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Foliar Acids Control Iron Chlorosis in Orange Trees

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ABSTRACT

Different foliar treatments were applied to evaluate the recovery of iron chlorosis of sweet orange trees (*Citrus sinensis* L. cv 'Darabi' grafted on *Citrus aurantifolia* L.) grown on a calcareous soil. The treatments were Fe (II) sulfate (500 mg Fe/L), sulfuric acid (200 mg/L), citric acid (1000 mg/L), nitric acid (1000 mg/L) and distilled water as a control. A non-ionic wetting agent was used in all treatments. Results shown that the use of frequent foliar sprays alleviated Fe chlorosis in orange trees. Sprays of Fe(II) sulfate increased the leaf chlorophyll and Fe content, and improved fruit quality compared to control trees. Sprays of citric acid also increased leaf chlorophyll and Fe concentrations and improved fruit size and quality. Sprays of nitric acid also increased leaf chlorophyll content and fruit size, but did not affect fruit quality. Sprays of sulfuric acid alone slightly increased leaf chlorophyll and Fe concentrations, without affecting fruit quality. These results suggest that foliar acids sprays with and without Fe could help to avoid yield and quality losses caused by Fe chlorosis in citrus orchards. Furthermore, these treatments could be done with relatively cheap materials such as solutions containing Fe (II) sulfate.

Keywords: citric acid, chlorophyll, Fe(II) sulfate, nitric acid, sulfuric acid

INTRODUCTION

Orange trees growing in calcareous soils may exhibit typical signs of iron (Fe) deficiency including yellowing of the tissue between veins of young leaves which is called iron chlorosis or lime-induced chlorosis (Abadia *et al.* 1985). Fe-deficient orange trees may produce light green to yellow leaves and those that are severely Fe-deficient become completely pale yellow in calcareous soils in the south of Iran.

Lime-induced chlorosis is a nutritional disorder in fruit tree orchards established on calcareous soils. In this situation, iron availability does not fulfill a plant's needs in spite of the high total soil Fe concentrations (Pestana *et al*. 2003). All plants need a continuous supply of iron during growth, because it is not translocated from mature leaves to developing ones and is classified as an immobile nutrient element (Mengel *et al*. 1994).

Root Fe uptake involves a reduction of Fe from Fe^{3+} to Fe^{2+} at the cell membrane of epidermal root cells; this reduction is catalyzed by the enzyme, ferric chelate reductase (Miller *et al*. 1994). After Fe is reduced in the roots, it is reoxidized back to Fe^{3+} in the apoplast where Fe^{3+} then binds with citric acid (Schmidt *et al*. 1999). Fe is then transported in the xylem from the roots to the leaves as ferric-citrate and re-reduced in the leaf apoplast to the Fe^{2+} form and is actively transported across the plasma membrane into the symplast where it is metabolized by the plant (Schmidt *et al*. 1999; Pestana *et al*. 2002). Because of the unavailability of Fe in calcareous soils (high pH) chlorosis is a major agricultural problem that results in diminished crop yields in an estimated 30% of calcareous soils worldwide (Sanz *et al.* 1994).

Nearly 12% of the world's agricultural soils are calcareous (FAO-AGL, 2007). Calcareous soils are characterized by a high pH due to large amounts of calcium carbonate, most of the Fe in these soils is unavailable for plant uptake because it is tightly bound to soil particles or precipitated as water-insoluble iron oxides (Wallace *et al*. 1992).

Soil applications of non-chelated Fe fertilizers do not prevent or alleviate Fe deficiency in orange trees grown in calcareous soils (Belkhodja *et al.* 1998). An effective method to prevent Fe deficiency in oranges grown on calcareous soil is by soil application of chelated Fe (Legaz *et al*. 1992; Mengel *et al.* 1994; Miller *et al*. 1994; Schmidt *et al*. 1999; Pestana *et al*. 2002). However, chelated Fe is very expensive and is by far the single highest fertilizer material cost for the production of orange trees on calcareous soils (Legaz *et al*. 1992).

