2. Mechanisms of penetration into the plant

The processes by which a nutrient solution applied to the foliage is ultimately utilized by the plant include foliar adsorption, cuticular penetration, uptake and absorption into the metabolically active cellular compartments in the leaf, then translocation and utilization of the absorbed nutrient by the plant. From a practical perspective it is often difficult to distinguish between these processes though many trials using the term 'foliar uptake' often refer to an increase in tissue nutrient content without directly measuring the relative biological benefit of the application to the plant as a whole. This confusion and imprecision greatly complicates the interpretation of both controlled environment/ laboratory and field experimentation and has undoubtedly resulted in inconsistent plant response and general uncertainty in predicting the efficacy of foliar treatments. Therefore the challenges facing practitioners of foliar fertilization and for researchers attempting to understand the factors that determine the efficacy of foliar fertilizers are great.

The aerial surface of the plant[1] is characterized by a complex and diverse array of specialized chemical and physical adaptations that serve to enhance plant tolerance to an extensive list of factors including unfavorable irradiation, temperatures, vapor pressure deficits, wind, herbivory, physical damage, dust, rain, pollutants, anthropogenic chemicals, insects and pathogens. Aerial plant surfaces and structures are also well adapted to control the passage of water vapor and gases, and to restrict the loss of nutrients, metabolites and water from the plant to the environment under unfavourable conditions. These characteristics of aerial plant surfaces that allow them to protect the plant from environmental stress and to regulate water, gas and nutrient exchange also provide the mechanisms affecting the uptake of foliar applied nutrients. Improvements in the efficacy and reproducibility of foliar fertilization requires knowledge of the chemical and physical attributes of plant surfaces and the processes of penetration into the plant.

Aerial plant surfaces are generally covered by a hydrophobic cuticle and very often possess modified epidermal cells such as trichomes or stomata. The outer surface of the cuticle is covered by waxes that may confer a hydrophobic character to the plant's surface. The degree of hydrophobicity and polarity of the plant surface is determined by the species, chemistry and topography which are also influenced by the epidermal cell structure at a microscopic level. Like leaves, fruits are also protected by a cuticle

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[1]For simplicity we will use the term 'aerial plant surfaces' to mean the external surfaces of all above ground plant organs including stems, leaves, trunks, fruits, reproductive and other above ground organs that can be targeted for foliar application.

and may contain epidermal structures such as stomata[2] or trichomes[3] that influence the transpiration pathway and contribute to its conductance of water (and nutrients) which are critical factor for fruit growth and quality (Gibert *et al.*, 2005; Morandi *et al.*, 2010).

A transverse section of a typical angiosperm leaf consists of a cuticle that covers the upper and lower epidermal cell layers enclosing the mesophyll as illustrated in Figure 2.1 with a microscopic image shown in Figure 2.2.E. Leaves differ in their structure between species but generally consist of palisade parenchyma near the upper epidermis and spongy parenchyma (also refered to as spongy mesophyll) between the palisade layer and the lower epidermis. There are large intercellular spaces among the mesophyll cells, especially in the spongy parenchyma (Epstein and Bloom, 2005). The epidermis is a compact layer with sometimes two or more layers of cells (Figure 2.2.F) and the principal features, related to nutrient and water transport, which characterize the epidermis are the cuticle and the stomata.
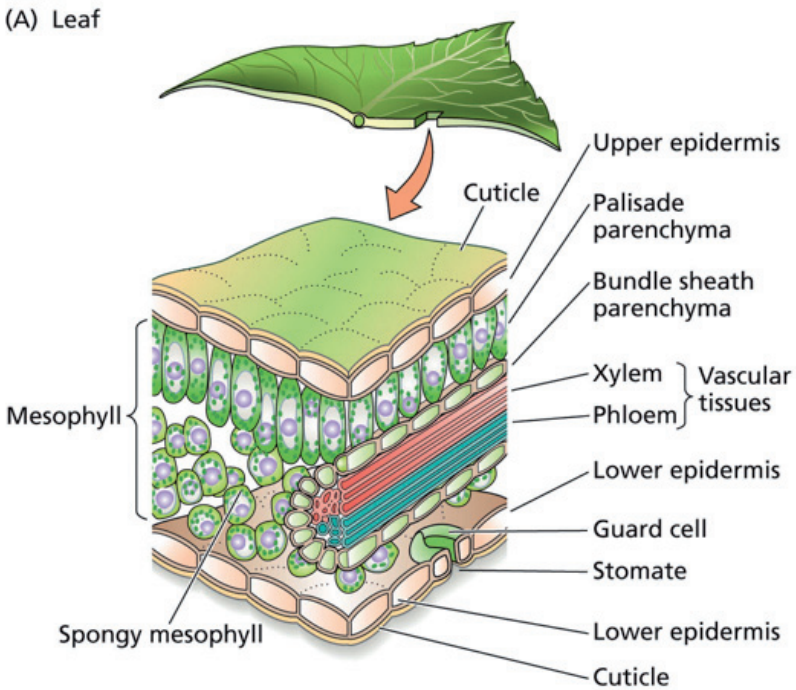

Figure 2.1. Typical structure of dicotyledonous leaf including vascular bundle in a leaf vein. (Reproduced with permission from Plant Physiology, 4th Edition, 2007, Sinauer Associates).

