Foliar iron fertilization of peach (*Prunus persica* (L.) Batsch): effects of iron compounds, surfactants and other adjuvants

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Abstract

Experiments to assess the capability of different combinations of Fe-compounds and adjuvants to provide iron (Fe) *via* foliar application to Fe-deficient plants have been carried out. A total of 80 formulations containing i) one of five Fe-compounds (FeSO₄·7H₂O, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA, Fe(III)-IDHA), ii) a surfactant (Mistol, alkyl-polyglucoside1 or alkyl-polyglucoside2) and iii) an adjuvant (glycerol, methanol or glycine-betaine) were studied with respect to leaf wetting ability and surface tension. From the initial formulations only 26 resulted in adequate leaf wetting, 20 with alkyl-polyglucoside2 and 3 each with Mistol and alkyl-polyglucoside1, and some of them (4 with alkyl-polyglucoside2, 1 with Mistol and 3 with alkyl-polyglucoside1) were found to have inadequate surface tension values for use as foliar fertilizers. In a second experiment, 20 formulations containing alkyl-polyglucoside2 and one each of the five Fe-compounds and adjuvants listed above, were used for a foliar experiment with Fe-deficient peach trees (*Prunus persica* (L.) Batsch) grown under field conditions. Iron-deficient shoots were sprayed only once and leaf re-greening was assessed over 6 weeks for leaf chlorophyll content (*via* SPAD measurements) and percentage of green leaf area (*via* image analysis). Foliar Fe

1 application always resulted in leaf chlorophyll increases, although different degrees of
2 re-greening were observed for the various Fe-compounds tested. Best results were
3 obtained after treatment with formulations containing (in a decreasing order): Fe(II)-
4 sulfate, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-IDHA and Fe(III)-DTPA. A positive
5 effect of adding glycerol, methanol or glycine-betaine was often observed, although
6 the effect depended on each Fe-containing compound, indicating the existence of
7 significant interactions between spray components. Results are of importance while
8 trying to critically evaluate the potential of Fe sprays as a viable strategy to remedy
9 plant Fe deficiency under field conditions.

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11
12 *Abbreviations:* EDTA – ethylenediaminetetraacetic acid; DTPA – diethylenetriamine
13 pentaacetic acid; IDHA – iminodisuccinic acid; Chl – chlorophyll; RH – relative
14 humidity

1 **Introduction**

2
3 Iron (Fe) deficiency chlorosis is a common nutritional disorder affecting plants, and a
4 limiting factor for fruit agricultural production in many areas of the world. Iron
5 deficiency impairs fruit quality and yield, and can ultimately lead to tree death
6 (Álvarez-Fernández et al., 2003, 2006). Fruit production under soil conditions leading
7 to Fe deficiency requires continuous (every year) treatment with Fe-containing
8 compounds. The most efficient practice to control Fe deficiency in fruit trees is
9 currently the supply of synthetic Fe(III)-chelates to the root system, although these
10 treatments are very costly. For instance, in the northeast of Spain approximately
11 45,000 ha of fruit crops with high economic value (peach and pear) require treatment
12 with Fe(III)-chelates, increasing growers' costs by 25 million € every year (Álvarez-
13 Fernández et al., 2004). The possibility to deliver small amounts of Fe to fruit trees *via*
14 foliar sprays could be a target-oriented, cheaper strategy to overcome Fe deficiency in
15 fruit crops, although variable responses to Fe sprays have been often reported (see
16 Abadía et al., 2002a; Fernández and Ebert, 2005, and references therein).
17 Development of suitable Fe spray formulations is currently hindered by the limited
18 understanding of the mechanisms involved in the penetration, translocation and
19 bioavailability of the Fe-containing solutions applied to the foliage (Fernández et al.,
20 2005).

21 The cuticle that covers all aerial plant parts is the limiting barrier for the exchange
22 of water and ions between the plant and the surrounding environment (Schönherr and
23 Schreiber, 2004). The cuticle is mainly composed of cutin, cuticular waxes (intra- and
24 epi-cuticular) and polysaccharides, and therefore shows both hydrophobic and
25 hydrophilic properties (Popp et al., 2005). Moreover, there is wide micro-structural
26 diversity among leaf surfaces and wax regeneration processes in living leaf surfaces
27 also occur (Barthlott and Neinhuis, 1997; Koch et al., 2004). In the four last decades,
28 research concerning foliar uptake of agrochemicals has chiefly focused on
29 investigating penetration through the cuticle (Schönherr, 2002). Non-charged molecules
30 are thought to cross cuticles by dissolving and diffusing in lipophilic domains made of
31 cutin and cuticular waxes (Schönherr et al. 2005), whereas ionic species, which are not
32 lipid-soluble and would be subsequently excluded from the lipophilic pathway, would
33 be capable of crossing lipid membranes only through aqueous pores (Schönherr,
34 2000). These pores are still poorly known, although they are thought to be very small

1 in size, with a radius of 0.45 nm found in *Citrus aurantium* L. polymer matrix
2 membranes (Schönherr 1976; Schönherr and Schreiber, 2004). Recently, it has been
3 observed that the size of molecular Ca species was less limiting for ionic species
4 (entering through aqueous pores) than for neutral species (entering through cutin and
5 waxes) (Schönherr and Schreiber, 2004). The process of cuticular water transport has
6 been recently investigated using an experimental design based on the formation of
7 AgCl precipitates in the cuticle polar pores (Schreiber et al., 2006). Cuticular water
8 sorption has also been attributed to a polysaccharide fraction with a high hydration
9 capacity (Domínguez and Heredia, 1999), which may consist of a reticulum of
10 microfibrils ramifying and stretching through the cuticular membrane (Jeffree, 1996).
11 On the other hand, the significance of the stomatal pathway regarding the penetration
12 of leaf-applied chemicals remains unclear, although there is evidence that it may
13 constitute an alternative route for the uptake of foliar sprays (Currier and Dybing,
14 1959; Eichert et al., 2002).

