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Corresponding author:

- **Name** Victoria Fernández
- **Address:** Plant Nutrition Department, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), Apdo. 13034, 50080 Zaragoza, Spain
- **Phone N°:** +34976716169
- **FAX N°:** +34976716145
- **E-mail:** victoria.fernandez@gmx.de

Foliar Fertilization of Peach (*Prunus persica* (L.) Batsch) with different Iron Formulations: Effects on Re-greening, Iron Concentration and Mineral Composition in Treated and Untreated Leaf Surfaces

Victoria Fernández^{1,3*}, Victor Del Río^{1,3}, Lorena Pumariño¹, Ernesto Igartua², Javier Abadía¹ & Anunciación Abadía¹

¹*Departamento de Nutrición Vegetal and* ²*Departamento de Genética y Producción Vegetal, Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), Apdo. 13034, E-50080 Zaragoza, Spain*

³*Both authors contributed equally to this work*

**Corresponding author*

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Abstract

A trial to assess the effects of applying several Fe-containing formulations on Fe-deficient (chlorotic) peach leaves was carried out under field conditions. Solutions consisting of an Fe-containing compound (FeSO₄·7H₂O, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA or Fe(III)-IDHA) and one of five different surfactant treatments (no surfactant, an organo-silicon, an ethoxylated oil, a non-ionic alkyl polyglucoside and a household detergent) were applied to one half of the leaf *via* dipping, first at the beginning of the trial and then after 4 weeks. The re-greening of treated and untreated leaf areas was estimated with a SPAD apparatus, on a weekly basis, during 8 weeks. At the end of the experimental period, leaves were detached, and tissue Fe, N, P, K, Ca, Mg, Mn, Zn and Cu concentrations were determined in Fe-treated and untreated leaf areas. Treatment with Fe-containing solutions always resulted in leaf chlorophyll (Chl) increases, which however significantly depended on the Fe-source, the surfactant-type and the combination between both formulation components. Untreated leaf zones experienced a Chl increase only in some cases, and this depended on the type of surfactant used. Iron application significantly increased the Fe concentration of

59 treated and untreated leaf areas, especially with some formulations. Foliar treatment
60 with Fe-containing solutions induced significant changes in the concentration of
61 several nutrients as compared to those found in Fe-deficient peach leaves, with
62 changes being similar in treated and untreated leaf areas, although in some elements
63 the extent of the changes was of a different magnitude in both materials. This indicates
64 that some leaf mineral composition changes typical of chlorotic leaves are dependent
65 on leaf Fe concentration rather than on leaf Chl levels. Results obtained are relevant to
66 help understand the factors involved in the penetration and bioavailability of leaf-
67 applied Fe, and to assess the potential of foliar Fe fertilization to control Fe deficiency
68 in fruit trees.

1. Introduction

Iron (Fe) deficiency chlorosis can impair fruit quality and yield and lead to early tree death (Álvarez-Fernández et al., 2003; Álvarez-Fernández et al., 2006). To ensure adequate economic agricultural returns in areas where soil conditions induce Fe chlorosis, plants must be treated with Fe-containing compounds on a regular basis. Iron chelate supply *via* fertigation or soil treatment is currently the most effective method to control Fe chlorosis under field conditions (Lucena, 2006). However, foliar Fe fertilisation could also be an economical and target-oriented strategy to cure plant Fe chlorosis, although recent reviews have shown that the response to Fe sprays could be variable, depending on plant species and experimental conditions (Fernández and Ebert, 2005; Fernández et al., 2005). The lack of understanding of the many factors relating to penetration, translocation and bioavailability of leaf applied, Fe-containing solutions has hindered the development of suitable spray formulations.

Aerial plant organs are covered by a continuous hydrophobic cuticle, which constitutes the interface between the plant and the surrounding environment (Schönherr, 2006). The cuticle is a chemically heterogeneous membrane of variable structure and composition, depending on many factors (Jeffree, 2006). It consists of an insoluble biopolymer matrix (cutin and/or cutan), with waxes both embedded (intracuticular) and deposited on the surface (epi-cuticular) (Heredia, 2003; Matas et al., 2005). Variable amounts of polysaccharide fibrils and pectin lamellae may extend from the cell wall, binding the biopolymer to the underlying tissue (Bargel et al., 2006; Jeffree, 2006). For decades, research concerning leaf uptake of agrochemicals has chiefly focused on cuticular penetration (Schönherr, 2006). Cuticles have been shown to be permeable to water and ions and also to polar compounds (Kerstiens, 2006; Schreiber, 2006). Two distinct penetration pathways in the cuticle have been suggested (Schlegel et al., 2005; Schönherr, 2006), with uncharged molecules dissolving and diffusing in lipophilic domains made of cutin and cuticular waxes (lipophilic pathway), and ionic species crossing lipid membranes through aqueous pores (Schönherr, 2006), micropores and spaces between molecules (Luque et al., 1995a). Hydrophilic pathways may in fact follow the spatial localization of a polysaccharide fraction with a high hydration capacity (Domínguez and Heredia, 1999). Stomata may also be important in the penetration of leaf-applied chemicals, although their role is not fully understood (Eichert et al., 2006).

