Foliar iron-fertilisation of fruit trees: present knowledge and future perspectives – a review

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SUMMARY
Iron (Fe)-deficiency is a common physiological disorder affecting fruit crops in many areas of the World. Foliar Fe-fertilisation is a common agricultural strategy to control Fe-deficiency under field conditions. However, variable responses to Fe sprays have often been described and foliar Fe-fertilisation cannot yet be considered a reliable strategy to control plant Fe-deficiency. The lack of understanding of some factors relating to the penetration, distribution, and bio-activity of leaf-applied, Fe-containing solutions hinders the development of effective Fe formulations for foliar treatment. The current state-of-the-art and future perspectives for foliar Fe-fertilisation, as a strategy to control Fe-deficiency in fruit crops, is discussed.

Iron (Fe)-deficiency chlorosis is a widespread physiological disorder affecting many fruit crops and is a limiting factor for production, especially under high pH, calcareous soil conditions, such as those prevailing in many agricultural areas with a Mediterranean climate. Typical symptoms of Fe-deficiency include the development of interveinal chlorosis, starting from the apical leaves, reduction of shoot growth, defoliation during the growing season and, ultimately, tree death (Rombolà and Tagliavini, 2006). Iron chlorosis has deleterious effects on fruit production, reducing the number of fruits per tree, fruit size, total yield, and affecting fruit quality parameters such as colour, firmness, or acidity (Álvarez-Fernández et al., 2003; 2006).

There is scientific evidence that Fe-fertilisation increases fruit quality and yield in many crops (Álvarez-Fernández et al., 2006). Iron-fertilisation is a standard agricultural practice in fruit production areas that suffer from plant Fe-deficiency. Strategies to alleviate Fe-chlorosis in fruit crops include: (i) the use of rootstocks tolerant to soil conditions that induce the development of the disorder and with improved Fe-uptake mechanisms; (ii) modifying soil characteristics; and/or (iii) treatment with Fe-substances via root, trunk, or canopy application(s) (Abadía et al., 2004; Lucena, 2006). Iron-fertilisation of roots is the most reliable and widely-used technique to control Fe-deficiency, and commercial Fe(III)-EDDHA-based products are the most effective fertilisers used to correct Fe-chlorosis under severe soil conditions (Lucena, 2006). However, such chemicals are expensive and may perform differently according to the particular Fe(III)-EDDHA formulation (Cerdán et al., 2007).

Foliar Fe-fertilisation could be a cheaper and more targeted strategy to correct plant Fe-chlorosis (Abadía et al., 2002; Álvarez-Fernández et al., 2004; Fernández et al., 2008a), but the response to Fe sprays has been shown to vary according to many plant-related, environmental, and physico-chemical factors (Fernández and Ebert, 2005). Problems of reproducibility and interpretation of results from foliar and cuticular Fe-application studies have been described (Fernández and Ebert, 2005). Our current limited understanding of the factors involved in the penetration, translocation, and bio-availability of leaf-applied Fe fertilisers makes it difficult to develop effective spray formulations for agricultural purposes. At present, foliar nutrition is only considered to be a valuable complement to the application of nutrients via the root system (Weinbaum, 1996).

In general, the penetration of Fe-containing solutions will be influenced by plant factors, environmental conditions, the nature of the spray solution, and the method of application (Currier and Dybing, 1959). Similarly, the roles of active and passive processes involved in the penetration and subsequent physiological effects of foliar-applied nutrient solutions remain controversial (Jyung and Wittwer, 1964; Zhang and Brown, 1999).

The effectiveness of leaf-applied, Fe-containing solutions is normally assessed on the basis of their re-greening capacity, tissue Fe-absorption rate, and Fe-translocation from the site of treatment (Fernández, 2004; Fernández et al., 2006; 2008a). Therefore, in response to foliar treatment with a Fe-containing solution, at least three distinct key processes can be distinguished, in theory, although they are difficult to separate from one another: (i) the penetration of foliar-applied Fe through the leaf surface; (ii) the distribution of Fe from the site of application; and (iii) the active involvement of exogenous Fe in physiological processes.

