* by comparing the efficiency of the transpiration barrier in different plant organs and addressing the functional significance of the inflated fruiting calyx for the cuticular water loss in fruits.

2 Results

Compositional, structural and functional cuticle attributes were analysed in leaf, inflated fruiting calyx and fruit of the four solanaceous species *Physalis alkekengi, P. ixocarpa, P. peruviana* and *N. physalodes* and the results are presented below:

2.1 Species-specific morphological traits of Physalis and Nicandra species

Physalis alkekengi, *P. peruviana*, *P. ixocarpa* and *N. physalodes* were herbaceous to arbustive plants with an inflated fruiting calyx hanging from the stem entirely enclosing the fruit (Fig. 1A). The inflated fruiting calyces and their enclosed fruits displayed impressive physical changes during development, especially related to their size, colour and water content (Fig. 1B and 1C). Fruit and calyx colouring were associated, and a papery dried calyx characterised the later stage of fruit maturation in the four plant species.

Before the inflated fruiting calyx dried out completely, its colour ranged from green in early stages to yellow in *P. peruviana* and *N. physalodes*. Ripe fruits of *P. peruviana* were bright orange, while *N. physalodes* had yellow-brownish fruits that were relatively dry. *Physalis ixocarpa* showed green or green-purple split calyx with ripe fruits in the same colour but slightly sticky. *Physalis alkekengi* had a striking variation in calyx and fruit pigmentation, which was initially green becoming gradually bright red, as well as its ripe fruit (Fig. 1C). Ripe fruits of the three *Physalis* species were glossy and fleshy (Fig. 1D). Nevertheless, these four plant species bore globose berries enclosed by an inflated and persistent calyx as a common morphological attribute.

The floral morphology was characterised by solitary and axillary flowers comprised by five anthers with longitudinal dehiscence, pentamerous perianth with gamopetalous sepals and corolla forming somewhat bell-shaped flowers in the Physalideae species and bell-shaped in *N. physalodes* (1D). Petals of *P. alkekengi* were white with yellow-green markings at the base whereas *P. peruviana* and *P. ixocarpa* had yellow petals with five striking purple or greenish spots at the base. *Nicandra physalodes* had a blue perianth with dark purple spots on a white base. The green leaf of the Physalideae species was ovate to broadly ovate, and sometimes wavy-margined in *P. ixocarpa* and *P. alkekengi*. In contrast, *Nicandra* leaves had an oval to elliptical laminar shape with sinuate margins. (Fig. 1E).

The surface area of the investigated plant organs was determined in order to relate the cuticular water loss to the transpiring area (Table 1). The highest surface area was found for leaves, and it was similar among the plant species with 30.4 ± 8.6 cm^2 (mean ± standard deviation, $p \geq 0.05$). *P. alkekengi* had the largest inflated fruiting calyx (27.1 ± 1.9 cm^2), which was two-fold larger ($F(3.40), 129.82, p < 0.001$) than the calyces of the other plant species (14. 4 ± 1.9 cm^2). The fruit had the lowest surface area among the plant organs investigated, and *Nicandra* produced the smallest fruits (5.3 ± 0.6 cm^2) among the plant species followed by *P. alkekengi* (9.8 ± 1.8 cm^2). The largest fruit surface was measured for *P. ixocarpa* (24.0 ± 3.7 cm^2) being from two- to fivefold higher than fruits of the other three plant species ($X^2(3) = 46.01, p < 0.05$).

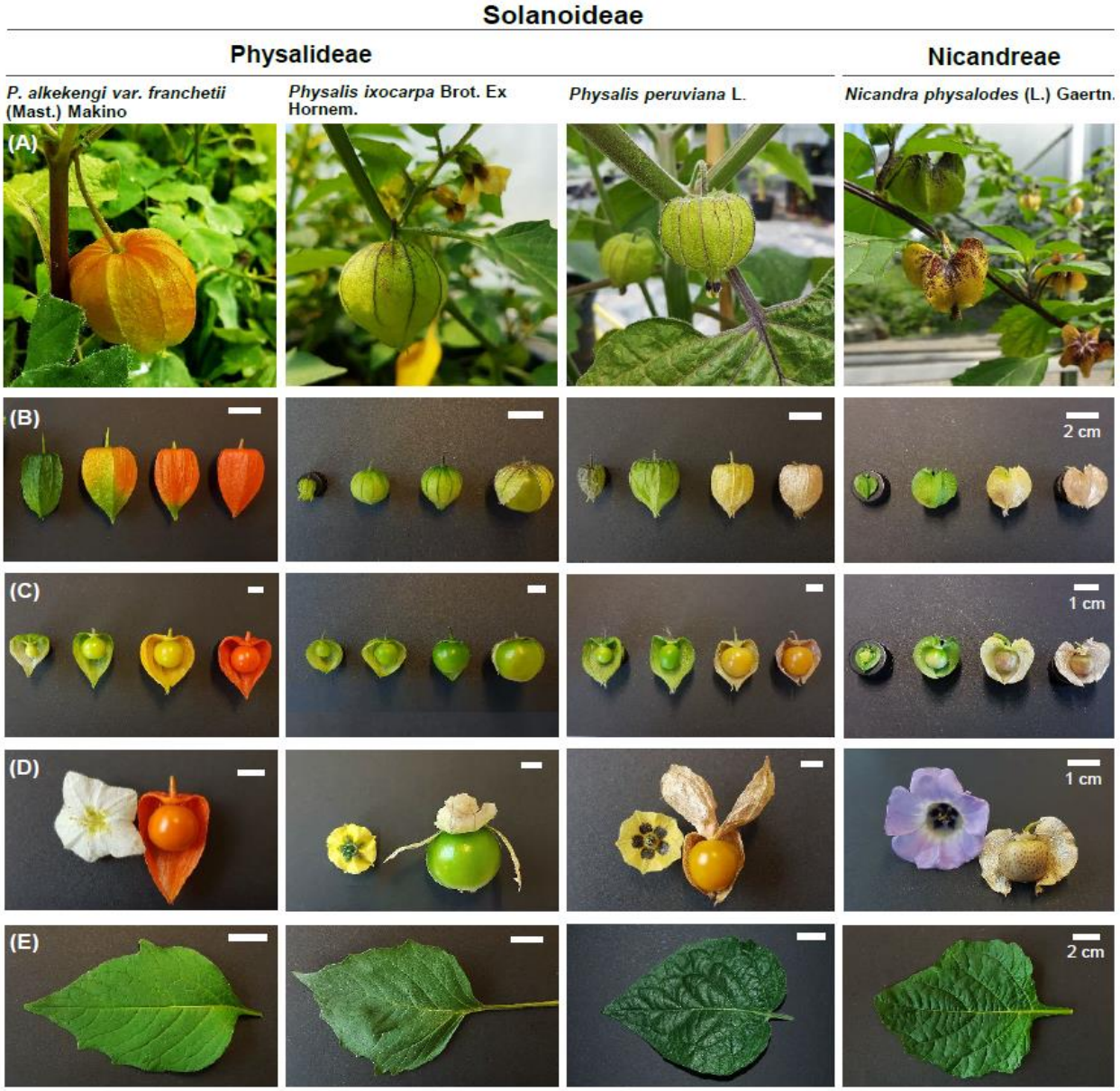


Fig. 1. Photographs of the plant species of the subfamily Solanoideae *P. alkekengi*, *P. ixocarpa*, *P. peruviana* (Physalideae tribe) and *N. physalodes* (Nicandreae tribe) showing fruits enclosed by the inflated fruiting calyx and attached to plant (A), developmental stages of the calyx (B) and fruits (C), ripe fruit and flower (D) and fully expanded leaf (E).

Table 1. Surface area of leaf, calyx and fruit of *P. alkekengi*, *P. ixocarpa*, *P. peruviana* and *N. physalodes*. Values represent the mean ± standard deviation (n ≥ 8). Different letters indicate a significant difference ($p < 0.05$) of the plant organ among plant species. Statistical differences were analysed by Anova or Kruskal-Wallis Anova test.

organs	surface area (cm^2)			
	P. alkekengi	*P. ixocarpa*	*P. peruviana*	*N. physalodes*
leaf	28.67 ± 9.92^{a}	30.81 ± 9.07^{a}	32.41 ± 6.82^{a}	30.46 ± 6.75^{a}
calyx	27.12 ± 1.82^{a}	13.56 ± 0.80^{b}	15.56 ± 2.10^{c}	13.91 ± 1.96^{bc}
fruit	9.78 ± 1.75^{b}	24.02 ± 3.70^{a}	12.08 ± 1.07^{ab}	5.26 ± 0.61^{c}

2.2 Organ-specific ultrastructural properties of the cuticular surface

Samples of leaves, inflated fruiting calyces and fruit cuticles were analysed by scanning electron microscopy (SEM) to assess the surface morphology of the plant organs. Microscopically, leaves similarly had stomata on both surfaces (Fig. 2). Simple, non-glandular and glandular trichomes were also observed in all plant species, but *N. physalodes* had only sparsely hairy leaf surfaces. *Physalis peruviana* leaves were more densely covered by trichomes than the other plant species, not only on leaves but also on the adaxial surface of the inflated fruiting calyx. Stomata were detected solely on the adaxial surface of the inflated fruiting calyx, but glandular trichomes were also present on the abaxial surface of *P. alkekengi* and *N. physalodes* calyces (Fig. 3). The examination of the ultrastructure of the leaf and the inflated fruiting calyx revealed surfaces covered by an epicuticular wax film. Irregular epicuticular wax granules and crystal-like platelets intermittently covered the abaxial and adaxial surfaces of leaves and calyces. Clusters of rodletes were also observed on the surface of *Nicandra* leaves.

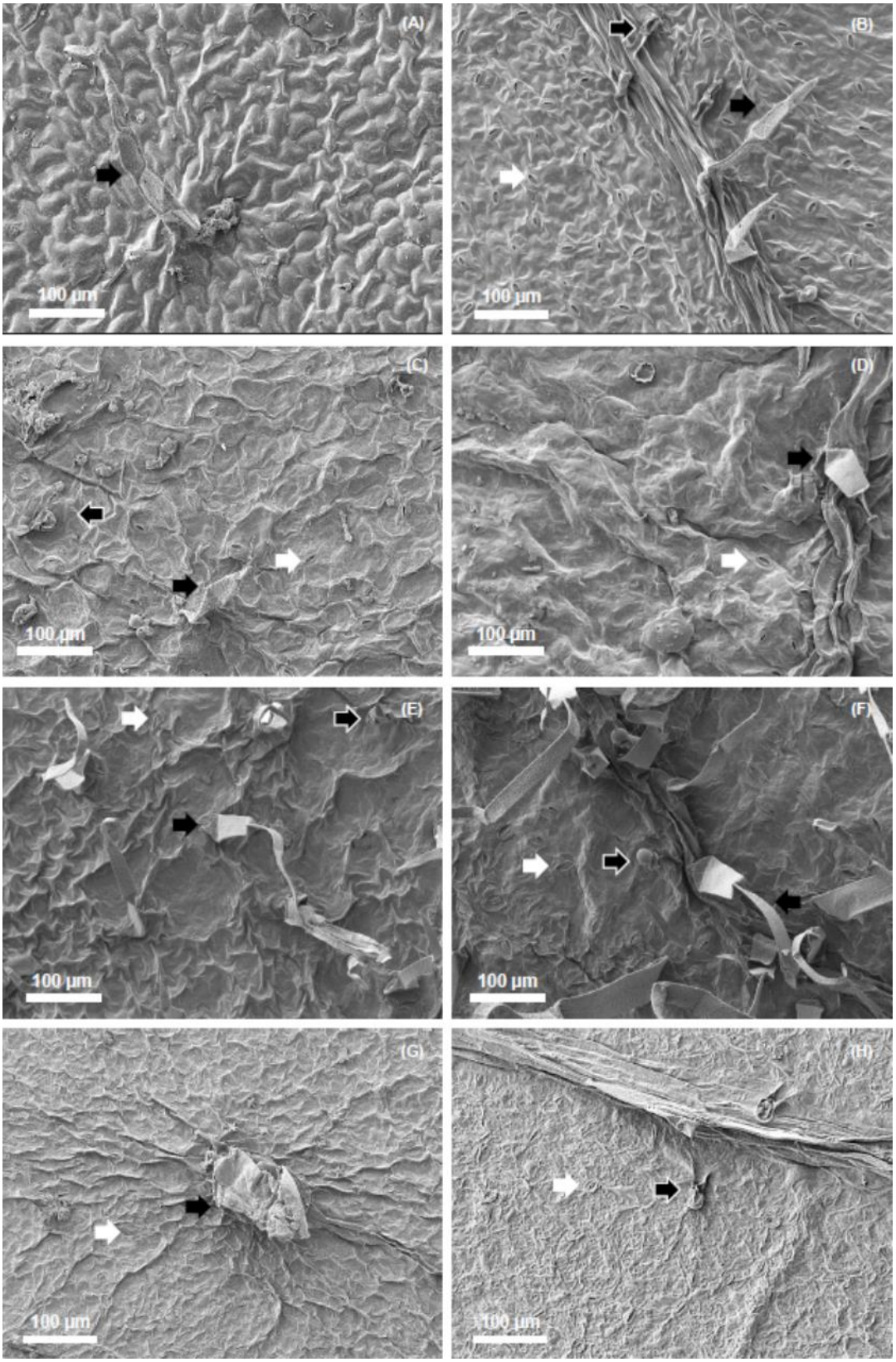


Fig. 2. SEM micrographs of the intact leaf (adaxial and abaxial surfaces) of *P. alkekengi* (A-B), *P. ixocarpa* (C-D), *P. peruviana* (E-F) and *N. physalodes* (G-H). Arrows indicate stomata (white), non-glandular trichomes (black), glandular trichomes (black and white).

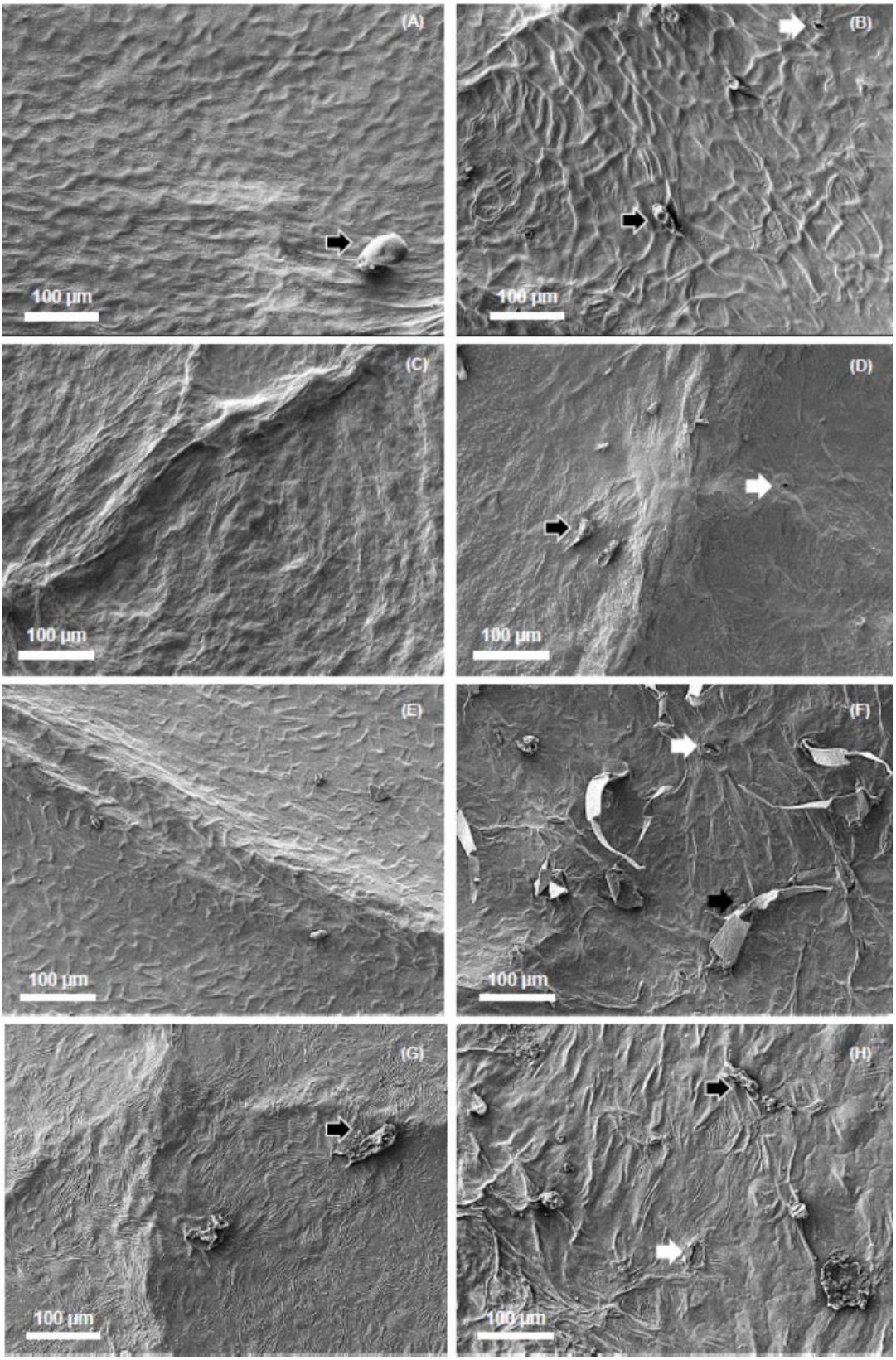


Fig. 3. SEM micrographs of inflated fruiting calyces (adaxial and abaxial surfaces) of *P. alkekengi* (A-B), *P. ixocarpa* (C-D), *P. peruviana* (E-F) and *N. physalodes* (G-H). Arrows indicate stomata (white), non-glandular trichomes (black), glandular trichomes (black and white).

The fruit surface of these four plant species was free of trichomes or stomata (Fig. 4). Based on the cuticular imprints from the inner surface of the fruit cuticle, the pavement cells of *P. alkekengi* fruits had straight cell boundaries giving a honeycomb-like pattern to its interior (Fig. 4A and 4B), while *P. ixocarpa* and *P. peruviana* had S-undulated anticlines (Fig. 4C to 4F). Notable features were papilla-like surface excrescence and some of their respective invaginations visible only on the inner side of the fruit cuticle surface of *P. ixocarpa*. Additionally, the surface of *N. physalodes* was strongly different from the other fruit cuticles (Fig. 4G and 4H). The outer cuticular surface had concave cells with a polygon-like pattern and was covered by an epicuticular wax film and granules irregularly distributed. Due to the nature of *N. physalodes* dry fruits, the cuticle was isolated together with the underlying lignified plant tissue being not possible to investigate the cuticular inner surface. However, sclereid cells with an isodiametric shape were observed on *N. physalodes* inner fruit surface as well as small pores that appear as dark circular objects in the SEM micrographs (Fig. 4H). On the outer surface, the pores were fully covered by an epicuticular wax film.

A smooth layer of epicuticular wax covered the surface of *Physalis* fruits with platelet-type wax crystals and irregular granules sparsely distributed. The cross-section of the fruit surfaces showed a cuticle thickness of 2.2 μm in *P. ixocarpa*, 5.6 μm in *P. peruviana* and 8.6 μm in *P. alkekengi* fruits cuticles (Fig. 5A to 5C). A thick palisade layer of macrosclereids (30.9 μm) was observed below the thin cuticle (0.7 μm) in the cross-section of *N. physalodes* fruit surface (Fig. 5D).

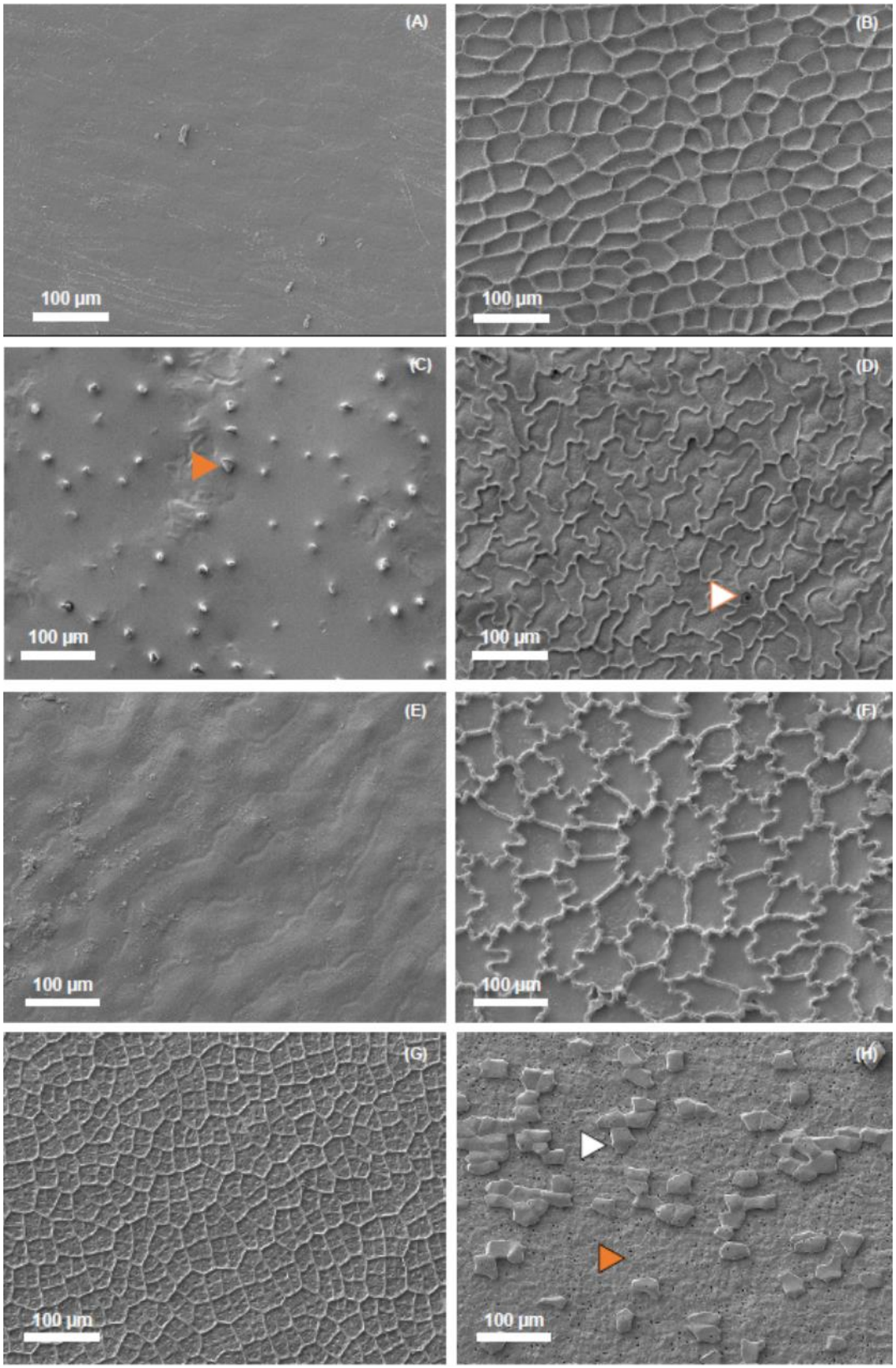


Fig. 4. SEM micrographs of fruit surfaces (outer and inner surface) of *P. alkekengi* (A-B), *P. ixocarpa* (C-D), *P. peruviana* (E-F) and *N. physalodes* (G-H). Arrowheads indicate cuticular papillae (full orange), cuticular pores (white and orange), sclereid cells (white and black) and epidermal pores (orange and black).

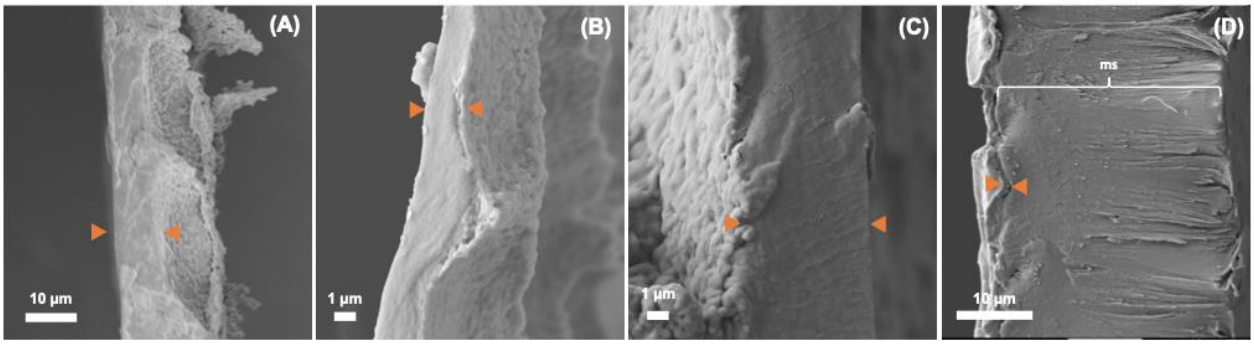


Fig. 5. Cross-section SEM micrographs of the isolated fruit surface of *P. alkekengi* (A), *P. ixocarpa* (B), *P. peruviana* (C) and *N. physalodes* (D). Orange arrowheads indicate fruit cuticle, ms: layer of macrosclereids.

2.3. Water permeability in leaf, inflated fruiting calyx and fruit of Physalis and Nicandra species

Minimum conductance of leaf and inflated fruiting calyx with maximal stomatal closure and fruit cuticular conductance for water were determined by relating the water loss rate to the surface area. At an intraspecific level, all plant species showed significant differences in water permeability among the plant organs investigated (Table 2). All three plant organs of *P. alkekengi* showed a different water permeability ($F(2.38), 31.12, p < 0.001$), with the highest conductance found in the calyx (7.4×10^{-5} m s^{-1}), followed by leaf (4.8×10^{-5} m s^{-1}) and the lowest value being detected in fruit (2.4×10^{-5} m s^{-1}). Similarly, *P. ixocarpa, P. peruviana* and *N. physalodes* calyces had a significantly higher water permeability compared to the other two plant organs tested ($p < 0.05$) but no statistical difference was found between water permeability of leaf and fruit ($p \geq 0.05$). In *P. ixocarpa* calyx, the minimum conductance was 18.4×10^{-5} m s^{-1}, almost sixfold higher ($X^2(2) = 28.22, p < 0.05$) compared to the average water permeability of leaf and fruit (3.3×10^{-5} m s^{-1}). On the other hand, the water permeability of *P. peruviana* calyx (16.0×10^{-5} m s^{-1}) was threefold higher ($F(2.29), 133.41, p < 0.001$) than that of leaf and fruit (mean of 4.6×10^{-5} m s^{-1}). Comparably, the water permeability of *N. physalodes* calyx was 26.8×10^{-5} m s^{-1}, while leaf and fruit had an average of 8.6×10^{-5} m s^{-1}, threefold lower than the value found for calyx ($F(2.28), 17.65, p < 0.001$).

Table 2. Water permeability of leaf, calyx and fruit of *P. alkekengi*, *P. ixocarpa*, *P. peruviana* and *N. physalodes*. Values represent the mean ± standard deviation (n ≥ 8). Different letters indicate a significant difference ($p < 0.05$) between the plant organs of the same plant species. Statistical differences were analysed by Anova or Kruskal-Wallis Anova test.

Plant species	Water permeability $\times 10^5$ (m s $^{-1}$)		
	leaf	calyx	fruit
P. alkekengi	4.77 ± 1.46^b	7.43 ± 1.99^a	2.44 ± 1.27^b
P. ixocarpa	5.06 ± 0.76^b	18.41 ± 7.34^a	1.85 ± 0.97^b
P. peruviana	4.40 ± 1.58^b	15.97 ± 2.25^a	4.84 ± 1.40^b
N. physalodes	5.32 ± 0.55^b	26.79 ± 14.07^a	11.18 ± 3.02^b

Interspecifically, minimum leaf conductance did not statistically differ among the four plant species studied ($p \geq 0.05$) being on average 4.9×10^{-5} m s $^{-1}$ (Fig. 6A). The calyx conductance of *P. ixocarpa, P. peruviana* and *N. physalodes* had a similar minimum conductance ($p \geq 0.05$) with a mean value of 19.6×10^{-5} m s $^{-1,}$ but it was significantly different for *P. alkekengi* (X^2 (3) = 23.28, $p < 0.001$), which had a lower value of 7.4×10^{-5} m s $^{-1}$ (Fig. 6B). The highest cuticular conductance was measured for *Nicandra* fruits (X^2 (3) = 38.62, $p < 0.05$). Fruits of *P. peruviana* had a twofold higher cuticular conductance (4.8×10^{-5} m s $^{-1}$) in comparison to *P. alkekengi* and *P. ixocarpa* fruits (Fig.6C), which were not statistically different (mean of 2.2×10^{-5} m s $^{-1}$, $p \geq 0.05$). The water permeability was different among the plant organs investigated. Inflated fruiting calyces had the highest conductance among the plant organs (F (2.135), 54.47, $p < 0.001$) but no difference was found between fruits and leaves ($p \geq 0.05$).

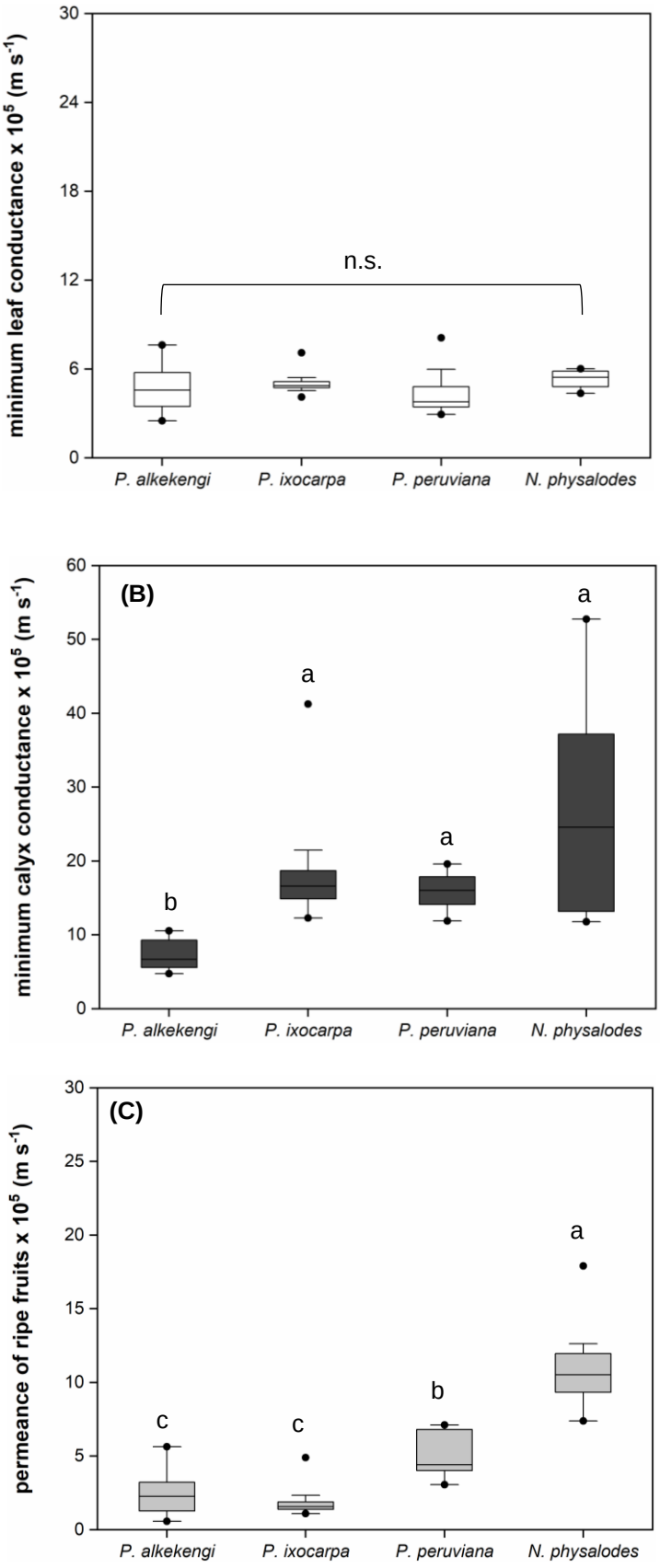


Fig. 6. Water permeabilities of leaf (A), inflated fruiting calyx (B), and fruits (C) ($n \geq 8$) of *Physalis* and *Nicandra* species. Different letters indicate a significant difference ($p < 0.05$) between plant species. Statistical differences were analysed by Anova or Kruskal-Wallis Anova test; n.s., not significant.

