

Foliar Nutrient Uptake – of Myths and Legends

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Keywords: foliar fertilization, cuticle, stomata, deliquescence relative humidity, ectodesmata, relative humidity, permeability

Abstract

Foliar fertilization is a common practice to quickly correct nutrient deficiencies, especially under conditions of limited soil nutrient availability. For a long time it has been a matter of debate how nutrients enter the leaves. It was assumed that uptake is restricted to the cuticle, while stomata were supposed to be impermeable to foliar-applied solutes. It was suggested that uptake occurs through “ectodesmata”, a concept that was later disproved but still persists in current textbooks and review articles. The current model assumes that polar solutes, such as nutrient salts, enter the cuticle through hydrophilic polar pores. Early estimations suggested that in isolated, dewaxed cuticles the pores are so small (diameter: 1 nm) that they exclude large molecules, such as sucrose or chelates, from foliar uptake. Meanwhile, new estimations using intact leaves indicate that these pores can be considerably larger (4-5 nm). It was also shown that solute uptake through stomata is in fact possible and that this stomatal penetration pathway can be as important as the cuticular pathway. These findings are in accordance with observations in the field that large molecules (e.g. Fe-chelates) can penetrate stomata-free upper leaf surfaces and that uptake rates of mineral nutrients are frequently larger through the stomata-bearing lower leaf surface than through the upper surface. A new concept is introduced which may help to improve our understanding of the effects of ambient air humidity on foliar uptake rates.

INTRODUCTION

Foliar fertilization is a common practice to supply crops with mineral nutrients, especially under conditions of limited soil nutrient availability. However, it has to be kept in mind that the aerial plant parts are designed to minimize exchange of matter with the environment rather than to absorb mineral nutrients. For this purpose, leaf surfaces are equipped with a water repellent lipophilic cuticle which forms a high resistance against the penetration of hydrophilic solutes such as mineral nutrients. Moreover, stomata are protected against the infiltration of liquid water (Schönherr and Bukovac, 1972). Foliar-applied nutrient thus have to overcome the barrier properties of leaf surfaces in order to be absorbed by leaves.

A second specific feature of leaf penetration by mineral nutrients is that, unlike root uptake, no active processes are involved and hence nutrient penetration of leaf surfaces is not selective. Strictly speaking, the term foliar “uptake” may thus be regarded as wrong and misleading because it more or less implies an active process. Because of its widespread use, the expression “uptake” will nevertheless be used in this paper to describe leaf penetration of solutes. The passive nature of the uptake process has an important consequence: any substance present on the leaf surface will penetrate the leaf surface as long as there is a concentration gradient across the leaf surface as the driving force of diffusion. If the resulting penetration rates are too high and incompatible with the plant’s metabolism, leaf damage (“leaf scorch”) will be the result, a phenomenon that can be quite often observed in practice. The efficacy of foliar spraying is thus challenged by both low uptake rates due to the barrier properties of leaf surfaces and excessive uptake

because of the passive uptake process. The main challenge of foliar spraying is to apply the optimum nutrient dose which corrects or prevents nutrient deficiency without causing leaf scorch.

Foliar uptake rates are controlled by the resistance of the leaf surface and by the nutrient concentration on the leaf. The solute concentrations in foliar sprays are usually not in equilibrium with the humidity of atmosphere. As a consequence, spray solutions will evaporate until this equilibrium is reached. It has been shown that the equilibrium concentrations of foliar-applied solutes present on the leaf surface depend on both the ambient relative humidity (RH) and the hygroscopicity of the solute (Fernández and Eichert, 2009). The degree of hygroscopicity of a solute can be expressed by the RH above which the salt dissolves in the water absorbed from the atmosphere. This threshold humidity is called “deliquescence relative humidity” (DRH) or “deliquescence point” (DQ). The interaction between RH and DRH of the solute controls whether the spray solution will dry out on the leaf surface (if $RH < DRH$) or not (if $RH > DRH$).

The pathways by which foliar-applied solutes penetrate leaf surface have been a matter of debate for a long time. While some research groups regarded the role of stomata as negligible and thus focused on the cuticle, others provided evidence that leaf-applied chemicals may use the stomatal route for penetration. Relative humidity affects the aperture of stomata and the hydration state of the cuticle, and both parameters are known to affect the permeability of leaf surfaces. However, the functional relationship between RH and leaf permeability is still not understood.