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[2]Stomata are pores surrounded by 2 guard cells that regulate their opening and closure which are present at high densities in leaves and are responsible for gaseous exchange and controlling water transpiration through the plant.
[3]Epidermal cell hair or bristle-like outgrowth.

The surface topography and transversal structure of a peach leaf and a fruit using Scanning Electron Microscopy (SEM) and optical microscopy after tissue staining is shown in Figure 2.2. Both the peach fruit and leaf surface stained with auramine O is covered by a cuticle that emits a green-yellow fluorescence when observed under UV light (Figure 2.2. C and D). The leaf has a cuticle protecting the abaxial (lower) and adaxial (upper) leaf side and the trichomes on the peach fruit surface are also covered by a cuticle. On the abaxial peach leaf surface, stomata are present (approximately 220 mm^{-2}) while only a few (approximately 3 mm^{-2}) occur beneath the trichomes covering the peach fruit (Figure 2.2. A and B) (Fernandez *et al.*, 2008a; Fernandez *et al.*, 2011). A
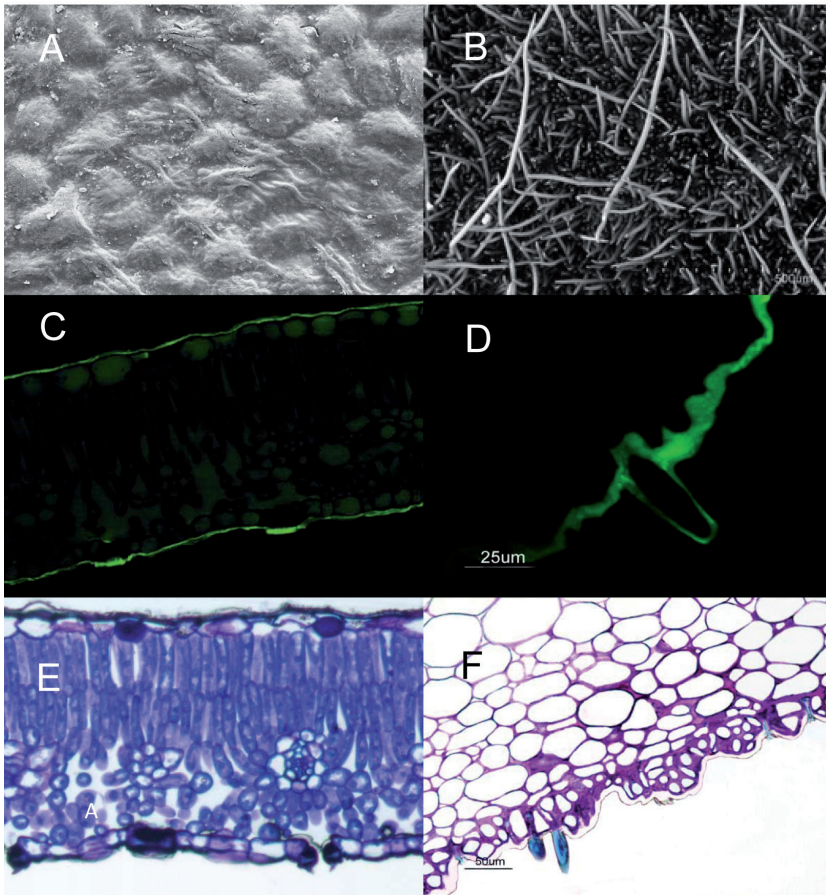

Figure 2.2. Micrographs of a peach leaf *versus* a peach fruit. Surface topography of a leaf (A) and fruit (B) observed by Scanning Electron Microscopy (SEM) (x400). Transversal sections of a peach leaf and a peach fruit after tissue staining with auramine O (UV light observation; C and D) and toluidine blue (light transmission; E and F) (micrographs A and B by V. Fernández; C and E by G. López-Casado; D and F by E. Domínguez).

layer of epidermal cells is observed beneath the abaxial and adaxial leaf cuticle and on top of the mesophyll cells (Figure 2.2. E). A multiserrate, disorganized epidermis with single-celled trichomes is found above the parenchyma cells and underneath the peach fruit surface (Figure 2.2. F).

When present in deciduous plant species, and always in evergreens, the leaves represent the majority of the total surface of the aerial part and will capture most of the spray applied and will also interact with rain water, fog or mist. While the primary function of the plant surface is to protect against dehydration, the permeability of plant surfaces to water and solutes may actually play a crucial eco-physiological role to absorb water under water-limiting conditions (Fernandez and Eichert, 2009; Limm *et al.*, 2009).

- All aerial plant parts are covered by a hydrophobic cuticle that limits the bi-directional exchange of water, solutes and gases between the plant and the surrounding environment.
- Epidermal structures such as stomata, trichomes or lenticels may occur on the surface of different plant organs and play important physiological roles.