An account of the state-of-the-art concerning foliar Fe-fertilisation of fruit trees and the key factors to be considered for the development of more effective Fe-containing formulations is provided in the following sections.

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GENERAL CONSIDERATIONS REGARDING THE PENETRATION OF LEAF-APPLIED SOLUTIONS, WITH SPECIAL REFERENCE TO IRON

The ability of plant leaves to exchange water and solutions with the surrounding environment was recognised more than 300 years ago (see Franke, 1986). The penetration of leaf-applied chemicals was found to take place via the cuticle, through cuticular cracks and imperfections, the stomata, leaf hairs, and other specialised epidermal cells (Tukey et al., 1961). The significance of the stomatal route vs. the cuticular pathway remains unclear and is still an area of controversy (Eichert and Goldbach, 2008). The majority of studies on foliar uptake carried out over the last decades have focussed on the penetration of lipophilic and hydrophilic solutions through the cuticle (Fernández et al., 2006).

Aerial plant organs are covered by a continuous hydrophobic cuticle, which constitutes a protective barrier against water loss and limits the penetration of leaf-applied chemicals (Schönherr, 2006). The cuticle is a chemically heterogeneous membrane, consisting of an insoluble biopolymer matrix with waxes embedded in, and deposited on the surface (Jeffree, 2006).

Water repellency has been shown to be related to the crystalloids of epicuticular wax that cover the cuticular surface in a micro-relief (Barthlott and Neinhuis, 1997). Both a wide micro-structural diversity, and the existence of wax regeneration processes in living leaf surfaces have been reported in the last decade (Barthlott and Neinhuis, 1997; Koch et al., 2004). Plant cuticles are permeable to water, electrolytes, and polar compounds (Kerstiens, 2006) and the occurrence of two distinct penetration pathways has been proposed (Schönherr, 2006). Neutral, non-charged molecules may cross cuticles by dissolving the leaf through aqueous pores (Schönherr, 2006), micro pores, and/or spaces between molecules (Luque et al., 1995). The radii of cuticular aqueous pores have been estimated to range from 0.3 nm (ivy leaves) to 1.2 nm (tomato fruit; Schönherr, 2006).

In an attempt to bring together findings concerning the penetration of ionic compounds through the cuticle, Schönherr (2000; 2001) proposed the following general principles: (i) since ionic compounds in solution are surrounded by water molecules, a diffusion pathway parallel to the lipophilic pathway must occur; (ii) air relative humidity (RH) could be a key factor in determining the rate of penetration of solutions; (iii) given the importance of RH, active ingredients, either hygroscopic or with low points of deliquescence, will promote foliar penetration; (iv) concentration would serve as driving force for penetration, and the absolute amount which penetrates would probably be proportional to concentration; (v) smaller molecules should penetrate faster than larger ones; (vi) cations and anions will penetrate in equivalent amounts; and (vii) effective wetting agents can greatly increase the rate of penetration of electrolyte solutions. In contrast, stomatal uptake may resemble diffusion in bulk water, and therefore should not be restricted by the factors mentioned above (Eichert et al., 2006).

Iron sprays are generally based on Fe-chelates or Fe-salt solutions and can be expected to be taken up via the cuticle or stomata. However, recent evidence showed that foliar uptake of Fe-containing solutions does not fully concur with the seven principles described above. Fernández et al. (2005; 2006; 2008a) and Schönherr et al. (2005) observed no correlation between molecular mass and penetration rate. The Fe(III)-chelates commonly used as Fe sprays have sizes ranging from 1.0 – 1.3 nm (maximum radii) and 0.5 – 0.8 nm (minimum radii; Fernández et al., 2008a). Iron complexes in solution will be larger, due to hydration, but will remain smaller than stomatal apertures (e.g., 20 – 30 μm pore size for pear and peach leaves). Chelates may also penetrate the leaf through the cuticular pathway, since the sizes of Fe-compounds and the pores may be comparable.