The overall objective of this project will be to evaluate foliar applications of weak (citric, ascorbic and dilute sulfuric) acids as cost-effective alternatives to the current standard practice of applying very expensive chelated iron to calcareous soil to prevent iron deficiency in tropical and subtropical fruit trees (Crane *et al*. 2007). The specific hypotheses to be tested are: 1) foliar applications of weak acids will decrease the pH of the leaf apoplast of trees grown in calcareous soils, thus increasing the bioavailability of iron and preventing or eliminating iron deficiency 2) incorporating the spraying of weak acids into an orchard management program will provide a more cost effective means of preventing iron deficiency in subtropical and tropical fruit crops than the current practice of applying chelated iron to the soil (Schaffer *et al*. 2007).

The potential exists for the use of foliar applications of acids as a low-cost alternative to expensive chelated Fe for preventing Fe deficiency in orange trees growing on calcareous soils (Tagliavini *et al*. 2000).

Therefore the purpose of this study was to test the effects of foliar applications of 3 acids and Fe (II) sulfate on re-greening and total Fe content of leaves of orange trees showing signs of Fe deficiency.

MATERIALS AND METHODS

A field experiment was carried out during 2007 to 2008 in 10-year-old orange trees (*Citrus sinensis* L. cv 'Darabi' grafted on to *Citrus aurantifolia* L.), which were randomly selected in an orchard planted on a calcareous soil (35% total $CaCo_3$, 0.5% active $CaCo_3$, 3% organic matter, pH 8.1, 54% clay, 28% silt and 18% sand) located in Fasa (South of Iran). At the beginning of the expe-

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Short Communication

riment all trees were similar in shape and size, and showed moderate Fe chlorosis.

Three different foliar spray treatments were applied. The treatments were Fe (II) sulfate (500 mg Fe /L), sulfuric acid (200 mg/L), citric acid (1000 mg/L), nitric acid (1000 mg/L) and distilled water as a control. The pH values of these solutions were 4.1, 1.9, 4.3, and 2.3, respectively. A non-ionic wetting agent was used in all treatments. Treatments started on 15 August, 2007, when the diameter of the fruit was approximately 30 mm, trees were treated three times during each year (August 15, 30 and 15 September in 2007 and 2008), a total of 6 times. Sprays were applied to whole tree. Treatments were applied with a manually operated sprayer by performing full wetting of the whole tree until the solution dropped from the foliage to the ground.

Total leaf chlorophyll content was determined at the beginning and at the end of the experiment two weeks after the last spray application, on the trees, using a SPAD-502 (Minolta, Osaka, Japan) each reading being obtained from 10 different leaves.

At the end of the assay (30 September) at least 30 healthy, recently expanded (second and third fully developed) leaves were randomly collected per tree were washed with tap water, followed by distilled water containing a non-ionic detergent, and then with HCl (0.01 M). Finally, three rinses were carried out with distilled water. Samples were dried at 70 C° for 48 h in an oven to determine Fe content using atomic absorption spectrometry GBC 310 (GBC Australia).

To study the effects of foliar application on fruit quality, at least 20 fruits were collected on 30 January from treated trees. Fresh weight, fruit diameter, juice content, total soluble solids (TSS), citric acid concentration and total acids (TA), were determined with standard methods (Ranagana 1977).

The effects of treatments were evaluated using analysis of variance and the means compared by Duncan's Multiple Range Test (DMRT) at the 95% significance level, using SAS software (SAS Version 6, 4th Edn).