2.1. Role of plant morphology and structure

The fundamental requirement for an effective foliar nutrient spray is that the active ingredient penetrates the plant surface so it can become metabolically active in the target cells where the nutrient is required. A foliar applied chemical may cross the plant leaf surface *via* the cuticle *per se*, along cuticular cracks or imperfections, or through modified epidermal structures such as stomata, trichomes or lenticels. The cuticle proves an effective barrier against the loss of water and yet, at the same time, it proves an equally effective one against the uptake of foliar applied chemicals. The presence of cuticular cracks or the occurrence of modified epidermal structures can contribute significantly to the rate of uptake of foliar nutrient sprays. The structure and composition of the plant leaf surface will be briefly described as a basis for understanding their role in the uptake and absorption of foliar applied nutrient sprays.

2.1.1. Cuticles and their specialized epidermal structures

The cuticle covering aerial plant parts is an extra-cellular layer composed of a biopolymer matrix with waxes embedded into (intra-cuticular), or deposited onto (epi-cuticular waxes), the surface (Heredia, 2003). On the inner side, a waxy substance called cutin is mixed with polysaccharide material from the epidermal cell wall, which is chiefly composed of cellulose, hemicellulose and pectin in a ratio similar to that found in plant cell walls. Therefore the cuticle itself can be considered as a 'cutinized' cell wall, which emphasizes the compositional and heterogeneous nature of this layer and its physiologically important interaction with the cell wall underneath (Dominguez *et al.*, 2011).

The cuticle matrix is commonly made of the bio-polyester cutin forming a network of cross-esterified hydroxy C_{16} and/or C_{18} fatty-acids (Kolattukudy, 1980). The composition of the biopolymer matrix may vary depending on the plant organ, species and genotypes, stage of development and growing conditions (Heredia, 2003; Kerstiens, 2010). While cutin is depolymerized and solubilized upon saponification, cuticles from some species may contain an alternative non-saponifiable and non-extractable polymer known as cutan, which yields a highly characteristic series of long chain n-alkenes and n-alkanes upon flash pyrolysis (Boom *et al.*, 2005; Deshmukh *et al.*, 2005; Villena *et al.*, 1999). Recently, Boom *et al.* (2005) determined the presence of cutan in cuticles of drought-tolerant species such as *Agave americana*, *Podocarpus sp.* or *Clusia rosea* and suggested that it might be a preserved biopolymer especially in xeromorphic (water storing) plants. Cutin is the only polymer present in cuticles of the fruits and leaves of many *Solanaceae* and *Citrus* species (Jeffree, 2006) whereas in *Beta vulgaris* cutan is the only polymer forming the leaf cuticular matrix (Jeffree, 2006). Variable proportions of cutin and cutan have been determined in cuticular membranes extracted from leaves of some plant species such as *Agave americana* (Villena *et al.*, 1999) and in some fruit types such as soft-fruit berries, apples and peppers (Jarvinen *et al.*, 2010; Johnson *et al.*, 2007).

The waxes present in the cuticle, either deposited onto, or embedded into, the cuticular matrix are mainly mixtures of long chain aliphatic molecules (mainly C_{20}-C_{40} n-alcohols, n-aldehydes, very long-chain fatty-acids and n-alkanes) and of aromatic (ring-chain) compounds (Samuels *et al.*, 2008). Wax composition has been observed to vary between different plant species and organs, the stage of development and the prevailing environmental conditions (Koch *et al.*, 2006; Kosma *et al.*, 2009).

As well as the cutin and/or cutan matrix and the waxes, variable amounts and types of phenolics may be present in the cuticle either in free form embedded in the matrix or chemically bound to cutin or waxes by ester or ether bonds (Karabourniotis and Liakopoulos, 2005). Hydroxycinnamic acid derivatives (e.g. ferulic, caffeic or p-coumaric acid), phenolic acids (e.g. vanillic acid) and flavonoids (e.g. naringenin) have been determined analytically in epicuticular wax and cuticle matrix extracts and observed by fluorescence microscopy (Karabourniotis and Liakopoulos, 2005; Liakopoulos *et al.*, 2001). Besides the major role of phenols in protection against biotic (microbes or herbivores) and abiotic (UV radiation, pollutants) stress factors, they are also involved in the attraction of pollinators (Liakopoulos *et al.*, 2001).

