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Re-greening of Citrus Leaves after $FeCl_2\,4H_2O$ Leaf Application

V. Fernández, A. Rohrbach and G. Ebert
(Fachgebiet Obstbau der Humboldt-Universität Berlin)

Summary

A leaf re-greening trial using both Fe-sufficient and deficient *Citrus madurensis* plants, was carried out over a period of 5 weeks. Attached green and chlorotic leaves were immersed in a 0.2 % $FeCl_2\,4H_2O$ solution containing 0.05 % Silwet L-7607 surfactant. Treatments were applied under light and dark conditions. Changes in leaf colour, chlorophyll content, and extractable Fe associated with $FeCl_2\,4H_2O$ supply were monitored. $FeCl_2\,4H_2O$ application resulted in higher chlorophyll contents and extractable Fe in chlorotic leaves. At the end of the experiment, the treated chlorotic leaves achieved similar rates of leaf chlorophyll content, colour and Fe-content to those of Fe-sufficient control leaves.

Zusammenfassung

Wiederergrünen von Zitrusblättern nach Applikation von $FeCl_2\,4H_2O$. Der Versuch zum Wiedererergrünen von chlorotischen Zitrusblättern wurde an *Citrus madurensis*-Pflanzen durchgeführt, die entweder Fe-Mangelsymptome aufwiesen oder ausreichend mit Fe versorgt worden waren. Am Baum befindliche Blätter wurden in eine 0,2 % $FeCl_2\,4H_2O$-Lösung getaucht, die zusätzlich 0,05 % Silwet L-7607 als Netzmittel enthielt. Über einen Zeitraum von 5 Wochen wurden Veränderungen der Blattfarbe, des Chlorophyllgehaltes und des extrahierbaren Fe-Gehaltes aufgezeichnet. Durch die $FeCl_2\,4H_2O$-Applikation kam es zu einer Erhöhung der Chlorophyll- und Fe-Gehalte in den chlorotischen Blättern. Am Ende des Versuches hatten die vormals chlorotischen Blätter vergleichbare Werte (Blattfarbe, Chlorophyll- und Fe-Gehalt) wie grüne Blätter ohne Fe-Mangel.

Introduction

Plant iron chlorosis is a world-wide problem in agriculture, which is often related to alkaline and calcareous soils (ANDERSON 1982). Such disorder can impair quality and yield of most horticultural crops and requires the use of expensive corrective measures, subsequently having economic significance. Application of Fe-containing foliar sprays to Fe-deficient plants is the most common practice under field conditions (HECHT-BUCHHOLZ and ORTMANN 1986). Moreover, several investigations reported that Fe fertiliser treatments to chlorotic plants grown in calcareous soils were more effective and economical while applied via the leaf in contrast to soil Fe applications (WITHEE and CARLSON 1959; MORTVEDT 1991). However, there is still a limited understanding of some key factors related to the success of foliar applied nutrient applications, such as the penetration mechanisms of water and electrolyte solutions (SCHÖNHERR 2000) or the regulation of leaf nutrient uptake (RÖMHELD and EL-FOULY 1999).

SCHÖNHERR (2001) stressed the importance of selecting appropriate electrolyte carrier compounds, as the single control strategy to favour the penetration of electrolyte solutions through plant leaves. Salts should be selected chiefly on the basis of having a low molecular weight, a high solubility, including a physiologically suitable accompanying ion and finally, presenting a low deliquescent point since penetration takes place when there is a liquid phase between the leaf surface and the salt residue (SCHÖNHERR 2001).

Beneficial effects in the form of increased leaf wetting, penetration and retention of foliar applied agrochemical formulations containing surface active agents have been often described (STOCK and HOLLOWAY 1993; TAMURA et al. 2001). Further on, the capacity of organosilicone surfactants such as Silwet L-77 to lower surface tension to below 30 mN m^{-1}, the threshold for stomatal infiltration to occur (SCHÖNHERR and BUKOVAC 1972), was recognised and used in some Fe foliar spray investigations (NEUMANN and PRINZ 1974; HORESH and LEVY 1981). Working with Fe-containing compounds, NEUMANN and PRINZ (1975) noted that the spreading properties of Silwet L-77 could be lost even some minutes after preparing the spray solution, as they recorded for Fe (III)-citrate (800 ppm Fe) plus 0.05 % L-77. More recently, KNOCHE et al. (1991) observed a rapid degradation of Silwet L-77 and Silwet L-7607 molecules in acid (pH 2 to 5) and alkaline (pH 9 to 10) solution, resulting in a loss of spreading ability over time.

