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Diagnosis and correction of iron chlorosis in fruit trees: a review

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Abstract

Several plant species grown in calcareous soils in arid and semiarid regions are iron-deficient, a condition known as lime-induced iron chlorosis, or simply as iron chlorosis. The nutritional status of perennial plants is commonly evaluated by leaf analysis. However, there is often no correlation between iron in leaves and degree of chlorosis, and therefore leaf analysis presents serious limitations as a technique to evaluate lime-induced iron chlorosis. Recently, a technique for the early prognosis of iron chlorosis based on floral analysis was developed for fruit trees to help prevent the development of iron deficiency and avoid losses in yield and quality. Correction of iron chlorosis is commonly carried out by massive applications of synthetic iron chelates to soils. Since iron is rapidly immobilised in the soil, this treatment has to be repeated each year, representing a major part of fertilizer costs. Environmental impacts of chelates in soils have not been properly investigated, but it is known that they also result in enhanced plant uptake of metals such as copper and nickel. Alternative, more environment-friendly treatments are being evaluated. In this article we concentrate on reviewing current methods to detect and correct iron chlorosis in fruit trees.

Key words: Floral analysis, foliar treatments, fruit trees, lime-induced chlorosis.

Introduction

Excesses or deficiencies of nutrients are a special concern in fruit trees where nutritional imbalances can affect the yield for more than a single season, since new growth depends on nutrient stored in the plant. Iron deficiency (iron chlorosis) is an important nutritional disorder in fruit trees that results not from a low level of iron in soils but from impaired acquisition and use of the metal by plants. The most prevalent cause of iron chlorosis in the Mediterranean area is the bicarbonate ion, which occurs in high levels in calcareous soils. It is estimated that from 20 to 50% of fruit trees in the Mediterranean basin suffer from iron chlorosis[1]. No single approach has been found to solve iron chlorosis satisfactory, making it one of the most complex nutritional deficiencies. Decreased yield and poor quality of fruit resulting from the deficiency justify the development of methods to diagnose and correct this disorder. Based on a correct diagnosis it is possible to select the right type and amount of fertilizer and thus recommend a rational fertilizer programme, taking into account the risks of negative environmental impacts that can result from excessive applications of some nutrients.

Diagnosis of Iron Chlorosis in Fruit Trees

Both soil and plant analyses can be used to diagnose iron chlorosis in fruit trees.

Soil analysis: Soil analysis is routinely used as the basis for fertilisation recommendations of annual crops. However, soil tests have limited value when applied to trees because the root system is deep and unevenly distributed, making it difficult to obtain a representative soil sample. Two major approaches can be taken to diagnose lime-induced iron chlorosis based on soil analysis (for a review see Hartwig and Loeppert[2]), i) to analyse for available iron using extractants capable of chelating the metal, and ii) to determine the lime content of the soil. Rootstocks are ranked according to their tolerance to active lime, but very often susceptible-rootstocks have other characteristics that make them more eligible for commercial operations, such as tolerance to diseases. For a review on tolerance of different rootstocks see Tagliavini and Rombolà[3].