Foliar uptake of agrochemicals is a complex process, which depends on the physico-chemical properties of the spray solution, the characteristics of the leaf surface, the type and concentration of the additives and the existing environmental conditions (Wang and Liu, 2007). An agrochemical active ingredient is often ineffective if applied to the target surface alone (Perkins et al., 2005), and the use of adjuvants can accelerate cuticular and foliar penetration (Liu, 2004; Schönherr et al., 2005). While it is clear that surfactants increase spray droplet retention and wetting by lowering surface tension, the effect of surface-active agents on the uptake of foliar sprays is very complex and the underlying mechanisms are not fully understood (Liu, 2004). Some surfactants have also been shown to have a plasticizing effect, promoting the fluidity or even solubilizing cuticular waxes, (Tamura et al., 2001; Perkins et al., 2005), while others may hydrate the cuticle and increase the permeability of the plasma membrane (Wang and Liu, 2007).

Concerning the effect of leaf applied Fe-containing formulations, a recent study supports a possible role for stomata in the penetration of Fe compounds, since uptake through the lower leaf side was higher as compared to the upper one (Schlegel et al., 2006). Schönherr et al. (2005) found no response to temperature and no correlation between molecular mass and penetration rates of several Fe-containing compounds. The penetration rate of Fe-chelates through cuticular membranes was found to decrease with increasing concentrations, as it has also been described for 2,4-D (Liu, 2004), leading to the suggestion that Fe chelates may reduce somehow the size of aqueous pores (Schönherr et al., 2005). Schönherr et al. (2005) and Schlegel et al. (2006) also concluded that 100% relative humidity (RH) was required for foliar penetration of Fe(III)-chelates and that addition of hygroscopic humectants may be an option to improve the performance of Fe-sprays. However, working with peach and pear trees grown on calcareous soils, the penetration and physiological response to several leaf applied, Fe-containing compounds have been demonstrated in practice (Álvarez-Fernández et al. 2004; Fernández et al., 2006).

This investigation was aimed at assessing the performance of five different surfactant formulations (no surfactant, an organo-silicon, an ethoxylated oil, a non-ionic alkyl polyglucoside and a household detergent) in combination with five commercially relevant Fe-containing compounds ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA or Fe(III)-IDHA) on re-greening-associated processes in Fe-deficient peach leaves under field conditions. This paper is the follow-up of a

previous study that used only one surfactant (the alkyl polyglucoside) and several adjuvants (glycerol, methanol or glycine-betaine) in combination with several Fe-containing compounds to re-green Fe-deficient peach leaves (Fernández et al. 2006). Treatments were applied *via* dipping instead of spraying to avoid cross-contamination between Fe-compounds and surfactants, and also to permit assessing of treatment effects on untreated leaf surfaces.

2. Materials and Methods

2.1. Treatment solutions: iron sources and surface-active agents

Treatment solutions (2 mM Fe) contained FeSO₄·7H₂O (Panreac, Barcelona, Spain), Fe(III)-EDTA, Fe(III)-DTPA, Fe(III)-IDHA or Fe(III)-citrate, dissolved in water type II analytical grade (obtained with an Elix 5 apparatus, Millipore, USA). The latter four compounds were synthesized in the laboratory by complexing free Fe(III) (FeCl₃, acidic AAS standard, Merck, Darmstadt, Germany) with the corresponding ligand at 1:1 (Fe:ligand) ratios, except for Fe(III)-citrate, where the ratio was 1:20. The chelating agents used were K₂EDTA·2H₂O (CAS No. 25102-12-9; Panreac), DTPA (CAS No. 67-43-6; Merck), IDHA (CAS No. 1445-38-83-0; Baypure CX 100 Solid, supplied by Lanxess, Leverkusen, Germany), and Na₃-citrate·2H₂O (CAS No. 6132-04-3; Sigma, St. Louis, Mo, USA). Once the ligand was complexed by the metal, the pH of the solution was adjusted to 5.5, a value for which all chelates used in this study are stable. In contrast, FeSO₄ solutions were kept at pH 4.0 to slow down the process of atmospheric oxidation and were applied immediately after preparation (Fernández et al., 2006).

Some characteristics of these Fe-compounds are summarized in Table 1. The maximum and minimum molecular *radii* (i.e. the maximum and minimum distance between distal atoms, after rotating the molecule along the 3 axes) of model crystalline Fe-chelates were estimated with the program ChemDraw 3D Ultra (Cambridge Scientific, UK) after search on the Cambridge Structural Database (CSD, The Cambridge Crystallographic Data Centre, Cambridge, UK). For simplicity, the estimation was made for Fe-containing compounds lacking the hydration shell that will probably have in aqueous solution.

Surface-active agents were tested at a concentration of 1 g l⁻¹, and included an organo-silicon compound, polyether-modified polysiloxane, from Goldschmidt

GmbH, Essen, Germany (thereafter called Surfactant S-1), an ethoxylated oil, obtained from Cognis, Düsseldorf, Germany (S-2), a non-ionic alkyl polyglucoside, obtained from Cognis, Düsseldorf, Germany (S-3) and the household detergent Mistol, a mixture of ionic and non-ionic surfactants, obtained from Henkel, Barcelona, Spain (S-4). For each Fe source, a control using no surfactant was also carried out.