Inorganic iron solutions are strongly pH dependent. At pH > 2, Fe (III) solutions begin to form colloidal gels which irreversibly polymerise already at pH 4 to 5. On the other hand, Fe (II) salts readily oxidise upon exposure to the atmosphere, the process occurring less rapidly with increasing acidity (COTTON et al. 1999).

All aerial parts of higher plants are covered by a hydrophobic cuticle, which chiefly protects plants from uncontrolled water and solute loss (RIEDERER and SCHREIBER 2001) and is the most important barrier for the penetration of leaf applied chemicals (TAMURA et

al. 2001). Plant cuticles are poly-electrolytes with isoelectric points around 3 (SCHÖNHERR and HUBER 1977). Subsequently, the ion exchange capacity of the cuticle can be affected by the pH of a leaf applied solution (CHAMEL 1996).

The aim of this experiment was to assess the re-greening potential of $FeCl_2 4H_2O$, which was selected due to its high solubility. The effect of light on penetration of this iron salt plus 0.05 % Silwet L-7607 was tested on chlorotic and green *Citrus madurensis* leaves and evaluated in terms of increments in leaf colour, chlorophyll and extractable Fe content.

Material and Methods

Plant material and pre-treatment

Citrus madurensis var. Loureiro plants were used in this investigation. The plants were grown in 51 containers filled with neutral quartz-sand and kept in the greenhouse at 20 °C and 60 to 80 % relative humidity.

Cuttings were pre-treated for 18 months via the root system prior to the first leaf treatment was given as described below. Plants were supplied a full strength ARNON and HOAGLAND (1952) solution, with and without Fe. The pH of the Fe-free nutrient solution was raised to 7.8 with $NaHCO_3$, while the solution applied to Fe-sufficient plants was maintained at pH 5.5. 18 months after the onset of the mentioned root treatments the Fe-free grown plants had already developed Fe deficiency symptoms and bore chlorotic leaves.

Leaf treatments

Six Fe-deficient and six Fe-sufficient *C. madurensis* plants were chosen and three sample leaves were taken per shoot. Every randomly selected shoot bore a light-treated, a dark-treated and a control leaf, which were located close to each other. The relative position of these three treatments within the shoot was randomly varied between the existing shoots. 30 light, dark and non-treated leaves were sampled, both for Fe-sufficient and deficient plants. Control leaves were dipped in water containing 0.05 % Silwet L-7607.

Two months old Fe-deficient and sufficient leaves were dipped in a 0.2 % $FeCl_2 4H_2O$ (Merck) distilled water solution, containing 0.05 % Silwet L-7607. The pH of the solution was raised to 4 with NaOH and treatments were applied immediately after preparation, so that a good spreading on the leaf was attained. The effect of light on the penetration of Fe(II)-chloride was tested by means of treating the leaves under light or dark conditions, having previously observed the degree of stomatal opening under the microscope. Leaves were treated in the first and third week after the beginning of the experiment.

Data collection and analysis

The re-greening effect of the Fe-salt was assessed with a Minolta CR 200b photometer on a weekly basis. Colour was measured following the L*a*b* system and four samples were taken per leaf, since we observed that leaf colour was not homogeneously distributed on the leaf.

The chlorophyll content of leaves was assessed prior treatment and by the end of the trial, following the method of MACKINNEY (1941). Chlorophyll concentration was measured from fresh tissue, after vacuum extraction in 80 % acetone and further spectrophotometric reading at 664 nm for chlorophyll a and 647 nm for chlorophyll b (SP8-300 UV/VIS Spectrophotometer, Pye Unicam).

The extractable Fe fraction of leaf tissue was measured according to the method of LLORENTE et al. (1976). To remove surface contamination, leaves were dipped in chloroform for 15 seconds and subsequently rinsed in two water baths. Then, the clean leaves were dried for 2 days at 65 °C. The dried leaf tissue was ground on a porcelain mortar and 0.5 g per sample were filled into test tubes. 10 ml 1 N HCl were added and samples were shaken for 4 h. The resulting solutions were filtered through 11 cm diameter, MN 615 1/4 Macherrey-Nagel filter paper and filled with distilled water to 25 ml in volumetric flasks. The concentration of extractable Fe in solution was determined by atomic absorption spectrophotometry (GBC 905 AA, Australia). Contact to any Fe containing material was avoided and flasks, test tubes, filter paper, funnels and caps were rinsed in 0.1 N HCl prior to use.