Many plant surfaces are pubescent[4] to a greater or lesser degree as shown in Figure 2.3. for soybean, maize and cherry leaf adaxial surfaces. According to Werker (2000), trichomes are defined as unicellular or multicellular appendages which originate from epidermal cells only, and which develop outwards from the surface of various plant organs. Scientific studies on these epidermal structures began in the 17th century with emphasis being placed either on individual trichomes or on the collective properties of the trichome layer referred to as the indumentum (Johnson, 1975). Trichomes can grow on all plant parts and are chiefly classified as "glandular" or "non-glandular". While "non-glandular" trichomes are distinguished by their morphology, different kinds of "glandular" trichomes are defined by the secretory materials they excrete, accumulate or
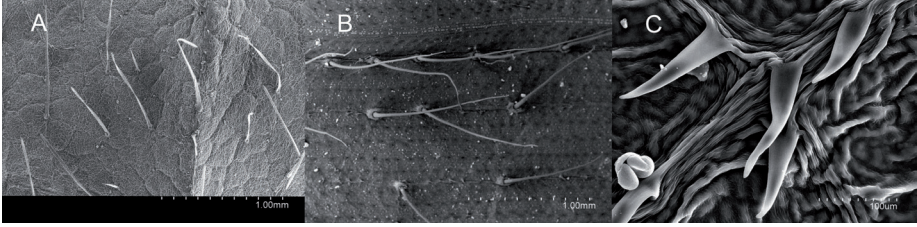
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[4]A surface covered by trichomes.

Figure 2.3. Adaxial surface of: (A) soybean; (B) maize; and (C) cherry leaf (Micrographs by V. Fernández, 2010).

absorb (Wagner *et al.*, 2004; Werker, 2000). "Non-glandular" trichomes exhibit a major variability in size, morphology and function and their presence is more prominent in plants thriving in dry habitats and usually on young plant organs (Fahn, 1986; Karabourniotis and Liakopoulos, 2005).

Stomata are modified epidermal cells that control leaf gaseous exchange and transpirational water losses. They are generally present on the abaxial leaf side but in some plant species (known as amphistomatic), including maize and soybean, they also occur on the upper leaf side (Eichert and Fernández, 2011). Stomata also occur in the epidermis of many fruits such as peaches, nectarines, plums or cherries though at lower densities compared to the leaves. Stomatal density, morphology and functionality may vary between different plant species and organs (Figure 2.4) and can be affected by
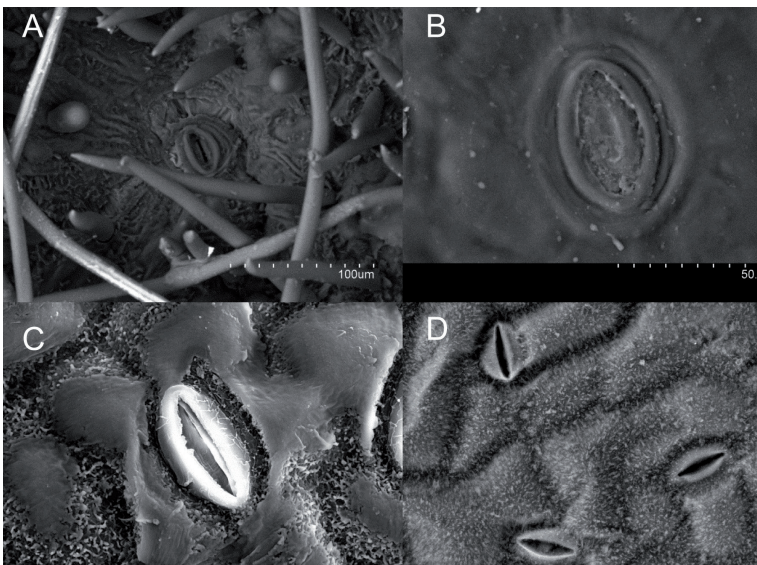

Figure 2.4. Scanning electron micrographs of stomata present on the surface of: (A) peach fruit; (B) cherry fruit; (C) rose abaxial leaf surface; and (D) broccoli abaxial leaf surface (Micrographs by V. Fernández, 2010).

stress factors such as nutrient deficiencies (Fernandez *et al.*, 2008a; Will *et al.*, 2011), or the prevailing environmental conditions such as light intensity and quality as illustrated by changes seen in plants growing in natural or artificial shade (Aranda *et al.*, 2001; Hunsche *et al.*, 2010).

Another example of epidermal structures that occur on plant surfaces are lenticels (Figure 2.5). Lenticels are macroscopic structures that may occur in stems, pedicels or fruit surfaces (e.g. they are present on the skin of fruits such as apple, pear or mango) once the periderm (cork) has formed. Their evolutionary origin has been linked to stomata, epidermal cracks and trichomes (Du Plooy *et al.*, 2006; Shaheen *et al.*, 1981).
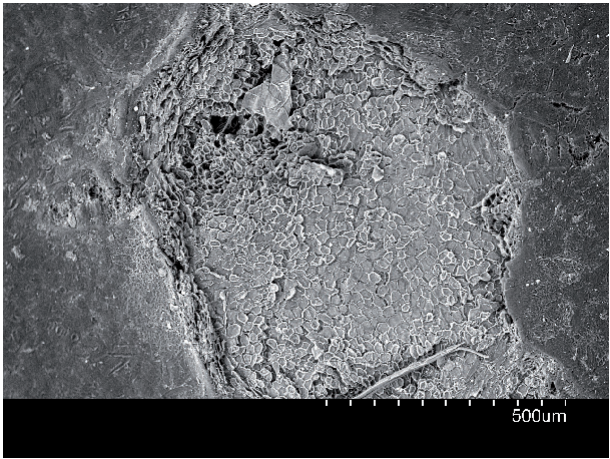

Figure 2.5. Scanning electron micrograph of a lenticel found on the surface of a "Golden Delicious" apple skin (Micrograph by V. Fernández, 2010).

