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Foliar water and solute absorption: an update

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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 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

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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 it 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 C₁₆ and/or C₁₈ 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 Mohl'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; Philippe *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 be 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 hydrotodes (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 microtomography. 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 μ l 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 than 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; Philippe *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 CO₂ 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 HgCl₂. 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 AgNO₃) 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

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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, hydrotodes, 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.

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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





