# PREDICTING THE OCCURRENCE OF IRON CHLOROSIS IN GRAPEVINE WITH TESTS BASED ON SOIL IRON FORMS

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## Abstract

**Aims:** Iron deficiency symptoms (leaf chlorosis and depressed growth) are common in grapevine growing on calcareous soils. The objective of this study was to investigate the relationships between these symptoms and the properties of Spanish vineyard soils.

**Methods and results:** Soils from thirty vineyards with leaf chlorosis were used to pot '110 Richter' rootstock for three seasons. Mean leaf chlorophyll concentration, as measured via SPAD, was positively correlated with the soil content in poorly crystalline Fe oxides, as estimated by extraction with various reagents, and negatively correlated with the contents in calcium carbonate equivalent and active lime. Iron deficiency affected plant growth but none of the measured growth variables was correlated with soil properties. The relationships between SPAD and acid ammonium oxalate-, citrate/ascorbate- and unbuffered hydroxylamine-extractable Fe conformed to the linear-plateau model, from which clear-cut critical levels (345, 425 and 8 mg kg$^{-1}$, respectively) could be established. This was not possible with tests based on the alkalinity properties (e.g. the calcite ion activity product), the IPC («Indice du pouvoir chlorosant»), and the DTPA-extractable Fe test, which generally exhibited a poor predictive value.

**Conclusion:** Tests based on reagents capable of extracting, in part or quantitatively, the most reactive forms of soil Fe were useful to estimate the risk of Fe chlorosis in grapevine. Acid ammonium oxalate, citrate/ascorbate and unbuffered hydroxylamine were the best extractants for this purpose.

**Significance and impact of study:** This study has shown the limited usefulness of tests based on the contents and reactivity of the soil carbonate to predict the occurrence of Fe chlorosis in grapevine; tests capable of estimating the contents of the labile soil Fe forms constitute the best alternative.

**Key words:** iron chlorosis, iron oxides, calcareous soils, grapevine

## Résumé

**Objectifs** : Les symptômes de carence en fer (chlorose des feuilles et ralentissement de la croissance) sont fréquentes chez la vigne poussant sur des sols calcaires. L'objectif de cette étude était d'étudier la relation entre ces symptômes et les propriétés des sols viticoles espagnols.

**Méthodes et résultats** : Des sols de trente vignobles avec une chlorose ferrique ont été utilisées pour cultiver le porte-greffe '110 Richter' en pots pendant trois saisons. La concentration moyenne en chlorophylle des feuilles, telle que mesurée par SPAD, a été positivement corrélée avec la teneur du sol en oxydes de fer amorphes, selon les estimations de l'extraction avec des réactifs différents, et négativement corrélée avec les teneurs en carbonate de calcium et en calcaire actif. La carence en fer a affecté la croissance des plantes, mais aucune des variables de croissance mesurées a été en corrélation avec les propriétés du sol. Les relations entre le SPAD et le fer extractible par l'oxalate d'ammonium, le citrate/ascorbate et l'hydroxylamine sans tampon peuvent être établies. Ce n'était pas possible avec les tests basés sur les propriétés liées à l'alcalinité (par exemple le produit ionique de la calcite), le IPC (Indice du pouvoir chlorosant) et le test de fer extractible au DTPA, qui ont généralement montré une faible valeur prédictive.

**Conclusion** : Les tests, basés sur des réactifs capables d'extraire, en partie ou quantitativement, les formes les plus réactives de fer, ont été utiles pour estimer le risque de la chlorose ferrique de la vigne. L'oxalate d'ammonium acide, le citrate/ascorbate et l'hydroxylamine ont été les meilleurs extractifs à cet effet.

**Signification et impact de l'étude** : Cette étude a montré l'utilité limitée des tests basés sur la teneur et la réactivité du carbonate du sol pour prédire l'apparition de la chlorose ferrique de la vigne. Des tests capables d'évaluer la teneur des formes labiles du fer constituent la meilleure alternative.