15 The pathways for Fe uptake in leaves are still poorly known (Fernández and Ebert,
16 2005). Using different Fe-compounds and *Populus x canescens* cuticular membranes,
17 Schönherr et al. (2005) observed that there was no correlation between molecular mass
18 and penetration rates, and also that temperature (from 15 to 35 °C) did not affect the
19 penetration process. Furthermore, the permeability of cuticular membranes decreased
20 with increasing concentrations of Fe-chelates, leading to the suggestion that Fe-
21 chelates themselves may somehow reduce the size of aqueous pores. A reduction of
22 the water conductance through fruit cuticles after Fe-treatments was also reported by
23 Beyer et al. (2002) and Weichert et al. (2004). It has been suggested that 100% relative
24 humidity (RH) would be required for a significant cuticular penetration of Fe-chelates,
25 and that addition of hygroscopic humectants would favor foliar uptake (Schönherr et
26 al., 2005). Evidence for the leaf penetration of several Fe-containing compounds was
27 also obtained in attached leaves of *Vicia faba* L., *Citrus madurensis* Lour. and
28 *Nicotiana tabacum* L. (Fernández, 2004; Fernández et al., 2005). Working with field
29 grown pear trees (*Pyrus communis* L.), Álvarez-Fernández et al. (2004) tested the re-
30 greening effect of various foliar treatments including Fe(II)-sulfate, Fe(III)-DTPA,
31 ascorbic and citric acid. Whilst substantial chlorophyll increments were always
32 associated with Fe supply, the authors concluded that with the current state of
33 knowledge, treatment with Fe sprays is still not an efficient alternative to the use of
34 soil applied Fe chelates.

Many factors involved in the processes of Fe uptake by leaves, transport and its distribution within the plant remain unclear, and in particular the significance of the leaf apoplast is not fully understood (Kosegarten et al., 2001; Larbi et al., 2001; Nikolic and Römheld, 2003). Iron is thought to be taken up from the apoplast by leaf cells through a plasma membrane-bound Fe(III) reductase (Brüggemann et al., 1993), which is light dependent and does not increase with Fe-deficiency (González Vallejo et al., 2000; Larbi et al., 2001), and may be regulated by changes in leaf apoplastic pH (Kosegarten et al., 2001; López-Millán et al., 2001). It has been hypothesized that apoplastic pH increases may depress the activity of the leaf plasma membrane-bound reductase, thereby hindering symplastic Fe uptake (Kosegarten et al., 2001; see Abadía et al 2002b, for a review).

Given the complex scene determining the efficiency of Fe sprays and aware of the existing constraints and opportunities, the aim of this investigation was to assess the re-greening effect of optimized Fe-containing solutions applied to Fe chlorotic peach leaves under field conditions as a means to understand the mechanisms involved on the penetration and uptake of leaf-applied Fe, taking into account different Fe compounds, surfactants and other adjuvants.

Materials and Methods

Iron containing formulations for foliar sprays: iron sources, surface-active agents and adjuvants

All treatment solutions contained Fe at concentrations of 2 mM, supplied as five different Fe-compounds: Fe(II)SO₄·7H₂O (Panreac, Barcelona, Spain), Fe(III)-EDTA, Fe(III)-DTPA, Fe(III)-IDHA or Fe(III)-citrate, all of them dissolved in water type II analytical grade (obtained with an Elix 5 apparatus, Millipore, USA). The latter four Fe(III)-compounds were synthesized in the laboratory by complexing Fe(III) (FeCl₃, acidic AAS standard, Merck, Darmstadt, Germany) with the corresponding ligand at 1:1 (Fe:ligand) ratios, excepting for Fe(III)-citrate, where the ratio was 1:20. The chelating agents employed were: K₂EDTA·2H₂O (Panreac), DTPA free acid (Merck), IDHA Na salt (Baypure CX 100 Solid, supplied by Lanxess, Leverkusen, Germany), and Na₃-citrate·2H₂O (Sigma, St. Louis, Mo, USA). All solutions were adjusted to pH 5.0 to avoid altering the ion exchange properties of the cuticle (Fernández et al., 2005).

excepting $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ formulations, which were kept at pH 4.0 to keep Fe soluble and retarding the process of atmospheric oxidation (Fernández and Ebert, 2006).

Four different surface-active agents (surfactants) were tested at a concentration of 1 g l^{-1} : Mistol (a mixture of ionic and non-ionic surfactants, Henkel, Barcelona, Spain), Silwet L-7607 (an organo-silicon surfactant, Witco Corporation, Tarrytown, NY, USA) and 2 nonionic alkyl-polyglucoside surfactants, capryl-glucoside and alkyl (8-16) glucoside, herein referred to as alkyl-glucoside1 and alkyl-glucoside2, respectively (supplied by Cognis, Düsseldorf, Germany).

Three different adjuvants were also tested: 1% glycerol (Sigma), 5% methanol (Panreac) and 5 g l^{-1} glycine-betaine (Fluka, Madrid, Spain). Such compounds may act as synergists or humectants and facilitate the process of leaf penetration (Hazen, 2000; Schönherr et al., 2005).

Methodologies to assess the physico-chemical characteristics of potential spray formulations: wetting ability and surface tension

We tested under laboratory conditions two physico-chemical characteristics, wetting ability and surface tension, in 80 different formulations containing Fe. These formulations contained: i) 2 mM Fe solutions, obtained with 5 different Fe-compounds, ii) 1 g l^{-1} surfactant (alkyl-glucoside1, alkyl-glucoside2, Mistol or no surfactant) and iii) an adjuvant (glycerol, methanol, glycine-betaine or no adjuvant). All 80 possible combinations, including the Fe-compounds alone, were freshly prepared prior to measurement.

Estimates of the rate of wetting (integrating wetting, spreading and retention) were obtained by dipping in the solutions fully expanded leaves, with a similar maturity stage, of the shrub *Evonymus japonicus* L. Leaves were harvested in the garden of the Aula Dei Experimental Station, CSIC (Zaragoza, Spain). These leaves are coriaceous and non-wettable, constituting therefore a good model for a worst-case scenario. Peach leaves are more wettable, but leaves of other fruit tree species affected by Fe deficiency such as citrus and pear would perform similarly to leaves of *E. japonicus*. Once the leaf was dipped in the solution, the average degree of wetting was recorded according to a visual rating scale. The wetting performance of formulations was classified in 4 grades as follows: (0) leaf completely dry, (1) approximately 2/3 of the

1 leaf surface wet, (2) leaf wet, but with some dry areas and (3) leaf completely wet. Six
2 homogeneous *E. japonicus* leaves were used per treatment.

3 Also, the surface tension of the formulations was determined with a torsion balance
4 apparatus for surface and interfacial tension measurement (Model OS, Worcs, UK).
5 Formulations tested included the 80 previously screened in the wetting experiment,
6 plus 20 more prepared with the surfactant Silwet L-7607, used as a low-surface
7 tension check. Four surface tension measurements were taken per formulation.