2.2. Field plant material

Twelve year-old peach trees (*Prunus persica* (L.) Batsch, cv. Miraflores, grafted on seedling) grown on calcareous soil (*Typical xerofluvent*, clay-loamy texture, with 30% total CaCO₃, 10% active CaCO₃, 7 mg kg⁻¹ DTPA-extractable Fe, 2.6% organic matter and pH 7.8 in water) were used. The flood irrigated orchard had a frame of 5 × 4 m and was located in Plasencia de Jalón (Zaragoza province, Aragón, Spain). The orchard was appropriately maintained in terms of nutrition, pruning and pest and disease control. Trees did not receive any exogenous Fe input for one year prior to the beginning of the foliar fertilization trial, and therefore developed Fe deficiency symptoms in springtime. The experiment was designed as a completely randomized block. Four trees with a similar leaf chlorosis level (average SPAD value of 14, corresponding to approximately 100 µmol chlorophyll (Chl) m⁻²) were selected at the beginning of the trial and then used for the treatments.

2.3. Foliar treatment with Fe-containing formulations

A total of 52 one-year old, medium size shoots, located at a height of approximately 1.5 meters from the ground level, were chosen in the 4 selected trees (13 shoots per tree). Prior to foliar fertilizer application, all shoots and leaves were adequately labelled with colour tape. The upper side of all leaves used for experimental purposes was marked in the middle with a black line, drawn with a felt-tip lab marker, as shown in Fig. 1. In each leaf, one half of the surface, i.e. from the tip to the middle black line (Fig. 1) was dipped for 10 s into one of the Fe-containing formulations. Drying of the wetted surface took place within five minutes after treatment. In every shoot, 30 young, fully expanded leaves were treated individually with a given formulation, and each formulation was applied to the leaves of two different shoots, each one in a different tree. The 26 treatments (5 Fe x 5 surfactant treatments, plus zero Fe in water) were applied first at the beginning of the experiment

and then repeated in the same leaves 4 weeks later. Solutions were applied on July 17th and on August 18th 2006, from 8:00 to 10:00 solar time. At the time of the treatment RH and temperature were approximately 60-80% and 20°C. During the night following foliar fertilization, RH values were in the range 60-85% in both treatments. Fruits in the orchard were harvested at the end of August.

2.4. Leaf re-greening assessment

The re-greening effect of the different Fe-carrier-surfactant combinations was evaluated for an 8-week period. Re-greening was assessed on the treated (i.e. the leaf surface which was dipped into Fe-containing solutions) and non-treated (i.e. the remaining half leaf which did not receive directly any exogenous Fe) leaf areas. Leaf Chl concentration was estimated with a SPAD Chl meter (Minolta 502, Osaka, Japan), using two measurements in the middle of the treated area and two more in the middle of the untreated area in each leaf (see below). For the Chl measurements, 10 labelled leaves per treatment (two groups of five in two different trees) were monitored over time. Initial SPAD-measurements were carried out 1 day before the first foliar treatment. SPAD measurements were converted into Chl concentration values by using a curve obtained experimentally (Fernández et al., 2006).

2.5. Leaf tissue mineral analysis

At the end of the experimental period, leaves were detached and the mineral element concentration of treated and untreated leaf areas was analyzed according to standard laboratory procedures (Igartua et al., 2000). Leaves were divided in two sections, discarding a 2-mm strip corresponding to the black marker line. Two groups of at least 30 treated leaves were sampled per treatment. Prior to processing, both leaf sides were carefully scrubbed with a brush and a 0.1% detergent (Mistol, Henkel) solution to remove surface contamination. The leaf midrib was not removed. Thereafter, leaves were washed thoroughly in tap water and then in ultrapure water. Results were expressed as percentage of dry weight (DW) for macronutrients (N, P, K, Ca and Mg) and as $\mu\text{g g}^{-1}$ DW for micronutrients (Fe, Cu, Mn and Zn).

2.6. Statistical analysis

Data were statistically evaluated by two-way analysis of variance (ANOVA)

with the program SPSS 11.0 to assess the significance of the main factors and interactions. Interaction effects were broken down into contributions of single levels of factors Fe and Surfactant, using the slice option in proc GLM in SAS v9 (SAS Institute Inc., Cary, NC, USA). Means were also compared using Duncan's test at $P < 0.05$ in order to find significant differences between treatments.

3. Results

3.1. Chlorophyll evolution following foliar treatment

The re-greening time-course of the Fe-treated and untreated leaf areas for the different treatments was assessed by the Chl percentage increase in relation to the initial Chl values (Fig. 2). In the treated leaf areas, Fe-containing formulations led to significant Chl increases over time, whereas leaves dipped in Fe-free solutions showed only a slight re-greening. Treated leaf areas experienced a rapid Chl increase after the 1st treatment, whereas the 2nd one induced a steep Chl increment during 2 weeks, with a minor dip in Chl content at week 7 and a large Chl increase in the last week of the experiment (Fig. 2A). In contrast, in the untreated leaf areas the first treatment had little effects on Chl content, whereas after the second treatment only slight increases in Chl were observed, somewhat larger than those found in areas dipped in solutions with zero-Fe (Fig. 2B).