The absorption of nutrient solutions by plant surfaces may occur via:
- The cuticle.
- Cuticular cracks and imperfections.
- Stomata, trichomes, lenticels.

2.1.2. Effect of topography: micro- and nano-structure of the plant surface

The topography of the plant surface, as determined by the composition and structure of the epi-cuticular waxes in glabrous (trichome-free) areas, or by the presence of trichomes or trichome layers in pubescent surfaces, will determine its properties and interactions with water, nutrient solutions, contaminants, micro-organisms, agrochemicals, etc.
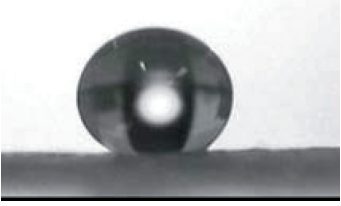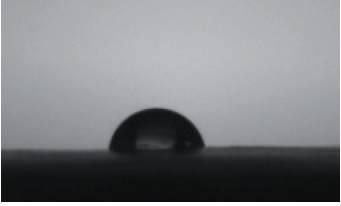
Plant organ and species	Average contact angle with pure H$_2$O (°)	Drop image
Adaxial side of *Eucalyptus globulus* leaf	140	
Adaxial side of *Ficus elastica* leaf	83	
'Calanda' Peach (*Prunus persica* L. Batsch)	130	
Apple (*Malus domestica* L. Borkh) fruit surface	84	

Figure 2.6. Average contact angles with pure water drops of the adaxial *Eucalyptus globulus* (A) and *Ficus elastica* (B) leaves; and peach (C) and apple (D) fruit surfaces (V. Fernández, 2011).

Plant surfaces have different degrees of wettability when in contact with water droplets as shown in Figure 2.6 for the leaves and fruits of four different plant species.

In the last decade, the water and contaminant repellent properties of plant surfaces with 'rough' topography have been described (Barthlott and Neinhuis, 1997; Wagner *et al.*, 2003) and different types of epicuticular waxes have been classified for several plant species (Barthlott *et al.*, 1998; Koch and Ensikat, 2008).

The presence of a micro- and nano-relief structures associated with the surfaces over the epidermal cells, and the chemical properties of the waxes deposited onto the leaf surface, may markedly increase its 'roughness' and surface area and will ultimately

determine the degree of polarity and hydrophobicity. Differences in surface polarity and hydrophobicity in relation to variable growing conditions, plant species, varieties and organs can be expected and these will have an influence on the effectiveness of foliar sprays. Fernández *et al.* (2011) examined the properties of a peach variety which is covered by a dense indumentum[5] as a model system for a pubescent plant surfaces. The peach skin investigated was found to be very hydrophobic with contact angles for water higher than 130°. Properties such as the surface free energy, polarity, and work of adhesion of the peach leaf surface was determined by means of estimating the contact angle of three liquids - water, glycerol and di-iodomethane. This methodology has proved a valuable tool for the characterization of plant surfaces and should be further explored and exploited for scientific and applied purposes (Figure 2.6).

2.2. Pathways and mechanisms of penetration

The structure and chemistry of the plant surface will affect the bi-directional diffusion of substances between the plant, the leaf surface and the surrounding environment and hence and therefore the rate of uptake of foliar fertilizers. In the following sections, the most significant plant surface penetration pathways of chemical sprays will be described, with emphasis on the mechanisms of cuticular permeability and stomatal uptake.

2.2.1. Cuticular permeability

The cuticle consists of three layers (Figure 2.7), namely (from the external to the internal surfaces of the plant organ), the epicuticular wax layer (EW), the cuticle proper (CP) and the cuticular layer (CL) (Jeffree, 2006).

The EW layer is the outermost and most hydrophobic component of the cuticle. The CP that lies beneath the epicuticular waxes contains mainly cutin and/or cutan and is by definition free of polysaccharides (Jeffree, 2006). The CL is located under the CP and consists of cutin/cutan, pectin and hemicelluloses that increase the polarity of this layer due to the presence of hydroxyl and carboxylic functional groups. The middle lamellae and pectin layer (ML) is situated beneath the CL. Variable amounts of polysaccharide fibrils and pectin lamellae may extend from the cell wall (CW), binding the cuticle to the underlying tissue (Jeffree, 2006).

A gradual increase in negative charge from the epicuticular wax to the pectin layer creates an electrochemical gradient that may increase the movement of cations and water molecules (Franke, 1967). The intra-cuticular waxes limit the exchange of water and solutes between the plant and the surrounding environment (Schreiber and Schönherr, 2009), while the epicuticular waxes influence the wettability (Holloway, 1969; Koch and Ensikat, 2008), light reflectance (Lenk *et al.*, 2007; Pfündel *et al.*, 2006) and surface properties of the plant organ.