8 9 *Field plant material*

10
11 Twenty year-old peach trees (*Prunus persica* (L.) Batsch, cv. Babygold 10, grafted on
12 seedling) grown on a flood-irrigated calcareous soil (*Typical xerofluvent*, clay-loamy
13 texture, with 31% total CaCO₃, 9.9% active CaCO₃, 7 mg kg⁻¹ DTPA-extractable Fe,
14 2.86% organic matter and pH 8.0 in water) were used. The orchard was located in the
15 Aula Dei Experimental Station, CSIC (Zaragoza, Spain), had a frame of 3 × 4 m, and
16 was appropriately maintained in terms of nutrition, pruning and pest and disease
17 control. However, trees did not receive any exogenous Fe input for 2 years prior to the
18 beginning of the foliar fertilization trial, and therefore developed Fe deficiency
19 symptoms in springtime. The experiment was designed as a completely randomized
20 block. Six trees with a similar leaf chlorosis level (average SPAD value of 14,
21 corresponding to approximately 100 μmol Chl m⁻²) were selected at the beginning of
22 the trial.

23 24 *Spray treatments with Fe formulations in peach trees growing in the field*

25
26 From the analysis of the physico-chemical properties it was decided to use in field
27 foliar spray trials only formulations containing alkyl-glucoside₂, since they had a good
28 wetting ability, low surface tension values and minimal interactions with Fe-
29 compounds. All these selected formulations had wetting rates of 3 and relatively low
30 surface tension values.

31 The re-greening effects of different Fe-compounds and adjuvants, using always
32 alkyl-glucoside₂ as a surfactant, were evaluated for a 6-week period after a single
33 foliar spray of 20 different formulations containing 1 g l⁻¹ alkyl-glucoside₂. Iron was
34 supplied at 2 mM concentrations as Fe(II)-sulfate, Fe(III)-citrate, Fe(III)-EDTA,

1 Fe(III)-DTPA or Fe(III)-IDHA. Also, three adjuvants (1% glycerol, 5% methanol and
2 5 g l⁻¹ glycine-betaine) were evaluated, as compared to pure Fe-compound sprays.
3 Solutions containing only adjuvants and alkyl-glucoside² as well as pure water were
4 also applied as controls.

5 Leaf sprays were applied with a commercial hand sprayer, both on the adaxial and
6 abaxial leaf surface, until full wetting (i.e., until solution run-off). Peach leaves are
7 known to have stomata only in the abaxial surface, and this was confirmed by our own
8 microscopic observations. However, both sides were treated to mimic usual field
9 spraying techniques. Treatments were applied from 6:00 to 8:00 solar time on June
10 20th, 2005 (a sunny day, with 70% RH and 20 °C at the time of treatment). Each of the
11 5 Fe-compounds was applied to one specific tree, which was separated from other Fe-
12 treatments by at least one non-treated tree to avoid cross-contamination between Fe-
13 compounds. In each tree, 4 different southwest oriented, sun exposed vegetative shoots
14 (not bearing fruits) were treated, each with a different adjuvant (glycerol, methanol,
15 glycine-betaine or no adjuvant). A separate tree was used to perform adjuvant-only (no
16 Fe) treatments in different branches as controls.

18 *Methodologies to estimate leaf re-greening: SPAD and image analysis*

20 Leaf re-greening after treatment was assessed by two different methods: i) measuring
21 the degree of intensity of leaf re-greening (using a SPAD apparatus) and ii) estimating
22 the percentage of green area having experienced significant re-greening (using a photo
23 scanner and image analysis software). Each methodology gives a different assessment
24 of the re-greening process.

25 In the first methodology, leaf chlorophyll (Chl) was monitored non-destructively
26 with a SPAD apparatus (Minolta 502, Osaka, Japan), 1 day before spray application
27 and then on a weekly basis during 6 weeks. This technique estimates the Chl
28 concentration from red light absorbance measurements in a column-shaped cross-
29 section of the leaf, with a 6 mm² base surface. To account for leaf heterogeneity,
30 averaged SPAD measurements from 4 different locations were taken per leaf. Seven
31 different leaves across the same shoot (excluding non-fully developed leaves) were
32 monitored per treatment. The SPAD method was calibrated by extracting pigments
33 from leaf disks (previously measured with the SPAD apparatus) with pure acetone and
34 then measuring Chl spectrophotometrically in the extracts (Abadía and Abadía, 1993).

1 The calibration curve correlating Chl concentration in peach leaves with SPAD values
2 was: $Y = -0.0002X^2 + 0.1942X - 4.865$ ($R^2 = 0.992$; Y and X were in SPAD units and
3 Chl in $\mu\text{mol m}^{-2}$, respectively).

4 A second methodology to assess leaf re-greening was based on the measurement of
5 green areas as a percentage of the total leaf surface at the end of the experiment, by
6 scanning (with an Epson Perfection 4870 Photo Scanner), once detached, the same
7 leaves used for SPAD readings in each treatment, and analyzing the digital images
8 obtained. The color distribution of the obtained images was analyzed with the image
9 analysis application Carnoy v.2.1 for Mac OS X (Schols et al., 2002). In each scanned
10 leaf photograph, total surface was determined by calibrating the image size by
11 standard image measurement techniques. Then, green areas were measured in each
12 leaf following a similar procedure, by selecting an appropriate grey-scale threshold
13 value. The resulting image was compared with the initial color image to ensure that
14 the discrimination of colors was carried out appropriately. The green and total leaf
15 area of 7 leaves per shoot was measured, and the image analysis was performed twice
16 per leaf image.

18 *Statistical analysis*

20 Data were statistically evaluated by two-way analysis of variance (ANOVA) with the
21 program SPSS 11.0 to assess the significance of the main factors and the significance
22 of interactions. Means were also compared using Duncan's test at $P < 0.05$ in order to
23 find significant differences between treatments.

25 **Results**

27 *Iron formulations differ in leaf wetting ability and surface tension*

29 A total of 80 Fe-containing formulations were prepared, based on all potential
30 combinations, including: i) different Fe-compounds at an Fe concentration of 2 mM
31 ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-IDHA); ii)
32 different surfactants (alkyl-glucoside1, alkyl-glucoside2, Mistol and no surfactant),
33 and iii) different adjuvants (glycerol, methanol, glycine-betaine and no adjuvant). The
34 degree of wetting was visually assessed as four different grades: full wetting (3), some

dry areas (2), one-third of the leaf dry (1) and no wetting at all (0). Optimal leaf wetting was observed with all 20 alkyl-glucoside₂-solutions, whereas only 3 alkyl-glucoside₁- and 3 Mistol-containing formulations led to full leaf surface wetting (Table 1). The 3 formulations giving optimal wetting with Mistol were Fe sulfate/betaine, Fe(III)-EDTA/methanol and Fe(III)-DTPA/methanol. The 3 formulations giving optimal wetting with alkyl-glucoside₁ were Fe(III)-DTPA/methanol, Fe(III)-DTPA/no adjuvant and Fe(III)-IDHA/glycerol. In all cases, solutions containing no surface-active agents had a wetting rate of 0.