**Mots clés** : chlorose ferrique, oxydes de fer, sols calcaires, vigne

## INTRODUCTION

Iron (Fe) deficiency symptoms (leaf chlorosis and depressed growth) are common in grapevine growing on calcareous soils (Gruber and Kosergarten, 2002; Díaz *et al.*, 2009). The problem is severe in many parts of the Mediterranean region, especially in Spain, where highly calcareous soils are widespread. It is therefore pertinent to estimate the risk of iron chlorosis in order to predict the performance of a plantation and select the appropriate rootstock. Many soil-testing laboratories use «active lime» (Drouineau, 1942), i.e. the highly reactive fraction of the soil calcite, for predicting this risk. This is implicitly based on the relationship between calcite reactivity and concentration of bicarbonate in the soil solution, which is in turn related to the severity of iron chlorosis (Boxma, 1972; Chaney, 1984; Mengel *et al.*, 1984; Romera *et al.*, 1992; Alcántara *et al.*, 2000). In fact, the active lime test is commonly used for sorting vine rootstocks (Champagnol, 1984). However, the key influence of reactive soil Fe phases on iron chlorosis was recognized almost forty years ago when the «indice de pouvoir chlorosant» (IPC) («chlorosing power index»), defined as the ratio between active lime and neutral $NH_4$-oxalate extractable Fe, was proposed (Juste and Pouget, 1972). In the eighties, the concentration in poorly crystalline Fe oxides was found to be a better predictor for occurrence of iron chlorosis in field crops than active lime or other soil alkalinity-related properties (Loeppert and Hallmark, 1985; Vempati and Loeppert, 1986). More recent studies on woody species such as peach (Yanguas *et al.*, 1997) or olive (Benítez *et al.*, 2002) support this contention. The purpose of this study was to determine which soil properties are related to the incidence of iron chlorosis in grapevine and to establish critical levels for the corresponding tests. To this end, a three-year pot experiment was established with a group of calcareous soils that ranged widely in alkalinity-related properties and extractable Fe forms.

## MATERIALS AND METHODS

### 1. Soil sampling and analysis

Thirty calcareous soils from vineyards located in Spanish wine producing areas with a denomination of origin (DO) (Rioja, Somontano, Penedès, Ribera del Duero, Vinos de Madrid, La Mancha, Montilla-Moriles, Condado de Huelva, and Jerez) were selected on the basis of the occurrence of leaf chlorosis at the time of sampling. Composite soil samples (ca. 100 kg) were collected at the depth of maximum root density (20-70 cm), air-dried, gently ground, and passed through a 1 cm sieve for the pot experiment. Subsamples of 1-1.5 kg were ground to < 2 mm for soil analysis.

Particle size distribution was determined by the pipette method following dispersion with Na hexametaphosphate, organic carbon (OC) by dichromate oxidation, pH (in 1:2.5 soil:water and soil:1 M KCl suspensions) with a glass electrode, and electrical conductivity (EC) in a 1:1 soil:water suspension with a conductivity meter. Cation exchange capacity (CEC) and available K were determined by extraction with 1 M $NH_4OAc$ buffered at pH 7. Available P was determined by extraction with 0.5 M $NaHCO_3$ buffered at pH 8.5 (Olsen *et al.*, 1954). Total $CaCO_3$ equivalent (CCE) was determined by weight loss after treating the soil with 6 M HCl (van Wesemael, 1955) and the active $CaCO_3$ equivalent or « active lime » with neutral $NH_4$-oxalate (Drouineau, 1942). The calcite ionic activity product (pIAP = -log calcite ionic activity) in the 1:1 soil:water extract was calculated using the VMINTEQA2 software (Visual MINTEQ 2.53, 2007) on the basis of the concentrations of the cations and anions in solution. In all solutions, $Ca^{2+}$ and $Mg^{2+}$ were determined by atomic absorption spectrometry, and $Na^+$ and $K^+$ by flame emission. $NO_3^-$ was determined by the flow injection method with photometric detection proposed by Ballesteros *et al.* (1997), which involves the reduction of nitrate to nitrite by using a cupperized Cd column and colorimetric determination of nitrites based on the Griess reaction. $Cl^-$ and $HCO_3^-$ were determined by titration with a CRISON Compact Titrator with 0.05 M AgCl and 0.0025 M $H_2SO_4$, respectively. The concentration of sulphate was estimated by subtracting the sum of the concentrations of these three anions from the sum of the concentrations of the four cations analyzed.

Diethylenetriaminepentacetic acid-extractable Fe ($Fe_{DTPA}$) was determined according to Lindsay and Norvell (1978). Hydroxylamine-extractable Fe ($Fe_{ha}$) was determined by extraction with non-buffered 0.5 M hydroxylamonium chloride at 298 K during 17 h at the 1:20 soil:extractant ratio (de Santiago and Delgado, 2006). Neutral $NH_4$ oxalate-extractable Fe ($Fe_{ox(pH7)}$) was determined in the active lime extracting solution. Acid $NH_4$ oxalate-extractable Fe was determined in two different ways: (a) according to Schwertmann (1964) except that the soil:solution ratio was 1:200 in order to prevent a significant pH increase in the extractant due to presence of carbonate (extractable Fe was designated as $Fe_{ox}$), and (b) according to the procedure of del Campillo and Torrent (1992a), in which the soil is treated with oxalic acid before oxalate extraction to neutralize active lime (extractable Fe was designated as « decalcified » $Fe_{ox}$, $Fe_{ox(d)}$). Citrate/ascorbate-extractable Fe ($Fe_{ca}$) was determined according to Reyes and Torrent (1997). Citrate/bicarbonate and citrate/bicarbonate/dithionite-extractable Fe ($Fe_{cb}$ and $Fe_d$, respectively) were determined according to Mehra and Jackson (1960) except that extraction was carried out at 25 °C for 16 h. All extractions were performed in triplicate at room

temperature (298 K) using 50-cm$^3$ polyethylene centrifuge flasks shaken in an end-over-end shaker at 3 s$^{-1}$. After extraction, the suspensions were centrifuged at 104 m s$^{-2}$ for 15 min and the supernatant analyzed for Fe either by atomic absorption spectrometry (DTPA and hydroxylamine extracts) or the colorimetric o-phenanthroline method (Olson and Ellis, 1982) (for the other extracts).

