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Lowering iron chlorosis of olive by soil application of iron sulfate or siderite

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Abstract About 70 % of the olive is grown on calcareous soils in Spain, most of which can induce iron (Fe) chlorosis. The main symptoms are interveinal yellowing of the young leaves, leaf necrosis, and reduction of leaf and shoot size, which can lead to major yield losses. Several strategies have been used to avoid Fe chlorosis such as the application of Fe chelates, but they are expensive. Here, we studied the effectiveness of synthetic siderite (FeCO_3) and Fe sulfate injected in the form of suspension/solution into the soil for correcting Fe chlorosis in olives. Experiments were established in spring 2009 for four growing seasons in ‘Manzanilla,’ ‘Ocal,’ ‘Arbequina,’ and ‘Picual’ orchards. The treatments were injections of (1) a siderite suspension; (2) a Fe sulfate solution supplied with K_2SO_4 to make up for the K present in the siderite suspension; (3) a K_2SO_4 solution, named Control-K; and (4) water, named Control-0. At the start of the experiment, the suspensions or solutions were injected into the soil at 10–20 points around the tree at the depth of maximum root density. Results show that Fe chlorosis was alleviated in ‘Manzanilla’ and ‘Picual’ since leaf chlorophyll concentration (as estimated by the soil plant analysis development (SPAD) value) in the trees treated with siderite or Fe sulfate was increased by 4–7 % relative to the control trees, and leaf weight was increased by 6–9 %, but not in the ‘Arbequina’ and ‘Ocal’ trees. This finding was explained by higher available Fe in Arbequina and Ocal soils. The antichlorosis effect of iron sulfate was persistent and slightly higher than that of siderite. Both iron sulfate and siderite are not susceptible to leaching due to the low solubility of Fe oxide phases. Thus, a single application of these fertilizers

constitutes a promising alternative to other Fe fertilizers, which require more than one application per year.

Keywords Iron chlorosis · Iron deficiency · Siderite · Iron sulfate · Calcareous soils · Olive tree

1 Introduction

Iron (Fe) deficiency chlorosis, which is characterized by interveinal yellowing of the leaf, is commonplace in plants growing on calcareous soils (Tagliavini and Rombolà 2001; Abadía et al. 2011). Iron chlorosis *sensu stricto* is often associated with other symptoms (such as leaf necrosis and reduction of leaf and shoot size) and can result in important yield losses (Fig. 1). Increasing Fe availability can be achieved through several strategies, including the supply of Fe compounds either to the soil near the root system, to the tree aerial part as foliage sprays, or by trunk or branch injections. Also, selection of tolerant cultivars is effective to prevent the problems of Fe deficiency in both annual and perennial species (Alcántara et al. 2003).

Correcting Fe chlorosis is difficult and costly. Iron chelates applied to the plant or to the soil are widely used for controlling this deficiency because they are an effective source of Fe (Álvarez-Fernández et al. 2004). Important progresses have been made on the preparation and use of the Fe chelates and natural Fe-complex fertilizers (Abadía et al. 2011; Lucena 2006); however, they are generally costly and have the important limitation of being easily washed from the soil. In addition, Fe chelates can trigger nutritional disorders because they can chelate Mn and reduce plant uptake of this element (Sánchez-Alcalá et al. 2012a).

Many different inorganic Fe sources have been used to alleviate Fe chlorosis. Insoluble products are generally ineffective in the short term, although good results have been

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Fig. 1 The photo on the *left* corresponds to a healthy olive, and the one on the *right*, an olive with iron chlorosis. The main symptoms are interveinal yellowing of the young leaves, leaf necrosis, and reduction of leaf and shoot size, and can result in important yield losses

obtained with some of these products if they are thoroughly mixed with the soil. This is the case with vivianite, an Fe(II) phosphate [$\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$] (Rosado et al. 2002; Díaz et al. 2010), and siderite, an Fe(II) carbonate (FeCO_3) (Sánchez-Alcalá et al. 2012a, b). The efficacy of these products is related to their ability to react with the soil carbonate to produce poorly crystalline Fe oxides (Roldán et al. 2002; Sánchez-Alcalá et al. 2012a), which are considered to be the main source of Fe to the plant (Loeppert and Hallmark 1985; del Campillo and Torrent 1992; de Santiago and Delgado 2006). The problem of these two salts is that they should be applied in the form of a suspension that must be continuously stirred to prevent uneven distribution of the product in the soil.