The obtained data were statistically analysed with the programme Statistica Base, Statsoft.

Results

Over a 5-week period, leaf colour changes of green and chlorotic leaves were assessed on a weekly basis (Fig. 2). It was observed that the colour variable b* was more strongly correlated to the chlorophyll a concentration than either the L* or a* colour components. The lower the b* value is, the greener is the measured leaf and subsequently, the higher is its chlorophyll content (Fig. 1). All chlorotic leaves turned markedly greener chiefly after the first and third Fe(II)-chloride treatments (i.e. week 1 and week 3). The treated Fe-sufficient leaves also became greener. 4 weeks after the beginning of the experiment, no significant difference between treated chlorotic and non-treated Fe-sufficient leaves could be found. Control Fe-deficient and Fe-sufficient leaves also re-greened after Fe(II)-chloride application to neighbouring leaves. With regard to leaf greening, no significant effect of the light regime in which the treatment was applied could be observed, neither for Fe-sufficient nor for chlorotic leaves.

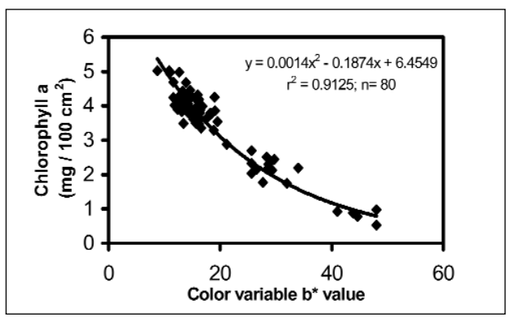


Fig. 1. Correlation between colour variable b* and chlorophyll a content for a range of green, semi-green and chlorotic *C. madurensis* leaves.

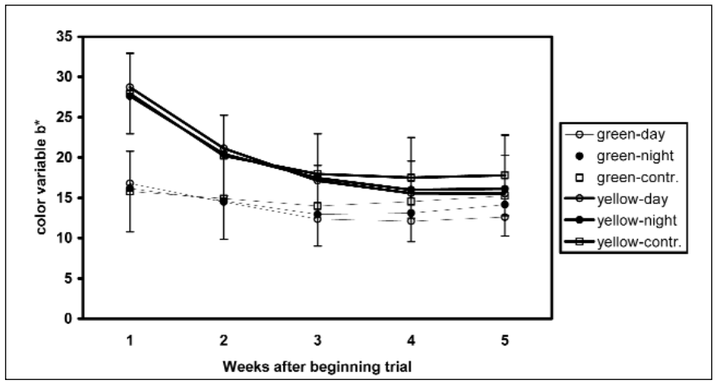


Fig. 2. Leaf colour changes after 0.2 % $FeCl_2 4H_2O$ plus 0.05 % Silwet L-7602 leaf application. Leaves were treated in 1st and 3nd week after the initiation of the experiment. Every point represents the mean of 20 colour values and bars relate to the standard deviation. The term "day" is used to refer to treatments given when majority of stomata were open, "night" relates to leaves treated under dark conditions. Control leaves were dipped in water.

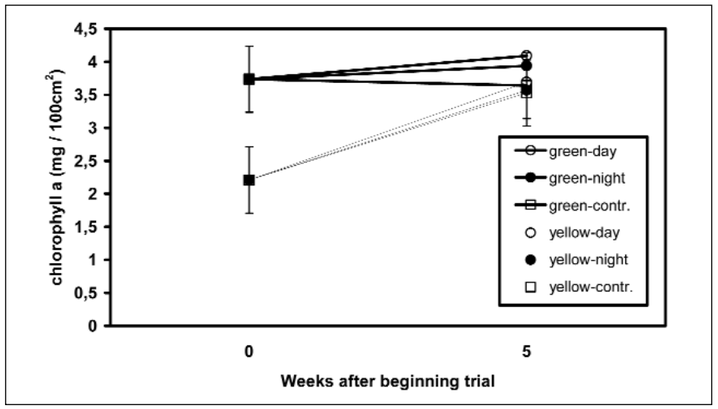


Fig. 3. Chlorophyll a evolution after $FeCl_2 4H_2O$ leaf supply. Data were collected prior treatment and five weeks after the first foliar Fe application. Point represent the mean of 10 leaves with two replications each.

