



## Long-term effectiveness of vivianite in reducing iron chlorosis in olive trees

R. Rosado, M.C. del Campillo<sup>1</sup>, M.A. Martínez, V. Barrón & J. Torrent

Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Apdo. 3048, 14080 Córdoba, Spain. <sup>1</sup>Corresponding author\*

Received 19 October 2000. Accepted in revised form 28 March 2001

**Key words:** calcareous soil, ferrous phosphate, iron chlorosis, olive tree, vivianite

### Abstract

Iron (Fe) chlorosis is common in olive (*Olea europaea* L.) trees growing on highly calcareous soils in Southern Spain, where generally causes reduction in yield, size and commercial value of the olives. The objective of this research was to study the effectiveness of synthetic vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ) to reduce Fe chlorosis in olive. Experiments were established in three orchards with cultivars 'Hojiblanco', 'Manzanillo', and 'Picual'. The design was a randomised block design with two or three treatments (control with no Fe fertiliser and vivianite at one or two rates). A vivianite suspension ( $0.05 \text{ kg dm}^{-3}$  water) was injected into the soil at 10–20 points around the tree at the depth of maximum root density (25–35 cm). The rates (and times of application) were 0.5 and 1  $\text{kg tree}^{-1}$  for 'Hojiblanco' (March 1997), 1  $\text{kg tree}^{-1}$  for 'Manzanillo' (March 1998), and 2  $\text{kg tree}^{-1}$  for 'Picual' (March 1998). The leaf chlorophyll content index (CCI) was estimated on the youngest expanded leaves by means of a Minolta apparatus (SPAD units). The colour index of the olives was estimated by visual comparison with a scale ranging from 1 (pale yellow) to 8 (normal green). For the period studied (July 1997–November 1999), the CCI of fertilised trees was, in general, significantly higher than that of control trees, and so was the case with the olive colour index. Olive yield, measured in the experimental fields with 'Hojiblanco' (in 1999) and 'Manzanillo' (in 1998 and 1999), was higher for the fertilised than for the control trees but differences were only significant in 1999. These results suggest that vivianite is effective to reduce Fe chlorosis for more than two seasons. Such effectiveness is probably due to the poorly crystalline Fe(III) oxides (which are good sources of Fe to plants) that result from the slow oxidation and incongruent dissolution of vivianite.

**Abbreviations:** ACCE – active calcium carbonate equivalent; CCE – calcium carbonate equivalent; CCI – chlorophyll content index; CEC – cation exchange capacity; EC – electrical conductivity;  $\text{Fe}_o$  – oxalate-extractable Fe;  $\text{Fe}_{ca}$  – citrate/ascorbate-extractable Fe

### Introduction

Iron chlorosis is commonly observed in olive trees cultivated on highly calcareous soils of Southern Spain. It is easily recognised by the typical interveinal yellowing of the young leaves and generally results in yield reduction, yellowing of the olives (which makes them of little commercial value for direct consumption), and decrease in the stability and chlorophyll and carotenoids contents of the virgin olive oil. This has nutritional significance, since carotenoids are anticar-

cinogenic and anti-ulcer agents (Mínguez-Mosquera et al., 1991).

Studies over the past ten years showed that vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ), mixed with, or injected into the soil at rates of about  $1 \text{ g kg}^{-1}$  was effective to prevent Fe chlorosis in chickpea (Eynard et al., 1992) and 'Picual' olive trees (Marta, 1999) grown in pots, and in pear trees cultivated following the usual farmers' practices (del Campillo et al., 1998). In pot experiments, vivianite was as effective as chelate (Fe-EDDHA) to prevent Fe chlorosis; in the experiments with pear trees, vivianite was effective even five years after application.

\* FAX No: +34-957-218440. E-mail: ef1cagam@uco.es

The potential value of vivianite as a source of Fe to plants is supported by its high Fe content (>30%) (Eynard et al., 1992) and the low price of the products needed for its synthesis. Moreover, vivianite suspensions are not toxic, can be readily prepared before application, and contain, when prepared in the simplest way, fertiliser N and P.

The objective of this research was to test the effectiveness of vivianite to prevent Fe chlorosis in olive trees. For this purpose, we carried out field experiments with three different olive cultivars, which were studied for two or three growing seasons.