Fe(II) sulfate is commonly used in foliar fertilization, spread in granulated form over the soil surface, or applied in the form of mixtures with organic materials. In most cases, the residual effects are limited; in addition, full recovery is not expected in foliar sprays (Tagliavini et al. 2000). The poor results obtained with these soluble inorganic Fe salts are attributed to their almost immediate transformation into insoluble Fe oxides. Obviously, this strongly limits mass flow and diffusion of Fe to the plant roots not close to the point where the fertilizer granule or solution is placed.

In summary, correction of Fe chlorosis is difficult, costly, and often yields poor results. A need thus exists for effective, cheap, and environmentally friendly Fe fertilizers. To the authors' knowledge, no experiments have been made in which a dilute Fe sulfate has been applied to a large volume of soil in order to place the products resulting from its transformation in the soil close to plant roots. The objectives of the present paper were to assess the effectiveness of a dilute Fe sulfate solution injected into the soil to correct Fe chlorosis in olive and compare it with a suspension of siderite, a product that has proven to be effective for olive growing on highly calcareous soils but is more expensive and difficult to prepare than the Fe sulfate solution.

2 Materials and methods

2.1 Plant and soil materials

The four experimental orchards were planted with 3-year-old 'Manzanilla,' 3-year-old 'Arbequina,' 14-year-old 'Ocal,' and 7-year-old 'Picual' olive trees and were located near the localities of Gilena ($37^\circ 14' \text{N}$, $4^\circ 54' \text{W}$), Aldea Quintana ($37^\circ 43' \text{N}$, $4^\circ 48' \text{W}$), Aldea Quintana ($37^\circ 43' \text{N}$, $4^\circ 48' \text{W}$), and Baena ($37^\circ 38' \text{N}$, $4^\circ 15' \text{W}$), respectively, in Andalusia, southern Spain. In all orchards, olive trees exhibited clear symptoms of Fe chlorosis but not of any other nutrient deficiency. The second and third orchards were irrigated in summer and managed with vegetation cover; the first and fourth orchards were rain fed and conventionally tilled. Thus, the orchards differed in cultivar, tree age, soil properties, and management in order to test the treatments in various environments.

Four soil samples per experimental orchard were collected at the depth where most roots were found (0–25 cm in the 'Manzanilla' and 'Arbequina' and 15–40 cm in the 'Ocal' and 'Picual' orchards). The samples were air-dried and ground to pass a 2-mm sieve. The properties of the soils from the four experimental orchards are shown in Table 1. All soils were strongly calcareous with calcium carbonate equivalent content (CCE), determined by weight loss upon treatment with 6 M HCl, that ranged from 470 to 710 g kg⁻¹ and active calcium carbonate equivalent or "active lime" (ACCE), determined by reaction with neutral NH_4 oxalate, that ranged from 177 to 290 g kg⁻¹. Clay content (Gee and Bauder 1986) ranged from 250 to 320 g kg⁻¹ and organic carbon (dichromate oxidation) from 0.6 to 1.2 g kg⁻¹. All pH values (1:2.5 soil/water suspension) were above 7.8, which is typical of calcareous soils, and electrical conductivity (1:5 soil/water suspension) was low (<0.16 dS m⁻¹). Only the 'Picual' soil showed a low level (7 mg kg⁻¹) in Olsen P (extraction with 0.5 M NaHCO_3 buffered at pH 8.5; Olsen et al. 1954). The 'Manzanilla' was

Table 1 Soil properties in the four experimental orchards

Cultivar	Depth cm	Clay g kg ⁻¹	Organic carbon	CCE	ACCE	pH	Electrical conductivity dS m ⁻¹	Olsen P mg kg ⁻¹	Available K	CEC cmol _(c) kg ⁻¹	Fe _d g kg ⁻¹	Fe _{ox}	Fe _{ca}	Fe _{DTPA} mg kg ⁻¹
‘Manzanilla’	0–25	250	1.2	640	278	7.8	0.16	21	52	21.8	2.5	0.25	0.66	2.2
‘Arbequina’	0–25	300	1.2	470	177	8.1	0.16	29	250	23.4	4.6	0.50	0.95	4.0
‘Ocal’	15–40	320	1.0	480	218	8.5	0.12	19	188	25.4	3.9	0.43	0.86	4.5
‘Picual’	15–40	270	0.6	710	290	8.6	0.08	7	131	17.0	2.8	0.33	1.27	2.6

