

# A Review of the Factors Influencing the Absorption and Efficacy of Lipophilic and Highly Water-Soluble Post-Emergence Herbicides

Fabio Stagnari

Department of Food Science, University of Teramo, Via Carlo Lerici, 1, I-64023, Teramo, Italy

Correspondence: [fstagnari@unite.it](mailto:fstagnari@unite.it)

## ABSTRACT

It is well known that post-emergence herbicide uptake into plant foliage and efficacy varies with plant and chemicals, and can be greatly influenced by adjuvants and environmental conditions. The penetration of herbicides into plant leaves is related to the physicochemical properties of the active ingredients, especially molecular size and lipophilicity. For a specific herbicide, uptake varies greatly with plant species and there is no simple method at the moment to quickly evaluate the leaf surface permeability of a plant. Furthermore, current evidence suggests that highly-water soluble, ionic herbicides may be more sensitive to some environmental conditions (low humidity and rapid drop drying) than lipophilic herbicides. Various adjuvants are being used to increase the penetration of herbicides into target plant foliage, but their effect varies with chemicals and plant species. The mechanisms of action of adjuvants in enhancing herbicide uptake remain unclear despite the effort made during the last three decades. A better understanding of the transcuticular transport of herbicides and the mode of action of adjuvants should lead to a more rational use of herbicides and minimize their negative impact on the environment.

**Keywords:** adjuvants, cuticular waxes, herbicide uptake, pesticides

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## INTRODUCTION

In the case of foliage-applied post-emergence herbicides, delivery of active ingredients to the target site depends on the efficiency of several inter-related factors including the efficiency of cuticle retention and penetration, absorption into the leaf tissues, translocation from the sites of absorption to the sites of action in cells, and biochemical and biophysical reactions in the target organisms (Kirkwood 1991). How each of these factors influences uptake and efficiency is only partially understood, despite an urgent demand for efficient use of pesticides. In fact, it is estimated that of the total amount of pesticides applied for weed control only a very small part (<0.1%) actually reaches the site of action (Pimentel 1995), with the larger proportion being lost via spray drift, off-target deposition, run-off, photodegradation and so on. It is clear that, although post-emergence

herbicides remain indispensable in agriculture in the foreseeable future, great potential still exists to improve their efficacy, and consequently their costs and impact on environment and food chain.

In agriculture plant cuticles often represent the major barrier, which has to be overcome, when herbicides are sprayed on to leaf surface and poor performance of foliar applied herbicides has been often attributed to limited cuticular penetration. Water and many herbicides and xenobiotics are non ionic, and with the exception of water are lipophilic. A very well established theory and a series of experimental techniques are available to predict and measure the permeability of the cuticle to water and to lipophilic molecules (Schönherr and Riederer 1989; Niederl *et al.* 1998). Significantly less is known about the permeability of the cuticle to water-soluble herbicides and ionic compounds in general, although recently significant progress has been

obtained in measuring cuticular penetration quantitatively (Schönherr 2000, 2001, 2002; Schlegel *et al.* 2004; Popp *et al.* 2005).

A concrete possibility to improve herbicides efficacy is the use of adjuvants. In fact it is well known that adjuvants, especially surfactants, can significantly improve the efficacy of herbicides in many ways (Bukovac *et al.* 1993; Foy 1993; Kirkwood 1993; Schönherr and Baur 1994). It is generally recognized that not all surfactants can increase the uptake of any herbicide. At present, there is no practical theory or comprehensive model that can predict quantitatively the effect of a particular surfactant on the uptake of any herbicide.

This review will first explain the structure of the plant cuticle with particular reference to the role of waxes. It will subsequently give an overview of the factors involved in the weed's foliar uptake which affect efficacy of lipophilic and highly water-soluble post-emergence herbicides. Particular attention will be given to the role of adjuvants in enhancing the performance of the active ingredients.

## CUTICLE STRUCTURE AND PROPERTIES

On the surface of the leaves of higher plants there is a layer of waxy material known as the cuticle. It is theorized that, after plants migrated from the sea onto land 400 million years ago, cuticles evolved as a means of reducing water loss to the surrounding air (Stewart 1993). Although cuticle composition and structure of modern higher plants vary greatly from species to species (Holloway 1982b), it is generally less than 1  $\mu\text{m}$  thick although it can vary from 0.1-20  $\mu\text{m}$  (Jeffree 1996) and it is translucent for photosynthetically active radiation. The major structural model is a bilayer cuticular membrane (CM) in which the two layers are distinguishable by their ontogeny, ultrastructure and chemical composition (Jeffree 1996). The inner layer is composed of cutin, long chain alkyl ketones, alcohols, embedded wax and closest to the cells, polysaccharides; the outer region consists of epicuticular wax. Among the constituents, lipids are dominant in terms of weight and the two major classes are insoluble high molecular weight polyester cutin and solid waxes. Some species contain also another type of biopolymer, cutan which unlike cutin is non-saponifiable (Nip *et al.* 1986).

Cutin forms the matrix of the cuticle and is a polyester-type biopolymer which is mainly composed of two families of hydroxy and hydroxyepoxy fatty acids derived from the most common cellular fatty acids, as well as from C16 saturated and C18 unsaturated fatty acids (Holloway 1982a). The chief members are 16-hydroxy and dihydroxy hexadecanoic acids. The C18 family is represented by unsubstituted and 18-hydroxy octadec-9-enoic acids, 18-hydroxy-9-epoxy, and 9,10,18-trihydroxy octadecanoic acids. The monomers are cross-linked by ester and, in some species, by nonester bonds, although the exact chemical nature has not been clarified (Riederer and Schönherr 1988). Among the

esterified fatty acids of the cutin there are free hydroxyl and carboxyl groups; thus there is an overall "hydrophilic/lipophilic balance" in cutin. The composition and amount per unit area of cutin vary considerably among plant species and may differ depending on plant organs and ontogenetic states (Riederer and Schönherr 1984, 1988).

Cuticular solid waxes are the second major components and comprise epicuticular and embedded waxes. The epicuticular waxes here are used in a broad sense to include, in addition to ester of long-chain fatty acids with long-chain primary alcohols, cyclic compounds such as pentacyclic triterpenoids (Riederer and Markstädter 1996). The aliphatic fraction of epicuticular waxes consists of a complex mixture of compounds having a hydrocarbon backbone with 21 to >40 carbon atoms. The major compound classes of plant cuticular waxes are *n*-alkanes (chain-length  $\text{C}_{21}\text{-C}_{35}$ ) and smaller proportions of iso and anteiso homologues. However, a small percentage (~10 to 20%) are other substances such as primary alcohols ( $\text{C}_{22}\text{-C}_{40}$ ), fatty acids ( $\text{C}_{20}\text{-C}_{24}$ ), aldehydes ( $\text{C}_{24}\text{-C}_{36}$ ), secondary alcohols ( $\text{C}_{21}\text{-C}_{35}$ ), with a tendency for mid-chain hydroxylation, ketones ( $\text{C}_{21}\text{-C}_{35}$ ),  $\beta$ -diketones ( $\text{C}_{22}\text{-C}_{36}$ ), and *n*-alkyl esters ( $\text{C}_{36}\text{-C}_{60}$ ) resulting from the combination of long-chain primary alcohols and fatty acids (Baker 1982). In many cases, one compound dominates the total composition of the cuticular wax. In addition to the aliphatic portion of the wax, various amounts of cyclic compounds, such as pentacyclic triterpene acids, triterpene alcohols, triterpene ketones, and triterpene esters, as well as hydro-xycinnamic acid derivatives, flavonoids, and their respective glycosides, may be present. The embedded or intracuticular waxes, consist principally of fatty acids (Baker 1982). However their exact composition seems to be uncertain due to the problems of interspecific variation in extraction yields, in surface yields, and discrimination in favour of certain wax constituents.