2.4 Post-floral contribution of the inflated fruiting calyx to the water permeability of fruits

The cuticular conductance of fruits covered by the inflated fruiting calyx was significantly lower than that of non-covered fruits in *P. ixocarpa* (U = 75, $p < 0.05$) and *P. peruviana* (*t* (10) = 7.24, $p < 0.001$, Fig. 7). After the inflated fruiting calyx removal, the cuticular conductance increased by a factor of 1.7 from 1.1×10^{-5} m s $^{-1}$ to 1.9×10^{-5} m s $^{-1}$ in *P. ixocarpa* fruits and by a factor of 2.3 in *P. peruviana* from 2.1×10^{-5} m s $^{-1}$ to 4.8×10^{-5} m s $^{-1}$. No significant effect of the calyx coating was found on the cuticular conductance of *P. alkekengi* and *N. physalodes* fruits ($p \geq 0.05$).

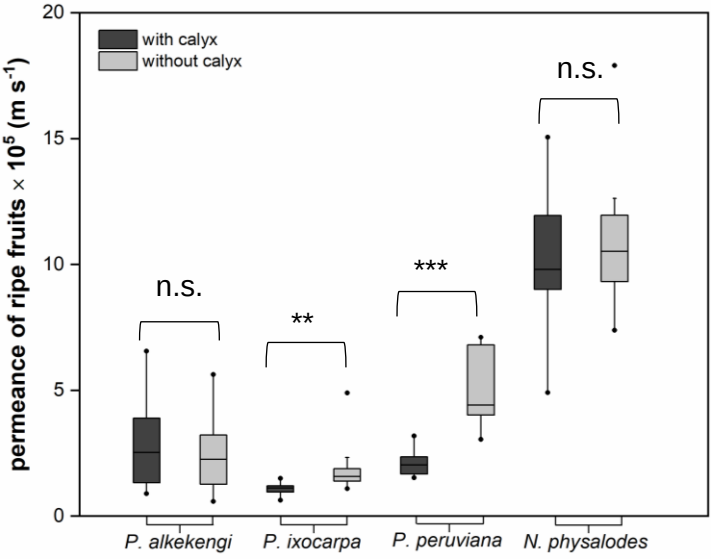


Fig. 7. Cuticular water permeance of fruits with and after calyx removal of *Physalis* and *Nicandra* species ($n \geq 11$). The asterisks indicate a significant difference (n.s., not significant, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) within the same plant species. Statistical differences were analysed by Mann-Whitney-*U*-Test or *t*-test.

2.5 Chemical analyses of the leaf, inflated fruiting calyx and fruit cuticular waxes of Physalis and Nicandra species

Chemical analysis of cuticular wax extracts from fully expanded leaves, inflated fruiting calyces and isolated cuticles of ripe fruits were analysed quantitatively and qualitatively by GC-FID and GC-MS. The cuticular waxes of *P. alkekengi*, *P. ixocarpa, P. peruviana* and *N. physalodes* were primarily composed of very-long-chain aliphatic compounds, regardless of the plant organ investigated, comprising more than 89% of the total cuticular waxes. The major aliphatic components identified were *n*-alkanes, primary alkanols and alkanoic acids. Minor amounts of aromatic and alicyclic components were also identified (≤ 11%) in most of the cuticular wax mixtures, mainly sterols, pentacyclic triterpenoids and tocopherols. The cuticular wax coverage of the plant organs ranged from 0.9 µg cm^{-2} for *N. physalodes* leaf to 25.1 µg cm^{-2} for *P. peruviana* fruit. The individual results of cuticular wax composition of leaf, calyx and fruit for *Physalis* and *Nicandra* species studied are presented below:

2.5.1 Cuticular waxes of Physalis alkekengi

Chemical analysis of *P. alkekengi* leaves, inflated fruiting calyces and fruits revealed a cuticular wax coverage of 0.91 µg cm^{-2}, 1.83 µg cm^{-2} and 8.39 µg cm^{-2}, respectively (Table 3). Very-long-chain aliphatic compounds were the main components found in the cuticular wax mixture of these three plant organs, comprising up to 97% of the total cuticular waxes (Fig.8A).

The major aliphatic component class identified in the leaf cuticular wax was primary alkanols (0.35 µg cm^{-2}), followed by *n*-alkanes (0.21 µg cm^{-2}), and alkanoic acids (0.17 µg cm^{-2}), representing 79% of the total leaf cuticular wax mixture. Branched *anteiso*-alkanes (0.04 µg cm^{-2}), alkanals (0.02 µg cm^{-2}), alkenyl esters (0.02 µg cm^{-2}) and alkyl esters (0.03 µg cm^{-2}) were also identified in minor amounts (≤ 4.5%).

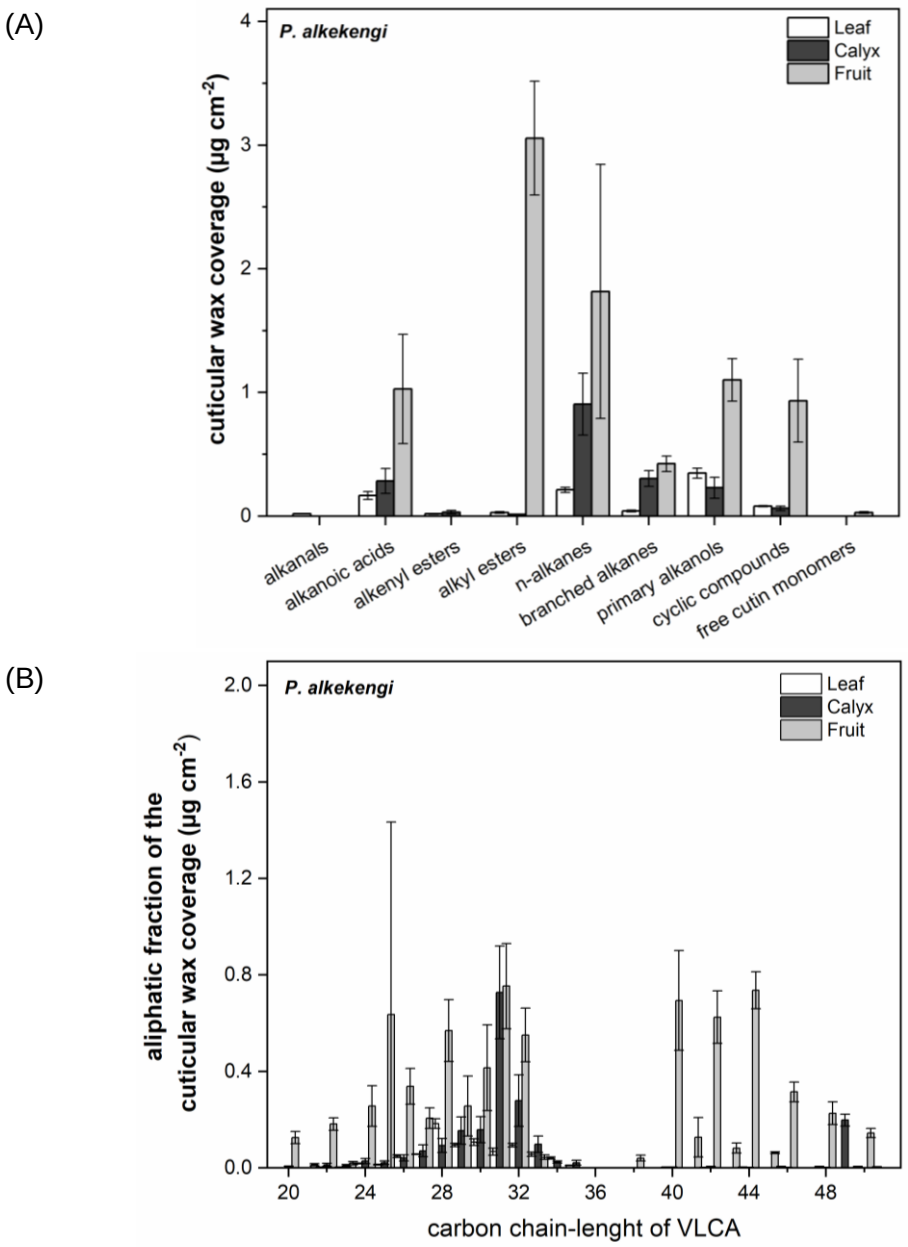


Fig. 8. Cuticular wax coverage (A) and carbon chain length distribution of the very-long-chain aliphatic compounds (VLCA; B) of *P. alkekengi* leaf, calyx and fruit. Values represent the mean ± standard deviation (n ≥ 4).

Calyx cuticular wax was mostly composed of *n*-alkanes (50% of the total cuticular wax), followed by *iso*- and *anteiso*-alkanes (17%, 0.30 µg cm^{-2}), alkanoic acids (16%, 0.28 µg cm^{-2}) and primary alkanols (13%, 0.23 µg cm^{-2}). Minor fractions (≤ 2%) of alkenyl esters (0.03 µg cm^{-2}) and alkyl esters (0.01 µg cm^{-2}) were also identified in the calyx cuticular wax mixture.

On the other hand, alkyl esters (36%, 3.06 ± 0.46 µg cm^{-2}) and *n*-alkanes (22%, 1.82 ± 1.03

µg cm^{-2}) were the most prominent classes in the cuticular wax of *P. alkekengi* fruit. Alkanoic acids (12%, 1.03 ± 0.44 µg cm^{-2}), primary alkanols (13%, 1.10 ± 0.17 µg cm^{-2}) and *anteiso*-alkanes (5%, 0.42 ± 0.06 µg cm^{-2}) made up the residual amount of the aliphatic wax fraction of fruits. In addition, traces of 9/10,ω-dihydroxy hexadecanoic acid (0.3%, 0.03 µg cm^{-2}), a free monomer of cutin, was also identified in the cuticular wax extract of fruits.

Carbon chain lengths of the very-long-chain aliphatic compounds ranged from C_{20} to C_{51} in leaves, from C_{20} to C_{48} in calyx and C_{20} to C_{50} in fruits (Fig.8B). The average chain length of the aliphatic fraction was 30 carbon atoms for *P. alkekengi* leaves and calyx and of 34 for *P. alkekengi* fruits.

Aromatic and alicyclic compounds, primarily tocopherols and sterols, were found in leaves, corresponding to 9% (0.08 µg cm^{-2}) of the total cuticular wax (Table 2). In calyces, β-sitosterol, stigmasterol and campesterol formed the alicyclic fraction (3%, 0.06 ± 0.02 µg cm^{-2}). The fruit had a more diverse composition of the alicyclic compounds being sterols (β-sitosterol, stigmasterol and campesterol) and pentacyclic triterpenoids (ursolic acid and oleanolic acid) the main alicyclic components found amounting to 11% of the fruit cuticular wax composition (0.93 ± 0.33 µg cm^{-2}).

Table 3. Cuticular wax composition of *P. alkekengi* leaf, inflated fruiting calyx and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *P. alkekengi* (µg cm^{-2})		
		leaf	calyx	fruit
alkanoic acids	20	0.001 ± 0.000	0.005 ± 0.002	0.126 ± 0.025
	21	0.000 ± 0.000	±	0.013 ± 0.005
	22	0.003 ± 0.001	0.006 ± 0.003	0.138 ± 0.019
	23	0.002 ± 0.000	0.005 ± 0.003	0.020 ± 0.007
	24	0.013 ± 0.002	0.019 ± 0.008	0.113 ± 0.039
	25	0.004 ± 0.000	0.010 ± 0.005	0.038 ± 0.011
	26	0.020 ± 0.003	0.020 ± 0.010	0.100 ± 0.033
	27	0.011 ± 0.002	0.016 ± 0.006	0.029 ± 0.017
	28	0.054 ± 0.010	0.033 ± 0.023	0.099 ± 0.076
	29	0.018 ± 0.005	0.017 ± 0.012	0.029 ± 0.028
	30	0.035 ± 0.010	0.037 ± 0.021	0.165 ± 0.154
	31	-	0.017 ± 0.009	0.061 ± 0.018
	32	0.004 ± 0.002	0.097 ± 0.101	0.097 ± 0.060
alkanals	33	0.015 ± 0.001	-	-
	35	0.004 ± 0.001	-	-
anteiso-alkanes	30	-	0.025 ± 0.009	0.067 ± 0.023
	31	-	0.009 ± 0.002	-
	32	0.022 ± 0.003	0.081 ± 0.008	0.356 ± 0.049
	33	±	0.028 ± 0.008	-
	34	0.019 ± 0.004	0.025 ± 0.004	-
	35	-	0.022 ± 0.010	-
iso-alkanes	29	-	0.023 ± 0.009	-
	31	-	0.059 ± 0.014	-
	32	-	0.008 ± 0.002	-
	33	-	0.024 ± 0.016	-
n-alkanes	25	0.006 ± 0.001	0.012 ± 0.005	0.574 ± 0.798
	26	0.006 ± 0.001	0.003 ± 0.001	0.031 ± 0.014
	27	0.029 ± 0.002	0.049 ± 0.019	0.130 ± 0.038
	28	0.012 ± 0.002	0.017 ± 0.006	-
	29	0.037 ± 0.006	0.107 ± 0.037	0.190 ± 0.096
	30	0.013 ± 0.001	0.042 ± 0.014	0.085 ± 0.032
	31	0.054 ± 0.014	0.643 ± 0.171	0.664 ± 0.154
	32	0.021 ± 0.005	0.038 ± 0.009	0.098 ± 0.029
	33	0.033 ± 0.006	-	0.045 ± 0.010
primary alkanols	21	0.000 ± 0.000	-	-
	22	0.001 ± 0.000	0.006 ± 0.003	0.044 ± 0.009
	23	0.001 ± 0.000	0.005 ± 0.002	-
	24	0.005 ± 0.001	0.010 ± 0.003	0.144 ± 0.049
	25	0.003 ± 0.001	-	0.024 ± 0.006
	26	0.023 ± 0.006	0.019 ± 0.006	0.207 ± 0.046
	27	0.016 ± 0.002	0.005 ± 0.001	0.047 ± 0.005
	28	0.118 ± 0.020	0.043 ± 0.011	0.471 ± 0.056

	29	0.040	±	0.003	0.007	±	0.003	0.038	±	0.003
	30	0.059	±	0.008	0.031	±	0.013	0.098	±	0.016
	31	0.013	±	0.002		-		0.029	±	0.010
	32	0.037	±	0.004	0.055	±	0.036		-	
	33	0.009	±	0.002	0.047	±	0.034		-	
	34	0.014	±	0.002		-			-	
	35	0.006	±	0.001		-			-	
	30		-		0.031	±	0.014		-	
alkenyl esters	32	0.011	±	0.002		-			-	
	34	0.007	±	0.001		-			-	
	38		-			-		0.041	±	0.012
	40	0.003	±	0.001	0.003	±	0.001	0.694	±	0.207
	41		±			±		0.127	±	0.082
	42	0.002	±	0.001	0.005	±	0.002	0.625	±	0.109
	43		±			±		0.082	±	0.021
	44	0.003	±	0.001	0.003	±	0.001	0.736	±	0.077
alkyl esters	45		±			±		0.063	±	0.004
	46	0.004	±	0.002	0.002	±	0.001	0.315	±	0.041
	47		-			-			-	
	48	0.005	±	0.002	0.002	±	0.000	0.227	±	0.047
	49	0.002	±	0.000		-			±	
	50	0.005	±	0.001		-		0.145	±	0.019
	51	0.004	±	0.001		-			-	
dihydroxy alkanoic acid	16		-			-		0.029	±	0.007
total aliphatics		0.832	±	0.051	1.775	±	0.459	7.455	±	1.487
alpha-tocopherol		0.015	±	0.004		-			-	
beta-tocopherol		0.007	±	0.002		-			-	
total cyclic aromatics		0.022	±	0.005		-			-	
β-sitosterol		0.032	±	0.003	0.026	±	0.012	0.461	±	0.169
stigmasterol		0.009	±	0.001	0.005	±	0.003	0.230	±	0.110
camposterol		0.017	±	0.003	0.030	±	0.012	0.221	±	0.049
oleanoic acid			-			-		0.016	±	0.009
ursolic acid			-			-		0.047	±	0.014
total alicyclics		0.103	±	0.004	0.062	±	0.018	0.975	±	0.335
total cuticular waxes		0.913	±	0.055	1.828	±	0.465	8.387	±	1.693

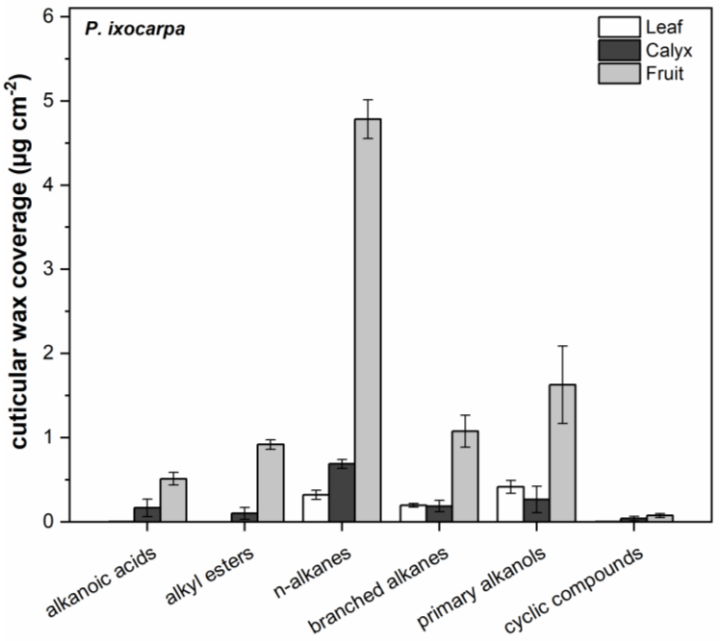
2.5.2 Cuticular waxes of Physalis ixocarpa

The total wax coverage of *P. ixocarpa* leaf, inflated fruiting calyx and fruit were 0.94 ± 0.02 µg cm^{-2}, 1.44 ± 0.46 µg cm^{-2} and 8.99 ± 0.72 µg cm^{-2}, respectively (Table 4). Very-long-chain aliphatic compounds were the main components found in the cuticular wax mixture of these three plant organs, comprising up to 99.7% of the total cuticular waxes.

The major aliphatic component class identified in the leaf cuticular wax was primary alkanols (0.42 ± 0.08 µg cm^{-2}) followed by *n*-alkanes (0.32 ± 0.06 µg cm^{-2}), representing 78% of the total leaf wax mixture (Fig.9A). Branched *anteis*o- and *iso*-alkanes comprised 21% of the aliphatic fraction (0.20 ± 0.02 µg cm^{-2}), and only traces of alkanoic acids (0.4%) were present in the leaf cuticular wax.

The cuticular waxes of *P. ixocarpa* calyx and fruit showed a similar aliphatic wax composition, being *n*-alkanes (48%, 0.69 µg cm^{-2} and 53%, 4.78 µg cm^{-2}, respectively) the most prominent component class, followed by primary alkanols (18% for both plant organs, 0.27 µg cm^{-2} and 1.63 µg cm^{-2}, respectively). Furthermore, calyx and fruit waxes were also composed of *anteiso*- and *iso*-alkanes (13%, 0.19 µg cm^{-2} and 12%, 1.08 µg cm^{-2}, respectively), alkanoic acids (11%, 017 µg cm^{-2} and 6%, 0.51 µg cm^{-2}, respectively) and alkyl esters (7%, 0.10 µg cm^{-2} and 10%, 0.92 µg cm^{-2}, respectively). Carbon chain lengths of the very-long-chain aliphatic compounds ranged from C_{20} to C_{34} in leaves, from C_{20} to C_{52} in calyx and C_{20} to C_{50} in fruits (Fig.9B). The average chain length of the aliphatic fraction was 29 carbon atoms for leaves, and 31 for calyx and fruits.

(A)



(B)

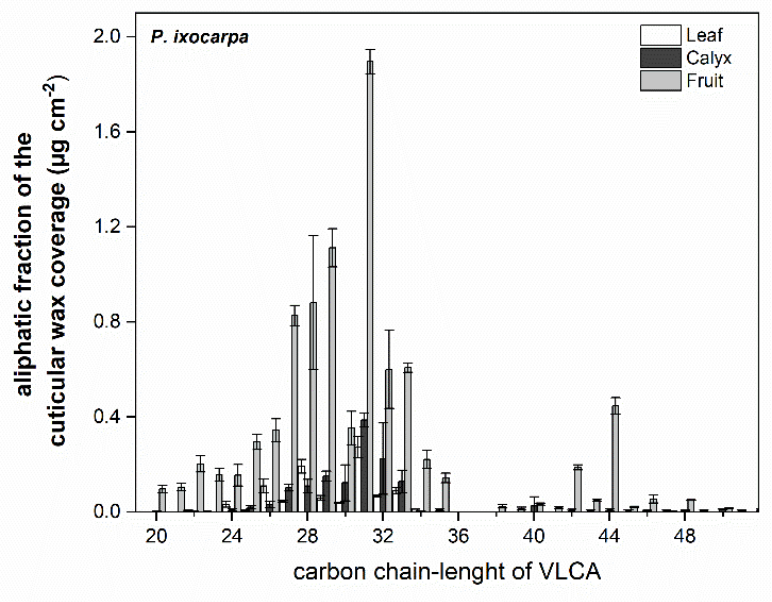


Fig. 9. Cuticular wax coverage (A) and carbon chain length distribution of the very-long-chain aliphatic compounds (VLCA; B) of *P. ixocarpa* leaf, calyx and fruit. Values represent the mean ± standard deviation ($n \geq 4$).

Campesterol was found only in traces in leaves, corresponding to 0.3% of the total cuticular waxes. In calyces, β-sitosterol, campesterol and ursolic acid formed the alicyclic fraction (3%, 0.04 ± 0.03 µg cm^{-2}). Ursolic acid was the only alicyclic component identified in fruit, comprising 1% (0.07 ± 0.02 µg cm^{-2}) of the cuticular wax composition (Table 4).

Table 4. Cuticular wax composition of *P. ixocarpa*. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *P. ixocarpa* (µg cm^{-2})		
		leaf	calyx	fruit
alkanoic acids	20	0.001 ± 0.000	0.002 ± 0.001	0.065 ± 0.007
	21	-	-	0.037 ± 0.008
	22	0.001 ± 0.001	0.002 ± 0.001	0.085 ± 0.010
	23	-	-	0.035 ± 0.009
	24	0.002 ± 0.001	0.003 ± 0.001	0.111 ± 0.017
	25	-	-	0.028 ± 0.007
	26	-	0.011 ± 0.007	0.041 ± 0.015
	27	-	-	0.010 ± 0.002
	28	-	0.014 ± 0.007	0.011 ± 0.008
	29	-	0.003 ± 0.002	0.016 ± 0.002
	30	-	0.013 ± 0.007	0.024 ± 0.006
	32	-	0.118 ± 0.099	0.026 ± 0.026
	33	-	-	0.012 ± 0.003
	34	-	-	0.011 ± 0.006
anteiso-alkanes	24	-	-	0.019 ± 0.010
	26	-	-	0.027 ± 0.005
	28	-	-	0.019 ± 0.003
	30	0.007 ± 0.000	0.009 ± 0.004	0.025 ± 0.005
	31	0.004 ± 0.000	0.005 ± 0.003	-
	32	0.034 ± 0.002	0.032 ± 0.019	0.026 ± 0.006
	33	0.003 ± 0.000	-	-
	34	0.009 ± 0.001	0.003 ± 0.002	0.045 ± 0.007
	35	-	0.009 ± 0.005	-
iso-alkanes	25	-	0.004 ± 0.003	0.017 ± 0.003
	27	-	0.005 ± 0.003	0.044 ± 0.009
	29	0.007 ± 0.001	0.021 ± 0.007	0.273 ± 0.056
	30	-	0.007 ± 0.006	-
	31	0.088 ± 0.015	0.057 ± 0.011	0.387 ± 0.090
	32	0.006 ± 0.001	0.006 ± 0.001	-
	33	0.039 ± 0.004	0.029 ± 0.010	0.168 ± 0.021
	35	-	-	0.028 ± 0.007
n-alkanes	21	-	-	0.057 ± 0.016
	22	-	-	0.060 ± 0.028
	23	-	-	0.118 ± 0.021
	25	-	0.014 ± 0.004	0.223 ± 0.022
	26	-	0.003 ± 0.001	0.191 ± 0.033
	27	0.021 ± 0.004	0.090 ± 0.012	0.741 ± 0.047
	28	0.003 ± 0.001	0.016 ± 0.003	0.169 ± 0.036
	29	0.045 ± 0.010	0.122 ± 0.013	0.791 ± 0.032
	30	0.011 ± 0.002	0.024 ± 0.004	0.170 ± 0.021
	31	0.178 ± 0.030	0.319 ± 0.017	1.498 ± 0.125
	32	0.014 ± 0.003	0.033 ± 0.007	0.228 ± 0.034

	33	0.048	±	0.008	0.066	±	0.007	0.389	± 0.034
	34		-			-		0.032	± 0.009
	35		-			-		0.116	± 0.013
	20		-			-		0.032	± 0.016
	21	0.000	±	0.000		-		0.010	± 0.002
	22	0.005	±	0.002	0.002	±	0.001	0.058	± 0.008
	23	0.002	±	0.001	0.001	±	0.001	0.003	± 0.003
	24	0.031	±	0.012	0.006	±	0.003	0.025	± 0.028
	25	0.007	±	0.002	0.002	±	0.001	0.028	± 0.009
	26	0.110	±	0.028	0.017	±	0.007	0.086	± 0.030
primary alkanols	27	0.025	±	0.005	0.008	±	0.002	0.032	± 0.012
	28	0.189	±	0.030	0.080	±	0.024	0.683	± 0.254
	29	0.007	±	0.001	0.005	±	0.001	0.033	± 0.013
	30	0.020	±	0.001	0.070	±	0.067	0.134	± 0.048
	31	0.003	±	0.000	0.007	±	0.005	0.011	± 0.009
	32	0.013	±	0.003	0.035	±	0.025	0.320	± 0.110
	33		-		0.033	±	0.032	0.039	± 0.009
	34	0.004	±	0.001		-		0.134	± 0.023
	38		-			-		0.023	± 0.007
	39		-			-		0.015	± 0.006
	40		-		0.025	±	0.040	0.034	± 0.005
	41		-			-		0.019	± 0.003
	42		-		0.007	±	0.005	0.189	± 0.011
	43		-		0.006	±	0.005	0.049	± 0.003
	44		-		0.007	±	0.005	0.446	± 0.035
alkyl esters	45		-		0.005	±	0.004	0.021	± 0.003
	46		-		0.006	±	0.002	0.054	± 0.017
	47		-		0.004	±	0.003	0.001	± 0.003
	48		-		0.008	±	0.003	0.050	± 0.002
	49		-		0.005	±	0.003		-
	50		-		0.011	±	0.003	0.016	± 0.001
	51		-		0.006	±	0.003		-
	52		-		0.011	±	0.003		-
total aliphatics		0.938	±	0.022	1.406	±	0.435	8.918	± 0.699
β-sitosterol			-		0.011	±	0.005		-
camposterol		0.003	±	0.001	0.007	±	0.003		-
ursolic acid			±		0.020	±	0.022	0.075	± 0.024
total alicyclics		0.003	±	0.001	0.038	±	0.028	0.075	± 0.024
total cuticular waxes		0.941	±	0.022	1.444	±	0.458	8.992	± 0.722

2.5.3 Cuticular waxes of Physalis peruviana

P. peruviana leaves, inflated fruiting calyces and fruits had a total cuticular wax coverage of 1.44 µg cm^{-2}, 0.56 µg cm^{-2} and 25.11 µg cm^{-2}, respectively. Very-long-chain aliphatic compounds were the main components found in the cuticular wax mixture of these three plant organs, comprising up to 99.8% of the total cuticular waxes (Table 5).

The primary aliphatic component class identified in the leaf cuticular wax mixture was *n*-alkanes (41%, 0.59 µg cm^{-2}), followed by primary alkanols (36%, 0.52 µg cm^{-2}) and *anteiso*- and *iso*-alkanes (20%, 0.28 µg cm^{-2}). Alkanoic acids, alkanals and alkyl esters were found only in minor amounts (≤ 2%).

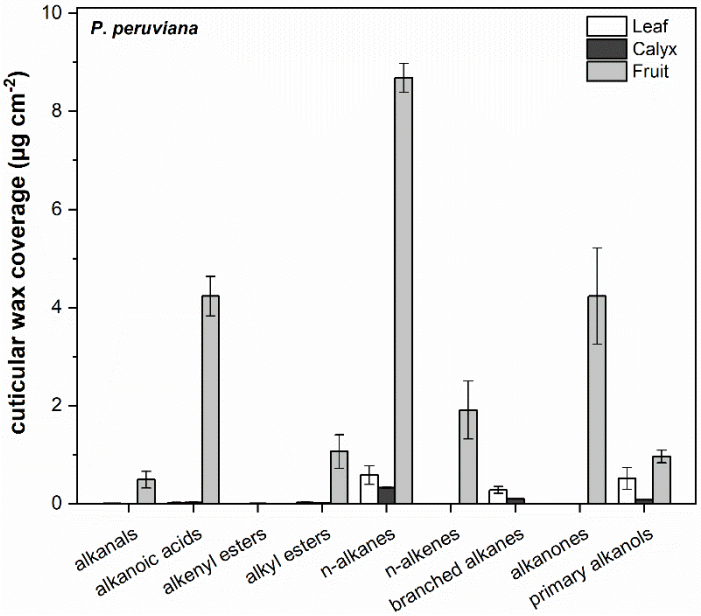
The most prominent aliphatic component class in the cuticular waxes of *P. peruviana* calyx was *n*-alkanes (59%, 0.33 µg cm^{-2}), followed by branched alkanes (17%, 0.10 µg cm^{-2}) and primary alkanols (14%, 0.08 µg cm^{-2}). In addition, alkanoic acids (5%, 0.03 µg cm^{-2}), alkenyl esters and alkyl esters (≤ 2%) were found in small amounts (Fig. 10A).