The present paper gives an overview of the historical development of the state of knowledge of leaf uptake. It starts with the concept of “ectodesmata”, specific structures in the cuticle proposed to be designed for foliar uptake, which was developed in the 1950s and 1960s and was later revoked, but still circulates in textbooks and reviews. This is followed by a summary of recent findings on the permeability of cuticles and stomata and by the introduction of a new concept to elucidate the effects RH on foliar uptake.

THE ECTODESMATA-HYPOTHESIS

“Ectodesmata” were thought to be cytoplasmic extensions of epidermal cells interfusing the cuticle (Schumacher and Lambertz, 1956; Franke, 1960) and to be involved in foliar absorption of hydrophilic solutes (Franke, 1964). They were discovered by a complex preparation procedure involving the incubation of leaves in a solution containing acids and $HgCl_2$ for several hours followed by various separation and cleaning steps and a final staining step in 10% H_2SO_4 . After this procedure Hg precipitates could be detected in the cuticles, especially over anticlinal epidermal cell walls and guard cells (Franke, 1964; Schönherr and Bukovac, 1970).

Later it was shown that the distribution of the precipitates was not related to epidermal cell structures, i.e., that they were not symplastic, and that they most probably did not trace a hydrophilic penetration pathway relevant for polar and charged molecules, because $HgCl_2$ is an uncharged molecule. Schönherr (2006) proposed that the Hg precipitates preferentially mark sites which are rich in non-ionic functional groups, such as aldehyde and phenolic hydroxyl groups, and/or sites rich in reducing substances.

The debate about ectodesmata in the late 1960s, whether they were symplastic or apoplasmic in nature, probably wasted the first opportunity to discover and develop the “pore” model, which will be described in the next section.

AQUEOUS POLAR CUTICULAR PORES

The cuticle is a hydrophobic, non-living skin covering most aerial parts of higher land plants. The backbone of the cuticle is provided by a three-dimensional polymer made of cutin, in which amorphous or crystalline lipids (waxes) are embedded.

Whereas small lipophilic solutes may easily penetrate the cuticle by dissolving in the waxes and diffusing through the voids of the cutin network, the penetration of hydrophilic solutes is strongly hindered by their very low solubility in the cuticle. For example, NH_4NO_3 is 10^7 times less soluble in the cuticle than in water (estimated by the

octanol-water partition coefficient), and estimations using other salts yield similar values. Nevertheless, mineral fertilizer salts can be taken up when applied to the leaf surface (without stomata), indicating that the model of solution and diffusion in the cuticle, which is valid for apolar, lipophilic solutes, cannot satisfactorily explain the cuticular uptake of hydrophilic solutes.

For this reason, the model of aqueous polar pores (Schönherr, 1976, 2000) was developed. According to this “pore model”, water can be absorbed by the cuticle and form clusters. If enough water is absorbed, the clusters may form an aqueous bridge within the lipophilic cuticle enabling hydrophilic solutes to diffuse between the outermost leaf surface and the epidermal cells. Polar pores are supposed to be located in the voids of the three-dimensional cutin polymer. Therefore, the size of these voids may limit the effective diameter of the pores and the molecules diffusing within them. It follows that there is probably a certain maximum size of solutes that may penetrate the cuticle, while molecules larger than the available size of the voids should be excluded.

The first attempt to estimate the effective pore size was published by Schönherr (1976). He compared the fluxes of water driven by diffusion and driven by a pressure gradient through isolated and dewaxed *Citrus × aurantium* cuticles. He assumed that any water transport through the cuticle is restricted to pores and utilized the ratio of both fluxes for the calculation of the pore diameter. This method yielded diameters of around 1 nm. For almost three decades this was the only available estimation of the size relations of pores and therefore gained currency in many papers and textbooks. However, such small pores would exclude large molecules such as chelated nutrients. Later it was shown that some penetration of water, a small uncharged molecule, may take place in the lipophilic domains of the cuticle and not only in pores (Schreiber et al., 2001). Fernández and Eichert (2009) reanalyzed Schönherr’s data and demonstrated that a 50% share of the lipophilic penetration route in water penetration would result in a pore diameter of 1.5 nm, while a 90% share would yield 3.5 nm. This latter value is in agreement with the pore diameters reported by Eichert and Goldbach (2008). They used a different method based on intact leaves, i.e., avoiding the isolation of cuticles, which may otherwise potentially lead to wrong or biased results (Fernández and Eichert, 2009). They followed a co-application approach measuring the penetration of two compounds simultaneously and found pore diameters of 4.0 to 4.8 nm in astomatous leaf surfaces of coffee (*Coffea arabica*) and poplar (*Populus × canadensis*), respectively.