## 2. Cropping Experiment and Plant Analysis

One-year-old rooted cuttings of *Vitis berlandieri* x *Vitis rupestris*, '110 Richter' grapevine rootstock were planted in May 2003 in pots containing about 12 kg of soil. This rootstock was chosen because it is widely used in Spain and, by virtue of being moderately susceptible to Fe chlorosis (Champagnol, 1984), a variable response to Fe deficiency is usually observed in the field (Reyes *et al*., 2006). A randomized block design was used with two treatments: «control» (soils with no Fe fertilizer added) and «fertilized» [soils treated with Fe (0.5, 1.0 and 1.5 g Fe-EDDHA with 4.8% Fe per pot in 2003, 2004 and 2005, respectively)]. Three replicates per treatment were prepared.

To prevent other nutrient deficiencies, a modified Hoagland nutrient solution without Fe [Ca(NO$_3$)$_2$ (2.5 mM), KNO$_3$ (2.5 mM), MgSO$_4$ (2 mM), KH$_2$PO$_4$ (1 mM), H$_3$BO$_3$ (50 µM), MnSO$_4$ (4 µM), CuSO$_4$ (1µM), ZnSO$_4$ (4 µM) and (NH$_4$)$_6$Mo$_7$O$_{24}$ (0.1 µM)] was applied at a rate 1 dm$^3$ per pot and month from May to October each year. Water was supplied every day by drip irrigation to maintain the soil near field capacity.

Chlorophyll meter readings were obtained with a Minolta SPAD-502 portable meter (Minolta Camera Co., Osaka, Japan). Previous experiments had shown that the concentration of ethanol-extractable chlorophyll per unit surface area determined according to Wintermans and Mots (1965) was highly correlated with the SPAD reading of that chlorophyll meter for the '110R' rootstock leaves ($r = 0.79$; $P < 0.001$).

The average SPAD value and mid rip length of the youngest fully expanded leaf of three randomly chosen shoots in each plant were recorded two times in 2003 and 2005 and three times in 2004. Fifteen leaf petioles from the mid shoot per plant were sampled at the end of each annual growth cycle (fall) for elemental analysis. Petioles were dried at 65° C for 72 h and digested with nitric/perchloric acid. 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 colorimetric method of Murphy and Riley (1962). Flowers collected in the spring 2005 were weighed. Vines were pruned each winter and the pruning wood weight was recorded.

In the spring of 2005, root penetration resistance (R), soil bulk density ($\rho_b$) and FeDTPA were measured. Root penetration resistance on two points in each pot was determined using a recording penetrometer with a cone angle of 30º and 1 cm$^2$ in area at a depth of 25 cm. DTPA-extractable Fe (Fe$_{DTPA}$) was determined in a composite soil sample taken at 25 cm depth. Soil bulk density three days after saturation was calculated from the volume and the weight of the soil in each pot. Correction for water content was made after measuring it with a Thetaprobe sensor (Eijkelkamp, Agrisearch Equipment).

## 3. Statistical analysis

The analysis of variance (ANOVA) and regression were performed with Statistix 8.0 (Analytical Software, Tallahassee, FL, USA). Means were separated using the LSD test. The relationships between the SPAD value and the different Fe forms were fitted to the linear-plateau segmented regression model using the software Curve Expert 1.3 (CurveExpert, 2005). Unless otherwise stated, the word «significant» is used here to indicate significance at the $P < 0.05$ level.

# RESULTS AND DISCUSSION

## 1. Soil properties

The studied soils ranged widely in properties as a result of differences in climate and parent material. The minimum, maximum, mean, standard deviation and median values for selected soil properties are shown in Table 1. Clay content ranged from 124 to 405 g kg$^{-1}$, organic carbon from 2 to 11 g kg$^{-1}$, and CEC from 7 to 32 cmol$_c$ kg$^{-1}$. All samples were calcareous (CCE, 180-763 g kg$^{-1}$; active lime, 36-315 g kg$^{-1}$), and pH in water and KCl ranged from 7.5 to 8.6 and 7.2 to 7.6, respectively.

The equilibrium ion activity product of calcite (pIAP) ranged between 8.08 and 9.01. In ten soil samples pIAP < 8.44 (indicating oversaturation in calcite), and three samples contained gypsum. Oversaturation in calcite can be attributed to the presence of non-crystalline small calcite particles and silicates (Suarez and Rhoades, 1982). The correlation between pIAP and organic carbon was negative ($r = - 0.75$, $P < 0.001$) suggesting that dissolved organic carbon can influence the precipitation of calcium carbonate (Amrheim *et al*., 1993). The pIAP was negatively correlated with ACCE ($r = - 0.36$; $P < 0.05$), consistent with the idea that the later is related to the reactivity of carbonate.