Fruit wax of *P. peruviana* was mostly composed of *n*-alkanes (35%, 8.68 µg cm^{-2}) followed by alkanoic acids and alkanones (17% for both plant organs, 4.23 µg cm^{-2} and 4.23 µg cm^{-2}, respectively). Additionally, fruit waxes were also constituted by *n*-alkenes (8%, 1.91 µg cm^{-2}), alkyl esters (4%, 1.07 µg cm^{-2}), primary alkanols (4%, 0.97 µg cm^{-2}) and alkanals (2%, 0.49 µg cm^{-2}).

Carbon chain lengths of the very-long-chain aliphatic compounds ranged from C_{20} to C_{50} in leaves, calyx and fruits (Fig. 10B). The average chain length of the aliphatic fraction was 30 carbon atoms for leaves, 31 for calyx and 28 for fruits. A homologous series of 12 unknown compounds were also observed in *P. peruviana* fruit cuticular waxes, and comprised 14% of the wax mixture (Table 5).

Only traces of campesterol represented the alicyclic fraction found in leaves, corresponding to 0.2% of the total cuticular waxes. In calyces, β-sitosterol, stigmasterol and campesterol formed the alicyclic fraction (1.5%). Cyclic components were not identified in fruit cuticular waxes.

(A)



(B)

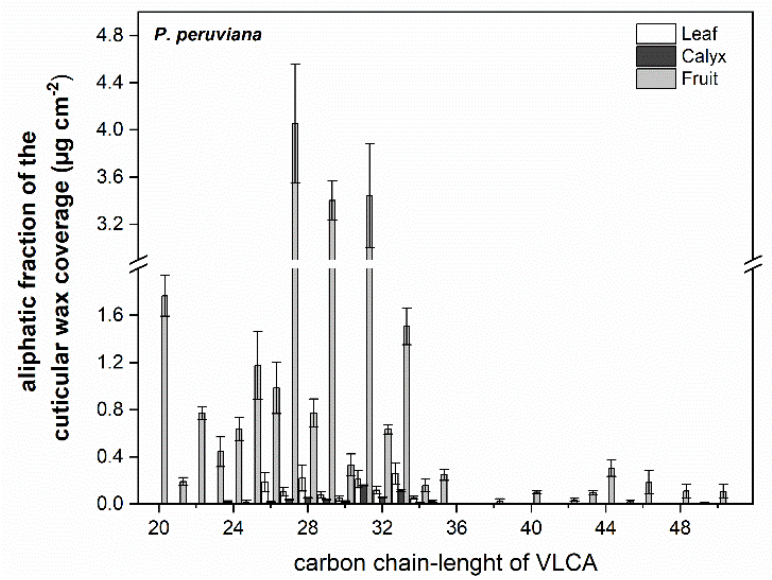


Fig. 10. Cuticular wax coverage (A) and carbon chain length distribution of the very-long-chain aliphatic compounds (VLCA; B) of *P. peruviana* leaf, calyx and fruit. Values represent the mean ± standard deviation (n ≥ 4).

Table 5. Cuticular wax composition of *P. peruviana*. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *P. peruviana* (µg cm^{-2})		
		leaf	calyx	fruit
alkanoic acids	20	0.003 ± 0.002	0.001 ± 0.000	1.686 ± 0.156
	21	-	0.000 ± 0.000	0.076 ± 0.027
	22	-	0.001 ± 0.001	0.682 ± 0.064
	23	-	-	0.081 ± 0.008
	24	-	0.004 ± 0.003	0.574 ± 0.093
	25	-	0.002 ± 0.000	0.047 ± 0.027
	26	0.003 ± 0.002	0.003 ± 0.001	0.548 ± 0.121
	27	-	-	0.030 ± 0.019
	28	-	0.003 ± 0.001	0.153 ± 0.008
	29	-	0.003 ± 0.000	0.018 ± 0.009
	30	0.006 ± 0.004	0.004 ± 0.004	0.121 ± 0.030
	31	-	0.001 ± 0.000	0.017 ± 0.016
	32	0.008 ± 0.005	0.003 ± 0.001	0.138 ± 0.017
	33	-	±	0.018 ± 0.017
	34	-	0.001 - 0.001	0.045 ± 0.025
alkanals	22	-	-	0.004 ± 0.007
	24	-	-	0.018 ± 0.007
	26	-	-	0.173 ± 0.116
	28	-	-	0.118 ± 0.038
	30	-	-	0.115 ± 0.052
	32	-	-	0.045 ± 0.019
	33	-	-	0.019 ± 0.003
	35	0.006 ± 0.004	-	-
anteiso-alkanes	28	-	0.000 ± 0.000	-
	30	0.008 ± 0.001	0.003 ± 0.000	-
	31	-	0.004 ± 0.001	-
	32	0.053 ± 0.008	0.014 ± 0.001	-
	33	0.008 ± 0.002	0.003 ± 0.000	-
	34	0.034 ± 0.006	0.007 ± 0.001	-
	35	0.017 ± 0.007	-	-
	36	0.002 ± 0.001	-	-
iso-alkanes	29	0.020 ± 0.005	0.005 ± 0.001	-
	30	-	0.002 ± 0.000	-
	31	0.059 ± 0.017	0.023 ± 0.001	-
	32	0.011 ± 0.003	0.007 ± 0.000	-
	33	0.065 ± 0.021	0.024 ± 0.001	-
	34	0.003 ± 0.001	0.003 ± 0.000	-
	35	-	0.002 ± 0.000	-
n-alkanes	21	-	-	0.067 ± 0.021
	23	-	-	0.076 ± 0.023
	25	-	0.001 ± 0.000	0.339 ± 0.027
	26	0.009 ± 0.007	0.002 ± 0.000	0.187 ± 0.023

	27	0.071	±	0.019	0.026	±	0.003	2.475	± 0.112
	28	0.015	±	0.004	0.008	±	0.001	0.216	± 0.039
	29	0.043	±	0.014	0.024	±	0.001	1.966	± 0.113
	30	0.013	±	0.006	0.009	±	0.000	-	
	31	0.210	±	0.073	0.129	±	0.007	2.517	± 0.051
	32	0.038	±	0.014	0.033	±	0.002	0.324	± 0.016
	33	0.181	±	0.068	0.085	±	0.008	0.438	± 0.007
	34	0.006	±	0.002	0.005	±	0.000	0.025	± 0.021
	35	-			0.007	±	0.001	0.053	± 0.014
	23	-			-			0.067	± 0.028
	25	-			-			0.044	± 0.010
	27	-			-			0.057	± 0.008
n-alkenes	29	-			-			0.155	± 0.059
	31	-			-			0.603	± 0.427
	33	-			-			0.842	± 0.092
	35	-			-			0.142	± 0.039
	21	-			-			0.048	± 0.015
	23	-			-			0.219	± 0.104
	25	-			-			0.746	± 0.266
	27	-			-			1.494	± 0.506
alkanones	29	-			-			1.262	± 0.200
	31	-			-			0.241	± 0.053
	33	-			-			0.172	± 0.183
	35	-			-			0.052	± 0.013
	20	-			-			0.080	± 0.027
	22	0.002	±	0.001	0.000	±	0.000	0.086	± 0.041
	24	0.021	±	0.008	0.001	±	0.001	0.044	± 0.016
	25	0.017	±	0.014	0.001	±	0.000	-	
	26	0.175	±	0.080	0.012	±	0.004	0.076	± 0.022
	27	0.035	±	0.018	0.009	±	0.001	-	
	28	0.208	±	0.107	0.040	±	0.005	0.286	± 0.066
primary alkanols	29	0.016	±	0.011	0.004	±	0.001	-	
	30	0.020	±	0.011	0.005	±	0.001	0.097	± 0.020
	31	0.003	±	0.002	0.003	±	0.003	0.062	± 0.035
	32	0.009	±	0.004	0.002	±	0.000	0.128	± 0.022
	33	0.003	±	0.001	0.001	±	0.000	0.035	± 0.012
	34	0.009	±	0.005	0.002	±	0.000	0.070	± 0.023
	35	-			-			-	
	36	-			-			-	
	25	-			0.002	±	0.001	-	
alkenyl esters	26	-			0.000	±	0.000	-	
	27	-			0.003	±	0.002	-	
	29	-			0.002	±	0.001	-	
	36	-			0.003	±	0.000	-	
alkyl esters	37	-			0.001	±	0.000	-	
	38	0.004	±	0.002	-			0.023	± 0.022
	39	-			0.001	±	0.000	-	

	40	0.003	±	0.002	0.001	±	0.000	0.101	± 0.014
	41		-		0.001	±	0.000		-
	42	0.004	±	0.003	0.001	±	0.000	0.117	± 0.037
	43		-		0.000	±	0.000	0.095	± 0.019
	44	0.002	±	0.002	0.001	±	0.000	0.304	± 0.069
	45		-			-		0.019	± 0.011
	46	0.002	±	0.002	0.001	±	0.000	0.183	± 0.098
	47	0.001	±	0.001		-		0.000	± 0.000
	48	0.003	±	0.003	0.001	±	0.000	0.110	± 0.060
	49	0.001	±	0.000		-		0.004	± 0.009
	50	0.003	±	0.003	0.001	±	0.000	0.109	± 0.056
homologous series of unkown compounds	1		-			-		0.027	± 0.018
	2		-			-		0.022	± 0.007
	3		-			-		0.028	± 0.016
	4		-			-		0.163	± 0.121
	5		-			-		0.934	± 0.189
	6		-			-		0.077	± 0.027
	7		-			-		1.147	± 0.245
	8		-			-		0.008	± 0.015
	9		-			-		0.749	± 0.198
	10		-			-		0.079	± 0.027
	11		-			-		0.231	± 0.075
	12		-			-		0.068	± 0.084
total aliphatics		1.434	±	0.497	0.553	±	0.014	25.115	± 2.329
β-sitosterol			-		0.003	±	0.000		-
stigmasterol			-		0.005	±	0.000		-
camposterol		0.003	±	0.002	0.002	±	0.000		-
total alicyclics		0.003	±	0.001	0.010	±	0.00		-
total cuticular waxes		1.437	±	0.498	0.563	±	0.014	25.115	± 2.329

2.5.4 Cuticular waxes of Nicandra physalodes

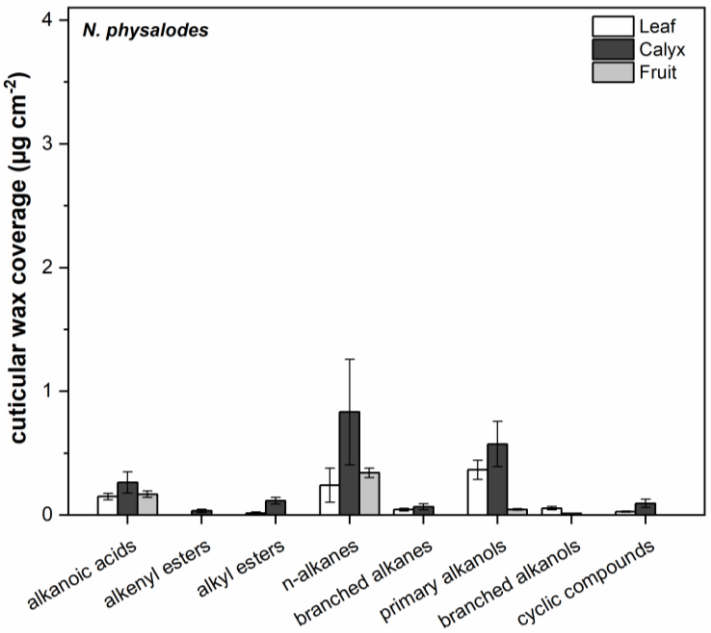
The total wax coverage of *N. physalodes* leaf, inflated fruiting calyx and fruit were 0.90 ± 0.26 µg cm^{-2}, 2.00 ± 0.65 µg cm^{-2} and 0.55 ± 0.04 µg cm^{-2}, respectively (Table 6). Very-long-chain aliphatic compounds were the main components found in the wax mixture of these three plant organs investigated (≥ 95%; Fig. 11A).

The major aliphatic component class identified in the leaf cuticular wax mixture was primary alkanols (41%, 0.37 ± 0.08 µg cm^{-2}) followed by *n*-alkanes (27%, 0.24 ± 0.14 µg cm^{-2}) and alkanoic acids (17%, 0.15 ± 0.02 µg cm^{-2}). Other compounds identified in leaves were branched alkanols (6%, 0.06 ± 0.01 µg cm^{-2}), branched *anteiso*- and *iso*- alkanes (5%, 0.04 ± 0.01 µg cm^{-2}) and alkyl esters (2%, 0.02 ± 0.01 µg cm^{-2}).

The cuticular waxes of *N. physalodes* calyx were mostly composed of *n*-alkanes (42%, 0.83 µg cm^{-2}) followed by primary alkanols (29%, 0.57 µg cm^{-2}). Alkanoic acids comprised 13% (0.26 µg cm^{-2}), alkyl esters represented 6% (0.12 µg cm^{-2}), and alkenyl ester and branched alkanols were found only in small fractions (≤ 2%). Fruit cuticular wax was solely composed of aliphatic compounds and showed a simple composition made up by *n*-alkanes (62%, 0.34 µg cm^{-2}), alkanoic acids (30%, 0.17 µg cm^{-2}) and primary alkanols (8%, 0.05 µg cm^{-2}). Carbon chain lengths of the very-long-chain aliphatic compounds ranged from C_{22} to C_{50} in leaves, from C_{20} to C_{51} in calyx and C_{20} to C_{35} in fruits (Fig. 11B). The average chain length of the aliphatic fraction was 28 carbon atoms for leaves and fruits and 29 for the calyx.

The alicyclic wax fraction of leaf and calyx was composed of the same components, but calyx had a slightly higher amount (5%, 0.09 µg cm^{-2}) compared to leaf (3%, 0.03µg cm^{-2}). The compounds identified in both plant organs were β-sitosterol, cholesterol, stigmasterol, campesterol and ursolic acid (Table 6).

(A)



(B)

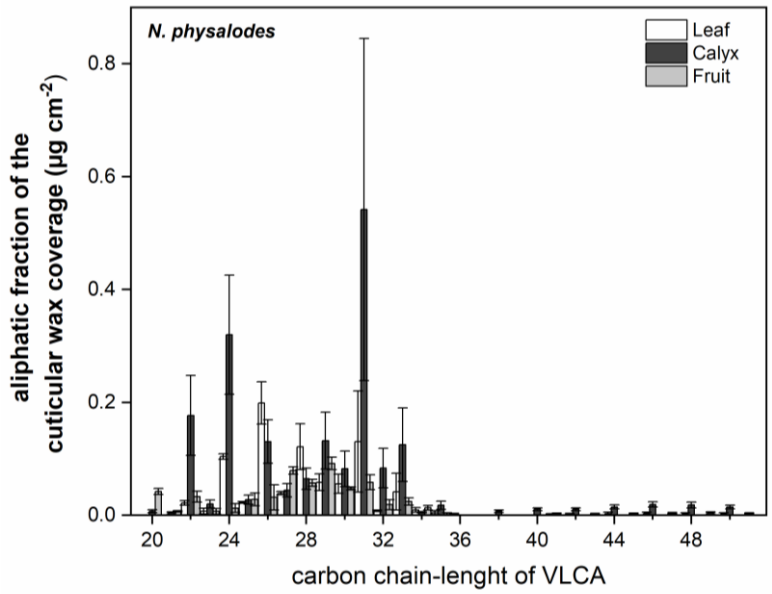


Fig. 11. Cuticular wax coverage (A) and carbon chain length distribution of the very-long-chain aliphatic compounds (VLCA; B) of *N. physalodes* leaf, calyx and fruit. Values represent the mean ± standard deviation ($n \geq 4$).

Table 6. Cuticular wax composition of *N. physalodes*. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *N. physalodes* (µg cm^{-2})		
		leaf	calyx	fruit
alkanoic acids	20	-	0.007 ± 0.003	0.039 ± 0.006
	21	-	0.002 ± 0.000	0.007 ± 0.001
	22	0.008 ± 0.001	0.018 ± 0.005	0.027 ± 0.008
	23	0.006 ± 0.005	0.009 ± 0.003	0.007 ± 0.005
	24	0.079 ± 0.007	0.089 ± 0.025	0.011 ± 0.006
	25	0.008 ± 0.002	0.010 ± 0.003	0.004 ± 0.001
	26	0.028 ± 0.011	0.042 ± 0.014	0.007 ± 0.005
	27	0.003 ± 0.001	0.003 ± 0.002	0.010 ± 0.001
	28	0.006 ± 0.002	0.023 ± 0.009	0.027 ± 0.009
	29	0.002 ± 0.002	0.003 ± 0.002	0.000 ± 0.000
	30	0.005 ± 0.003	0.022 ± 0.014	0.017 ± 0.002
	32	0.002 ± 0.001	0.013 ± 0.009	0.010 ± 0.004
	33	0.003 ± 0.001	0.023 ± 0.015	-
	34	-	-	0.001 ± 0.002
anteiso-alkanes	30	0.004 ± 0.001	-	-
	31	-	0.008 ± 0.004	-
	32	0.005 ± 0.001	0.014 ± 0.005	-
	33	-	0.013 ± 0.005	-
	34	0.002 ± 0.001	-	-
	35	0.005 ± 0.003	0.018 ± 0.007	-
iso-alkanes	27	0.002 ± 0.001	-	-
	29	0.006 ± 0.001	-	-
	31	0.013 ± 0.004	0.014 ± 0.004	-
	33	0.006 ± 0.002	±	-
n-alkanes	25	0.006 ± 0.001	0.004 ± 0.001	0.024 ± 0.011
	26	0.004 ± 0.001	0.002 ± 0.000	0.024 ± 0.023
	27	0.017 ± 0.004	0.034 ± 0.009	0.069 ± 0.006
	28	-	-	0.019 ± 0.010
	29	0.044 ± 0.012	0.129 ± 0.050	0.092 ± 0.011
	30	0.011 ± 0.004	0.030 ± 0.016	0.012 ± 0.001
	31	0.114 ± 0.086	0.513 ± 0.296	0.058 ± 0.013
	32	0.011 ± 0.008	0.032 ± 0.016	0.007 ± 0.006
	33	0.031 ± 0.031	0.089 ± 0.047	0.024 ± 0.006
	34	0.004 ± 0.001	-	0.009 ± 0.003
	35	-	-	0.002 ± 0.003
branched alkanols	26	0.010 ± 0.002	0.002 ± 0.001	-
	27	0.003 ± 0.001	-	-
	28	0.026 ± 0.008	0.005 ± 0.002	-
	29	0.002 ± 0.000	-	-
	30	0.012 ± 0.005	0.005 ± 0.002	-
	32	0.004 ± 0.000	-	-
primary alkanols	20	-	-	0.004 ± 0.002

	21		-		0.003	±	0.001		-	
	22	0.014	±	0.003	0.155	±	0.065	0.006	±	0.002
	23	0.002	±	0.000	0.012	±	0.005		-	
	24	0.025	±	0.003	0.213	±	0.075	0.002	±	0.004
	25	0.009	±	0.002	0.014	±	0.005		-	
	26	0.157	±	0.032	0.071	±	0.025	0.001	±	0.001
	27	0.015	±	0.004	0.007	±	0.003		-	
	28	0.089	±	0.031	0.037	±	0.011	0.011	±	0.001
	29	0.005	±	0.001		-			-	
	30	0.023	±	0.005	0.025	±	0.006	0.018	±	0.002
	31	0.003	±	0.001	0.006	±	0.003		-	
	32	0.016	±	0.005	0.025	±	0.015	0.001	±	0.002
	33	0.002	±	0.000		-			-	
	34	0.004	±	0.002	0.005	±	0.001	0.003	±	0.003
	36	0.003	±	0.001		-			-	
	22		-		0.004	±	0.002		-	
alkenyl esters	24		-		0.017	±	0.008		-	
	26		-		0.014	±	0.004		-	
	38		-		0.007	±	0.002		-	
	40		-		0.011	±	0.002		-	
	41	0.002	±	0.000	0.003	±	0.001		-	
	42	0.002	±	0.001	0.011	±	0.002		-	
	43		-		0.003	±	0.001		-	
	44	0.003	±	0.002	0.015	±	0.004		-	
alkyl esters	45		-		0.003	±	0.001		-	
	46	0.003	±	0.002	0.019	±	0.005		-	
	47		-		0.004	±	0.001		-	
	48	0.002	±	0.002	0.018	±	0.005		-	
	49		-		0.005	±	0.001		-	
	50	0.003	±	0.002	0.015	±	0.003		-	
	51		-		0.003	±	0.001		-	
total aliphatics		0.873	±	0.257	1.901	±	0.616	0.554	±	0.044
β-sitosterol		0.007	±	0.001	0.018	±	0.006		-	
cholesterol		0.003	±	0.000	0.017	±	0.009		-	
stigmasterol		0.007	±	0.000	0.024	±	0.007		-	
camposterol		0.007	±	0.003	0.027	±	0.009		-	
ursolic acid		0.004	±	0.001	0.009	±	0.004		-	
total alicyclics		0.028	±	0.004	0.094	±	0.033		-	
total cuticular waxes		0.900	±	0.256	1.996	±	0.649	0.554	±	0.044

2.6 Chemical composition of the cutin matrix of fruits of Physalis and Nicandra species

Cutin monomers of the isolated fruit cuticles were analysed by GC-FID and GC-MS, showing the presence of aliphatic and aromatic cutin acids and primary alkanols. The cutin monomers amounted to 570.72 µg cm^{-2} in *P. alkekengi*, 168.05 µg cm^{-2} in *P. ixocarpa*, 333. 60 µg cm^{-2} in *P. peruviana* and the lower cutin deposition was exhibited by *N. physalodes* fruits with 81.77 µg cm^{-2} (Table 7).

The chain length distribution of the saturated and unsaturated aliphatic cutin monomers ranged from 16 and 32 carbon atoms and was predominated by C_{16} and C_{18} ω-hydroxy alkanoic acid derivatives. The cutin matrix was primarily composed of 9/10,ω-dihydroxy C_{16} and C_{18} alkanoic acids, 9/10-oxo ω-hydroxy C_{16} alkanoic acid, 9,10-epoxy ω-hydroxy C_{18} alkanoic acid and 9,10,ω-trihydroxy C_{18} alkanoic acid in different quantities. The ratio between C_{16} and C_{18} aliphatic cutin acids was different among the fruits. *P. alkekengi* and *N. physalodes* were dominated by C_{16} cutin acids, whereas *P. ixocarpa* and *P. peruviana* had a comparable higher amount of C_{18} cutin acids. Furthermore, the proportion of unsaturated C_{16} and C_{18} aliphatic cutin acids varied among the plant species with the lowest percentage of 1% of total cutin monomers found in *N. physalodes* and being between 5% and 8% in the *Physalis* species.

The amount of primary alkanols (< 1%), alkanoic acids (≤ 4%) and ω-hydroxy alkanoic acids (< 5%) was relatively small in the fruit cutin matrix and in the same range for all *Physalis* and *Nicandra* species. In contrast, ω-hydroxy alkanoic acid derivatives ranged from 87% to 92% of total cutin monomers in *P. ixocarpa*, *P. peruviana* and *N. physalodes* but only 76% in *P. alkekengi*. In the case of *P. alkekengi*, the proportion of α,ω-dicarboxylic acids and aromatic hydroxy cinnamic acids was enhanced with 12% and 5%, respectively. The amount of these both compound classes was considerably lower in *P. ixocarpa*, *P. peruviana* and *N. physalodes* (< 7% and < 2%). The fruit cutin matrix of the three *Physalis* species exhibited 4-hydroxy cinnamic acid as predominant hydroxy cinnamic acid, which was not detected for *N. physalodes*, which had mainly 3,4-dihydroxy cinnamic acid.

Table 7. Fruit cutin monomers of *Physalis* and *Nicandra* species. Each value represent the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Cutin monomeric coverage of fruits (µg cm^{-2})			
		P. alkekengi	*P. ixocarpa*	*P. peruviana*	*N. physalodes*
alkanoic acids	16	2.47 ± 0.82	0.58 ± 0.03	5.72 ± 0.30	1.23 ± 0.77
	18:2	-	0.29 ± 0.08	0.76 ± 0.27	-
	18:1	-	0.33 ± 0.08	-	-
	18	1.46 ± 0.67	0.67 ± 0.13	1.03 ± 0.42	0.40 ± 0.23
	20	0.93 ± 0.16	0.29 ± 0.08	0.26 ± 0.07	0.08 ± 0.04
	22	0.73 ± 0.17	0.05 ± 0.01	0.09 ± 0.01	0.22 ± 0.19
	24	1.81 ± 0.26	-	-	0.06 ± 0.02
	26	2.33 ± 0.34	-	-	0.32 ± 0.04
	28	1.53 ± 0.17	-	-	0.47 ± 0.08
	30	0.66 ± 0.23	0.02 ± 0.02	0.06 ± 0.04	0.38 ± 0.05
	32	0.04 ± 0.02	0.11 ± 0.03	0.04 ± 0.01	0.12 ± 0.06
primary alkanols	16	0.16 ± 0.10	0.08 ± 0.05	0.20 ± 0.01	-
	18	0.40 ± 0.09	0.28 ± 0.05	0.66 ± 0.13	0.22 ± 0.07
	20	0.74 ± 0.17	0.11 ± 0.03	0.41 ± 0.12	-
	22	0.40 ± 0.14	0.14 ± 0.04	0.24 ± 0.37	-
	24	0.16 ± 0.16	-	-	-
	26	0.28 ± 0.07	0.28 ± 0.06	0.23 ± 0.05	-
	28	0.58 ± 0.07	0.09 ± 0.04	0.14 ± 0.03	-
	30	0.32 ± 0.17	0.10 ± 0.11	0.05 ± 0.02	-
	32	0.09 ± 0.08	0.05 ± 0.02	0.04 ± 0.02	-
ω-hydroxy alkanoic acid	16:1	0.49 ± 0.28	0.10 ± 0.04	-	0.29 ± 0.07
	16	8.82 ± 0.77	0.62 ± 0.03	1.24 ± 0.11	2.95 ± 0.47
	18:2	2.03 ± 0.42	3.29 ± 0.64	5.18 ± 0.39	-
	18:1	15.62 ± 3.03	1.48 ± 0.34	0.09 ± 0.03	-
	18	0.31 ± 0.20	0.20 ± 0.02	0.14 ± 0.06	0.06 ± 0.04
9/10 oxo ω-hydroxy alkanoic acid	16	19.34 ± 5.32	9.50 ± 1.46	93.93 ± 8.63	0.42 ± 0.33
9/10, ω-dihydroxy alkanoic acid	16	386.34 ± 27.81	23.14 ± 2.27	33.90 ± 2.14	64.95 ± 4.24

Table 7. Fruit cutin monomers of *Physalis* and *Nicandra* species. Each value represent the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Cutin monomeric coverage of fruits (µg cm^{-2})			
		P. alkekengi	*P. ixocarpa*	*P. peruviana*	*N. physalodes*
9, 10 epoxy ω-hydroxy alkanoic acid	18	9.80 ± 1.38	10.17 ± 2.16	62.27 ± 8.57	4.51 ± 0.34
	18:1	-	-	2.03 ± 0.70	-
	18	-	59.89 ± 14.89	6.08 ± 1.42	-
9, 10, ω-trihydroxy alkanoic acid	16	-	-	-	-
	18:1	8.31 ± 1.72	4.87 ± 1.63	16.50 ± 5.98	0.92 ± 0.34
	18	8.99 ± 0.95	47.57 ± 9.93	73.88 ± 6.93	0.96 ± 0.24
α, ω-dicarboxylic acid	16	1.63 ± 0.20	0.09 ± 0.01	0.45 ± 0.15	-
9/10-hydroxy α, ω-dicarboxylic acid	16	5.90 ± 0.42	1.67 ± 0.44	15.68 ± 0.83	2.78 ± 0.50
	18	53.16 ± 5.57	0.45 ± 0.04	-	-
9, 10 epoxy α, ω-dicarboxylic acid	18	5.61 ± 1.04	0.92 ± 0.62	6.64 ± 0.91	-
4-hydroxy cinnamic acid		22.32 ± 1.68	0.47 ± 0.04	3.08 ± 0.66	-
3, 4-dihydroxy cinnamic acid		5.27 ± 1.90	0.05 ± 0.02	2.57 ± 0.06	0.34 ± 0.06
3-methoxy 4-hydroxy cinnamic acid		1.69 ± 0.97	0.11 ± 0.05	0.00 ± 0.00	0.09 ± 0.02
total cutin monomers		570.72 ± 31.82	168.05 ± 32.71	333.60 ± 33.59	81.77 ± 5.52

3 Discussion

The focus of this comparative study was to investigate the compositional, structural and functional cuticle attributes in leaf, inflated fruiting calyx and fruit of *Physalis* and *Nicandra species*. These plant species are cultivated due to their attractive reproductive parts, medicinal and culinary uses. *P. alkekengi* and *N. physalodes* are largely used as ornamental plants, while *P. ixocarpa* and *P. peruviana* are primarily cultivated for their edible fruits (Silva & Agra, 2005; Medina-Meldrano, 2015).

3.1 Morphological characterization and traits of Physalis and Nicandra species

Among the morphological traits shared by the *Physalis* and *Nicandra* species stood out the herbaceous to arbustive habit, the pentamerous androecium, the anthers with longitudinal dehiscence and the berry fruit type enclosed by an inflated and persistent calyx, as previously reported by Silva & Agra, (2005). The most distinguishable characters among the plant species were related to their reproductive parts, reflecting the flower and fruit diversity of the Solanaceae family (Knapp, 2002; Knapp et al. 2004). Shape, form, size and colour of flowers, inflated fruiting calyx and fruits were notably different among the plant species and were especially distinctive in the later stages of calyx and fruit development.

Species-specific characteristics were investigated in leaves, inflated fruiting calyces and fruits. The surface area was different among the plant organs investigated, being the largest surface area found for leaf, followed by calyx and fruit, respectively. These four plant species had a similar leaf surface area, but calyx and fruit size were different. The photosynthetic capability of the persistent green calyx was already demonstrated for some plant species, comprising a significant source of assimilates, which can contribute to fruit and seed development especially during the first days of growth (Fischer, 1995; Herrera, 2005; Li et al., 2019).

However, in this study, a larger inflated fruiting calyx did not result in a higher fruit volume. *P. alkekengi* had the largest calyx but produced the smallest fruits among the Physalideae species.