The quantitative coverage and qualitative composition of the total wax as well as those of the epicuticular layer may vary according to a number of exogenous and endogenous factors. They may vary with season, as it was observed in *Baccharis linearis* where in the winter the production of epicuticular waxes is at minimum level with the relative percentage of less polar compounds (waxes and hydrophobic solids) that increases in comparison to the other seasons, while in the summer the production of epicuticular waxes is very high with polar solids (triterpenes, flavonoids, diterpeneacids) present in higher concentrations (Gülz and Müller 1992; Faini *et al.* 1999; Jenks *et al.* 2002). The temperature has been demonstrated to induce significant changes in cuticular composition: low temperatures increases leaf epicuticular waxes content, while increasing air temperature decreases leaf epicuticular waxes content in particular the aldehyde accumulation (Faini *et al.* 1999; Dodd and Afzal-Rafii 2000; Dodd and Poveda 2003). The light have also been proven to influence the composition of total waxes (Reed and Tukey 1982; Steinmüller and Tevini 1985; Upadhyaya and Furness 1994; Shepherd *et al.* 1995). Barnes *et al.*

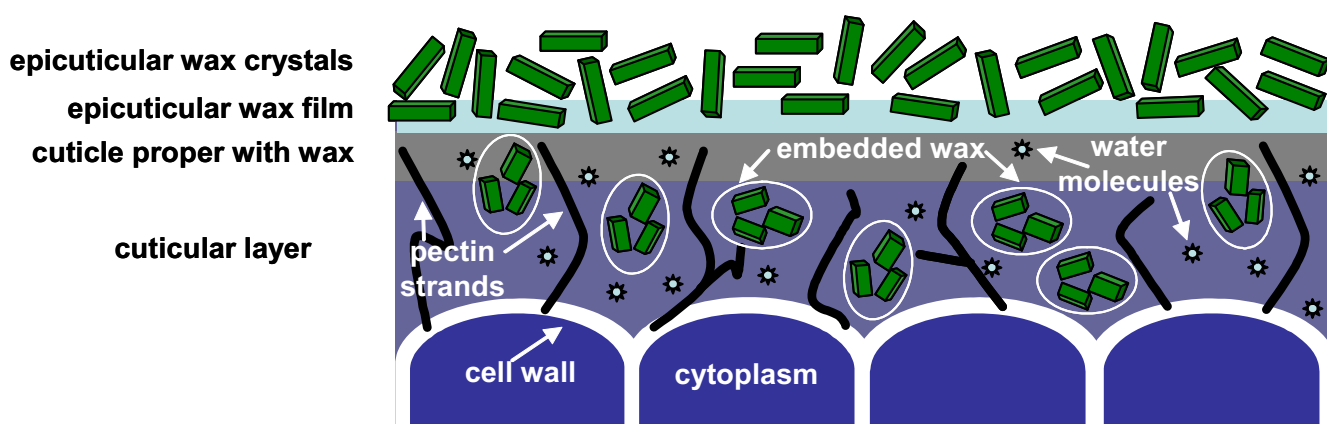


Fig. 1 Scheme showing the layering of the plant cuticle. The features displayed are not to scale.

(1996) found that in *Nicotiana tabacum* L. leaves that UV-B radiation reduces the amount of wax deposited on the adaxial leaf surface and modifies wax composition; the increased branching and synthesis of shorter n-alkane and br-alkane homologues provides further evidence of a direct and highly specific action of UV-B radiation on wax biosynthesis (Steinmüller and Tevini 1985). In general low light intensity decreases epicuticular waxes content compared with medium light intensity and increases fatty acid and aldehyde content while decreases primary alcohols and esters (Shepherd *et al.* 1995). Erosion by wind and rain can also influence wax amounts and the density of cuticular wax blooms (Baker and Hunt 1986; Hadley and Smith 1989). So far five basic types of cuticle have been identified: smooth, ridged, papillose, glaucous (having an additional covering of microcrystalline wax), and glandular where trichomes are present in high number and comprise the main "surface" of the leaf (Holloway 1993). The presence of epicuticular waxes, trichomes, leaf venations causes leaf surface microroughness (Fig. 1).

The plant cuticle has an overall negative charge at physiological pH values and the iso-electric pH of many cuticles is about 3 (Bukovac and Norris 1968).

### MODEL SYSTEMS FOR UPTAKE OF FOLIAR APPLIED HERBICIDES

Numerous approaches have been used to study foliar-applied herbicides uptake. Among the studies carried out at intact plant level two experimental techniques are commonly used. The first one consisting in spraying herbicide and observing the effects, the second one involving the use of radiolabelled active ingredients and measuring the amounts taken up by the leaves. In the former, the rates of herbicide uptake and dose-response curves are not known and the response depends on many other factors besides foliar penetration. In the latter, radiolabelled, active ingredients are typically applied as small water solution droplets, and since water evaporates quickly and solutes penetrate into the leaves, the concentration of solutes in the droplets changes in an unknown manner. Moreover, the amount of the radiolabelled active ingredient which have not yet penetrated the cuticle is estimated by washing the treated area with water or solvent or by using a stripping technique. Using this latter approach, solutes contained in epicuticular waxes are removed as well, while solutes in the cuticle are considered to have penetrated. These procedures make the term "foliar penetration" rather ambiguous because it is not well established which fraction of the solute is contained in the compartments on the leaf. The problems associated with these types of experiments mean that the rates of penetration cannot be related to properties of cuticles and active ingredients, since rates of penetration are ambiguous and the driving forces are not known.

Intact plants are complex systems and such studies may be of limited values in understanding the mechanisms involved in foliar penetration. Attempts to overcome these limitations have led to the use of cuticles isolated from leaves and fruits as model systems, excised leaf, leaf discs and recrystallised cuticular waxes.

With isolated cuticles a separation of variables has been accomplished and rates of penetration have successfully been related to the properties solutes and molecules. Working with isolated plant cuticles does however present some limitations. Namely astomatous cuticles (stable enough for handling) can be isolated only from a very limited number of plant species, permeability of isolated cuticles tend to decrease during storage, and isolated cuticles are a static system which makes it difficult to investigate dynamic aspects of cuticular permeability. Nevertheless, it represents a very powerful analytical tool. When working with isolated plant cuticles variables may be imposed without altering physiological systems; detailed kinetics of penetration can be readily monitored and interaction between penetrant and cuticle identified and quantified. Data obtained with such

systems are useful in defining the penetration process and quantitative transport parameters (e.g. partition, diffusion, and permeability coefficients) can be calculated.

Studies on excised leaf, although with significant limitations, have led to important findings on uptake of organic compounds (Schreiber and Schönherr 1992a, 1992b, 1993).

In the studies with leaf disc, glass ring are fixed with lipophilic adhesives to the surface of intact leaf discs and comparatively large volumes of donor solution pipetted into the glass rings. The area across which uptake occurs and the concentration of pesticide in the donor solution are kept constant during the course of the experiment; however this method is limited to relatively hydrophilic herbicides, since lipophilic compounds tend to adsorb to the lipophilic adhesive.

The use of recrystallised leaf waxes has been reported by Schreiber and Schönherr (1993b), Schreiber (1995) and Schreiber and Riederer (1996). The latter, from their investigations, concluded that: the model system using recrystallised leaf waxes reflected transport properties of intact CM, the barrier properties of intact cuticles were almost solely a function of cuticular waxes, the waxy-transport limiting barrier of the cuticle functioned like a sorption diffusion-membrane. This experimental system could be used to determine cuticular permeability in the case of stomatal cuticles or when cuticle isolation is not possible.

Undoubtedly, several *in-vitro* studies, using isolated cuticles, isolated waxes and detached leaves, have been useful to develop experimental models enabling calculation of solute flow across the CM using partition, diffusion coefficients and rate constants of desorption. Moreover, *in-vitro* studies on interaction adjuvants/active ingredients/plant cuticles have released important practical implications about solubility and diffusivity of AI in the cuticle waxes and about the influence of adjuvants in herbicide foliar uptake. For example, Schönherr (1993) studying the effects of alcohols, glycols and ethoxylated alcohols on the mobility of 2,4-D in isolated plant cuticles found that alcohols, and ethoxylated alcohols are sorbed in cuticular waxes and plasticize them and those having 6 and 10 carbon atoms are powerful accelerator adjuvants. Schönherr and Baur (1996) examined the effects of some accelerator adjuvants on penetration of 2,4-D on isolated cuticles; they observed that providing maximum driving forces are realised, rates of penetration of organic compounds can be further increase by adding accelerator adjuvants though they are influenced by properties of the wax, molar volumes of accelerators and intrinsic accelerating properties of the adjuvants. *In-vitro* studies have also been determinant to understand the role of temperature on cuticular wax and mobilities of organic solutes in cuticular membranes. In studies carried out on cuticular membranes isolated from mature leaves of a range of a species, (Baur *et al.* 1997) over a temperature range of 15 to 78°C solute mobilities increased >1000-fold and variability between cuticles as well as the effect of the molecular size of active ingredients decreased with increasing temperatures. Thanks to *in-vitro* approaches, knowledge on selectivity of plant cuticles to the mobilities of organic molecules and ionic compounds have been largely improved. It was convincingly shown that for organic substances a 4-fold increase in molecular weight resulted in a decrease of the mobility by a factor >1000 (Buchholz *et al.* 1998) and that also charged molecules can diffuse across isolated cuticles (Schönherr 2000, 2001, 2002; Schlegel *et al.* 2004).

