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Floral analysis as a tool to diagnose iron chlorosis in orange trees

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Abstract

A three-year field experiment was conducted in a commercial orange grove [*Citrus sinensis* (L.) Osb. cv. 'Valencia late' grafted on Citrange Troyer] established on a calcareous soil in the south of Portugal, to investigate if flower analysis could be used to diagnose lime-induced iron chlorosis. In April, during full bloom, flowers and leaves were collected from 20 trees. Leaf samples were again collected from the same trees in May, June, July and August. Total chlorophyll was estimated in all the leaves sampled for foliar analysis, using a SPAD-502 apparatus. Leaves and flowers were analysed for N, P, K, Ca, Mg, Fe, Zn, Mn and Cu. Principal Component Analysis was used to evaluate the variation of nutrient concentrations in flowers, and linear regressions were established between these and the chlorophyll content of leaves 90 days after full bloom. Evaluation of the best-fit equation was carried out using separate data obtained from other groves. Variation in the pattern of floral mineral composition in the flowers showed contrasts between the increase in N, P and K and that of Ca, Fe and Zn, while the concentration of Mg, Mn and Ca varied synchronously. The ratio of Mg:Zn in flowers explained about half of the variation of chlorophyll in leaves later in the season. A ratio below 100 indicated that trees would develop iron chlorosis, while with a ratio above 200 leaves would remain green. An early prognosis of iron chlorosis based on floral analysis can benefit growers, since it allows them to apply treatments in time to prevent loss of fruit yield and quality due to iron chlorosis.

Introduction

The most prevalent cause of lime-induced iron chlorosis in the Mediterranean area is the bicarbonate ion, which occurs in high levels in calcareous soils. Small annual precipitation (< 500 mm), typical of these regions with arid and semi-arid climates, increases iron chlorosis. It is estimated that from 20 to 50% of fruit trees in the Mediterranean basin suffer from iron chlorosis (Jaegger et al., 2000).

Leaf analysis integrates all the factors that might influence soil nutrient availability 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 [for a review see Abadía et al. (2002)].

Another limitation of leaf analysis is that the sampling date recommended for fruit trees is late in the growing season, generally very close to harvest. At this point it is no longer possible to correct nutritional disorders in time to avoid decreases in yield (Sanz and Montañés, 1995). It is therefore important to develop alternative methods to diagnose iron deficiency in fruit

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trees so that remedial action can be taken before yield is affected.

As a novel approach for the prognosis of iron deficiency in pear trees Sanz et al. (1993) proposed methods based on the mineral composition of flowers. These authors stated that floral analysis could be used to determine the nutritional status of crops at an early stage, since the mineral composition of flowers at full bloom is often related to the nutrient content in leaves taken later in the season. Flower analysis has now been developed for a number of deciduous fruit trees: pear (Sanz et al., 1993, 1994), peach (Belkhodja et al., 1998; Igartua et al., 2000; Sanz et al., 1997b), nectarine (Toselli et al., 2000), apple (Morales et al., 1998; Sanz et al., 1998) and almond (Bouranis et al., 2001). Igartua et al. (2000) used principal component analysis to develop a model for identifying iron chlorosis in peach leaves using the ratio of concentrations of K and Zn in the flowers. The ratio explained 27 to 29% of leaf chlorophyll content.

In this work, we tested the possibility of using the mineral composition of flowers as a tool to predict iron chlorosis in orange trees, which are evergreen. Furthermore, we used the approach of Igartua et al. (2000) to develop and test a statistical model for predicting the chlorophyll content of citrus leaves.

Material and methods

The experimental site was on a commercial grove located in southern Portugal (37° 03′ N, 8° 23′ W; Algarve). When the experiment began, the orange trees [*Citrus sinensis* L. Osb. cv. 'Valencia late' grafted on Citrange troyer (*Poncirus trifoliata* L. Raf. × *Citrus sinensis* L. Osb.)] were 12 years old.

The soil was a calcareous sandy clay loam (Calcaric Fluvisol) with (0–30 cm layer): 2.1% organic matter (Walkley and Black, 1934), 17% total calcium carbonate, 9% active lime (Drouineau, 1942), and a pH in water (1:25) of 7.7. The soil had 166 mg P kg^{-1} (Olsen and Sommers, 1982) and 398 mg K kg^{-1} (Isaac and Kerber, 1971).