On the other hand, *P. ixocarpa* had a calyx surface area almost twofold smaller than that of fruit. This difference was responsible for the rupture of the inflated fruiting calyx observed at the ripe stage of *P. ixocarpa* fruit. In fact, the energy resources produced by the persistent calyx may not be translocated to other parts of the fruit, remaining in the sepals or being exported to other metabolic sinks (Herrera, 2005).

The surface of leaves and calyces were microscopically similar among plant species. The main difference was related to their indumentum. Leaves were similarly amphistomatic, bearing both glandular and non-glandular trichomes, and were covered by an epicuticular wax film. *P. peruviana* leaf and calyx surfaces were easily distinguishable from the other plant species by its longer and dense non-glandular trichomes, especially on the abaxial side, while *N. physalodes* had only a sparsely hairy leaf surface. On the other hand, the presence of trichomes on the adaxial surface of the calyx was observed exclusively in *P. alkekengi* and *N. physalodes*. Indeed, trichomes are important plant taxonomic features, and their presence is related to plant resistance against herbivory and tolerance to abiotic stress and, thus, enhancing plant fitness (Dalin et al., 2008; Karinho-Betancourt & Nunez-Farfán, 2015).

Likewise found for morphological features, the most recognisable microscopic traits among the plant organs and plant species were found in fruit surface. The four investigated plant species were similarly characterised by the absence of trichomes and stomata on their fruit surface. The fruit surface *N. physalodes* had concave cells, a common structure in dry surfaces such as seeds, and it is probably a result of cell shrinking due to water loss of the cells (Koch et al., 2008) during *Nicandra* fruit maturation. *Physalis ixocarpa* was unique for being covered with papilla-like projections on the fruit surface, a feature also documented by Dyki et al., 1997, with a shape similar to the cylindrical papillae as reported in *Callicostellopsis meridensis* leaves (Pilotrichaceae; Duarte-Silva et al., 2013). Papillate surfaces were also identified on fruits of *Anabasis* species and *Dysphania bhutanica* (Amaranthaceae; Sukhorukov, 2008, 2012).

3.2 Differences of water permeability between Physalis and Nicandra species

While leaves showed a similar water permeability, the green-inflated fruiting calyx lost higher amounts of water among the plant organs studied. Similarly, Díaz-Pérez (1998) reported that the calyx comprises the main route for water loss in *Solanum melongena* fruits (Solanaceae), accounting for at least 60% of fruit transpiration. Furthermore, the higher cuticular conductance presented by *Nicandra* fruit might be related to its seed dispersion strategy since the fleshy berry reverts to a dry fruit during maturation and lately irregularly burst, releasing its numerous seeds. Since no stomata were identified on fruit and were only sparsely present on the abaxial calyx surfaces, the desiccation process observed in *Nicandra* most likely relies on the cuticular water loss. This is in line with the findings for water permeability of these two plant organs in *N. physalodes*.

The results showed that, intraspecifically, leaves and fruits of *Physalis* and *Nicandra* species had similar transpiration barrier properties, with green-calyx presenting the less effective water transpiration barrier among the plant organs. Interspecifically, *P. alkekengi* calyx had the most effective transpiration barrier with 7.4×10^{-5} m s $^{-1}$ among the plant species, while *P. alkekengi* and *P. ixocarpa* fruits with about 2.2×10^{-5} m s $^{-1}$ showed the best performance against fruit cuticular water loss.

3.3 The inflated fruiting calyx and its effect of the cuticular water transpiration of fruits

The calyx-removal experiment was conducted in order to investigate the functional significance of the inflated fruiting calyx for the cuticular water conductance of ripe fruits. Although *P. peruviana* fruit had a higher cuticular conductance compared to its congener plant species, its cuticular water loss was the most affected by the calyx removal followed by *P. ixocarpa* fruits. Whilst the green-inflated fruiting calyx contributes to transpirational water loss of fruits, it probably plays the opposite role after its dehydration in those two plant species, since its removal increased the cuticular water loss of ripe fruits by a factor of two. The mechanism behind these findings might

result from the reduction of the evaporative demand inside the calyx structure, probably due to the maintenance of high relative air humidity and low air temperature that in turn would reduce fruit driving force to water loss in comparison to the atmosphere outside of the inflated fruiting calyx. Indeed, Fischer (1995) registered temperatures up to 5°C lower inside of the inflated fruiting calyx structure in comparison to the surrounding atmosphere, corroborating this hypothesis. It was recently shown for *Physalis pubescens* (synonym: *Physalis floridana*; Solanaceae) that the inflated fruiting calyx favours the formation of a microclimate inside its structure, enabling the fruit development and maturation under a low-temperature environment (Li et al., 2019). The results herein found for *P. peruviana* suggest that its calyx have a similar mechanism showed by *P. pubescens* since the presence of this post-floral structure significantly affected the cuticular water loss of *P. peruviana* fruits.

In addition to the minimisation of water loss, recent studies have attributed a role for the fruit cuticle in the modulation of fruit quality (Lara et al., 2015), and the calyx protection, with its own cuticular properties, can enhance fruit cuticle performance in *Physalis* fruits. Indeed, previous studies on *P. peruviana* fruits demonstrated that fruits without calyx had higher weight loss and ethylene production than fruits with calyx (Balaguera-López et al., 2014). Fruits with the dried calyx could last for up to 30 days after harvesting when stored at low temperatures (Novoa et al., 2006). On the other hand, *P. ixocarpa* calyx might contribute differently to the reduced water loss rate in ripe fruits. Once the *P. ixocarpa* fruit fills almost completely the calyx space at the ripe stage, this structure may act as a natural coating after dehydration, comprising a physical barrier against fruit cuticular water loss. In this way, the findings of the present study indicate that the dry inflated fruiting calyx has a post-maturation function, significantly reducing cuticular water loss of ripe fruits in *P. ixocarpa* and *P. peruviana*.

The inflated fruiting calyx is a supplementary plant organ of the fruit, thus having several functions, including protection against pathogens and herbivory, mechanical damages and promoting fruit and seed dispersal by water and wind (Fischer et al., 1997; Herrera, 2005; Li et al., 2019). These are likely the functions of this structure for *P. alkekengi* and *N. physalodes* ripe fruits since no

effect of the calyx removal was detected for the fruit cuticular water conductance in these two plant species. In the field, *P. alkekengi* inflated fruiting calyx gradually decays by rain and wind, revealing a delicate skeletal network formed by the dry veins of the calyx. In this scenario, the shiny red fruit became visible and attractive for frugivore animals. *Nicandra* is an introduced and ruderal plant species elsewhere, considered a weed plant in several parts of the world corroborating this idea (Cohen, 1970; Horton, 1979; de Carvalho et al., 2008). The shovel-like calyx lobes of *N. physalodes* favour the mechanical dispersion of the seeds by wind, thus, increasing plant fitness (Seale & Nakayama, 2020).

As a physical barrier, one of the main functions of the cuticle is to prevent uncontrolled water loss to the atmosphere. The transpiration barrier of the cuticle is primarily provided by its complex mixture of cuticular waxes, which was mostly composed of very-long-chain aliphatic compounds in the plant species studied, comprising more than 89% of the total cuticular waxes, regardless of the plant organ. Although the cuticular waxes coating the surfaces of the plant organs had specific compositions, the major aliphatic compound classes identified in the wax mixture of *Physalis* and *Nicandra* were *n*-alkanes and primary alkanols, but *P. alkekengi* fruits had alkyl esters as the most abundant component. This result is in accordance with other cuticular studies on leaves and fruits of solanaceous species (Haliński et al., 2009; Leide et al., 2011; da Silva et al., 2012). However, it is different from the findings for plant species from other families, such as *Prunus avium* (Rosaceae; Peschel et al., 2007), *Malus domestica* (Rosaceae; Leide et al., 2018), *Olea europeae* (Oleaceae; Huang et al., 2017) and *Vitis vinifera* (Vitaceae; Casado & Heredia, 1999), which are predominantly composed of pentacyclic triterpenoids. While pentacyclic triterpenoids are mostly associated with plant-insect interactions (Eigenhrode & Espelie, 1995) and stabilisation of the cuticle under extreme climate conditions (Schuster et al., 2016), very-long-chain aliphatic compounds primarily contribute to the cuticular transpiration barrier (Jetter & Riederer, 2016).

3.4 Cuticular waxes differences between Physalis and Nicandra species

In the solanaceous species studied, alicyclic compounds were found in moderate amounts of up to 9% in leaves and calyces that are plant organs directly exposed to biotic and abiotic stresses, whereas fruits, which develop protected inside the inflated fruiting calyx structure, had only traces or no alicyclic compounds. Interestingly, *P. alkekengi,* the only studied plant species that grows under temperate conditions and fructify during the fall season in the northern hemisphere, had 11% of cyclic compounds in its total fruit cuticular wax composition. These results corroborate the hypothesis that alicyclic compounds play a significant role in protection against herbivory or pathogen infection, or maintenance of cuticular structural integrity and stability under extreme environmental conditions (Buschhaus & Jetter, 2012; Schuster, 2016).

Except for *Nicandra*, fruits had the highest wax accumulation among the plant organs investigated, being the largest quantity of cuticular waxes found in *P. peruviana* fruit with about 25.1 µg cm^{-2}, while the thicker fruit cuticle was observed for *P. alkekengi* with 8.6 µm. This difference between cuticle thickness and cuticular wax coverage for *P. alkekengi* fruit originated from the amount of its cutin matrix, which was up to sevenfold higher compared to the amount of the other three plant species.

The predominant cutin monomers in fruits were C_{16} and C_{18} type, mainly 9/10,ω-dihydroxy hexadecanoic acid in *P. alkekengi* and *N. physalodes*, 9,10-epoxy ω-hydroxy octadecanoic acid in *P. ixocarpa* and 9/10-oxo ω-hydroxy hexadecanoic acid in *P. peruviana* fruits. Comparatively, the C_{16}/C_{18} cutin acid ratio was different. The cutin composition of *P. alkekengi* and *N. physalodes* comprises a C_{16} type, while *P. ixocarpa* and *P peruviana* represent a C_{18} type (Holloway, 1982).

However, similarly to the acyclic components, the cutin monomers, wax and cutin total amounts, cuticle thickness do not primarily contribute to the efficacy of the cuticular transpiration barrier (Jetter & Riederer, 2016). Nevertheless, it is proposed that the cutin polymer provides a supporting framework to the cuticular waxes and imparts plasticity to the cuticle, playing an

important role in the construction of an effective transpiration barrier (Kosma & Jenks, 2007; López-Casado et al., 2007).

4 Conclusion

To the author's best knowledge, this was the first comparative study focused on structural, chemical and functional analyses of the plant cuticle of different vegetative and reproductive plant organs from plant species bearing the inflated fruiting calyx as a special morphological trait. The results revealed a species-specific and an organ-specific pattern for water permeability and chemical composition for *P. alkekengi*, *P. ixocarpa*, *P. peruviana* and *N. physalodes* leaves, inflated fruiting calyces and fruits. Furthermore, it was shown that the inflated fruiting calyx significantly reduces the cuticular water transpiration in *P. ixocarpa* and *P. peruviana* fruits. In these two *Physalis* species, the inflated fruiting calyx structure potentially provides a microclimate that increases ripe fruit performance by reducing its cuticular water transpiration.

Chapter III. Variability of water permeability of leaves and fruits within the Solanaceae family

1 Introduction

Most land plants have a limited ability to cope with severe desiccation. Even after drought-induced stomatal closure, water is passively lost through aerial plant surfaces, resulting in their desiccation and death, when water scarcity is sufficiently extended. A crucial evolutionary survival tool for reducing uncontrolled water loss is the plant cuticle, a continuous extracellular layer synthetized by the epidermal cells (Goodwin & Jenks, 2005; Burghardt & Riederer, 2006). Together with the stomata, the plant cuticle is as a ubiquitous feature among land plants to regulate plant water status (Croxdale, 2001; Reina-Pinto & Yephremov, 2009; Budke et al., 2012).

Due to its extracellular position, the cuticle acts as a natural interface between aerial plant organs and their surrounding environment and, thus, plays a vital role in the survival of land plants (Kerstiens, G, 1996d; Heredia & Dominguez, 2009). This hydrophobic waxy layer covers and protects primary organs of lower and higher land plants, including leaves, stems, flowers, and fruits (Jeffre, 2006; Koch et al., 2008; Samuels et al., 2008; Buschhaus & Jetter, 2011). In addition to its primary role as the foremost barrier to transpirational water loss, the plant cuticle has multiple secondary functions in plant development and physiology, including conferring resistance to a wide range of biotic and abiotic stresses (Buschhaus & Jetter, 2011; Domínguez et al., 2011; Yeats & Rose, 2013). It is chemically composed of an insoluble polymer, the cutin matrix, and associated solvent-soluble lipids, named cuticular waxes, but polysaccharides, phenolic compounds and cutan can also be present (Guzmán-Delgado et al., 2016).

Cuticular traits can vary enormously across plant species, cultivars and even within individuals in an organ- and tissue-specific manner (Matzke & Riederer, 1991; Kerstiens, 1996c; Bonaventure et al. 2004; Xu et al., 2014; Jetter et al., 2006; Leide et al., 2018). In particular, cuticular water permeability is highly variable among plant species with values ranging over

three orders of magnitude, from 10^{-7} ms^{-1} to 10^{-4} ms^{-1}. Given the crucial role of the cuticular water permeability in determining the minimum and inevitable water loss from plant surfaces (Riederer & Schreiber, 2001), comprehensively addressing this parameter is essential to broaden our understanding of the different adaptations of plants to drought.

Despite its importance for plant fitness and survival under water-limited conditions, cuticular functionalities have been studied only in a limited number of plant species (Lara et al., 2014; Fernandéz et al., 2017; Zarrouk et al., 2018). In this context, species from the Solanaceae family comprise an excellent opportunity to bridge the gaps in knowledge on cuticle biology by offering a wide range of working resources. Solanaceous plant species, or nightshades, inhabit a broad range of ecosystems, ranging from annual and perennial herbs to shrubs and even trees and exhibit a notable diversity of dispersal attributes, including animal and non-animal vectors (D'Arcy, 1991; Hunziker, 2001; Knapp, 2002; Olmstead, 2013; Vorontsova et al., 2013; Barboza et al., 2016). This cosmopolitan plant family comprises between 3000 and 4000 plant species within 90 genera, including important food crops of economic and historical value to humans, such as *Solanum tuberosum* and *Solanum lycopersicum*, medicinal plants and ornamentals, as *Nicotiana tabacum* and *Solandra grandiflora*, and even model organisms used in biological studies (Knapp et al., 2004; Wilf et al., 2017).

The minimum conductance of leaves is well discussed in the cuticle-related literature, whereas the permeability of fruits was examined to a lesser extent (Becker, 1986; Parsons et al., 2013; Leide et al., 2018; Diarte et al., 2019). Fleshy fruits, especially *S. lycopersicum*, have stood out as a valuable model of research (Martin & Rose, 2014); still, there are no studies on the cuticular properties of dry fruits. Furthermore, comparative investigations of different organs from the same plant species are still scarce (Huang, 2017; Huang et al., 2017). Thus, this study focused on the function of the plant cuticle as a barrier to water loss in species from the Solanaceae family and aimed to shed light on possible similarities among phylogenetically related plant species. For that, the cuticular function of leaves and fruits were compared among twenty-seven solanaceous species belonging to ten different genera to determine potential

species and organ specificities. In addition to the species investigated in the chapters I and II, the cuticular structure was further investigated in eleven solanaceous representatives. Furthermore, the cuticle function as transpiration barrier to water loss was put into perspective of the respective life forms, fruit types and climatic origin of plant species (Table 1).

2 Results

2.1 Surface morphology of solanaceous leaves and fruits

Plant surface properties of *Nicandra, Physalis* and *Solanum* genera were previously described in the chapters I and II. In addition, this chapter investigated morphological traits of leaves and fruits belonging to the genera *Atropa*, *Cestrum*, *Datura*, *Hyoscyamus*, *Nicotiana* and *Solandra*, plus two additional *Solanum* species (Fig. 1).

The solanaceous leaves were microscopically diverse with side- and species-specific densities of stomata and trichomes (Fig. 2). Stomata were observed on both adaxial and abaxial leaf surfaces, except for *A. bella-donna*, *C. elegans* and *S. grandiflora*, which were hypostomatic, with stomata located only on the abaxial surface. Leaves were densely covered by non-glandular and glandular trichomes, with the only exception for *Solandra grandiflora*, which has glabrous leaf surfaces. All other solanaceous species had trichomes on the abaxial and adaxial sides, mainly simple and capitate glandular trichomes. Non-stalked glandular trichomes were observed solely on *S. lycocarpum* surfaces. Stellate trichomes were observed exclusively on S. *lycocarpum* and *S. sisymbriifolium*. *Cestrum elegans* and *C. parqui* were the only leaves bearing y-shaped trichomes.

Table 1. Plant species of the Solanaceae family characterised based on their climatic origin according to Köppen-Geiger climate classification updated by Beck et al. (2018), life habit of the species (annual or perennial - evergreen or deciduous), and fruit type (fleshy or dry fruits).

	plant species	climatic origin	life forms	fruit type
1	*Atropa bella-donna* L.	temperate	perennial deciduous	fleshy
	Atropa bella-donna var. *lutea* Döll			
2	*Capsicum annuum* L. cv. 'Kapia'	arid	annual	fleshy
3	*Cestrum elegans* (Brongn. ex Neumann) Schltdl.	arid	perennial evergreen	fleshy
4	*Cestrum parqui* (Lam.) L'Hér	arid	perennial deciduous	fleshy
5	*Datura innoxia* Mill.	tropical	perennial evergreen	dry
6	*Hyoscyamus albus* L.	temperate	annual	dry
7	*Hyoscyamus niger* L.	temperate	annual	dry
8	*Nicandra physalodes* Gaertn.	equatorial	annual	fleshy
9	*Nicotiana tabacum* L. cv. 'Izmir'	tropical	annual	dry
10	*Physalis alkekengi* L.	temperate	perennial deciduous	fleshy
11	*Physalis ixocarpa* Brot. ex. Hornem	arid	annual	fleshy
12	*Physalis peruviana* L.	tropical	perennial evergreen	fleshy
13	*Solandra grandiflora* Sw	tropical	perennial evergreen	fleshy
14	*Solanum burchellii* Dunal	arid	perennial evergreen	fleshy
15	*Solanum dulcamara* L.	temperate	perennial evergreen	fleshy
16	*Solanum linnaeanum* Hepper & P.-M.L. Jaeger	arid	perennial evergreen	fleshy
17	*Solanum lycocarpum* St.Hill	tropical	perennial evergreen	fleshy
18	*Solanum lycopersicum* L. cv. 'Benarys Gartenfreude'	tropical	perennial evergreen	fleshy
	Solanum lycopersicum L. cv. 'John Baer'			
	Solanum lycopersicum L. cv. 'Pearson'	l		
19	*Solanum melongena* L. cv. 'Slim Jim'	tropical	perennial evergreen	fleshy
20	*Solanum muricatum* Aiton	arid	perennial evergreen	fleshy
21	*Solanum pennellii* Correll	arid	perennial evergreen	fleshy
22	*Solanum pseudocapsicum* L.	temperate	perennial evergreen	fleshy
23	*Solanum quitoense* Lam.	tropical	perennial deciduous	fleshy
24	*Solanum retroflexum* Dunal	arid	annual	fleshy
25	*Solanum sisymbriifolium* Lam.	temperate	annual to semi-perennial	fleshy
26	*Solanum tuberosum* subsp. *andigena* (Juz. & Bukasov) Hawkes	temperate	perennial deciduous	fleshy
27	*Solanum virginianum* L.	arid	annual	fleshy

Smooth to irregular folding cuticular surfaces were observed in the solanaceous leaves. Leaves of *A. bella-donna* were distinguishable by a highly folded surface and marked wavy epidermal cells contours. Irregular folding was also observed in *C. parqui* surface, although in a less extent than observed to *Atropa*, and mainly restricted to trichomes and stomata vicinity. A cuticular wax film or layer covered the leaf surfaces, and epicuticular wax granules irregularly distributed were also observed in most of the species. Platelet and rodlet-like crystals were also found but only in *A. bella-donna* var. *lutea*, *C. parqui*, *H. albus*, and *S. grandiflora* leaves. Occasionally, it was observed the cuticular waxes completely covering the stomata in *A. bella-donna* and densely covering the outer ledges of the stomata in *C. parqui*.

Fruits of the solanaceous investigated were mostly characterized by a wax film covering their smooth surface with any explicit crystalloid sculptures, but wax granules occasionally covering the surface. Outstanding features were an irregular folding cuticle observed in *C. elegans* and *C. parqui* surfaces, and marked epidermal cell contours with papillae-like structures were observed in *A. bella-donna* fruits. Fruits of both *Hyoscyamus* species and *N. tabacum* were characterized by a very thin cuticle. Broken trichomes were observed on *S. sisymbriifolium* fruit surface, as well as numerous glandular and non-glandular trichomes in *D. innoxia*. No stomata or lenticels were observed on the fruit surfaces.

Fig. 1. Morphological diversity of leaves, fruits and flowers of solanaceous species belonging to the genera *Atropa*, *Cestrum*, *Datura*, *Hyoscyamus*, *Nicotiana*, *Solandra* and *Solanum*. *S. lycocarpum* and *S. sysimbriifolium* were the only species bearing prickles on the leaf surfaces. Only leaves were investigated for *S. grandiflora* and *S. lycocarpum*. Scale bar = 1 cm.

2.2 Organ-specific variation in functional traits of leaf and fruit among solanaceous species

Different functional attributes were measured in fully expanded leaves and ripe fruits to characterize both plant organs. To verify a relationship between the organ traits and plant life strategies, fresh and dry weights, total and specific surface areas, and water content were related to the plant organs, life forms (annual or perennial evergreen / deciduous) and fruit type (fleshy or dry fruits).

2.2.1 Fresh and dry weight

Fresh weight of the solanaceous leaves varied from 0.10 g in *S. burchellii* to 3.58 g in *N. tabacum*, while the fresh weight of fruits ranged from 0.57 g in *C. parqui* to 103.84 g in *S. muricatum*. A difference of 15-fold was found between the fresh weight of the organs, that was about 1.06 g in leaves and 16.27 g in fruits ($U = 563.000$, $p < 0.05$, Fig.3a). Likewise, dry weight was 8.5 times lower in leaves compared to fruits, averaging 0.19 g and 1.61 g, respectively ($U = 620.000$, $p < 0.01$, Fig.3b).

2.2.2 Total and specific surface area

The total leaf surface area ranged from 2.99 cm^2 in *S. burchellii* to 120.21 cm^2 in *N. tabacum*, averaging 35.12 cm^2. Fruit surface area varied from 1.96 cm^2 in *C. parqui* to 122.24 cm^2 in *S. muricatum*, with about 25.27 cm^2 and, thus, had lower surface area than that of leaves ($U = 218.000$, $p < 0.01$, Fig.3c). Similarly, a lower specific surface area was found for fruits with 2.51 $m^2\ kg^{-1}$ in comparison to leaves with 21.19 $m^2\ kg^{-1}$ ($U = 1.000$, $p < 0.001$, Fig.3d).

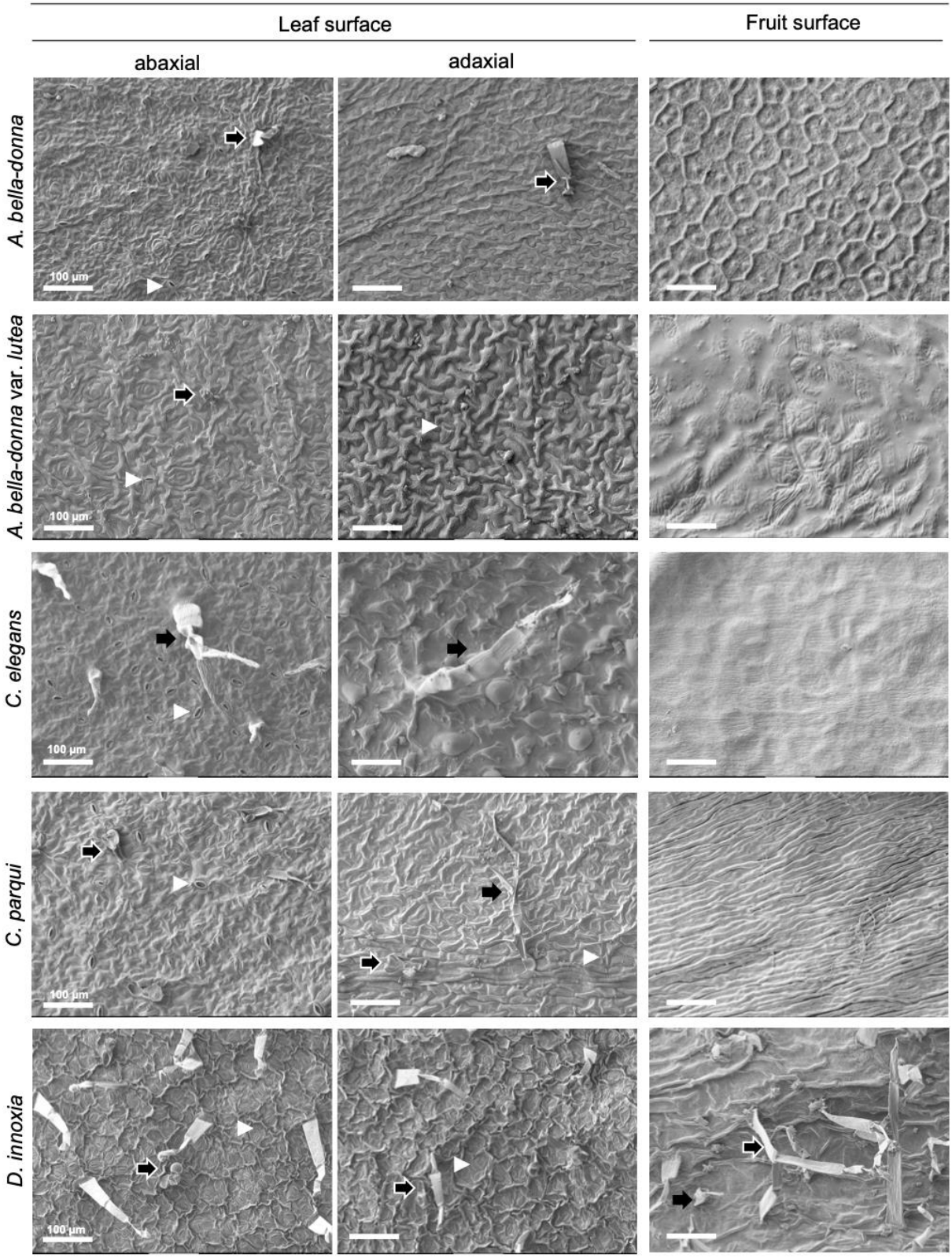


Fig. 2. Scanning electron micrographs from intact leaf surfaces (abaxial and adaxial) and outer fruit cuticles of solanaceous representatives. Arrowheads indicate stomata (white). Arrows indicate non-glandular trichomes (black) and glandular trichomes (white contour and black filling).

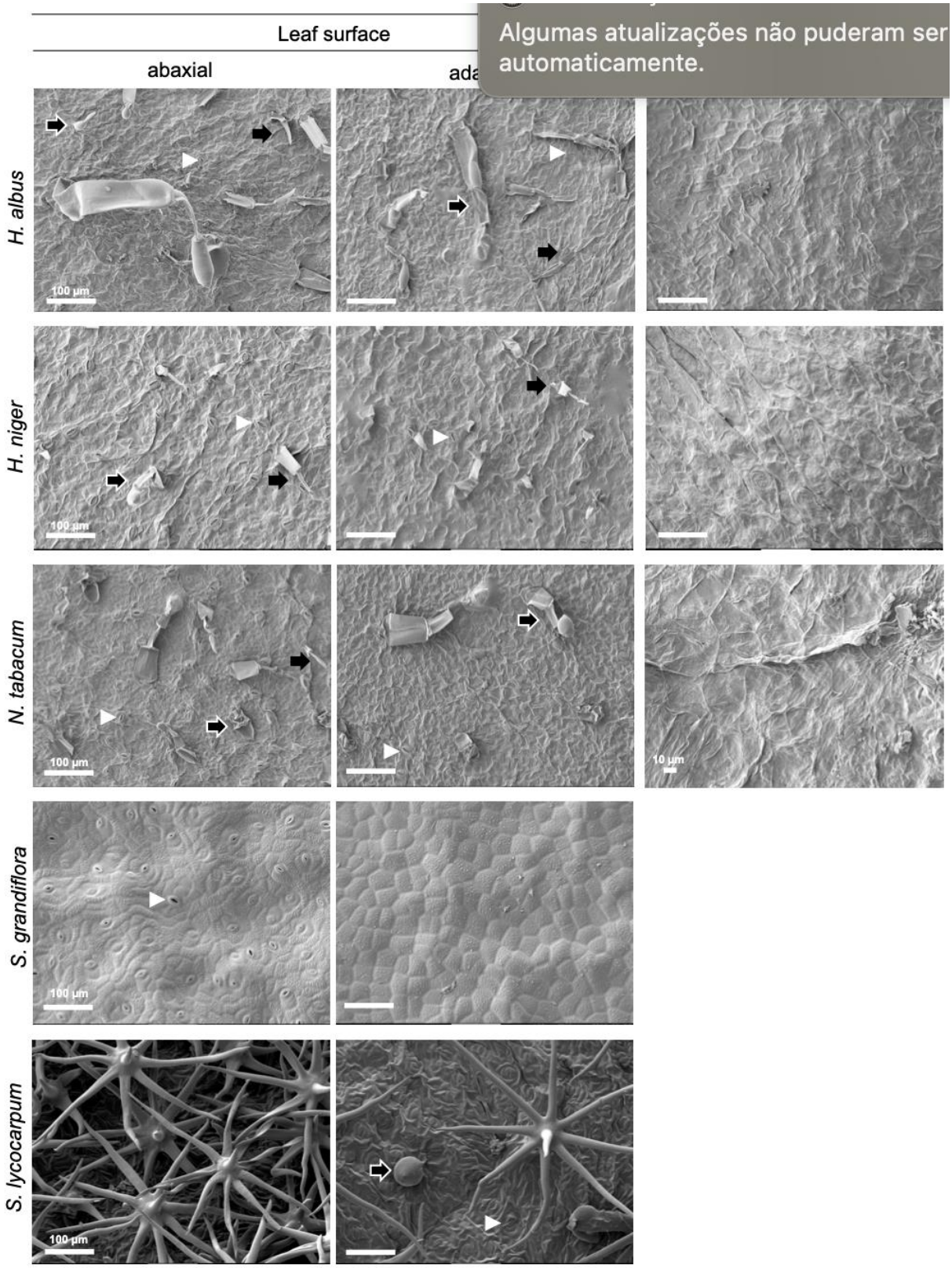


Fig. 2. (continued) Scanning electron micrographs from intact leaf surfaces (abaxial and adaxial) and outer fruit cuticles of solanaceous representatives. Arrowheads indicate stomata (white). Arrows indicate non-glandular trichomes (black) and glandular trichomes (white contour and black filling).