Surface tension measurements were carried out immediately after solution preparation (Figure 1). Formulations tested were the 80 used in the wetting experiment plus 20 more built with the surfactant Silwet L-7607, used as a low-surface tension check. In the absence of surface-active agents, Fe-containing solutions had surface tension values similar to that of distilled water (i.e. above 60 mN m⁻¹), significantly higher than those measured for all surfactant-containing formulations. According to the hypothesis of Schönherr and Bukovac (1972), a threshold value of 30 mN m⁻¹ should be considered limiting for wetting purposes. Addition of both Mistol and alkyl-glucoside₁ at rates of 1 g l⁻¹ caused significant decreases in surface tension, which were markedly affected by the nature of the Fe-compound. When using Mistol, Fe(III)-EDTA and Fe(III)-IDHA solutions still had a surface tension higher than 34 mN m⁻¹, indicating the occurrence of interactions between the ionic surfactant and the negatively-charged Fe(III)-chelate molecules, whereas solutions including the other three Fe-compounds led to surface tension values of approximately 30 mN m⁻¹. The non-ionic surface active agent alkyl-glucoside₁ also provided low surface tension values (ca. 30 mN m⁻¹) with three Fe compounds, whereas tensions of approximately 36 mN m⁻¹ were measured for Fe(III)-IDHA and Fe(III)-DTPA formulations, indicating the occurrence of interactions with the two latter Fe-compounds. Alkyl-glucoside₂ gave low surface tensions with most Fe-containing formulations (ca. 30 mN m⁻¹), excepting for Fe(III)-DTPA solutions, which had a tension of 34 mN m⁻¹. Finally, significantly lower surface tension values (ca. 24 mN m⁻¹) were measured for all Fe-compounds when using the organo-silicon surfactant Silwet L-7607. These surface tension values are similar to those reported by Knoche et al. (1991) and Neumann and Prinz (1975). Addition of glycerol, methanol or glycine-betaine did not alter significantly surface tension of solutions including Fe-compounds and surfactants (data not shown).

1 In conclusion, alkyl-glucoside2 was selected as the most suitable surface-active
2 agent for foliar spray formulations among those tested, since in most cases it provided
3 both optimal wetting and low surface tension. Silwet L-7607 was not chosen since it
4 has been reported to degrade at pH values below 5 (Knoche et al., 1991) and is also
5 known to markedly interact with Fe compounds such as Fe(III)-citrate (Neumann and
6 Prinz, 1975), in both cases leading to a rapid increase in surface tension over time after
7 solution preparation.

8 The effect of different concentrations of alkyl-glucoside2 on surface tension was
9 also assessed (Figure 2). Surface tension decreased linearly from concentrations of
10 0.001 to approximately 0.02% alkyl-glucoside2. Concentrations higher than 0.02%
11 and up to 5% led to a surface tension of approximately 30 mN m⁻¹. Therefore, an
12 alkyl-glucoside2 concentration of 0.1% (1 g l⁻¹) was used in further experiments; this
13 concentration is in agreement with the values suggested elsewhere for other
14 surfactants (Schönherr, 2001).

15
16 *Peach leaf re-greening effects of a single spray application with different Fe-*
17 *compounds and adjuvants*

18
19 The re-greening effects of a single foliar spray with different Fe-compounds and
20 adjuvants, using always alkyl-glucoside2 as a surfactant, are shown in Tables 2 and 3.
21 The two-way analysis of variance (Table 2) showed that the effects of Fe compounds
22 on three parameters used to assess leaf re-greening, i.e., final leaf Chl concentration
23 and Chl increase (both measured with a SPAD meter) and final percentage of green
24 leaf area (estimated *via* image analysis) were highly significant ($P \leq 0.001$). The best Fe
25 compound used was Fe(II)-sulfate, with other compounds being less effective,
26 whereas no significant re-greening was obtained in the absence of Fe in the spray
27 formulations (Table 3a). The highest Chl increases 6 weeks after treatment were found
28 with Fe(II)-sulfate, followed by Fe(III)-citrate, Fe(III)-IDHA, Fe(III)-EDTA, Fe(III)-
29 DTPA and alkyl-glucoside2-only (Table 3a). When comparing the extent of the re-
30 greened surface the ranking was quite similar, with the highest values being found
31 with Fe(II)-sulfate, followed by Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA, Fe(III)-
32 IDHA and alkyl-glucoside2-only (Table 3a). In leaves treated with surfactant and
33 adjuvants but without Fe, a significant part of the surface could be still considered as

green (21% of the total surface, corresponding to the major leaf veins), a percentage similar to that found in Fe-deficient, untreated leaves (not shown).

The effect of the different adjuvants, on the other hand, was only significant at $P \leq 0.05$ when considering the final Chl increase and the percentage of green leaf area (Table 3b). A strong interaction ($P \leq 0.001$) between the Fe-compounds and adjuvants was also found (see below for a detailed description) (Table 2).

The time-course of re-greening after the foliar treatment with different Fe-containing compounds, expressed as a percentage increase in relation to the initial Chl values, is shown in Figure 3. With all Fe-containing compounds, leaf Chl concentrations increased gradually in the first 2 weeks after the treatment, and in the case of Fe(II)-sulfate the increase continued for two more weeks (Figure 3). In contrast, in leaves sprayed with Fe-free solutions Chl increased only slightly (less than 10%) in the first week and showed a decrease from week 3 on, to show at the end of the experimental period a 5% increase as compared to the initial values.

Interactions between Fe compounds and adjuvants in the re-greening of peach leaves

The interactions between the different Fe-compounds (all of the applied with alkyl-glucoside²) and adjuvants (glycerol, methanol, glycine-betaine or none) on both the leaf Chl level and the percentage of green leaf area are shown in detail in Figure 4. Data indicate that the interactions between Fe-compounds and adjuvants were very marked.

The best re-greening results were obtained with Fe(II)-sulfate. When using this compound, the best re-greening effect (a $95 \mu\text{mol m}^{-2}$ increase in Chl with 78% of the leaf green at the end of the experiment) was obtained using the adjuvant methanol. However, glycerol and glycine-betaine also led to green leaf surface values of approximately 80%, a value higher than that obtained without adjuvants (56%), although the intensity of the re-greening obtained did not reach that obtained with methanol. Even when using no adjuvants, Fe sulfate led to major increases in both Chl content and percentage of green area over the values found in the Fe-untreated controls. It should be kept in mind that in Fe-deficient, untreated leaves the percentage of green area was approximately 21%.