Root penetration resistance ranged from 1.4 to 3.9 MPa. Bulk density (0.9-1.4 kg dm$^{-3}$) and active lime were negatively correlated ($r = - 0.72$, $P < 0.001$), which suggests that active lime influences soil porosity.

**Table 1. Selected soil properties.**

| Property | Minimum | Maximum | Mean | Standard deviation | Median |
|---|---|---|---|---|---|
| Clay (g kg$^{-1}$) | 124 | 405 | 262 | 88 | 266 |
| Organic carbon (g kg$^{-1}$) | 1,6 | 11,1 | 5,8 | 2,4 | 5,8 |
| EC (dS m$^{-1}$) | 0,21 | 3,27 | 0,61 | 0,75 | 0,37 |
| CEC (cmol$_c$ kg$^{-1}$) | 6,8 | 32,2 | 17,1 | 7,6 | 18,0 |
| K (cmol$_c$ kg$^{-1}$) | 0,09 | 1,6 | 0,57 | 0,38 | 0,49 |
| P (mg kg$^{-1}$) | 1,2 | 57,4 | 19,9 | 15,4 | 17,0 |
| **Alkalinity-related properties** | | | | | |
| pH$_{H2O}$ (1:2.5) | 7,5 | 8,6 | 8,2 | 0,3 | 8,2 |
| pH$_{KCl}$ (1:2.5) | 7,2 | 7,6 | 7,4 | 0,1 | 7,4 |
| CCE (g kg$^{-1}$) | 180 | 763 | 538 | 140 | 548 |
| Active lime (g kg$^{-1}$) | 36 | 315 | 199 | 76 | 215 |
| HCO$_3^-$ (meq dm$^{-3}$) | 1,13 | 3,56 | 2,14 | 0,66 | 2,14 |
| pIAP (meq dm$^{-3}$) | 8,08 | 9,01 | 8,55 | 0,23 | 8,44 |
| **Extractable Fe forms**[b] | | | | | |
| Fe$_{DTPA}$ (mg kg$^{-1}$) | 1,4 | 6,2 | 3,3 | 1,2 | 3,5 |
| Fe$_{ha}$ (mg kg$^{-1}$) | 0,1 | 45,4 | 7,0 | 9,6 | 4,0 |
| Fe$_{ox(pH7)}$ (mg kg$^{-1}$) | 4,4 | 48,9 | 14,9 | 11,3 | 11,0 |
| Fe$_{cb}$ (mg kg$^{-1}$) | 17,6 | 90,2 | 53 | 22,7 | 52,2 |
| Fe$_{ox(d)}$ (mg kg$^{-1}$) | 55 | 1319 | 257 | 243 | 207 |
| Fe$_{ox}$ (mg kg$^{-1}$) | 92 | 1255 | 357 | 248 | 325 |
| Fe$_{ca}$ (mg kg$^{-1}$) | 142 | 1250 | 449 | 250 | 406 |
| Fe$_d$ (g kg$^{-1}$) | 0,7 | 12,0 | 3,4 | 2,4 | 2,7 |
| **Properties of the pot soil**[c] | | | | | |
| $\rho_b$ (kg dm$^{-3}$) | 0,9 | 1,4 | 1,2 | 0,2 | 1,2 |
| R (MPa) | 1,4 | 3,9 | 2,4 | 0,6 | 2,3 |
| Fe$_{DTPA}$ (mg kg$^{-1}$) | 2 | 13,6 | 5,5 | 2,3 | 5,4 |

[a]EC, Electrical conductivity in the 1:1 soil water extract; CEC cation exchange capacity; K, available K; P, available P (Olsen P); CCE, calcium carbonate equivalent; pIAP, -log calcite ion activity product.
[b]Subscripts for the Fe forms: DTPA, dietylenetriaminepentacetic acid-extractable; ha, hydroxylamine non-buffered; ox (pH 7), ox (d), and ox, neutral, «decalcified» and acid NH4-oxalate extractable, respectively; cb = citrate/bicarbonate-extractable; ca, citrate/ascorbate; d = citrate/bicarbonate/dithionite-extractable. [c]$_{r_b}$, bulk density; R, root penetration resistance.

The soils also differed widely in the contents of the various Fe forms. The Fe$_{ox}$/Fe$_d$ ratio, which measures the proportion of Fe oxides in poorly crystalline forms (mainly ferrihydrite) was low (< 0.30), as is the case with soils of the Mediterranean region (Torrent *et al.*, 1980). Fe$_{ca}$ ranged between 142 and 1250 mg kg$^{-1}$ and Fe$_{ox}$ between 92 and 1255 mg kg$^{-1}$. There was a significant correlation between Fe$_{ox}$ and Fe$_{ca}$ ($r = 0.70$; $P < 0.001$) because both extractants exhibit selectivity for the poorly crystalline Fe oxides (Reyes and Torrent, 1997). The amounts of Fe extracted by ammonium oxalate according to the two different method used (Fe$_{ox}$ and Fe$_{ox(d)}$) are strongly correlated ($r = 0.91$, $P < 0.001$). Fe$_{ox(d)}$ is on average 70% of Feox, probably because of differences in the dissolution of calcite that was cementing aggregates in which Fe oxides were occluded. Indeed, Fe$_{ox(pH7)}$ represented on average only ~5% of Fe$_{ox}$ because the dissolution of non crystalline iron oxides is greatly affected by the proton and oxalate concentrations (Schwertmann, 1991). The values of Fe$_{cb}$ were on average 3.5 times greater than those of Fe$_{ox(pH7)}$, probably because citrate is a more

