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Foliar water and solute absorption: an update

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Running title: Foliar water and solute absorption

SUMMARY

The absorption of water and solutes by plant leaves has been recognised since more than two centuries. Given the polar nature of water and solutes, the mechanisms of foliar uptake have been proposed to be similar for water and electrolytes, including nutrient solutions. Research efforts since the XIX Century focussed on characterising the properties of cuticles and applying foliar sprays to crop plants as tool for improving crop nutrition. This was accompanied by the development of hundreds of studies aimed at characterising the chemical and structural nature of plant cuticles from different species and the mechanisms of cuticular and, to a lower extent, stomatal penetration of water and solutes. The processes involved are complex and will be affected by multiple environmental, physico-chemical and physiological factors which are only partially clear to date. During the last decades, there is growing evidence that water transport across leaf surfaces of native species may contribute to water balances (absorption and loss) at an ecosystem level. Given the potential importance of foliar water absorption for many plant species and ecosystems as shown in recent studies, the aim of this review is to first integrate current knowledge on plant surface composition, structure, wettability and physico-chemical interactions with surface-deposited matter. The different mechanisms of foliar absorption of water and electrolytes and experimental procedures for tracing the uptake process are discussed before posing several outstanding questions which should be tackled in future studies.

Box 1. Summary

- Plant leaves from several species can absorb water and solutes as demonstrated in hundreds of agronomic and ecophysiological studies
- Plant surfaces have been found to be chemically and structurally heterogeneous and this affects wetting and foliar absorption of water and solutes
- Recent foliar water absorption studies performed in different areas of the world, provide evidence for the widespread occurrence of this phenomenon which can contribute to plant water economy
- The main foliar water and solute absorption pathways can be related to stomata, the cuticle, trichomes, veins and other epidermal structures but the mechanism are still not fully characterised
- The process of foliar absorption of water and chiefly solutes has been extensively examined since more than one century and experimental methods are prone to artefacts and misinterpretation

INTRODUCTION

Plant surfaces have a key role in protection against biotic and abiotic stress factors such as water loss (Schuster *et al*., 2017; Kane *et al*., 2020), excess UV radiation (Krauss *et al*., 1997; Liakoura *et al*., 1999) or insect and pathogen attack (Gorb and Gorb, 2017; Ziv *et al*., 2018). The surface of organs with primary growth is covered with an epidermis which may contain modified cell structures such as stomata or trichomes (Javelle *et al.,* 2011). The outermost surface of epidermal cells is meant to be covered by a lipid-rich cell wall part named cuticle (Riederer and Müller, 2006). Aerial organs such as leaves (Tanaka *et al*., 2004; Kosma *et al*., 2010), fruits (Buda *et al*., 2009; Veličković *et al*., 2014), stems (Shumborski *et al*., 2016), flowers (Mazurek *et al*., 2017; Somaratne *et al*., 2017) or developing organs (Ingram and Nawrath, 2017) are covered with a cuticle, as shown in Figure 1. The cuticle is generally located at the external, periclinal, epidermal cell wall, but can also be found in anticlinal walls (Javelle *et al*., 2011), stomatal chambers (Wullschleger and Oosterhuis, 1989) and guard cell walls as shown in Figure 1 c to f.

The structure and composition of plant surfaces, together with the capacity of leaves to absorb water and solutes, have been a matter of scientific interest for more than two centuries (Kannan and Chamemel, 1986; Fernández and Eichert, 2009; Fernández *et al.*, 2016). On the other hand, several studies evaluated leaf cuticular transpiration (e.g., Kerstiens, 2006; Schuster *et al.*, 2017) and nutrient leaching (e.g., Tukey, 1970; Sohrt et al., 2019). While absorption, transpiration and leaching of water and/or solutes are associated with transport phenomena across plant surfaces, their potential relationship is still unknown, and has not been considered in a holistic way likely due to experimental constraints. It can be reckoned that the bidirectional transport of water and solutes in the cuticle may follow a similar pathway, but for modelling such process it will be necessary to gain a better understanding of cuticle structure and composition, also regarding the surface of veins, bundle sheath extensions or trichomes.

In recent years, the contribution of foliar water uptake to water economy in native species of different world ecosystems is becoming a topic of raising interest for plant physiologists and ecologists (e.g., Munné-Bosch, 2010; Dawson and Goldsmith, 2018; Guzmán-Delgado *et al.*, 2018, 2020; Berry *et al.*, 2019; Holanda *et al.*, 2019; Schreel and Steppe, 2020). When critically reading most of the existing foliar fertilization studies, it it is possible to recognise the great response variability, limited trial reproducibility and lack of detail on key factors affecting foliar absorption processes, such as the prevailing environmental conditions during trial development (Fernández and Eichert, 2009). Foliar fertilisers are increasingly used worldwide, but their efficacy may vary because of many constraints related to the complex physico-chemical, physiological and environmental factors affecting the rate of foliar absorption of solutions (Fernández and Eichert, 2009). Hundreds of foliar permeability studies were carried out since more than one century but they still not enable the optimisation of foliar treatments due to major knowledge gaps. The situation is well reflected with the popular "spray and pray" saying which may be extrapolated to the overall foliar water absorption scenario as: "wait for fog, dew or rain and pray". In the case of foliar water absorption experiments, the lack of awareness on plant surface composition and structure is particularly astonishing, together with basically ignoring the exiting foliar penetration literature. The mechanisms of foliar absorption of different species have been evaluated following various experimental approaches in many studies carried out since more than one century. This review is

hence focussed on providing an overview of the state-of-the-art on plant surface structure, chemical composition, wettability and permeability to water and solutes, considering methodological approaches and future perspectives. A broader approach considering the potential relationship between foliar water absorption and transpiration has been avoided, because it would require a great degree of speculation due the lack of integrative studies, but this is however an important aspect which should be born in mind for future foliar water and solute transport investigations.

THE CUTICLE AS OUTERMOST STRUCTURE COVERING AERIAL PLANT ORGANS

In general, all aerial plant surfaces such as leaves, stems, flowers or fruits with primary growth are covered with a cuticle (Jeffree, 2006; see Figure 1 as an example) which serves many protecting roles against biotic and abiotic stress factors as described above. Interestingly, the occurrence of a cuticle covering the cap of *Arabidopsis thaliana* roots has been recently shown (Berhin *et al*., 2019) which suggests that the cuticle is not only be present in aerial plant organs. The barrier properties of the cuticle against stress factors will be linked to its structure and chemical composition at the micro- and nano-scale level, but ii is difficult to establish a clear association between these factors due to multiple experimental constraints.

A main feature of the cuticle is that it is generally rich in lipids, such as waxes, cutin and/or cutan polymers, and may also contain minor phenolic and mineral element amounts (Guzmán-Delgado *et al*., 2016; Segado *et al*., 2016; Lara *et al*., 2019; Philippe *et al.,* 2020a). Waxes may be present on the cuticle surface (epicuticular) or embedded in it (intra-cuticular; Domínguez *et al.,* 2011). However, there is controversy on the contribution of epi- versus intra-cuticular waxes as main barrier for preventing transpiration losses (Jetter and Riederer, 2016; Zeisler-Diehl *et al*., 2018; Zhang *et al*., 2020), and it could be reckoned that this may vary e.g., depending on species, organs or environmental conditions. Cutin is a polyester formed by C_{16} and/or C_{18} hydroxi-fatty acids formed in epidermal cells (Yeats and Rose, 2013; Philippe *et al*., 2020b; Segado *et al*., 2020) abundantly found in the cuticle of aerial organs of many plant species. However, an alternative insoluble and non-saponifiable compound named cutan has been found to occur in the leaf and fruit cuticle of various species (e.g., Schmidt Schönherr, 1982; Villena *et al.,* 1999; Johnson *et al.* 2007; Guzmán-Delgado *et al.,* 2016)

which has been recently classified according to its degradability after gradual chemical treatment (Leide *et al*., 2020). An important role of phenolics in cuticle structure, biomechanics and function has been described in several studies (Karabourniotis and Liakopoulos, 2006; Domínguez *et al*., 2009). Based on von Mohĺ's (1842, 1847) hypotheses, the cuticle has been traditionally understood as a lipid-rich layer which is independent from the epidermal cell wall underneath. However, recent studies showed the presence of cell wall polysaccharides in the leaf cuticle of several species (Guzmán *et al*., 2014a,b; Hama *et al*., 2017, 2019) and also in tomato fruit cuticles (Karabourniotis and Liakopoulos, 2006; Segado *et al*., 2016, 2020; Phiilippe *et al*., 2020a). The cuticle may be hence interpreted as a specialised part of the primary cell wall, somehow analogous to a lignified secondary or a suberized cell wall (Niklas *et al*., 2017).