Iron(III)-citrate was quite effective in increasing the percentage of green surface, with all formulations leading to values in the range 62-74%. However, this Fe-source

1 did not always lead to large Chl concentration increases. When considering the Chl
2 increase the best formulation was that containing glycerol, followed by the
3 formulation not containing adjuvants. Both methanol and glycine-betaine led to
4 smaller Chl increases.

5 Iron(III) synthetic chelates were generally less effective than Fe(II)-sulfate. Using
6 Fe(III)-EDTA led to the best results, either without adjuvants or with glycerol and
7 glycine-betaine, when considering the percentage of green leaf area (56-68%),
8 whereas methanol had a detrimental effect when compared to the treatment not
9 including adjuvants. However, using glycerol and glycine-betaine led to higher
10 increases in Chl content as compared to the no-adjuvant control. The fact that the
11 treatment with no adjuvants produced an increase in the green area with almost no
12 increase in total Chl content suggests that the re-greening was only superficial. In this
13 case, methanol did slightly increase the Chl content.

14 The chelates Fe(III)-DTPA and Fe(III)-IDHA were even less effective than Fe(III)-
15 EDTA. Using Fe(III)-DTPA, all formulations led to similar percentages of green area
16 (41-40%). However, only the addition of methanol increased significantly the leaf Chl
17 content as compared to the rest of treatments. With regard to Fe(III)-IDHA, the
18 application of glycerol led to the highest green area rates (57%), followed by glycine-
19 betaine and methanol (48 and 43%, respectively). Glycerol also induced the highest
20 Chl increases with Fe(III)-IDHA, followed by the formulation containing glycine-
21 betaine. In contrast, application of Fe(III)-IDHA alone or in combination with
22 methanol led to very small Chl increases.

23 24 **Discussion**

25
26 Clear evidence for the re-greening of Fe-deficient peach leaves was gained after a
27 single treatment with different foliar Fe treatments. Despite the variable plant
28 responses to foliar Fe fertilization reported in the literature (see review by Fernández
29 and Ebert, 2005), several investigations have described the beneficial effects of foliar
30 Fe sprays application to Fe-deficient fruit crops such as citrus, pear, peach, apple,
31 mango, plum and almond, in terms of increasing leaf Chl concentration and improving
32 fruit yield and quality (Kadman and Gazit, 1984; Sanz et al., 1992; Abadía et al.,
33 2002a; Álvarez-Fernández et al., 2004; Álvarez-Fernández et al., 2006). Results
34 obtained in the present study support the idea that maximizing the chances for leaf

1 penetration *via* optimizing spray formulations and application practices may improve
2 significantly the efficiency of foliar Fe fertilization.

3 One of the conclusions of this investigation is that the physico-chemical
4 characteristics must be taken into account when carrying out foliar spray studies. Out
5 of the initial 80 possible Fe-compound/surfactant/adjuvant combinations, only 26 can
6 be considered appropriate for foliar sprays with respect to optimal leaf wetting, and 8
7 of them (4 with alkylglucoside2, 3 with alkylglucoside1 and 1 more with Mistol) had
8 values higher than 30 mN m⁻¹ (Figure 1). A low surface tension will enable an intimate
9 contact between the leaf surface and the solution, as well as facilitate the spontaneous
10 infiltration of stomatal cavities (Schönherr and Bukovac, 1972) and other possible
11 hydrophilic domains of the cuticular layer. To our knowledge, this is the first study in
12 which of the physico-chemical properties of Fe sprays have been considered *a priori*
13 as key factors determining the success of foliar Fe fertilization.

14 Among the surfactants tested, alkyl-glucoside2, a non-ionic, alkyl (8-16) glucoside,
15 was found to provide the best physico-chemical properties for use in foliar Fe fertilizer
16 formulations, with optimal characteristics for leaf wetting and good surface tension
17 values (excepting for formulations including Fe(III)-DTPA), as well as providing a
18 relatively low degree of deleterious interactions with Fe-compounds. This non-
19 phytotoxic, biodegradable surfactant has not been used before, to our knowledge, in Fe
20 foliar fertilization studies. Results indicate that this surfactant and other similar
21 compounds could improve markedly the effectiveness of Fe foliar fertilization.

22 With regard to the Fe-containing compounds applied in this study, the best results
23 were recorded for Fe(II)-sulfate supplemented with alkyl-glucoside2 and methanol,
24 with other Fe(II)-sulfate formulations giving also good results. The mechanism of leaf
25 Fe(II) penetration is still unknown, although the Fe(II)-ion has been recently shown to
26 penetrate *Vicia faba* leaves at a higher rate than most of the Fe-compounds tested
27 (Fernández et al., 2005). Iron sulfate was also the best Fe-compound in field foliar
28 fertilization experiments carried out with pear trees (Álvarez-Fernández et al., 2004).
29 The low molecular mass of the Fe(II) ion (56 g mol⁻¹) may facilitate foliar penetration
30 *via* stomata or hydrophilic cuticular pathways, although in theory it may readily
31 precipitate and/or interact with the negative charges of cuticular and apoplastic
32 components. Iron(II) may be oxidized and then chelated in the apoplast by endogenous
33 ligands such as citrate or other organic acids and nicotianamine. Subsequently, it may

1 follow reduction by the plasma membrane Fe(III)-chelate reductase, or may directly
2 enter the cell via a Fe(II)-transporters as suggested by Álvarez-Fernández et al. (2004).

3 The second best re-greening Fe-compound was Fe(III)-citrate (245 g mol^{-1}
4 molecular mass) alone or in combination with glycerol, followed by Fe(III)-EDTA
5 (428 g mol^{-1}) also in the presence of glycerol. Both Fe-compounds have been reported
6 to penetrate bean leaves, but at a lower rate than Fe(II)-sulfate (Fernández et al.,
7 2005). Iron(III)-citrate foliar application has been found to induce positive effects in
8 pear (Álvarez-Fernández et al., 2004) and bean (Fernández et al., 2005). Iron(III)-
9 DTPA and Fe(III)-IDHA, with molecular masses of 449 and 392 g mol^{-1} ,
10 respectively, also induced leaf re-greening but to a lower extent than the rest of Fe-
11 compounds tested. The poor effect of Fe(III)-DTPA formulations is likely associated
12 with their relatively high surface tension values. The lack of correlation found between
13 the molecular mass of leaf-applied Fe-chelates and the re-greening efficiency is in
14 agreement with recent findings (Fernández 2004; Schönherr et al. 2005), and would be
15 in line with some mechanisms proposed for foliar uptake, including leaf penetration
16 *via* stomata or through cuticular hydrophilic pathways. Molecular mass has also been
17 shown to be less limiting for Ca-containing compounds when diffusion takes place
18 through hydrophilic than through lipophilic domains (Schönherr and Schreiber, 2004).