Chlorophyll concentration was measured before Fe was supplied to leaves and five weeks after the first Fe(II)-chloride treatment. Initially, there was a significant difference in chorophyll a concentration between the Fe-supplied and Fe-depleted plants, which would be subsequently treated with Fe. By the end of the test period, all chlorotic leaves showed similar chlorophyll a contents as compared to control green leaves. Chlorotic treated and non-treated leaves experienced a similar increase in leaf chlorophyll content. No apparent light effect in relation to chlorophyll concentration was observed, neither for Fe-sufficient nor for chlorotic plants (Fig. 3).

The proportion of leaf Fe solubilised in 1 N HCl after 4 hours shaking, was measured before treatment and 5 and 10 weeks after the beginning of the experiment. Fe-deficient and sufficient plants showed significantly different extractable Fe contents. Fe(II)-chloride application to leaves increased the proportion of leaf soluble Fe for all treated leaves. This increment was more pronounced after the first 5 weeks of the trial. Fe-treated green leaves also showed increased extractable Fe contents. A potential light effect could be observed with regard to light-treated, Fe-deficient leaves which showed a very high soluble Fe concentration after 5 weeks, being within the range of the treated green leaves. However, the extractable Fe of light-treated, chlorotic leaves did not significantly increase after 10 weeks in relation to the values obtained for the fifth week. All other treated leaves showed higher extractable Fe contents after 10 weeks in contrast to the values measured 5 weeks after the first Fe application. Control Fe-deficient leaves showed a minor increase in leaf soluble Fe for the 10-week interval. The level of soluble Fe of control green leaves was similar at any stage of the experiment and did not appear to be affected by Fe application to neighbouring leaves (Fig. 4).

Discussion

The effect of $FeCl_2 4H_2O$ application to Fe-sufficient and deficient *C. madurensis* plants was assessed in this investigation. This Fe(II)-salt was selected on the basis of its high solubility (160 g/100 cm^3 versus 48 g/100 cm^3 of $FeSO_4$ $7H_2O$) and low molecular weight (198.81 g mol^{-1}). GUEST and CHAPMAN (1949) tested an array of inorganic Fe containing compounds in combination with several surface active agents and reported good re-greening of chlorotic citrus leaves after 0.17 % $FeCl_2$ $4H_2O$ application. They also observed a moderate to severe degree of leaf injury in the form of necrotic spots and blotches. Subsequently, we developed a pre-experiment including several $FeCl_2 4H_2O$ concentrations and found that 0.2 % (i.e. 562 ppm Fe) did not cause any damage to *C. madurensis* leaves.

The pH of the Fe solution, which was prepared with distilled water, was raised to 4 not to affect the ion ex-

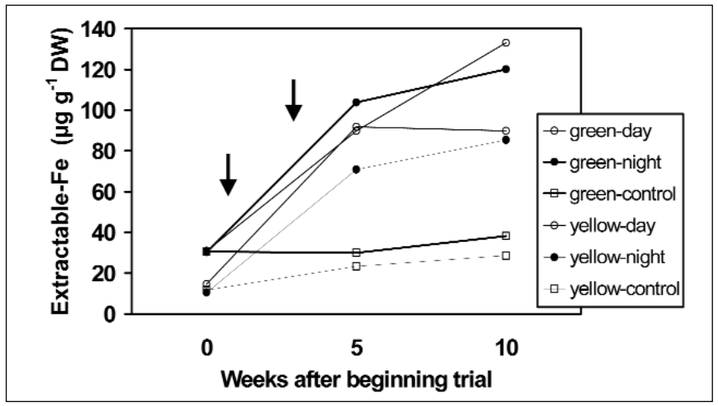


Fig. 4. Extractable Fe concentration in citrus leaves after $FeCl_2 4H_2O$ leaf supply. Mean values representing 2 replications of ten leaf samples each. Arrows indicate the applications in the first and in the third week of the trial.

change properties of the leaf surface. Once the solution was put to pH 4, the surfactant was added aware that the desirable characteristics of Silwet L-7607 could be lost within 1/2 h after the time of preparation as reported by NEUMANN and PRINZ (1975) for 800 ppm Fe supplied as $FeSO_4 7H_2O$. On the other hand, the more acid the Fe(II) solution is, the less rapidly oxidation upon exposure to ambient oxygen takes place. The resulting $FeCl_2 4H_2O$ solution at pH 4 had an orange coloration indicating the presence of Fe(III) species. Pure Fe(II) solutions including no complexing agents are turquoise in colour due to the presence of the hexaquo Fe(II) ion. However, in acid solution the hexaquo ion is easily converted to Fe(III) by the presence of molecular oxygen (SILVER 1993). Thereby, it appeared obvious that the 0.2 % $FeCl_2 4H_2O$ solution did not have the theoretically present 562 ppm Fe available for leaf penetration purposes from the very moment of preparation. This conclusion was supported by some experiments we carried out while aiming at assessing the rate of oxidation of several Fe(II) salts as affected by pH of the solution, water and time of exposure to ambient air (data not shown).