Plant analysis: Iron chlorosis can be identified by visual symptoms, a fast and economic method. Several authors proposed the use of visual scores, from 0 (without symptoms) to 5 (trees with dead branches and white young leaves)[4,5]. The degree of chlorosis can now be rapidly quantified by the evaluation of chlorophyll content using a SPAD apparatus, which measures leaf transmittance at two wavelengths - 650 and 950 nm. However, by the time symptoms become apparent it is often too late to prevent the negative effects of the disorder on yield and fruit quality. Chemical plant analysis, in particular leaf analysis, is still the most common method used for diagnostic purposes in trees, and is based on the relationship between growth rate of plants and nutrient content[6,8]. Tissue analysis offers a number of advantages as well as some challenges. Leaf analysis integrates all the factors that might influence nutrient availability in the soil and plant uptake, and pinpoints the nutritional balance of the plant at the time of sampling. However, the use of leaf analysis presents limitations when applied to lime-induced chlorosis, since in many field-grown plants there is no correlation between leaf iron concentration and the degree of chlorosis expressed as chlorophyll content[9-11]. Moreover, iron concentration in chlorotic leaves, expressed on a dry weight basis, is frequently even greater than in green leaves[11,13]. This was called the "chlorosis paradox" by Römheld[14] and results from the inactivation of iron in leaves or from an inhibition of leaf growth due to iron chlorosis[13,15]. The analysis of an "active" pool of iron in leaves (usually identified with Fe (II)), using extractants such as acetic, nitric and hydrochloric acids, 2,2' bipyridyl and o-phenanthroline, is frequently mentioned[16-19]. However, according to Abadía[11] this method does not solve the problem adequately because these extractants may also remove some Fe (III) from leaves, such as the iron in phytoferritin. Another limitation of leaf analysis is the fact that the sampling date recommended for fruit trees is late in the growing season, generally very close to harvest. For example, at the recommended date for foliar analysis of peach, 120 days after full bloom, most of the varieties grown in the Mediterranean area are already harvested or are very close to harvest[20]. At this point it is no longer possible to correct nutritional disorders in time to avoid decreases in yield[6]. As a novel approach for the prognosis of iron deficiency in pear trees Sanz[21] proposed methods based on the mineral composition of flowers. Flower analysis has now been developed for a number of fruit trees: pear[7,22], peach[6,20,23,24], nectarine[25], apple[13,26], walnut[27], olive[28], pistachio[29], almond[30], and citrus[9]. The main advantage of analysing flowers over leaves is that the evaluation can take place earlier in the season. In both deciduous and evergreen fruit trees, using floral analysis it is possible to detect and correct any deficiencies before fruit set, thus giving sufficient time for nutrient amendments to improve yield and fruit quality [20,23,24,26,31].

To diagnose iron chlorosis based on the iron content of flowers, results must allow the prediction of leaf chlorophyll later in the season. This has indeed been demonstrated for several species, such as nectarine, apple, peach, and orange[25,26,32,33]. For example, after long-term experiments Sanz et al.[33] stated that the probability of iron chlorosis developing in peach trees is large when the concentration of iron in flowers is less than 160 mg kg^{-1} dry weight. The interpretation of floral analysis is as complex as when leaf analysis is carried out, and requires similar tools to obtain a correct diagnosis. Rather than the use of a singular concentration, nutrient balances are now being investigated in the search for a good indicator of iron chlorosis. The pattern of iron accumulation in fruit trees seems to depend on their life cycle. In deciduous species (nectarine, peach, pear and apple trees), the mean concentration of iron is greater in flowers than in leaves, contrary to what is observed in orange[9,32,34]. Significantly, the correlation coefficient for the relationship between iron in the flowers and the chlorophyll in the leaves is positive for deciduous trees but negative for orange (cv. 'Valencia late'). While in deciduous trees flowering occurs before vegetative growth, full bloom in citrus is concurrent with new vegetative growth. Young leaves are thus likely to act as strong sinks for iron in citrus, and compete with translocation towards flowers. Probably as a result of these differences, the concentrations or ratios of nutrients that can be used as indexes vary between species.

Examples of nutrients and balances related to iron chlorosis are the increase in potassium content and in the K:Ca ratio resulting from lime-induced chlorosis in flowers of peach[24]. Moreover, while the iron concentration in flowers of peach fluctuates from year to year, a major problem when using this element for the prognosis of the chlorosis later in the year, the concentrations of potassium and zinc and the K:Zn ratio in flowers had consistent values from year to year, making them more likely candidates for indicators of iron chlorosis[20,35]. A K:Zn ratio over 450 in flowers at full bloom is likely to be associated with the development of iron chlorosis in peach (leaf chlorophyll concentrations below 200 µmol m^{-2}) 120 days later, while chlorosis is unlikely to develop with a ratio below 375[20,35]. Using mineral analysis the level of a nutrient is determined but it is seldom possible to distinguish metabolic (active) forms from non-active[36]. To overcome this difficulty, some researchers have measured key enzymatic activities to diagnose iron chlorosis in fruit trees. Garcia and Galindo[37] proposed the use of chlorophyllase activity as a biochemical indicator of manganese and iron deficiencies in citrus. In leaves of lemon, iron deficiency decreases peroxidase, catalase and some superoxide dismutase activities[38], enzymes that are part of the intrinsic enzymatic defensive system required for the detoxification of superoxide radicals. Further research is clearly needed before a reliable indicator of iron chlorosis can be used consistently for diagnostic purposes in fruit trees.