19 Interactions between Fe-compounds, surfactants and adjuvants are extremely
20 important, and in many cases they could inactivate the possible effect of foliar
21 fertilizer preparations. First, significant interactions were seen when measuring surface
22 tensions. For instance, formulations including Fe(III)-IDHA/Mistol, Fe(III)-
23 IDHA/alkyl-glucoside1, Fe(III)-DTPA/alkyl-glucoside1 and DTPA/alkyl-glucoside1
24 had surface tension values higher than 33 mN m^{-1} . A loss in surface tension and leaf
25 wetting in the presence of Fe-containing compounds has also been described for the
26 surfactant Silwet L-77 (Neumann and Prinz, 1975; Horesh and Levy, 1981; Knoche et
27 al., 1991). Whereas interactions between the ionic Mistol molecules and the negatively
28 charged Fe-chelates or Fe-salts can be expected, the nature of the interactions between
29 the non-ionic alkyl-polyglucoside surfactants and some of the Fe-compounds used
30 remains unclear and deserves further study. Second, major interactions between the
31 various Fe-compounds and adjuvants in the presence of alkyl-glucoside2 on peach leaf
32 re-greening have also been observed, as summarized in Table 4. Using all three
33 adjuvants in addition to alkyl-glucoside2 induced significant beneficial effects,
34 depending on every particular Fe-carrier, whereas treatment with Fe-free adjuvant

1 solutions did neither increase the percentage of leaf green area nor induce Chl content
2 increases. In association with Fe(II)-sulfate, methanol promoted a more intense Chl
3 increase than that found with glycerol and glycine-betaine, without causing any
4 supplementary effect on the extent of leaf surface undergoing re-greening, suggesting
5 that the addition of methanol facilitates Fe availability to cells located deep inside the
6 leaf. A beneficial effect of applying diluted methanol solutions in combination with
7 foliar Fe sprays has been described by Nonomura et al. (1995).

8 Mechanisms acting during the process of plant cuticular penetration of Fe-
9 compounds include interactions of a variable nature (e.g. electro-chemical or osmotic),
10 which may induce the clogging of hydrophilic pathways (Beyer et al., 2002; Weichert
11 et al., 2004), as supported by the relatively lower penetration rate of more concentrated
12 versus more diluted Fe-containing solutions (Fernández, 2004; Schönherr et al., 2005).
13 Consequently, all Fe-compounds were applied in this study at a relatively low
14 concentration (i.e. 2 mM Fe), in contrast to most published Fe spray trials (Fernández
15 and Ebert, 2005). Also, and whereas it has been concluded that 100% RH would be
16 required for the penetration of Fe-chelates (Schönherr et al., 2005), a physiological
17 response has been observed in this study for all Fe-chelates applied to chlorotic peach
18 leaves under field conditions, confirming previous reports with other crops (Abadía et
19 al., 2002; Álvarez-Fernández et al., 2004). These results may imply that a successful
20 leaf penetration of Fe could have taken place during droplet drying. The adjuvants
21 tested in this trial caused significant improvements on the performance of Fe-
22 containing solutions, probably by facilitating the leaf penetration process in terms of
23 “plasticizing” (solubilizing) the cuticle (methanol) or maintaining Fe sprays in a liquid
24 form for a longer period due to their hygroscopicity (glycerol and glycine-betaine).

25 Some treatments, such as Fe(III)-EDTA with alkylglucoside² and without
26 adjuvants, induced increases in the percentage of green leaf area without leading to
27 significant increases in the leaf Chl content. This would suggest that re-greening in
28 these treatments was only superficial, in good agreement with the general belief of
29 growers that in many cases only a “painting” effect is achieved after foliar Fe
30 treatments. This finding also indicates that SPAD and image analysis are
31 complementary methods to assess re-greening.

32 In conclusion, re-greening of Fe-deficient peach leaves was achieved in this study
33 after foliar treatment with some optimized Fe-sprays, and addition of adjuvants to Fe
34 sprays significantly improved leaf re-greening as compared to Fe-carrier solutions

1 alone. Since the process of leaf penetration and subsequent Fe delivery to the cell is
2 very complex, further trials to investigate the mechanisms involved in foliar Fe
3 penetration and leaf cell uptake should be carried out in the future. These experiments
4 should consider the possibility of stomatal and cuticular Fe penetration using Fe-
5 deficient leaves, as well as introduce new techniques to investigate the spatial
6 characteristics of the uptake of Fe-substances, including microanalysis of Fe-
7 compounds and others. These studies will help optimizing Fe spray formulations to
8 make foliar fertilization a reliable strategy in the future to control fruit tree Fe
9 deficiency.

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22 **References**

- 23 Abadía J and Abadía A 1993 Iron and Plant Pigments. In Iron Chelation in Plants and
24 Soil Microorganisms. Ed. L L Barton and B C Hemming. pp 327-343. Academic
25 Press, New York. ISBN 0-12-079870-0.
- 26 Abadía J, Álvarez-Fernández A, Morales F, Sanz M and Abadía A 2002a Correction
27 of iron chlorosis by foliar sprays. *Acta Hortic.* 594, 115-121.
- 28 Abadía J, López-Millán A-F, Rombolà A D, Abadía A 2002b Organic acids and Fe
29 deficiency: A review. *Plant Soil* 241, 75-86.
- 30 Álvarez-Fernández A, Paniagua P, Abadía J and Abadía A 2003 Effects of Fe
31 deficiency chlorosis on yield and fruit quality in peach (*Prunus persica* L.
32 Batsch). *J. Agric. Food Chem.* 51, 5738-5744.
- 33 Álvarez-Fernández A, García-Laviña P, Fidalgo J, Abadía J and Abadía A 2004 Foliar