The climate of the region is typically Mediterranean. Air temperature (minimum, maximum and mean) and precipitation were recorded every month from January 1996 to March 1999. Total precipitation during year 1 was about 1030 mm whereas during years 2 and 3 only 594 mm and 298 mm were recorded, respectively (Figure 1). Compared with the mean values (514 mm) reported between 1964 and 1980 (INMG, 1991), 1996 was atypical with more rain in January, March and December. In contrast, seasonal variation of temperatures was similar during the three years of the experiment (Figure 1).

Mineral composition of leaves and flowers

Twenty orange trees were randomly selected and labelled in the spring of 1996. The experiment was carried out over three years, from April 1996 to March 1999. Each year, at full bloom (more than 75% of flowers open), 30 flowers and 30 newly expanded leaves were randomly collected per tree, irrespective of the type of the branches, in all canopy orientations. Flowers were taken from the distal part of the branches and the leaves sampled were those located at the second or third position from the tip.

Before nutrient analysis, the leaf chlorophyll concentration was estimated with a SPAD-502 meter (Minolta Co., Osaka, Japan) in all leaves sampled. SPAD values were converted into chlorophyll concentration (μmol m^{-2}) by using the calibration equation:

$$y = 0.15x^2 + 1.49x + 85 \quad (R^2 = 0.94;\ P < 0.0001;\ n = 16), \tag{1}$$

where y is the chlorophyll concentration and x the SPAD value in leaves (Pestana et al., 2001). After the removal of the petiole and main vein, leaves were washed with tap water, followed by distilled water containing a non-ionic detergent, then with 10 mmol HCl dm^{-3} and finally three rinses with distilled water. Flowers, including petals, sepals, reproductive parts, bracts and peduncles were washed with distilled water only. Leaves and flowers were dried at 60 °C for 48 h. Plant material was ground, ashed at 450 °C, and digested in 10 cm^3 HCl 1 mol dm^{-3}. Standardized procedures (A.O.A.C., 1990) were used to measure nutrient concentrations. Nitrogen was analysed by the Kjeldahl method, phosphorus was determined colorimetrically by the molybdo-vanadate method, potassium was measured by flame photometry, and Mg, Ca, Fe, Mn, Cu and Zn were measured by atomic absorption spectrometry.

Statistical analysis

Our experiment had a completely randomised design, and the values obtained for each tree and each parameter were considered independent replications. The number of trees sampled fell from 20 to 18 in the last year because the grower removed two trees. The

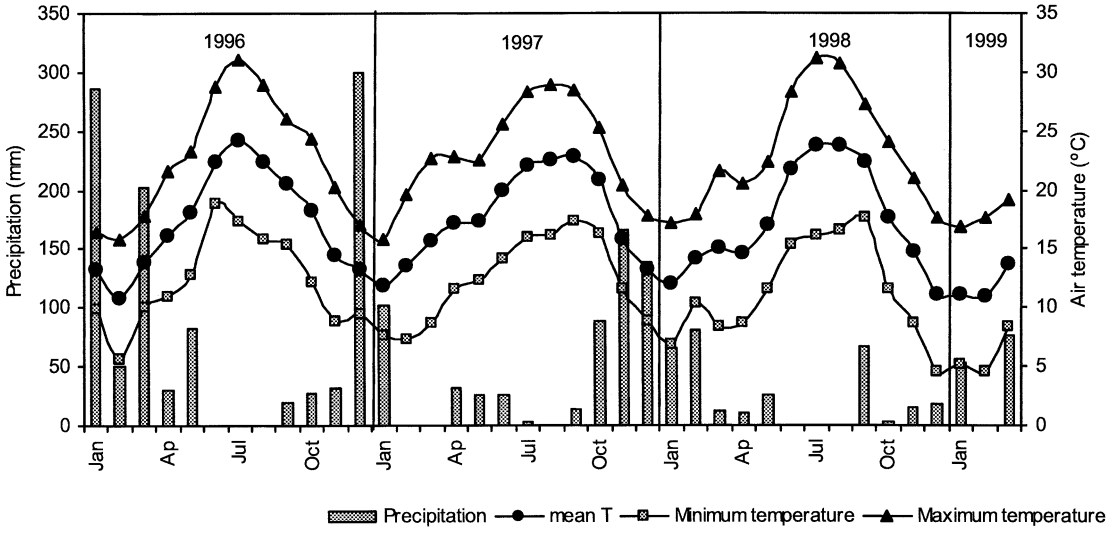

Figure 1. Seasonal variation of monthly air temperatures (mean, maximum, and minimum; °C) and precipitation (mm) registered from January 1996 to March 1999.