The relationship between cuticular structure and chemical composition is unclear to date and its analysis is not easy to approach. Experimental difficulties for assessing this relationship stem from the fact that the cuticle is a composite membrane made of compounds with hydrophilic (i.e., chiefly polysaccharides) and hydrophobic (mainly waxes and cutin) components and functional groups which are heterogeneously arranged also at the nano-scale level (Fernández *et al*., 2016). The fine structure of cuticle cross-sections observed by transmission electron microscopy (TEM) has been analysed in various studies which attempted to gain insight into the link between chemical composition and structure (e.g., Wattendorff and Holloway, 1980, 1982; Mérida *et al*., 1981; Krüger *et al*., 1996; Guzmán *et al*., 2014a,b). Proper observation of this part of the epidermal cell wall is not simple, and TEM tissue preparation processes like fixation or staining may influence the degree of contrast and occurrence or electron lucent or dense areas in samples, and also the risk of artefacts (Krüger *et al.,* 1996). An example of cuticle cross-sections of different organs and epidermal structures is provided in Figure 1, where a *Quercus Ilex* leaf trichome (a) a rose petal (b), the guard cells and stomatal pores of leaves of *Cucumis sativus* and *Ulmus minor* (c-f). are shown in TEM micrographs prepared as described by Guzmán *et al*. (2014a). Observation of the thin cuticle covering the trichome, the rose petal or guard cells (Figure 1) requires higher magnification for examining its fine structure. Chiefly in the cuticle of the holm-oak trichome and flower petal, a reticulate pattern can be observed, with the presence of polysaccharides as electron-dense areas and pectin as a dark continuous zone often

visible underneath the cuticle (see the trichome in Figure 1a, as an example). Recently, Schreel *et al.* (2020) analysed the structure of *Fagus sylvatica* leaf trichomes in relation to their capacity to absorb water. In a low magnification TEM micrograph of a thin-section having folds which are artefacts looking like dark pores, trichome surfaces were surprisingly interpreted to by covered with pectin as the outermost cuticular layer, a substance known to form hydrogels (Zwieniecki *et al.*, 2001). Pectin is an important primary cell wall and middle lamella constituent (Bidhendi *et al.*, 2020) which has also been detected in plant cuticles (Guzmán *et al.*, 2014b; Segado *et al.*, 2016). However, Schreel *et al.* (2020) suggested the occurrence of pectin as outermost cuticle chemical constituent and this will have to be verified in future plant surface characterisation studies. When analysing the composition of cuticular waxes of trichomes compared to epidermal pavement cells of *Arabidopsis* leaves and stems, Hegebarth *et al.* (2016) determined variations in wax chemical composition and chain length distribution. In Figure 1a, an irregular deposition of lipids (electron translucent areas with grey to white colours in TEM micrographs) in the cuticle covering a holm-oak trichome is observed providing evidence for the chemical and structural heterogeneity of this epidermal cell wall part.

PLANT SURFACE COMPOSITION AND STRUCTURE AFFECTS WETTABILITY AND PERMEABILITY

Plant surfaces have been found to have a major degree of topographical heterogeneity as potentially provided by micro-scale roughness (e.g., presence of trichomes, papillae or encrypted stomata) and/or nano-scale roughness associated with epicuticular waxes or cuticular folds (Koch *et al.*, 2008; Koch and Barthlott, 2009; Barthlott *et al.*, 2017). While plant surface roughness has been assessed in few studies (e.g., Chowdhury *et al.*, 2005; Bediaf *et al.*, 2015), the distribution of chemical compounds in cuticle surfaces is unknown and both factors will affect contact phenomena with e.g., surface deposited water, aerosol particles or microorganisms (Fernández and Khayet, 2015). Interest on leaf wettability as affected with epicuticular wax composition and structure or the addition of surface-active agents (surfactants) dates back to the 1940s (Ebeling, 1939; Fogg, 1947; Holloway, 1969a,b; Rentschler, 1971). Structural observations of leaves of

highly water repellent *Nelumbo nucifera* and other species (Barthlott and Neinhuis, 1997; Neinhuis and Barthlott, 1997; Barthlott *et al*., 1998), triggered the development of wettability studies focussing on biomimetics (Barthlott et al., 2017).

Water or aqueous solutions deposited as e.g., rain, foliar sprays, fog or dew will interact with the surface of leaves, leading to potentially high or low contact angles, in addition to drop adherence or repellence (Fernández *et al*., 2017), as summarised in Figure 2. Provided that there is adherence of liquid drops to the surface of leaves, the resulting contact angles will be due to the combination of surface chemistry and structure, as noted above. Lower contact angles of water or agrochemical sprays will increase the area of contact between the liquid and the leaf surface and potentially favour the process of foliar absorption, as shown for *Dracaena draco* leaves (Jura-Morawiec and Marcinkiewicz, 2020). On the contrary, surfaces having high contact angles and even drop repellence like the adaxial leaf side of wheat (Fernández *et al.,* 2014b) or *Nelumbo nucifera* (Barthlott and Neinhuis, 1997) will have no chance for foliar penetration to occur, unless a surfactant for lowering the surface tension of water is added to the formulation (Fernández and Eichert, 2009). This implies that wettability (i.e., measured as the contact angle of drops of a liquid with a solid surface) is a prerequisite for foliar penetration to take place (Figure 2). Two water condensation mechanisms may be expected to occur in leaves in response to dew or fog exposure, namely, film-wise condensation or drop-wise condensation (Fernández *et al*., 2014a). Drop condensation mechanisms will also depend on leaf surface chemical composition and roughness, low surface free energy (hydrophobic) materials forming discrete liquid droplets (Miljkovic and Wang, 2013), especially on highly unwettable surfaces with low contact angle hysteresis (Ahlers *et al*., 2019; Cha *et al*., 2020). Thereby, water condensation of dew or fog may be expected to occur as films in rather wettable surfaces and hydrophilic areas or as drops in unwettable leaf surfaces (Figure 2). Hence, leaf water condensation can contribute to fog harvesting and water delivery to the roots (Ebner *et al.*, 2011; Konrad *et al*., 2015, Rosado and Holder, 2013).

The importance of leaf wetting has also been considered in few ecophysiological studies that assessed how foliar deposition of fog (Hanba *et al.,* 2004; Yokoyama *et al*., 2019) or sprinkler irrigation (Urrego-Pereira *et al.*, 2013) affected the rate of photosynthesis and transpiration, as recently discussed by Binks et al. (2020). The

process of stomatal uptake is however complex and heterogeneous (Eichert *et al*., 2008), as described below. In summary, while adequate wetting preliminary favours the process of absorption as described in Figure 2, it does not ensure that water and electrolyte solutions may cross the plant epidermis, as discussed in the following paragraphs.

FOLIAR ABSORPTION PATHWAYS FOR WATER AND SOLUTES

For approaching the transport of water and solutes across plant surfaces, the importance of thermodynamic aspects related to the affinity or not between cuticle /cell wall constituents and diffusing substances such as water or electrolytes, should be preliminary considered. While all molecules will be subjected to van der Waals (dispersive or apolar) forces, water and solutes which have significant non-dispersive (including polar) and hydrogen (H)-bonding interactions, will have affinity for cell wall polysaccharides and no or limited affinity for lipids present in the cuticle (Khayet and Fernández, 2012). This will be taken into account when discussing about foliar water and solute uptake mechanisms in the sections below. Nonetheless, additional factors such as cuticle and/or cell wall porosity, nano-structure or length of the diffusion pathway will affect transport phenomena across plant surfaces, but these aspects are difficult to characterise experimentally and remain unclear so far.

The absorption and subsequent utilisation and/or accumulation of atmospheric water by aerial plant organs (chiefly leaves) have been reported specially for xerophytes, halophytes and species subjected to temporary drought (Stone *et al*., 1950; Munné-Bosch *et al*., 1999; Limm *et al*., 2009; Eller *et al*., 2013; Wang *et al*., 2016; Jura-Morawiec and Marcinkiewicz, 2020). However, few more specific investigations evaluated the contribution of various foliar epidermal structures to the uptake of surface-deposited water, such as hydatodes (Martin and von Willert, 2000), scales (Wang *et al*., 2016) but chiefly trichomes (Grammatikopoulos and Manetas, 1994; Papini *et al.,* 2010; Fernández *et al*., 2014a; Pina *et al.,* 2016; Li *et al.*, 2018a,b; Li et *al.*, 2019, Schreel *et al*., 2020). For example, Li *et al.* (2018a,b; 2019) evaluated the absorption of foliar-applied zinc (Zn, with no surfactant)) provided as Zn-sulphate and Zn-nanoparticles, by synchrotron-based X-ray fluorescence microscopy and nanoscale secondary ion mass spectrometry. They observed an accumulation of foliar-applied Zn in some glandular trichomes of

soybean (*Glycine max*), but not in the 7 different types of glandular and non-glandular trichomes found in tomato (*Solanum lycopersicum*) leaves (Li *et al.,* 2018a). In the case of Zn foliar application to sunflower (*Helianthus annuus*), Zn absorption was chiefly associated with non-glandular trichomes which often occurred in bundle sheath extensions that further enabled Zn transport to the vascular bundles (Li *et al*., 2019). Recently, Schreel *et al.* (2020) gained evidence for the absorption of water and solutes by beech (*Fagus sylvatica*) leaf vein trichomes using Synchrotron-based micro-tomography. When analysing the anatomical features of leaves of 12 xeromorphic species and 5 mesomorphic species, Fahn (1986) observed that the base of the trichomes occurring in xeromorphic species was stained with Sudan IV, indicating that cell walls were cutinised. By contrast, trichomes of leaves of the mesic species analysed appeared to have a primary cell wall which may enable water movement out of the trichomes, unlike the apoplastic barrier occurring in cutinised cell walls of xeromorphic trichomes (Fahn, 1986). Similarly, Fernández *et al*. (2011) observed that the base of peach fruit trichomes was cutinised, such highly pubescent fruit surface being highly unwettable by water drops. The occurrence of extremely hydrophilic (e.g., Benz and Martin, 2006; Grammatikopoulos and Manetas, 1994; Schreel *et al*., 2020) or hydrophobic (Fernández *et al*., 2011) trichomes has been shown in few plant species and organs. Kim et al (2017) discussed about the importance of water absorption and wettability of trichomes and trichome clusters for cacti survival. By carrying out leaf wettability measurements and trichome structure observations, they concluded that trichomes and trichome clusters can contribute to fog and dew collection, with the subsequent absorption of water prior to evaporation. Future studies should hence analyse the structure and composition of trichomes and trichome surfaces at various scales, and their influence on water and solute transport, surface wettability and water adherence or repellence, as evaluated by some authors (Brewer *et al.,* 1991; Smith and McClean 1989; Fernández *et al*., 2014a, 2017). For example, a different degree of wettability and potential water and solute transport capacity may be expected for glandular versus non-glandular trichomes, and dead compared to alive trichome cells (Karabourniotis *et al*., 2020)

On the other hand, the surface of veins and minor venations like bundle sheath extensions, may be chemically and structurally different to other leaf lamina areas,

having also different rates of wettability and bidirectional transport of water and solutes. To assess this hypothesis and using beech as model species, Bahamonde *et al.* (2018) estimated the wettability, surface free energy and permeability to 150 mM calcium (Ca) chloride of vein versus green lamina areas, by depositing 3 µl drops with a micro-syringe. Veins of beech and *Quercus petraea* leaves were found to have lower contact angles with water, and evidence for foliar Ca absorption was only gained after the deposition of Ca-chloride drops on to the veins of beech. This suggests that beech leaf veins are chemically and structurally different to the rest of the leaf lamina, and that this tissue is more permeable to water and solutes that other epidermal zones. This may also occur with bundle sheath extensions of heterobaric leaves which can enable the transport of water and solutes (Wylie, 1943, 1952), and contribute to water economy (Nikolopoulos *et al.*, 2002). The transport of foliar-applied Zn in sunflower after trichome absorption was observed via bundle sheath extensions (Li *et al.*, 2019), and trichomes often occur in this minor leaf venation of some species (e.g., in *Quercus ilex*; Fernández *et al.*, 2014a).