Although with some limitations, *in-vivo* studies have complemented *in-vitro* research providing an overview of the steps which ultimately results in target-site delivery. Principally, this type of research has enabled examination of the uptake and distribution of <sup>14</sup>C-labelled organic solutes with the role of the cuticle being less clearly focused. By studying a range of compounds of varying lipophilic solubility and surfactants of varying ethylene oxide Holloway *et al.* (1992) and Gaskin and Holloway (1992) provided data which supported the predictive response model originally proposed by Holloway and Stock (1990) and modified by

Stock *et al.* (1993) which allow optimisation of surfactant choice. Many experiments conducted at the whole plant level have been very useful in evaluating foliar absorption, translocation and efficacy of many active ingredients (Carlson and Burnside 1984; Striebig 1988; Anderson *et al.* 1993; Steckel *et al.* 1997). Furthermore the influence of environmental conditions on post-emergence herbicide efficacy could be detected thanks to whole plant bioassays (Wills and McWorther 1985; Kudsk *et al.* 1990; Oosterhuis *et al.* 1991; Snipes and Wills 1994; Coetzer *et al.* 2001 Ramsey *et al.* 2002).

Although *in-vitro* and *in-vivo* approaches have been invaluable in understanding the interaction between plant cuticle and absorption of organic compounds, future studies directed to bridge the apparent gap between the foliar uptake of active ingredients by whole plants and *in-vitro* cuticle model systems are needed.

## PATHWAYS FOR UPTAKE OF FOLIAR APPLIED HERBICIDES

Cuticular penetration of foliar applied herbicides as well as crop protection agents involves a large number of variables and it is a much more complex process than uptake of chemicals from diffuse environmental sources. Uptake of herbicides proceeds from non-aqueous formulation residues and both the composition and volumes of these residues are likely to change with time; interaction between active ingredients and formulation constituents can play a major role in penetration; permeabilities of cuticles from different species can differ greatly; adjuvants and environmental conditions affect both driving forces of penetration and permeabilities of cuticles.

Plant cuticles are heterogeneous membranes and rates of diffusion of herbicides are governed by low solubility and low mobility in the waxy outer surface of the cuticle. Evidence of structural and functional heterogeneity of cuticles have been found in studies on double refraction polarised light and sorption of dyes across them (Sitte and Renner 1963; Holloway 1982; Tyree *et al.* 1990). Additional evidence for cuticle heterogeneity was obtained from bilateral desorption of 2,4-D from CM (Schönherr and Riederer 1988). With homogeneous membranes, rates of desorption would be equal on both sides and half of the total amount of 2,4-D would be desorbed from each surface. From total amount of 2,4-D sorbed initially in the CM of four plant species, 68-92% was desorbed from the inner surfaces and only 2-5% from the outer surfaces thus confirming high asymmetry of the cuticles.

Diffusion through the cuticle is a mass transport process that resembles transport of heat or charge. Although diffusion is a continuous event, for study and analysis cuticular penetration may be considered as consisting of three component processes: sorption on the one side, diffusion through, and desorption on the other side from the cuticle (Barrie 1968; Bukovac and Petracek 1993).

The amount of a substance that diffuses per unit area and time depends on the properties of the barrier and on the driving force. The basic equation for modelling cuticular penetration is

$$J = D \times \left( \frac{K}{\Delta X} \right) \times (C^2 - C^1) = D \times K \times \left( \frac{\Delta C}{\Delta X} \right) \quad (1)$$

where  $J$  is the flux (mole) that diffuses per unit area ( $m^2$ ) and time (s),  $D$  represents a proportionality constant, the diffusion coefficient of the compound in the cuticle,  $K$  the partition coefficient,  $\Delta C$  is concentration gradient between the inner and outer compartment,  $\Delta X$  the thickness of the cuticle.

**Sorption.** Sorption, a term used instead of absorption and adsorption because it is not specific and does not imply the location or the nature of the interaction. It represents the

initial interaction of an applied herbicide with the plant cuticle. It is usually quantified by the difference method (Kipling 1965) or directly by measuring the concentration of an applied herbicide in the cuticle after removal of the surface material. Data can be expressed as a) amount of solute per mass of cuticle or b) distribution or partition coefficient ( $K$ ) which is the ratio of the concentration of solute in the cuticle to the concentration in the solvent at equilibrium:

$$K_{\text{cuticle/solvent}} = \frac{\text{Concentration in the cuticle}}{\text{Concentration in solvent}} \quad (2)$$

$K$  first governs the ability of an herbicide to diffuse into the cuticle and if the solute is more soluble in the cuticle than in solvent, then  $K > 1$ . Plant cuticles are lipid membranes so it is very useful to define the lipophilic/hydrophilic properties of herbicides in order to understand their penetration properties across cuticles. These characteristics can be approximated by the water solubility of the herbicide, but a more accurate assessment is carried out by determining the 1-octanol/water partition coefficient ( $K_{o/w}$ ) (Hansch and Leo 1979). Water solubilities and the  $K_{o/w}$  are inversely proportional (Leo *et al.* 1971; Mackay *et al.* 1980); lipophilic herbicides have high  $K_{o/w}$  values while hydrophilic herbicides have low  $K_{o/w}$  values which are often expressed as  $\log K_{o/w}$  with commercial herbicides falling within the range of -5 (very hydrophilic) to +5 (very lipophilic).

Direct experimental measurement of the cuticle/solvent partition coefficient  $K_{c/w}$  is feasible only for a small fraction of the large number of chemicals. Therefore, a quantitative property-property relationship (QPPR) was established between the  $K_{c/w}$  and the  $K_{o/w}$  (Schönherr and Riederer 1989)

$$\log K_{\text{cuticle/solvent}} = 0.057 + 0.970 \times \log K_{o/w} \quad (r = 0.987) \quad (3)$$

**Transcuticular transport.** Most of the data on cuticular penetration of foliar-applied herbicides have been generated using various methods, among which are the determination of cuticle/water partition coefficient and the determination of permeances using steady-state diffusion in the system water/cuticular membrane/water. In these studies (infinite dose-system studies) the kinetics of diffusion across cuticle is readily determined. Flow divided by the product of the area ( $A$ ) through which diffusion is taking place and the concentration gradient across the cuticle ( $\Delta C$ ) yields permeances ( $P$ ):

$$P = \frac{F}{A} \times \Delta C \quad (4)$$

$P$  is also obtained by the following equation:

$$P = D \times \frac{K_{m/l}}{\Delta X} \quad (5)$$

$P$  is a useful parameter, since it characterises the permeability of membranes of unspecific thickness for a given pesticide molecules. It contains information about solute mobility ( $D$ ), differential solubility of solute in the membrane and in adjacent solutions ( $K_{m/l}$ ) and the lengths of the diffusion path in the membrane ( $\Delta X$ ). Recent studies have modified equation 5 by adding the term tortuosity of the diffusional path length ( $\tau$ ):

$$P = D \times \frac{K_{m/l}}{\Delta X} \times \tau \quad (6)$$

The diffusion coefficient ( $D$ ) is a measure of the mobility of the compound within the cuticle. In homogeneous

membrane it can be calculated as (Helfferich 1962):

$$D = \frac{(\Delta X)^2}{6 \times te} \quad (7)$$

where  $\Delta X$  is the thickness of the cuticle and  $te$  is the hold-up time defined as the intercept of the extension of the steady-state straight line with the time axis on a plot of total amount diffused versus time.

The concentration gradient ( $\Delta C$ ) of post-emergence herbicides between the inner (receive phase) and the outer surface (donor side) of the cuticle represents the driving force in cuticular penetration. The receiver phase is represented by water in the epidermal cell wall. The maximum concentration of active ingredients (a.i.) in the cell wall depends on their water solubility, on the concentration of the a.i. in the cuticle and on the cuticle/cell/wall penetration coefficient. The driving force on the donor side depend on the concentration in the formulate residue ( $C_{fr}$ ) and on the differential solubility of a.i. in the formulation residue and in the wax of the outer side of cuticle, as expressed by the partition wax/formulation residue ( $K_{w/fr}$ ). Driving force is maximum when the formulate residue is a saturated solution of active ingredients.

New techniques for cuticular penetration analysis of foliar-applied herbicides have also been used, called simultaneous bilateral desorption (BIDE), unilateral desorption from the outer surface (UDOS) and simulation of foliar uptake (SOFU). The membrane model is laminar made-up of a thin outer layer normally called "limiting skin" and a thick inner layer called sorption compartment (soco). In BIDE, cuticular membranes are loaded with radio-labelled solutes and, after drying, they are inserted into an apparatus which permits simultaneous desorption from the inner and the outer surface of the CM separately (Schönherr and Riederer 1988). Desorption is initiated by suddenly adding to both surfaces of the CM a phospholipids suspension as desorption medium.

Procedures used in UDOS (Bauer and Schönherr 1992) are very similar, but with this technique all the solute sorbed in CM must diffuse through the limiting skin. The UDOS method is limited to a very lipophilic solutes with a rate constant of penetration ( $K > 100$ ). In SOFU experiments, discrete amounts of radiolabeled solutes (and surfactants) are applied to the outer surface of the CM. The water evaporates quickly leaving a solution of solute in a neat hydrated surfactant. From this residue, surfactant homologues and radio-labelled solutes diffuse into and through the CM, each at its individual velocity. Eventually, they are collected in the receiver solution and are removed from the system. This process leads to decreasing amount of both solutes and surfactants in the surface and to transient concentration gradients in the CM.