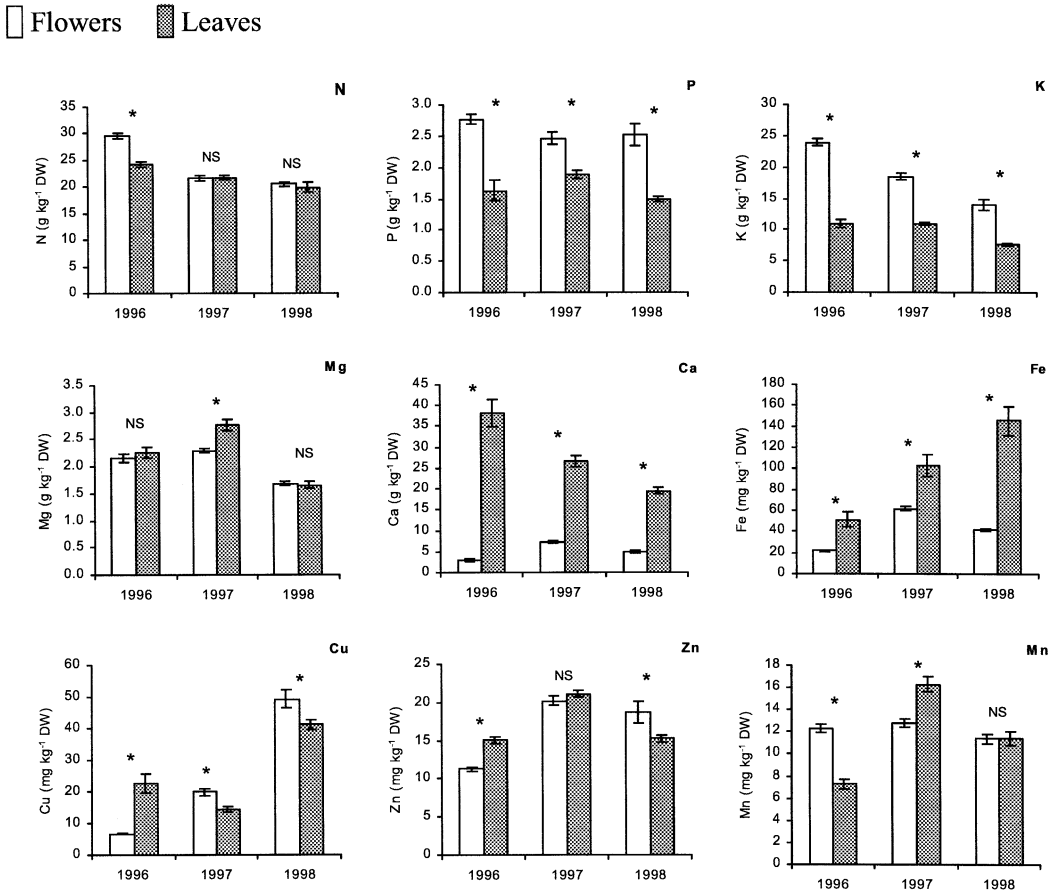

Figure 2. Mean concentration and standard error of several nutrients in orange flowers (light columns) and leaves (dark columns) taken during full bloom in 1996, 1997 and 1998. NS – non significant; * - significantly different at $P > 0.95$, as estimated using the Duncan multiple range test.