Cuticular absorption pathways for water and solutes

As discussed in the previous section, aerial plant surfaces of organs with primary growth are meant to be covered with a cuticle, and recent studies showed the presence of cell wall polysaccharides as major chemical constituents (Guzmán *et al.*, 2014a, Segado *et al.*, 2016; Hama *et al.*, 2017; Phiilippe *et al.*, 2020a). The cuticle from different species, organs or developmental stages may have different degrees of lipidisation in qualitative and quantitative terms (Fernández *et al.*, 2016), as observed in Figure 1. Before considering the permeability of the plant cuticle to water and solutes, it must be highlighted its cell wall nature as noted before. The primary cell wall is structurally formed by cellulose fibrils embedded in a hydrated matrix of pectin, hemicellulose and proteins (Cosgrove, 2016). Considering the cuticle as a modified cell wall (Niklas *et al.*, 2017), potentially variable amounts of lipids (which are chiefly apolar) and polysaccharides (which provide non-dispersive/ polar and H-bonding interactions) may be found e.g., in different species, developmental stages organs, or epidermal structures (Fernández *et al.*, 2016). The permeability of the cell wall or the cuticle to water and solutes is the product of their solubility, which is a thermodynamic parameter reflecting

the interactions between the diffusing compound/s and the cuticle/cell wall, and their diffusivity through the cuticle/cell wall matrix. Diffusivity is a kinetic parameter associated with the molecular size of the compound and the structure of the matrix (Fernández *et al.*, 2016, Khayet and Fernández, 2012). To date the structure and chemical heterogeneity at the nanoscale is poorly understood and this hinders the development of models for the proper prediction of cuticular permeability. However, the cuticle can be considered a dense membrane (Fernández *et al.*, 2016) which excludes the occurrence of micro-pores or continuous pores, as hypothesized by some researchers (Schönherr, 1976, 2006; Riederer, 2006; Tredenick et al., 2017). Chiefly based on trials carried out with isolated cuticles from few plant species, it has been suggested that polar substances and water penetrate via different mechanisms to those of rather apolar compounds, such as many herbicides, insecticides or fungicides (Schreiber, 2006; Schreiber and Schönherr, 2009), an idea which was however challenged by Fernández and Eichert (2009).

The process of diffusion of water and solutes in the cuticle is still not fully characterized and has been related to the occurrence of "aqueous pores" (Schönherr, 2006). The existence of these cuticular pores has never been microscopically observed and has been questioned by various authors (e.g., Aponte and Baur, 2014; Fernández *et al.*, 2016; Riederer, 2006). An alternative hypothesis could be that water and solute cuticular transport may be associated with polar functional groups of cuticular chemical constituents (chiefly hydrophilic polysaccharides; Chamel *et al.*, 1991; Fernández *et al.*, 2016; Reina *et al.*, 2001; Riederer, 2006). Several studies showed that water sorption to polar functional groups increase the volume of the cuticle leading to swelling, and that this may be associated with transport mechanisms (Arand *et al.*, 2010; Chamel *et al.*, 1991; Luque *et al.*, 1995; Riederer, 2006). The processes of cuticle swelling or shrinkage may be influenced by environmental variables, such as relative humidity (RH) and temperature (Figure 3). Another factor which may affect the process of transport of water and solutes across the cell wall or the cuticle, is solution pH, as recently shown by Aponte and Baur (2018). Increased water permeability and cation exchange capacity above pH 3 (the isoelectric point of cuticles described by Schönherr and Hubert (1977), may enable the attraction of water molecules, with formation of hydration shells which may ultimately lead to cuticular transport (Aponte and Baur, 2018). It must be however

noted that the existing cuticular water sorption studies have been exclusively developed with cellulase- and pectinase-isolated cuticles which may lead to artefacts and misleading interpretations (Fernández and Eichert, 2009; Fernández *et al*., 2016). Hence, it can be probably expected that the state of hydration of the cuticle of intact organs may have been seriously underestimated to date. The major influence of cuticle hydration on water and solute transport across the cuticle following a tortuous pathway, has been referred to as a "dynamic aqueous continuum" (Beyer *et al*., 2005; or "dynamic polar continuum" (Fernández *et al*., 2017). An important feature of cuticle transport of chiefly apolar (lipophilic) or rather polar (hydrophilic) compounds is size selectivity (Aponte and Baur, 2014). Molecular size constraints for diffusion may be linked to molecular spacing and thermodynamic interactions between cuticular constituents and the diffusing chemicals. For the leaf and fruit cuticle of few species, size limits of 0.3 to 4.8 nm diameter have been indirectly estimated as diffusion threshold, for hydrophilic (e.g., water or nutrient sources) or rather hydrophobic (e.g., herbicides or fungicides) substances (Beyer *et al*., 2005; Eichert and Goldbach, 2008; Luque *et al*., 1995; Popp *et al*., 2005; Schönherr, 1976). However, the cuticle of some epidermal surface areas may be chemically and structurally irregular (as observed in trichomes and trichome scars of the *Quercus ilex* adaxial leaf sides, Fernández *et al*., 2014a), favouring the penetration of water and solutes as shown to occur in underground hypocotyl tissues of mung bean (*Vigna radiata*; Aponte and Baur, 2014). The hypocotyl underground surface was more hydrophilic and permeable to water and solutes than a cuticle, being also less size-limiting and maybe closer to a primary cell wall. The limiting size threshold for the diffusion of molecules was found to be 1.5 nm (Aponte and Baur, 2014) which is within the range reported for plant cuticles, as described above.

Transport of water and solutes through stomata

For many decades, the role of stomata in the processes of foliar water and solute uptake had been a subject of controversial debate. Initially, it was assumed that solutions may enter stomata spontaneously by infiltration, i.e. by mass flow through open stomata. This view was supported by a multitude of studies indicating that the presence, density or degree of aperture of stomatal pores affected penetration rates of foliar-applied substances (e.g. Schönherr and Bukovac, 1978; Eichert *et al*., 1998; Eichert and

Goldbach, 2008, Burkhardt *et al*., 2012). The possibility of spontaneous infiltration of leaves by aqueous solutions, however, would imply that leaves bearing stomata on their adaxial (i.e., upper) surface should be regularly infiltrated by water during precipitation events. From an ecophysiological viewpoint, it is clear that infiltration of the leaf mesophyll by precipitation events must be prevented, because this would limit photosynthesis due to the restricted diffusion of CO_2 in water as compared to air (Brewer *et al*., 1991). The argumentation against spontaneous infiltration of stomata was substantiated by Schönherr and Bukovac (1972), who hypothesised that the specific architecture of stomata prevents capillary infiltration of aqueous solutions. They (Schönherr and Bukovac 1972) emphasised that stomatal infiltration of foliar-applied solutions may only occur after exerting external pressure, or by adding to the solution certain kind of surfactants (Field and Bishop, 1988; Zabkiewicz *et al*., 1993)

Meanwhile, it became evident that the apparent contradiction between theory ("infiltration of stomata by mass flow is impossible") and experimental evidence ("foliar uptake of solutes is promoted by the presence, density or degree of aperture of stomata") was caused by the misconception of the underlying physical mechanisms of stomatal penetration. While solute uptake by infiltration of solutions assumes that solutes penetrate stomata together with the (aqueous) solvent, solute transport by diffusion may take place independently of the solvent. It was shown that solutes and even small nano-particles most probably penetrated the stomatal pore by diffusion along the surface of guard cells (Eichert *et al*., 2008; Eichert and Goldbach, 2008). It was demonstrated that this stomatal penetration pathway may enable much faster penetration rates of ionic solutes as compared to cuticular penetration (Eichert *et al*., 2008), and even be the exclusive pathway for certain substances, such as nano- particles (Eichert and Goldbach, 2008).

Diffusion of water-soluble solutes requires the existence of an aqueous diffusion medium. The diffusion of foliar-applied solutes along the surface of guard cells thus indicates the presence of liquid water lining the cell surface. It is known that the surface of guard cells is, like ordinary epidermal cells, covered by a cuticle (Wullschleger and Oosterhuis, 1989) which in combination with surface roughness (see Figures 1c and d, for a smooth versus a rough guard cell surface), may prevent the presence of substantial amounts of liquid water in the stomatal pore (see Figure 3). However, it was shown that

not all stomata contribute to the uptake of foliar-applied solutes (Eichert and Burkhardt, 2001; Eichert *et al.,* 2008; Eichert and Goldbach, 2008). Therefore, it was concluded that external processes may increase the wettability of the guard cell surface of individual stomata, "activating" them for solute transport (Eichert and Burkhardt, 2001; Eichert *et al.*, 2008; Fernández and Eichert, 2009; Burkhardt *et al.*,2012). Different processes were proposed to be involved in the activation of individual stomata for the diffusive exchange of matter across leaf surfaces, namely: presence of bacteria (Eichert *et al.*, 2008) or fungal hyphae (Burgess and Dawson, 2004), epistomatal mucilage (Westhoff *et al.*, 2009) and hygroscopic particles (Burkhardt *et al.*, 2012; Basi *et al.*, 2014).