3.2. Effect of Fe-containing compounds and different surfactants

The significance of the re-greening effects of the Fe-compound and surfactant types and their interactions were tested in treated and untreated leaf areas, taking into account the final Chl, Chl increases and tissue Fe concentrations (Table 2). In treated leaf areas, two-way ANOVA indicated highly significant effects (at $P \leq 0.001$) for the type of Fe compound, the surfactant-type and the interaction Fe-compound x surfactant. The significance of the interaction arises from most Fe sources and surfactants. For untreated leaf areas, surfactant-type had a highly significant effect ($P \leq 0.001$) for all parameters, whereas the effect of the Fe-compound was highly significant for final Chl and Fe concentration and significant ($P \leq 0.05$) for Chl increases. The effect of the interaction between Fe-compounds and surfactants was highly significant ($P \leq 0.001$) for final Chl, and Fe concentrations, and significant ($P \leq$

0.05) with regard to Chl increases. In this case, the significance of the interactions was mainly associated with Fe(III)-DTPA and Fe(III)-citrate, and also with the surfactants S-1 and S-3. No significant re-greening was observed when the zero-Fe treatment was applied to the leaf.

Changes in leaf Chl and Fe concentration associated with each formulation are shown in Fig. 3. With Fe-sulfate, the highest Chl increases in treated areas were found in combination with S-1 and S-2, whereas S-3 and surfactant-free solutions led to intermediate values and S-4 led to the lowest one. In untreated leaf areas, the highest Chl increases were found with S-1 or in surfactant-free solutions, with all other treatments leading to smaller increases, similar to the value found in Fe-deficient leaves treated with zero-Fe. In treated leaf areas, Fe-sulfate led to the highest Fe concentrations with S-2, followed by S-3 and S-1, with the S-4 and the surfactant-free solution leading to the lowest values. In untreated leaf areas, the highest tissue Fe concentrations were found with S-1 and S-2, followed by S-3 and the surfactant-free solutions. With S-4, Fe in untreated areas was within the range found in Fe-deficient leaves treated with zero-Fe.

Iron(III)-IDHA treated leaf areas had the highest Chl increase in combination with S-1, followed by S-2, with S-4, with surfactant-free solutions and S-3 giving lower values. The highest Chl increases in untreated leaf areas were found with S-1 and S-2, followed by S-4 and surfactant-free solutions. Untreated areas of leaves fertilized with S-3 and Iron(III)-IDHA did not show any re-greening. Treated leaf areas had the largest Fe concentration values with S-3, followed by S-2 and S-1, and then S-4 and the surfactant-free solutions. In the untreated areas, the largest Fe concentration was found with S-2 and S3, followed by S-1, whereas S-4 and surfactant-free solutions led to tissue Fe concentrations similar to those found in Fe-deficient leaves treated with zero-Fe.

Iron(III)-citrate led to the highest Chl increases in treated leaf areas with S-1, followed by S-2, S-3 and S-4. Surfactant-free solutions induced a lower Chl increase, but still significantly higher than that measured for chlorotic leaves treated with zero-Fe. With regard to untreated leaf areas, the highest Chl increases were also recorded for S-1 solutions, with all other treatments leading to smaller increases. Iron(III)-citrate formulations led to the highest tissue Fe concentrations in treated leaf areas with S-1, followed by surfactant-free solutions, S-2 and S-3, whereas the Fe concentrations in S-4-treated leaves were lower. In untreated leaf areas, the highest

tissue Fe concentration was also with S-1, with all other treatments leading to similar low values, excepting for S-4, which led to tissue Fe values in line with those found in chlorotic leaves treated with zero-Fe.

Iron(III)-EDTA induced the highest Chl increases in treated areas with S-1 and S-2, while S-3, S-4 and pure water led to similar values. The highest Chl increases in untreated areas were also measured with S-1, S-2 and S-3, whereas S-4 and the surfactant-free treatment did not lead to changes when compared to Fe-deficient leaves treated with zero-Fe. The highest tissue Fe concentrations in treated leaf areas were obtained with S-1, followed by S-3 and S-2. Surfactant-free solutions were capable to increase leaf Fe, whereas with S-4 Fe concentrations were similar to those found in chlorotic leaves treated with zero-Fe. In untreated leaf areas, the highest Fe concentrations were found for S-1, S-2 and S3, followed by S-4 and the no-surfactant treatment.

The highest Chl increase of Fe(III)-DTPA treated leaf areas was found with S-1, followed by S-2 and S-3, with S-4 and the surfactant-free treatment leading to smaller increases. The highest Chl increase in untreated areas was for S-1, followed by S-3, the surfactant-free treatment and S-2, whereas S-4 did not induce Chl changes as compared to the value recorded for control leaves. In treated areas the highest tissue Fe concentrations were found with S-1, followed by S-2, S-4 and S-3 and the surfactant-free treatment. The highest Fe concentration in untreated areas was with S-1, followed by S-2 and S-3, with S-4 and surfactant-free solutions leading to Fe concentrations similar to those measured in chlorotic leaves treated with zero-Fe.