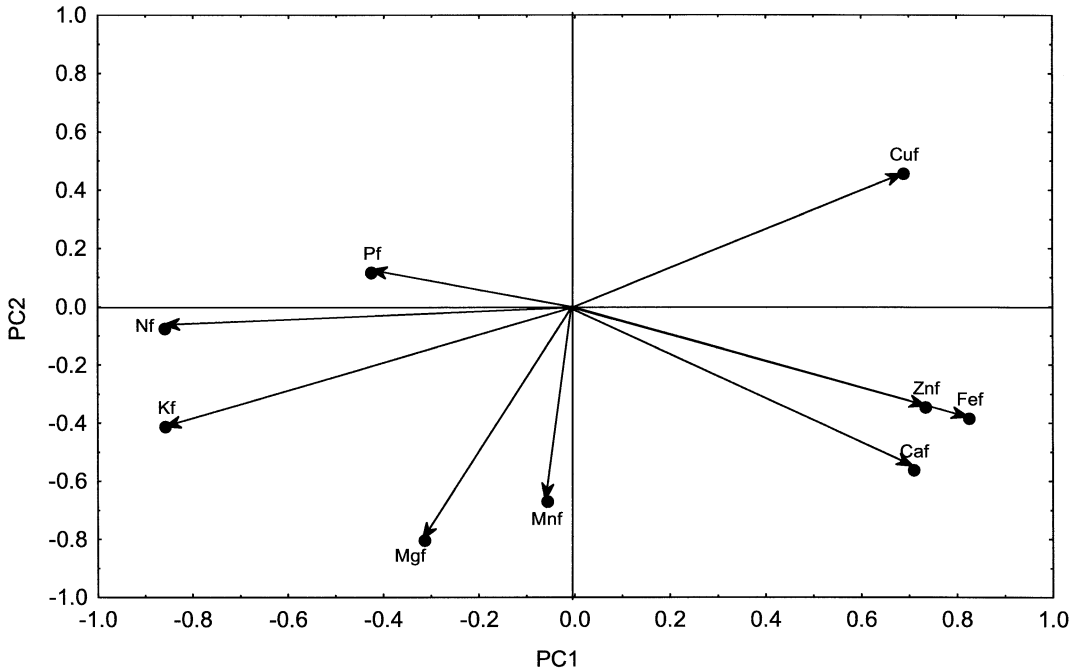

Figure 3. Principal component analysis of nutrient concentrations (N, P, K, Ca, Mg, Fe, Cu, Zn and Mn) in flowers (f) of orange trees. Each vectors represents the loadings of variables (nutrients) in each principal component. Loadings represent the relative contribution of each nutrient to that principal component. PC1 – first principal component; PC2 – second principal component.

means were compared by analysis of variance and by using the Duncan Multiple Range Test at $P \leq 0.05$.

The identification of the main nutritional patterns and the study of the variability of the three-year database were done by Principal Component Analysis. This is an exploratory multivariate statistical method that reduces many variables to a small number of new derived variables. Principal components contain the same information as the original variables but have the advantage of being mutually uncorrelated so that there is no redundant information between them. Using this procedure it is also possible to reveal associations in the data that cannot be found by analysing each variable separately. Each extracted component or factor accounts for part of the variation of all data sets, and is associated to an eigenvalue. The eigenvalue associated with each eigenvector is a measure of the variance within variables of the corresponding principal component. The eigenvectors can be used to calculate new values, called scores, for each observation on each principal component. The scores can be positioned on a plot to identify the cases that contributed more towards the formation of the axis (Legendre and Legendre, 1998). For the interpretation of data, only the components with eigenvalues greater than one were kept, in agreement with the Kaiser criteria (Legendre and Legendre, 1998).

To test if flowers could be used to diagnose iron chlorosis, floral nutrient concentrations registered in full bloom were correlated with chlorophyll concentrations measured later in the season. Several multiple regression models of the form: $Y = b_0 + b_1X_1 + b_2X_2 + \ldots + b_nX_n$ were tested. The inclusion of variables ($X_1 \ldots X_n$) in the models was done by stepwise backward selection to produce regression models without serious multi-collinearity. Such models have already been used to estimate relationships between yield and leaf mineral composition in carob trees (Correia et al., 2002).

To evaluate the reliability of the best-fit equation, 20 trees selected from three different orchards were used. The concentrations of Mg and Zn in flowers and the chlorophyll content of leaves 90 days after full bloom were determined as described previously. The plants analysed were orange trees [*Citrus sinensis* L. Osb. cv. 'Valencia late' and cv. 'Navelina'] and *Citrus clementina* hort. ex hort. Tanaka cv. 'Hernandina'.

Results

The mineral composition of orange flowers at full bloom (April) is shown in Table 1. The mineral composition of flowers was compared with the mineral composition of leaves sampled at the same time (Figure 2). The concentrations of P and K in the leaves were lower than in flowers but the opposite was true for Ca and Fe. Potassium concentration in leaves ranged between 4 to 12 g kg^{-1} dry weight and P concentration ranged between 1.0 to 2.5 g kg^{-1} dry weight. The Ca concentration in leaves was only between three to seven g kg^{-1} dry weight. The iron concentrations in flowers ranged from 22 to 62 mg Fe kg^{-1} dry weight, but in leaves the range was larger, from 51 to 203 mg Fe kg^{-1} dry weight. Flowers and leaves had similar concentrations of N and Mg, except in 1996 for N and 1997 for Mg. There was no recognizable pattern for Cu, Zn, Mn and Zn during this period. The statistical analysis of variations in the nutrient concentrations in flowers for the three years of the experiment produced a dominant first principal component (PC1), which explained 44% of the total variance (Figure 3). The second component (PC2) explained 23% of the variance with further components explaining less than 9%. This analysis indicated that data could be summarized in two dimensions. Increases in N, P and K concentrations were put in contrast to Ca, Fe and Zn along PC1. However, the contribution of P was small. The PC2 reflected the coordinated increases in Mg, Ca and Mn concentrations.