effective Fe$^{3+}$ complexing agent than oxalate at neutral or alkaline pH.

DTPA-extractable Fe, which is considered to be an estimate of the most labile Fe forms in calcareous soils (Lindsay and Norvell, 1978), ranged between 1.4 and 6.2 mg kg$^{-1}$. As expected, the correlation between Fe$_{DTPA}$ and the tests that basically quantify the non-crystalline Fe oxides was positive and significant ($r = 0.68$, $P < 0.001$ for Fe$_{ox}$, and $r = 0.70$, $P < 0.001$ for Fe$_{ca}$). Fe$_{ha}$ was of the same order of magnitude as, and strongly correlated ($r = 0.81$, $P < 0.001$) with Fe$_{ox(pH7)}$. This suggests that the unbuffered hydroxylamine and the neutral oxalate solutions extract Fe from the same sources. Fe$_{ha}$ was strongly correlated with Fe$_{ox(d)}$ ($r = 0.90$, $P < 0.001$), Fe$_{ox}$ ($r = 0.74$, P < 0.001) and Fe$_{ca}$ ($r = 0.70$, $P < 0.001$) but only dissolved about 2% of the Fe in poorly crystalline Fe oxides as estimated by the acid oxalate and citrate/ascorbate extractants.

In summary, the amount of Fe extracted by the various reagents used in this study decreased in the order

$Fe_d \gg Fe_{ca} > Fe_{ox} > Fe_{ox(d)} > Fe_{cb} > Fe_{ox(pH\,7)} > Fe_{ha} > Fe_{DTPA}$.

The amounts of Fe extracted by reductants and complexing reagents depend on the pH of the extracting solution (Reyes and Torrent, 1997). This explains the negative correlation that we observed between $Fe_{ha}$, $Fe_{ox}$, and $Fe_{ox\,(d)}$ and the CCE content of the soil (detailed values not shown). The strongest relationships between CCE and extractable Fe forms were observed for $Fe_{ox\,(d)}$ and $Fe_{ha}$. These two tests showed thus to be the most sensitive to the pH buffering capacity of the soil.

## 2. Iron deficiency symptoms in plants

Control plants showed leaf chlorosis three months after transplantation, with SPAD values ranging from 18 to 28 in August and from 15 to 30 in October 2003. The SPAD values of the leaves of control plants were significantly smaller that those of the Fe-fertilized ones not only in 2003 but also in 2004 and 2005 (Table 2). The values of leaf length, and pruning wood and flower weight of control plants were always smaller than those of Fe-fertilized plants (Table 2). No correlation was found between the SPAD value and either leaf length, pruning wood or flower weight. The greatest differences between control and Fe-fertilized plants were found for the first year probably because in the second and third year root and thus plant growth was limited by the limited volume of the pots (~12 dm³).

Mineral element concentrations in petioles were greater in 2003 and 2004 than the critical levels established by Benton Jones *et al.* (1991) (no measurements were made in 2005). Therefore, the small concentration of chlorophyll in the younger leaves and reduced plant growth could be attributed to Fe deficiency.

The Fe concentration in control plants was significantly greater than that in Fe-fertilized plants in 2003, but the opposite was true in 2004. The fact that chlorotic leaves exhibit greater Fe concentration than the non-chlorotic leaves is referred to as the « chlorosis paradox » (Häusling *et al.*, 1985; Bavaresco *et al.*, 1999; Römheld, 2000). The chlorosis paradox can be generally attributed to the reduction of growth associated with Fe chlorosis (Nikolic and Römheld, 2002). For 2003, our results support the occurrence of the chlorosis paradox for petiole rather than leaf blade [we analyzed petiole because of several advantages, e.g. ease of handling and little need of washing (Christensen, 1984)]. However, significant relationships were previously found between the concentrations of Fe in petiole and leaf blade for 'Zalema/161-49C', 'Palomino/140R' and 'Pedro Ximénez/140R' plants