All data are means of four samples

CCE calcium carbonate equivalent, ACCE active calcium carbonate equivalent (active lime), CEC cation exchange capacity, Fe_d dithionite-extractable Fe, Fe_{ox} oxalate-extractable Fe, Fe_{ca} citrate/ascorbate-extractable Fe, Fe_{DTPA} diethylenetriaminepentaacetic acid-extractable Fe

low in available K (determined by extraction with 1 M NH₄OAc buffered at pH 7) (52 g kg⁻¹). Cation exchange capacity (CEC) was medium (17–25.4 cmol_c kg⁻¹). The value of acid oxalate-extractable Fe (Fe_{ox}), which was determined according to Schwertmann (1964) but using only 250 mg of soil in 50 mL of extractant to keep pH close to 3, was below the critical level of 0.35 g kg⁻¹ proposed by Benítez et al. (2002) for the ‘Manzanilla’ and ‘Picual’ but above it in the ‘Arbequina’ and ‘Ocal’ soils. The range in diethylenetriaminepentaacetic acid-extractable Fe (Fe_{DTPA}; Lindsay and Norvell 1978) was 2.2–4.5 mg kg⁻¹, the ‘Manzanilla’ and ‘Picual’ soils being the ones with Fe_{DTPA} below the critical level of 4 mg kg⁻¹ proposed by Lindsay and Norvell (1978). These two soils were also the ones with the lowest citrate/bicarbonate/dithionite-extractable Fe (Fe_d; Mehra and Jackson 1960); however, the ‘Picual’ soil exhibited the highest citrate/ascorbate-extractable Fe (Fe_{ca}; Reyes and Torrent 1997).

2.2 Experimental design and treatments

A completely randomized block design with eight blocks (six for cv. Ocal) and one tree per plot was established in spring 2009 in the ‘Manzanilla,’ ‘Arbequina,’ and ‘Ocal’ orchards. A completely randomized design with six replications and one tree per plot was established in spring 2010 in the ‘Picual’ orchard.

The four experimental treatments consisted in injecting into the soil an equal volume of a suspension of siderite, a solution of Fe sulfate (FeSO₄·7H₂O), a solution of K₂SO₄, or water. These treatments were designed as “Siderite,” “Fe-sulfate,” “Control-K,” and “Control-0,” respectively. The Control-0 treatment was only applied in the ‘Ocal’ and ‘Picual’ orchards.

The siderite suspension was prepared by dissolving 4.25 kg of FeSO₄·7H₂O and 2.12 kg of K₂CO₃ (‘Manzanilla’ and ‘Arbequina’ orchards) or 4.50 kg of FeSO₄·7H₂O and 2.25 kg of K₂CO₃ (‘Picual’ and ‘Ocal’ orchards) in 100 L of water. The resulting dark green suspension, which contained about 1.75 and 1.85 kg of siderite, respectively, was

continuously stirred to keep the siderite particles in suspension. For the “Fe-sulfate” treatment, an amount of FeSO₄·7H₂O equal to that used in the “Siderite” treatment plus an amount of K₂SO₄ aimed at supplementing the solution with K was used (1.00 kg for ‘Manzanilla’ and ‘Arbequina’ and 2.80 kg for ‘Picual’ and ‘Ocal’) in order to prepare 100 L of solution. For the “Control-K” treatment, only K₂SO₄ was used in equal amounts to those used in the corresponding “Fe-sulfate” treatment.

In each orchard, the volume of the siderite suspension or diluted Fe sulfate solution applied to each tree was that corresponding to the Fe and K doses shown in Table 2. The suspensions of siderite and diluted Fe sulfate were injected into the soil immediately after preparation by means of a T-shaped injector connected to the 100-L tank in 0.5–2-L portions at 5–15 points at a distance of 0.5 and 1.5 m from the tree trunk and a depth where root density was highest, i.e., about 15–35 cm. In addition, the local olive growers applied to the soil about 0.5–1 kg N year⁻¹ tree⁻¹ and potassium applied as foliar spray (2.5 % K₂SO₄).