## Materials and methods

### *Experimental fields and vivianite application*

Three olive orchards located in Andalusia (Southern Spain) were selected for this study. Trees in these or in adjacent orchards had shown symptoms of chlorosis from 1995 to 1997, as evidence by interveinal yellowing of the youngest leaves. This yellowing diminished or disappeared upon treatment with Fe fertilisers (either Fe chelate applied to the soil or Fe(III) salts injected into the tree trunk).

The location, age and cultivar of the three orchards were, respectively: (1) Province of Seville, one-year-old 'Hojiblanco'; (2) Province of Málaga, twenty-year-old 'Manzanillo', and (3) Province of Jaén, eighty-years-old 'Picual'. For each orchard, composite samples from the surface horizon (0–15 cm) and from the horizon with maximum root density (generally, at a depth of 15–35 cm) were collected. Each composite sample was the combination of four subsamples. Soil samples were air-dried and passed through a 2 mm sieve before analysis. Organic matter was determined by dichromate oxidation (Walkley and Black, 1934), particle soil distribution analysis was determined by the pipette method, and cation exchange capacity (CEC) by the method of 1 M NH<sub>4</sub>OAc buffered at pH 7. Soil pH was measured in a 1:2 soil:water mixture and electrical conductivity (EC) was measured in a 1:5 soil:water extract. Total CaCO<sub>3</sub> equivalent (CCE) was determined by weight loss after treating 2 g of sample with 6 M HCl. The active calcium carbonate equivalent (ACCE) was determined with NH<sub>4</sub>-oxalate as described by Drouineau (1942). The acid oxalate-extractable Fe (Fe<sub>o</sub>) was determined according to Schwertmann (1964), with 0.25 g of sample in 50 cm<sup>3</sup> of 0.2 M NH<sub>4</sub> oxalate at pH 3. The citrate/ascorbate-

extractable Fe (Fe<sub>ca</sub>) was determined according to Reyes and Torrent (1997).

A randomised block design was used with (1) two blocks, three treatments (control with no Fe fertiliser, 0.5, and 1 kg of vivianite tree<sup>-1</sup>), and four trees per plot in the 'Hojiblanco' orchard; (2) five blocks, two treatments (control with no Fe fertiliser and 1 kg of vivianite tree<sup>-1</sup>), and two trees per plot in the 'Manzanillo' orchard, and (3) five blocks, two treatments (control with no Fe fertiliser and 2 kg of vivianite tree<sup>-1</sup>), and two trees per plot in the 'Picual' orchard. In this orchard a third treatment with Fe chelate (50 g Fe-EDDHA tree<sup>-1</sup> applied to the soil in April 1999) was added. After the experiments, the statistical analysis was carried out using the Costat Program (CoHort Software, Minneapolis, MN). Means were separated by the LSD (Least Significant Difference) test ( $P < 0.10$ ).

### *Vivianite application*

Vivianite, in the form of suspension, was applied in March 1997, March 1998, and March 1998 to the 'Hojiblanco', 'Manzanillo', and 'Picual' fields, respectively. In the 'Hojiblanco' field, vivianite powder was suspended in water at a concentration of 0.05 kg dm<sup>-3</sup>. This vivianite had been previously synthesised by bringing 100 dm<sup>3</sup> of a solution containing 16 kg of FeSO<sub>4</sub>·7H<sub>2</sub>O and 3.6 kg of H<sub>3</sub>PO<sub>4</sub> to pH 6.5 with KOH. The resulting suspension of vivianite was washed several times with water until salt free (EC < 0.2 dS m<sup>-1</sup>), air-dried and finally stored in plastic bags. In the 'Manzanillo' and 'Picual' orchards the vivianite suspension was prepared directly in the field by dissolving first 2.5 kg of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> in a tank with 100 dm<sup>3</sup> of water. Then, 7.5 kg of FeSO<sub>4</sub>·7H<sub>2</sub>O were added in several steps while stirring. After dissolution of this salt, a gelatinous suspension formed which rapidly transformed into a thin suspension of partly oxidized vivianite with a pale greenish-blue colour. This suspension contained about 0.05 kg vivianite dm<sup>-3</sup> in the form of particles of 2–10 μm, which were kept in suspension by gentle stirring.