**Table 2. Properties of the control and Fe - EDDHA fertilized plants (n = 90).**

| | 2003 | | 2004 | | 2005 | |
|---|---|---|---|---|---|---|
| | Control | Fe-EDDHA | Control | Fe-EDDHA | Control | Fe-EDDHA |
| **SPAD** | | | | | | |
| Jun | | | 18,5 a | 20,4 b | 19,6 | |
| Aug | 22,7 a | 26,6 b | 19,4 a | 22,1 b | 21,0 a | 23,7 b |
| Oct | 23,9 a | 26,1 b | 17,7 a | 20,7 b | | |
| Mean | 23,3 a | 26,4 b | 18,5 a | 21,1 b | 20,3 | |
| **Leaf length (cm)** | | | | | | |
| Jun | | | 4,1 a | 4,1 a | 3,8 | |
| Aug | 4,1 a | 4,3 a | 3,2 a | 3,5 b | 3,6 a | 3,9 b |
| Oct | 3,5 a | 3,7 b | 3,1 a | 3,3 a | | |
| Mean | 3,8 a | 4,0 b | 3,5 a | 3,7 b | 3,7 | |
| **Pruning wood (g)** | 13,7 a | 16,7 b | 32,7 a | 34,2 a | 25,2 a | 29,4 a |
| **Flower (g)** | | | | | 1,0 a | 1,1 a |
| **Minerals elements** | | | | | | |
| P (g kg⁻¹) | 1,6 a | 1,7 a | 2,8 a | 3,3 a | | |
| K (g kg⁻¹) | 32 b | 27 a | 29 b | 25 a | | |
| Ca (g kg⁻¹) | 29 a | 27 a | 35 a | 40 b | | |
| Mg (g kg⁻¹) | 3,8 a | 3,6 a | 5,3 a | 5,6 a | | |
| Fe (mg kg⁻¹) | 113 b | 94 a | 138 a | 209 b | | |
| Cu (mg kg⁻¹) | 6 a | 16 b | 11 a | 19 b | | |
| Mn (mg kg⁻¹) | 98 b | 50 a | 153 b | 83 a | | |
| Zn (mg kg⁻¹) | 55 b | 49 a | 52 a | 61 b | | |

[a]For each year and row values followed by the same letter are not significantly different at the 0.05 probability level according to the LSD test.

growing in the field (I. Díaz, unpublished results); this suggests that the chlorosis paradox we observed in 2003 for the petiole can be reasonably translated into chlorosis paradox for the leaf blade. In any case, it should be noted that reduction of growth was significant only in 2003 and Fe concentration in chlorotic vines was not correlated with any measured growth parameter.

The concentration of Mn was significantly greater in control than in Fe-fertilized plants. The negative effect of Fe-EDDHA on Mn uptake has been reported (Lucena *et al.*, 1987). However, this effect can probably be discarded because previous pot experiments with '110 Richter' rootstock showed no significant differences in Mn uptake between plants fertilized with Fe-EDDHA and plants fertilized with vivianite (an Fe fertilizer with no complexing reagent) (Díaz *et al.*, 2010). It is possible that the enhanced excretion of protons and increased reducing capacity of the roots in Fe-deficient plants, and the consequent acidification of the rhizosphere, might contribute to increase the concentration of $Mn^{2+}$ in solution and thus its uptake by roots (Venkatraju and Marschner, 1981; Marschner, 1995; Moraghan and Freeman 1978).

Differences in the concentrations of Ca and Zn between control and Fe-fertilized plants were inconsistent in the studied years. The concentrations of P and Mg were not affected by the application of the Fe fertilizer.

### 3. Relationships between iron deficiency symptoms and soil properties

No significant correlations were found for any of the growth variables and soil properties, probably because factors different from the indigenous Fe availability affected growth. Pot volume, bulk density, and root penetration resistance were likely to be influential in this respect.

The SPAD value was significantly ($P < 0.01$) correlated with CCE and the various forms of extractable Fe except for $Fe_{cb}$ in 2003. However, in 2005, the SPAD value was only correlated with active lime and $Fe_{ha}$ (Table 3). The average SPAD value for the 2003-2005 period was significantly correlated with CCE, active lime, $Fe_{DTPA}$, $Fe_{ha}$, $Fe_{ox(d)}$, $Fe_{ox}$, $Fe_{ca}$ and $Fe_d$. The modest significance ($P < 0.05$) of many of these linear relationships can be attributed to the plant response to increasing concentrations of labile Fe in soil. Generally, this response can be described by the linear-plateau model (i.e. one connecting a linear regression with a flat plateau). For several forms of extractable Fe, the proportion of the variance in SPAD accounted for by this model was greater than that accounted for by the linear regression model. Figures 1a, 1b, and 1c present the results of applying this model for $Fe_{ox(d)}$, $Fe_{ha}$, and $Fe_{ca}$, respectively, which are the tests that provided the best results. The good

performance of these tests can be attributed to their ability to dissolve the poorly crystalline Fe oxides -which generally constitute the most labile fraction of the soil Fe- either quantitatively (as is the case with $NH_4$ oxalate and or citrate/ascorbate) or in a proportion controlled by the soil's buffer capacity [as is the case of non-buffered hydroxylamine (de Santiago *et al.*, 2006)]. These results are consistent with those of previous studies on various crops [sorghum (Loeppert and Hallmark, 1985), soybean (Morris *et al.*, 1990), chickpea and sunflower (del Campillo and Torrent, 1992b), olive (Benítez *et al.*, 2002), grapevine (Reyes *et al.*, 2006) and lupin (de Santiago and Delgado, 2006)]. In summary, the phytoavailability of Fe in calcareous appears to be mainly controlled by the poorly crystalline Fe oxides.