- fertilization to control iron chlorosis in pear (*Pyrus communis* L.) trees. Plant Soil 263, 5-15.
- Álvarez-Fernández A, Abadía J and Abadía A 2006 Iron deficiency, fruit yield and quality. In Iron Nutrition in Plants and Rhizospheric microorganisms. Eds. L L Barton and J Abadía. pp. 85-101, Springer, Dordrecht, The Netherlands. ISBN-10 1-4020-4742-8.
- Barthlott W and Neinhuis C 1997 Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202, 1-8.
- Beyer M, Peschel S, Weichert H, Knoche M 2002 Studies on water transport through the sweet cherry fruit surface: VII Fe^{3+} and Al^{3+} reduce conductance for water uptake. J. Agric. Food Chem. 50, 7600-7608.
- Brüggemann W, Maas-Kantel K and Moog P R 1993 Iron uptake by leaf mesophyll cells: the role of the plasma-membrane bound ferric-chelate reductase. Planta 190, 151–155.
- Currier H B and Dybing C D 1959 Foliar penetration of herbicides. Review and present status. Weeds 7, 195–213.
- Domínguez E and Heredia A 1999 Water hydration in cutinized cell walls: a physico-chemical analysis. Biochim. Biophys. Acta 1426, 168-176.
- Eichert T, Burkhardt J and Goldbach H E 2002 Some factors controlling stomatal uptake. Acta Hortic. 594, 85–90.
- Fernández V 2004 Investigations on foliar iron application to plants - a new approach. Shaker Verlag, Aachen, Germany, 171 pp.
- Fernández V and Ebert G 2005 Foliar iron fertilization- a critical review. J. Plant Nutr. 28, 2113–2124.
- Fernández V, Ebert G and Winkelmann G 2005 The use of microbial siderophores for foliar iron application studies. Plant Soil 272, 245–252.
- Hazen J L 2000 Adjuvants – terminology, classification, and chemistry. Weed Technol. 14, 773-784.
- Horesh I and Levy Y 1981 Response of iron-deficient citrus trees to foliar iron sprays with a low-surface-tension surfactant. Sci. Hortic. 15, 227–233.
- Jansen L L, Gentner W A and Shaw W C 1961 Effect of surfactants on the herbicidal activity of several herbicides in aqueous spray systems. Weeds 9, 381–405.

- 1 Jeffree C E 1996 Structure and ontogeny of plant cuticles. In Plant cuticles: an
2 integrated functional approach. Ed. G Kerstiens. pp. 33-82. Bios Scientific
3 Publishers: Oxford, UK
- 4 Kadman A and Gazit S 1984 The problem of iron deficiency in mango trees and
5 experiments to cure it in Israel. J. Plant Nutr. 7, 283–290.
- 6 Knoche M, Tamura H and Bukovac M J 1991 Stability of the organosilicone surfactant
7 Silwet L–77 in growth regulator sprays. HortScience 26, 1498–1500.
- 8 Koch K, Neinhuis C, Ensikat H J and Barthlott W 2004 Self assembly of epicuticular
9 waxes on living plant surfaces imaged by atomic force microscopy (AFM). J.
10 Exp. Bot. 55, 711–718.
- 11 Kosegarten H, Hoffmann B and Mengel K 2001 The paramount influence of nitrate in
12 increasing apoplastic pH of young sunflower leaves to induce Fe deficiency
13 chlorosis, and the re-greening effect brought about by acidic foliar sprays. J. Plant
14 Nutr. Soil Sci. 164, 155–163.
- 15 Larbi A, Morales F, López-Millán A F, Gogorcena Y, Abadía A, Moog P R and
16 Abadía J 2001 Technical Advance: Reduction of Fe(III)-chelates by mesophyll
17 leaf disks of sugar beet. Multi-component origin and effects of Fe deficiency.
18 Plant Cell Physiol. 42, 94–105.
- 19 López-Millán A F, Morales F, Abadía A and Abadía J 2001 Changes induced by iron
20 deficiency in the composition of the leaf apoplastic fluid from field-grown pear
21 (*Pyrus communis* L.) trees. J. Exp. Bot. 52, 1489–1498.
- 22 Neumann M and Prinz R 1975 The reduction by surfactants of leaf burn resulting from
23 foliar sprays and a salt-induced inhibition of the effect. J. Sci. Food Agric. 26,
24 909–914.
- 25 Nikolic M and Römheld V 2003 Nitrate does not result in iron inactivation in the
26 apoplast of sunflower leaves. Plant Physiol. 132, 1303–1314.
- 27 Nonomura A M, Nishio J N and Benson A A 1995 Stimulated growth and correction
28 of Fe-deficiency with trunk- and foliar-applied methanol-soluble nutrient
29 amendments. In Iron nutrition of soils and plants. Ed. J Abadía. pp. 329-333,
30 Dordrecht, Kluwer Academic Publishers.
- 31 Popp C, Burghardt M, Friedmann A and Rieder, M. 2005 Characterization of
32 hydrophilic and lipophilic pathways of *Hedera helix* L. cuticular membranes:

1 permeation of water and uncharged organic compounds. J. Exp. Bot. 56, 2797–
2 2806.

3 Rombolà, A D, Brüggemann W, Tagliavini M, Marangoni B and Moog P R 2000 Iron
4 source affects iron reduction and re-greening of kiwifruit (*Actinidia deliciosa*)
5 leaves. J. Plant Nutr. 23, 1751–1765.

6 Sanz M, Caverio J and Abadía J 1992 Iron chlorosis in the Ebro River Basin, Spain. J.
7 Plant Nutr. 15, 1971–1981.

8 Schols P, Dessein S, D'Hondt K, Huysmans S and Smets E 2002 Carnoy: a new
9 digital measurement tool for palynology. Grana 41, 124–126.

10 Schönherr J 1976 Water permeability of isolated cuticular membranes: the effect of
11 pH and cations on diffusion, hydrodynamic permeability and size of polar pores in
12 the cutin matrix. Planta 128, 113–126.

13 Schönherr J 2000 Calcium chloride penetrates plant cuticles via aqueous pores. Planta
14 212, 112–118.

15 Schönherr J 2001 Cuticular penetration of calcium salts: effects of humidity, anions,
16 and adjuvants. J. Plant Nutr. Soil Sci. 164, 225–231.

17 Schönherr J 2002 A mechanistic analysis of penetration of glyphosate salts across
18 astomatous cuticular membranes. Pest Manag. Sci. 58, 343–351.

19 Schönherr J, Bukovac M 1972 Penetration of stomata by liquids. Dependence on
20 surface tension, wettability and stomatal morphology. Plant Physiol. 49, 813–819.

21 Schönherr J and Schreiber L 2004 Size selectivity of aqueous pores in astomatous
22 cuticular membranes isolated from *Populus canescens* (Aiton) Sm. leaves. Planta
23 219, 405–411.

24 Schönherr J, Fernández V and Schreiber L 2005 Rates of cuticular penetration of
25 chelated Fe^{III}: Role of humidity, concentration, adjuvants, temperature and type of
26 chelate. J. Agr. Food Chem. 53, 4484–4492.

27 Schreiber L, Elshatshat S, Koch K, Lin J and Santrucek J 2006 AgCl precipitates in
28 isolated cuticular membranes reduce rates of cuticular transpiration. Planta 223,
29 283–290.