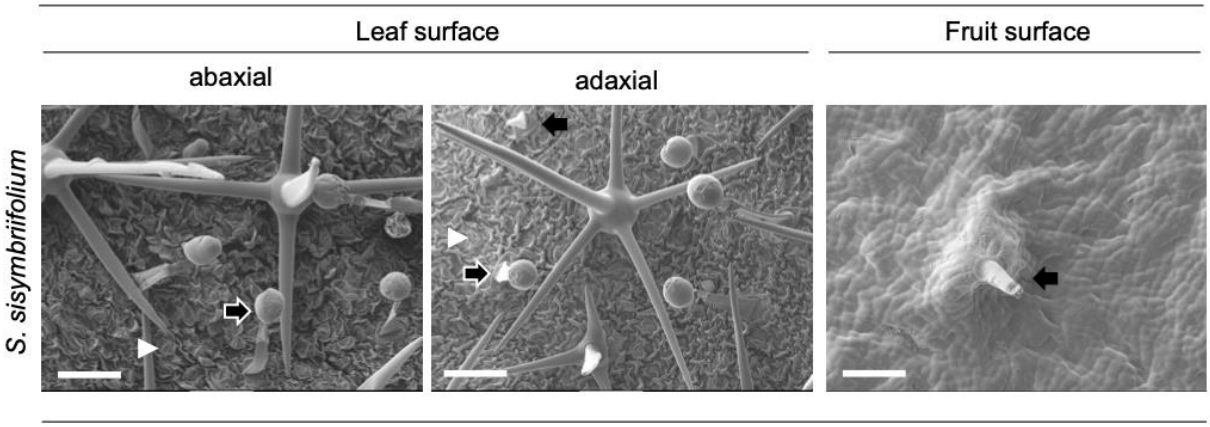


Fig. 2. (continued) Scanning electron micrographs from intact leaf surfaces (abaxial and adaxial) and outer fruit cuticles of solanaceous representatives. Arrowheads indicate stomata (white). Arrows indicate non-glandular trichomes (black) and glandular trichomes (white contour and black filling).

2.2.3 Leaf and fruit water content

Leaf water content ranged from 63% in *S. burchellii* to 88% in *S. lycopersicum* cv. 'John Baer', having a lower variation than fruits, which ranged from 34% in *N. physalodes* to 95% in *S. lycopersicum* cv. 'Benarys Gartenfreude'. Regardless of the considerable variation between organs, the water content of leaves and fruits were statistically similar ($p > 0.05$, Fig.3e). Due to its very low water content at the mature stage, the berry fruits of *N. physalodes* were included in the category of "dry fruits" in the following analyses.

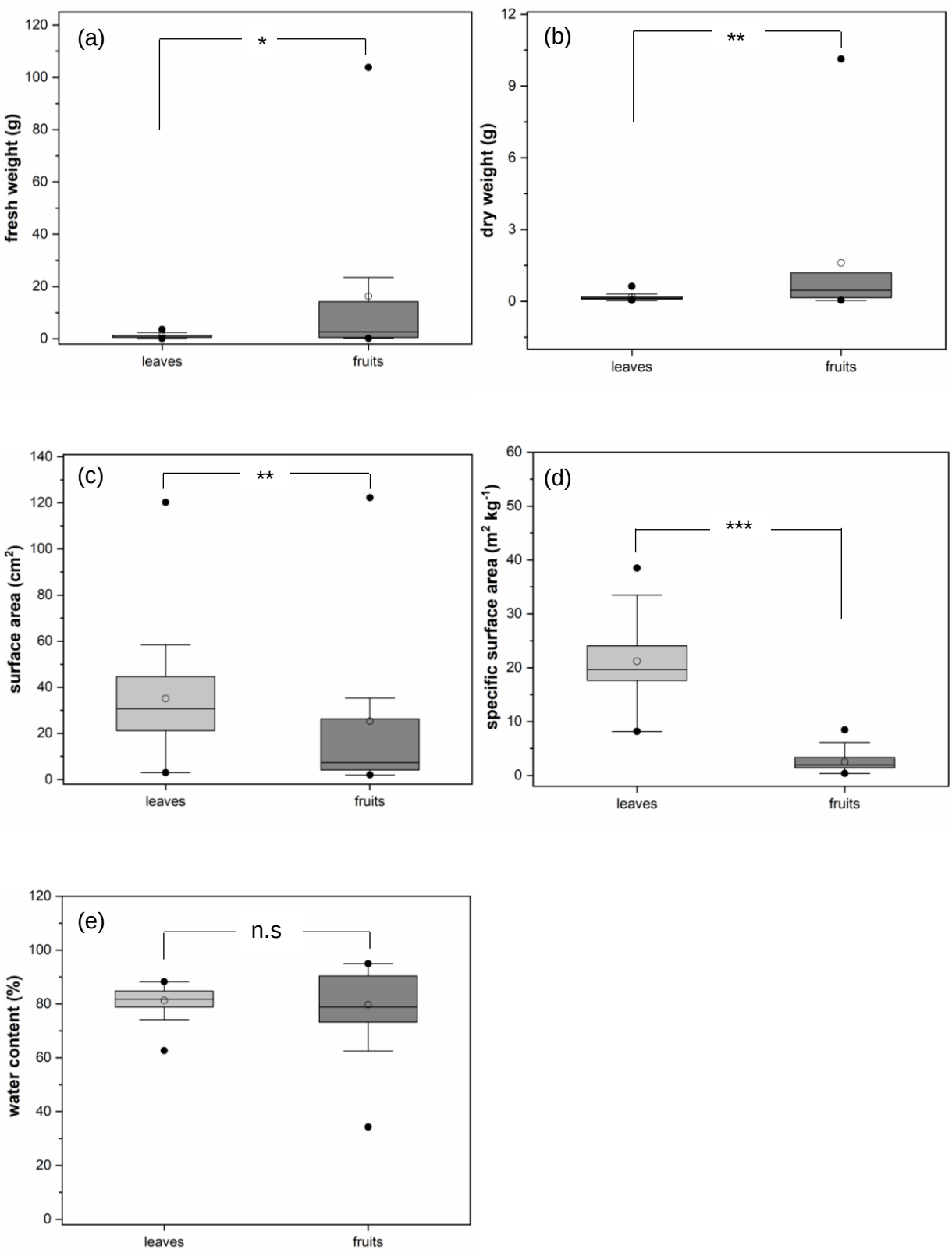


Fig. 3. Functional traits of solanaceous species (n= 30 representatives for leaves, n = 28 representatives for fruits). Fresh weight (a), dry weight (b), total surface area (c), specific surface area (d), and relative water content (e) of fully expanded leaves and ripe fruits. Closed circles indicate maximum and minimum values. Solid line within the box indicates the median, while open circles mark the mean. Statistical differences: Mann-Whitney-U-test; n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

2.2.4 *Functional traits and life strategies of the solanaceous species*

Despite the great functional diversity found across the solanaceous species, leaf functional traits did not differ among life forms (annual, perennial evergreen or deciduous, $p > 0.05$). Similarly, fruit traits were similar between fruit types ($p > 0.05$), except for water content, that was significantly lower in fleshy fruits compared to dry fruits (Fig. 4, $U = 18.000$, $p < 0.05$).

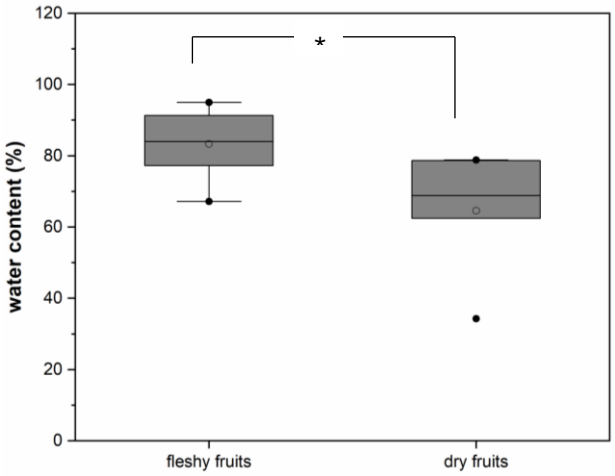


Fig 4. Relative water content between fleshy and dry fruits (n = 28 solanaceous representatives). Closed circles indicate maximum and minimum values. Solid line within the box indicates the median, while open circles mark the mean. Statistical differences: Mann-Whitney-U-test; *, p = 0.019.

2.3 Organ-specific water permeability within solanaceous plant species

At an organ-specific level, most solanaceous plant species had significant differences of water permeability between fully expanded leaves and ripe fruits, as showed by the leaf-to-fruit ratio within plant species (Table 2). Leaves had higher permeability in comparison to fruits in *A. bella-donna, P. alkekengi, P. ixocarpa, S. burchellii, S. dulcamara, S. linnaeanum, S. melongena, S. muricatum, S. pennellii, S. pseudocapsicum, S. sisymbriifolium* and *S. virginianum*. The opposite was detected for the following species: *A. bella-donna* var. *lutea, C. annuum, C. elegans, C. parqui, H. albus, H. niger, N. physalodes, N. tabacum, S. lycopersicum* cv. 'Benarys Gartenfreude' and *S. quitoense*, in which leaves had lower permeability than fruits. Furthermore, no difference

was found between the water permeability of the two organs in *D. innoxia*, *P. peruviana*, *S. lycopersicum* cv. 'John Baer' and 'Pearson', *S. retroflexum* and *S. tuberosum*.

Table 2. Cuticular water permeability of fully expanded leaves and ripe fruits of solanaceous representatives. Each value represents the mean ± standard deviation of leaves ($n \geq 8$) and fruits ($n \geq 6$). Statistical analysis of differences: *t*-test or Mann-Whitney-U-test; n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

plant species	**water permeability × 10^5 (m s^{-1})**		
	leaf	**fruit**	**ratio$_{leaf:\ fruit}$**
Atropa bella-donna	4.94 ± 1.08	3.05 ± 1.38	1.6**
Atropa bella-donna var. *lutea*	1.85 ± 0.49	2.04 ± 0.78	0.9*
Capsicum annuum	6.05 ± 1.49	8.06 ± 0.74	0.8*
Cestrum elegans	2.77 ± 0.64	13.25 ± 1.94	0.2***
Cestrum parqui	2.51 ± 0.53	8.81 ± 0.93	0.3***
Datura innoxia	7.89 ± 2.14	7.55 ± 1.22	1.1 ns
Hyoscyamus albus	4.00 ± 0.55	18.88 ± 7.83	0.2**
Hyoscyamus niger	3.23 ± 1.13	28.44 ± 8.58	0.1*
Nicandra physalodes	5.32 ± 0.55	11.18 ± 3.02	0.5***
Nicotiana tabacum	7.73 ± 2.10	34.98 ± 9.75	0.2**
Physalis alkekengi	4.77 ± 1.46	2.44 ± 1.27	2.0***
Physalis ixocarpa	5.06 ± 0.76	1.85 ± 0.97	2.7***
Physalis peruviana	4.40 ± 1.58	4.84 ± 1.40	0.9 ns
Solandra grandiflora	0.35 ± 0.08	-	-
Solanum burchellii	5.16 ± 1.24	3.82 ± 1.61	1.4*
Solanum dulcamara	3.85 ± 1.04	0.64 ± 0.19	6.0***
Solanum linnaeanum	2.64 ± 0.88	1.10 ± 0.59	2.4**
Solanum lycocarpum	6.61 ± 0.72	-	-
Solanum lycopersicum cv. 'Benarys Gartenfreude'	3.05 ± 0.56	6.01 ± 2.29	0.5*
Solanum lycopersicum cv. 'John Baer'	10.07 ± 3.33	9.64 ± 3.88	1.0 ns
Solanum lycopersicum cv. 'Pearson'	8.99 ± 3.87	6.63 ± 2.45	1.4 ns
Solanum melongena	12.87 ± 3.75	2.64 ± 0.66	4.9***
Solanum muricatum	31.54 ± 7.27	3.48 ± 1.11	9.0***
Solanum pennellii	4.17 ± 0.59	2.09 ± 0.64	2.0*
Solanum pseudocapsicum	6.43 ± 1.11	2.02 ± 0.44	3.2**
Solanum quitoense	3.62 ± 0.96	6.68 ± 1.13	0.5*
Solanum retroflexum	3.42 ± 0.74	4.33 ± 1.39	0.8 ns
Solanum sisymbriifolium	4.35 ± 1.26	2.02 ± 0.72	2.2***
Solanum tuberosum subsp. *andigena*	7.39 ± 1.16	6.72 ± 1.19	1.1 ns
Solanum virginianum	5.22 ± 1.08	1.73 ± 0.77	3.0**

2.4 Minimum water conductance of solanaceous leaves: different life forms

The minimum water conductance of leaves was significantly different among the species investigated (Table 2). To identify a relationship between plant life strategies and cuticular water loss, the minimum conductances were put into perspective of the respective life forms of the solanaceous species (annual, perennial evergreen and perennial deciduous). However, a relationship was not detected ($p > 0.05$, Fig. 5).

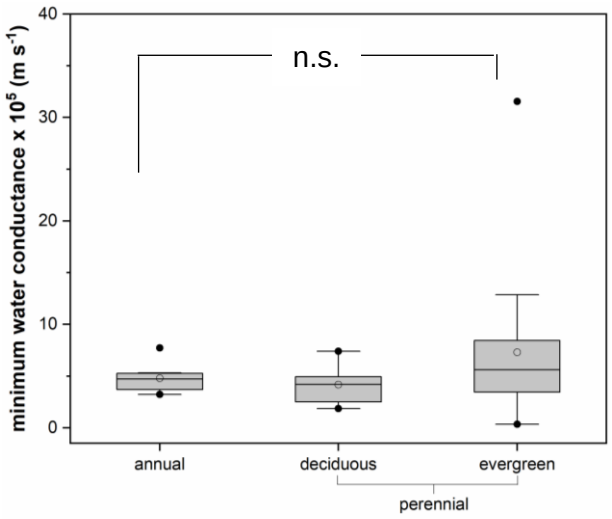


Fig. 5. Minimum water conductance of solanaceous leaves obtained from drying curves at 25°C and dark conditions, grouped by life forms in annual (n= 9) and perennial (deciduous, n= 6, or evergreen, n=15). Closed circles indicate maximum and minimum values. Solid line within the box indicates the median, while open circles mark the mean. Statistical analyses: Kruskal-Wallis test, (X^2 (2) = 1.897, p = 0.387); n.s., not significant.

A Kruskal-Wallis test followed by a post-hoc analysis ranked the minimum leaf conductances in sixteen homogeneous subsets characterized by a high species-specific variation (X^2 (29) = 251.56, $p < 0.001$). Values ranged over two orders of magnitude from 10^{-6} m s^{-1} to 10^{-4} m s^{-1} and varied up to strikingly 90-fold among the plant species. The lowest permeability was found in leaves of the evergreen *S. grandiflora* (0.35×10^{-5} m s^{-1}), followed by the deciduous *A. bella-donna* (1.85×10^{-5} m s^{-1}), and the highest in *S. muricatum*, an evergreen (31.54×10^{-5} m s^{-1}). These three species were the only ones belonging to unique subsets. The minimum conductances of the other 27 solanaceous leaves presented a lower variability and were more

consistent compared to the highest and lowest values. A variability with a factor of 5 was found among these groups with values ranging from 2.51 × 10^{-5} m s^{-1} in *C. parqui*, a deciduous plant species, to 12.85 × 10^{-5} m s^{-1} in *S. melongena*, an evergreen species. Despite being statistically similar, these values resulted in thirteen subsets intersecting each other, and no clear pattern of permeability distribution was identified within each subset.

2.5 Cuticular water permeability of solanaceous fruits: different fruit types

The cuticular water permeability varied considerably among the solanaceous fruits investigated. Similar to leaves, the post-hoc analysis after a Kruskal-Wallis test ranked the fruit permeabilities in sixteen homogeneous subsets (X^2 (27) = 339.32, p < 0.001). The subsets were characterized by a high species-specific variation of the cuticular water permeability, ranging two orders of magnitude from 10^{-6} m s^{-1} to 10^{-4} m s^{-1}, and up to 55-fold among the plant species.

The comparison among fruits resulted in more significantly different subsets than that of observed for leaves. Significantly lower values were found for the fleshy fruit of *S. dulcamara*, which had the lowest cuticular water permeability among the solanaceous fruits, followed by the fleshy fruit of *S. linnaeanum*, with values of 0.64 × 10^{-5} m s^{-1} and 1.10 × 10^{-5} m s^{-1}, respectively. The dry fruits of *N. tabacum* and *H. niger* were statistically similar, averaging 31.71 × 10^{-5} m s^{-1}, and corresponding to the highest permeabilities among the fruits. Dry fruits of *H. albus* and the fleshy fruit of *C. elegans* had permeabilities of about 15.58 × 10^{-5} m s^{-1}, and comprised the group of plants with the second-highest permeabilities. Despite not being significantly different from each other, the remaining permeabilities ranged by about 5-fold among the species (from 1.73 × 10^{-5} m s^{-1} in *S. virginianum* to 8.59 × 10^{-5} m s^{-1} in *C. parqui*).

Based on the results of the homogenous subsets, a distinct distribution of permeability was observed among the solanaceous fruits. While fleshy fruits had a high variability and were distributed in different subsets, species with dry fruits were concentrated between the 12th and 16th subsets, and therefore among the higher permeabilities found. Thus, the water permeability

of the solanaceous fruits was further classified according to their respective fruit types in fleshy fruits and dry fruits, to investigate whether this trait may influence cuticular water permeability. As a result, the permeability of fleshy fruits, with about 4.51 × 10^{-5} m s^{-1}, was 4.5 times lower than that of dry fruits, with about 20.21 × 10^{-5} m s^{-1} (U = 106.000, p < 0.001, Fig.6).

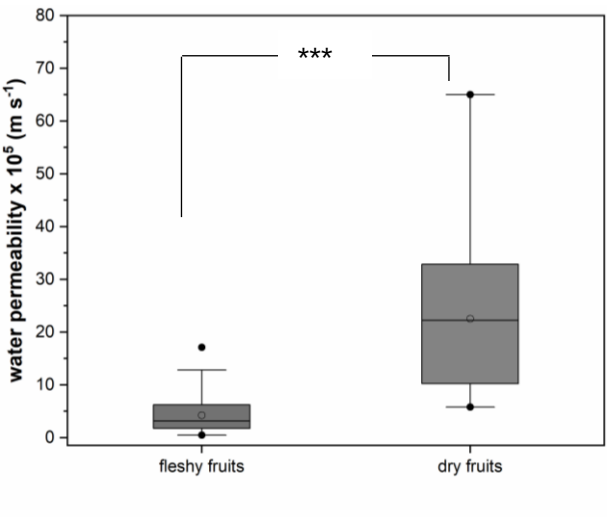


Fig. 6 Cuticular water permeability of ripe fruits of 28 solanaceous representatives grouped according to their fruit type. Closed circles indicate maximum and minimum values. Solid line within the box indicates the median, while open circles mark the mean; fleshy fruits, n = 23; dry fruits, n = 5. Statistical analysis of differences: Mann-Whitney-U-test, ****, p < 0.001.

2.6 Variety-specific water permeability within solanaceous plant species

This study additionally investigated the water permeability of the pale leaves and yellow fruits of *A. bella-donna* variety *lutea*, and among three cultivars of *S. lycopersicum*, named cv. 'Benarys Gartenfreude', cv. 'John Baer' and cv. 'Pearson'.

Interestingly, differences were found between the varieties and cultivars analysed. While the minimum leaf conductance for *A. bella-donna* was about 1.85 × 10^{-5} m s^{-1}, its variety *lutea* had a minimum water conductance of 4.94 × 10^{-5} m s^{-1}, comprising difference of almost three-fold (t(22) = 8.644, p < 0.001). Nevertheless, a significant difference was not found between fruits, which had a cuticular water permeability of about 2.55 × 10^{-5} m s^{-1} (p = 0.58). Furthermore, leaves of *S. lycopersicum* cv. 'Benarys Gartenfreude' (3.05 × 10^{-5} m s^{-1}) had a three-fold lower minimum water

conductance than the other two *S. lycopersicum* cultivars, 'John Baer' and 'Pearson' ($F(2) = 43.439$, $p < 0.001$). Again, no difference was found among fruits ($p = 0.63$).

2.7 Origin-specific water permeability within solanaceous plant species

The classification of the solanaceous plant species according to their climatic origin, where the plant species grow naturally, resulted in three clusters: arid, temperate and tropical (Fig. 7). Nevertheless, no difference was found neither for minimum leaf conductance nor for fruit permeance among each climate of origin ($p > 0.05$).

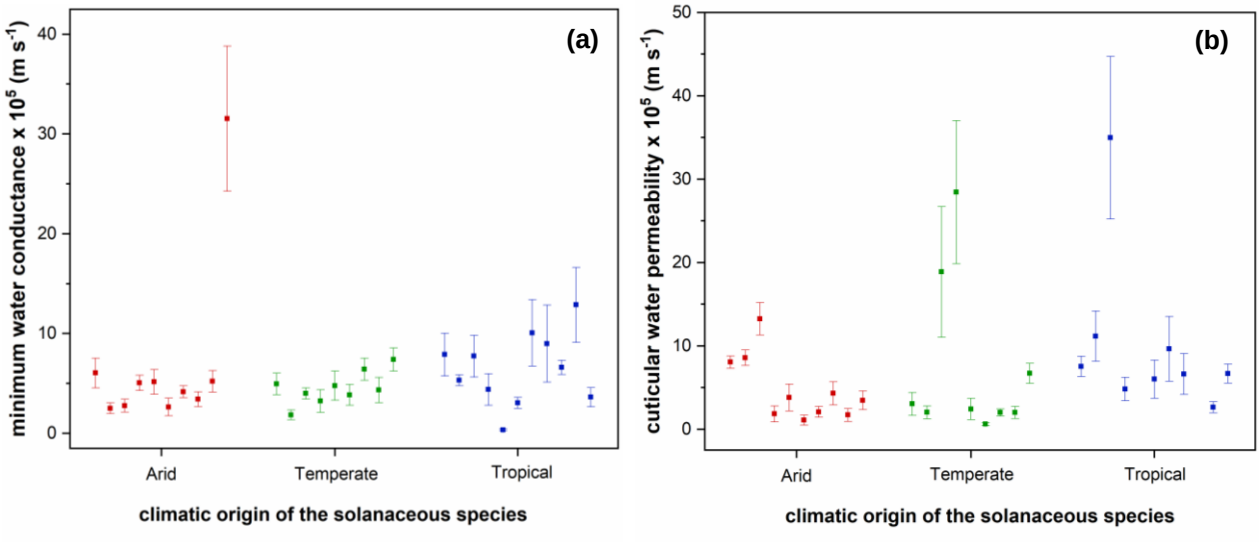


Fig. 7. Distribution of the cuticular water permeabilities of solanaceous representatives according to the climatic origin of plant species. Values represent the mean ± standard deviation of the minimum water conductance of fully expanded leaves (a, $n \geq 8$) and cuticular water permeability of ripe fruits (b, $n \geq 6$).

3 Discussion

The Solanaceae family is one of the largest and most complex family of angiosperms widely distributed in temperate and tropical regions. South America is its main centre of diversity and endemism, but a secondary centre occurs in East Africa (D'Arcy, 1991; Knapp, 2002; Olmstead et al.,2008, 2012; Vorontsova et al., 2013). So far, most studies on cuticular biosynthesis, composition, structure and biomechanical properties of solanaceous plant species have focused on *S. lycopersicum*, *Solanum melongena* and *Capsicum* species, occasionally including its wild relatives (Leide et al., 2007, 2011; López-Casado et al., 2007; Isaacson et al., 2009; Haliński et al., 2011, 2013, 2015; Segado et al., 2016). However, water permeability from solanaceous leaves and fruits is scarcely documented, being the data available mostly related to the domesticated plant species cited above (Schuster, 2016; Huang, 2017). Therefore, the ecophysiological function of the plant cuticle within the Solanaceae family was investigated, aiming to reveal similarities among phylogenetically related plant species.

3.1 Structural and functional traits of leaves and fruits

Microscopically, the solanaceous species investigated in this chapter were similar to the ones previously described in chapters I and II, reflecting the close phylogenetic relationship among the solanaceous species and genera. Most of the leaf surfaces were amphistomatic, indumented with glandular and non-glandular trichomes and with a generally lower density of stomata on the adaxial surface, which is in accordance with previous leaf epidermal studies within the Solanaceae family (Ahmad, 1964; Adedeji et al., 2007; Zhigila et al., 2015). Specific features were observed in the *Solanum* species of the *Leptostemonum* subgenus, *S. burchellii*, *S. melongena*, *S. quitoense*, *S. linnaeanum, S. sisymbriifolium, S. lycocarpum* and *S. virginianum*. Prickles and numerous non-glandular stellate trichomes were observed in the *Leptostemonum* species, which are features related to their common name of "spiny solanums" (Stern et al., 2011). The only glabrous leaves were found for the tropical vine *S. grandiflora*, which is a common feature of this species, but its leaves can be seldom found as pubescent (Bernardello & Hunziker, 1987).

Additionally, *C. annum* is also reported as possessing glabrous leaves (Wahua et al., 2013). The majority of the solanaceous fruits had a cuticular surface free of stomata or lenticels. Exceptions were observed in *S. muricatum*, which had stomata, and in *S. tuberosum* with lenticels heavily covered by waxes on its fruit surface.

The species-specific variation in leaf and fruit functional attributes is believed to result from coadaptative adjustments to selective pressures, such as seed-dispersers and herbivory. Furthermore, adaptative convergences related to environmental constraints, such as water stress, and phylogeny are also reported by Cipollini and Levey (1991) and Silva et al. (2015). In this study, the variation and relationships between several plant functional attributes and life strategies of the solanaceous species were investigated, revealing contrasting patterns between leaves and fruits. Leaves had lower fresh and dry weight compared to fruits, while the opposite was observed for total and specific leaf surface area of the solanaceous species. Conversely, the relative water content of both organs was similar.

3.2 Differences in cuticular water loss among solanaceous plants

The cuticular water permeability obtained from the solanaceous plant species showed a high species-specific and even cultivar-specific variability, which was not only detected in leaves but also in fruits. In the present study, the water permeability varied up by a factor of 100, with the highest and lowest permeabilities being found in distinct species and organs. Values ranged from 3.51×10^{-6} m s^{-1} in *S. grandiflora* leaves to 3.50×10^{-4} m s^{-1} in *N. tabacum* fruits, and, therefore, covered nearly the whole spectrum measured for permeability so far (Riederer & Schreiber, 2001; Schuster et al., 2017). Variety- and cultivar-specific differences were also observed for minimum leaf conductances of *A. bella-donna* and *S. lycopersicum*, respectively. Differences between cultivars of the same plant species were already described previously, reflecting the variability and multifunctional nature of the plant cuticle (Leide et al., 2011, 2018; Diarte et al., 2019). Despite the considerable differences between the highest and lowest cuticular water loss rates, a lower variation of the permeabilities was found most of the fruits and leaves investigated. About 90% of

the solanaceous leaves and of 75% the fruits had permeabilities ranging by about 5-fold, averaging at 5.43 × 10^{-5} m s^{-1} for leaves and 5.16 × 10^{-5} m s^{-1} for fruits. This lower variability suggests similarities of the cuticular transpiration barrier among these species.

Given the critical role of cuticular water permeability in stress responses and plant adaptability to environmental conditions, leaf and fruit water permeability of solanaceous plant species were put into perspective of their climatic origin. Nevertheless, no difference was found between the permeability of leaves or fruits across the different climates investigated (arid, temperate, and tropical). This lack of difference can be attributed to the limited number of species studied and the relatively low variability of permeabilities found for most of the solanaceous species.

3.3 Organ-specificity of the cuticular water permeability

An asymmetrical distribution was detected for the leaf and fruit water permeability within species. Although previous studies indicated that fruits generally have higher cuticular water loss than leaves, the water permeability of organs was species-dependent in solanaceous plant species (Schreiber & Riederer, 1996; Riederer & Schreiber, 2001; Huang, 2017). The leaf-to-fruit ratio of water permeability showed that the highest water permeability can be found in leaves or fruits or can even be similar between both organs. Thus, these findings indicate that comparisons between organs from different plant species can result in misinterpretation of the functional specificity of the plant cuticle.

The minimum leaf water conductance was about 6.01 × 10^{-5} m s^{-1}, ranging from 0.35 × 10^{-5} m s^{-1} in *S. grandiflora*, a perennial vine with hipostomatic leaf surfaces, to 31.54 × 10^{-5} m s^{-1} in *S. muricatum*, a perennial shrub with amphistomatic leaves. In a recent review of Schuster et al. (2017), the highest leaf cuticular permeability reported among 160 species was of 2.86 × 10^{-4} m s^{-1} for *Pinus pumila* (Pinaceae), an evergreen, coniferous shrub with needle-like leaves (Nagano et al., 2009). Thus, the solanaceous leaves of *Solanum muricatum* had a permeability higher than previously reported.

Lower cuticular water losses were detected predominantly for fleshy fruits of the genera *Solanum, Physalis* and *Atropa*. On the other hand, higher cuticular water permeabilities were mostly measured in dry fruits from *Datura*, *Hyoscyamus*, *Nicandra* and *Nicotiana* genera. Cuticular water permeability of fruits varied from 6.39×10^{-6} m s^{-1} (in the fleshy fruit of *S. dulcamara*) to 3.49×10^{-4} m s^{-1} (in the dry fruit of *N. tabacum*), averaging at 7.32×10^{-5} m s^{-1}. Similarly to leaves, the values found for fruit cuticular permeability was beyond the range described in previous studies from 0.9×10^{-5} m s^{-1} to 2.0×10^{-4} m s^{-1} for *S. lycopersicum* and *C. annum* fruits, respectively (Riederer & Schreiber, 2001; Leide et al., 2007).

Although stomata was not observed in *N. tabacum* fruit surface in the present study, a previous report showed that its fruits have stomata on the epicarp (Dave et al., 1981). The increased transpirational water loss measured in stomatous surfaces might result from incomplete stomatal closure, which occurs in some species, overestimating the minimum water conductance measured by the gravimetric method (Schuster et al., 2017). This might be the case for *S. muricatum* leaves and *N. tabacum* fruits. Nevertheless, further studies need to be done in order to determine the actual contribution of the residual stomatal water loss to the overall cuticular transpiration.

3.4 Plant functional traits and life strategies of the solanaceous: organ-specific cuticular functions

Whereas annual species have an all-or-nothing colonization strategy due to their short life cycles, perennial species are focused on storage and growth, presenting different life spans. Within the perennial category, evergreen species use their leaves during the whole growing season, while deciduous species shed their leaves as a stress tolerance strategy (Wullschleger et al., 2014). Although evergreen species generally have a longer leaf life span than deciduous species, overlapping or even shorter longevities can be seen, especially in tropical rain forests, due to continuous production and senescence of leaves (van Ommen Kloeke et al., 2012). Since the functional traits within life forms were similar, this might indicate that these traits are genetically

determined and that the leaf longevity amongst the solanaceous investigated is not significantly different.