Schönherr et al. (2005) and Fernández (2004) detected a negative correlation between the concentration of Fe(III)-chelates applied and the penetration rate, expressed as a percentage of the amount applied, which nevertheless represented a high amount of Fe penetrating the leaf or the cuticle. A similar effect has been described for the penetration of foliar-applied K (Chamel, 1996) and other elements (Tukey et al., 1961; Middleton and Sanderson, 1965) and also for 2,4-dichlorophenoxyacetic acid (2,4-D; Liu, 2004). Chamel (1996) hypothesised that the decrease in relative penetration rate with higher K concentrations may have been due to progressive saturation of the sites for uptake. A similar phenomenon was observed with Fe(III)-chelates, and it was suggested that high concentrations of these compounds may reduce the size of the aqueous pores (Schönherr et al., 2005). A reduction in water conductance through fruit cuticles after FeCl$_3$ treatment was reported by Weichert and Knoche (2006), which may have been caused by a competition for water between Fe(III) and ionised carboxyl groups in the cuticle, leading to partial dehydration of the pore (Schönherr, 2006). However, a different mechanism could be expected for the Fe(III)-chelates used in foliar sprays, since they have high stability constants (log$_{10}$ K$^{\text{(Fe)}}$ generally ranging from 10 to 25), and most of them are negatively-charged between pH 5 and pH 7 (the normal pH range of foliar sprays). It may be possible that, under the prevailing pH, concentration, and photo-reduction conditions, the functional groups in the Fe(III)-chelates could interact with each other, forming polynuclear Fe-complexes (Rich and Morel, 1990), and also with other formulation ingredients, components of the cuticle, and/or the walls limiting the apoplastic space.

FACTORS AFFECTING THE EFFICIENCY OF IRON-CONTAINING SOLUTIONS

Environmental factors

Environmental factors such as RH, temperature, and light will play a role with regard to droplet drying, cuticular hydration, and the physiological state of the plant (Currier and Dybing, 1959), among other parameters. Under field conditions, there is a continuous interaction between such factors, which will provoke diverse physiological responses. Thus, the results from field studies on foliar fertilisation may differ from those
obtained with annual plants and/or under controlled environmental conditions.

As described above, RH could be a key factor influencing the penetration of foliar sprays. At a high RH, cuticular permeability may be increased through hydration, and the drying of salt deposits will be delayed (Currier and Dybing, 1959; Schön herr, 2001). Substances with deliquescent points below the prevailing RH will remain as solutes, and leaf penetration will continue. In a study developed using cuticular membranes of astomatous Populus × canescens and different Fe-compounds, Schön herr et al. (2005) reported that the cuticle penetration rate was insignificant at RH ≤ 90%. These authors raised doubts about the usefulness of Fe-sprays under the arid and semi-arid conditions found in most of the areas of the World affected by Fe-chlorosis. However, foliar Fe-fertilisation trials carried out at RHs ranging from 30 – 60% showed the beneficial effects of applying Fe-substances to Fe-deficient fruit crops such as citrus, pear, peach, apple, mango, plum, and almond, both in terms of leaf re-greening and improved fruit yield and quality (Fernández et al., 2006; 2008a).

Currier and Dybing (1959) mentioned the positive effects of moderately warm temperatures in stimulating foliar penetration through changes in physiological processes (e.g., increased photosynthesis and translocation) and in the physico-chemical factors of the spray solution. Cuticular penetration of Fe-containing solutions at 100% RH did not seem to be affected by temperature changes ranging from 15° – 35°C (Schön herr et al., 2005).

Light is known to stimulate stomatal opening and various physiological processes such as photosynthesis or xylem flux (Currier and Dybing, 1959; Jyung and Wittwer, 1964), which may influence foliar Fe-uptake at some stage. Since Fe is thought to be taken up into the symplast via a light-dependent, plasma membrane-bound Fe(III) reductase, light may stimulate a sink-to-source Fe gradient within the leaf tissue, thereby promoting Fe penetration. Light has been shown to increase the rate of Fe-uptake and the distribution of leaf-applied Fe-containing solutions (Fernández, 2004; Schlegel et al., 2006; Wallihan et al., 1964).