The occurrence of water films onto the surface of guard cells may not only enable the exchange of solutes, but also of liquid water between the leaf surface and the mesophyll. This process may account for the uptake of water by the foliage, as described in recent ecophysiological studies (see above). Water movement into the leaves requires that the gradient of water potential is directed into the leaf interior, i.e., that the atmosphere is (almost) saturated with water vapour and/or that, due to precipitation or spray treatment, water drops are temporarily present onto the leaf surface. The pathways of foliar water uptake under such conditions are still not understood, and recently it was speculated that leaves may absorb water by "reverse transpiration", i.e., by diffusion of water vapour through stomata into the leaf interior (Vesala *et al.*, 2017, Binks *et al.*, 2019; Guzmán-Delgado *et al.*, 2020). On the other hand, movement of liquid water as water films present in stomatal pores will probably also result in substantial uptake rates. Moreover, contrary to reverse transpiration, this mechanism will also be available when the atmosphere is under-saturated with water, as long as liquid drops are present onto the leaves. This is a typical scenario during precipitation events and after foliar spraying with fertilisers or water-based agrochemical solutions or emulsions. In this situation, the retained drops are "over-saturated" with water and hence evaporation will start. However, as long as the evaporation process continues, water may enter the leaves in liquid form by diffusion either in stomatal water films or through the cuticle.

TECHNIQUES FOR TRACING WATER AND SOLUTE FOLIAR ABSORPTION

In the last decades, several approaches have been developed with the aim of characterising the pathways of foliar uptake of water and solutes. For example, a method for assessing foliar water uptake based on immersing leaves (with the petiole sealed with paraffin) in water for 60 minutes, and measuring water potentials before and after immersion, has been used in some studies (e.g., Goldsmith *et al.*, 2013; Gotsch *et al.*, 2015). This procedure has various potential drawbacks because leaves will never be naturally exposed to an aqueous environment. Processes ultimately leading to uncontrolled water uptake by immersed leaves, such as stomatal opening, leaching of mineral elements and soluble compounds (Guzmán-Delgado *et al.*, 2016), or cuticle component alterations (Kitamura, 2011) may occur, leading to artefacts and misleading ecological conclusions. Besides studying water uptake on a quantitative basis, the identification of pathways for foliar water uptake has been the main focus of many studies. A broad range of methods and experimental setups have been deployed and adapted to changing concepts and hypotheses. Generally, the identification of the exact location of entry points of substances into leaves requires the visualisation of the process. Therefore, different imaging methods have been utilised, most of them based on radiolabelled or fluorescent tracers or the precipitation and visualisation of insoluble salts within the penetration route.

Tracing foliar uptake: challenges and drawbacks

There are three main challenges in the development and application of a suitable methodology for the purpose of tracking mechanisms of foliar uptake (see also Fernández and Eichert, 2009): Firstly, for the unequivocal identification of penetration pathways, the studied substance must be "caught in the act", i.e., while still in the process of movement within the leaf surface. Secondly, because many imaging techniques require the use of easily detectable tracers as substitutes for target substances, such as fertilisers or agrochemicals, it has to be ensured that the physico-chemical properties of the selected tracers match those of the substances under consideration. Thirdly, it has to be made sure that the employed detection method itself does not change the nature of the leaf surface and the penetration pathways.

When evaluating the existing body of literature on the subject of penetration pathways, it becomes clear that most of the published studies did not fully take into account the abovementioned requirements. The first prerequisite, the detection of the substance directly in its uptake route, is particularly difficult to be fulfilled. This is mainly caused by technical limitations due to the low spatial resolution of visualisation procedures. This applies to auto-radiographic methods, which have been frequently used in the 1950s and 1960s (e.g. Barrier and Loomis, 1957; Franke 1964). Furthermore, these methods required complex sample preparation steps and usually long exposure times. Like all optical microscopy methods, conventional fluorescence microscopy has a limited resolution of 0.5 µm, hence hampering the exact localisation of tracers. The problem is furthermore aggravated by the fact that fluorescent tracers tend to outshine their surroundings, making the exact localisation of the tracer very difficult (Fernández and Eichert, 2009).

The second prerequisite is of exceeding relevance in studies based on fluorescent tracers. These compounds are quite large organic molecules, many of them, such as fluorescein, bear acidic groups and thus pH-dependent charges (Martin and Lindqvist, 1975). As a consequence, these molecules may be present in different chemical forms at the same time and change their speciation depending on environmental conditions. Due to their relatively high molecular mass and molar volume, their suitability as proxies for small ions such as metal cations, may be limited because the diffusion of larger molecules is more strongly affected by the size-limiting constraints of the penetration routes in the cuticle than smaller compounds (Schönherr and Schreiber, 2004). This is of particular relevance for the visualisation of pathways for foliar water uptake (see section below).

The third prerequisite, i.e., that the detection method itself must not affect the penetration pathways, is an important drawback of many experimental approaches. In many studies foliar uptake was studied with isolated cuticles which were mounted in diffusion chambers for evaluating the rate of solute penetration (Schönherr, 2006). Apart from the fact that only few species can be used for leaf cuticle isolation and diffusion experiments, this approach is additionally restricted to leaf surfaces without stomata. Hence, these studies neglect the possible contribution of the stomatal uptake pathway. Furthermore, it is likely that the process of cuticle isolation, which is based on

the enzymatic separation from the epidermal cell wall using cellulase and pectinase, will alter cuticular barrier properties (Fernández and Eichert, 2009). Considering that our view on the nature and composition of the cuticle is currently changing (see above), this risk is becoming even more evident. If the entire cuticle is viewed as a modified cell wall (Fernández *et al.*, 2016), applying enzymes decomposing cell wall components will probably have tremendous effects on the barrier properties of the obtained isolated cuticles.

Another group of methods immanently bearing the risk of artefacts relies on the formation of precipitates as indicators of foliar penetration pathways. In the 1960s a series of studies aimed at visualizing structures which were called "ectodesmata" (e.g., Franke, 1967). The experiments were based on the visual detection of silver (Hg) precipitates (Schönherr and Bukovac, 1970) after external application of $HgCl_2$. The procedure of visualisation was rather complex involving the treatment of leaves with concentrated acids and ethanol, followed by a range of washing steps. Such intensive leaf chemical handling may substantially affect the chemical composition, structure and barrier properties of leaf surfaces which may be significantly altered during treatment. Moreover, it was later shown that the formation of Hg precipitates did not indicate the location of penetration pathways, but only the occurrence of areas in the cuticles inducing the reduction of Hg ions (Schönherr, 2006). A similar method using AgCl precipitates as indicators of penetration pathways was employed for example by Schreiber *et al.* (2006) and more recently by Schreel et al. (2020). Here, Ag^+ ions were applied externally (as $AgNO_3$) and after reaction with Cl^- ions the resulting precipitates were thought to indicate the penetration pathway of Ag^+ ions as proxies for metallic cations. Apart from the risk of Ag toxicity, it is very likely that the precipitation of AgCl rather indicates locations with high native concentrations of Cl^- in the leaf surface, e.g. in the vicinity of stomata or in trichomes, than preferential sites of Ag^+ uptake (Fernández and Eichert, 2009). A general drawback of precipitation methods is the fact that the growth and formation of precipitates within the treated leaf tissues which may affect their structural integrity (Fernández and Eichert, 2009). Initialisation of precipitation may form crystallisation nuclei resulting in attraction and accumulation of large quantities of precipitates. The associated volume increase may finally cause mechanical damage to the leaf structure and confound the results.

The ultimate challenge of tracking water uptake routes

In the past, transport of water out of leaves by stomatal and/or cuticular transpiration has been intensively studied. The opposite direction of water transport, however, i.e., foliar water uptake has been largely neglected for a long time and considered to be important only under certain conditions. Meanwhile, foliar water uptake is receiving more and more attention in ecophysiological research, and is currently considered to be important not only for certain species and arid habitats, but rather a phenomenon of global importance (Berry *et al.*, 2019).

In the literature, there seems to be no clear agreement on the routes of water transport in the cuticle. Whereas some researchers assumed that water is exclusively transported following the very same route(s) of hydrophilic solutes, i.e., in stomata (Burkhardt, 2010) and pathways in the cuticle called "aqueous pores" or similar (Schönherr, 1976), others assumed that water may also diffuse in the cuticle independently of these specific polar pathways (Schreiber, 2005). At first sight, it might appear strange that water could (also) be transported in a route separate from water-soluble compounds. However, considering the different physico-chemical features of both substances, the occurrence of at least partially-separated routes appears likely. The water molecule is small and neutral, whereas water-soluble solutes are much bigger, often charged, and surrounded by a hydration shell consisting of several water molecules. Hence, even small ions, such as metal cations, are much bigger than water molecules due to the water molecules present in their surrounding hydration shell. It is therefore likely that water molecules may indeed access the so-called lipophilic pathway, while larger and/or neutral substances are excluded from this route. Some authors assumed that this lipophilic pathway is the most important (Schreiber et al. 2001) or even the only relevant route (Schönherr, 2000) for water movement in the cuticle. It can be hence hypothesised that regardless of their polar and apolar components (note that all compounds will be subjected to van der Waals interactions as described above), molecules and ions may diffuse in the chemically and structurally heterogeneous cuticle, following a tortuous pathway but these mechanisms should be analysed in future studies.

Bearing this in mind, it becomes evident that attempts to track cuticular water uptake routes by following the penetration routes of ionic or fluorescent tracers (e.g. Schreel *et al*., 2020) are *a priori* foredoomed. Both hydrated metallic ions and fluorescent tracers are much bigger than water molecules, and many of the fluorescent tracers are also ionic, excluding them from pathways available for small, neutral water molecules. Therefore, the routes of tracer uptake across leaf surfaces may not provide any sort of evidence for the concomitant routes of water uptake. Moreover, water uptake by reverse transpiration, i.e. by uptake of water vapour, is an uptake pathway which is exclusively available for water but not for the dissolved tracers.