3.3. Mineral element concentrations after foliar treatment

The effect of foliar Fe-fertilization on the concentration of macro- and microelements was also assessed (Table 3). With regard to the macroelement composition of treated areas, N concentrations were significantly lower than those found in Fe-deficient leaves. In the untreated leaf parts the N decrease was even larger. The P concentrations were also lower in leaves fertilized than in Fe-deficient leaves, and this effect was more pronounced in the untreated areas. Similarly, foliar Fe application induced leaf K concentrations of 1.8 to 2.0% DW, both in the treated and untreated leaf areas, compared to the value of 2.9% DW found in chlorotic leaves. In contrast, Mg concentrations were larger in fertilized leaves (both in the treated and

untreated leaf areas) than in chlorotic ones. Foliar Fe supply also induced large increases in leaf Ca, and the effect was also more pronounced in untreated areas.

As far as micronutrients are concerned, Mn concentrations in treated areas were significantly lower than those found in untreated areas and chlorotic leaves, while Zn concentrations in treated and untreated leaf areas were somewhat lower than those found in Fe-deficient leaves (Table 3). In the case of Cu, concentrations in treated and untreated leaf areas were lower than those found in Fe-deficient leaves, with the exception of leaf areas dipped in Fe-DTPA.

Since only minor differences were found between fertilizer formulations, the overall average concentration changes (including all five surfactant and five Fe-compound treatments) in treated and untreated leaf areas are also summarized in Fig. 4, taking as reference values found in chlorotic leaves. Treated leaf areas (black bars) had major increases not only in Fe, but also in Ca and Mg. Remarkably, leaf parts not dipped into the Fe-fertilizers (white bars) had also increases in Fe and Mg, and very large increases in Ca. Treated leaf areas also had major decreases in K and moderate decreases in N, P, Mn, Cu and Zn, whereas untreated leaf areas also had major decreases in the same elements, with the exception of Mn, whose concentrations were similar to those found in chlorotic leaves.

4. Discussion

Iron foliar fertilization (with 2 mM Fe) led to significant re-greening of Fe-deficient, chlorotic leaves, confirming data obtained in previous studies (Fernández et al., 2006). However, the degree of re-greening was markedly dependent on fertilizer formulation, confirming the variability of the response to Fe foliar sprays (see reviews by Abadía et al., 2002; Fernández, 2004; Fernández and Ebert, 2005). Both surfactant-type and Fe-source were relevant in determining formulation efficiency, with the type of surfactant being the most important factor. Three different commercial surface-active agents were tested: an organosilicon compound proposed to promote stomatal infiltration (S-1; Schlegel et al., 2006), an oil-seed rape derivative (S-2; Haefs et al., 2002) and an alkyl-polyglucoside (S-3; Fernández et al., 2006). Also, a household detergent commonly used for Fe-fertilization in Spain (S-4), and pure water solutions (i.e. no surfactant at all) were used as controls. With all Fe-compounds, the surfactant that led to better re-greening was S-1, although leaf burn was observed when used in

combination with most Fe-compounds (excepting for Fe(III)-IDHA), as described in other studies with organo-silicons (e.g. Neumann and Prinz, 1975). The rest of surfactants did not cause phytotoxicity symptoms at the concentration used (1 g L^{-1}). Surfactant 2 gave results comparable to S-1 with only two Fe-products, Fe(II)-sulfate and Fe(III)-EDTA, whereas S-3 gave acceptable results with Fe(II)-sulfate, Fe(III)-citrate and Fe(III)-DTPA, but not with Fe(III)-IDHA and Fe(III)-EDTA. Results with S-4 were generally similar to those obtained with no surfactant, excepting for the case of Fe(III)-citrate. Results obtained demonstrate the key role of surface-active agents in designing efficient spray formulations, both with regard to leaf penetration and re-translocation of Fe.

With respect to the Fe-compounds tested, all of them could be efficient in re-greening treated leaf areas, provided they are supplemented with an appropriate surfactant. However, in the absence of surfactants, all Fe-compounds were also capable of penetrating leaves, although under these conditions re-greening occurred in isolated spots across the leaf surface. Compounds which performed more constantly with all surfactants tested were Fe(II)-sulfate, Fe(III)-DTPA and Fe(III)-citrate. Conversely, the efficiency of products such as Fe(III)-IDHA and Fe(III)-EDTA depended heavily on the type of surfactant. Therefore, the interaction between surface-active agents and Fe-compounds must be always considered in designing Fe-formulations, and deserve further investigation.