The lipophilic and hydrophobic nature of the structural components of the cuticle make it an effective barrier against the diffusion of hydrophilic, polar compounds. However, lipophilic and a-polar compounds may penetrate the hydrophobic cuticular
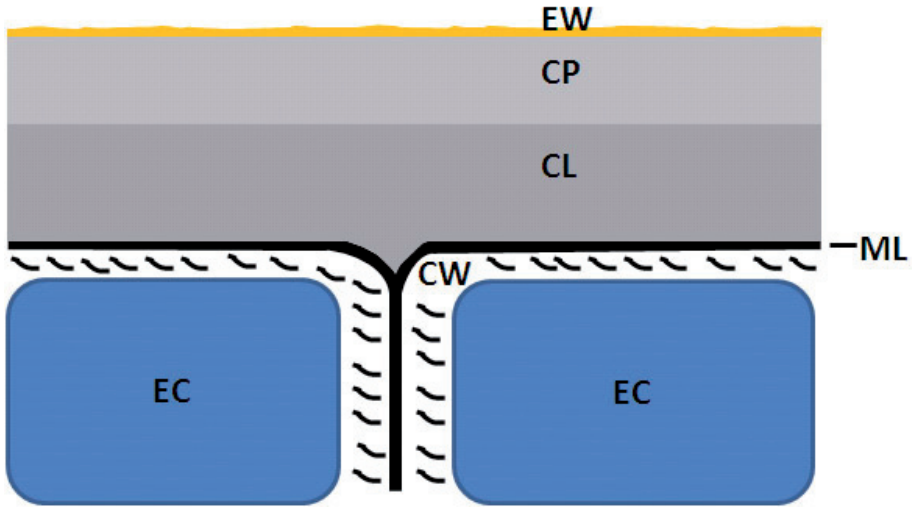
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[5]A covering of trichomes.

Figure 2.7. Schematic representation of the general structure of the plant cuticle covering two adjacent epidermal cells (EC) separated from each other by the middle lamellae and pectinaceous layer (ML) and the cell wall (CW). Epicuticular waxes (EW) are deposited onto the cuticle proper (CP) which is mainly composed of a biopolymer matrix and intra-cuticular waxes. The cuticular layer (CL) chiefly contains cutin and/or cutan and polysaccharides of the CW.

membrane at high rates compared to polar electrolyte solutions which have not had surface-active agents added to them (Fernandez and Eichert, 2009). Indeed, several studies provide evidence for the penetration of polar solutes through intact astomatous cuticles by direct and indirect means (Heredia, 2003; Riederer and Schreiber, 2001; Tyree *et al.*, 1992).

Experimental evidence has shown that plant cuticles are asymmetric membranes with a gradient of fine structure and waxes from the outer to the inner surface. Plant cuticles have a large inner sorption compartment consisting mainly of the biopolymer matrix (cutin and/or cutan) and a comparably smaller (≤10% of total volume) outer compartment where waxes predominate (Schönherr and Riederer, 1988; Tyree *et al.*, 1990).

The current state of knowledge on the mechanisms of penetration of polar solutes and apolar lipophilic substances through the cuticle will be briefly discussed in the following paragraphs.

The cuticle is an asymmetric membrane composed mainly of 3 layers:
- The epicuticular wax layer.
- The cuticle proper, chiefly made of cutin/cutan and intracuticular waxes.
- The cuticular layer, containing cutin/cutan and polysaccharide material.

Permeability of lipophilic, apolar compounds

The penetration of lipophilic[6], apolar substances through the plant cuticle has been proposed to follow a dissolution-diffusion process (Riederer and Friedmann, 2006). This model implies that the movement of a lipophilic, apolar molecule from a solution deposited onto the plant surface into the cuticle precedes the diffusion of the molecule through the cuticle (Riederer and Friedmann, 2006). The diffusion of a lipophilic molecule has been proposed to be governed by partitioning and its penetration rate will be proportional to the solubility and mobility of the compound in the cuticle (Riederer, 1995; Schreiber, 2006). At a molecular level, both the dissolution and diffusion of a molecule in the cuticle can be viewed as passing into and between voids in the polymer matrix arising by molecular motion (Elshatshat *et al.*, 2007).

Taking into account Fick's first law, the diffusive flux (J; molm^{-2}s^{-1}) is related to the concentration gradient with solutes moving from regions of high to low concentration with a magnitude that is proportional to the concentration gradient (spatial derivative). According to the cuticular diffusion model, which has been thoroughly explained by Riederer and Friedmann (2006), the diffusive flux J is proportional to the mass transfer coefficient P (i.e. the permeance of the membrane; m s^{-1}) multiplied by the concentration difference between the inner and the outer sides of the cuticle:

$$J = P * (C_i - C_o)$$

where: C_i is the concentration (mol m^{-3}) at the inner side of the cuticle and C_o is the concentration in the outer side of the cuticle.