All the models where plant cuticles are normally considered as solubility/mobility barriers work well for lipophilic molecules. When the principles established with these molecules are extended to water-soluble, ionic herbicides with  $K_{o/w}$  coefficient  $< 1$ , the model predicts much lower rates of permeance than observed (Price 1983). As results of this discrepancy, it was suggested that ionic compounds crossed the cuticle by a different pathway which is hydrophilic or polar in nature. In contrast to lipophilic molecules ionic compounds have only recently been extensively analysed for cuticular permeability (Schönherr 2000, 2001; Schönherr and Lubert 2001; Schönherr 2002; Schlegel *et al.* 2004; Schönherr and Schreiber 2004).

Since charged molecules are not soluble in the lipophilic cutin because of their hydration shells (Stein 1967), and due to the fact that the experiments convincingly shown that these molecules can diffuse across cuticles, it must be calculated that charged compounds use alternative routes of diffusion. It was postulated in the late 1970's that polar paths of diffusion must exist, as already emerged from a long-standing debate that theorized polar pores for cuticular

permeability to water and polar solutes (Lyshede 1978; Hock 1979; Majer-Mercker 1979). Using dewaxed leaf cuticles from 14 different plant families, 27 out of 30 taxa were found to have "ubiquitous" visible, discrete, natural cuticular pores and transcuticular canals (Miller 1985). Charged organic molecules penetrate CM independently of temperature (Schönherr 2001; Schönherr and Lubert 2001) and plasticizers (Schönherr 2000) and only slightly affected by wax extraction (Schönherr 2000). Wax extraction of ivy, pepper, holly and tomato cuticles only slightly enhanced glyphosate movement while lipophilic isoproturon movement was greatly increased (Santier and Chamel 1998).

Size selectivity for penetration of ionic compounds is significantly less pronounced than that for lipophilic molecules. A 4-fold increase in molecular weight resulted in a 2-fold decrease in rate of cuticular penetration of  $Ca^{+2}$  for *Vicia faba* leaves (Schegel *et al.* 2004), whereas size selectivity of lipophilic compounds was three order of magnitude higher in this range of molecular weights (Schreiber 2005). Below a critical threshold value of the molar volume, amounting to  $110 \text{ cm}^3 \text{ mol}^{-1}$ , diffusion across hydrophilic pathways is even faster than diffusion across lipophilic pathways (Popp *et al.* 2005). The radius of polar pores in cuticular membranes is specific for plant species.

The polar domains forming the postulated polar path of diffusion and those sites of the lipophilic cuticle sensitive to humidity could be formed by polar non-esterified hydroxyl and carboxyl groups (Schönherr and Huber 1977). This is confirmed by recent experiments showing that the effect of humidity on cuticular transpiration was reduced by 50% after methylation of carboxy groups in plant cuticles (Schreiber *et al.* 2001). Alternatively, polar paths within the lipophilic plant cuticles could consist of a reticulum of polysaccharide microfibrils ramifying and stretching through the cuticular membrane (Jeffrey 1996). The mean apparent pore radius seems to be of 0.3 nm with a standard deviation of 0.02 nm in *Hedera helix* leaf cuticular membranes (Popp *et al.* 2005).

## FACTORS AFFECTING UPTAKE OF FOLIAR APPLIED HERBICIDES

### Factor concerning xenobiotics

#### Lipophilicity

Lipophilicity is probably the single most important property of herbicides related to foliar uptake. It indicates the solubility of the penetrating compounds within the transport-limiting barrier of the plant cuticle and it is normally described as the partition coefficient between the plant cuticle or the wax phase of the cuticle and the adjacent aqueous phase ( $K_{c/w}$  and  $K_{w/w}$ ) (Schoherr and Riederer 1989). Due to the lipoidal nature of the epicuticular wax and the cuticle, foliar uptake tends to increase with increasing lipophilicity of the chemicals. Lipophilicity of compounds greatly affects permeability coefficients (P) of membranes, since P is proportional to cuticle/water partition coefficient ( $K_{c/w}$ ) and to solute mobility in cuticular waxes (Kerler and Schonerr 1988; Schönherr and Riederer 1989; Schönherr and Baur 1994). Since for lipophilic herbicides cuticular waxes form the main limiting barrier of the cuticular membrane, as permeance increases by up to three order of magnitude when cuticular waxes are extracted (Schönherr Baur 1994), as well as the initial compartment during sorption process (Riederer and Schreiber 1995) it has been suggested that  $K_{w/w}$  would describe better than  $K_{c/w}$  the sorption of lipophilic herbicide into cuticular membranes (Schönherr and Baur 1994). Conversely in transport through cuticles  $K_{c/w}$  is much more realistic due to the fact that cuticular transport across the whole membrane.

In the past the use of octanol:water partition coefficient ( $K_{o/w}$ ) to estimate lipid solubility and therefore cuticular sorption of herbicide molecules appeared of limited values (Chamel 1986). New findings on foliar sorption of lipophi-

lic herbicides have clearly demonstrated that  $K_{o/w}$  can be successfully used as good predictors for  $K_{c/w}$  (Kerler and Schonerr 1988) as well as for  $K_{w/w}$  although this coefficient results about one order of magnitude lower than  $K_{c/w}$  (Popp *et al.* 2005). For foliar applied herbicides,  $K_{o/w}$  has been shown to be a critical factor influencing directly or indirectly the process leading to tissue accumulation at target sites (Bromilow and Chamberlain 1991). High correlation between the amount of sorbed herbicides with their  $K_{o/w}$  was found by Evelyne *et al.* (1992) who studied the sorption process of five phenylureas by tomato and pepper fruits cuticles.  $K_{c/w}$  and  $K_{w/w}$  are useful indicators to an higher or lower herbicide accumulation at least during the earlier phase following herbicide application (sorption process), when excess of water is present (Baur *et al.* 1997) but the solid state restricts the validity of correlation between  $K_{c/w}$  and  $K_{o/w}$  to moderate amounts sorbed where  $K_{c/w}$  is below 10% mass of the cuticle (Schönherr and Riederer 1989).

However,  $K_{o/w}$  is not the only property that determines the foliar penetration. Some authors (Baker *et al.* 1992) found, on the uptake data obtained with 26 chemicals and 4 plant species, only a moderate correlation ( $R^2$ : 24-45%) between  $K_{o/w}$  and foliar uptake of chemicals. Therefore it would be wrong to conclude that all lipophilic compounds are taken up faster by plant foliage than hydrophilic ones. For hydrophilic compounds  $K_{o/w}$ ,  $K_{w/w}$  cannot be used as predictors for sorption process into cuticular membranes, as transport across cuticular wax barrier is not relevant.

### Size (molecular weight)

According to the first experiments on molecular weight and size of chemicals, the molecular size and spatial configuration did not seem to influence cuticle penetration (Goodman and Addy 1962). Later, Whitecross and Mercer (1972) observed that the permeability of the cuticle to the strain chain alcohols and amides was directly proportional to the size of the molecule, with the exception of the first member of each series. It was also reported that small changes in molecular structure greatly influenced efficiency of transcuticular movement: the addition of the butyl carbamoyl group at the 2-position of carbendazim was seen to increase uptake through isolated cuticles (Solel and Edgington 1973). Bauer and Schönherr (1992) while investigating the effect of solute size on mobilities of 11 not aliphatic compounds in plant cuticles, found a good correlation ( $r=0.87$ ) between  $\log$  of first order rate constant of desorption ( $K^*$ ) and the logarithms of the molar volume ( $\log V_x$ ), where  $K^*$  is directly proportional to the diffusion coefficient. These results were confirmed by Schreiber and Schönherr (1993) who observed good correlation between  $\log D$  and  $\log V_x$ , with different slopes for aromatic and aliphatic compounds, in studies on diffusion coefficients in barley leaf wax. In isolated cuticle from *Citrus* and *Pyrus* leaves and *Capsicum* fruits an increase in the molar volume of  $100 \text{ cm}^3 \text{ mol}^{-1}$  decreased solute mobility by almost 10-fold (Baur *et al.* 1996). Baur *et al.* (1997) found a very pronounced dependence of solute mobilities on molar volumes, especially at low temperatures, and the mobility of the small molecules of salicylic acid ( $V_x = 99 \text{ cm}^3 \text{ mol}^{-1}$ ) in *Citrus grandis* leaf cuticular membrane was higher by a factor of more than 100 than that of the larger molecule, cholesterol ( $V_x = 349 \text{ cm}^3 \text{ mol}^{-1}$ ) (Baur *et al.* 1999).