2.3 Plant measurements and analysis

In May, July, and October of each year, between 20 and 30 shoots of the current season were selected around each tree at about 150–180 cm above the soil surface, and one fully

Table 2 Fe and K doses for the different experimental orchards and treatments

Treatment	‘Manzanilla’		‘Arbequina’		‘Ocal’		‘Picual’	
	Fe g tree ⁻¹	K	Fe	K	Fe	K	Fe	K
Control-0	Not applied		Not applied		0	0	0	0
Control-K	0	90	0	90	0	630	0	380
Siderite	170	240	170	240	450	630	300	380
Fe-sulfate	170	90	170	90	450	630	300	380

expanded leaf longer than 3 cm was collected from each shoot. The leaves were kept in a cool box and transported to the lab within the next 5 h. Then, the leaves were weighted, and leaf chlorophyll concentration was estimated using the soil plant analysis development (SPAD) 502 portable chlorophyll meter (Minolta Camera, Osaka, Japan). To validate the use of the SPAD apparatus, 30 leaves with different degrees of Fe chlorosis were collected from each cultivar. In each leaf, SPAD readings were taken, and a piece of the middle of the leaf ($\sim 1 \text{ cm}^2$) was cut, weighed, and placed in a tube with 10 cm^3 of methanol for 24 h in the dark. The absorbance of the extract was measured at 665 nm (chlorophyll *a*) and 650 nm (chlorophyll *b*), and the chlorophyll content per unit of leaf weight was calculated according to Holden (1976). SPAD units and leaf chlorophyll concentration per unit weight were highly correlated ($r = 0.91^{***}$ for ‘Manzanilla’, $r = 0.94^{***}$ for ‘Arbequina’, $r = 0.89^{***}$ for ‘Ocal’, and $r = 0.92^{***}$ for ‘Picual’). Previous experiments with olive trees growing in the field had shown that SPAD units were strongly correlated with chlorophyll content (milligrams per square centimeter) (Benítez et al. 2002).

In the second half of July, 20 shoots of the previous year were randomly selected around the tree, and one leaf was taken from the central part of each shoot to analyze mineral element concentration. Leaves were washed with distilled water with 0.01 % Tween 20, dried at 60°C for 70 h before grinding with a mill, and finally digested with nitric/perchloric acid (Zazoski and Burau 1977). Ca, Mg, Fe, Mn, Cu, and Zn in solution were determined by atomic absorption spectrophotometry, K by flame emission, and P with the molybdenum blue color method of Murphy and Riley (1962). The N concentration was determined by direct combustion of the plant material, using a EuroVector EA-3000 Elemental Analyzer (EuroVector SpA, Milan, Italy).

The trunk perimeter 50 cm above the ground and canopy volume (estimated as the volume of a sphere with a mean diameter calculated from the height and two perpendicular diameters of the canopy) were measured at the beginning of October each year. Yield was recorded in the 2010–2012 period for the ‘Picual’ and in 2010 for the ‘Ocal’ orchard.

2.4 Experimental oxidation of Fe salts in the laboratory

Portions of 20 cm^3 of siderite suspensions and Fe sulfate and K_2SO_4 solutions prepared as in the field (but using deionized water) were injected with a syringe into 50 g of the soil collected from each experimental orchard and placed in a cylindrical glass funnel. Then, the funnel was covered with punctured parafilm for 48 h to allow air exchange but not water loss. Then, the soil was removed from the funnel, air-dried, ground, and Fe_{ox} , Fe_{ca} , and Fe_{DTPA} determined as before. Then, a portion of the soil in each funnel was subjected

to successive cycles of wetting (field capacity) and drying in a room at 24°C and Fe_{ox} , Fe_{ca} , and Fe_{DTPA} determined after three and ten of these cycles. This experiment was performed in duplicate for the different soil $\times$ treatment combinations.

2.5 Statistical analyses

The analysis of variance (ANOVA) was performed with Statistix 9.0 (Analytical Software, Tallahassee, FL, USA) and based on a randomized complete block design in three experimental orchards (‘Manzanilla,’ ‘Arbequina,’ and ‘Ocal’) with eight, eight, and six blocks, respectively, and completely randomized design in ‘Picual’ orchard with six replications. The experimental unit was one olive tree in all fields. Means were separated via the LSD test. Pearson correlation was used to relate measured variables. The word “significant” is used here to indicate significance at the $P < 0.05$ level.