ROLE OF STOMATA IN FOLIAR UPTAKE

From the beginning of mechanistic research on foliar uptake there was evidence that stomata play an important role in foliar penetration. Uptake rates were often reported to be higher through lower, stomata-bearing leaf surfaces than through stomata-free upper leaf surfaces (e.g., Will et al., 2012). It was also reported that the number of stomata per leaf (e.g., Schönherr and Bukovac, 1978), or the aperture of stomata (e.g., Eichert et al., 1998; Eichert and Burkhardt, 2001) affected uptake rates. On the other hand it was clear that the infiltration of stomata by mass-flow of the foliar-applied solution did not occur (unless the surface tension was lowered by very effective surface active ingredients.) The debate on the role of stomata in foliar uptake was virtually terminated when Schönherr and Bukovac (1972) elaborated that the infiltration of stomata by aqueous solutions is impossible for physical reasons. Consequently the striking contradiction between theory (“stomatal uptake is impossible”) and observation (“stomata play an important role”) remained unresolved for the next three decades.

The key to the puzzle was discovered when foliar uptake of nano-sized particles was studied. It was observed that the particles penetrated stomata by diffusion on the surface of stomatal pores and not by mass-flow (Eichert et al., 2008). The percentage of stomata involved in foliar uptake is usually small while at the same time the quantitative contribution of stomatal penetration to overall uptake can be substantial (Eichert and Goldbach, 2008; Eichert et al., 2008).

EFFECTS OF RELATIVE HUMIDITY ON FOLIAR UPTAKE

Leaf uptake is controlled by (i) the concentration gradient between the leaf surface and the leaf interior as the driving force of penetration and (ii) the permeability of the leaf surface. For an individual plant, both parameters are not constant but affected by the environment, and air humidity is one of the most important influencing factors.

The solutions sprayed onto leaves usually have relative low solute concentrations and will thus evaporate to establish equilibrium with the humidity of the atmosphere. Evaporation will stop as soon as this equilibrium is reached. Evaporation from the spray droplets causes an increase in solute concentrations, and since this increases the driving force of penetration, uptake rates will increase proportionally. Whether the solutions dry out completely or are still liquid in equilibrium with the atmospheric RH, depends on the particular salt and its hygroscopicity. The equilibrium solute concentration is also dependent on these two parameters. A spray solution containing NH_4NO_3 will not dry out completely as long as RH is above 61%, whereas an $(\text{NH}_4)_2\text{SO}_4$ solution will dry at RH below 80% (Fig. 1). These threshold values are called deliquescent humidity (DRH). In addition, Figure 1 shows that the equilibrium concentrations reached in spray solutions on leaf surfaces can be high and exceed the initial concentrations of the spray solutions (which are usually well below 1 mol L^{-1} or mol kg^{-1}).

Air humidity also affects the hydration status of the cuticle and thus its permeability for hydrophilic solutes. It was shown that a decrease in RH from 100% to 50% decreases the permeability of pear cuticles by a factor of about 100 (Fernández and Eichert, 2009). In dry air less water is absorbed by the cuticles than in moist air. Therefore, the probability that water clusters in the cuticle build up a hydraulic connection across the cuticle is strongly decreased – in other words: in dry air there are less aqueous pores than in moist air (Eichert and Fernández, 2012). Air humidity may also affect the aperture of stomata but there are many other controlling parameters such as light intensity and the water status of the leaf. Hence, no straightforward relationship between RH and aperture can be deduced, generally high stomatal apertures are more likely to occur at high RH than at low RH.

Taken together it becomes clear that RH affects both the concentration gradient and the permeability of leaf surfaces, but in opposite directions. Whereas the permeability increases with increasing RH, the solute concentrations decrease above the DRH of the solute (Fig. 1). The question is now, how these two effects together affect foliar uptake rates. Uptake rates can be calculated after

$$J = F/A = \Delta c * P \quad (\text{Equation 1})$$

Here, J is the penetration rate ($\text{mol m}^{-2} \text{ s}^{-1}$), F is the steady state flow (mol s^{-1}), A is the contact area (m^2), Δc is the concentration difference (mol m^{-3}) across the leaf surface and P is the permeability coefficient of the leaf surface (m s^{-1}). Equation 1 was used to estimate the effect of RH on the uptake of a hypothetical salt with a DRH of 50% and $c = 15 \text{ mol L}^{-1}$ saturation concentration (Fig. 2a). Furthermore, different scenarios of the effect of RH on overall permeability of the leaf surface were assumed (Fig. 2b). Figure 2c shows that, depending on the RH-permeability scenario, different maximal penetration rates were obtained which occurred at different RH values. An exponential increase in leaf permeability with increasing RH is a feature of the cuticular pathway (Fernández and Eichert, 2009). In this scenario, penetration peaks at relatively high RH and relatively low maximal penetration rates are achieved (Fig. 2c, solid line). The linear and saturation-like relationships between RH and permeability (Fig. 2b) may be a feature of the stomatal pathway. These result in much higher penetration rates at lower RH (Fig. 2c). In all scenarios it is evident that at very high RH penetration rates decrease strongly because the driving force approaches 0.