Correction of Iron Chlorosis in Fruit Trees

The correction of iron chlorosis in plants grown on calcareous soils is an old problem with no easy solution. Until rootstocks tolerant to iron chlorosis and with other favourable agronomical characteristics become available, the prevention or correction of iron chlorosis is of paramount importance to fruit growers. Obviously, the need to correct iron chlorosis is related to its effects on yield, fruit size and quality, and consequently to decreases in the growers' profits. In a recent review, Tagliavini et al.[39] summarized the economical impact of iron chlorosis in kiwi, peach and pear orchards established on calcareous soils in Italy, Spain and Greece and concluded that yield losses were directly related to the intensity of iron chlorosis. A significant proportion of peaches and kiwifruit were unsuitable for the market. However, Sanz et al.[23] found that iron chlorosis only affected peach quality when visual symptoms were obvious, corresponding to a severe deficiency. El-Kassa[40] reported the negative effect of iron chlorosis on gross yield and fruit quality of lime, resulting in smaller fruits that were more acidic and contained less ascorbic acid. The correction of iron chlorosis with sprays containing iron resulted in larger oranges representing a gain of more than 35% in the gross income of the farmer[31]. Furthermore, iron chlorosis can lead to a delay in fruit ripening in orange and peach[31-33,41]. The treatments already tested for the correction of iron chlorosis can be applied directly to soils or as foliar sprays.

Treatments applied to soils: The correction of iron chlorosis in trees grown on calcareous soils is normally achieved by the application of Fe (III)-chelates such as iron ethylenediaminedi-o-hydroxyphenylacetate (Fe EDDHA) to the soil[42]. The efficacy of treatments with Fe-EDDHA is related to the great stability of this chelate, even when the soil pH is above 9, preventing the precipitation of iron[43,44]. In contrast, the stability of iron ethylenediamine tetraacetate (Fe-EDTA) decreases above pH 6.5, resulting in the exchange of iron by other cations, such as Ca^{2+}, Zn^{2+} and Cu^{2+}, and in the precipitation of iron. Therefore, the application of Fe-EDTA to alkaline soils is not effective[45,46]. In orchards with drip irrigation iron can be applied by fertigation but in others the application of Fe-chelates is time consuming since they are placed around each individual tree, normally in the spring between the beginning of flowering and full bloom[47]. This practice is very expensive and has to be repeated every year because iron is rapidly immobilized in the soil or leached out of the root zone. Tagliavini et al.[39] estimated a cost of 250 Euros per hectare, accounting for up to 60 % of total fertilizer costs. Moreover, chelating agents remain in the soil after Fe^{2+} uptake by plants, and become available to react with other metals, such as manganese, copper and nickel, thus increasing their bioavailability[48].

Several studies have attempted to overcome iron chlorosis with soil treatments that do not involve synthetic chelates. Iglesias et al.[49] effectively prevented iron chlorosis in pear trees grown in a calcareous soil, by injecting a synthetic Fe (II) phosphate ($Fe(PO_4)_2.8H_2O$) in the soil. The addition of Fe (II) sulphate alone to calcareous soils is not effective since iron precipitates and becomes unavailable to plants[50], but its effectiveness can be enhanced when added with organic matter. Organic matter can prevent or correct lime-induced chlorosis due to complexation and solubilisation of iron [51], though the efficacy of the treatment depends on the organic matter composition, capacity to complex iron, and stability of the Fe chelates formed[52]. In a pear orchard established on a calcareous soil Tagliavini et al.[39] obtained the recovery from iron chlorosis with the application of blood meal or of compost enriched with $FeSO_4$. The use of industrial by-products and wastes has also been tested in herbaceous species with varying degrees of success[52]. According to Alva[53] iron humate, a by-product of the drinking water decolourisation process, was an effective source of iron for citrus trees planted on alkaline soils. In a follow-up study Alva and Obreza[54] reported the increase in growth, leaf iron concentration, and fruit yield following the application of iron humate. Incorporation of sewage sludge and a hydrogel significantly improved the growth of apple seedlings[55]. Pérez-Sanz et al.[56] tested the effectiveness of iron-enriched sewage sludge as an alternative to synthetic Fe chelates for the correction of iron chlorosis in orange (cv. 'Navelina') and peach (cv. 'Sudanell'). Though the treatment increased peach size it failed to improve yield both in peach and orange.