3 Results and discussion

3.1 Plant performance

At the beginning of the experiment, all trees exhibited symptoms of Fe chlorosis, and no significant differences in SPAD between the trees selected for the different treatments were observed. SPAD readings generally increased during the growing season (spring to autumn) because leaves become thicker and older, and it should be noted that SPAD is a transmittance measurement (data not shown). Potassium did not seem to influence SPAD, as evident from the absence of significant differences in SPAD between “Control-0” and “Control-K” trees in those orchards where both treatments were applied (‘Ocal’ and ‘Picual’). Either siderite, Fe-sulfate, or both had a positive effect on SPAD that was significant at most times for ‘Manzanilla’ and ‘Picual’ and only in July 2009 for ‘Arbequina’ and May 2009 and 2012 for ‘Ocal.’ The mean SPAD values for each growing season and for the 3 or 4 years (Fig. 2, left) reflect a significant superiority of Fe-sulfate over siderite in the two orchards where Fe fertilizers demonstrated to be effective to alleviate chlorosis (‘Manzanilla’ and ‘Picual’). The SPAD values of Fe sulfate-fertilized trees were about 7 and 6 % higher than those of control trees in ‘Manzanilla’ and ‘Picual,’ respectively. The SPAD values of siderite-fertilized trees were 4 % higher than those of the control trees in ‘Manzanilla.’ Visual inspection showed that these increases were reflected in an improvement in the overall health of the tree.

As can be seen in Fig. 2 (right), mean leaf weight was positively influenced by the application of one or both Fe fertilizers in ‘Manzanilla’ and ‘Picual’ (two seasons) and ‘Ocal’ (one season) but not in ‘Arbequina.’ The mean leaf weight for the 2010, 2011, and 2012 seasons was significantly

Fig. 2 Mean soil plant analysis development (SPAD) (left) and mean leaf weight (right) values for each cultivar growing season and mean for 3 of 4 years (mean $\pm$ standard error, where absent, bars fall

within symbols). *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively. Blue line Control-0, black line Control-K, orange line Siderite, green line Fe-sulfate

influenced by both fertilizers in 'Manzanilla' and by siderite in 'Picual,' with an increase of 6–9 % relative to the control trees. These results are thus in line with those on SPAD.

Fertilization with Fe had no significant effect on canopy volume, trunk perimeter, annual trunk perimeter increment, and cumulative yield (data not shown). Canopy volume and trunk perimeter were proportional to tree age, except in 'Ocal' because these trees were severely pruned during the experiment. Significant differences in yield were found in 2010 and 2012 (but not in cumulative yield) in 'Picual.'

Leaf mineral element concentrations (data not shown) were always above the critical levels compiled by Fernández-Escobar (2008), except for K in 'Manzanilla' trees because the available K content of its soil was low (52 g kg^{-1}). It should be noted that leaf Mn concentration in control trees in 2009, 2010, and 2011 for 'Manzanilla' and 2009 for 'Ocal' was significantly higher than that in trees fertilized with Fe because Fe-deficient conditions increase the outflow of protons and the reducing capacity of root cells, thereby contributing to the reduction and dissolution not only of Fe,

Table 3 Fe_{ox} (oxalate-extractable Fe), Fe_{ca} (citrate/ascorbate-extractable Fe), and Fe_{DTPA} (diethylenetriaminopentaacetic acid-extractable Fe) in the four soils following the specified treatment and after three and ten wetting and drying cycles