Tracing foliar uptake: conclusions

Any attempt to elucidate the routes of foliar uptake requires profound knowledge, both about the properties, availability and constraints of the diffusing pathway(s) and of the physico-chemical nature of the permeating substance. It is very important to be aware of the fundamental difference between properties of the solvent, which is usually water, and the solute, which may be more or less apolar or polar, be ionic or neutral, and considerably differ in terms of molecular size. It is of extreme importance to take into account that the degree of polarity of a given compound has to be evaluated on a continuous scale. In this context, any black or white approach is prone to fail. This is important, for example, for some fertilisers such as urea and boric acid, which are both rather small, neutral molecules and may thus penetrate the cuticle to a substantial degree by the same pathway as lipophilic compounds. The same applies to foliar water uptake. In contrast to many of the substances which are supplied as aqueous solutions, water molecules themselves acting as a solvent, are very small and uncharged. This leads to the rather counterintuitive conclusion that water may also have access to the so-called lipophilic penetration pathway, whereas solutes may be excluded from this route.

CONCLUDING REMARKS AND OUTSTANDING QUESTIONS

The absorption of water and solutes by the foliage has been demonstrated in many studies, and recent research efforts point towards its ecological significance for plant ecosystems (e.g., Dawson and Goldsmith, 2018; Schreel and Steppe, 2020). The potential of leaves to absorb chemicals applied in liquid form has been actually exploited

in agriculture since more than one century, and foliar nutrient sprays are commonly used in commercial agriculture fertilisation programs worldwide (Fernández and Eichert, 2009). However, knowledge on the composition and structure of plant surfaces and the mechanism of foliar absorption is still fragmentary and requires a proper understanding of the physico-chemical principles involved as preliminary requisite. Owing to the manifold constraints and great potential for the occurrence of experimental artefacts and misleading interpretations when analysing plant surfaces and their permeability to water and solutes, an array of questions remain open. For coming up with solid permeability models it will be necessary to know the actual nano-scale chemical and structural arrangement of cuticular and cell wall components and how may they vary in different surface structures such as guard cells, trichomes or veins. How may plant surfaces vary in chemical and structural terms during plant ontogeny and how they may be affected by different environmental conditions and stress factors? How are the mechanisms of foliar penetration of water and solutes through intact cuticles, stomata, trichomes, veins, hydatodes, scales or other epidermal structures? Which pathway may be relatively more important, for example, for each species or stage of development? Which new approaches and technologies may be used for assessing plant surface composition, structure barrier properties and permeability to water and solutes? What is the actual significance of foliar water absorption for plant ecosystems worldwide? How do leaf surface micro- and nano- roughness and chemical composition affect the mechanisms of condensation of dew or fog? How do environmental conditions affect plant surface structure, chemical composition and the mechanisms of absorption of water and solutes?

In summary, more knowledge on the physico-chemical properties of plant surfaces is required for assessing the process of foliar absorption of water and solutes, a phenomenon which has been evaluated in many studies but that it is difficult to trace and prone to experimental artefacts.

Box 2. Open Questions

- What is the nano-scale chemical and structural arrangement of cuticular chemical components?
- What is the actual significance of foliar water absorption for plant ecosystems worldwide?
- How are the mechanisms of foliar penetration of water and solutes through cuticles, stomata, trichomes, veins and other epidermal structures of e.g., different species or developmental stages, and which pathway may be relatively more important?
- Which additional technologies may be used for assessing plant surface composition, structure and barrier properties?
- How do environmental conditions affect plant surface structure, chemical composition and the mechanisms of absorption of water and solutes?

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahlers, M., Buck-Emden, A. and Bart, H.J. (2019) Is dropwise condensation feasible? A review on surface modifications for continuous dropwise condensation and a profitability analysis. *J. Adv. Res.* **16**, 1-13.

Aponte, J. and Baur, P. (2014) Transport properties of the mung bean (*Vigna radiata*) non-aerial hypocotyl membrane: permselectivity to hydrophilic compounds, *Pest. Manag. Sci.* **70**, 148-155.

Aponte, J. and Baur, P. (2018) The role of pH for ionic solute uptake by the non-aerial hypocotyl of mung bean plants. J. *Plant Dis. Prot*. **125**, 433-442.

Arand, K., Stock, D., Burghardt, M. and Riederer, M. (2010) pH-dependent permeation of amino acids through isolated ivy cuticles is affected by cuticular water sorption and hydration shell size of the solute, *J. Exp. Bot*., **61**(14), 3865-3873.

Bahamonde, H. A., Gil, L. and Fernández, V. (2018) Surface Properties and permeability to calcium chloride of *Fagus sylvatica* and *Quercus petraea* leaves of different canopy heights. *Front. Plant Sci.* **9,** 494.

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

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I. and Wilhelmi, H. (1998) Classification and terminology of plant epicuticular waxes. *Bot. j. Linn. Soc.* **126**(3), 237-260.

Barthlott, W., Mail, M., Bhushan, B. and Koch, K. (2017) Plant surfaces: structures and functions for biomimetic innovations. *Nano-Micro Letters* **9**(2), 23.

Barrier, G.E. and Loomis, W.E. (1957) Absorption and translocation of 2,4-dichlorophenoxyacetic acid and P[32] by leaves. *Plant Physiol*. **32**, 225–231.

Basi, S., Burkhardt, J., Noga, G. and Hunsche, M. (2014) Hygroscopic salts support the stomatal penetration of glyphosate and influence its biological efficacy. *Weed Biol. Manag.* **14**(3), 186-197.

Bediaf, H., Sabre, R., Journaux, L. and Cointault, F. (2015) Comparison of leaf surface roughness analysis methods by sensitivity to noise analysis. *Biosystems Eng*. **136,** 77-86.

Benz, B.W. and Martin, C.E. (2006) Foliar trichomes, boundary layers, and gas exchange in 12 species of epiphytic *Tillandsia* (Bromeliaceae). *J. Plant Physiol.* **163**, 648–656.

Berhin, A., de Bellis, D., Franke, R.B., Buono, R.A., Nowack, M.K. and Nawrath, C. (2019). The root cap cuticle: A cell wall structure for seedling establishment and lateral root formation. *Cell* **176**(6), 1367-1378.

Berry, Z.C., Emery, N.C., Gotsch, S.G. and Goldsmith, G.R. (2019) Foliar water uptake: processes, pathways, and integration into plant water budgets. *Plant Cell Environ.* **42**(2), 410-423.

Beyer, M., Lau, S. and Knoche, M. (2005) Studies on water transport through the sweet cherry fruit surface: IX. Comparing permeability in water uptake and transpiration. *Planta* **220**, 474–485

Bidhendi, A.J., Chebli, Y. and Geitmann, A. (2020) Fluorescence visualization of cellulose and pectin in the primary plant cell wall. *J. Microscop.* 278(3), 164-181.

Binks, O., Coughlin, I., Mencuccini, M. and Meir, P. (2020) Equivalence of foliar water uptake and stomatal conductance? *Plant Cell Environ*. **43**(2), 524-528.

Burkhardt, J. (2010) Hygroscopic particles on leaves: nutrients or desiccants? *Ecol. Monographs* **80**(3), 369-399.

Burkhardt, J., Basi, S., Pariyar, S. and Hunsche, M. (2012) Stomatal penetration by aqueous solutions–an update involving leaf surface particles. *New Phytol*. **196**(3), 774–787.

Buda, G.J., Isaacson, T., Matas, A.J., Paolillo, D.J. and Rose, J.K. (2009) Three-dimensional imaging of plant cuticle architecture using confocal scanning laser microscopy. *Plant J*. **60**(2), 378-385.

Burgess, S.S.O. and Dawson, T.E. (2004) The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. *Plant Cell Environ.* **27**(8), 1023–1034.

Brewer, C.A., Smith, W.K. and Vogelmann, T.C. (1991) Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant Cell Environ.* **14**, 955–962

Cha, H., Vahabi, H., Wu, A., Chavan, S., Kim, M.K., Sett, S., Bosch S.A., Wang W., Kota A.K. and Miljkovic, N. (2020) Dropwise condensation on solid hydrophilic surfaces. *Science Adv*. 6(2), eaax0746.

Chamel, A., Pineri, M. and Escoubes, M. (1991) Quantitative determination of water sorption by plant cuticles, *Plant Cell Environ*., **14**, 87–95.

Chowdhury, A.B.M.N.U., Jepson, P.C., Ford, M.G. and Frampton, G.K. (2005) The role of cuticular waxes and surface roughness in determining the insecticidal efficacy of deltamethrin and dimethoate applied as emulsifiable concentrates to leaf surfaces. *Internat. J. Pest Manag*. **51**(4), 253-263.

Cosgrove D.J. (2016) Plant cell wall extensibility: connecting plant cell growth with cell wall structure, mechanics, and the action of wall-modifying enzymes. *J. Exp. Bot.* **67**(2), 463-476.

Dawson, T.E., and Goldsmith, G.R. (2018) The value of wet leaves. *New Phytol*. **219**, 1156-1169

Domínguez, E., España, L., López-Casado, G., Cuartero, J. and Heredia, A. (2009) Biomechanics of isolated tomato (*Solanum lycopersicum*) fruit cuticles during ripening: the role of flavonoids. *Funct. Plant Biol.* **36**(7), 613-620.

Domínguez, E., Heredia-Guerrero, J.A. and Heredia, A. (2011) The biophysical design of plant cuticles: an overview. *New Phytol*. *189*(4), 938-949.

Ebeling, W. (1939) The role of surface tension and contact angle in the performance of sprays. *Hilgardia* **12**, 665–698.

