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Research article

Evaluation of different iron compounds in chlorotic Italian lemon trees (*Citrus lemon*)

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Abstract

The severe deficiency of iron or ferric chlorosis is a serious problem of most citrus trees established in calcareous soils, as a result of the low availability of iron in these soils and the poor uptake and limited transport of this nutrient in trees. The objective of this study was to evaluate the response of chlorotic Italian lemon trees (*Citrus lemon*) to the application of iron compounds to roots and stems. On comparing the effects of aqueous solutions of ferric citrate, ferrous sulphate and FeEDDHA chelate, applied to 20% of the roots grown in soil and sand, of trees that were planted in pots containing calcareous soil, it was observed that the chelate fully corrected ferric chlorosis, while citrate and sulphate did not solve the problem. EDDHA induced the root uptake of iron as well as the movement of the nutrient up to the leaves. With the use of injections of ferric solutions into the secondary stem of adult trees, ferric citrate corrected chlorosis but ferrous sulphate did not. The citrate ion expanded the mobility of iron within the plant, from the injection points up to the leaves, whereas the sulphate ion did not sufficiently improve the movement of iron towards the leaf mesophyll.

Keywords: Citrus trees; Calcareous soils; Ferric citrate; Ferrous sulphate; FeEDDHA

1. Introduction

In most calcareous soils, the supply of iron for plants is low. This leads to having poor yields in sensitive crops like citrus trees, and bad-quality fruits. One third of the Earth's surface is made up of calcareous soils [1] which shows the importance of studying the deficiency of iron in crops. The citrus trees planted in these soils often show signs of severe iron deficiency or iron chlorosis because of the high content of carbonates that generates alkalinity and a high concentration of bicarbonate ions in the soil solution, which in turn cause a low availability of iron for plants [17]. Although iron is the fourth most abundant element of the earth's crust [1] the deficiency of this element is a common problem of practically all the vegetal species developed in calcareous soils.

Iron chlorosis is caused by a deterioration in the uptake and transport of iron within the plant [13], which is in turn caused by the presence of the bicarbonate ion in the apoplast [20] that increases the pH and causes the precipitation of iron in that hole. The bicarbonate ion even limits the translocation of iron from the veins and apoplast to the cytoplasm of the leaf cells [6]. To be transported, iron must be complexed with chelates. Benavides [1], Tiffin [16], and Brown and Jolley [2] point out that Fe^{3+} is transported by the xylem to the aerial parts of plants under the form of ferric citrate. Clark et al. [4] showed that with the same concentration citrate is the greatest competitor among various organic acids, like malic acid, to form compounds with iron in the xylem. In a study by Yehuda et al. [19] in which various chelates were compared as carriers of iron within plants of cucumbers, tomatoes, barley and maize, it was found that the translocation of ^{59}Fe from the

Abbreviations: FeEDDHA, Fe^{3+}-ethylenediamine di-(*o*-hydroxyphenylacetate); DTPA, diethylene-triamine penta-acetic acid.

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roots up to the points of growth was higher when iron was applied to the roots together with a fungus siderophore called Rhizoferrin and EDDHA (ethylene-diamine-dihydroxy-phenylacetic acid) compared to the use of the same accompanied by EDTA (ethylene-diamine-tetraacetic acid) [12].

In some cases both chlorotic and non chlorotic leaves have a similar concentration of total iron, which has been called "the paradox of iron chlorosis" [14]. This phenomenon depends partly on the fact that iron chlorosis reduces or inhibits the growth of the leaf [13] which leads to a higher concentration of the element in leaves. Chlorosis may even occur in leaves with an adequate concentration of iron; tissues may have an apparently adequate level of total iron, but be physiologically deficient of this element [13]. In chlorotic leaves the existence of inactive iron has been proved by various authors [7,10,15]. This indicates that in calcareous soils part of the iron coming from the roots does not pass through the plasma membrane of the leaf cells, but is deposited in the apoplast of the mesophyll as a result of the alkaline pH, a high rate of oxidation of Fe^{2+} and a low activity of the Fe reductase enzyme [14]. Wiren and Grusak [18] found that the immobilization of iron in the apoplast may occur even if there is an adequate external supply of iron.