Cultivar	Treatment	Fe _{ox} g kg ⁻¹	Fe _{ox} 3 wet-dry	Fe _{ox} 10 wet-dry	Fe _{ca}	Fe _{ca} 10 wet-dry	Fe _{DTPA} mg kg ⁻¹	Fe _{DTPA} 3 wet-dry	Fe _{DTPA} 10 wet-dry
'Manzanilla'	Control-K	0.25±0.00 b	0.20±0.00 b	0.19±0.01 b	0.66±0.02 b	0.64±0.06 b	2.2±0.2 c	2.0±0.1 b	2.1±0.1 c
	Siderite	0.46±0.01 a	0.46±0.02 a	0.40±0.02 a	0.98±0.00 a	0.88±0.02 a	14.0±0.7 b	8.3±0.8 a	6.9±0.0 b
	Fe sulfate	0.54±0.07 a	0.51±0.03 a	0.38±0.03 a	0.98±0.06 a	0.85±0.00 a	16.7±0.5 a	11.1±1.0 a	9.1±0.5 a
	<i>P</i> -ANOVA	0.0318	0.0032	0.0105	0.0116	0.0336	0.0005	0.0065	0.0010
'Arbequina'	Control-K	0.50±0.02	0.41±0.00 b	0.44±0.00 b	0.95±0.02	0.78±0.08	4.0±0.5 b	2.8±0.1 b	3.3±0.1 c
	Siderite	0.67±0.01	0.61±0.02 a	0.63±0.01 a	1.23±0.02	0.97±0.00	14.7±0.6 a	8.4±0.1 a	7.2±0.2 b
	Fe sulfate	0.74±0.08	0.68±0.07 a	0.70±0.06 a	1.30±0.12	1.07±0.16	17.6±2.8 a	11.2±2.1 a	10.4±0.0 a
	<i>P</i> -ANOVA	0.0826	0.0432	0.0327	0.0795	0.2617	0.0206	0.0335	0.0032
'Ocal'	Control-K	0.43±0.04 c	0.35±0.01 c	0.37±0.01 c	0.86±0.05 b	0.71±0.04 b	4.5±0.2 c	3.5±0.0 c	3.5±0.3 c
	Siderite	0.58±0.01 b	0.55±0.01 b	0.57±0.03 b	1.07±0.05 ab	0.98±0.04 a	15.1±0.8 b	9.15±0.3 b	7.7±0.5 b
	Fe sulfate	0.70±0.01 a	0.62±0.00 a	0.65±0.01 a	1.20±0.04 a	1.03±0.00 a	19.7±0.6 a	13.2±0.2 a	10.5±0.3 a
	<i>P</i> -ANOVA	0.0087	0.0001	0.0029	0.0328	0.0123	0.0007	0.0001	0.0023
'Picual'	Control-K	0.33±0.00 b	0.31±0.00 b	0.29±0.00 b	1.27±0.01	0.92±0.04 b	2.6±0.0 c	2.3±0.1 c	2.1±0.2 c
	Siderite	0.67±0.03 a	0.62±0.02 a	0.60±0.03 a	1.61±0.06	1.34±0.01 a	17.0±1.1 b	8.9±0.6 b	7.1±0.8 b
	Fe sulfate	0.74±0.04 a	0.69±0.04 a	0.64±0.03 a	1.67±0.24	1.32±0.01 a	20.8±0.5 a	12.8±1.0 a	10.3±0.0 a
	<i>P</i> -ANOVA	0.0044	0.0043	0.0045	0.2434	0.0025	0.0070	0.0033	0.0023

Different letters in the same column and cultivar indicate significant differences ($P < 0.05$, LSD test) between treatments

but also of Mn (Moraghan and Freeman 1978). No differences were found in leaf Fe concentration (which ranged from 19 to 80 mg kg⁻¹) because this element can accumulate in leaves even under conditions of deficiency (a fact known as the “chlorosis paradox”; Römheld 2000).

3.2 Changes in Fe forms in soil incubated with Fe fertilizers

Table 3 shows the values of Fe_{ox}, Fe_{ca}, and Fe_{DTPA} in the four soils incubated with siderite and Fe sulfate after the start of the experiment and three and ten (only ten for Fe_{ca}) cycles of wetting and drying. Upon addition of Fe, Fe_{ox} increased from 0.2 to 0.4 g kg⁻¹, Fe_{ca} from 0.2 to 0.4 g kg⁻¹, and Fe_{DTPA} from 11 to 18 mg kg⁻¹. The ANOVA showed a significant effect of treatment on the amount of extractable Fe for all time × Fe form combinations in the ‘Manzanilla’ and ‘Ocal’ soils, in five out of eight combinations in the ‘Arbequina’ soil, and seven out of eight combinations in the ‘Picual’ soil. The lack of significance occurred mostly for Fe_{ca}, probably because citrate/ascorbate is capable of extracting substantially more Fe than oxalate. Recovery of applied Fe generally decreased with the number of wetting and drying cycles, the effect being particularly marked for Fe_{DTPA}. Iron applied in the form of sulfate was generally more available to the different extractants than that applied in the form of siderite; this effect was particularly evident for Fe_{DTPA} where significant evidences occurred in 9 out of 12 time × soil combinations.