The role of stomata in the process of leaf nutrient penetration has been subject of much debate (TURRELL 1947; GREENE and BUKOVAC 1974; EICHERT and BURKHARDT 2001). According to SCHÖNHERR and BUKOVAC (1972), stomatal infiltration of a foliar-applied solution is only possible when the surface tension of the solution is below 30 mN m^{-1}. HORESH and LEVY (1981) showed that an aqueous solution of 0.2 % $FeSO_4 7H_2O$ plus 0.05 % Silwet L-7607 had a surface tension of 21 mN m^{-1}. However, they applied 0.1 % Silwet L-77 to Fe-containing solutions for spraying citrus leaves, because in some preliminary tests they observed that L-77 concentrations lower than 0.1 % proved inefficient. We decided to use a concentration of 0.05 % surfactant as shown by NEUMANN and PRINZ (1975), since applications would be given by dipping the leaves and such a surface active agent amount in a Fe solution formed a film on the leaf surface and had a surface tension of 21 mN m^{-1}. Adsorption to the leaf or de-activation upon contact with the leaf surface as suggested by HORESH and LEVY (1981) and the negative effect of trivalent ions with regard to the spreading ability (NEUMANN and PRINZ 1975), are factors which have been related to reducing the effective concentration of organosilicone surfactants in solution. However, such observations disregarded the effect of pH on the stability of the surfactant molecules as shown by KNOCHE et al. (1991) and require further investigation.

WALLIHAN et al. (1964) reported positive re-greening effects after the application of 0.1 % Vatsol and several Fe-containing compounds to citrus leaves when stomata were open and expressed their results in terms of chlorophyll increments and leaf-greening. In contrast to this report, we did not measure any significant chlorophyll or colour change between leaves treated when the stomata were open or closed, neither for green nor for chlorotic leaves. A significant effect of the Fe treatment could be observed with regard to the extractable Fe and the high soluble Fe concentration of chlorotic leaves treated when stomata were open after 5 weeks, potentially attributed to stomatal infiltration of the applied solution. Such results may be due the low surfactant concentration used, since the ability of organosilicone surfactants to promote stomatal infiltration is linked to droplet spreading and both phenomena are related to surfactant concentration (KNOCHE 1994). Furthermore, Silwet L-7607 was reported to be less effective than Silwet L-77 with regard to inducing stomatal infitration (STEVENS et al. 1992) but more effective than L-77 in relation to promoting cuticular penetration (STEVENS et al. 1991).

In this study, the effect of the lower and upper side of the leaf was not taken into account since the whole leaf was treated.

In the light of the obtained colour and chlorophyll results, it may be possible that there was Fe migration within the shoot, since both green and chlorotic non-treated leaves also re-greened. Such minor amount of retranslocated Fe may be enough to increase leaf chlorophyll level but did reflect in little changes with regard to leaf extractable Fe. This chlorophyll increment could also be due to leaf maturity as shown by LLORENTE et al. (1976) for lemon trees.

Fe-sufficient leaves supplied with Fe also significantly increased their extractable Fe level, showing no apparent control mechanism to prevent the penetration of excess Fe into the leaf.

In conclusion, the use of Fe(II)-salts as foliar sprays may yield variable and uncertain results due to the many unknown factors involved, such as the age of the solid salt to use or every local water pH value. Measurement of leaf chlorophyll appeared to give a very rough estimation of the amount of Fe which penetrated the leaf. Knowledge about the existing Fe concentration per leaf area, both within the leaf and covering a Fe-treated leaf, will be useful to estimate leaf penetration rates.

Finally, no significant effect of light was found in relation to the penetration of *C. madurensis* leaves by means of dipping the whole leaf in an Fe(II)-chloride plus 0.05 % Silwet L-7607 solution. More research regarding the effect of surfactants, suitable Fe carriers or the mechanisms of Fe penetration through plant leaves is required prior to ensuring success of Fe foliar sprays.

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Adress of authors: Victoria Fernandez, Alexander Rohrbach and Georg Ebert (corresponding author), Fachgebiet Obstbau, Humboldt-Universität zu Berlin, Albrecht-Thaer-Weg 3, D-14195 Berlin, Germany, e-mail: georg.ebert@rz-huberlin.de.