Ebner, M., Miranda, T. and Roth-Nebelsick, A. (2011) Efficient fog harvesting by Stipagrostis sabulicola (Namib dune bushman grass). *J. Arid Environ*. **75**(6), 524-531.

Eichert, T., Goldbach, H.E. and Burkhardt, J. (1998) Evidence for the uptake of large anions through stomatal pores. *Bot. Acta* **111**(6), 461–6.

Eichert, T. and Burkhardt, J. (2001) Quantification of stomatal uptake of ionic solutes using a new model system. *J. Exp. Bot.* **52**, 771–781.

Eichert, T., Burkhardt, J. and Goldbach, H. E. (2002) Some factors controlling stomatal uptake. *Acta Hort*. **594**, 85–90.

Eichert, T. and Goldbach, H.E. (2008) Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces–further evidence for a stomatal pathway. *Physiol. Plant*. **132**(4), 491–502.

Eichert, T., Kurtz, A., Steiner, U. and Goldbach, H.E. (2008) Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. *Physiol. Plant.* **134**(1), 151–60.

Eller, C.B., Lima, A.L. and Oliveira, R.S. (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *New Phytol.* **199**(1), 151-162.

Fahn, A. (1986) Structural and functional properties of trichomes of xeromorphic leaves. *Ann. Bot*. **57**(5), 631-637.

Fernández, V. and Eichert, T. (2009) Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Crit. Rev. Plant Sci.* **28**(1–2), 36–68.

Fernández, V., Khayet, M., Montero-Prado, P., Heredia-Guerrero, J.A., Liakoloulos, G., Karabourniotis, G., Del Río, V., Domínguez, E., Tacchini, I., Nerín, C., Val, J. and Heredia, A. (2011) New insights into the properties of pubescent surfaces: peach fruit as model. *Plant Physiol*. **156**(4), 2098-2108

Fernández, V., Sancho-Knapik, D., Guzmán, P., Peguero-Pina, J.J., Gil, L., Karabourniotis, G., Khayet, M., Fasseas, C., Heredia-Guerrero, J.A., Heredia, A. and Gil-Pelegrin, E. (2014a) Wettability, polarity and water absorption of *Quercus ilex* leaves: effect of leaf side and age. *Plant Physiol*. **166**, 168-180.

Fernández, V., Guzmán, P., Peirce, C. A. E., McBeath, T. M., Khayet, M., and McLaughlin M. J. (2014b). Effect of wheat phosphorus status on leaf surface properties and permeability to foliar applied phosphorus. *Plant Soil* **384**, 7-20

Fernandez, V. and Khayet, M. (2015) Evaluation of the surface free energy of plant surfaces: towards standardizing the procedure. *Front. Plant Sci.* **6**, 510.

Fernández, V., Guzmán-Delgado, P., Graça, J., Santos, S. and Gil, L. (2016) Cuticle structure in relation to chemical composition: re-assessing the prevailing model. *Front. Plant Sci*. **7**, 427.

Fernández, V., Bahamonde, H. A., Peguero-Pina, J.J., Gil-Pelegrín, E., Sancho-Knapik, D., Gil, L., Goldbach H.E. and Eichert, T. (2017) Physico-chemical properties of plant cuticles and their functional and ecological significance*. J. Exp. Bot.* **68**(19), 5293-5306.

Field, R.J. and Bishop, N.G. (1988) Promotion of stomatal infiltration of glyphosate by an organosilicone surfactant reduces the critical rainfall period. *Pest. Sci*. **24**, 55-62.

Fogg, G.E. (1947) Quantitative studies on the wetting of leaves by water. *Proc. R. Soc. Lond.* B **134** (877), 503–522.

Franke, W. (1964) Role of guard cells in foliar absorption. *Nature* **202**, 1236- 1237.

Franke, W. (1967) Mechanisms of foliar penetration of solutions. *Ann. Rev. Plant Physiol.* **18**, 281–300.

Goldsmith, G.R., Matzke, N.J. and Dawson, T.E. (2013) The incidence and implications of clouds for cloud forest plant water relations. *Ecol. Lett.* 16(3), 307-314.

Gorb, E.V. and Gorb, S.N. (2017) Anti-adhesive effects of plant wax coverage on insect attachment. *J. Exp. Bot.* **68**(19), 5323-5337.

Gotsch, S.G., Nadkarni, N., Darby, A., Glunk, A., Dix, M., Davidson, K. and Dawson, T.E. (2015) Life in the treetops: ecophysiological strategies of canopy epiphytes in a tropical montane cloud forest. *Ecol. Monographs* **85**(3), 393-412.

Grammatikopoulos, G. and Manetas, Y. (1994) Direct absorption of water by hairy leaves of *Phlomis fruticosa* and its contribution to drought avoidance**.** *Can. J. Bot.* **72,** 1805–1811

Guzmán, P., Fernández, V., Graça, J., Cabral, V., Kayali, N., Khayet, M. and Gil, L (2014a) Chemical and structural analysis of *Eucalyptus globulus* and *E. camaldulensis* leaf cuticles: a lipidized cell wall region. *Front. Plant Sci*. **5**, 481.

Guzmán, P., Fernández, V., García, M.L., Khayet, M., Fernández, A. and Gil, L. (2014b) Localization of polysaccharides in isolated and intact cuticles of eucalypt, poplar and pear leaves by enzyme-gold labeling. *Plant Physiol. Biochem*. **76**, 1-6.

Guzmán-Delgado, P., Graça, J., Cabral, V., Gil, L. and Fernández, V. (2016) The presence of cutan limits the interpretation of cuticular chemistry and structure: *Ficus elastica* leaf as an example. *Physiol. Plantarum* **157**(2), 205-220.

Guzmán-Delgado, P., Mason Earles, J. and Zwieniecki, M.A. (2018) Insight into the physiological role of water absorption via the leaf surface from a rehydration kinetics perspective. *Plant Cell Environ*. **41** (8), 1886–1894.

Guzmán-Delgado, P., Laca, E. and Zwieniecki, M. (2020) Unravelling foliar water uptake pathways: the contribution of stomata and the cuticle. Authorea, Aug 10th, DOI: 10.22541/au.159708705.55227474.

Hama, T., Kouchi, A., Watanabe, N., Enami, S., Shimoaka, T. and Hasegawa, T. (2017) In situ nondestructive analysis of *Kalanchoe pinnata* leaf surface structure by polarization-modulation infrared reflection-absorption spectroscopy. *J. Phys. Chem. B* **121**, 11124–11131.

Hama, T., Seki, K., Ishibashi, A., Miyazaki, A., Kouchi, A., Watanabe, N., Shimoaka, T. and Hasegawa, T. (2019) Probing the molecular structure and orientation of the leaf surface of *Brassica oleracea* L. by polarization modulation-infrared reflection-absorption spectroscopy. *Plant Cell Physiol***.** **60**(7), 1567-1580.

Hanba, Y.T., Moriya, A. and Kimura, K. (2004) Effect of leaf surface wetness and wettability on photosynthesis in bean and pea. *Plant Cell Environ*. **27**(4), 413-421.

Hegebarth, D., Buschhaus, C., Wu, M., Bird, D. and Jetter, R. (2016) The composition of surface wax on trichomes of *Arabidopsis thaliana* differs from wax on other epidermal cells. *Plant J.* **88**(5), 762-774.

Henslow, J.S. (1831) On the examination of a hybrid Digitalis. *Trans. Camb.. Phil. Soc*. **4**, 257-278.

Holanda, A.E.R., Souza, B.C., Carvalho, E.C.D., Oliveira, R. S., Martins, F. R., Muniz, C. R., Costa R.C. and Soares, A. A. (2019) How do leaf wetting events affect gas exchange and leaf lifespan of plants from seasonally dry tropical vegetation? *Plant Biol*. **21**(6), 1097-1109.

Holloway, P.J. (1969a) Chemistry of leaf waxes in relation to wetting. *J. Sci. Food Agr. **20**(2), 124-128.

Holloway, P.J. (1969b) The effects of superficial wax on leaf wettability. *Ann. Appl. Biol.* **63**(1), 145-153.

Ingram, G. and Nawrath, C. (2017) The roles of the cuticle in plant development: organ adhesions and beyond. *J. Exp. Bot*. **68**(19), 5307-5321.

Javelle, M., Vernoud, V., Rogowsky, P.M. and Ingram, G.C. (2011) Epidermis: the formation and functions of a fundamental plant tissue. *New Phytol.* **189**(1), 17-39.

Jeffree, C.E. (2006) "The fine structure of the plant cuticle," in: Biology of the plant cuticle, Annual Plant Reviews, vol. 23, eds. M. Riederer, C. Müller (Oxford: Blackwell Publishing), 11-125.

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

Johnson, E.J., Dorot, O., Liu, J., Chefetz, B. and Xing, B. (2007) Spectroscopic characterization of aliphatic moieties in four plant cuticles. *Commun Soil Sci. Plant Anal.* 38, 2461–2478

Jura-Morawiec, J. and Marcinkiewicz, J. (2020) Wettability, water absorption and water storage in rosette leaves of the dragon tree (*Dracaena draco* L.). *Planta* **252**(2), 1-8.

Kannan S. and Charnel A. (1986) Foliar absorption and transport of inorganic nutrients, Crit. Rev. Plant Sci. **4** (4), 341-375

Kane, C.N., Jordan, G.J., Jansen, S. and McAdam, S.A.M. (2020) A permeable cuticle, not open stomata, Is the primary source of water Loss from expanding leaves. *Front. Plant Sci.* 11, 774.

Karabourniotis, G. and Liakopoulos, G. (2006) Phenolic compounds in plant cuticles: Physiological and ecological aspects. Advances in Plant Physiology Vol. 8, A. Hemantaranjan (ed.), Scientific Publishers, India, 33-47.

Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D. and Bresta, P. (2020) Protective and defensive roles of non-glandular trichomes against multiple stresses: Structure–function coordination. *J. Forestry Res.* **31**(1), 1-12.