In 1994 it was pointed out that correlation between  $\log K^*$  (or  $\log D$ ) and  $\log V_x$  is purely descriptive, while the slopes and y-intercepts of the graphs  $\log K^*$  vs.  $V_x$  have definite physical meaning (Schönherr and Baur 1994). The y-intercept represents the mobility of a hypothetical molecule of zero molar volume while the slope of the graph ( $\beta'$ ) is a measure of size selectivity of the barrier. On this base, experimental studies that measured the rate constants of desorption of solutes across the transport-limiting barrier of cuticular membranes for lipophilic pathways, showed that the size selectivity varied only a little between plant species obtaining an average size of  $0.0095 \text{ mol cm}^{-3}$  (Buchholz *et*

*al.* 1998). Similar size selectivities of cuticular membranes were also found for four species (*Citrus grandis* L., *Camellia assamica* L., *Ilex paraguayensis* St. Hil., *Prunus armeniaca* L., *Pyrus communis* L.) with an average size selectivity of  $8.9 \pm 2.4 \times 10^{-3} \text{ mol cm}^{-3}$ . In a more recent investigation (Popp *et al.* 2005) the registered values of size selectivities of cuticular membranes confirmed the findings reported by the previous studies ( $0.0088 \text{ mol cm}^{-3}$ ).

The size selectivity of cuticular membranes can be interpreted by the free volume theory claiming an exponential distribution of the free volume size. Accordingly, diffusion in the lipophilic pathway takes place in holes which temporarily emerge by the segmental motion of the aliphatic alkyl chains of cutin and wax fractions.

The plasmodesmata of plants can only allow free circulation of molecules smaller than 1 kDa (Oparka and Roberts 2001). It is just not a coincidence that most foliar-applied herbicide have a molecular weight between 100 and 500 (Tice 2001) as many out-ranged molecules would be eliminated at the screening stage if they cannot enter plant leaves or move between cells.

However, results from old studies suggests that other characteristics of the molecule such as polar nature of constituents atoms, potential hydrogen bonding ability and possible steric effects could interfere with the transport through cuticle (Darlington and Cirulis 1963).

### pH

Most post-emergence herbicides are weak organic acid compounds and the dissociation of the acid groups depends on the pH of the medium. The degree of dissociation influences the hydrophilic-lipophilic properties of the molecule to remain in a polar phase or to partition into a non polar phase (Norris and Bukovac 1972). At pH values lower than the pK, the molecules are primarily undissociated and more lipophilic, whereas at the pH values above pK, they are ionized and more hydrophilic. This influence the route for herbicide entry in the cuticles (Briggs and Bromilow 1994). The undissociated and hence more lipophilic form would be able to penetrate the cuticular barrier by simple molecular diffusion through the dominant lipophilic components, the ionized and more hydrophilic form due to their low partitioning into and through cuticle would have a lower rate of movement. Evidence that sorption of non-dissociated species is favoured and that little, if any, of the dissociated species is sorbed has given by Riederer and Schönherr (1984) and by Shafer and Bukovac (1989). Sorption and/or penetration of 2,4-D, DPA, dicamba and 2,4-D increases as the pH decreases (Bukovac and Norris 1968; Norris and Bukovac 1972; Schönherr 1976; Chamel 1986). It was also revealed with MCPA and MCPB that the degree of dissociation of the herbicide is important (Kirkwood *et al.* 1972), though some contradictions appeared in the results of these authors. However, most of the data supporting the abovementioned statement were mostly obtained with plant tissues immersed or cultured cells incubated in a solution of unformulated weak acid herbicides. Few reports deal with the effect of carrier pH on the uptake of weak acid herbicides, generally water-soluble salts, applied into plant foliage. Liu (2004) demonstrated that only when bentazone acid was used at extremely low concentration (0.002%), uptake into broad bean leaves was higher at lower pH whereas with increased concentration of bentazone acid or with application as sodium salt lower carrier pH did not provide greater uptake. Lower carrier pH produce more non-dissociated molecules more lipophilic but also with lower water solubility and could crystallize on the leaf surface after droplet dry-down, hence unavailable for uptake. The importance of a.i. solubilization was also observed for other herbicides. The dry particulate formulation of sulfonylureas are poorly soluble in acid spray mixtures, but if pH is increased, the particles readily dissolve and activity is enhanced (Woznica *et al.* 1996). Nevertheless, the optimum carrier pH to achieve maximum uptake for each class of weak acid pesticides remains to be

determined.

### Factor regarding cuticles

Cuticular waxes form the transport-limiting barrier of cuticular membranes (Rieder and Schreiber 1995). Permeabilities of cuticles from different plant species can vary greatly. Rieder and Schönherr (1985) found that 2,4-D permeance of cuticular membranes isolated from ten plant species varied with a factor of  $272 \text{ m s}^{-1}$ . Also in the case of isoproturon, removal of superficial waxes of isolated cuticles in different species had contrasting effect on the diffusion of the herbicide: a maximum diffusion was observed for pepper and tomato fruit cuticles, while no increase was found for rubber cuticles (Santier and Chamel 1998). From the arguments above, it follows that mobility (D) and solubility (K) in the waxes and hence quantity, chemical composition and/or physical arrangement of the wax cuticle for the various plant species are responsible for the most of the differences observed, while the path lengths ( $\Delta X$ ) make only minor contribution. This is confirmed by other studies which obtained no significant correlation between cuticle thickness and penetration of 2,4-D and some other compounds either for undewaxed cuticles or after chloroform extraction of waxes (Norris 1974).

The effect of extraction of cuticular waxes are to be solely attributed to an increase in the mobility of a molecule of zero molar volume, and it does not affect the size selectivity of the lipophilic pathway (Popp *et al.* 2005). This value can be regarded as a measure for the tortuosity of the diffusion path ( $\tau$ ) (Riederer and Schreiber 1995). In *Hedera helix*, cuticular waxes cause an extension of the diffusional pathway of 273. Since the size selectivity of the lipophilic pathway differs only slightly between plant species (Buchholz *et al.* 1998), tortuosity of the diffusional path length can be considered as the main reason for the species-specific variability of cuticular permeances (Baur *et al.* 1999).

With regard to wax amount of the cuticles, it seems better to consider the density of waxes within cuticles expressed as a percentage of the cuticle dry weight rather than the wax amount per unit surface area. This is supported by the fact that for ivy and tomato cuticles wax amount is about the same on a surface basis but it differs by a factor 3 when expressed on a weight basis and the penetration rates of isoproturon differed by a factor of 3.8 between this two species (Santier and Chamel 1998). Probably physical arrangement of the wax cuticle plays a more important role than wax amount and chemical composition in explaining differences in herbicide cuticular penetration among different species. In fact some studies report that in fruit cuticles isoproturon penetration increased considerably after the removal of superficial waxes while remained very low for rubber and ivy cuticles after 30 s cuticle immersion in chloroform (Santier and Chamel 1998). After this treatment the wax fraction remaining in the cuticles was 47 and 66% respectively; conversely, a greater increase was observed for holly cuticles after the same treatment, although 70% of soluble waxes were still present in the cuticle.

Differences in cuticle structure might explain some variation in cuticle permeability. It has been shown that diffusion of different organic compounds in cuticular membranes of various species occurs in region having similar physicochemical properties (Baur *et al.* 1997) and it appears that even water follow the same route, as suggested by several observations (Niederl *et al.* 1998). Studies in which the estimates of tortuosity was obtained with organic compounds varying widely in physicochemical properties, indicates that there is no compounds-specific path (length) in the cuticle or it has only a minor effect on solute mobilities (Baur *et al.* 1999). The increase tortuosity due to waxes depends on the crystallinity of waxes within the limiting skin which alternates with amorphous wax. Diffusion across the transport-limiting barrier is thought to occur only in the amorphous wax phase which provides a tortuous diffusion path across the wax while crystalline phase

can be regarded as an excluded volume with respect to diffusion (Schreiber *et al.* 1996).

The cuticle proper of CM is often found to consist of electron-dense and lucent lamellae with a number varying among species (Jeffree 1996); in these cases the increased path length for diffusion might be related to these lamellae since they form surface-parallel barriers that can increase the tortuosity. This effect was observed by Wattendorff and Holloway (1984) who observed that in the cuticle proper of *Agave americana* leaves, potassium permanganate diffuse much more rapidly in periclinal direction than in anticlinal. It is however not clear yet whether the electron-lucent lamellae contains crystalline waxes.

### External factors

#### Temperature

Plants are exposed to widely differing temperatures which, depending on environmental factors like wind velocity and insulation, on the leaf surface can be more than  $10^\circ\text{C}$  or lower than ambient temperatures (Lange and Lange 1963; Mellor *et al.* 1964; Kreeb 1974). Even under temperate climatic conditions the annual variation of leaf surface temperature may range from  $-20^\circ\text{C}$  to  $50^\circ\text{C}$  (Nito *et al.* 1979).