The prevention of lime-induced chlorosis by acidification of the entire root zone is unrealistic[57]. Broadcast application of small amounts of strong acids to calcareous soils does not significantly decrease pH, and may have negative effects, namely phytotoxity and increased soil salinity[58]. In contrast, local acidification of small volumes of soil is possible and can significantly improve the nutritional status of fruit trees. Horesh et al.[51] corrected lime-induced chlorosis in citrus with the application of a peat-plug with iron sulphate to small volumes of soil close to the trees. Application of elemental sulphur, banded on both sides of tree rows, allowed for excellent chlorosis control in peach trees and simultaneously improved the availability of phosphorus, manganese and zinc to plants[57]. In calcareous soils nitrogen nutrition is predominantly based on nitrate even when ammonium is applied due to rapid nitrification, but rhizosphere acidification due to cation uptake by plants can still be achieved when nitrification inhibitors are used with urea or ammonium[57]. Recently, a promising technique based on the Controlled Uptake Long Term Ammonium Nutrition (CULTAN) cropping system established by Sommer[59] has been adapted to prevent and control lime induced iron chlorosis[1].

Treatments applied to trees: Foliar sprays can be a cheaper and environment friendly alternative to soil treatments to control iron chlorosis. Applying iron compounds or acid solutions to shoots bypasses the inhibitory effects of soil bicarbonate on iron uptake and translocation[60]. Release of iron immobilized in the plant can also be achieved[57,61]. The success of treatments with iron compounds depends on their capacity to penetrate the cuticle, travel through the apoplastic free space and cross the plasmalemma of leaf cells to reach the cytoplasm[62]. The foliar application of Fe (II) sulphate increased leaf chlorophyll content in kiwi[62] and citrus[31,32,63,64]. Though this treatment can improve fruit size and quality, as observed in orange[31,41,64,65], the positive effects obtained on leaf chlorophyll content did not always translate into increased yield, because the translocation of the applied iron into developing new leaves or fruits can be small[66]. Several authors tested foliar applications of iron chelates to plants such as orange[31,40,41,66], tangerine[65], grape[67], and kiwi[39,62]. The foliar application of chelates can be less efficient than soil application, due to limited uptake by aerial parts[66], but the results obtained by Rombolà et al.[62] suggest that leaves of field-grown kiwi were able to reduce the Fe (III) from diethylenetriaminepentaacetic acid (DTPA) and take it up into mesophyll cells. This is also true for citrus (orange and tangerine) since the recovery from iron chlorosis symptoms was obtained after frequent foliar sprays with Fe (III) from Fe-EDDHA[31,41,64,65]. Other treatments that can be applied directly to trees are products that promote the activity of the Fe-chelate reductase present in the plasmalemma of leaf mesophyll cells. Examples are dilute solutions of mineral or organic acids, hormones, alcohols and urea. Acid treatments release the iron immobilized within the plant by changing apoplastic pH[57]. Sprays with sulphuric, citric and ascorbic acids on their own have been assayed in kiwi, pear and orange, but resulted in an incomplete recovery of the symptoms of iron chlorosis[31,41,64,65]. Application of substances that stimulate proton pumps located in the plasmalemma should also alleviate iron chlorosis, based on the concept outlined by Mengel[60] that the pH of leaf apoplast affects the activity of the Fe (III)-chelate reductase. Mengel et al.[68] treated chlorotic maize leaves with sprays containing fusicoccin and indole-3-acetic acid (IAA). Tagliavini et al.[39] applied IAA (50 μmol L^{-1}) to kiwi grown in calcareous soils, which resulted in enhanced chlorophyll content. However, different results are obtained when the same product is applied to different species. These may derive from differences in leaf permeability, dependent on cuticle composition and thickness, and response mechanisms to iron deficiency[62]. Table 1 summarizes the re-greening effects obtained by foliar application of several compounds to fruit trees. According to Tagliavini et al.[39] the activation of iron pools in chlorotic leaves rarely results in a full recovery from iron chlorosis because part of the iron is inactivated on the outside of mesophyll cells. Therefore, foliar treatments are only effective in situations with slight or moderate symptoms of iron chlorosis, and the *effect is short-lived requiring repeated applications to maintain the re-greening of leaves[62].*