The linear-plateau model allows one to establish a critical level above which response to the application of Fe fertilizers is unlikely; this level is given by the abscissa of the intersection point of the two lines. In this study, the critical level for the `110 R´ rootstock was 345 mg $kg^{-1}$ for $Fe_{ox(d)}$, 425 mg $kg^{-1}$ or $Fe_{ca}$, and 8 mg $kg^{-1}$ for $Fe_{ha}$. It is worth noting that the critical level of $Fe_{ox(d)}$ for grapevine was equivalent to that for olive trees (350 mg $kg^{-1}$; Benítez *et al.*, 2002).

FeDTPA was significantly correlated with the SPAD value in 2003 ($r = 0.54$, $P < 0.01$) and with the mean SPAD value ($r = 0.38$, $P < 0.05$). However, the linear-plateau model could not describe the SPAD-$Fe_{DTPA}$ relationship, for which a linear relationship was more useful (Figure 1d). Lindsay and Norvell (1978) proposed DTPA as predictor of the iron chlorosis risk in calcareous soils and 4.5 mg $kg^{-1}$ as the critical level. The data in Figure 1d do not allow one to establish a clear-cut critical level but suggest that 5 mg $kg^{-1}$ could be taken as a conservative critical level.

Our results suggest that the tests based on extraction with oxalate, hydroxylamine, and citrate/ascorbate are useful and slightly better than $Fe_{DTPA}$ as predictors of the risk of Fe chlorosis and in the ability to establish the corresponding critical level. Interestingly, though, the predictive value of $Fe_{DTPA}$ was greater after than before cropping (Table 3), probably because the soil content in available Fe had increased (Figure 2). This increase probably resulted from the complexing effect of root exudates and from the mineralization of organic matter.

From the alkalinity properties, the SPAD in 2003 was negatively correlated with CCE, and the SPAD in 2004, SPAD in 2005 and the mean SPAD were negatively correlated with active lime (Table 3). It should also be noted that the positively correlation between SPAD in 2005 and $\rho_b$ can derive from the negative correlation between $\rho_b$ and active lime ($r = -0.72$, $P < 0.001$). As indicated before, experiments with nutrient solutions

clearly show the negative effect of the bicarbonate ion on iron chlorosis (Boxma, 1972; Chaney, 1984; Mengel *et al.*, 1984; Romera *et al.*, 1992; Alcántara *et al.*, 2000). However, in this study, neither bicarbonate concentration nor pIAP were correlated with the SPAD value or any the growth-related variables.
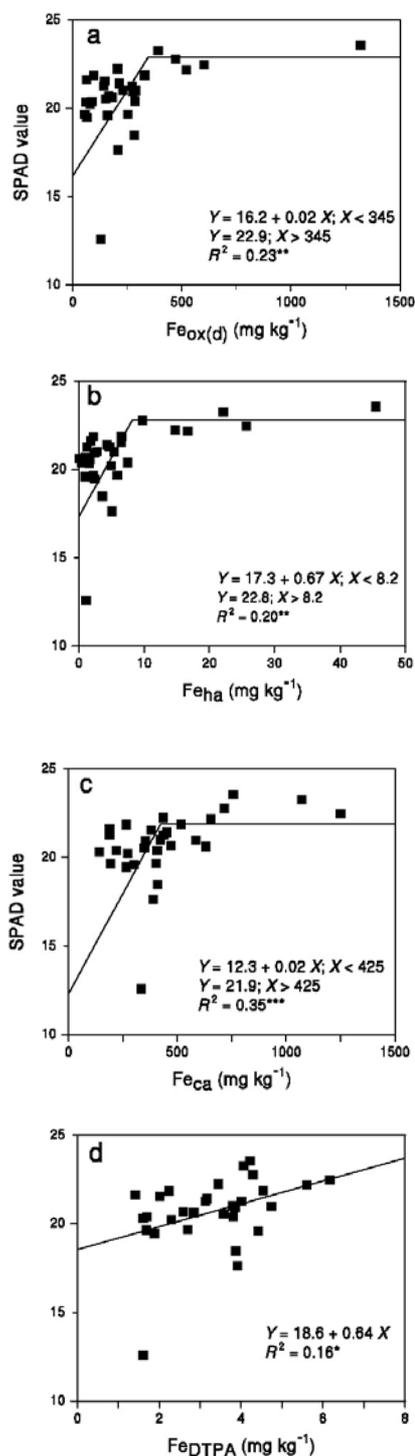
The values of the IPC ranged from 54 to 10950 (mean, 2470 g $g^{-1}$). In contrast, the IPC values calculated by Juste and Pouget (1972) were in the range between 0 and 280 g $g^{-1}$. Neither the SPAD nor the growth-related variables were correlated with the IPC. Therefore, this index does not seem to be useful to predict the occurrence of iron chlorosis in grapevine, consistent with the observations of Morlat and Courbé (1981).