Iron concentrations in treated leaf areas can provide information about penetration mechanisms. Iron concentration increased markedly in treated areas, in most cases reaching values higher than $150 \text{ } \mu\text{g g}^{-1} \text{ DW}$, and in some instances approaching values similar or even higher than those found in Fe-sufficient peach leaves ($240 \text{ } \mu\text{g g}^{-1} \text{ DW}$; Abadía et al., 1985; Belhodkja et al., 1998). However, Fe concentration increases did not fully correlate with re-greening rates. From our results, it appears that Fe-compounds of different size can penetrate the leaf in the absence of surfactants, although generally at a lower rate than those found with surfactants, and also that differences in penetration cannot be explained only by molecular size. The Fe(III)-chelates used have sizes ranging from 1.0 to 1.3 nm (maximum *radii*) and 0.5 to 0.8 nm (minimum *radii*), whereas Fe(II) could have a Van der Waals radius close to 0.2 nm. Even keeping in mind that the molecules will likely be larger in solution due to hydration, these Fe-compounds are much smaller than peach leaf stomatal pores (approximately $15\text{-}26 \text{ } \mu\text{m}$). With respect to the cuticle, a polar pathway has been

proposed to consist of aqueous pores (Schönherr, 2006) or an aqueous continuum (Beyer et al., 2005). The *radii* of cuticular aqueous pores have been estimated to be approximately (in nm) 0.3-0.45 in leaves, and 0.7-1.2 in fruits (Luque et al., 1995b; Beyer et al., 2005; Popp et al., 2005; Schönherr, 2006), although new data point out to considerable larger pores, up to 3 nm *radii*, in leaves of different plant species (Eichert and Goldbach, 2008). From our data we cannot rule out the possibility that Fe-compounds may penetrate the leaf *via* a cuticular hydrophilic pathway, given that the sizes of Fe-compounds and pores could be comparable. Further studies to measure the pore *radii* of peach leaf cuticles versus the molecular size of Fe-chelates would be necessary to shed light on this issue. Iron-chelates may penetrate the cuticle through a different mechanism, for instance via a tortuous solubilization process in the polymer matrix. It should also be borne in mind the possibility that upon the prevailing, e.g. pH, concentration and photo-reduction conditions, functional groups in Fe(III)-chelates can interact with each other forming polynuclear Fe-complexes (Rich and Morel, 1990), and also with cuticle (Weichert and Knoche, 2006), cell wall and apoplastic (Bienfait and Scheffers, 1992) components.

Iron concentrations in untreated areas could reflect the extent of re-distribution and re-translocation of this element within the leaf. Surprisingly, in many cases leaf areas not dipped into Fe-fertilizer solutions also had major Fe concentration increases in the presence of surfactants, a fact which could be attributed to Fe re-translocation from treated to untreated leaf areas, although these Fe increases did not result in major re-greening. This occurred with solutions containing surfactants S-1, S-2 and S-3 and all five Fe-compounds used. In most cases, Fe concentrations increases in untreated areas were not as large as those found in treated ones, but with some formulations increases in both leaf areas were in a similar range (e.g. with Fe(III)-DTPA). Using no surfactant, Fe increases in untreated areas were only found with Fe(II)-sulfate and Fe(III)-citrate. It is unlikely that Fe concentration increases in untreated areas may result from surface mass flow movement of Fe compounds at the moment of application, because the treated leaf surface dried within a few minutes. The night following application RH was 65-85%, well below the deliquescence point (>90% RH) measured by Schönherr et al. (2005) for several Fe-chelates. The low surface tension of S-1 solutions may have enabled that the liquid progressed from the treated leaf surface above limiting area at the time of treatment, but this could not be observed visually. Chlorophyll increases above 50% of the initial value were found only with

Fe(III)-DTPA and Fe(III)-citrate plus S-1. Although Chl values reflect the average concentration in the middle part of the untreated area, more intense Chl increases were found close to the limit of the application zone (data not shown). This effect was more pronounced with surfactant S-1. The lack of biological effect of fairly high tissue Fe concentrations suggests that Fe could be immobilized in inactive forms, similarly to the “chlorosis paradox” phenomenon described in Fe-deficient leaves of field-grown plants (Morales et al., 1998).

In treated leaf areas, Fe foliar fertilization led to decreases in the concentrations of K, N, P and all micronutrients, which became similar to those found in Fe-sufficient peach leaves, both in treated and untreated areas (Belkhodja et al., 1998). This may reflect the normalization of leaf metabolic processes as a result of re-greening. In Fe-treated leaf areas, there were also significant increases in the concentrations of Mg and especially Ca, which were much higher than the standard values described for peach (Westwood, 1978). In untreated areas Fe foliar fertilization also induced decreases in K and increases in Mg similar to those found in treated ones, and even larger decreases in P and N and increases in Ca than those occurring in treated areas. The differences in the extent of N decrease in treated and untreated areas may be related to the need for this element to build Rubisco during re-greening. Results suggest that the increase in leaf Fe upon Fe foliar fertilization led to a switch in nutrient composition, from a high K-N-P/low Ca-Mg to a high Ca-Mg/low K-N-P state. All these data also indicate that mineral composition changes usually found in Fe-deficient leaves could be associated with leaf Fe concentration rather than with leaf chlorosis itself.