Khayet, M. and Fernández, V. (2012) Estimation of the solubility parameters of model plant surfaces and agrochemicals: a valuable tool for understanding plant surface interactions. *Theor. Biol. Med. Mod*. **9**, 45.

Kerstiens, G. (2006) Water transport in plant cuticles: an update. *J. Exp. Bot.* **57**(11), 2493-2499.

Kim, K., Kim, H., Ho Park, S. and Joon Lee, S. (2017) Hydraulic strategy of cactus trichome for absorption and storage of water under arid environment. *Front. Plant Sci.* **8**, 1777.

Kitamura, H. (2011) A case study on leaching characteristics of *Quercus glauca* leaves in Southern Kyusyu stream water. *Bull. Minamikyushu Univ*. **41A**, 43-52.

Koch, K., Bhushan, B. and Barthlott, W. (2008) Diversity of structure, morphology and wetting of plant surfaces. *Soft Matter* **4**(10), 1943-1963.

Koch, K. and Barthlott, W. (2009) Superhydrophobic and superhydrophilic plant surfaces: an inspiration for biomimetic materials. *Phil. Trans. Series A, Math. Phys. Eng. Sci.* **367**, 1487–1509.

Konrad, W., Burkhardt, J., Ebner, M. and Roth-Nebelsick, A. (2015) Leaf pubescence as a possibility to increase water use efficiency by promoting condensation. *Ecohydrol*. **8**(3), 480-492.

Kosma, D.K., Nemacheck, J.A., Jenks, M.A. and Williams, C.E. (2010) Changes in properties of wheat leaf cuticle during interactions with Hessian fly. *Plant J*. **63**(1), 31-43.

Krauss, P., Markstädter, C. and Riederer, M. (1997) Attenuation of UV radiation by plant cuticles from woody species. *Plant Cell Environ*., **20**(8), 1079-1085.

Krüger, H.K., Van Rensburg, L. and Peacock, J. (1996) Cuticular membrane fine structure of *Nicotiana tabacum* L. leaves. *Ann. Bot*. **77**(1), 11-16.

Lara, I., Heredia, A. and Domínguez, E. (2019) Shelf life potential and the fruit cuticle: the unexpected player. *Front. Plant Sci*. **10**, 770.

Leide, J., Nierop, K.G., Deininger, A.C., Staiger, S., Riederer, M. and de Leeuw, J.W. (2020) Leaf cuticle analyses: Implications for the existence of cutan/non-ester cutin and its biosynthetic origin. *Ann. Bot.* **126**(1), 141–162,

Liakoura, V., Stavrianakou, S., Liakopoulos, G., Karabourniotis, G. and Manetas, Y. (1999) Effects of UV-B radiation on *Olea europaea*: comparisons between a greenhouse and a field experiment. *Tree Physiol.* **19**(13), 905-908.

Li, C., Wang, P., Lombi, E., Cheng, M., Tang, C., Howard, D.L., Menzies, N.W. and Kopittke, P.M. (2018a), Absorption of foliar-applied Zn fertilizers by trichomes in soybean and tomato, *J. Exp. Bot*. **69**(10), 2717-2729.

Li, C., Wang, P, Lombi, E., Wu, J., Blamey, F.P.C., Fernández, V., Howard, D.L., Menzies, N.W. and Kopittke, P.M. (2018b) Absorption of foliar applied Zn is decreased in Zn deficient sunflower (*Helianthus annuus*) due to changes in leaf properties, *Plant Soil* **433**, 309–322.

Li, C., Wang, P., van der Ent, A., Cheng, M., Jiang, H., Lund Read, T., Lombi, E., Tang, C., de Jonge, M.D., Menzies, N. W. and Kopittke, P.M. (2019) Absorption of foliar-applied Zn in sunflower *(Helianthus annuus)*: importance of the cuticle, stomata and trichomes, *Ann. Bot.* **123**, 57-68.

Limm, E.B., Simonin, K.A., Bothman, A.G. and Dawson, T.E. (2009) Foliar water uptake: a common water acquisition strategy for plants of the redwood forest. *Oecologia* **161**, 449-459.

Luque, P., Bruque, S. and Heredia, A. (1995) Water permeability of isolated cuticular membranes: a structural analysis, *Arch. Biochem. Biophys.* **317**, 417–422.

Martin, M.M. and Lindqvist, L. (1975) The pH dependence of fluorescein fluorescence. *J. Lumin.* **10**, 381–390.

Martin, C.E. and von Willert, A.D. (2000) Leaf epidermal hydathodes and the ecophysiological consequences of foliar water uptake in species of Crassula from the Namib Desert in southern Africa. *Plant Biol.* **2**(02), 229-242.

Mazurek, S., Garroum, I., Daraspe, J., De Bellis, D., Olsson, V., Mucciolo, A., Butenko M.A., Humbel, B.H. and Nawrath, C. (2017) Connecting the molecular structure of cutin to ultrastructure and physical properties of the cuticle in petals of Arabidopsis. *Plant Physiol.* **173**(2), 1146-1163.

Mérida, T., Schönherr, J. and Schmidt, H.W. (1981) Fine structure of plant cuticles in relation to water permeability: The fine structure of the cuticle of *Clivia miniata* Reg. leaves. *Planta* **152**, 259±267.

Miljkovic, N. and Wang, E.N. (2013) Condensation heat transfer on superhydrophobic surfaces. *MRS Bulletin* **38**(5), 397-406.

Munné-Bosch, S., Nogués, S. and Alegre, L. (1999) Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions. *New Phytol*. **144**, 109-119.

Munné-Bosch, S. (2010) Direct foliar absorption of rainfall water and its biological significance in dryland ecosystems. *J. Arid Environ*. **74**(3), 417-418.

Neinhuis, C. and Barthlott, W. (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. *Ann. Bot.* **79**(6), 667-677.

Niklas, K.J., Cobb, E.D. and Matas, A.J. (2017) The evolution of hydrophobic cell wall biopolymers: from algae to angiosperms. *J. Exp. Bot*. **68**(19), 5261-5269.

Nikolopoulos, D., Liakopoulos, G., Drossopoulos, I. and Karabourniotis, G. (2002) The relationship between anatomy and photosynthetic performance of heterobaric leaves. *Plant Physiol.* **129**(1), 235-243.

Papini, A., Tani, G., Di Falco, P. and Brighigna, L. (2010) The ultrastructure of the development of *Tillandsia* (Bromeliaceae) trichome. *Flora* **205**(2), 94-100.

Philippe, G., Geneix, N., Petit, J., Guillon, F., Sandt, C., Rothan, C., Lahaye M., MarionD. and Bakan, B. (2020a) Assembly of tomato fruit cuticles: a cross-talk between the cutin polyester and cell wall polysaccharides. *New Phytol.* **226**(3), 809-822.

Philippe, G., Sørensen, I., Jiao, C., Sun, X., Fei, Z., Domozych, D.S. and Rose, J.K. (2020b) Cutin and suberin: assembly and origins of specialized lipidic cell wall scaffolds. *Curr. Op. Plant Biol*. **55**, 11-20.

Pina, A.L.C.B., Zandavalli, R.B., Oliveira, R.S., Martins, F.R. and Soares, A.A. (2016) Dew absorption by the leaf trichomes of *Combretum leprosum* in the Brazilian semiarid region. *Funct Plant Biol.* **43**(9), 851-861.

Popp, C., Burghardt, M., Friedmann, A. and Riederer, M. (2005) Characterization of hydrophilic and lipophilic pathways of Hedera helix L. cuticular membranes: permeation of water and uncharged organic compounds, *J. Exp. Bot*. **56**, 2797–2806

Reina, J. J., Domínguez, E. and Heredia, A. (2001) Water sorption–desorption in conifer cuticles: the role of lignin, *Physiol. Plantarum* **112**(3), 372-378.

Rentschler, I. (1971) Die The wettability of leaf surfaces and the submicroscopic structure of their wax. *Planta* **96**, 119-135.

Riederer, M. and Muller C. (2006) Biology of the plant cuticle. Annual Plant Reviews Vol. 23, Oxford: Blackwell.

Riederer, M. (2006) Thermodynamics of the water permeability of plant cuticles: characterization of the polar pathway. *J. Exp. Bot.* **57**(12), 2937–2942.

Rosado, B.H., and Holder, C.D. (2013) The significance of leaf water repellency in ecohydrological research: a review. *Ecohydrol.* **6**(1), 150-161.

Segado, P., Domínguez, E. and Heredia, A. (2016) Ultrastructure of the epidermal cell wall and cuticle of tomato fruit (*Solanum lycopersicum* L.) during development. *Plant Physiol.* **170**, 935–946.

Segado, P., Heredia-Guerrero, J.A., Heredia, A. and Domínguez, E. (2020) Cutinsomes and CUTIN SYNTHASE1 function sequentially in tomato fruit cutin deposition in fruits. *Plant Physiol.,* **183**(4), 1622–1637.

Schmidt, H.W., and Schönherr, J. (1982) Development of plant cuticles: occurrence and role of non-ester bonds in cutin of *Clivia miniata* Reg. leaves. *Planta* **156**(4), 380-384.

Smith W.K. and McClean T.M. (1989). Adaptive relationship between leaf water repellency, stomatal distribution, and gas exchange. *Am. J. Bot.* **76**, 465–469.

Schönherr, J. (1976) Water permeability of isolated cuticular membranes: the effect of pH and cations on diffusion, hydrodynamic permeability and size of polar pores in the cutin matrix. *Planta* **128**(2), 113–26.

Schönherr, J. (2006) Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. *J. Exp. Bot*. **57**, 2471–2491.

Schönherr, J. and Bukovac M.J. (1970) The nature of precipitates formed in the outer cell wall following fixation of leaf tissue with Gilson solution. *Planta* **92**, 202–207.

Schönherr, J. and Bukovac, M.J. (1972) Penetration of stomata by liquids: dependence on surface tension, wettability, and stomatal morphology. *Plant Physiol*. **49**, 813–819.