The suspension of vivianite (either prepared from the powder or synthesised directly in the field) was injected into the soil using a T-shaped injector connected to a tank. The pressure and the volume of suspension applied were controlled with the injector. The suspension was injected into the soil at a depth of 25–35 cm (where active root density was high) at 10–20 points regularly distributed below the tree canopy. At each

Table 1. Selected properties of samples from surface (A) and subsurface (B) soil horizons

Cultivar	Horizon	Organic matter	Clay	CCE <sup>a</sup>	ACCE <sup>a</sup>	CEC <sup>a</sup>	pH (H <sub>2</sub> O)	EC <sup>a</sup> (1:5)	Fe forms <sup>a</sup>	
									Fe <sub>o</sub>	Fe <sub>ca</sub>
	cm	g kg <sup>-1</sup>			cmol <sub>c</sub> kg <sup>-1</sup>			dS m <sup>-1</sup>	g kg <sup>-1</sup>	
'Hojiblanco'	0-15	9	278	500	310	25	8.4	0.12	0.30	0.45
'Manzanillo'	0-15	20	217	656	252	13	8.2	0.19	0.10	0.18
'Manzanillo'	15-35	10	238	770	260	11	8.6	0.14	0.17	0.36
'Picual'	0-15	10	262	593	242	21	8.1	0.24	0.28	0.58
'Picual'	15-35	11	275	675	287	19	8.3	0.12	0.21	0.47

<sup>a</sup>Abbreviations: CCE, calcium carbonate equivalent; ACCE, active calcium carbonate equivalent; CEC, cation exchange capacity; EC, electrical conductivity; Fe<sub>o</sub>, oxalate-extractable Fe; Fe<sub>ca</sub>, citrate/ascorbate-extractable Fe.

Table 2. Olive colour index of 'Manzanillo' and 'Picual' olive trees<sup>a</sup>

Cultivar	1998		1999		
	Control (-Fe)	Fertilised with vivianite	Control (-Fe)	Fertilised with vivianite	Fertilised with Fe chelate
'Manzanillo'	6.1a	7.4b	4.4a	6.5b	
'Picual'			3.9a	5.8b	6.0b

<sup>a</sup>Means in the same row followed by the same letter are not significantly different ( $P < 0.10$ ).

point, between 0.5 and 2 dm<sup>3</sup> of suspension were injected. Injection was carried out when soil moisture content was between wilting point and field capacity.

### Plant analyses

At selected times, 30 randomly picked shoots were taken from each tree at about 1.60 m from the soil surface. From each shoot, the chlorophyll content index (CCI) of the youngest fully expanded leaf was estimated by a Minolta apparatus (SPAD units). In the 'Manzanillo', and 'Picual' fields, the CCI was measured before applying vivianite (March 1998), and in May, July and October 1998, and May, July and November 1999. In the 'Hojiblanco' field, CCI was also measured in July and October 1997 (vivianite had been applied in March 1997). We discarded using leaf Fe concentration as a measure of the Fe status in the olive trees because leaf Fe concentration is not generally well correlated with leaf chlorophyll content under field conditions. This so-called "chlorosis paradox" (Morales et al., 1998; Römheld, 1997) is likely due to the effect that Fe chlorosis has on leaf growth.

The colour of the olives was measured in September 1998 and September 1999 for the 'Manzanillo' and in September 1999 for the 'Picual' trees. For each tree, the degree of chlorosis of 16 randomly picked olives

was estimated using a visual scale from 1 (pale yellow to 8 (normal green). Olive yield was measured in the 'Hojiblanco' (1999) and 'Manzanillo' (1998 and 1999) fields.

## Results

### Soil properties

Selected soil properties of the experimental fields are summarised in Table 1. Organic matter ranged from 9 to 20 g kg<sup>-1</sup>. The CCE varied between 500 and 675 g kg<sup>-1</sup> and the ACCE between 242 and 310 g kg<sup>-1</sup>, consistent with a pH range of 8.1–8.6. The contents in Fe<sub>o</sub> and Fe<sub>ca</sub>, which can be used as estimates of the content in poorly crystalline Fe oxides (Reyes and Torrent, 1997), were low (Fe<sub>o</sub> = 0.10–0.30 g kg<sup>-1</sup>; Fe<sub>ca</sub> = 0.18–0.58 g kg<sup>-1</sup>). These values are below the critical values that separate soils that induce Fe chlorosis in olive from those that do not (0.35 g Fe<sub>o</sub> kg<sup>-1</sup> and 0.60 g Fe<sub>ca</sub> kg<sup>-1</sup>) (Benítez et al., 2000).