The leaves of trees with an iron deficiency have a low supply of this element as of the apoplast [8]. According to Mengel and Geurtzen [9], the critical moment in the distribution of iron to the leaves is in the transport of the element from the veins and the apoplast up to the inside of cells, since when Fe^{3+} reaches the apoplast of leaves it is reduced, before its uptake by the cells of the mesophyll [11]. Therefore, a high pH in these tissues as a result of the uptake of bicarbonate inhibits transport and leads to the precipitation of iron in that space. Fleming et al. [6] say the bicarbonate ion is the main factor limiting the translocation of iron from the veins and the apoplast to the cell cytoplasm of the rest of the leaf. Wiren and Grusak [18] pointed out that the immobilization of iron in the apoplast of leaves is due to the high level of pH. One of the main factors limiting iron uptake by the roots was the presence of bicarbonate in the soil solution as this ion caused a considerable accumulation of the metal in the pea roots because of its precipitation in the external part of the plasma membrane of the root cells [20].

The objective of the present study was to evaluate the response of chlorotic Italian lemon trees (*Citrus lemon*) to the application of the iron compounds ferric citrate, ferrous sulphate and FeEDDHA to roots and secondary stems.

2. Methods

2.1. Root iron uptake

Six-month-old chlorotic trees were planted in pots of an 8.0-litre capacity, filled with calcareous soil from Tamaulipas, in the northeast of Mexico, (clay loam texture, pH 8.1, total carbonates 420 g kg^{-1}, active carbonate 208 g kg^{-1}, organic matter 3.7%, Fe-DTPA 2.3 mg kg^{-1}, Zn-DTPA 0.9 mg kg^{-1}, Cu-DTPA 1.2 mg kg^{-1}, Mn-DTPA 4.3 mg kg^{-1}). Climate in the region is warm sub humid with rain in summer, an annual precipitation average of 625 mm and temperature medium of 22.5 °C. The pots were set under a transparent ceiling at environmental temperature. Approximately 20% of the roots of these trees were grown in the soil and in a 1.5-litre container, filled with sand and placed in the lower part of the pot. Aqueous solutions (0.1% Fe) of ferrous sulphate, ferric citrate and FeEDDHA were applied to the roots growing in the sand every 4 days during 24 days; the dose was 100 ml solution per plant. This experiment was made to supply iron in solution to the roots developed in the sand below the pot containing soil; also to determinate if iron applied is taken up by the part of roots grown in sand and transported up to the leaves, through of the part of roots grown in calcareous soil. Three pots as control without iron solutions were established. The FeEDDHA was applied as a commercial product with 5.0% ortho-ortho isomer content. The trees were fertilized in the soil with 100 ml NH_4NO_3 0.1 M and K_2HPO_4 0.05 M per pot, every 2 weeks. Seven months after the last application of ferric solutions, showing no response to the attempt of having iron reach to the roots, the same chlorotic plants treated with ferric citrate, ferrous sulphate, and those untreated were used in a second test, after removing the sand from the roots and washing them thoroughly. The same iron salts were supplied again during 24 days, now in an aerated aqueous solution keeping the pH at 6.5, but without the sand substratum in order to evaluate the iron uptake by the roots directly from the solution without being influenced by the sand layer. In both tests the response of plants to iron applications was evaluated by determining foliar iron and setting one foliar rate to estimate the content of chlorophyll, called SPAD reading. To measure the content of iron, leaves of four months old were sampled, dried at 65 °C, ground and digested with nitric and perchloric acids [3]. The sampling was made after 60 days of the first application of the element in order to allow the leaves to mature. Iron was quantified by using a spectrometer of atomic absorption. The chlorophyll rate was measured with a Portable Chlorophyll Meter Minolta SPAD-502 (Minolta Corp., Ramsey, NJ). The pots were placed in a completely random design with three repetitions to make variance analysis and Tukey's test to data.

2.2. Injections of iron solutions in the stems

To compare the effect of ferric citrate and ferrous sulphate on the mobility of iron within the adult Italian lemon trees, solutions of 1.25% of these salts were supplied to the secondary stems, under field conditions. These solutions were applied only once. Three chlorotic trees were chosen; in each one of them three similar branches were selected. The two iron treatments mentioned were applied to secondary stems of two branches, while one branch was left untreated as control in each tree. The distance between the points of injection and the chlorotic leaves, pre-selected to be observed and evaluated with SPAD readings (four leaves per branch), was 1.5 m. Solutions were applied by drilling holes of 0.63 cm in diameter and 3.0 cm depth in the secondary stems. Immediately after