Temperature is the predominant physical factor influencing the permeance of a barrier. Two terms contributing to the permeance (see equation 5) are temperature dependent: the diffusion coefficient (D) of a molecule which increases with temperature, and the partition coefficient between the membrane and the adjacent phases which decreases with temperature. In fact, the Arrhenius equation for diffusion states that

$$D = D_0 e^{-\frac{E_D}{RT}} \quad (8)$$

where D ( $\text{m}^2 \text{s}^{-1}$ ) is the diffusion coefficient at temperature T (K),  $D_0$  the pre-exponential factor which is equal to D at infinite temperature,  $E_D$  ( $\text{J mol}^{-1}$ ) the activation energy for diffusion which is a measure of the energy expended against the cohesive forces of the medium in which diffusion occur, and R ( $\text{J mol}^{-1} \text{K}^{-1}$ ) the gas constant. While the partition coefficient is temperature-dependent according to

$$K = K_0 e^{-\frac{\Delta H_K}{RT}} \quad (9)$$

with  $K_0$  standing for the partition coefficient at infinite temperature and  $\Delta H_K$  for the enthalpy of phase transfer during partitioning. Taking into account equation 5 and 9 it follows that the temperature dependence of permeance is given by

$$p = p_0 e^{-\frac{E_p}{RT}} \quad (10)$$

where

$$E_p = E_D + \Delta H_K \quad (11)$$

Linear regression analysis of  $\ln p$  versus  $1/T$  yields  $E_p$  from the slope and  $p_0$  from the y-intercept. Temperature has a stronger effect on the diffusion than on partitioning; that is why permeances generally increase with increasing temperatures.

Also the rate constant of desorption results temperature-dependent with the relationship that can be quantified using the Arrhenius equation (Baur and Schönherr 1995)

$$K^* = K_0^* e^{-\frac{E_D}{RT}} \quad (12)$$

where  $K^*$  being the first rate constant of desorption,  $K_0^*$  is the pre-exponential factor. By plotting  $\log K^*$  ( $s^{-1}$ ) versus  $1/T$ , the activation energy of diffusion ( $E_D$ ) can be calculated from the slope of the graph

$$E_D = -1 \times \text{slope} \times R \times 2.3 \quad (13)$$

In a recent comparative study involving leaf cuticles, cuticular permeance for water increased by roughly a factor of 2 in the temperature range from 15°C to 35°C; higher temperatures reaching up to 50°C enhanced cuticular water permeability by approximately one order of magnitude. Assuming that water preferentially uses polysaccharide pathway, as recently reported by different studies (Schreiber and Schönherr 2004; Popp *et al.* 2005; Shi *et al.* 2005),  $E_p$  adds up the activation energy of diffusion in the aqueous phase of the polysaccharide strands and the enthalpy of water/polysaccharide interactions. Water/polysaccharide interactions are exothermic while the diffusion of water molecules within the strands consumes energy. As  $E_D$  and  $\Delta H_K$  can be assumed to have opposite signs, the activation energy of the permeation process  $E_p$  will be lower than  $E_D$  and can ever reach 0 when  $E_D = -\Delta H_K$ . Some studies (Riederer 2006) have shown that at critical temperature critical degree of swelling may occur and cause opening of new and "stiffer" regions of the polysaccharides for the diffusion of water (Damstra 1986). This conclusion agrees with the preceding observations of Schreiber and Schönherr (1990) who found a sharp change in the thermal expansion coefficient of water-saturated cuticles at about 35°C which they ascribed to (volume) changes in the polysaccharide fraction(s); the mechanical strain exerted by these changes may open up additional polar pathways in plant cuticular membranes. These new pathways may contribute to higher water permeances since the diffusion cross-section increases. The additional pathways for diffusion can be accessed only at a higher cost of activation energy  $E_p$ .

The influence of temperature on foliar uptake of herbicide and organic compounds in general has not been studied systematically (Hartley and Graham-Bryce 1980). Rate constants of foliar uptake, estimated by McCall (1988), increased by a factor of ten between 13 and 30°C. Additional reports show that foliar uptake of organic compounds (such post-emergence herbicides) increased with temperature (Kirkwood 1987), but permeability or diffusion coefficients were not measured and the data cannot be rigorously analysed or generalised. Following studies on temperature effects on mobilities of organic compounds in cuticles were restricted to 15-35°C (Baur and Schönherr 1995; Baur *et al.* 1996b).

In the study of Baur *et al.* (1997) in the CM of several plant species, solid/liquid or glass/rubber phase transitions which affect transport properties, did not occur in the temperature range of 20-70°C. This could be explained taking into account that even in more uniform synthetic polymers many phase transitions of unknown origin have been observed without consequences for transport properties (Elias 1993).

## Humidity

The effect of humidity on the efficacy of herbicides is an area that has received little systematic scientific study under field and controlled environment growth room conditions. This lack of research most relates to difficulty in designing experiments and controlled environment growth chambers where water content of air is held constant while eliminating the effect of temperature on herbicide uptake. Temperature and humidity have both a profound effect on herbicide uptake, and although humidity and temperature interactions can vary from species to species, many researchers found that humidity has a greater influence on herbicide efficacy than temperature (Jordan 1977; Anderson *et al.* 1993; Skuterud *et al.* 1998; Shaw *et al.* 2000; Coetzer *et al.*

2001). Anderson *et al.* (1993) following the evaluation of glufosinate ammonium efficacy on barley (*Hordeum vulgare*) and green foxtail (*Setaria faberi*) at various combinations of high and low humidity and temperatures regimes, reported that humidity had a greater impact on efficacy than temperature. Skuterud *et al.* (1998) found that for predicting herbicide efficacy (mixture of ioxynil, dichlorprop, and MCPA), humidity was more important than wind or temperature. Humidity also is proven to have a beneficial effect on penetration of ions like that of water (Schönherr 2000, 2001, 2002).

One of the first papers where the control of humidity was successfully considered into herbicide uptake studies was that by Cook *et al.* (1977) where although actual humidity values were not reported, low and high humidity treatments were used to examine the effect of humidity on uptake of aminotriazole into bean leaves.

While it is clear that environmental variables have a profound effect on the uptake and efficacy of foliar applied herbicides, there is little information on the exact mechanisms involved in the influence of humidity on herbicide performance (Baur 1999). The two most common explanations of improved efficacy in foliar applied herbicide at high humidity are: increased cuticle hydration, and delayed droplet drying. By their temporal relationship with spray event, these two variables can be separated: if increased cuticle hydration most improves efficacy at high humidity then the humidity must be kept high for some period before spraying; if delaying droplet drying most improves efficacy then the humidity must be high for some periods after spraying.

Probably the reason which is at the base of the increased penetration of herbicide, and more in general molecules and ions, due to increased cuticle hydration thanks to high humidity levels, regards cuticular transpiration rate. In fact it has been shown that cuticular transpiration is significantly affected by humidity (Schreiber *et al.* 2001): when humidity at the outer side of the cuticle was increased from 0 to 100%, cuticular transpiration was increased by a factor of 2-3. This indicates that water molecules can be absorbed by the lipophilic cuticle and cause swelling, which in turn leads to an increase of the cuticular transpiration. The part of the cuticle which is most sensitive to the absorption of water is represented by the polar domains in the transport-limiting barrier of the cuticle (besides the lipophilic domains). With increasing humidity, increasing amounts of water are absorbed by these polar domains and, as consequence, cuticular permeability increases.

Several investigators have suggested that uptake and efficacy are affected more by high humidity after spraying than before (Cook *et al.* 1977; Lym 1992; Ramsey *et al.* 2002). According to these researchers, one of the chief benefits of high humidity was the prevention of herbicide crystallization, which depletes the concentration of the active ingredient in the solution, thereby decreasing penetration. These findings agree with those of others who found that herbicide deposits with good uptake and efficacy were associated with amorphous or gel-like deposits with no crystals (Hess *et al.* 1981; MacIsaac *et al.* 1991).

Probably, to evaluate the actual influence of humidity and temperature on herbicide uptake, an absolute measure for air moisture that account for the effect of temperature must be used (Ramsey *et al.* 2005); until this will not be taken into account, the effect of atmospheric water on foliar herbicide uptake will not be clearly understood.

## Adjuvants

When dealing with foliar uptake of herbicides it is difficult to ignore adjuvants. Although most of the synthetic adjuvants were diffused only a few decades ago, the use of adjuvants dated back as early as about 200 years ago. An adjuvant is any compound that is added to an herbicide formulation or tank mix to facilitate the mixing, application, or effectiveness of that herbicide. An adjuvant generally is required to obtain the full potential of a post-emergence herbi-



cide or to allow their full potential under various application practices.

Adjuvants may be classified in a variety of ways, such as by their function (activator or utility), chemistry (such as organosilicones), or source (vegetable or petroleum oils) (Penner 2000). Classification on the basis of their function provide for activator adjuvants or utility adjuvants. In the first group are surfactants (non-ionic, ionic, amphoteric), oil adjuvants (petroleum oil concentrates, vegetable oils), and ammonium fertilisers; in the second group are wetting agents (spreaders), dyes, drift control and foaming agents, thickening agents, deposition agents (stickers), water conditioners, compatibility agents, pH buffers, humectants, defoaming and antifoam agents, UV absorbents.