### Effectiveness of vivianite

The time course of the CCI is shown in Figure 1. Control trees showed clear visual symptoms of Fe chlorosis through the period of sampling in the three

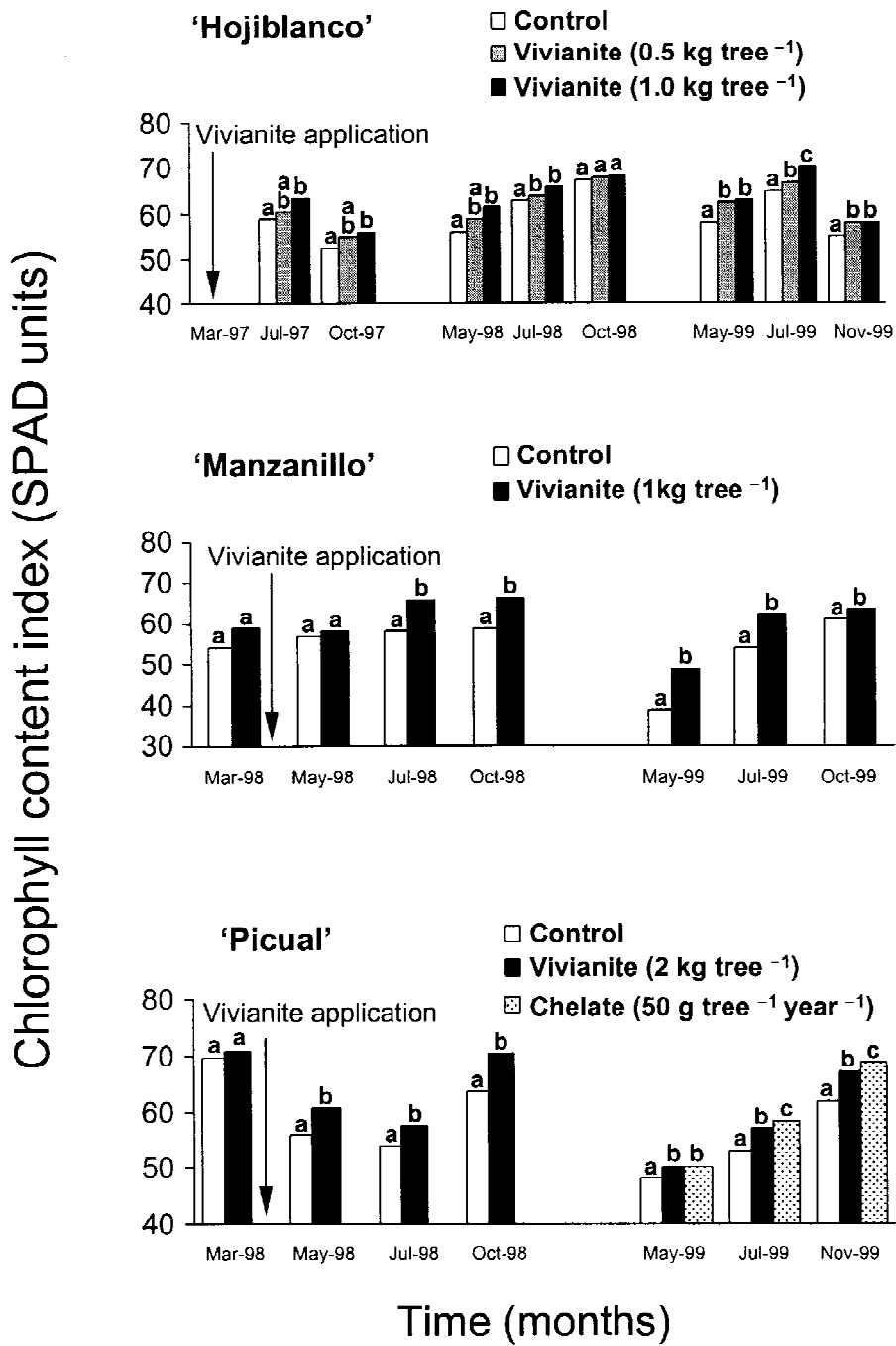


Figure 1. Time course of the leaf chlorophyll content index (SPAD units) for 'Hojiblanco', 'Manzanillo', and 'Picual' olive trees for different Fe fertiliser treatments. For the same cultivar and time of measurement, values marked with the same letter are not significantly different ( $P < 0.10$ ).