The relative water content was the only trait to differ between fruit types, with about 83% and 65%, in fleshy and dry fruits, respectively. Among the dry fruits investigated, *Nicotiana* has capsular fruit, while the spiny fruit of *Datura* is considered a reversion to a capsule (Pabón-Mora & Litt, 2011). Fruits of the *Hyoscyamus* and *Nicandra* species develop inside of an accrescent calyx. *Hyoscyamus* has highly modified berries called pyxidia, which are usually classified as a type of capsule. *Nicandra* has a berry with sclerified inclusions in the fleshy portion of the fruit, as already shown on chapter II. Although starting as fleshy, *Nicandra* berries dried out during fruit development becoming hard and fractured, mostly near calyx insertion, thus differing from the common berry definition of “soft indehiscent fruits” (Knapp, 2002; Chiarini & Barboza, 2007). Indeed, Kaniewsky, (1965) even suggested that *N. physalodes* should not be considered a berry, since its pericarp is comparable to the ones of the non-capsular dehiscent fruits of *Solanum mortonii* and *Solanum homalospermum*. According to the classification proposed by Chiarini & Barboza, (2007), and based on the observations of this study, *N. physalodes* fruits would better suit the definition of a foraminicidal capsule, a dry or slightly fleshy fruit with a thin pericarp that cracks irregularly at the senescent phase, than that of a berry.

Altogether, fruits of these species commonly release their seeds after the drying of the pericarp, usually accompanied by a change in fruit colour from green to dull yellow-brown. Capsular fruits split open at maturity due to tissue contraction and seeds are dispersed by wind. In non-capsular dehiscent fruits, such as the pyxidium, the sclerenchyma cells are most likely responsible for the rupture of the pericarp, triggered by changes in temperature and humidity (Knapp, 2002; Chiarini & Barboza, 2007).

Whilst morphological, developmental and evolutionary analyses of dry and fleshy fruits are extensively studied within Solanaceae, the physiology behind transpirational water losses and desiccation during the maturation of dry fruits is still poorly discussed, if it is examined at all.

Interestingly, most of the dry fruits here investigated (*D. innoxia*, *H. albus*, *H. niger* and *N. physalodes*) were free of stomata or lenticels and, together with *N. tabacum,* showed about to 5-fold higher cuticular water loss than fleshy fruits. Contrary to the primary function of the cuticle as a protection against water loss observed in leaves and fleshy fruits, the reduced cuticular transpiration barrier properties in the dry fruits might be a physiological adaptation of seed dispersion. It is suggested that the low water content verified in the dry fruits primarily results from their elevated cuticular permeance, which leads to pericarp dehydration and rupture, and release of seeds in the solanaceous investigated.

4 Conclusion

In summary, this is the first comparative study on the efficiency of the cuticular transpiration barrier of leaves and fruits from a large number of closely related plant species belonging to the Solanaceae family. Genus-, species- and organ-specificities were found for cuticular water loss among the solanaceous plant species investigated. Additionally, differences were detected between distinct varieties of the same plant species. Especially, the fruit type-specificity observed suggest that the properties of the plant cuticle can have significant consequences in fruits with contrasting mechanisms of seed dispersion. In fleshy fruits, it can be a determinant in fruit quality properties, such as fruit water content and consistency, gloss, colour and texture, thus favouring the attraction of seed-dispersing animals. On the other hand, it might contribute to the drying process of the pericarp, which leads to seed dispersion in dry fruits. Although the water permeability ranged over 100-times between the lowest and the highest values, more than 75% of the minimum leaf conductances and fruit water permeabilities investigated varied only by a factor of 5, result similar to the findings of Schuster et al., (2017) for leaf permeabilities. Altogether, the functional variability found in these plant species may relate to the waterproofing properties of the plant cuticle and, thus, might have an essential role in leaf and fruit performances.

General discussion

The plant cuticle, a hydrophobic waxy layer synthesized by epidermal cells, was a key innovation to prevent water loss from aerial surfaces in early plants. This continuous extracellular layer covers and protects primary organs of lower and higher land plants, including stems, leaves, flowers, and fruits (Jefree, 2006; Koch et al., 2008; Samuels et al., 2008; Buschhaus & Jetter, 2011). This ubiquitous character among land plants is exceptionally complex and its properties, such as structure and composition, can vary widely among and within plant species (Croxdale, 2001; Riederer & Muller, 2006; Reina-Pinto & Yephremov, 2009; Budke et al., 2012; Zarrouk et al., 2018).

So far, most cuticle-related studies have focused on species-specific variations in plants, mainly vegetative parts of cultivated species. Additionally, the present study examined the cuticular traits on an organ-specific level and among non-cultivated species. For that, phylogenetically related plant species of the Solanaceae family were investigated to identify structural, chemical and functional cuticular attributes. This study comprised twenty-seven species of ten different genera, including three different cultivars of *Solanum lycopersicum* (cv. ‘Benarys Gartenfreude’, cv. ‘John Baer’ and cv. ‘Pearson’) and one variety of *Atropa bella-donna* (var. *lutea*). Species of the Solanaceae are found throughout all temperate and tropical climates, yet undoubtedly the western hemisphere contains the greatest biodiversity of the family (Olmstead et al., 2008). The solanaceous species here investigated are native from five different continents (North and South America, Africa, Asia and Europe), thus, well representing the worldwide distribution and the distinct adaptations to natural habitats and climatic conditions observed for the family.

The cuticular permeability of solanaceous species

Under stress conditions that lead to stomatal closure, the path of epidermal water loss is mainly restricted to the plant cuticle, being this passive movement of water called cuticular transpiration. Water permeability obtained from stomatous cuticles is named minimum water conductance and corresponds to the lowest cuticular water loss when stomata are maximally closed in response to plant dehydration (Kerstiens, 1996a; Veraverberke, 2003b). On the other hand, values obtained from astomatous systems, such as astomatous fruits, is termed cuticular water permeance. Water permeability refers to both minimum conductance and cuticular permeance (Kerstiens, 1996a; Schuster et al., 2017). In this study, minimum conductance and permeance for water were used as an indicator of the cuticular transpiration barrier efficiency in leaves, inflated fruiting calyces and fruits.

The cuticular permeability of leaves and fruits revealed a species-dependent pattern, suggesting different strategies for adaptation to drought among the solanaceous plants. Values varied up to strikingly one hundred-fold among the species. Permeabilities ranged from 0.35×10^{-5} m s^{-1} in leaves of the perennial vine *Solandra grandiflora* to 34.98×10^{-5} m s^{-1} in capsular fruits of annual herb *Nicotiana tabacum*, both species being native from North and South America and adapted to tropical climates. The literature review shows that cuticular permeability varies from 2.5×10^{-7} m s^{-1} in *Zamioculcas zamiifolia* to 4.0×10^{-3} m s^{-1} in *Ipomoea batatas*, which is in accordance with the range found for the solanaceous species (Karbulková et al., 2008; Zobayed et al., 2000; Schuster et al., 2017). Previous studies comparing plant organs of different species showed that fruits generally have higher permeabilities than leaves (Schreiber & Riederer, 1996; Riederer & Schreiber, 2001; Huang, 2017). However, this assumption cannot be assumed when comparing permeabilities of leaves and fruits of the same plant species, as shown by the results obtained for the solanaceous species.

Plant adaptations to their environment might determine the distinct organ-specificities observed in the solanaceous species, such as responses to drought stress, as well as by their life-

strategies, e.g. the life-span of their organs (Gratani, 2014). Overall, it is concluded that cuticle-related comparisons between organs from different plant species, which comprise the major studies available so far, can result in misinterpretation of the functional specificity of the cuticle within species. Therefore, adopting integrative approaches, considering the particularities of each plant species, is essential to broadening our understanding of the mechanisms that determine cuticle properties in plants.

Cuticular wax coverage of solanaceous leaves and fruits

The plant cuticle is a multi-functional structure with a complex and heterogeneous nature, basically composed of a cutin polymer associated with cuticular waxes. The cuticular waxes comprise the true barrier against passive water loss and diffusion of solutes across the cuticle, and its properties are affected by the compositional characteristics and spatial arrangement of the wax mixture (Schönherr & Riederer, 1989; Riederer & Schneider, 1990; Veraverbeke et al., 2003a; Jeffree, 2006). Yet, how cuticular wax amount and composition are correlated with water permeability is still unclear (Schönherr, 1982, Schreiber & Riederer, 1996; Kerstiens, 2006). Therefore, the chemical composition of the plant cuticle was investigated in a wide range of solanaceous species aiming to contribute to filling this gap in knowledge.

Leaves, inflated fruiting calyces and fruits were chemically characterized by a pronounced variety of amount and composition of the cuticular waxes. Wax coverage was species-specific and ranged up to 62-fold. The lowest and highest wax amounts were found for fruits, which ranged from 0.55 µg cm^{-2} (*Nicandra physalodes*) to 33.99 µg cm^{-2} (*Solanum pennellii*). Consequently, wax amount of fruits was more variable than leaves and inflated calyces. Considerable variations of wax load were also reported for other solanaceous fruits (Yeats et al., 2012; Parsons et al., 2013).

The wax load leaves ranged over 32 fold, from 0.90 µg cm^{-2} (*N. physalodes*) to 28.42 µg cm^{-2} (*Solanum burchellii*). The inflated calyx of the *Physalis* and *Nicandra* species had a wax amount

raging from 0.56 µg cm^{-2} in *P. peruviana* to 2.00 µg cm^{-2} in *N. physalodes*. Overall, these results are within the range previously reported for leaves, from 0.4 µg cm^{-2} (*Morus alba*) to 160 µg cm^{-2} (*Argania spinosa*; Bouzoubaâ et al., 2006; Mamrutha et al., 2010).

Interestingly, the lowest wax coverage of leaves and fruits and the highest of the inflated calyx was found in the same species, *N. physalodes*; an annual species considered a weed in several areas of the world (Hawton, 1976; Ronchi & Silva, 2006). The wax coverage of leaves and fruits of *Nicandra* had a difference of about 1.6-fold. Similarly, the cuticular permeabilities between the two *Nicandra* organs were contrasting by a factor of 2. While *Nicandra* leaves had a minimum conductance of 5.32×10^{-5} m s^{-1}, its fruits had cuticular permeance of 11.18×10^{-5} m s^{-1}, thus suggesting that higher cuticular wax amounts could be related with a more efficient transpiration barrier on a species-specific level. A similar pattern of higher wax coverage occurring in organs with lower permeability was observed for other solanaceous species (*Physalis alkekengi*, *Physalis ixocarpa*, *Solanum dulcamara*, *Solanum linnaeanum*, *Solanum muricatum*, *Solanum pennellii*, and *Solanum pseudocapsicum*). Nevertheless, no correlation was found between wax load and water permeability of the solanaceous species investigated.

Wax composition of solanaceous leaves and fruits

The cuticular waxes consist of a complex mixture of homologues series of very-long-chain aliphatic compounds, and variable amounts of alicyclic compounds, like sterols and pentacyclic triterpenoids (Kolattukudy, 1970; Walton, 1990; Jetter et al., 2006; Heredia & Dominguez, 2009). In the solanaceous species studied, the qualitative composition of cuticular waxes was species- and organ-dependent. Yet, all species had very-long-chain aliphatic compounds as the main wax fraction, comprising more than 66% of the total wax of leaves and fruits. The cuticular waxes of the solanaceous were primarily dominated by *n*-alkanes, which comprised up to 80% of the wax composition. Other aliphatic components frequently found were primary alkanols, alkanoic acids and *iso*- and *anteiso*-alkanes. The lipid composition of solanaceous surfaces has been studied for several solanaceous species, mainly from the *Solanum* genus, and have shown that *n*-alkanes

are the main components found in their cuticular wax mixture, which agrees with the findings of the present study (Szafranek & Synak, 2006; Haliński et al., 2009; 2011; da Silva et al., 2012; Bolger et al., 2014; Haliński et al., 2015).

Prior research has shown that very-long-chain aliphatic compounds, such as *n*-alkanes, are more important for the formation of the cuticular transpiration barrier. In contrast, alicyclic components are proposed to establish less efficient transpiration barriers resulting in a more permeable pathway for water (Riederer & Schneider, 1990; Riederer & Schreiber, 1995; Kosma et al. 2009; Jetter & Riederer, 2016). Hence, the amount of alicyclic components was positively correlated with water permeability of fruits in the *Solanum* species here investigated. Although no correlation was found for leaves, *S. muricatum*, which had the highest minimum conductance, also had the highest aromatic wax fraction, which was mainly composed by phenylmethyl esters and tocopherols. A similar result was found in *Capsicum* species by Parsons et al., (2012), in which the amount of alicyclics (triterpenoids plus sterols) showed a positive correlation with the water loss rate. No correlation between particular aliphatic components and water permeability was found for the solanaceous species. Still, the abundance of *n*-alkanes in the wax mixture of the solanaceous species might influence the different transpiration barrier properties of their leaves and fruits cuticles.

Differences of cuticular water permeability of wild and cultivated solanaceous species

The domestication of plants was one of the most significant cultural and evolutionary transitions in human history. As such, we still rely on crops that were domesticated more than 10,000 years ago, with a relatively limited number of crop species being of critical importance to modern societies. Despite its crucial importance, in most cases, little is known about the adaptation of plants under domestication (Ross-Ibarra, 2007; Larsons et al., 2014).

The phenotypic variations driven by artificial selection is considered an inappropriate analogy for understanding plants adaptation in nature (Ross-Ibarra, 2007; Larsons et al., 2014). In this

context, the domestication and selection of desirable traits (such as appearance, aroma, texture and colour) may unconsciously have been modified cuticular properties with important consequences for pivotal functions of plant surfaces (Parsons, 2012; Lara et al., 2014, 2015; Martin & Rose, 2014; Zarrouk et al., 2018). Therefore, the cuticle of cultivated species might be an example of an abnormal, artificial model rather than a representation of natural adaptation of plants to drought (Ross-Ibarra, 2007; Fernández et al., 2016).

Cuticle-related plant properties were investigated in fruits and leaves of twelve species of the *Solanum* genus aiming to identify the specificities of the cuticle among cultivated plant species in relation to wild species. As a result, structural, chemical and functional cuticular diversities were found among wild, minor cultivated and major cultivated species.

A wide range of total cuticular wax coverage, ranging up to 23-fold among the species, and varied cuticular chemical composition and average chain length of the aliphatic wax fraction characterized leaves and fruits of the wild and cultivated species. Cuticular waxes primarily composed of very-long-chain aliphatic compounds ranged from 1.24 µg cm^{-2} in leaves of the cultivated *S. tuberosum* to 28.42 µg cm^{-2} in leaves of the wild *S. burchellii*. In fruits, cuticular wax load varied from 3.40 µg cm^{-2} in the cultivated *S. melongena* to 33.99 µg cm^{-2} in the wild *S. pennellii* fruits. Additionally, the average chain length of the aliphatic wax fraction varied from 29.91 to 33.17 carbon atoms in leaves and from 28.11 to 35.34 carbon atoms in fruits.

Aliphatic wax compounds comprised more than 66% of the cuticular wax amount in leaves and fruits. Alkanals, alkanoic acids, alkyl esters, *n*-alkanes, branched alkanes, and primary alkanols were identified in different proportions showing an organ- and species-specific. The primary aliphatic compound identified in *Solanum* leaves and fruits was *n*-alkanes, comprising from 36% to 80% of the total cuticular waxes. Similarly to the wax coverage and composition, the distribution of water permeability was different between organs, with species generally presenting higher cuticular water losses in leaves in comparison to fruits, whereas the contrary was observed solely for two cultivated species (*S: lycopersicum* cv. ‘Benarys Gartenfreude’ and *S. quitoense*).

Considering the degree of domestication of the *Solanum* species, leaves and fruits of wild *Solanum* species had water permeabilities up to 3-fold lower in comparison to the cultivated ones. Especially for fruits, a gradient of water permeability was identified according to their cultivation status: the higher is the domestication grade, the higher is the water permeability. Wild *Solanum* fruits had a cuticular permeability of about 2.21×10^{-5} m s^{-1}, whereas minor cultivated ones averaged at 3.92×10^{-5} m s^{-1}, and by last, fruits of major cultivated species had a water permeability of 6.45×10^{-5} m s^{-1}. In accordance, it is known that breeding programs and the selection of organoleptic properties are mainly targeted to fruits in these cultivated species (Prohens et al., 2003; Rodríguez-Burruezo, 2011; Sim et al., 2011). Hence, these results indicate that the domestication process likely impaired cuticular properties, e.g. by altering cuticular wax quantity and quality, leading to the increased water permeability verified in the cultivated species. Furthermore, these alterations may not be only limited to the targeted organs of artificial selection, but might also be extended to other plant parts, as suggested by the lower water permeability of leaves of wild species in relation to the cultivated ones.

Solanaceous species as model plants for cuticular studies

Cuticular transpiration has been investigated in a variety of plant species due to its physiological relevance, but *Arabidopsis thaliana* leaves and more recently *Solanum lycopersicum* fruits has stood out as model plants in the cuticle research (Domínguez et al., 2017). Still, the increasing interest in the different roles of the plant cuticle has brought attention to other species, such as *Capsicum annum* (Parson et al., 2013), *Malus domestica* (Veraverbeke et al., 2003a, Leide et al., 2018) *Prunus avium* (Peschel et al., 2007; Belge et al., 2014), *Prunus domestica* (Peschel et al., 2007), and *Prunus laurocerasus* (Diarte et al., 2020), and *Solanum melongena* (Haliński et al., 2011).

Overall, this study additionally showed that species of the Solanaceae family comprise a valuable resource for the investigation of many cuticular properties. Some plant species newly characterized in this study, such as the crop wild relatives of *S. melongena* (*Solanum burchellii,*

Solanum linnaeanum, and *Solanum virginianum*), might better represent the natural adaptations of the cuticle to avoiding water loss in comparison to the plant species commonly used in cuticle-related studies, e.g. cultivated species. Fruits of these solanaceous species are astomatous and covered by a robust cuticle, being easily isolated by enzymatic solution. Moreover, their fruit and leaf cuticles are structurally, chemically, and functionally diverse favouring elaborate experimental designs. In this way, the investigated solanaceous species, especially the wild ones, represent a promising platform for cuticle-related studies and use in breeding programs aiming to improve fruit quality traits.

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Appendices

Appendix 1. Cuticular wax composition of *S.burchellii* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S.burchellii* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	-	0.125 ± 0.018
	22	-	0.101 ± 0.025
	23	-	0.182 ± 0.049
alkanals	34	-	0.295 ± 0.064
	35	-	0.438 ± 0.032
anteiso-alkanes	30	0.094 ± 0.024	-
	31	0.073 ± 0.014	-
	32	2.731 ± 0.652	0.271 ± 0.033
	33	0.584 ± 0.107	-
	34	2.443 ± 0.514	-
	35	0.195 ± 0.046	-
iso-alkanes	31	0.112 ± 0.032	-
	33	0.656 ± 0.159	-
	35	0.178 ± 0.049	-
n-alkanes	25	-	0.260 ± 0.054
	27	-	0.410 ± 0.084
	28	-	0.219 ± 0.052
	29	-	0.792 ± 0.107
	30	-	0.442 ± 0.048
	31	2.737 ± 0.516	6.614 ± 0.821
	32	1.017 ± 0.129	1.650 ± 0.104
	33	15.937 ± 3.315	5.150 ± 0.875
	34	0.624 ± 0.108	-
	35	0.542 ± 0.118	-
primary alkanols	24	-	0.359 ± 0.094
	25	-	0.211 ± 0.076
	26	-	1.000 ± 0.262
	27	-	0.336 ± 0.072
	28	-	4.291 ± 0.894
	29	-	0.681 ± 0.101
	30	-	1.052 ± 0.105
alkyl esters	35	0.190 ± 0.044	-
	36	-	0.291 ± 0.052
	42	-	0.476 ± 0.097
	44	-	0.815 ± 0.161
total aliphatics		28.112 ± 5.619	26.459 ± 3.245
phenylmethyl ester	32	0.155 ± 0.066	-
	34	0.148 ± 0.066	-
β-sitosterol		-	0.301 ± 0.042

total cyclics	0.303	±	0.129	0.301	±	0.042
total cuticular waxes	28.415	±	5.741	26.760	±	3.241

Appendix 2. Cuticular wax composition of *S. dulcamara* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S.dulcamara* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.005 ± 0.002	0.067 ± 0.011
	21	0.003 ± 0.001	0.057 ± 0.014
	22	0.004 ± 0.002	0.244 ± 0.052
	23	0.004 ± 0.001	0.171 ± 0.030
	24	0.018 ± 0.006	0.554 ± 0.166
	25	0.028 ± 0.036	0.103 ± 0.026
	26	0.040 ± 0.011	0.108 ± 0.031
	27	0.021 ± 0.005	0.065 ± 0.036
	28	0.115 ± 0.045	0.070 ± 0.008
	29	0.025 ± 0.008	0.108 ± 0.100
	30	0.046 ± 0.014	-
	31	0.016 ± 0.005	-
	32	0.017 ± 0.004	-
	33	0.005 ± 0.001	-
alkanals	28	0.008 ± 0.001	-
	30	0.008 ± 0.001	-
	34	0.008 ± 0.002	-
	35	0.007 ± 0.001	-
anteiso-alkanes	30	0.013 ± 0.003	-
	31	0.015 ± 0.003	-
	32	0.035 ± 0.008	-
	33	0.009 ± 0.002	-
	34	0.046 ± 0.009	-
	35	0.012 ± 0.003	-
	36	0.013 ± 0.002	-
iso-alkanes	29	0.011 ± 0.004	-
	30	0.008 ± 0.002	-
	31	0.032 ± 0.008	-
	32	0.008 ± 0.002	-
	33	0.046 ± 0.012	-
	35	0.014 ± 0.003	-
n-alkanes	25	0.007 ± 0.001	0.033 ± 0.013
	26	0.007 ± 0.002	0.067 ± 0.023
	27	0.101 ± 0.036	0.163 ± 0.025
	28	0.017 ± 0.005	0.144 ± 0.045
	29	0.195 ± 0.033	0.843 ± 0.112
	30	0.056 ± 0.009	0.234 ± 0.027
	31	0.393 ± 0.088	0.677 ± 0.051
	32	0.072 ± 0.012	0.125 ± 0.019
	33	0.116 ± 0.033	0.198 ± 0.027
	34	0.018 ± 0.003	-

Compound class	Carbon chain length	Wax coverage of *S.dulcamara* (µg cm^{-2})	
		leaf	fruit
	35	0.020 ± 0.003	-
primary alkanols	22	0.003 ± 0.001	-
	24	0.005 ± 0.001	-
	26	0.007 ± 0.002	-
	27	0.017 ± 0.005	-
	28	0.057 ± 0.020	0.055 ± 0.011
	29	0.040 ± 0.015	0.088 ± 0.025
	30	0.051 ± 0.015	0.148 ± 0.023
	31	0.020 ± 0.006	0.115 ± 0.026
	32	0.038 ± 0.004	0.074 ± 0.007
	33	0.015 ± 0.002	-
	34	0.009 ± 0.003	-
alkyl esters	44	0.007 ± 0.003	-
	46	0.008 ± 0.002	-
	48	0.008 ± 0.001	-
	49	0.005 ± 0.001	-
	50	0.012 ± 0.004	-
	51	0.008 ± 0.002	-
total aliphatics (%)		1.960 ± 0.220	4.510 ± 0.470
coumaric acid	26	0.026 ± 0.011	-
	27	0.019 ± 0.009	-
	28	0.016 ± 0.007	-
	30	0.003 ± 0.001	-
phenylmethyl ester	28	0.061 ± 0.035	-
	29	0.009 ± 0.004	-
	30	0.039 ± 0.018	-
	31	0.012 ± 0.004	-
	32	0.034 ± 0.011	-
	33	0.009 ± 0.002	-
	34	0.018 ± 0.009	-
β-sitosterol		0.012 ± 0.004	-
alpha-tocopherol		0.008 ± 0.001	-
total cyclics		0.220 ± 0.120	-
dihydroxy alkanoic acid	16	-	0.190 ± 0.070
total cutiular waxes		2.210 ± 0.310	4.700 ± 0.510

Appendix 3. Cuticular wax composition of *S. linnaeanum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. linnaeanum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.005 ± 0.004	0.280 ± 0.131
	21	-	0.129 ± 0.047
	22	0.005 ± 0.002	0.316 ± 0.108
	23	-	0.276 ± 0.084
	24	0.021 ± 0.010	0.454 ± 0.203
	25	0.015 ± 0.007	0.271 ± 0.069
	26	0.040 ± 0.016	0.392 ± 0.168
	27	0.018 ± 0.007	0.229 ± 0.078
	28	0.078 ± 0.015	0.471 ± 0.215
	29	0.025 ± 0.007	0.197 ± 0.067
	30	0.098 ± 0.017	0.394 ± 0.169
	31	0.088 ± 0.027	0.157 ± 0.051
	32	0.218 ± 0.045	0.243 ± 0.095
	33	0.093 ± 0.033	0.096 ± 0.025
	34	-	0.063 ± 0.016
alkanals	30	-	0.097 ± 0.034
	33	-	0.119 ± 0.032
	35	0.062 ± 0.018	-
anteiso-alkanes	32	0.073 ± 0.016	-
	34	0.063 ± 0.010	-
	36	0.051 ± 0.006	-
iso-alkanes	29	0.023 ± 0.012	0.227 ± 0.046
	31	0.140 ± 0.028	0.112 ± 0.014
	32	0.026 ± 0.013	-
	33	0.214 ± 0.041	0.124 ± 0.029
	34	0.025 ± 0.005	-
	35	0.064 ± 0.012	0.039 ± 0.013
n-alkanes	25	0.009 ± 0.003	0.133 ± 0.035
	26	0.006 ± 0.004	0.058 ± 0.021
	27	0.113 ± 0.057	0.358 ± 0.096
	28	0.015 ± 0.008	0.200 ± 0.044
	29	0.097 ± 0.028	0.544 ± 0.134
	30	0.021 ± 0.005	0.273 ± 0.084
	31	0.400 ± 0.088	1.903 ± 0.458
	32	0.223 ± 0.094	0.694 ± 0.206
	33	1.802 ± 0.529	0.763 ± 0.232
	34	0.100 ± 0.026	-
	35	0.308 ± 0.061	-
primary alkanols	22	0.004 ± 0.002	-
	24	0.009 ± 0.005	-
	25	0.004 ± 0.001	-

Compound class	Carbon chain length	Wax coverage of *S. linnaeanum* (µg cm^{-2})	
		leaf	fruit
	26	0.152 ± 0.101	0.144 ± 0.066
	27	0.095 ± 0.043	0.088 ± 0.028
	28	0.934 ± 0.388	0.357 ± 0.125
	29	0.167 ± 0.027	0.087 ± 0.028
	30	0.166 ± 0.034	0.138 ± 0.034
	31	0.017 ± 0.003	-
	33	0.019 ± 0.006	-
	34	0.021 ± 0.007	-
alkyl esters	41	0.010 ± 0.002	±
	42	0.020 ± 0.008	0.053 ± 0.013
	43	-	0.055 ± 0.020
	44	0.027 ± 0.006	0.119 ± 0.042
	45	0.014 ± 0.009	0.095 ± 0.023
	46	0.035 ± 0.009	0.129 ± 0.044
	47	0.023 ± 0.006	-
	48	0.055 ± 0.014	0.118 ± 0.051
	49	0.016 ± 0.005	-
	50	0.024 ± 0.005	0.108 ± 0.040
	51	0.079 ± 0.021	-
total aliphatics		6.430 ± 1.182	11.103 ± 2.782
coumaric acid	26	0.019 ± 0.006	-
	28	0.035 ± 0.011	-
	30	0.052 ± 0.018	-
phenylmethyl ester	30	0.039 ± 0.011	-
	32	0.063 ± 0.021	-
	33	0.018 ± 0.005	-
	34	0.032 ± 0.011	-
campesterol		0.033 ± 0.010	0.065 ± 0.029
cholesterol		0.162 ± 0.016	0.080 ± 0.022
naringenin		-	0.148 ± 0.058
β-sitosterol		0.135 ± 0.028	0.195 ± 0.066
stigmasterol		0.098 ± 0.033	0.064 ± 0.024
α-tocopherol		0.208 ± 0.076	-
β-tocopherol		0.052 ± 0.024	-
δ-tocopherol		0.005 ± 0.001	-
total cyclics		0.952 ± 0.172	0.553 ± 0.190
dihydroxy alkanoic acid	16	-	0.289 ± 0.056
trihydroxy octadecenoic acid	18	-	0.973 ± 0.164
total cuticular waxes		7.382 ± 1.342	12.918 ± 3.079

Appendix 4. Cuticular wax composition of *S. virginianum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. virginianum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.019 ± 0.002	0.055 ± 0.022
	21	0.004 ± 0.001	0.111 ± 0.052
	22	0.010 ± 0.003	0.039 ± 0.018
	23	0.005 ± 0.001	0.059 ± 0.030
	24	0.013 ± 0.003	0.048 ± 0.032
	25	0.005 ± 0.001	0.035 ± 0.013
	26	0.014 ± 0.003	0.034 ± 0.015
	27	0.008 ± 0.001	0.025 ± 0.010
	28	0.113 ± 0.066	0.126 ± 0.045
	29	0.021 ± 0.004	-
	30	0.045 ± 0.012	-
	31	0.050 ± 0.003	-
	32	0.134 ± 0.051	-
	33	0.031 ± 0.011	-
	34	0.064 ± 0.029	-
	35	0.006 ± 0.002	-
alkanals	33	-	0.073 ± 0.010
	35	0.053 ± 0.009	-
	36	-	0.026 ± 0.006
anteiso-alkanes	32	0.032 ± 0.004	-
	34	0.019 ± 0.004	-
	36	0.024 ± 0.003	-
iso-alkanes	31	0.009 ± 0.002	
	32	0.013 ± 0.002	-
	33	0.103 ± 0.022	0.045 ± 0.013
	34	0.031 ± 0.007	-
	35	0.031 ± 0.008	-
n-alkanes	25	0.002 ± 0.001	-
	27	0.009 ± 0.003	-
	29	0.034 ± 0.011	0.201 ± 0.072
	30	0.026 ± 0.007	0.231 ± 0.034
	31	0.564 ± 0.197	0.875 ± 0.298
	32	0.132 ± 0.045	0.429 ± 0.099
	33	2.183 ± 0.403	0.502 ± 0.153
	34	0.156 ± 0.017	-
	35	0.146 ± 0.009	-
primary alkanols	22	0.002 ± 0.000	-
	24	0.004 ± 0.001	-
	26	0.005 ± 0.000	-
	29	0.031 ± 0.007	-
	30	0.013 ± 0.003	-