30 Weichert H, von Jagemann C, Peschel S, Knoche M, Neumann D and Erfurth W 2004
31 Studies on water transport through the sweet cherry fruit surface: VIII. Effect of

- 1 selected cations on water uptake and fruit cracking J. Am. Soc. Hortic. Sci. 129,
- 2 781-788.

Tables

Table 1. Number of Fe-containing formulations leading to various degrees of leaf wetting (ranging from 0 to 3) with regard to the surfactant added. A total of 80 formulations were tested. The experiment was carried out with 6 replications and results were always consistent.

Table 2. Two-way ANOVA analysis of data corresponding to final Chl concentration and Chl increase (both estimated from SPAD measurements) and green area per leaf (estimated from image analysis), 6 weeks after the spray treatments with several Fe-compounds and different adjuvants.

Table 3. Final Chl concentration and Chl increase (both estimated from SPAD measurements) and green area per leaf (estimated from image analysis), 6 weeks after the spray treatments with several Fe-compounds and different adjuvants. All treatment solutions contained the surfactant alkyl-glucoside² at a concentration of 0.1%. The upper part of the Table (a) corresponds to the average of the 4 adjuvant treatments for a given Fe-carrier $\pm$ SE (7 replications, 4 treatments, n=28). Data on the lower part of the Table (b) are the means of 5 Fe-compound treatments for a given adjuvant $\pm$ SE (n=28). Different letters within each column indicate different levels of significance according to Duncan's t test ($P \leq 0.05$).

1 *Table 4.* Summary of the interactions between Fe-containing compounds (Fe(II)-
2 sulfate, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-IDHA) and adjuvants
3 (glycerol, methanol and glycine-betaine) as compared to adjuvant-free Fe sprays.
4 Improvements were assessed with respect to both Chl increases and extent of leaf area
5 re-greening. All solutions contained 0.1% alkyl-glucoside².
6

Figure captions

Figure 1. Surface tension of Fe-containing solutions (Fe(II)-sulfate, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-IDHA) plus 1 g l⁻¹ surfactant (Mistol, alkyl-glucoside1, alkyl-glucoside2, Silwet L-7607 or no surfactant, respectively). Solutions were freshly prepared and surface tension was measured immediately. Results are means $\pm$ SD (n=4).

Figure 2. Effect of alkyl-glucoside2 concentration (w/v) on equilibrium surface tension (measured 2 h after solution preparation; solution pH 5.0). Results are means $\pm$ SD (n=4).

Figure 3. Evolution of the leaf Chl increases in Fe-deficient peach leaves after treatment with Fe sprays (Fe(II)-sulfate, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-IDHA) plus 0.1% alkyl-glucoside2. Data represent mean Chl increases (%) in relation to the initial Chl concentration for each Fe treatment. Results are the average of the 4 adjuvant treatments for a given Fe-carrier $\pm$ SD (n=28).

Figure 4. Final Chl increase (measured *via* SPAD measurements) and green area per leaf (estimated *via* image analysis), 6 weeks after the spray treatments with several Fe-compounds (from top to bottom, Fe(II)-sulfate, Fe(III)-citrate, Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-IDHA) and different adjuvants (none, glycerol, methanol or glycine-betaine). All treatment solutions contained the surfactant alkyl-glucoside2 at a concentration of 0.1%. It should be kept in mind that in Fe-deficient, untreated leaves the percentage of green area was approximately 21%. Values are means $\pm$ SD (n=28).

1 Table 1

Surfactant	N° of formulations			
	Wetting rate 3	Wetting rate 2	Wetting rate 1	Wetting rate 0
None	0	0	0	20
Mistol	3	8	6	3
alkyl-glucoside1	3	8	6	3
alkyl-glucoside2	20	0	0	0

2

3

4 Table 2

<i>Significance</i>	Final Chl concentration (in $\mu\text{mol m}^{-2}$)	Chl increase (week 6-week 0; in $\mu\text{mol m}^{-2}$)	Green area per leaf (in %)
<i>Fe-treatment</i>	***	***	***
<i>Adjuvant-type</i>	NS	*	*
<i>Fe-treatments x adjuvants</i>	***	***	***

5

^aNS, not significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; ***, significant at $P \leq 0.001$.

6

7 Table 3

Iron Treatment	Final Chl concentration (in $\mu\text{mol m}^{-2}$)	Chl increase (week 6-week 0; in $\mu\text{mol m}^{-2}$)	Green area per leaf (in %)
(a) No Fe	81.6 $\pm$ 4.3 a	-7.6 $\pm$ 3.1 a	20.8 $\pm$ 3.0 a
Fe-sulfate	181.1 $\pm$ 9.0 d	68.0 $\pm$ 7.0 d	81.7 $\pm$ 1.9 e
Fe-citrate	139.5 $\pm$ 4.3 c	30.9 $\pm$ 3.5 c	65.9 $\pm$ 2.9 d
Fe-EDTA	126.4 $\pm$ 4.9 b	22.0 $\pm$ 3.0 bc	54.8 $\pm$ 3.4 c
Fe-DTPA	102.5 $\pm$ 5.2 ab	15.7 $\pm$ 2.0 bc	44.7 $\pm$ 2.9 b
Fe-IDHA	127.7 $\pm$ 3.5 b	13.6 $\pm$ 1.5 b	44.4 $\pm$ 3.0 b
Adjuvant- type	Final Chl concentration (in $\mu\text{mol m}^{-2}$)	Chl increase (week 6- week 0; in $\mu\text{mol m}^{-2}$)	Green area per leaf (in %)
(b) No adjuvant	127.4 $\pm$ 4.1 a	16.9 $\pm$ 2.0 a	52.0 $\pm$ 2.1 a
glycerol	127.1 $\pm$ 4.5 a	35.2 $\pm$ 4.0 b	63.6 $\pm$ 2.3 b
methanol	134.6 $\pm$ 4.5 a	33.6 $\pm$ 3.8 b	51.7 $\pm$ 2.3 a
glycine- betaine	121.8 $\pm$ 4.5 a	29.3 $\pm$ 2.5 b	59.6 $\pm$ 2.3 b

8

1 Table 4

Fe-carrier	Adjuvant		
	Glycerol	Methanol	Glycine-betaine
Fe(II)-sulfate	- ^a	++ ^c	+ ^b
Fe(III)-citrate	++	-	-
Fe(III)-EDTA	++	+	+
Fe(III)-DTPA	-	++	-
Fe(III)-IDHA	++	-	+

^a - : no significant improvement

^b + : there was a positive effect

^c ++ : there was a highly positive effect

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4 Fig 1

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1 Fig 2

4 Fig 3

1 Fig 4

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