Activator adjuvants work to enhance the activity of the herbicide, often by increasing rates of absorption of the herbicide into the target plant. Utility adjuvants, sometimes called spray modifiers, work by altering the physical or chemical characteristics of the spray mixture to improve its ease of application, its ability to remain on the plant surface rather than rolling off, or its persistence in the environment (McWhorter 1982).

However, it is recognized now that the mechanisms of action of adjuvant in enhancing pesticide uptake are very complex and only partially understood due to two main reasons: the issue is complex and there is no one simple or unifying mechanism (different adjuvants do different things to different agrochemicals on different target species) (Stock and Holloway 1993); it is a subject of great importance for agrochemical industry, but much less so in plant science. Nevertheless, it seems that adjuvants can affect each of the following steps in the uptake herbicide process: a) at leaf surface level, b) on the transcuticular diffusion and c) on the permeability of the plasma membrane.

a) The presence of adjuvant in the spray mixture may have multiple functions on the leaf surface. These include: increasing contact area of deposit especially on waxy species, dissolving or disrupting epicuticular waxes, solubilising herbicide a.i. in deposit, preventing or delaying crystal formation in the droplet residue, delaying droplet drying through an hygroscopic effect, promote uptake of solutions via stomatal infiltration.

Addition of appropriate adjuvant to herbicide formulation can produce improvement in wetting and spreading properties. This function is directly related to reduction of surface tension and thus is unquestionable. This parameter is most relevant to surfactant-based adjuvants. It is known that anionic surfactants with small lipophilic head groups migrate rapidly and can therefore reduce dynamic surface tension. Migration time of non ionic surfactants tends to be longer. Molecular architecture clearly has an impact, with more compact branch-chained molecules having greater capacity to reduce surface tension than long flexible linear chains (Varadaraj *et al.* 1991). In addition to surface tension, the dilational modulus has also received attention as a parameter for retention efficiency (Reekmans 1998). The dilational modulus  $E_0$  of a solution is defined as  $E_0 = A\gamma/dA$ , where  $A$  = initial interface area,  $d\gamma$  = initial change in surface tension, and  $dA$  = change in interface area during expansion. If the value for this parameter of a liquid is low, lessening the tendency of a spray droplet to be reflected when they distort and expand along the leaf surface during impaction. Although increasing the effective contact area of a deposit would be expected to increase the diffusion of a solute through the cuticle, experiments using single droplets have demonstrated that there is no general correlation between the efficiency of adjuvant-induced uptake and the apparent contact area of the corresponding deposit.

Dissolving or disrupting the covering of wax on leaf surface was thought being a mechanism of action of surfactants. Some results of the studies on the effect of alcohols, and monodisperse ethoxylated alcohols on the mobility of 2,4-D in isolated plant cuticles suggest that these surfactants are sorbed in cuticular waxes and plasticize them. However, many of the attempts to confirm surfactant-

induced damage on cuticular waxes have failed to detect any dramatic changes in waxy morphology or distribution, even after a surfactant has physically penetrated through the wax layer.

Solubilising effect of surfactants on herbicides have also been suggested as a possible way that uptake could be facilitated from a foliar deposit, since it is known that their solubility can be increased in aqueous surfactant-containing solutions (Nassetta *et al.* 1991). Few attempts have been made to examine the solubilisation capacity of a surfactant for an herbicide in relation to its ability to enhance the foliar uptake of the same compound. Although solubilisation probably makes a contribution to the transport of herbicides to its initial site of absorption, current evidence suggests that it is unlikely to play a dominant role in uptake enhancement.

It is often assumed that the foliar uptake of an herbicide from a crystalline deposit will be less than that from an amorphous one. There are many papers in the open literature (Hess *et al.* 1986; Hess and Falk 1990) that indicate that production of a crystalline deposit of an herbicide is detrimental to subsequent penetration. With this regard, while studying the effect of formulations on foliar uptake and on the morphology of glyphosate deposits on the leaves of tartary buck-wheat, MacIsaac *et al.* (1991) observed that the addition of "Tween 20" (polyoxyethylene sorbitan monolaurate) resulted in the formation of a more amorphous, non-crystalline deposit, whereas a crystalline deposit was formed with glyphosate alone. For surfactant adjuvants, the internal structure of an amorphous deposit may vary significantly. As spray droplets dry down, surfactants pass through different packing phases, including hexagonal, cubic, and lamellar structures. Cubic phases are known to be the most viscous. As high-viscosity media limit diffusion of solutes, then the packing of surfactant molecules in foliar deposits could limit availability of the a.i. diffusing from the foliar residue. The packing structure will depend upon humidity and the presence of electrolytes and other formulants, such as glycols. It is, therefore, difficult to predict adjuvant behaviour because of the numerous interactions (besides environmental factors). However, whilst surfactant addition will undoubtedly ensure that there is an intimate contact between the agrochemical, in a non-crystalline state, and the cuticle surface, foliar uptake from an amorphous deposit may be little different from that of the crystalline form, may be less or enhanced.

When the adverse effect of rapid droplet drying at low humidity on efficacy was realized, humectants were employed to keep droplets moist (Mitchell and Hamner 1944; Marth *et al.* 1945; Rice 1948). Although the early studies by Marth *et al.* (1945) were performed without the benefit of modern adjuvants, their conclusions about the importance of maintaining aqueous qualities of the herbicide droplet/deposit are still relevant today. Following these initial studies, there were several reports of humectants improving herbicide efficacy (Holly 1956; Hughes 1968; Babiker and Duncan 1975). Penetration of 2,4-D from droplets ceased upon drying, whereas the presence of glycerol kept the drop moist and uptake continued for may hours (Holly 1956). At low humidity, Babiker and Duncan (1975) found that 1% glycerol doubled uptake of asulam (with 0.2% Tween 20) into bean leaves. Similar findings were reported by Prasad *et al.* (1967). On the other hand many examples of scepticism emerged from several studies. Holloway and Edgerton (1992) indicated that glycerol and polyethylene glycerol (PEG) 300 did not increase uptake of difenzoquat and 2,4-D into wild oat. In commenting on the lack of humectant effect in that research Stock and Holloway (1993) stated that "humectancy clearly is not the only factor involved. Also, it is likely to be unimportant in aiding the penetration of more lipophilic molecules, which account for the majority of pesticidal compounds". Field *et al.* when discussed results about a research study involving glyphosate, surfactant Silwet L-77 and 6% glycerol, stated "glycerol slowed the drying of deposit, but it seems unlikely that its positive effect was simply related to this physical phenomenon".

Before the end of 1980s it was thought that stomata did not constitute an important pathway for pesticide uptake. Now convincing evidence is available that solutions of agrochemicals penetrate directly into leaves via open stomata. This can be achieved with the addition of organosilicone surfactants which can reduce the surface tension of aqueous solutions to as low as 22 mN/m (Buick *et al.* 1992). The use of an abscisic pre-treatment, which artificially closed the stomata, confirmed the stomata route as being the principal contributor to Silwet L-77 enhanced triclopyr triethyl-ammonium uptake into bean (Buick *et al.* 1992). Stomatal uptake of herbicides varies greatly with plant species, depends on the concentration of silicone surfactants (0.5% being usually required) and is characterized by extremely rapid uptake of the herbicides within 10 min of application (the route has been visualised by microscopic examination using fluorescent dyes). Stomatal uptake of post-emergence herbicides occurs fast and could thus reduce the rain-free time of herbicide applications.

b) With respect to the effect on transcuticular diffusion, theories of adjuvant action on mechanisms transport processes regards principally changes in solubility relationships and partition processes favourable to penetration of the agrochemical. For surfactants it has been speculated that they increase the penetration of hydrophilic compounds by hydrating the cuticle, whereas they accelerate the uptake of lipophilic chemicals by increasing the fluidity (or reducing the viscosity) of the cuticular wax (Hess and Foy 2000). There are many reports showing ethoxylated surfactants improving herbicide uptake into whole plants and isolated tissues (Stevens and Bukovac 1987; Coret and Chamel 1993; Stock and Holloway 1993; Stagnari *et al.* 2006a, 2007). Rates of penetration will be proportional to the permeances (and driving forces) of cuticles which are in turn related to their partition and diffusion coefficients. So in the presence of surfactants the partition system changing from water-cuticle to (water + surfactant)-(cuticle + surfactant) would increase solubility of the agrochemical in the lipophilic cuticle; obviously, surfactant composition and concentration would be important here. In fact, Stock and Holloway (1993) indicated for NIS a structure-activity relation in which ethylene oxide (EO) content plays a fundamental role; nonionic surfactants with low EO content units, which are good spreaders and have low surface tension, favoured the uptake of lipophilic herbicides, whereas uptake of water-soluble herbicides is best with nonionic surfactants of higher EO content (10-20) which have poor spreading properties.