RESULTS AND DISCUSSION

Spray of Fe (II) sulfate was effective in controlling Fe chlorosis in orange trees. The leaf chlorophyll content in the trees treated with Fe (II) sulfate was greater than that of in other treatments and control (**Fig. 1**). Spray of nitric acid also increased leaf chlorophyll more than citric and sulfuric acid treatments and control (**Fig. 1**). Spray of citric acid also increased leaf chlorophyll content compared to trees that received sulfuric acid treatments and control (**Fig. 1**). Sulfuric acid alone slightly increased leaf chlorophyll concentrations compared with control trees (**Fig. 1**)

These results suggest that in chlorotic leaves, which have a lower amount of Fe compared with green leaves, a part of the Fe is still stored outside the mesophyll cells. As citric, sulphuric and nitric acids caused some regreening it is possible that this storage may be due to high apoplastic pH, high rates of oxidation of Fe(II) or low Fe reduction activity (Legaz *et al*. 1992; Mengel *et al.* 1994; Miller *et al.* 1994). The Fe (II) sulfate sprays significantly increased the concentration of Fe in leaves. The well accepted phenomenon that chlorotic and green leaves have similar total Fe concentrations has been termed "the Fe-chlorosis paradox" (Rombola *et al*. 2000). Schmidt and Romheld (1999) suggested that this phenomenon depends, at least in part, on the fact that Fe chlorosis impairs leaf expansion growth; this would lead to a relative increase in Fe concentration, but not in the total content of Fe in the chlorotic leaf. If "the Fe-chlorosis paradox" would depend on a "dilution" effect, then the chlorotic leaves, while having a similar concentration of Fe, should show higher concentrations than other nutrients. Fe (II) sulfate, due to its acidic pH, may remobilize Fe pools in chlorotic leaves as well as act as an Fe source. These simultaneous effects may explain both the regreening of the treated leaves and the increased Fe concentrations (**Fig. 2**).

Sprays of citric acid significantly increased Fe concentrations compared trees with nitric and sulfuric acid treatments and control (**Fig. 2**). Nitric acid significantly increased Fe concentrations compared with trees with sulfuric acid treatments and control (**Fig. 2**). In the chlorotic leaf, the existence of Fe pools which are somehow inactivated has been demonstrated by several authors (Mengel 1994; Tagliavini *et al*. 1995), a fact suggesting that in calcareous soils, part of the Fe coming from the roots does not pass the leaf plasma membrane and may be confined to the apoplast.

Our data show that foliar treatments affected both fruit quality characteristics and fruit size in Darabi orange trees (**Table 1**).

The Fe(II) sulfate sprays significantly decreased the concentrations of citric acid in juice compared to the control. The applications of Fe (II) sulfate resulted in significantly larger fruits, with greater fresh mass, diameter and total juice content when compared both with the controls and with the trees treated with other acid treatments (**Table 2**).

Reducing the apoplastic pH of iron-chlorotic leaves with foliar applications of dilute acids such as sulfuric, citric, or nitric acid, resulted in a decrease in the leaf apoplast pH and "re-greening" of leaves of some fruit crops including kiwifruit (Tagliavini *et al.*1995), orange (Pestana *et*

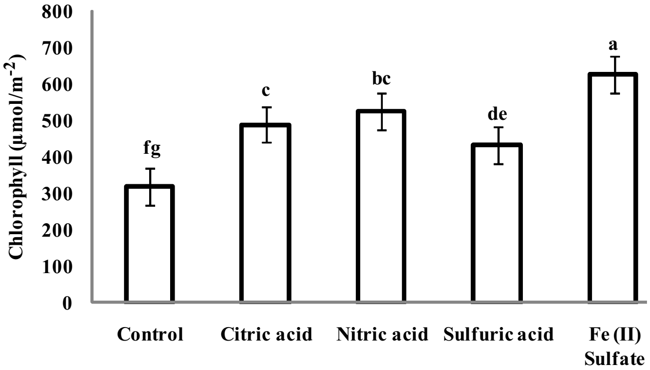

Fig. 1 Changes in total leaf chlorophyll (µmol/m^{-2}) in orange trees as affected by foliar sprays. For each date, means followed by the same letter are not significantly different at the 95% probability level (DMRT). Statistical analysis is shown only for the dates when significant differences were obtained.