The prevailing environmental conditions will also affect leaf morphology and structure in terms of, for example, cuticle thickness or the amount and/or composition of waxes (Currier and Dybing, 1959; Koch et al., 2004).

**Plant-related factors**

The uptake of leaf-applied chemicals can be expected to vary according to the particular plant species, variety, and growing conditions used, which will ultimately determine factors such as leaf morphology, structure, and the rates of physiological processes.

Both the upper and lower leaf surfaces are involved in the process of foliar uptake. Several investigators have reported on the penetrability of the lower epidermis vs. the upper epidermis, which will be governed chiefly by stomatal and cuticular variations between the two leaf surfaces (Currier and Dybing, 1959; Wójcik, 2004).

The structure and composition of the cuticle, as well as the morphology, distribution, and sizes of the stomata and leaf hairs, differ between plant species and may influence foliar uptake processes. Variations in cuticular structure and composition will result in differences in leaf wettability, retention and penetration of substances. Selective permeability of cuticles around the cuticular edges of guard cells has often been reported (Eichert et al., 2001; Schlegel et al., 2006; Schön herr, 2006).

Young, partially-expanded leaves are more penetrable than fully-expanded leaves (Sargent and Blackman, 1962). The stomata present in old leaves may fail to open (Turner and Begg, 1973) or, in the case of citrus leaves, may develop “plugs” (Turrell, 1947), thereby leading to lower penetration rates compared to younger leaves.

Factors relating to the physiological state of the plant such as root temperature, root osmotic potential, or nutrient status, may also modulate the effectiveness of foliar fertilisation (Weinbaum, 1996). Regarding Fe-chlorosis in fruit trees, structural (Maldonado-Torres et al., 2006) and morphological (Fernández et al., 2008b) changes have been observed, as illustrated in Figure 1 A, B for Fe-sufficient and Fe–deficient adaxial leaf surfaces in cherry trees, respectively. Iron-deficient
leaves have an altered surface appearance. Differences related to Fe-chlorosis with regard to the cuticle and waxes have been observed in peach, cherry, sugarbeet, and pear, and work is in progress to determine the significance of such variations in terms of physiological processes and the foliar uptake of Fe-fertilisers (Fernández et al., 2008b).

Effect of formulation adjuvants

The possibility of improving foliar uptake of Fe-containing solutions has long been recognised (Fernández and Ebert, 2005). According to Schönherr et al. (2005), the addition of hygroscopic humectants may be the only option to improve the performance of Fe-sprays in arid and semi-arid areas. Surfactants increase spray droplet retention and wetting, but their effect on the uptake of foliar sprays is complex and remains unclear (Liu, 2004). There is evidence that interactions between formulation components occur, but currently it is not possible to predict, a priori, the performance of a leaf-applied agrochemical in combination with a particular surfactant (Liu, 2004).

An example of the interactions between Fe-compounds and surfactants, as observed by Neumann and Prinz (1975), is illustrated in Table I. Iron concentrations in leaf tissue after foliar Fe-fertilisation were lower when using solutions of Fe(III)-EDTA in pure water than when using the same Fe compound and any of three different surfactants. Also, Surfactant 1 led to the highest tissue concentrations of Fe, suggesting the occurrence of different interactions between Fe-compounds and surfactants, at some stage (Fernández et al., 2008a).

In an attempt to trace the interactions between surfactant and Fe(III)-chelate molecules, surfactant and Fe-containing mixtures were analysed by electrospray-ionisation time-of-flight mass spectrometry (ESI-TOF MS). This technique enables accurate identification and quantification of ionisable compounds by using exact mass/charge (m/z) ratio determinations (Álvarez-Fernández et al., 2007). It is also an interesting tool with which to analyse surfactants because it provides qualitative information on surfactant structure concerning its molecular weight distribution and functional end-group identification.