Table 3. Yield of 'Hojiblanco' and 'Manzanillo' olive trees<sup>a</sup>

Cultivar	1998		1999		
	Control (-Fe)	1.0 kg vivianite tree <sup>-1</sup>	Control (-Fe)	0.5 kg vivianite tree <sup>-1</sup>	1.0 kg vivianite tree <sup>-1</sup>
			kg tree <sup>-1</sup>		
'Hojiblanco'			4.9a	6.8a	10.4b
'Manzanillo'	21.3a	29.7a	18.3a		26.9b

<sup>a</sup>Means in the same row followed by the same letter are not significantly different ( $P < 0.10$ ).

experimental fields. However, the CCI of the leaves of chlorotic trees was not uniform during the growing season. It generally increased from spring to autumn, apparently because leaves became older and thicker (it must be noted that the SPAD units are transmission measurements). When it was visually evident that leaf growth had taken place in autumn (e.g., October 1997 and November 1999 for 'Hojiblanco'), the SPAD readings were lower in autumn than in summer.

In 18 out of the 20 measurements made at different fields and times, the CCI for the vivianite-treated trees was significantly ( $P < 0.10$ ) higher than the CCI for the control trees. For the 'Hojiblanco' field, the lower vivianite application rate (0.5 kg tree<sup>-1</sup>) resulted in a significant CCI increase only in four of the eight measurements.

Figure 1 shows that the relative difference in CCI between the treated and control trees did not depend on the time of sampling. This makes it difficult to select the best sampling time to observe differences in experiments aimed at studying the effect of vivianite and other Fe amendments on olive.

Despite their higher CCI relative to control trees, most vivianite-treated trees showed some visual degree of Fe chlorosis, indicating that vivianite was not totally effective to prevent Fe chlorosis. However, in the 'Picual' field, where the results of applying vivianite and Fe chelate were compared, chelate was, in terms of CCI, only a little superior to vivianite applied two years before.

The olive colour index measured in 'Manzanillo' (1998 and 1999) and 'Picual' (1999) was significantly higher for fertilised than for control trees (Table 2). No significant differences in this index were observed between vivianite- and Fe chelate-treated trees in the 'Picual' field in 1999 (Table 2).

Olives were harvested in September 1998 and September 1999 in the 'Manzanillo' field and in September 1999 in the 'Hojiblanco' field. The vivianite-fertilised trees always yielded more olives

than the control trees but the differences were significant only in 1999 (Table 3).

## Discussion

Vivianite is effective in reducing Fe chlorosis in olive trees, as indicated by the CCI data (Figure 1) and the olive colour index (Table 2). This effect is reflected in the higher yield of vivianite-treated relative to control trees (Table 3). Because all experimental plots (including the control ones) were fertilized by the farmers with N and P at rates much higher than those contained in the vivianite suspension, differences between vivianite-treated and control plots cannot be ascribed to the effect of fertilizer N or P.

Our results suggest that vivianite is almost as effective as Fe chelate in reducing Fe chlorosis in olive. In addition, the anti-chlorotic effect of vivianite lasts seemingly for more than two years. This is consistent with the aforementioned experiments with pear trees, for which the application of vivianite was effective to reduce Fe chlorosis for five growing seasons.

We hypothesize that the effectiveness of vivianite in reducing Fe chlorosis lies in the type of alteration products that are formed upon its oxidation and incongruent dissolution. The hydrolysis of Fe salts in the presence of phosphate (as it is the case with vivianite) yields poorly crystalline Fe oxides, such as ferrihydrite (Schwertmann and Taylor, 1989) or lepidocrocite (Cumplido et al., 2000; Gálvez et al., 1999). Poorly crystalline Fe oxides are good sources of Fe to plants, as suggested by the negative correlation between degree of Fe chlorosis in different plants and soil Fe<sub>o</sub> or Fe<sub>ca</sub> content (del Campillo and Torrent, 1992; Loepfert et al., 1988; Yanguas et al., 1997), and pot experiments with ferrihydrite as an Fe amendment (Vempati and Loepfert, 1986). Although the solubility of vivianite at the typical alkaline pH of calcareous soils is low, its incongruent dissolution is likely to be

favoured by removal of its phosphate as a result of: (1) root uptake; (2) adsorption on active soil surfaces (Fe oxides, calcite), and (3) precipitation as insoluble Ca phosphates. Continuous passage of phosphate to these sinks probably determines a slow and continuous formation of poorly crystalline Fe oxides; this would explain the observed long-term effectiveness of vivianite in reducing Fe chlorosis.