5. Conclusion

All Fe-compounds can be effective in promoting re-greening, provided an adequate surface-active agent is used in the formulation. In fact, the type of surfactant is a key factor in the efficiency of the Fe foliar fertilizer, and three of them, i.e., the organo-silicon, the ethoxylated oil and the non-ionic alkyl polyglucoside, showed some efficacy depending on the Fe-compound used. Foliar Fe application increased Fe concentrations both in treated and untreated areas, whereas re-greening occurred preferentially in treated vs. untreated areas. Iron application changed dramatically the mineral composition of Fe-deficient leaves, both in Fe-treated and untreated areas, leading to element concentrations fairly similar to those of Fe-sufficient leaves. Given the complex scenario ruling the performance of Fe spray formulations, more research

efforts should be carried out in the future to elucidate the mechanisms of foliar penetration, Fe allocation and its metabolism within the plant.

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599 Fig. 1. Picture of a treated leaf, 8 weeks after the first foliar Fe treatment. The leaf was
600 dipped in a solution containing 2mM Fe(III)-IDHA and 0.1% surfactant S-1, only from
601 the tip to the black marker line. In this specific treatment, re-greening also progressed
602 from the black mark towards the leaf base.

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Fig. 2. Time-course of Chl changes in leaves treated with Fe-containing solutions. (A) leaf surfaces dipped in the different Fe formulations. (B) untreated leaf surfaces. Treatments were applied on weeks 0 and 4 as indicated by arrows. Data for each Fe-compound are means $\pm$ SD of all surfactant treatments (5 surfactants, 10 replications, n=50).

Fig. 3. Final Chl increments (Week 8-Week 0; $\mu\text{mol m}^{-2}$) and tissue Fe concentrations ($\mu\text{g g}^{-1}$ DW) in treated and untreated leaf areas. Data are means $\pm$ SD (n=10 for Chl and 2 for Fe).

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622 Fig. 4. Mineral element changes resulting from foliar Fe application, as compared to
623 the elemental concentrations found in Fe-deficient leaves. Data are means $\pm$ SD of all
624 five surfactant and five Fe-compound treatments, with 2 replications each (n=50).

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Tables

Table 1

Stability constants, molecular weight, and maximum and minimum molecular *radii* of the Fe-compounds used in foliar fertilization treatments.

Characteristics of the Fe-compounds used in foliar fertilization treatments

Fe-containing compound	Stability constant (in aqueous solution, at 25°C and ionic strength 0.1 M)	Molecular mass (g mol ⁻¹)	Mean maximum molecular radius (nm)	Mean minimum molecular radius (nm)
Fe(II)-sulfate	-	278	0.2	0.2
Fe(III)-IDHA*	10 ¹⁶	392	0.97	0.78
Fe(III)-EDTA**	10 ²⁵	428	0.96	0.76
Fe(III)-DTPA***	10 ²⁸	449	1.08	0.91
Fe-(III)-citrate**	10 ¹²	245	1.26	0.52

* Personal communication, Lanxess GmbH

** According to Furia (1972)

*** According to van Damm et al. (1999)

Table 2

Two-way ANOVA analysis of data corresponding to final Chl concentration and Chl increase (both estimated from SPAD measurements) and leaf tissue Fe, in treated and untreated leaf areas, 8 weeks after treatment with different Fe-compounds and surfactants.

	Treated leaf area			Treated leaf area		
	Leaf tissue Fe	Final Chl concentration	Chl increase	Leaf tissue Fe	Final Chl concentration	Chl increase
Fe-compound	***	***	***	***	***	*
Surfactant-type	***	***	***	***	***	***
Fe-compound × surfactant-type	***	***	***	***	***	*
Fe(II)-sulfate	***	***	***	***	*	ns
Fe(III)-IDHA	***	***	***	***	ns	ns
Fe(III)-EDTA	***	***	***	***	*	ns
Fe(III)-DTPA	***	***	***	***	***	***
Fe-(III)-citrate	***	***	***	***	**	**
S-1	***	***	***	***	***	***
S-2	***	***	ns	***	ns	ns
S-3	***	***	***	***	***	ns
S-4	***	***	***	***	ns	ns
None	***	***	**	***	*	ns

ns: no significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$

Table 3

Final leaf concentrations of N, P, K, Mg, Ca (% DW) and Fe, Mn, Cu and Zn ($\mu\text{g g}^{-1}$ DW), in treated and untreated leaf areas, 8 weeks after treatments with several Fe-compounds. Data correspond to the average of the 5 surfactant treatments for a given Fe-compound (means $\pm$ SE, 5 treatments, 2 replications each, $n=10$). Data were compared with the composition of Fe-deficient leaves in the same orchard. Different letters within each column indicate means are different according to Duncan's t test ($P \leq 0.05$).