3.3 General discussion

The results of this study demonstrated that siderite suspensions injected into the soil led in some cases to an alleviation of chlorosis symptoms, consistent with previous pot experiments with herbaceous crops (Sánchez-Alcalá et al. 2012a) and field experiments in olive orchards with other varieties of olive (Sánchez-Alcalá et al. 2012b). The latter experiments and the present study also indicate that the beneficial effect of a single application of siderite can persist for several growing seasons. This represents an advantage over the use of Fe chelates, which are easily washed from the soil. Siderite can be considered as a slow-release Fe fertilizer because it dissolves incongruently in soil to yield mainly poorly crystalline, small-sized lepidocrocite particles (Sánchez-Alcalá et al. 2012a), which cannot be washed and whose Fe is more phytoavailable than that of crystalline Fe oxides.

Iron sulfate has been used in different ways to prevent Fe chlorosis without positive persistent effects. Direct application to the soil surface is largely ineffective because it is rapidly oxidized and hydrolyzed to Fe oxides that are retained in the first few millimeters of the soil and are thus largely unavailable to the plant roots (Abadía et al. 2011). In the present study, the Fe sulfate solution was injected into a large

volume of soil in the zone of greatest root density, and the Fe²⁺ ions could then react with the abundant calcite surfaces of these highly calcareous soils. This reaction would likely result in calcite being partly coated with lepidocrocite crystals as suggested by the experiments of Loeppert and Hossner (1984) and Clarke et al. (1985). This probably explains the superiority of Fe sulfate solutions over siderite suspensions because the lepidocrocite particles resulting from the alteration of siderite are concentrated where the initial siderite grain was and not distributed over the calcite surfaces as is the case with the Fe sulfate solutions. This obviously affects the number of Fe oxide particles that are close to the plant roots.

The different response of ‘Manzanilla’ and ‘Picual’ on one side and ‘Arbequina’ and ‘Ocal’ to the Fe fertilizers on the other was consistent with the Fe_{ox} and Fe_{DTPA} values of the corresponding soils. So, Fe_{ox} and Fe_{DTPA} in ‘Manzanilla’ and ‘Picual’ soils are below and the ‘Arbequina’ and ‘Ocal’ soils are close or above the respective critical values of 0.35 g Fe_{ox} kg⁻¹ for olive (Benítez et al. 2002) and 4 mg Fe_{DTPA} kg⁻¹ for most crops (Lindsay and Norvell 1978). By contrast, Fe_{ca} had little predictive value, even though the citrate/ascorbate reagent appears to extract the same forms of Fe as oxalate, specifically the poorly crystalline Fe oxides (Reyes and Torrent 1997). Another possible reason is that the two orchards where there was no response were those managed with vegetation cover. Gramineous plants grown under Fe deficiency excrete phytosiderophores to the soil, which can bind Fe and constitute a good source of this element, as recent studies have demonstrated (Abadía et al. 2004).

Finally, it should be noted that the positive effect of Fe fertilizers on SPAD and leaf weight (Fig. 2) was not reflected in other plant growth parameters or oil yield. This can be explained by the lagged response of olive to nutrient stress because of built-up nutrient reserves, which are likely to increase with tree age.

4 Conclusions

Our results demonstrate the efficacy of siderite suspensions and clearly suggest the high potential of Fe sulfate solutions injected into the soil to increase Fe bioavailability in calcareous soils. Despite the scarce differences between Fe-fertilized and control trees, our results are consistent with our laboratory experiments. This calls for further research on the use of these Fe fertilizers, which have on the other hand the advantage of being readily prepared in a stirred tank and injected into the soil with a variety of devices. They are nontoxic and have no apparent adverse effects caused by the salinity of the solution. In addition, they are not susceptible to leaching because of the poor solubility of the Fe oxide phases resulting from their reaction with the soil.

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