### Acknowledgements

This study was financed by the Spanish C.I.C.Y.T., Project OL196–2183. The authors thank V. Cejas, F. Díaz Tresgallo and M. Pastor for their valuable help in establishing the experimental fields.

### References

- Benítez M L, Pedrajas V M, del Campillo M C and Torrent J 2000 Iron chlorosis in olive in relation to soil properties. *Nutr. Cycl. Agroecosyst.* (*In press*).
- Cumplido J, Barrón V and Torrent J 2000 Effect of phosphate on the formation of nanophase lepidocrocite from Fe(II) sulfate. *Clays Clay Miner.* 48, 503–510.
- del Campillo M C and Torrent J 1992 Predicting the incidence of Fe chlorosis in calcareous soils of southern Spain. *Commun. Soil Sci. Plant Anal.* 23, 399–416.
- del Campillo M C, Barrón V, Torrent J, Iglesias L, Dalmau R and Marcé X 1998 Fertilisation with Fe(II)-phosphate effectively prevents iron chlorosis in pear trees. *In Abstracts. XXV International Horticultural Congress (IHC)*, p 194. Brussels, Belgium.
- Drouineau G 1942 Dosage rapide du calcaire actif du sol: nouvelles données sur la separation et la nature des fractions calcaires. *Ann. Agron.* 12, 441–450.
- Eynard A, del Campillo M C, Barrón V and Torrent J 1992 Use of vivianite ( $\text{Fe}_3(\text{PO})_2 \cdot 8\text{H}_2\text{O}$ ) to prevent iron chlorosis in calcareous soils. *Fert. Res.* 31, 61–67.
- Gálvez N, Barrón V and Torrent J 1999 Effect of phosphate on the crystallization of hematite, goethite and lepidocrocite from ferrihydrite. *Clays Clay Min.* 47, 304–311.
- Loeppert R H, Geiger S C, Hartwig R C and Morris D R 1988 A comparison of indigenous soil factors influencing the Fe-deficiency chlorosis of sorghum and soybean in calcareous soils. *J. Plant Nutr.* 11, 1841–1492.
- Marta J 1999 Respuesta del olivo a diferentes fuentes de hierro en suelos y substratos calcáreos. M.Sc. thesis, Universidad de Córdoba, Spain, 76 p.
- Mínguez- Mosquera M I, Rejano-Navarro L, Gandul-Rojas B, Sánchez-Gómez A H and Garrido-Fernández J 1991 Color-pigment correlation in virgin olive oil. *J. Am. Oil Chem. Soc.* 68, 332–336.
- Morales, F, Grasa R, Abadía A and Abadía J 1998 Iron chlorosis paradox in fruit trees. *J. Plant Nutr.* 21, 815–825.
- Reyes I and Torrent J 1997 Citrate-ascorbate as a highly selective extractant for poorly crystalline iron oxides. *Soil Sci. Soc. Am. J.* 61, 1647–1654.
- Römheld V 1997 The chlorosis paradox: Fe inactivation in leaves as a secondary event in Fe deficiency chlorosis. *In Abstracts, 9th International Symposium on Iron Nutrition and Interactions in Plants*, p 10. Hohenheim, Stuttgart, Germany.
- Schwertmann U and Taylor R M 1989 Iron oxides. *In Minerals in Soils Environments*. Eds. J B Dixon and S B Weed. pp 379–438. Soil Science Society of America, Madison, Wisconsin.
- Schwertmann U 1964 Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. *Z. Pflanzenernähr. Düng. Bodenk.* 105, 194–202.
- Vempati R K and Loeppert R H 1986 Synthetic ferrihydrite as a potential Fe amendment in calcareous soils. *J. Plant Nutr.* 9, 1039–1052.
- Walkley A and Black I A 1934 An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38.
- Yangüas R, del Campillo M C and Torrent J 1997 Predicción de la incidencia de la clorosis férrica en melocotonero cultivado en suelos calcáreos. *Agrochimica* 41, 120–129.