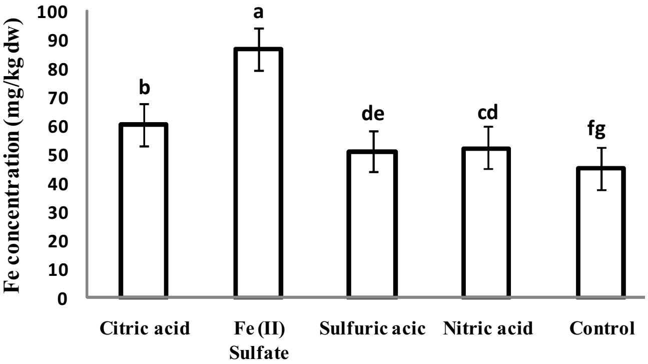

Fig. 2 Fe concentration of leaves from citrus trees at the end of the experiment, following different foliar applications. For each date, means followed by the same letter are not significantly different at the 95% probability level (DMRT). Statistical analysis is shown only for the dates when significant differences were obtained.

Table 1 Effect of foliar applications made to control iron chlorosis on quality of Darabi orange fruit.

	Control	Sulfuric acid	Nitric acid	Citric acid	Fe Sulfate
TSS (BRIX)	8.8 cde	9.25 bc	8.99 bcd	9.43 b	11.28 a
TA (mg/l)	2.26 de	2.009 cd	1.98 bc	1.93 ab	1.67 a
VC (mg/l)	7.2 fg	10.88 de	11.04 bc	11.12 b	12.48 a

For each plant part, means in a row followed by the same letter are not significantly different at the 95% probability level (DMRT).

Table 2 Effect of foliar applications made to control iron chlorosis on diameter, weight and juice weight of Darabi orange fruit.

	Control	Sulfuric acid	Nitric acid	Citric acid	Fe Sulfate
Fruits diameter (mm)	71.75 f	77.95 de	80.05 ab	77.23 cd	82.38 a
Fruits weight (g)	103.8 e	107.2 d	119.4 ab	114 c	120.8 a
Juice weight (ml)	40 ef	42.6 e	47.2 ab	44.6 cd	48.8 a

For each plant part, means in a row followed by the same letter are not significantly different at the 95% probability level (DMRT).

al. 2002), pear (Garcia-Lavina *et al*. 2000) growing in calcareous soil. Preliminary research on lychee, carambola, pond apple, and avocado using weak acids with ferrous iron resulted in higher leaf ferrous iron content than leaves treated with acid only (Crane *et al*. 2007), or non-treated control plants.

Chelated iron can represent up to 80% of the total fertilizer cost and up to 50% of the total agricultural chemical costs for some tropical fruit crops. This project will be to evaluate foliar applications of weak acids as low cost alternatives to applying expensive chelated iron to the soil to prevent iron deficiency in tropical fruit crops (Avocado, lychee, carambola) grown in calcareous soils (Crane *et al*. 2007). Thus, the potential exists for the use of foliar applications of weak acids as a low-cost alternative to expensive chelated iron for preventing iron deficiency in avocado trees growing on calcareous soils.

CONCLUSIONS

The results of the present research indicate that frequent foliar sprays are able to alleviate Fe chlorosis in orange tree orchards. Sprays of Fe (II) sulfate increased the concentrations of chlorophyll and Fe in leaves and significantly improved fruit size and quality over those of fruits in control trees. Sprays of citric acid also increased leaf chlorophyll and Fe concentrations and improved fruit quality, and increased fruit size. Sprays of nitric acid also increased leaf chlorophyll and increased fruit size, but did not improve fruit quality, sprays of sulfuric acid alone slightly increased leaf chlorophyll and Fe concentrations, without improving fruit quality.

These results suggest that foliar sprays with Fe could help reduce yield and quality losses caused by Fe chlorosis in citrus orchards. Furthermore, these treatments could be applied with relatively cheap materials such as solutions containing Fe (II) sulfate.

In the present experiment, treatments were applied during fruit growth and maturation. The impact of controlling Fe chlorosis prior to fruit formation on fruit yield and quality also deserves further investigation.

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