Under certain experimental conditions, the mobility of a molecule can be predicted by calculating the permeance which is a value specific to a given molecule and a particular cuticular membrane (Riederer and Friedmann, 2006). The permeance (P m s^{-1}) is expressed as:

$$P = D * K * l^{-1}$$

where: D (m^2 s^{-1}) is the diffusion coefficient in the cuticle; K the partition coefficient which is the ratio between the equilibrium molar concentrations in the cuticle and in the solution at the cuticle surface; and l (m) which is the path length of diffusion through the cuticle. The diffusion path length may be tortuous and much larger than the cuticle thickness which is determined by the waxes embedded in the polymer matrix (Baur *et al.*, 1999; Schönherr and Baur, 1994) and by the spatial disposition of cutin and/or cutan molecules (Fernandez and Eichert, 2009). The diffusion coefficient D also depends on the temperature and fluid viscosity of the foliar nutrient solution and size of the chemical molecules it contains.

Methods to predict the mobility of lipophilic, apolar compounds through the cuticle of a few species that enable the enzymatic isolation of astomatous (adaxial) cuticles have been developed in recent decades (Riederer and Friedmann, 2006; Schreiber, 2006; Schreiber and Schönherr, 2009).

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[6]Compounds which are soluble in oils, fats, or organic solvents.

Experimental evidence has shown that the cuticle is highly size selective (Buchholz *et al.*, 1998) and that it may act as a "molecular sieve". The size of voids have been found to follow a log-normal distribution that may be in the same order of magnitude as some agrochemicals which may be limiting their diffusion through the cuticle (Schreiber and Schönherr, 2009).

Permeability of hydrophilic, electrolytes

The permeability of cuticles to solutes has been investigated using astomatous isolated cuticles using the same methodology used to assess the penetration of apolar, lipophilic substances (Schreiber and Schönherr, 2009). In the absence of surface-active agents solutions of ionic, hydrophilic[7] compounds have generally been found to penetrate the cuticle at a lower rate compared to lipophilic, apolar compounds. This finding is probably explained by the lipophilic nature of the cuticular constituents as well as the ease with which lipophilic compounds will diffuse owing to their higher solubility in such media as compared to hydrophilics. However, some authors have suggested that the rate of penetration of electrolytes determined experimentally is too high to be explained by simple dis-solution and diffusion in the cuticle and have proposed that hydrophilic solutes may penetrate through the cuticle *via* a physically distinct pathway, along what have been called "polar, aqueous or water-filled pores" (Schönherr, 2006; Schreiber, 2005; Schreiber and Schönherr, 2009).

It has been hypothesized that such pores may arise from the absorption of water molecules onto polar moieties located in the cuticular layer (Schönherr, 2000; Schreiber, 2005), such as unesterified carboxyl groups (Schönherr and Bukovac, 1972); ester and hydroxylic groups (Chamel *et al.*, 1991) in the cutin network; and carboxylic groups of pectic cell wall material (Kerstiens, 2010; Schönherr and Huber, 1977). However, no conclusive experimental evidence has been found so far to support the presence of such "aqueous pores" in cuticles as they are not visible or identifiable with current microscope technologies (Fernandez and Eichert, 2009).

However the size of the "aqueous pores" of a few plant species has been indirectly derived from permeability trials using astomatous, adaxial cuticles. Diameters of about 1 nm were calculated for de-waxed isolated citrus cuticles (Schönherr, 1976), and isolated ivy (*Hedera* helix) cuticles (Popp *et al.*, 2005). Furthermore, pore diameters ranging from 4 to 5 nm have been calculated from permeability trials carried out with intact coffee and poplar leaves (Eichert and Goldbach, 2008).

- Lipophilic, apolar compounds have been proposed to penetrate cuticles by a solution-diffusion process.
- The mechanisms of penetration by hydrophilic, polar compounds are not fully elucidated yet.

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[7] Water miscible/soluble compounds such as mineral salts, chelates or complexes.

Permeability of stomata and other plant surface structures

The potential contribution of stomata to the penetration of leaf-applied chemicals has been a matter of controversy for many decades (Dybing and Currier, 1961; Schönherr and Bukovac, 1978; Turrell, 1947) and is still not fully understood (Fernandez and Eichert, 2009). Early studies aimed at assessing the process of stomatal uptake suggested that it may occur *via* infiltration i.e. the mass flow of foliar-applied solutions into the leaf interior through the open stomata (Dybing and Currier, 1961; Turrell, 1947); Middleton and Sanderson, 1965). However, Schönherr and Bukovac (1972) showed that the spontaneous infiltration of an open stoma by a foliar-applied aqueous solution could not occur in the absence of an external pressure or a surface-active agent that could lower the surface tension of the solution below a certain threshold (set to 30 mN m^{-1}). Subsequently, many studies have provided evidence for increased uptake rates of plant surfaces where stomata are present, especially when the prevailing experimental conditions were favourable to the opening of the stomatal pores (Eichert and Burkhardt, 2001; Fernandez and Eichert, 2009). Investigations carried out on leaves containing stomata only on the abaxial leaf surface demonstrated higher foliar penetration rates through the abaxial as compared to the adaxial side (Eichert and Goldbach, 2008; Kannan, 2010). Since this observation contradicts the premise of Schönherr and Bukovac (1972) that the higher penetration rates associated with stomatal opening could not be due to the mass flow through the stomatal pores unless the solution's surface tension is below 30 mN m^{-1}, several different hypotheses have been proposed to explain these subsequent observations. For instance, the higher penetration rates in the presence of stomata have been attributed to the increased permeability of the peristomatal cuticle and the guard cells (Sargent and Blackman, 1962; Schlegel and Schönherr, 2002; Schlegel *et al.*, 2005; Schönherr and Bukovac, 1978) but no conclusive evidence supporting this has been forthcoming so far (Fernandez and Eichert, 2009).