CONCLUSIONS

Foliar fertilization is a common agricultural and horticultural practice, but how it actually works is still poorly understood. It is now clear that both the cuticle and stomata

offer penetration pathways for the uptake of hydrophilic solutes and that both pathways are relevant. However, it is still not possible to easily quantify the contribution of both pathways to overall uptake.

Penetration rates can be predicted from the product of the concentration gradient across the leaf surface and the permeability of the surface - and both factors are controlled by RH. Depending on the RH-permeability function of the pathways, different patterns of RH-uptake curves will result. Solving Equation 1 for P , i.e., measuring penetration rates at constant RH and calculating the solute concentrations on the leaf surface may thus enable the estimation of leaf permeabilities and their dependence on RH. This may finally be used as a tool to gain further insight into the relative importance of both available pathways.

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Figures

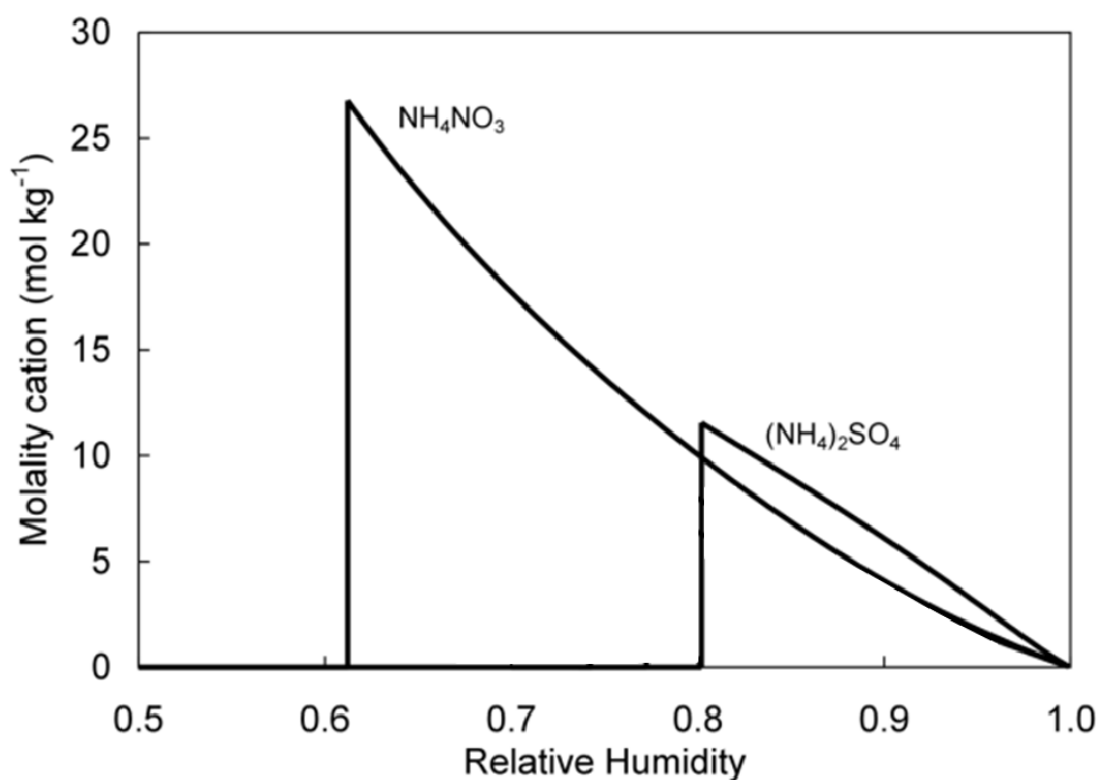


Fig. 1. Equilibrium concentrations of NH₄NO₃ and (NH₄)₂SO₄ solutions as a function of the relative humidity (RH) of the surrounding air. The RH at which the concentration suddenly drops to zero is the deliquescence relative humidity (DRH) or deliquescence point of the salt. Redrawn after Fernández and Eichert (2009).