Schönherr, J. and Huber, R. (1977) Plant cuticles are polyelectrolytes with isoelectric points around three, *Plant Physiol*. **59**, 145–150.

Schönherr, J. and Bukovac, M.J. (1978) Foliar penetration of succinic acid-2,2-dimethylhydrazide: Mechanisms and rate limiting step. *Physiol. Plant*. **42**, 243-251.

Schönherr, J. and Schreiber, L. (2004) Size selectivity of aqueous pores in astomatous cuticular membranes isolated from *Populus canescens* (Aiton) Sm. leaves. *Planta* **219**, 405–411.

Schreel, J.D. and Steppe, K. (2020) Foliar Water Uptake in Trees: Negligible or Necessary? *Trends Plant Sci*. **25**(6) 590-603.

Schreel, J.D., Leroux, O., Goossens, W., Brodersen, C., Rubinstein, A. and Steppe, K. (2020). Identifying the pathways for foliar water uptake in beech (*Fagus sylvatica* L.): a major role for trichomes. *Plant J.* **103** (2),769-780.

Schreiber, L. and Schönherr, J. (2009) Water and Solute Permeability of Plant Cuticles.

Springer, Berlin.

Schreiber, L. (2005) Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. *Ann. Bot.* **95**, 1069–73.

Schreiber, L. (2006) Review of sorption and diffusion of lipophilic molecules in cuticular waxes and the effects of accelerators on solute mobilities. *J. Exp. Bot*. **57**, 2515–2523.

Schreiber, L., Skrabs, M., Hartmann, K.D., Diamantopoulos, P., Simanova, E. and Santrucek, J. (2001) Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. *Planta* **214**, 274–282.

Schuster, A.C., Burghardt, M. and Riederer, M. (2017) The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions? *J. Exp. Bot*. **68(**19), 5271-5279.

Sohrt, J., Uhlig, D., Kaiser, K., von Blanckenburg, F., Siemens, J., Seeger, S., Frick, D.A., Krüger, J., Lang, F. and Weiler M. (2019) Phosphorus fluxes in a temperate forested watershed: canopy leaching, runoff sources, and In-stream transformation. *Front. For. Glob. Change* **2**, 85.

Somaratne, Y., Tian, Y., Zhang, H., Wang, M., Huo, Y., Cao, F., Zhao, L. and Chen, H. (2017) ABNORMAL POLLEN VACUOLATION1 (APV1) is required for male fertility by contributing to anther cuticle and pollen exine formation in maize. *Plant J*. **90**(1), 96-110.

Stone, E.C., Went, F.W. and Young, C.L. (1950) Water absorption from the atmosphere by plants growing in dry soil. *Science* **111** (I2890), 546-548.

Shumborski, S.J., Samuels, A.L. and Bird, D.A. (2016). Fine structure of the Arabidopsis stem cuticle: effects of fixation and changes over development. *Planta* **244**(4), 843-851.

Tanaka, T., Tanaka, H., Machida, C., Watanabe, M. and Machida, Y. (2004) A new method for rapid visualization of defects in leaf cuticle reveals five intrinsic patterns of surface defects in Arabidopsis. *Plant J*. **37**(1), 139-146.

Tredenick, E.C., Farrell, T.W., Forster, W.A. and Psaltis, S.T. (2017) Nonlinear porous diffusion modelling of hydrophilic Ionic agrochemicals in astomatous plant cuticle aqueous pores: a mechanistic approach. *Front. Plant Sci*. **8**, 746.

Tukey, H.B. Jr (1970) The leaching of substance from plants. *Ann. Rev. Plant Physiol*. **21**, 305–324.

Urrego-Pereira, Y.F., Martínez-Cob, A., Fernández, V. and Cavero, J. (2013) Daytime Sprinkler Irrigation Effects on Net Photosynthesis of Maize and Alfalfa. *Agron. J.* **105**, 1515-1528.

Veličković, D., Herdier, H., Philippe, G., Marion, D., Rogniaux, H. and Bakan, B. (2014) Matrix-assisted laser desorption/ionization mass spectrometry imaging: a powerful tool for probing the molecular topology of plant cutin polymer. *Plant J*. **80**(5), 926-935.

Vesala, T., Sevanto, S., Gronholm, T., Salmon, Y., Nikinmaa, E., Hari, P. and Holtta, T. (2017) Effect of leaf water potential on internal humidity and CO_2 dissolution: Reverse transpiration and improved water use efficiency under negative pressure. *Front. Plant Sci*. **8**, 54.

von Mohl, H. (1842) "Über die Cuticula der Gewächse", in Linnaea, vol 16, ed von D. F. C. Schlechtendal, (Schwetschke und Sohn: Halle), 401-416.

von Mohl, H. (1847) Untersuchungen der Frage: bildet die Cellulose die Grundlage sämmtlicher vegetabilischen Membranen. *Bot. Zeit.* **5**, 497-505.

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

Wang, X., Xiao, H., Cheng, Y. and Ren, J. (2016) Leaf epidermal water-absorbing scales and their absorption of unsaturated atmospheric water in *Reaumuria soongorica*, a desert plant from the northwest arid region of China. *J. Arid Environ*. **128**, 17-29.

Wattendorff, J. and Holloway, P.J. (1980) Studies on the ultrastructure and histochemistry of plant cuticles. The cuticular membrane of *Agave americana* L. in situ. **Ann. Bot** 46, 13-28.

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

Westhoff, M., Zimmermann, D., Zimmermann, G., Gessner, P., Wegner, L., Bentrup, F.W. and Zimmermann, U. (2009) Distribution and function of epistomatal mucilage plugs. *Protoplasma* 235, 101-105.

Wullschleger, S.D., and Oosterhuis, D.M. (1989) The occurrence of an internal cuticle in cotton (*Gossypium hirsutum* L.) leaf stomates. *Environ. Exp. Bot.* **29**(2), 229-235.

Wylie, R.B. (1943) The role of the epidermis in foliar organization and its relations to the minor venation. *Am. J. Bot*. **30** (4), 273–280.

Wylie, R.B. (1952) The bundle sheath extension in leaves of dicotyledons. *Am J. Bot*. **39**(9), 645-651

Yeats, T.H. and Rose, J.K.C. (2013) The formation and function of plant cuticles. *Plant Physiol.* **163**, 5-20

Yokoyama, G., Yasutake, D., Tanizaki, T. and Kitano, M. (2019) Leaf wetting mitigates midday depression of photosynthesis in tomato plants. *Photosynthetica* **57**(3), 740-747.

Zabkiewicz, J.A., Stevens, P.J.G., Forster, W.A. and Steele, K.D. (1993) Foliar uptake of organosilicone surfactant oligomers into bean leaf in the presence and absence of glyphosate. *Pestic. Sci*. 38, 135-143.

Zeisler-Diehl, V., Müller, Y. and Schreiber, L. (2018) Epicuticular wax on leaf cuticles does not establish the transpiration barrier, which is essentially formed by intracuticular wax. *J. Plant Physiol.* **227**, 66–74

Zhang, Y., Chen, X., Du, Z., Zhang, W., Devkota, A.R., Chen, Z., Chen, C., Sun, W. and Chen, M. (2020) A proposed method for simultaneous measurement of cuticular transpiration from different leaf surfaces in *Camellia sinensis*. *Front. Plant Sci*. **11**, 420.

Ziv, C., Zhao, Z., Gao, Y.G. and Xia, Y. (2018) Multifunctional roles of plant cuticle during plant-pathogen interactions. *Front. Plant Sci*. **9**, 1088.

Zwieniecki, M. A., Melcher, P.J. and Holbrook, N.M. (2001) Hydrogel control of xylem hydraulic resistance in plants. *Science* **291**(5506), 1059-1062.

Figure Captions

Figure 1. Examples of the characteristics of different plant surfaces covered with a cuticle.

Transmission electron micrographs of cross-sections of: (a) adaxial leaf trichome of a *Quercus ilex* leaf, (b) cuticular folds on the adaxial surface of a rose petal, (c, e) *Cucumis sativus* leaf stoma with a smooth and thin cuticle covering the surface of the stomatal pore and guard cells (e), and (d, f) *Ulmus minor* stoma having rough guard cell and stomatal pore surfaces due to the occurrence of cuticular folds (d). GC, Guard cell; SC, stomatal cavity; CW, cell wall; C, cuticle

Figure 2. Effect of plant surface wettability on water and solute surface interactions and foliar absorption potential.

Figure 3. Pathways for the penetration of hydrophilic solutes across leaf surfaces.

Water (blue) may be deposited on to the cuticle (a, b) or stomata (c), enabling the diffusion in to the leaf interior of hydrophilic solutes. At low relative humidity (RH), water in the cuticle is present mostly in the inner regions adjacent to the epidermal cells, whereas in the outermost regions only little water is sorbed (a). Only if RH is high or underneath a drop of liquid water, the cuticle absorbs enough water from the outer side to create continuous aqueous connections crossing the cuticle (b). In some stomata, water clusters may be present and form thin water films creating diffusion pathways for the penetration of solutes by diffusion (c). Since RH within the stomatal pores is generally increased by transpiration, these stomatal water films may exist at lower external RH and in the absence of liquid water drops on the leaves

Unwettable: $\vartheta \geq 90º$

- Drop adhesion unless very unwettable & apolar surface
- Reduced liquid-leaf contact area
- Film- or drop-wise condensation of fog or dew

Very unwettable (e.g., $\vartheta \geq 130º$)

- Drop repulsion or adhesion
- Limited or no liquid-leaf contact

Lower foliar *absorption potential* with increasing ϑ

Wettable: $\vartheta < 90º$

- Drop adhesion
- High liquid-leaf contact area
- Film-wise condensation of fog or dew

Higher foliar absorption potential

RH low

Covered by water or RH high

Cuticle

(a)

(b)

Epidermis

(c)

Stoma