## CONCLUSIONS

The present study reinforces the idea that tests based on extraction of the labile soil Fe forms are more useful than those based on alkalinity properties such as active lime to predict the occurrence of Fe chlorosis in susceptible plants. For the `110 R´ rootstock, the best extractants for this purpose were acid ammonium oxalate, citrate/ascorbate and hydroxylamine, for which the respective critical levels were 345, 425 and 8 mg $kg^{-1}$. A lower predictive value was found for DTPA-extractable Fe; the «Indice du pouvoir chlorosant» (IPC) showed no predictive value for the soils population studied.

**Figure 1 - Relationship between mean SPAD value and Fe$_{ox (d)}$ (a), Fe$_{ha}$ (b), Fe$_{ca}$ (c), and Fe$_{DTPA}$ (d).**

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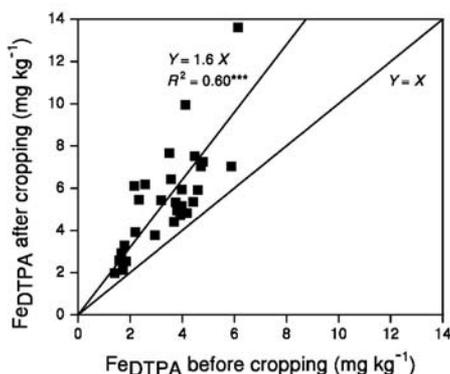
**Figure 2 - Relationship between the amounts of Fe$_{DTPA}$ extracted from the soil sample after and before cropping.**

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**Table 3. Correlation coefficients between SPAD values and soil properties.**

| Property[a] | SPAD | | | |
|---|---|---|---|---|
| | **2003** | **2004** | **2005** | **Mean** |
| **Clay** | 0,03 | -0,10 | -0,17 | -0,09 |
| **Organic carbon** | 0,03 | -0,10 | -0,31 | -0,14 |
| **EC** | 0.49** | 0,10 | 0,25 | 0,33 |
| **CEC** | 0,05 | -0,12 | -0,25 | -0,12 |
| **K** | 0,18 | 0,07 | -0,31 | 0,06 |
| **P** | -0,18 | -0,14 | -0,11 | -0,21 |
| | | | | |
| **Alkalinity-related properties** | | | | |
| **pH$_{H2O}$ (1:2.5)** | -0,35 | 0,04 | 0,04 | -0,11 |
| **pH$_{KCl}$ (1:2.5)** | -0,36* | 0,05 | 0,14 | -0,07 |
| **CCE** | -0,57*** | -0,34 | -0,26 | -0,46** |
| **Active lime** | -0,21 | -0,41* | -0,46** | -0,42* |
| **HCO$_3^-$** | -0,17 | -0,18 | -0,33 | -0,26 |
| **pIAP** | -0,11 | 0,20 | 0,25 | 0,13 |
| | | | | |
| **Extractable Fe forms[b]** | | | | |
| **Fe$_{DTPA}$** | 0.54** | 0,20 | 0,27 | 0.39* |
| **Fe$_{ha}$** | 0.65*** | 0,25 | 0.38* | 0.50** |
| **Fe$_{ox (pH 7)}$** | 0.51** | 0,11 | 0,24 | 0,33 |
| **Fe$_{cb}$** | 0,29 | 0,00 | -0,07 | 0,08 |
| **Fe$_{ox (d)}$** | 0.64*** | 0,20 | 0,28 | 0.44* |
| **Fe$_{ox}$** | 0.59*** | 0,21 | 0,32 | 0.43* |
| **Fe$_{ca}$** | 0.53** | 0,26 | 0,32 | 0.43* |
| **Fe$_d$** | 0.53** | 0,26 | 0,24 | 0.40* |
| | | | | |
| **Properties in the soil of pots[c]** | | | | |
| **ρ$_b$** | 0,08 | 0,32 | 0.49* | 0,34 |
| **R** | 0,09 | 0,21 | 0,17 | 0,18 |
| **Fe$_{DTPA}$** | 0.56** | 0.35* | 0.38* | 0.51** |

[a]EC, Electrical conductivity in the 1:1 soil water extract; CEC cation exchange capacity; K, available K; P, available P (Olsen P); CCE, calcium carbonate equivalent; pIAP, -log calcite ion activity product.
[b]Subscripts for the Fe forms: DTPA, dietylenetriaminepentacetic acid-extractable; ha, hydroxylamine non-buffered; ox (pH 7), ox (d), and ox, neutral, «decalcified» and acid NH4-oxalate extractable, respectively; cb = citrate/bicarbonate-extractable; ca, citrate/ascorbate; d = citrate/bicarbonate/dithionite-extractable. [c]ρ$_b$, bulk density; R, root penetration resistance.

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