Other treatments: Data presented by several authors[69,70] show that injection of ferrous sulphate into tree trunks can correct iron chlorosis, but this is an expensive procedure and the wounds that are caused in the tree represent an increased risk of bacterial or viral infections. On calcareous soils with only small concentrations of active lime, the use of an integrated management system can be effective in dealing with iron chlorosis. Minimal tillage, especially during the rainy season, allows the establishment of grasses that improve soil infiltration and hydraulic conductivity, and release phytosiderophores to the rizosphere[71,72]. These effects improve soil aeration and iron chelation increasing the bioavailability of the nutrient. Tillage seems to be necessary only when there is a strong competition for nutrients and water between grasses and fruit trees. Another practice that can be implemented is the use of fertilizers with acidic reactions, like potassium sulphate[60]. Several authors[73] claim that siderophores are an important source of iron for plants growing on calcareous soils. Root colonization by Pseudomonas fluorescens and Glomus mosseae led to an increase in leaf iron in grape (cv. 'Chardonnay') grafted on a chlorosis susceptible rootstock[74].

Conclusions and Outlook

Undoubtedly, there has been a major improvement in the understanding of lime-induced iron chlorosis over the last 15 years. Nevertheless, several aspects remain unclear, especially when related to fruit trees grown under field conditions. Genetically improved chlorosis resistant rootstocks still offer the best solution to iron chlorosis, but this is a long-term approach. Meanwhile, there is a need for new methods to diagnose and correct this nutritional disorder. Flower analysis appears to offer major advantages such that it may substitute for leaf analysis in diagnosis of iron chlorosis, but more information is needed before it can be used to assess the nutritional status of all fruit trees. A greater understanding of the involvement of hormones on the adaptive mechanisms to iron chlorosis in tolerant species is needed, including the study of wild species well adapted to iron starvation. To overcome iron chlorosis, additional attention should be paid to the use of mixed crops and to application of organic residues to soil. More emphasis should also be put into the management of calcareous soils. The use of an integrated management system to correct iron chlorosis should consider economic,ecological and social aspects. Orchard management techniques are sustainable only if they represent an advantage for fruit growers, and the studies on iron chlorosis should include the effects on fruit quality and yield.

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Table 1. The re-greening effects obtained by the foliar application of several compounds at different concentrations to some fruit trees.

Species	Compounds	Concentration	Re-greening	Authors
Kiwi	Fe (III) DTPA	72 mg Fe L^{-1}	Total	[39,62,75]
	Citric acid	2 g L^{-1}	Partial	
	Fe (II) sulphate	207 mg Fe L^{-1}	Total	
	Sulphuric acid	100 mg L^{-1}	Partial	
	Indole-3-acetic acid	50 mmol L^{-1}	Partial	
	Fe (III) malate	1 mM; 3 mM	Partial	
	Fe (III) citrate	1 mM; 3 mM	Partial	
	Fe (III) DTPA	2 mM Fe	Total	
	(Fe + Mn) EDTA	10 g L^{-1} +10 g L^{-1}	Partial	[76]
Pear	Fe (II) sulphate	207 mg Fe L^{-1}	Partial	[39,62,75]
	Ascorbic acid	2 g L^{-1}	Partial	[77]
	Sulphuric acid	0.55 g L^{-1}	Partial	
	Fe (III) DTPA	199 mg Fe L^{-1}	Total	
	Fe (II) sulphate	500 mg Fe L^{-1}	Total	
Apple	Polyflavonoid Fe	9.6 % Fe DW	Total	[78]
Orange	Fe (III) EDDHA	120 mg Fe L^{-1}	Total	[31,41]
	Fe (II) sulphate	500 mg Fe L^{-1}	Total	
	Sulphuric acid	0.5 mM	Partial	
Tangerine	Fe (III) EDDHA	120 mg Fe L^{-1}	Total	[65]
	Fe (II) sulphate	500 mg Fe L^{-1}	Total	
	Sulphuric acid	0.5 mM	Partial	