Compound class	Carbon chain length	Wax coverage of *S. virginianum* (µg cm^{-2})	
		leaf	fruit
alkyl esters	31	0.030 ± 0.009	-
	33	0.021 ± 0.003	-
	34	0.015 ± 0.003	-
	40	0.016 ± 0.009	-
	42	0.038 ± 0.024	-
	44	0.043 ± 0.032	-
	46	0.043 ± 0.030	-
	48	0.042 ± 0.025	-
	49	0.032 ± 0.006	-
	50	0.031 ± 0.013	-
	51	0.027 ± 0.012	-
total aliphatics		4.501 ± 0.970	2.914 ± 0.734
phenylmethyl ester	30	0.062 ± 0.013	-
	31	0.017 ± 0.006	-
	32	0.084 ± 0.035	-
	33	0.022 ± 0.010	-
	34	0.045 ± 0.021	-
campesterol		0.159 ± 0.023	0.084 ± 0.020
cholesterol		0.014 ± 0.003	-
β-sitosterol		0.191 ± 0.029	0.151 ± 0.018
stigmasterol		0.141 ± 0.036	0.359 ± 0.038
total cyclics		0.734 ± 0.104	0.595 ± 0.048
total cuticular waxes		5.234 ± 1.060	3.509 ± 0.777

Appendix 5. Cuticular wax composition of *S. pennellii* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. pennellii* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	-	0.107 ± 0.015
	21	-	0.018 ± 0.003
	22	-	0.051 ± 0.008
	24	-	0.092 ± 0.019
	27	-	0.124 ± 0.030
	28	-	0.171 ± 0.087
	29	-	0.075 ± 0.024
	30	-	0.317 ± 0.206
	32	-	0.231 ± 0.160
	34	-	0.049 ± 0.028
alkanals	33	-	0.391 ± 0.114
anteiso-alkanes	30	-	0.401 - 0.086
	32	0.108 ± 0.019	0.205 - 0.029
	33	0.032 ± 0.011	-
	34	0.044 ± 0.010	-
iso-alkanes	27	0.022 ± 0.011	0.040 ± 0.016
	29	0.284 ± 0.049	0.476 ± 0.136
	30	0.096 ± 0.018	0.108 ± 0.014
	31	1.164 ± 0.143	0.880 ± 0.164
	32	0.186 ± 0.046	-
	33	0.830 ± 0.219	0.355 ± 0.055
	34	0.029 ± 0.011	-
	35	0.038 ± 0.013	-
n-alkanes	25	-	0.036 ± 0.006
	26	0.013 ± 0.002	0.043 ± 0.006
	27	0.165 ± 0.028	1.210 ± 0.237
	28	0.051 ± 0.007	0.463 ± 0.075
	29	0.364 ± 0.028	7.765 ± 1.371
	30	0.130 ± 0.034	0.843 ± 0.075
	31	1.980 ± 0.431	14.336 ± 2.240
	32	0.268 ± 0.110	0.696 ± 0.095
	33	0.873 ± 0.365	2.130 ± 0.344
	34	0.039 ± 0.016	-
	35	0.043 ± 0.016	-
primary alkanols	22	-	0.018 ± 0.005
	24	-	0.026 ± 0.017
	26	0.023 ± 0.010	0.064 ± 0.031
	28	0.065 ± 0.021	0.174 ± 0.079
	30	0.019 ± 0.003	0.152 ± 0.060
	31	0.012 ± 0.004	-
	32	0.015 - 0.004	0.179 ± 0.051

Compound class	Carbon chain length	Wax coverage of *S. pennellii* (µg cm^{-2})	
		leaf	fruit
	33	0.015 ± 0.004	-
	34	0.019 ± 0.006	-
alkyl esters	40	-	0.148 ± 0.071
	42	-	0.235 ± 0.078
	44	-	0.390 ± 0.130
	46	-	0.317 ± 0.113
	48	-	0.211 ± 0.038
total aliphatics		6.929 ± 1.367	33.523 ± 5.643
β-amyrin		-	0.213 ± 0.047
stigmasterol		0.021 ± 0.006	-
ursolic acid		0.017 ± 0.008	0.253 ± 0.114
total cyclics		0.038 ± 0.014	0.466 ± 0.095
total cuticular waxes		6.967 ± 1.379	33.989 ± 5.703

Appendix 6. Cuticular wax composition of *S. muricatum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. muricatum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.004 ± 0.003	0.146 ± 0.018
	21	-	0.014 ± 0.002
	22	0.003 ± 0.001	0.062 ± 0.001
	23	0.002 ± 0.001	0.038 ± 0.001
	24	0.020 ± 0.005	0.168 ± 0.024
	25	0.006 ± 0.002	0.073 ± 0.011
	26	0.021 ± 0.003	0.191 ± 0.036
	27	0.005 ± 0.001	0.045 ± 0.007
	28	0.052 ± 0.003	0.083 ± 0.014
	29	0.010 ± 0.001	0.037 ± 0.007
	30	0.032 ± 0.008	0.070 ± 0.009
	31	-	0.063 ± 0.005
	32	0.018 ± 0.008	0.209 ± 0.025
	33	-	0.048 ± 0.007
	34	0.002 ± 0.001	0.100 ± 0.017
alkanals	33	-	0.612 ± 0.097
	34	-	0.153 ± 0.029
	35	-	0.348 ± 0.061
anteiso-alkanes	32	0.033 ± 0.011	-
	33	0.016 ± 0.004	-
	34	0.049 ± 0.016	0.031 ± 0.004
	35	0.053 ± 0.005	-
	36	0.006 ± 0.002	-
iso-alkanes	31	0.036 ± 0.008	-
	32	0.006 ± 0.001	-
	33	0.062 ± 0.013	0.018 ± 0.002
	34	0.004 ± 0.001	-
	35	0.017 ± 0.002	-
n-alkanes	25	-	0.117 ± 0.040
	26	0.002 ± 0.001	0.019 ± 0.002
	27	0.059 ± 0.016	0.109 ± 0.021
	28	0.019 ± 0.007	0.060 ± 0.025
	29	0.133 ± 0.018	0.347 ± 0.044
	30	0.051 ± 0.006	0.166 ± 0.023
	31	0.725 ± 0.071	1.943 ± 0.415
	32	0.105 ± 0.014	0.381 ± 0.072
	33	0.328 ± 0.038	0.966 ± 0.268
	34	0.021 ± 0.003	0.066 ± 0.014
	36	0.016 ± 0.002	-
primary alkanols	20	-	0.023 ± 0.008
	21	0.001 ± 0.000	-

Compound class	Carbon chain length	Wax coverage of *S. muricatum* (µg cm^{-2})	
		leaf	fruit
	22	0.017 ± 0.007	0.024 ± 0.004
	23	0.003 ± 0.001	-
	24	0.030 ± 0.009	-
	25	0.013 ± 0.003	0.012 ± 0.002
	26	0.030 ± 0.006	0.063 ± 0.016
	27	0.017 ± 0.005	0.032 ± 0.004
	28	0.083 ± 0.012	0.101 ± 0.019
	29	0.031 ± 0.004	0.049 ± 0.007
	30	0.057 ± 0.007	0.043 ± 0.006
	31	0.027 ± 0.005	0.046 ± 0.010
	32	0.031 ± 0.004	0.024 ± 0.003
	33	0.008 ± 0.001	-
	34	0.009 ± 0.001	-
alkyl esters	38	0.010 ± 0.003	-
	40	0.008 ± 0.002	-
	42	0.011 ± 0.003	0.082 ± 0.016
	43	-	0.043 ± 0.006
	44	0.015 ± 0.003	0.121 ± 0.019
	45	0.005 ± 0.001	0.043 ± 0.007
	46	0.019 ± 0.004	0.067 ± 0.019
	47	0.083 ± 0.025	-
	48	0.020 ± 0.004	-
	49	0.011 ± 0.004	-
	50	0.026 ± 0.009	-
	51	0.014 ± 0.003	-
total aliphatics		2.493 ± 0.286	7.455 ± 1.111
phenylmethyl ester	28	0.022 ± 0.005	-
	29	0.005 ± 0.001	-
	30	0.021 ± 0.003	-
	31	0.006 ± 0.001	-
	32	0.045 ± 0.003	-
	33	0.009 ± 0.001	-
	34	0.021 ± 0.003	-
β-amyrin		-	0.059 ± 0.013
β-sitosterol		-	0.110 ± 0.028
α-tocopherol		0.002 ± 0.001	-
β-tocopherol		0.445 ± 0.116	-
δ-tocopherol		0.011 ± 0.005	-
total cyclics		0.587 ± 0.117	0.169 ± 0.040
dihydroxy alkanoic acid	16	-	0.090 ± 0.014
trihydroxy octadecenoic acid	18	-	0.154 ± 0.021

Compound class	Carbon chain length	Wax coverage of *S. muricatum* (µg cm^{-2})	
		leaf	fruit
total cuticular waxes		3.081 ± 0.322	7.867 ± 1.157

Appendix 7. Cuticular wax composition of *S. pseudocapsicum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. pseudocapsicum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.004 ± 0.003	0.146 ± 0.018
	24	-	0.014 ± 0.002
	25	0.003 ± 0.001	0.062 ± 0.001
	26	0.002 ± 0.001	0.038 ± 0.001
	27	0.020 ± 0.005	0.168 ± 0.024
	28	0.006 ± 0.002	0.073 ± 0.011
	29	0.021 ± 0.003	0.191 ± 0.036
	30	0.005 ± 0.001	0.045 ± 0.007
	31	0.052 ± 0.003	0.083 ± 0.014
	32	0.010 ± 0.001	0.037 ± 0.007
	33	0.032 ± 0.008	0.070 ± 0.009
alkanals	32	-	0.063 ± 0.005
	33	0.018 ± 0.008	0.209 ± 0.025
anteiso-alkanes	32	-	0.048 ± 0.007
	34	0.002 ± 0.001	0.100 ± 0.017
iso-alkanes	31	-	0.612 ± 0.097
	32	-	0.153 ± 0.029
	33	-	0.348 ± 0.061
n-alkanes	25	0.033 ± 0.011	-
	26	0.016 ± 0.004	-
	27	0.049 ± 0.016	0.031 ± 0.004
	28	0.053 ± 0.005	-
	29	0.006 ± 0.002	-
	30	0.036 ± 0.008	-
	31	0.006 ± 0.001	-
	32	0.062 ± 0.013	0.018 ± 0.002
	33	0.004 ± 0.001	-
primary alkanols	26	0.017 ± 0.002	-
	27	-	0.117 ± 0.040
	28	0.002 ± 0.001	0.019 ± 0.002
	29	0.059 ± 0.016	0.109 ± 0.021
	30	0.019 ± 0.007	0.060 ± 0.025
	31	0.133 ± 0.018	0.347 ± 0.044
	32	0.051 ± 0.006	0.166 ± 0.023
	33	0.725 ± 0.071	1.943 ± 0.415
alkyl esters	37	0.105 ± 0.014	0.381 ± 0.072
	38	0.328 ± 0.038	0.966 ± 0.268
	42	0.021 ± 0.003	0.066 ± 0.014
total aliphatics		0.016 ± 0.002	-
α-amyrin		-	0.023 ± 0.008

Compound class	Carbon chain length	Wax coverage of *S. pseudocapsicum* (µg cm^{-2})	
		leaf	fruit
β-amyrin		0.001 ± 0.000	-
δ-amyrin		0.017 ± 0.007	0.024 ± 0.004
camposterol		0.003 ± 0.001	-
cholesterol		0.030 ± 0.009	-
naringenin		0.013 ± 0.003	0.012 ± 0.002
stigmasterol		0.030 ± 0.006	0.063 ± 0.016
β-sitosterol		0.017 ± 0.005	0.032 ± 0.004
α-tocopherol		0.083 ± 0.012	0.101 ± 0.019
β-tocopherol		0.031 ± 0.004	0.049 ± 0.007
ɣ-tocopherol		0.057 ± 0.007	0.043 ± 0.006
total cyclics		0.027 ± 0.005	0.046 ± 0.010
total cuticular waxes		3.081 ± 0.322	7.867 ± 1.157

Appendix 8. Cuticular wax composition of *S. quitoense* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. quitoense* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.007 ± 0.003	1.576 ± 0.346
	21	0.024 ± 0.008	0.161 ± 0.012
	22	0.014 ± 0.006	1.099 ± 0.202
	23	0.024 ± 0.006	0.393 ± 0.064
	24	0.046 ± 0.014	1.017 ± 0.248
	25	0.055 ± 0.015	0.210 ± 0.027
	26	0.063 ± 0.013	0.265 ± 0.028
	27	0.131 ± 0.026	0.191 ± 0.025
	28	0.043 ± 0.009	0.336 ± 0.054
	29	0.348 ± 0.068	0.139 ± 0.017
	30	0.044 ± 0.012	0.226 ± 0.056
	31	0.073 ± 0.018	-
	32	0.023 ± 0.006	0.113 ± 0.016
alkanals	23	0.018 ± 0.006	-
	24	0.035 ± 0.011	-
	25	0.040 ± 0.011	-
	26	0.049 ± 0.011	-
	27	0.098 ± 0.027	-
	28	0.039 ± 0.010	-
	29	0.283 ± 0.080	-
	30	0.032 ± 0.010	-
	31	0.080 ± 0.027	-
	32	0.028 ± 0.012	-
	34	0.022 ± 0.005	-
anteiso-alkanes	34	0.018 ± 0.007	-
	35	0.085 ± 0.040	-
	36	0.016 ± 0.004	-
iso-alkanes	27	0.009 ± 0.003	0.078 ± 0.004
	28	0.008 ± 0.003	-
	29	0.104 ± 0.038	-
	30	0.017 ± 0.006	-
	31	0.277 ± 0.082	0.120 ± 0.022
	32	0.051 ± 0.013	-
	33	0.632 ± 0.151	0.286 ± 0.039
	34	0.086 ± 0.029	-
	35	0.219 ± 0.038	0.130 ± 0.031
	36	0.023 ± 0.004	-
	37	0.053 ± 0.012	-
n-alkanes	25	0.009 ± 0.003	0.971 ± 0.231
	26	0.027 ± 0.008	0.482 ± 0.032
	27	0.355 ± 0.083	1.596 ± 0.091

Compound class	Carbon chain length	Wax coverage of *S. quitoense* (µg cm^{-2})	
		leaf	fruit
	28	0.096 ± 0.024	0.398 ± 0.076
	29	0.390 ± 0.107	0.531 ± 0.054
	30	0.073 ± 0.021	0.372 ± 0.036
	31	0.931 ± 0.266	0.635 ± 0.063
	32	0.238 ± 0.079	0.526 ± 0.099
	33	3.181 ± 0.797	0.451 ± 0.081
	34	0.340 ± 0.097	-
	35	3.671 ± 0.907	-
	36	0.086 ± 0.021	-
	37	0.300 ± 0.073	-
n-alkenes	31	0.046 ± 0.034	-
	32	0.018 ± 0.008	-
	33	0.098 ± 0.070	-
	34	0.028 ± 0.014	-
	35	0.228 ± 0.162	-
	36	0.023 ± 0.007	-
	37	0.091 ± 0.036	-
primary alkanols	26	-	0.065 ± 0.016
	27	-	0.051 ± 0.009
	28	0.017 ± 0.004	0.079 ± 0.015
	30	0.021 ± 0.007	-
	32	0.017 ± 0.005	-
	33	0.021 ± 0.005	-
alkyl esters	37	0.015 ± 0.003	-
	38	0.022 ± 0.004	-
	39	0.014 ± 0.005	-
	40	0.016 ± 0.002	-
	41	0.014 ± 0.006	-
	42	-	0.068 ± 0.017
	44	-	0.135 ± 0.042
total aliphatics		13.602 ± 3.252	12.700 ± 1.103
campesterol		-	0.164 ± 0.018
cholesterol		-	0.138 ± 0.021
naringenin		-	0.624 ± 0.107
β-sitosterol		-	0.390 ± 0.029
δ-sitosterol		-	0.250 ± 0.065
total cyclics		±	1.565 ± 0.138
dihydroxy alkanoic acid	16	-	0.492 ± 0.057
trihydroxy octadecenoic acid	18	-	0.167 ± 0.028
total cuticular waxes		13.602 ± 3.252	14.924 ± 1.268

Appendix 9. Cuticular wax composition of *S. retroflexum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. retroflexum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	-	0.050 ± 0.019
	21	-	0.024 ± 0.010
	22	-	0.046 ± 0.011
	23	-	0.032 ± 0.013
	24	-	0.083 ± 0.032
	25	-	0.043 ± 0.015
	26	-	0.053 ± 0.027
	28	0.006 ± 0.001	-
	30	0.005 ± 0.001	-
	32	0.005 ± 0.001	-
alkanals	33	-	0.114 ± 0.016
anteiso-alkanes	30	-	0.027 ± 0.003
	31	-	0.043 ± 0.005
	32	0.097 ± 0.015	0.104 ± 0.006
	34	0.062 ± 0.008	0.065 ± 0.003
	35	0.005 ± 0.001	-
	36	0.011 ± 0.002	-
iso-alkanes	30	-	0.027 ± 0.003
	31	0.148 ± 0.030	0.148 ± 0.011
	32	0.017 ± 0.001	0.036 ± 0.004
	33	0.147 ± 0.027	0.078 ± 0.003
	34	0.008 ± 0.001	-
	35	0.017 ± 0.002	-
n-alkanes	25	-	0.234 ± 0.068
	26	-	0.083 ± 0.016
	27	-	0.544 ± 0.167
	28	-	0.259 ± 0.066
	29	0.190 ± 0.037	1.618 ± 0.445
	30	0.064 ± 0.013	0.236 ± 0.023
	31	0.531 ± 0.073	0.803 ± 0.056
	32	0.058 ± 0.012	0.116 ± 0.021
	33	0.319 ± 0.094	0.138 ± 0.010
	34	0.015 ± 0.003	-
	35	0.028 ± 0.004	-
	36	0.009 ± 0.001	-
primary alkanols	26	0.080 ± 0.010	-
	28	0.050 ± 0.012	-
	29	0.006 ± 0.001	-
	30	0.010 ± 0.001	0.042 ± 0.010
	32	0.016 ± 0.003	-
	33	0.006 ± 0.001	-

Compound class	Carbon chain length	Wax coverage of *S. retroflexum* (µg cm^{-2})	
		leaf	fruit
	34	0.014 ± 0.002	-
alkyl esters	41	0.008 ± 0.002	-
	42	0.010 ± 0.002	-
	44	0.010 ± 0.001	-
	46	0.012 ± 0.002	-
	48	0.015 ± 0.004	-
	50	0.016 ± 0.004	-
total aliphatics		1.994 ± 0.107	5.042 ± 0.699
phenylmethyl esters	26	0.066 ± 0.012	-
	30	0.013 ± 0.004	-
	32	0.010 ± 0.003	-
β-sitosterol		0.056 ± 0.019	0.078 ± 0.004
total cyclics		0.145 ± 0.025	0.078 ± 0.004
total cuticular waxes		2.139 ± 0.126	5.120 ± 0.698

Appendix 10. Cuticular wax composition of *S. lycopersicum* cv. 'Benarys Gartenfreude' leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'Benarys Gartenfreude' (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.003 ± 0.001	0.025 ± 0.018
	21	-	0.005 ± 0.001
	22	0.004 ± 0.003	0.025 ± 0.031
	23	0.001 ± 0.000	0.010 ± 0.008
	24	0.012 ± 0.006	0.068 ± 0.117
	25	0.004 ± 0.002	0.008 ± 0.003
	26	0.026 ± 0.016	0.035 ± 0.043
	27	-	0.009 ± 0.002
	28	0.014 ± 0.014	0.387 ± 0.062
	29	0.003 ± 0.001	0.040 ± 0.007
	30	0.013 ± 0.005	0.425 ± 0.242
	31	-	0.024 ± 0.010
	32	0.014 ± 0.004	0.353 ± 0.128
	33	-	0.030 ± 0.021
	34	-	0.013 ± 0.005
alkanals	32	-	0.055 ± 0.025
anteiso-alkanes	30	0.015 ± 0.004	-
	32	0.127 ± 0.035	0.010 ± 0.003
	34	0.008 ± 0.002	-
	35	0.012 ± 0.002	-
iso-alkanes	29	0.012 ± 0.003	-
	31	0.118 ± 0.023	0.104 ± 0.062
	32	0.015 ± 0.004	-
	33	0.047 ± 0.008	-
n-alkanes	25	0.006 ± 0.002	0.015 ± 0.006
	26	0.003 ± 0.001	0.010 ± 0.004
	27	0.069 ± 0.015	0.131 ± 0.133
	28	0.002 ± 0.001	0.118 ± 0.065
	29	0.125 ± 0.027	3.113 ± 2.094
	30	0.043 ± 0.010	0.285 ± 0.036
	31	0.903 ± 0.178	7.311 ± 1.840
	32	0.134 ± 0.035	0.299 ± 0.221
	33	0.253 ± 0.055	0.451 ± 0.244
	34	0.007 ± 0.002	0.024 ± 0.008
	35	-	0.039 ± 0.005
alkanonas	33	-	0.117 ± 0.168
primary alkanols	22	0.001 ± 0.001	0.048 ± 0.046
	23	-	0.006 ± 0.003
	24	0.003 ± 0.002	0.098 ± 0.100
	25	0.001 ± 0.001	0.030 ± 0.040
	26	0.016 ± 0.007	0.107 ± 0.088

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'Benarys Gartenfreude' (µg cm^{-2})	
		leaf	fruit
	27	0.004 ± 0.001	0.066 ± 0.041
	28	0.023 ± 0.009	0.017 ± 0.003
	29	-	0.037 ± 0.033
	30	0.010 ± 0.002	1.145 ± 0.280
	31	0.003 ± 0.001	0.026 ± 0.009
	32	-	0.176 ± 0.046
	33	0.004 ± 0.001	0.018 ± 0.006
	34	0.008 ± 0.003	0.062 ± 0.019
alkyl esters	38	-	0.117 ± 0.087
	39	-	0.010 ± 0.005
	40	-	0.229 ± 0.205
	41	-	0.020 ± 0.010
	42	0.002 ± 0.001	0.284 ± 0.230
	43	-	0.075 ± 0.053
	44	0.003 ± 0.001	0.396 ± 0.307
	45	-	0.103 ± 0.063
	46	0.002 ± 0.001	0.410 ± 0.353
	47	-	0.074 ± 0.041
	48	0.001 ± 0.000	0.299 ± 0.239
	49	-	0.056 ± 0.035
	50	0.006 ± 0.003	0.160 ± 0.118
	51	-	0.069 ± 0.038
	52	-	0.112 ± 0.071
total aliphatics		2.078 ± 0.423	17.791 ± 7.313
α-amyrin		0.038 ± 0.009	0.237 ± 0.209
β-amyrin		0.063 ± 0.014	0.540 ± 0.293
δ-amyrin		0.048 ± 0.012	0.816 ± 0.364
camposterol		-	-
cholesterol		0.025 ± 0.005	0.034 ± 0.031
naringenin		-	0.079 ± 0.104
β-sitosterol		-	0.059 ± 0.035
stigmasterol		-	0.740 ± 0.728
ɣ-tocopherol		-	0.021 ± 0.008
lupeol		0.022 ± 0.004	0.086 ± 0.027
total cyclics		0.196 ± 0.042	2.612 ± 0.647
dihydroxy alkanoic acid	16	-	0.017 ± 0.002
total cuticular waxes		2.274 ± 0.462	20.421 ± 7.930

Appendix 11. Cuticular wax composition of *S. lycopersicum* cv. 'John Baer' leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'John Baer' (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.002 ± 0.001	0.048 ± 0.016
	21	-	0.025 ± 0.004
	22	-	0.069 ± 0.019
	23	-	0.159 ± 0.013
	24	0.005 ± 0.001	0.764 ± 0.086
	25	-	0.109 ± 0.013
	26	0.007 ± 0.001	0.183 ± 0.053
	29	-	0.075 ± 0.018
	30	0.008 ± 0.003	0.151 ± 0.103
	31	-	0.117 ± 0.016
	32	-	0.223 ± 0.169
	33	-	0.064 ± 0.012
	34	-	0.077 ± 0.042
alkanals	33	-	0.305 ± 0.025
anteiso-alkanes	30	0.012 ± 0.005	-
	31	0.009 ± 0.002	-
	32	0.101 ± 0.032	-
	34	0.017 ± 0.006	-
iso-alkanes	29	0.016 ± 0.008	-
	30	0.006 ± 0.001	-
	31	0.160 ± 0.045	0.221 ± 0.033
	32	0.017 ± 0.002	-
	33	0.051 ± 0.021	-
n-alkanes	25	-	0.033 ± 0.005
	26	0.005 ± 0.001	-
	27	0.055 ± 0.015	0.094 ± 0.087
	28	0.012 ± 0.002	0.051 ± 0.014
	29	0.107 ± 0.012	1.139 ± 0.435
	30	0.036 ± 0.003	0.506 ± 0.143
	31	0.784 ± 0.076	3.752 ± 1.439
	32	0.082 ± 0.009	0.647 ± 0.189
	33	0.178 ± 0.086	0.692 ± 0.688
primary alkanols	22	-	0.026 ± 0.008
	23	-	0.042 ± 0.005
	24	-	0.047 ± 0.014
	26	0.006 ± 0.002	0.031 ± 0.018
	27	0.004 ± 0.001	0.044 ± 0.028
	28	0.012 ± 0.004	0.053 ± 0.047
	29	-	0.086 ± 0.028
	30	0.009 ± 0.002	0.122 ± 0.032
	31	-	0.117 ± 0.007

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'John Baer' (µg cm^{-2})	
		leaf	fruit
	32	0.011 ± 0.004	0.235 ± 0.088
	33	-	0.084 ± 0.023
	34	-	0.145 ± 0.058
alkyl esters	39	-	0.087 ± 0.031
	46	0.010 ± 0.002	-
total aliphatics		1.721 ± 0.168	10.625 ± 2.887
alpha-amyrin		0.062 ± 0.066	0.467 ± 0.068
beta-amyrin		0.077 ± 0.028	0.342 ± 0.046
delta-amyrin		0.049 ± 0.007	0.858 ± 0.115
camposterol		-	-
cholesterol		0.004 ± 0.001	-
beta-sitosterol		0.021 ± 0.006	-
stigmasterol		0.010 ± 0.002	-
total cyclics		0.223 ± 0.089	1.666 ± 0.165
dihydroxy alkanoic acid	16	-	0.319 ± 0.082
total cuticular waxes		1.945 ± 0.107	12.610 ± 2.897

Appendix 12. Cuticular wax composition of *S. lycopersicum* cv. 'Pearson' leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'Pearson' (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.002 ± 0.001	0.022 ± 0.007
	22	-	0.025 ± 0.009
	23	-	0.054 ± 0.013
	24	0.005 ± 0.001	0.310 ± 0.193
	25	-	0.032 ± 0.017
	26	0.007 ± 0.002	0.049 ± 0.022
	28	-	0.083 ± 0.011
	30	0.009 ± 0.002	0.077 ± 0.015
	32	0.008 ± 0.002	0.074 ± 0.017
anteiso-alkanes	30	0.027 ± 0.006	-
	31	0.016 ± 0.004	-
	32	0.201 ± 0.032	-
	34	0.010 ± 0.001	-
iso-alkanes	29	0.020 ± 0.004	-
	30	0.009 ± 0.004	-
	31	0.198 ± 0.009	0.133 ± 0.030
	32	0.023 ± 0.004	-
	33	0.104 ± 0.054	-
n-alkanes	25	0.017 ± 0.022	0.027 ± 0.007
	27	0.053 ± 0.007	0.050 ± 0.011
	28	0.005 ± 0.001	±
	29	0.139 ± 0.010	1.157 ± 0.369
	30	0.051 ± 0.002	0.479 ± 0.107
	31	1.153 ± 0.116	2.538 ± 0.582
	32	0.137 ± 0.029	0.532 ± 0.093
	33	0.229 ± 0.041	0.523 ± 0.131
primary alkanols	22	0.002 ± 0.001	0.014 ± 0.004
	23	-	0.028 ± 0.005
	24	0.006 ± 0.003	-
	25	0.001 ± 0.001	-
	26	0.016 ± 0.009	0.019 ± 0.004
	28	0.020 ± 0.008	0.045 ± 0.009
	30	0.009 ± 0.002	0.045 ± 0.006
	31	0.003 ± 0.001	0.025 ± 0.008
	32	0.012 ± 0.002	0.187 ± 0.012
	33	0.003 ± 0.000	0.160 ± 0.055
	34	0.008 ± 0.002	-
alkyl esters	37	-	0.028 ± 0.006
	39	-	0.052 ± 0.019
	40	-	0.018 ± 0.006
	44	-	0.020 ± 0.008

Compound class	Carbon chain length	Wax coverage of *S. lycopersicum* cv. 'Pearson' (µg cm^{-2})	
		leaf	fruit
	46	0.003 ± 0.001	0.038 ± 0.018
	50	0.007 ± 0.002	-
total aliphatics		2.512 ± 0.098	6.843 ± 1.451
α-amyrin		0.036 ± 0.004	0.987 ± 0.164
β-amyrin		0.041 ± 0.008	0.603 ± 0.064
δ-amyrin		0.043 ± 0.006	1.576 ± 0.268
lupeol		0.025 ± 0.002	-
triterpenoide 1		-	0.092 ± 0.017
triterpenoide 2		-	0.049 ± 0.011
triterpenoide 3		-	0.093 ± 0.018
total cyclics		0.146 ± 0.018	3.399 ± 0.532
dihydroxy alkanoic acid	16	-	0.129 ± 0.065
total cuticular waxes		2.658 ± 0.100	10.371 ± 1.952

Appendix 13. Cuticular wax composition of *S. melongena* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

Compound class	Carbon chain length	Wax coverage of *S. melongena* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	0.004 ± 0.002	0.078 ± 0.022
	21	-	0.020 ± 0.005
	22	0.004 ± 0.001	0.021 ± 0.008
	23	0.001 ± 0.001	0.048 ± 0.015
	24	0.012 ± 0.007	0.037 ± 0.016
	25	0.005 ± 0.003	0.023 ± 0.012
	26	0.038 ± 0.012	0.036 ± 0.022
	27	0.006 ± 0.002	0.035 ± 0.009
	28	0.054 ± 0.022	0.068 ± 0.027
	29	0.009 ± 0.005	-
	30	0.046 ± 0.016	0.054 ± 0.019
	31	0.020 ± 0.013	-
	32	0.086 ± 0.027	0.033 ± 0.015
	33	0.015 ± 0.004	-
	34	0.043 ± 0.009	-
	35	0.010 ± 0.001	-
alkanals	26	0.013 ± 0.004	-
	28	0.016 ± 0.008	-
	33	-	0.127 ± 0.013
	34	-	0.053 ± 0.004
	35	0.014 ± 0.001	0.079 ± 0.007
	37	0.017 ± 0.002	-
anteiso-alkanes	31	0.009 ± 0.001	-
	32	0.194 ± 0.017	0.080 ± 0.017
	33	0.091 ± 0.016	-
	34	0.465 ± 0.044	0.151 ± 0.034
	35	0.042 ± 0.004	-
	36	0.128 ± 0.015	-
iso-alkanes	31	0.037 ± 0.009	-
	33	0.153 ± 0.055	-
	34	0.012 ± 0.002	-
	35	0.082 ± 0.006	-
	35	0.007 ± 0.001	-
n-alkanes	27	0.036 ± 0.008	-
	29	0.054 ± 0.010	0.134 ± 0.023
	30	0.017 ± 0.003	0.096 ± 0.018
	31	0.590 ± 0.151	0.677 ± 0.136
	32	0.227 ± 0.040	0.215 ± 0.033
	33	1.685 ± 0.221	0.347 ± 0.052
	34	0.136 ± 0.008	-