	Treated leaf area								
	Macroelements (% DW)					Microelements ($\mu\text{g g}^{-1}$ DW)			
	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
Fe-deficient leaves	3.88 $\pm$ 0.04 ^e	0.24 $\pm$ 0.01 ^c	2.91 $\pm$ 0.07 ^c	0.62 $\pm$ 0.01 ^a	2.37 $\pm$ 0.01 ^a	93.4 $\pm$ 2.1 ^a	44.0 $\pm$ 0.9 ^d	26.0 $\pm$ 0.8 ^d	16.5 $\pm$ 0.8 ^b
Fe(II)-sulfate	3.49 $\pm$ 0.02 ^d	0.21 $\pm$ 0.01 ^a	1.96 $\pm$ 0.03 ^{ab}	0.75 $\pm$ 0.01 ^b	3.14 $\pm$ 0.03 ^b	192.1 $\pm$ 3.2 ^d	33.7 $\pm$ 0.5 ^a	18.8 $\pm$ 0.2 ^a	11.5 $\pm$ 0.3 ^a
Fe(III)-IDHA	3.40 $\pm$ 0.02 ^c	0.23 $\pm$ 0.01 ^b	2.03 $\pm$ 0.03 ^b	0.80 $\pm$ 0.01 ^d	3.36 $\pm$ 0.02 ^{cd}	165.9 $\pm$ 3.7 ^c	37.6 $\pm$ 0.4 ^c	20.1 $\pm$ 0.2 ^b	11.7 $\pm$ 0.3 ^a
Fe(III)-EDTA	3.29 $\pm$ 0.03 ^b	0.21 $\pm$ 0.01 ^a	1.87 $\pm$ 0.03 ^a	0.76 $\pm$ 0.01 ^{bc}	3.31 $\pm$ 0.03 ^c	166.3 $\pm$ 5.2 ^c	35.2 $\pm$ 0.1 ^b	19.0 $\pm$ 0.1 ^a	12.3 $\pm$ 0.3 ^a
Fe(III)-DTPA	3.19 $\pm$ 0.02 ^a	0.21 $\pm$ 0.01 ^a	1.90 $\pm$ 0.04 ^a	0.79 $\pm$ 0.01 ^d	3.53 $\pm$ 0.02 ^e	138.2 $\pm$ 1.7 ^b	37.0 $\pm$ 0.7 ^c	23.8 $\pm$ 0.7 ^c	18.6 $\pm$ 1.1 ^c
Fe(III)-citrate	3.30 $\pm$ 0.01 ^b	0.21 $\pm$ 0.01 ^a	1.88 $\pm$ 0.01 ^a	0.77 $\pm$ 0.01 ^c	3.42 $\pm$ 0.03 ^d	161.8 $\pm$ 3.0 ^c	36.2 $\pm$ 0.5 ^{bc}	18.9 $\pm$ 0.3 ^a	12.0 $\pm$ 0.4 ^a
	Untreated leaf area								
	Macroelements (% DW)					Microelements ($\mu\text{g g}^{-1}$ DW)			
	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
Fe-deficient leaves	3.88 $\pm$ 0.04 ^c	0.24 $\pm$ 0.01 ^b	2.91 $\pm$ 0.1 ^c	0.62 $\pm$ 0.01 ^a	2.37 $\pm$ 0.01 ^a	93.4 $\pm$ 2.1 ^a	44.0 $\pm$ 0.9 ^a	26.0 $\pm$ 0.8 ^c	16.5 $\pm$ 0.8 ^c
Fe(II)-sulfate	2.55 $\pm$ 0.01 ^b	0.19 $\pm$ 0.01 ^a	1.95 $\pm$ 0.02 ^b	0.72 $\pm$ 0.01 ^c	4.98 $\pm$ 0.02 ^d	145.7 $\pm$ 5.2 ^c	43.1 $\pm$ 0.5 ^a	18.5 $\pm$ 0.1 ^b	10.9 $\pm$ 0.3 ^a
Fe(III)-IDHA	2.54 $\pm$ 0.01 ^b	0.19 $\pm$ 0.01 ^a	2.01 $\pm$ 0.02 ^b	0.70 $\pm$ 0.01 ^c	4.91 $\pm$ 0.03 ^{cd}	141.9 $\pm$ 4.9 ^c	45.2 $\pm$ 0.7 ^a	17.7 $\pm$ 0.1 ^a	11.2 $\pm$ 0.3 ^a
Fe(III)-EDTA	2.55 $\pm$ 0.02 ^b	0.18 $\pm$ 0.01 ^a	1.82 $\pm$ 0.02 ^a	0.68 $\pm$ 0.01 ^b	4.83 $\pm$ 0.03 ^c	118.7 $\pm$ 2.8 ^b	44.9 $\pm$ 0.5 ^a	17.6 $\pm$ 0.1 ^a	11.5 $\pm$ 0.3 ^a
Fe(III)-DTPA	2.32 $\pm$ 0.02 ^a	0.18 $\pm$ 0.01 ^a	1.96 $\pm$ 0.07 ^b	0.74 $\pm$ 0.01 ^d	4.73 $\pm$ 0.08 ^b	122.1 $\pm$ 3.2 ^b	43.9 $\pm$ 0.4 ^a	18.9 $\pm$ 0.2 ^b	12.2 $\pm$ 0.4 ^b
Fe(III)-citrate	2.34 $\pm$ 0.02 ^a	0.18 $\pm$ 0.01 ^a	1.93 $\pm$ 0.01 ^b	0.77 $\pm$ 0.01 ^c	5.19 $\pm$ 0.02 ^e	123.8 $\pm$ 2.4 ^b	47.1 $\pm$ 0.9 ^b	17.9 $\pm$ 0.3 ^a	11.9 $\pm$ 0.4 ^{ab}