The direct contribution of stomata to the process of penetration by foliar-applied aqueous solutions in the absence of surface-active agents has been subsequently re-assessed (Eichert *et al.*, 1998) in investigations on stomatal uptake performed with water-suspended hydrophilic particles (43 nm and 1 μm diameter respectively) using confocal laser scanning microscopy which demonstrated that the treatment solution passed through the stomata by diffusing along the walls of the stomatal pores (Eichert and Goldbach, 2008). This process was reported to be slow and size selective since particles with a diameter of 1 μm were excluded while the 43 nm particles passed into the pores.

The mechanisms of solute movement into fruits has received only limited investigation though several studies have estimated the permeability of apples to Ca solutions either with intact fruits (Mason *et al.*, 1974; Van Goor, 1973), fruit discs (Schlegel and Schönherr, 2002) or isolated cuticular membranes (Chamel, 1989; Glenn and Poovaiah, 1985; Harker and Ferguson, 1988; Harker and Ferguson, 1991). Schlegel and Schönherr (2002) reported a major contribution of stomata and trichomes to the uptake of surface-applied Ca-containing solutions during the early developmental stages of fruits. However after June drop the disappearance of stomata and trichomes

and the sealing of the remaining scars by cutin and waxes may significantly reduce the permeability of fruit surfaces.

There have been a few instances of the assessment of the contribution of trichomes or lenticels on fruits to the process of uptake of surface-applied nutrient solutions. Harker and Ferguson (1988) and others (Glenn and Poovaiah, 1985; Harker and Ferguson, 1991) suggested that lenticels in mature apples were preferential sites for the uptake of Ca solutions through the fruit surface though this possibility has not been assessed in detail so far.

- Stomata may play a major role in the absorption of nutrient solutions applied to the foliage.
- The mechanisms of stomatal penetration by pure water are not yet fully elucidated but recent evidence points towards a process of diffusion along the stomatal pore walls.
- Addition of certain surfactants to the nutrient solution formulation leads to the infiltration of stomata (Chapter 3).

2.3. Conclusions

The state-of-the-art concerning the process of uptake of solutions by plant surfaces has been described in Chapter 2. Plants are covered by a hydrophobic cuticle that controls the loss of water, solutes and gases to the environment though conversely it also prevents their unrestrained entry into the plant interior. The structural and chemical features of the plant surface render it difficult to wetting and therefore permeation by a surface-applied polar nutrient solution. In the light of the current state of knowledge, the following certainties, uncertainties and opportunities for the application of foliar fertilizers can be addressed.

Certainties
- Plant surfaces are permeable to nutrient solutions.
- The ease by which a nutrient solution may penetrate into the plant interior will depend on the characteristics of the plant surface, which may vary with organ, species, variety and growing conditions, and on the properties of the foliar spray formulation applied.
- Plant surfaces usually possess a hydrophobic coating provided by the epicuticular waxes.
- The micro- and nano-relief associated with the structure of the epidermal cells, and the epicuticular waxes deposited onto the surface, together with the chemical composition of these waxes, will determine the polarity and hydrophobicity of each particular plant surface.

- Epidermal structures such as stomata and lenticels, which can be present on the leaves and fruits surfaces, are permeable to surface-applied solutions and may play a significant role in its uptake.
- Apolar, lipophilic substances have been found to cross cuticles via a solution-diffusion process.

Uncertainties

- The mechanisms of cuticular penetration of polar, hydrophilic compounds (i.e. those relating to the uptake of aqueous foliar fertilizers) are currently not fully understood.
- The contribution of the stomatal pathway to the foliar uptake process should be further elucidated as well as the role of other epidermal structures such as trichomes and lenticels.
- Improvingthe effectiveness of foliar fertilizers will require a better understanding of the contact phenomena at the interface between the liquid (i.e. the foliar fertilizer formulation) and the solid (i.e. the plant surface).
- The effectiveness of foliar nutrient treatments will improve once the mechanisms of foliar uptake are better understood.

Opportunities

- Multiple scientific experiments and applied studies carried out in the last century have shown that plant surfaces are permeable to foliar nutrient fertilizers.
- This permeability presents the opportunity to supply nutrients to plant tissues and organs, bye passing root uptake and translocation mechanisms which may limit the nutrient supply of the plant under certain growing conditions.
- Foliar fertilization has great potential and should be further explored and exploited in the future.