Compound class	Carbon chain length	Wax coverage of *S. melongena* (µg cm^{-2})	
		leaf	fruit
	35	0.868 ± 0.066	-
	36	0.114 ± 0.010	-
primary alkanols	22	0.001 ± 0.001	-
	24	0.005 ± 0.004	-
	25	0.009 ± 0.001	-
	26	0.029 ± 0.018	-
	27	0.032 ± 0.013	-
	28	0.210 ± 0.135	0.024 ± 0.010
	29	0.041 ± 0.033	-
	30	0.039 ± 0.019	0.019 ± 0.014
	31	0.005 ± 0.002	-
	32	0.015 ± 0.004	-
	33	0.008 ± 0.003	-
	34	0.011 ± 0.002	-
alkyl esters	37	-	0.020 ± 0.010
	38	-	0.019 ± 0.004
	42	0.012 ± 0.005	-
	44	0.019 ± 0.013	-
	45	0.005 ± 0.003	-
	46	0.022 ± 0.014	-
	47	0.012 ± 0.004	-
	48	0.029 ± 0.017	-
	49	0.011 ± 0.004	-
	50	0.021 ± 0.017	-
	51	0.021 ± 0.005	-
total aliphatics		5.917 ± 0.643	2.495 - 0.355
coumaric acid	26	0.010 ± 0.001	-
	28	0.024 ± 0.005	-
	30	0.097 ± 0.022	-
	31	0.020 ± 0.005	-
	32	0.043 ± 0.008	-
phenylmethyl esters	28	0.009 ± 0.003	-
	30	0.023 ± 0.010	-
	31	0.007 ± 0.002	-
	32	0.058 ± 0.022	-
	33	0.011 ± 0.002	-
	34	0.038 ± 0.010	-
	35	0.007 ± 0.003	-
	36	0.005 ± 0.001	-
δ-sitosterol		-	0.037 ± 0.008
stigmasterol		0.020 ± 0.005	-
β-tocopherol		0.032 ± 0.013	-
δ-tocopherol		0.002 0.001	

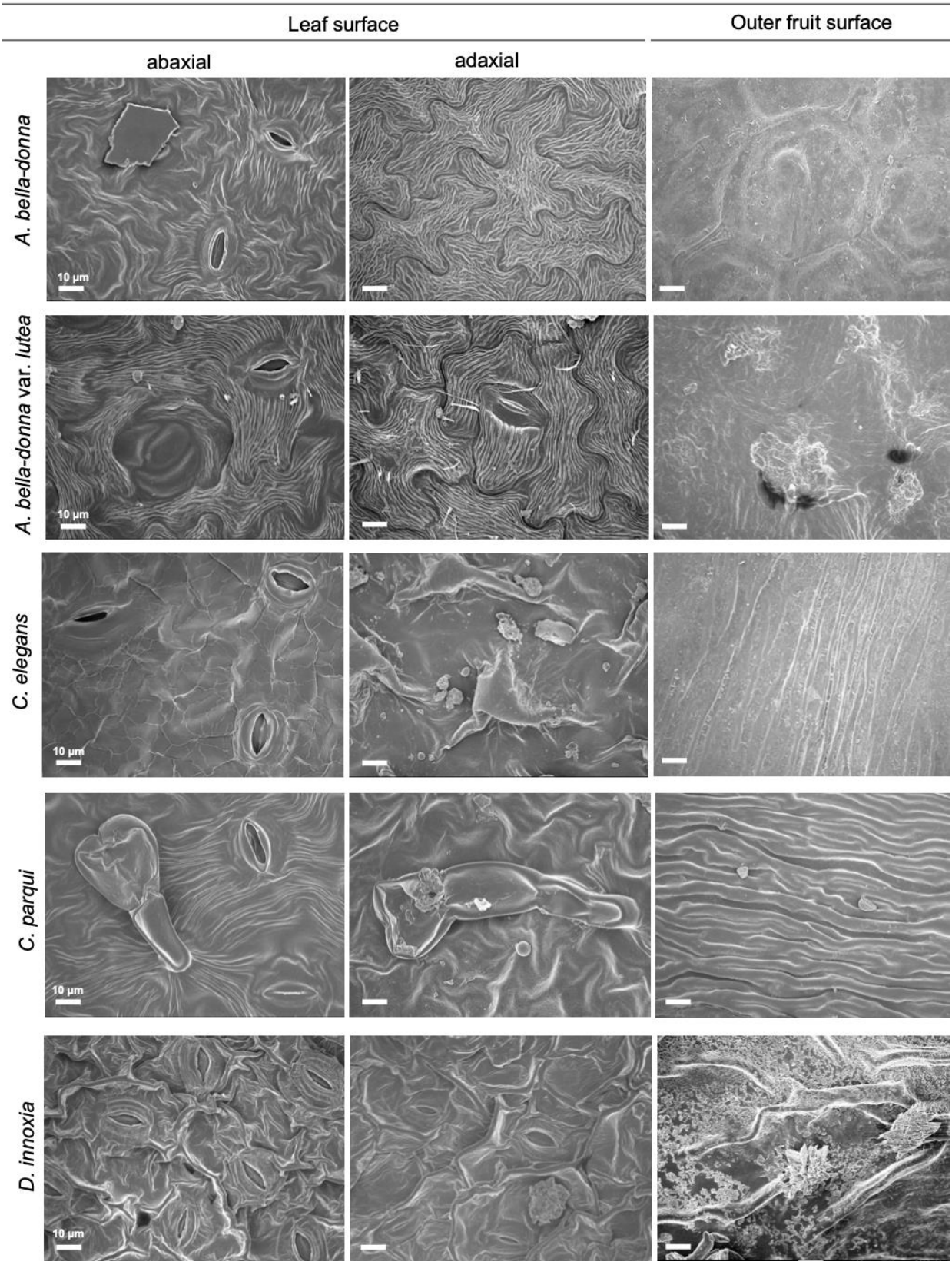
Compound class	Carbon chain length	Wax coverage of *S. melongena* (µg cm^{-2})	
		leaf	fruit
total cyclics		0.403 ± 0.071	0.037 ± 0.008
dihydroxy alkanoic acid	16	-	0.330 ± 0.132
trihydroxy octadecenoic acid	18	-	0.543 ± 0.430
total cuticular waxes		6.320 ± 0.678	3.404 ± 0.873

Appendix 14. Cuticular wax composition of *S. tuberosum* leaf and fruit. Each value represents the mean ± standard deviation (n ≥ 4).

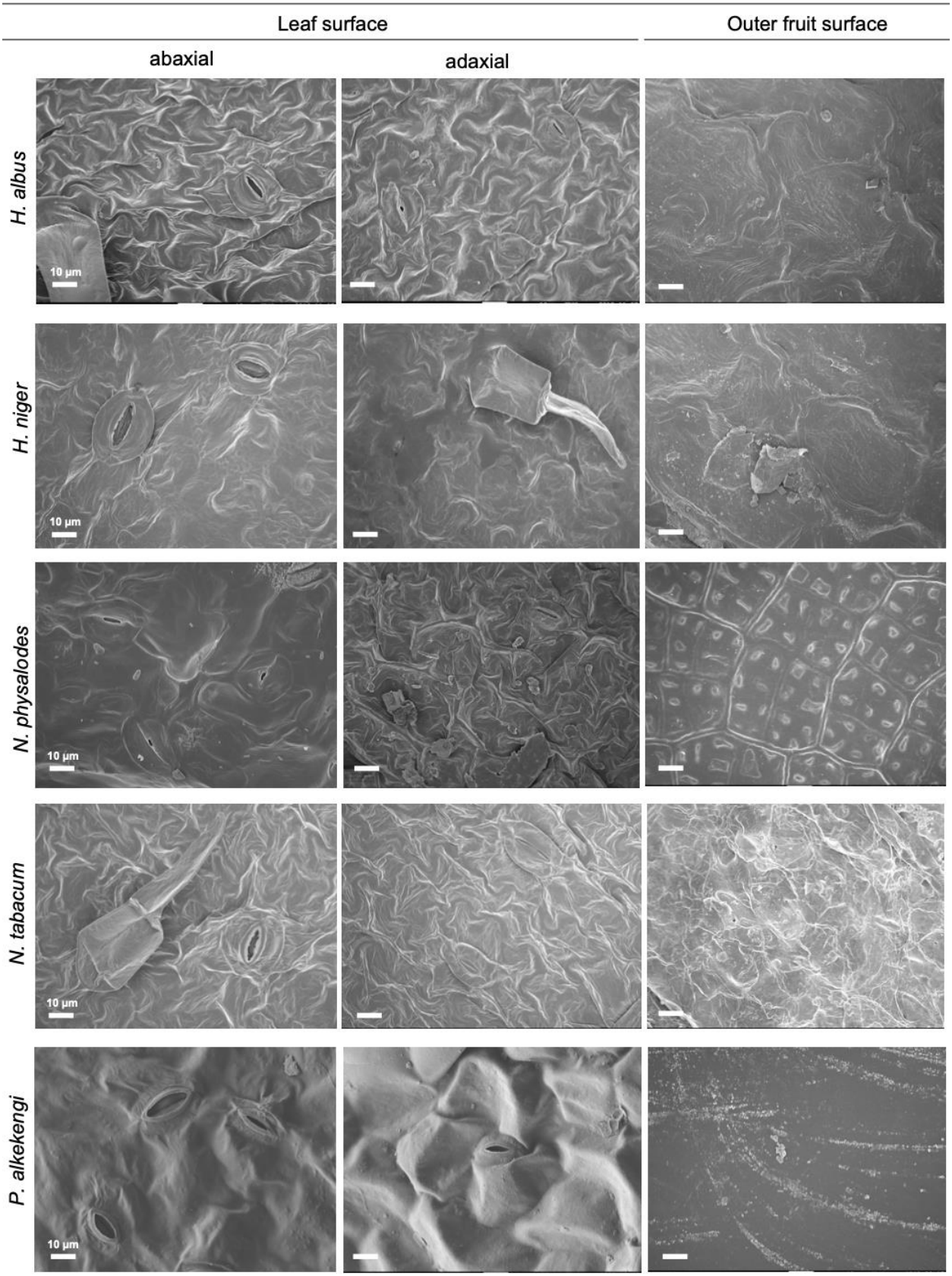
Compound class	Carbon chain length	Wax coverage of *S. tuberosum* (µg cm^{-2})	
		leaf	fruit
alkanoic acids	20	-	0.257 ± 0.049
	21	-	0.054 ± 0.011
	22	0.002 ± 0.001	0.244 ± 0.013
	23	-	0.121 ± 0.007
	24	0.003 ± 0.002	0.427 ± 0.048
	25	-	0.057 ± 0.006
	26	0.002 ± 0.000	0.233 ± 0.065
	27	-	0.055 ± 0.005
	28	-	0.115 ± 0.022
	29	0.005 ± 0.002	0.103 ± 0.016
	30	-	0.205 ± 0.043
	31	-	0.089 ± 0.014
	32	-	0.122 ± 0.036
alkanals	33	-	0.154 ± 0.041
anteiso-alkanes	28	0.002 ± 0.000	-
	30	0.013 ± 0.003	-
	31	0.010 ± 0.002	-
	32	0.063 ± 0.005	0.083 ± 0.019
	33		0.186 ± 0.052
	34	0.019 ± 0.004	0.124 ± 0.023
	35	0.016 ± 0.005	-
iso-alkanes	27	0.002 ± 0.001	-
	29	0.024 ± 0.010	0.074 ± 0.006
	30	0.005 ± 0.001	-
	31	0.137 ± 0.022	0.180 ± 0.031
	32	0.010 ± 0.001	-
	33	0.061 ± 0.003	0.091 ± 0.039
n-alkanes	25	0.020 ± 0.008	-
	27	0.095 ± 0.009	-
	28	0.013 ± 0.002	-
	29	0.104 ± 0.015	0.484 ± 0.104
	30	0.022 ± 0.007	0.133 ± 0.029
	31	0.281 ± 0.087	0.775 ± 0.176
	32	0.024 ± 0.010	-
	33	0.048 ± 0.015	-
primary alkanols	22	0.004 ± 0.001	0.162 ± 0.035
	23	0.001 ± 0.000	0.039 ± 0.001
	24	0.024 ± 0.010	0.177 ± 0.098
	25	0.008 ± 0.003	0.045 ± 0.012
	26	0.067 ± 0.026	0.167 ± 0.118
	27	0.013 ± 0.001	-

Compound class	Carbon chain length	Wax coverage of *S. tuberosum* (µg cm^{-2})	
		leaf	fruit
alkyl esters	28	0.075 ± 0.010	0.080 ± 0.067
	29	0.006 ± 0.001	-
	30	0.014 ± 0.001	0.097 ± 0.035
	32	0.004 ± 0.001	-
	36	0.002 ± 0.001	0.389 ± 0.178
	37	0.002 ± 0.001	0.068 ± 0.021
	38	0.003 ± 0.001	2.103 ± 0.873
	39	0.002 ± 0.000	0.173 ± 0.043
	40	-	1.912 ± 0.662
	41	-	0.175 ± 0.051
	42	0.003 ± 0.001	0.960 ± 0.345
	43	-	0.103 ± 0.032
	44	0.004 ± 0.001	0.591 ± 0.229
	45	-	0.103 ± 0.031
	46	0.004 ± 0.001	0.424 ± 0.183
	47	-	0.101 ± 0.027
	48	0.003 ± 0.001	0.242 ± 0.121
total aliphatics		1.220 ± 0.072	12.475 ± 2.912
α-amyrin		-	0.095 ± 0.028
stigmasterol		0.010 ± 0.006	-
β-sitosterol		0.007 ± 0.001	0.123 ± 0.031
total cyclics		0.017 ± 0.007	0.218 ± 0.057
dihydroxy alkanoic acid	16	-	1.285 ± 0.104
trihydroxy octadecenoic acid	18	-	0.130 ± 0.014
total cuticular waxes		1.238 ± 0.078	14.108 ± 3.053

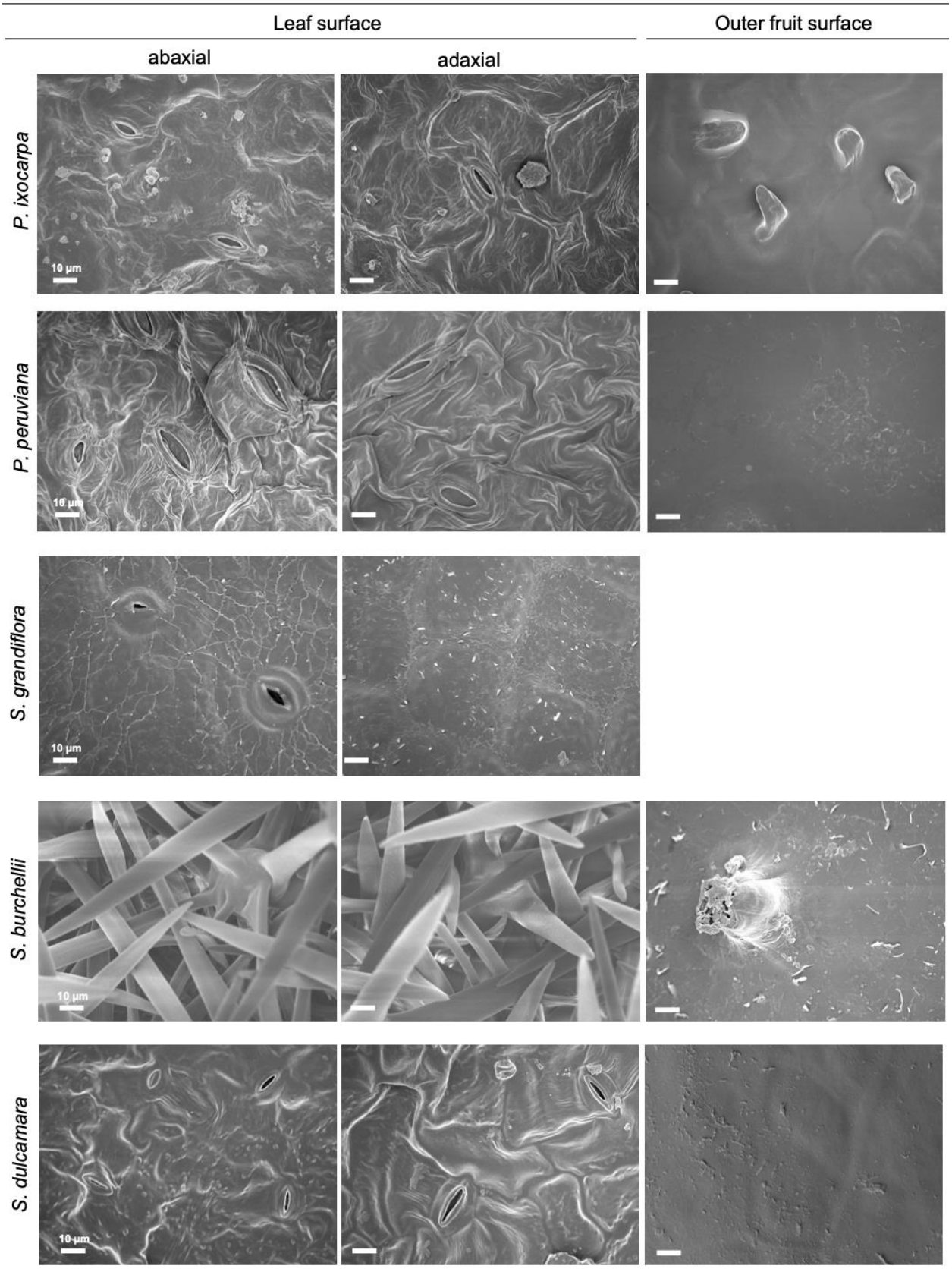
Appendix 15. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



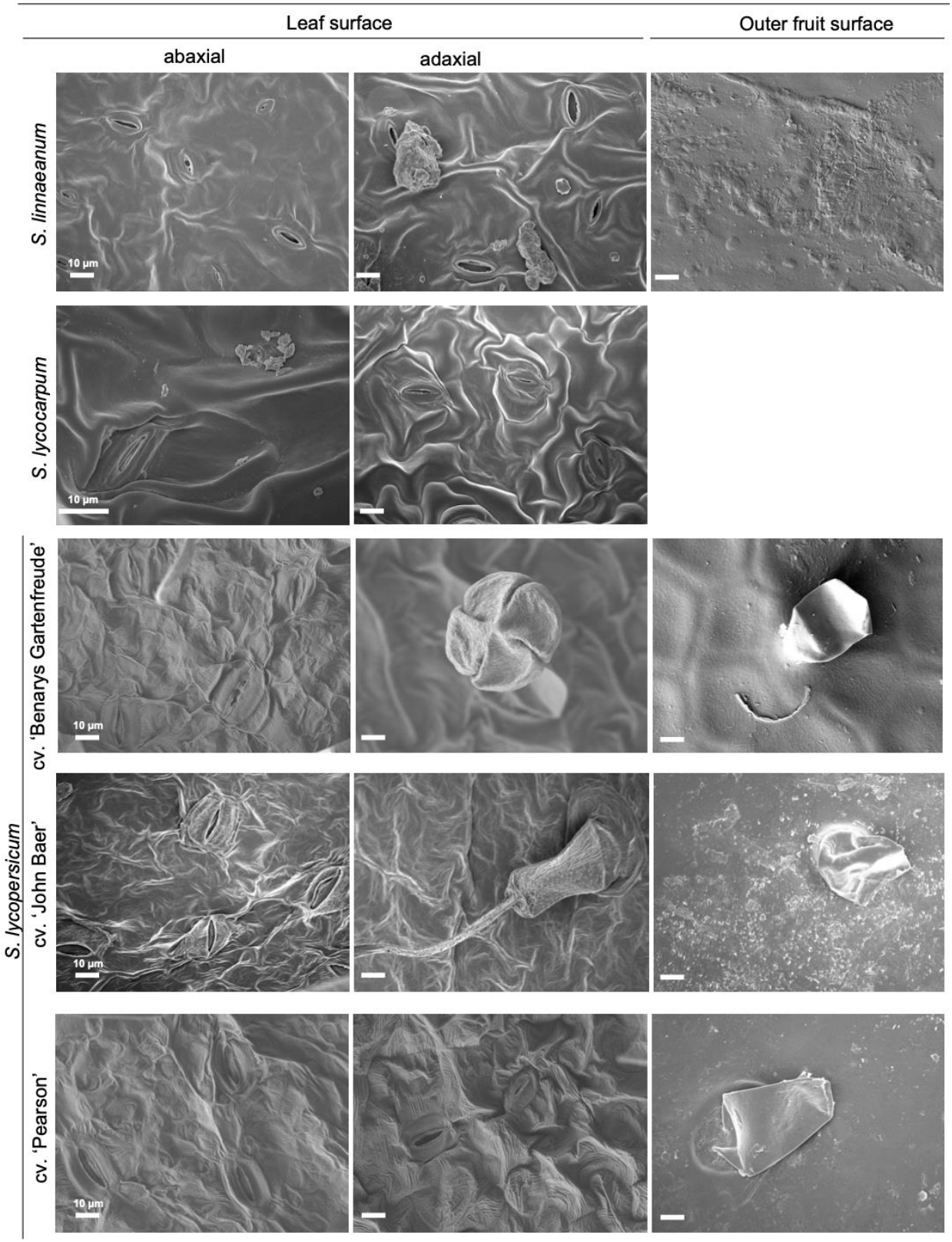
Appendix 16. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



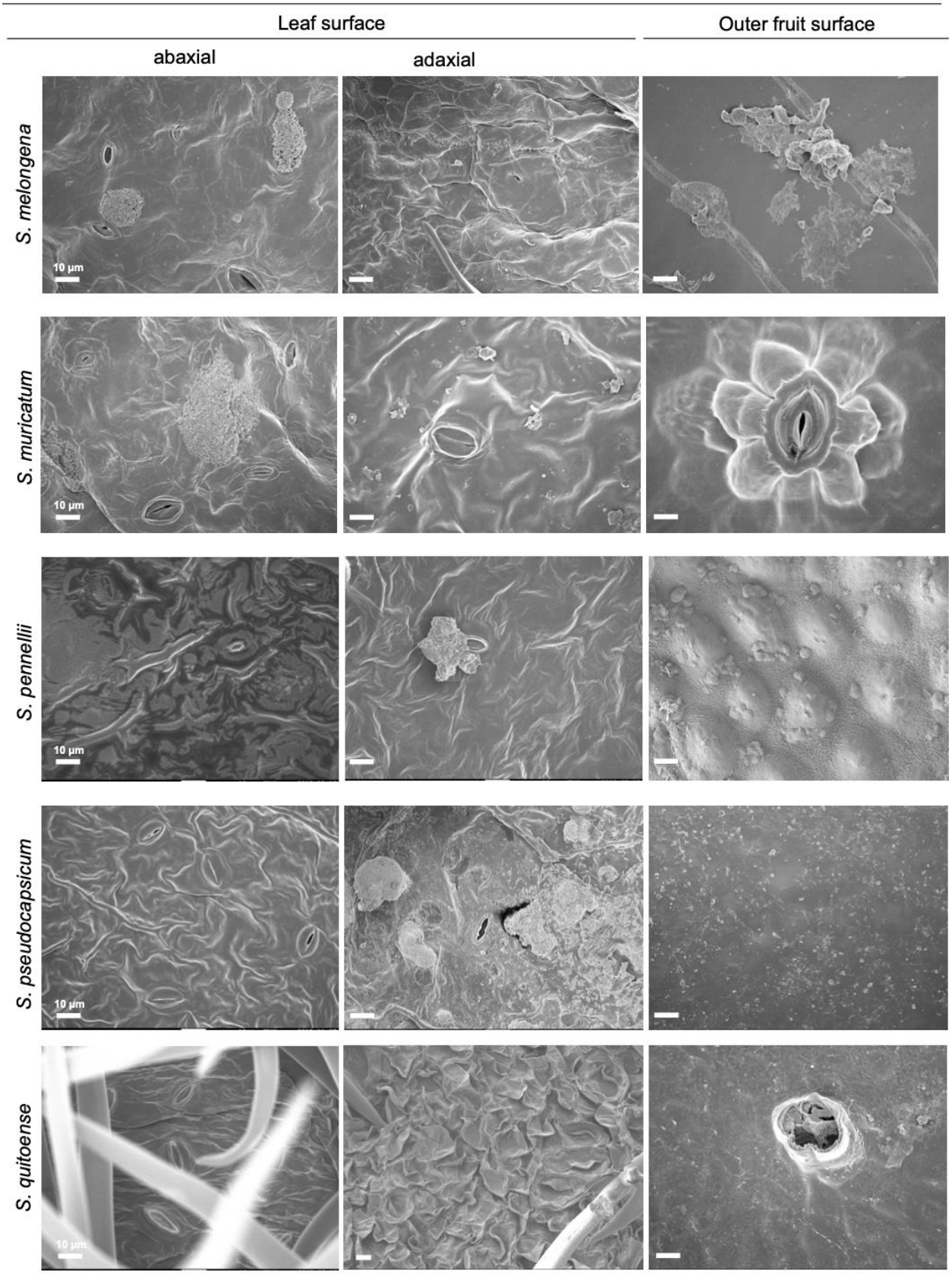
Appendix 17. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



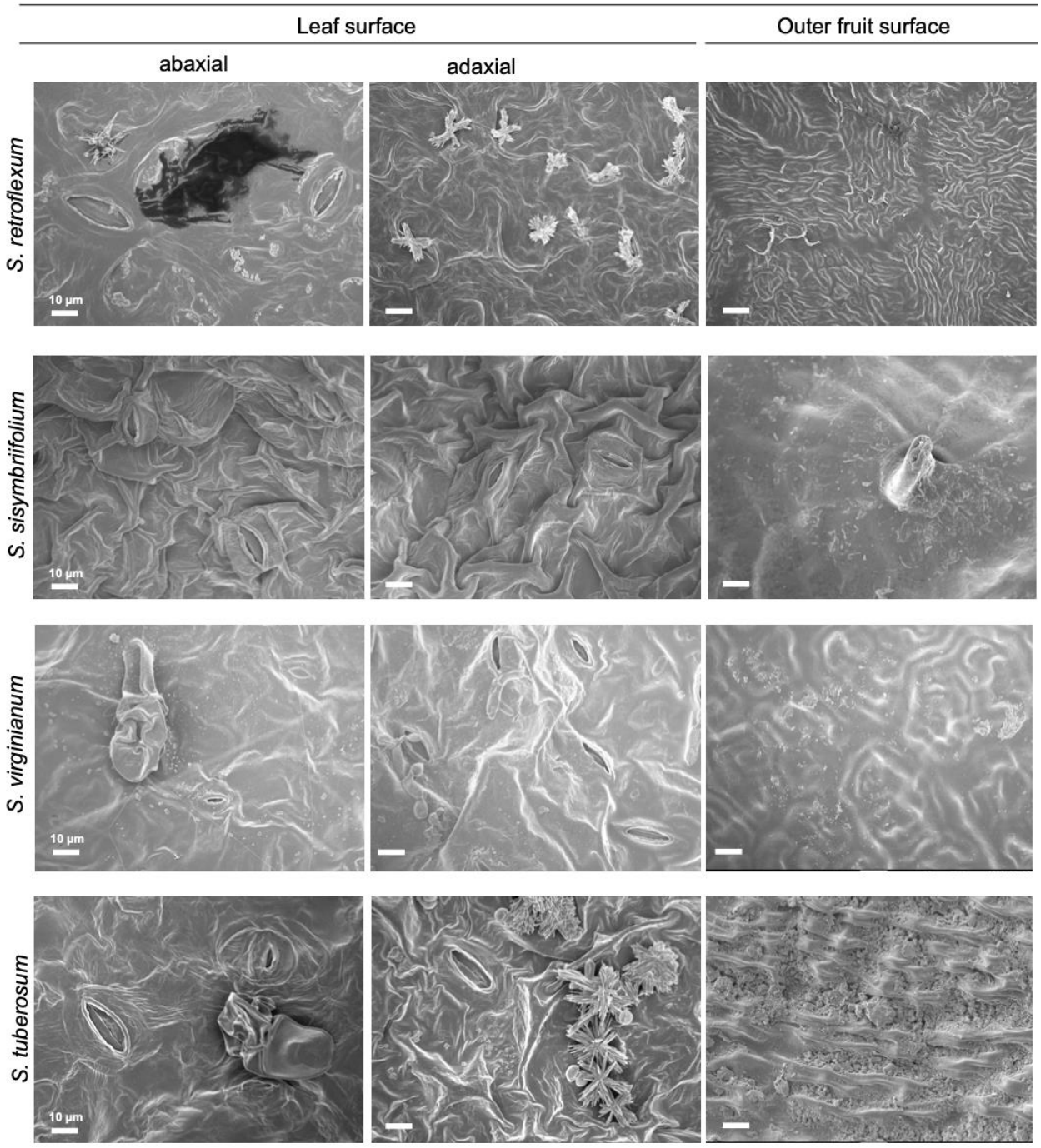
Appendix 18. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



Appendix 19. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



Appendix 20. Scanning electron micrographs from leaf (adaxial and abaxial surfaces) and fruit cuticles (outer side) of solanaceous species, scale bar = 10 µm.



Publications list

Diarte, C., **de Souza, A. X**., Staiger, S., Deininger, A. C., Bueno, A., Burghardt, M., Graell, J., Riederer, M., Lara, I. Leide, J. (2020). Compositional, structural and functional cuticle analysis of *Prunus laurocerasus* L. sheds light on cuticular barrier plasticity. *Plant Physiology and Biochemistry*.

Leide, J.; **de Souza, A. X**.; Papp, I.; Riederer, M. (2018). Specific characteristics of the apple fruit cuticle: Investigation of early and late season cultivars ‘Prima’; and ‘Florina’; (*Malus domestica* Borkh.). *Scientia Horticulturae*., v.229, 137-147.

Manuscripts in preparation

de Souza, A. X; Bueno, A.; Leide, J.; Riederer, M. Chemical and functional analyses of the plant cuticle in Solanaceae species bearing inflated calyx. ***in preparation***

de Souza, A. X; Leide, J.; Riederer, M. Comparative analysis of the cuticular transpiration barrier of wild and cultivated *Solanum* species. ***in preparation***

Affidavit

I hereby confirm that my thesis entitled “Ecophysiological adaptations of the cuticular water permeability within the Solanaceae family” 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 “Ecophysiological adaptations of the cuticular water permeability within the Solanaceae family“ eigenständig, d.h. insbesondere selbstständig 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.

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