With regards on mechanism of action for low EO-content surfactants, there is evidence of the altering action of the physical properties of the cuticle. Coret and Chamel (1995) discovered, through measurements of cuticle-wax melting point and melting enthalpy, that low-EO nonylphenol surfactants can increase fluidity of cuticle waxes, allowing greater diffusion of chlorotoluron through the cuticle. In contrast, a nonylphenol surfactant containing 20 EO units, which poorly enhanced chlorotoluron uptake, had no effect on cuticular wax fluidity (Coret and Chamel 1995). This difference in wax fluidity may be related to their ability to penetrate the cuticle. Other mechanism of action have been proposed for low EO surfactants. Schreiber (1995) and Schreiber *et al.* (1996) proposed that surfactants induce an unspecific and reversible "plasticizing" interaction with the cuticular wax that enhances solubility of the herbicide in the wax. Since the effect was reversible, the surfactant did not alter the molecular structure of the wax (solubilize the wax or change its crystalline nature). At concentration above the critical micelle concentration (CMC), however, these surfactants may retard the rate of cuticular penetration of lipophilic solutes (Schreiber and Schönherr 1993b). This can occur when the solute is more soluble in the surfactant micelles than in water; substantial amounts of a. i. may partition into these micelles resulting in a reduced partition coefficient between the cuticle and the deposit ("K depression"). High EO content surfactants have been shown to in-

crease water permeability of cuticles than low EO surfactants (Riederer and Schönherr 1990; Coret and Chamel 1993). So hydration for the polar pathway by surfactants seems plausible.

The effect of different lipophilic moieties on ethoxylated surfactants has also been examined although studies on the effect of surfactant hydrophobes which include numerous structures and alkyl chain lengths, are few. When comparing surfactants with identical EO content, but with different lipophilic component such as octylphenol vs. nonylphenol vs. aliphatic alcohols, variation in the lipophilic portion of the surfactant had no significant influence of the uptake of chlorotoluron. In contrast, glyphosate uptake was affected by the same lipophilic components (Coret and Chamel 1993) and resulted greatest with octylphenol and nonylphenol and least with aliphatic alcohols. Whereas Gaskin and Holloway (Gaskin and Holloway 1992) observed that glyphosate uptake into wheat and bean was improved more by linear alcohol surfactants than by nonylphenol surfactants. Holloway and Edgerton (1992) obtained similar results on the uptake of difenzoquat into wild oat foliage. However, it is still unclear whether a C12/C16 linear alkane is the most efficient surfactant hydrophobe for the uptake of all herbicides.

Substances other than surfactants can influence the uptake of herbicides through the cuticles. Vegetable and mineral oils can enhance efficacy of many classes of herbicides, such as aryloxyphenoxy-propionates, cyclohexanediones, triazines, bentazone, phenoxy acids, imidazolinones, sulfonylureas (Foy 1993; Gauvrit and Cabanne 1993; Bunting *et al.* 2004; Stagnari and Onofri 2002; Stagnari *et al.* 2006b). Although the use of oils as adjuvants to increase the efficacy of post-emergence herbicides is well documented, little attention has been given to the understanding of their mode of action. It is believed that their effect may be related to the ability of the oils to partition into the cuticle thus resulting in an increased fluidity of the cuticular components, as has been previously observed for non-ionic surfactants. In fact, the penetration of vegetable oils into plants was extensive (Mercier *et al.* 1997) and that of mineral oils also has been demonstrated by Tan *et al.* (2005). The mechanism of action in enhancing the uptake of lipophilic chemicals may be similar to that of low EO surfactants (Hess and Foy 2000). However, this may not explain their beneficial effect on the uptake of hydrophilic compounds. In the latter case, another mode of action, such as humectant effect, has been proposed (Sharma and Singh 2000). Generally, methylated seed oils exhibit a greater effect on herbicide uptake than non-modified seed oils, but differences between seed oils and mineral oils varies with herbicides and weed species (Gauvrit and Cabanne 1993). Ammonium salts have also been used as herbicide adjuvants for a long time. Ammonium salts can improve the efficacy of many herbicides including glyphosate, glufosinate, 2,4-D, bentazone, imidazolinones, sulfonylurea and so on (Foy 1993; Kirkwood 1993; Gauvrit 2003; Ramsdale *et al.* 2003).

c) The possible effect of adjuvants on the permeability of plasma membrane has drawn little attention until now. Although the effect of adjuvants on the cuticle and the spray droplet is of critical importance, the plasma membrane as a site of action of certain adjuvants may also warrant consideration. It is well established that surfactants are able to penetrate into plant foliage, they have a profound effect on plant cell membrane *in vitro*, affecting their permeability at low concentrations and their structural integrity at high concentration. Wade *et al.* (1993) reported that Mon 0818, a tallow ammine surfactant, increased the diffusion of glyphosate into isolated plasma membrane vesicles, suggesting that surfactant could increase the membrane permeability. The exact mechanism by which amine surfactants enhance penetration is unknown, since use of other surfactants classes will also results in increased membrane permeability. In contrast to non-ionic or anionic surfactants, it has been postulated that the cationic amine surfactants may exhibit sufficient polarity to diffuse out of the cuticle and interact with

plasma membrane. The surfactants may insert within the lipids of the membrane causing an unspecific and incomplete solubilization. Partial solubilization would allow enhanced penetration glyphosate into the symplastic system without destroying the membrane integrity.

It is also demonstrated that ammonium sulfate increased the uptake of glyphosate by isolated protoplasts of quackgrass (*Agropyron repens* L.) (de Ruiter and Meinen 1996), and the absorption of imazetaphyr by quackgrass and maize cell suspension cultures (Gronwald *et al.* 1993). This implies that ammonium ions could either alter the permeability of the plasma membrane directly, or facilitate chemical uptake by reducing the pH of the incubation medium in the case of weak acid herbicides.

However, it remains difficult to estimate the relative contribution of increasing the permeability of plasma membrane to the overall uptake enhancement provided by adjuvants.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Several studies carried out on the absorption of post-emergence herbicides into the plant foliage, and especially recent developments, have helped our understanding on the mechanisms of uptake of such molecules into the leaves and on the factors influencing their absorption, translocation and efficacy.

The importance of the cuticle as the first, if not the most important, barrier to penetration of xenobiotics and its structure and composition have been largely documented. However, further work will have to deal with the localization and analysis of that fraction of total cuticular waxes that actually determines the barrier properties of the cuticle. Progress on this route will also depend on the elucidation of the physical structure of cuticular waxes and how this property is influenced by the mixing behaviour of the mix constituent. In recent investigations the thickness as well as chemical composition of a thin film of epicuticular waxes on the leaves of some species were assessed. It remains to be determined how far these findings are specific for these plant systems or whether they can be repeated for other species.

The availability of quantitative data on permeability of the cuticle to non-ionic post-emergence herbicides, mostly lipophilic, have allowed experimentally as well as theoretically well-established models and the development of techniques to predict and measure the absorption, movement and desorption of these molecules within the plant cuticle. With regards to ionic post-emergence herbicides, although now there is good new evidence for the old hypothesis about polar paths of diffusions across plant cuticles, the chemical nature of this polar paths still remain unsolved. Future work on the permeability of plant cuticles will be needed to solve this question and that concerning on what extent polar paths of diffusions are characteristics of the cuticle of all plant species. In order to improve uptake of ionic post-emergence herbicides into leaves polar path of diffusion will form preferential sites of uptake. The same argument accounts for promoters used to induce gene expression in transgenic plants. Promoters which are polar or charged will have to diffuse via polar pores in the cuticle.

The characteristics proper of the molecule (lipophilicity, molecular size and pH of the medium) have been demonstrated to have an important influence on the uptake of post-emergence herbicide as well as environmental conditions. Among the latter, the effect of temperature and especially of humidity have received little systematic scientific study under field and controlled environment growth conditions. Current evidence suggests that highly-water soluble, ionic herbicides may be more sensitive to low humidity and rapid drop drying than lipophilic herbicides. The paucity of research in this area most likely relates to difficulty in designing experiments and controlled environment growth chambers; therefore in the future it is essential that the effect of atmospheric water content and temperature on herbi-

cide uptake be measured.

There is no doubt that adjuvants have played an important role and still have an important role to play in a foreseeable future in increasing post-emergence herbicide efficacy. However, a rational use of adjuvants requires a better understanding of their mode of function; although extensive experimental data are available about the effect of different surfactants on the uptake of various herbicides, the mechanism of interactions remains obscure and the optimum combination of solute and adjuvant have to be found empirically. Future efforts should be directed in the following directions: studies which combines with modern analytical and microscopic techniques to elucidate the complex interactions between pesticides, adjuvants, and plants in the uptake process; more attention should be given to oil adjuvants, as great potential still exists to optimize their performance by chemical modifications and biological testing; non-environmentally friendly surfactants should be eliminated from the market in the future; research on adjuvant mixtures should be further promoted due to the complexity of the foliar uptake process which requires several properties from a single adjuvant molecule.

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