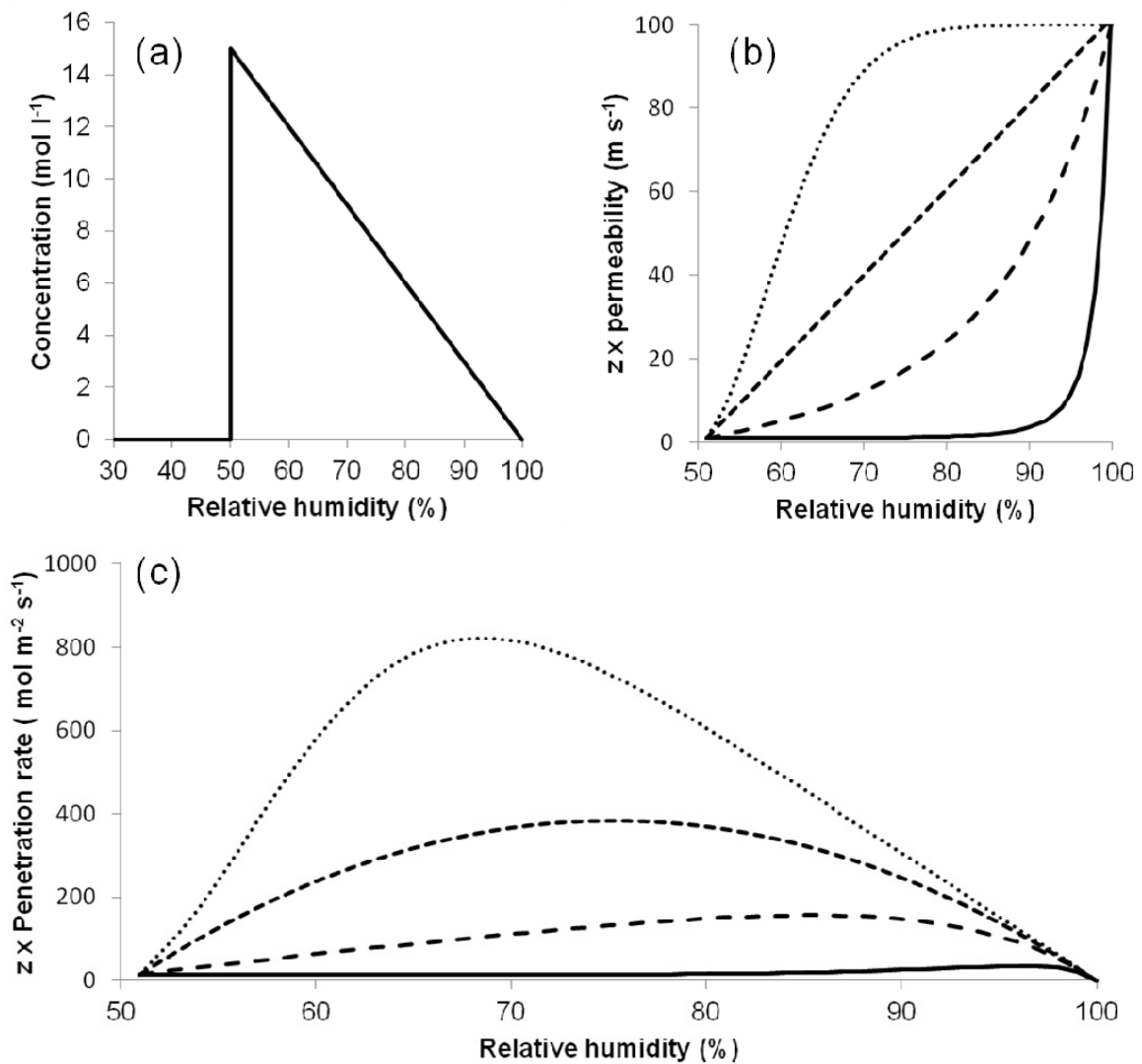


Fig. 2. Theoretical effects of relative humidity (RH) on foliar uptake. (a) Concentration profile of a hypothetical solute with a deliquescence relative humidity of 50% and saturation concentration of 15 mol L⁻¹. (b) Possible permeability profiles of leaf surfaces as a function of RH in relative units. The values at 50% RH and 100% RH were arbitrarily set to 0 and 100, respectively, so the factor z was included. (c) Penetration profile of the solute depicted in (a) for the different permeability profiles shown in (b).