The mass spectrum of Fe(III)-EDTA obtained with ESI-TOF MS in negative ion mode is represented in Figure 2A. The major peak corresponds to the \([\text{M} – \text{H}]^-\) molecular ion at an \(\text{m}/\text{z}\) value of 344.0. The mass spectrum of a non-ionic surfactant (an organosilicon) is shown in Figure 2B. Since MS with an electrospray ionisation source detects only ionic or ionisable molecules, no signal was detected in the mass spectrum with the non-ionic surfactant. The mass spectrum of Fe(III)-EDTA supplemented with the surfactant is represented in Figure 2C. A major peak at an \(\text{m}/\text{z}\) value of 344.0, corresponding to the \([\text{M} – \text{H}]^-\) molecular ion of the Fe(III)-EDTA chelate, was found once again. However, the occurrence of a negatively-charged polymer in the \(\text{m}/\text{z}\) range of 550 – 1,000 was detected. The \(\text{m}/\text{z}\) distance between monomeric units was 14, which is typical of ethylene-glycol and propylene-glycol hetero-polymers. These results show that the non-ionic

<table>
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<tr>
<th>Surfactant treatment</th>
<th>Fe concentration (mg g⁻¹ DW)</th>
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<tr>
<td>Surfactant 1†</td>
<td>245.3 ± 6.1 c</td>
</tr>
<tr>
<td>Surfactant 2</td>
<td>173.0 ± 3.5 b</td>
</tr>
<tr>
<td>Surfactant 3</td>
<td>185.1 ± 4.1 b</td>
</tr>
<tr>
<td>Pure water (no surfactant)</td>
<td>134.0 ± 3.1 a</td>
</tr>
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†Mean values followed by a different lower-case letter indicate different levels of significance according to Duncan’s multiple range test \((P \leq 0.05)\). Data are means ± SE \((n = 2; 30 leaves per sample)\).

Surfactant 1 was an organosilicon Surfactant 2 was an ethoxylated oil, and Surfactant 3 was an alkyl polyglycoside.
surfactant was ionised due to the presence of the Fe(III)-EDTA chelate.

Usually, Fe(III)-chelates are prepared by addition of FeCl₃ to the corresponding chelating agent, in this case K₂EDTA. Therefore, the hypothesis that Cl⁻ ions may be responsible for the ionisation of the polymer was subsequently tested. The mass spectrum of FeCl₃ plus 0.1% (v/v) Surfactant 1 is represented in Figure 2D. An identical polymer to the one observed in Figure 2C was formed. These results suggest that the Cl⁻ ions present in the Fe(III)-chelate solution may induce ionisation of the surfactant. The same polymer was observed in the mass spectrum of Fe(III)-EDTA synthesised from Na₂EDTA and FeCl₃ with Surfactant 1 (data not shown).

In summary, ions derived from the synthesis of the Fe(III)-chelate could induce ionisation of non-ionic surfactants due to “salting out” effects (Mackay, 1997), as shown above. This could affect the performance of surfactants as adjuvants in foliar sprays. Research is in progress to understand the interactions between Fe-substances and surfactants suitable for foliar application.

CONCLUSIONS AND FUTURE PERSPECTIVES

The performance of Fe-sprays is affected by many plant-related, environmental, and physico-chemical factors, which are currently not fully understood. Research should focus on investigating the potential interactions between formulation components using modern analytical techniques such as those described above. Efforts should be made to understand the relevance of the physico-chemical properties of spray solutions to design optimised Fe-containing formulations, and the significance of changes in the leaf surface in relation to the foliar uptake of agrochemicals. The process of penetration of a leaf-applied, Fe-containing solutions is not fully understood and should be investigated further, since foliar uptake is a prerequisite for leaf-cell Fe utilisation. Research on suitable foliar treatment strategies to ensure optimal plant coverage should also proceed. Similarly, information on plant Fe metabolism will facilitate the selection of bio-active Fe-containing compounds. The role of physiological processes and environmental factors in foliar Fe uptake and distribution should also be investigated further using intact leaves and following a holistic approach.

In summary, more knowledge relating to the role of Fe in plants, and on the effects of environmental, plant physiological, or leaf morphological factors, adopting a multi-disciplinary approach, is required for the development of effective Fe-spray formulations to correct widespread Fe-deficiency in fruit trees.

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