The scores for each tree (1 to 20) along PC1 and PC2 were also analysed (Figure 4). The main patterns identified along the two principal components (PC1 and PC2) were mainly related with differences between years rather than differences between trees. Along PC1 the values obtained were consistent for each year, but in 1996 the values were opposite to those obtained in 1997 and 1998. Consequently, in 1996 the trees had the greatest concentrations of N, P and K in flowers and the smallest concentrations of Ca, Fe, Cu and Mn. In relation to PC2 it is evident that in 1997 the trees presented the highest values of Mg, Ca and Mn and the opposite was observed in 1998. Leaf chlorophyll concentrations were estimated from April to August. The chlorophyll concentrations ranged between 333 and 552 μmol m^{-2} throughout the three years of the experiment, with the lowest values always measured in April. July was the month in which a stronger relationship between chlorophyll and the mineral composition of flowers was found (data not shown).

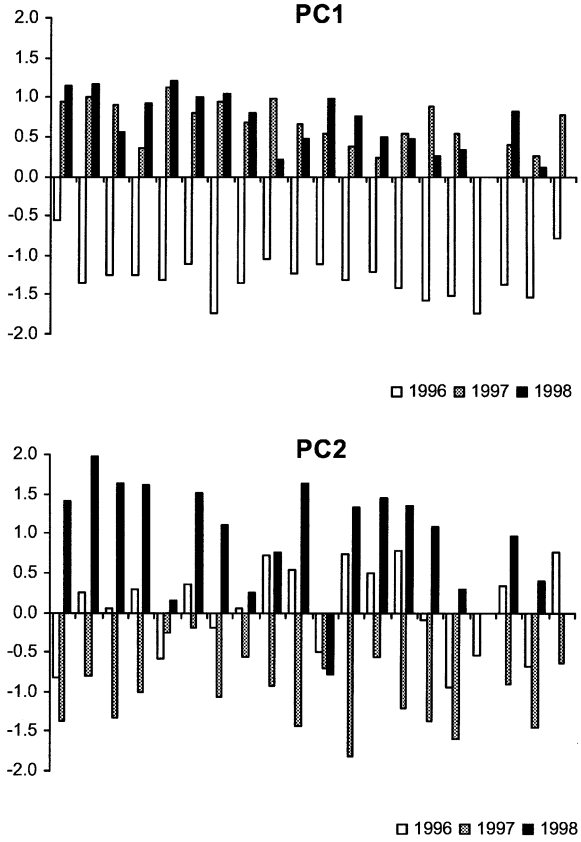

Figure 4. Scores obtained for each tree (1 to 20) along PC1 and PC2, which resulted from Principal Component Analysis of floral mineral composition. Each set of three columns together represent a given tree (1–20 from left to right) for each of three years. PC1 – first principal component; PC2 – second principal component.

The mean chlorophyll concentration in July decreased from years one to three (Table 1). Presumably, this was due to the decreasing precipitation over the three years, and its associated influence on iron bioavailability in the soil.

Several regression models were tested to estimate the chlorophyll concentration in July (90 days after full bloom) based on the mineral composition of flowers. The best model obtained was only dependent on the concentrations of Mg and Zn:

$$y = (442.3 \pm 53.3) + (79.2 \pm 22.9)\,\mathrm{Mg} + (-10.2 \pm 1.8)\,\mathrm{Zn}, \quad (2)$$

where y is the chlorophyll concentration (μmol m^{-2}) in July and Mg and Zn are the concentrations of these nutrients in flowers. Though highly significant ($P < 0.0001$, $n = 55$, $R^2 = 0.44$) the model has a relatively

Table 1. Mean concentration and standard deviation (STD) for several nutrients in orange flowers collected in April at full bloom (FB). Mean chlorophyll concentration and standard deviation in leaves collected at full bloom and at 30, 60, 90 and 120 days after full bloom (DAFB)

Nutrient	Year			
	1996 ($n = 20$) Mean ± STD	1997 ($n = 20$) Mean ± STD	1998 ($n = 18$) Mean ± STD	1996–1998 ($n = 58$) Mean ± STD
N (g kg^{-1} DW)	29.7 ± 2.4	21.6 ± 2.7	20.5 ± 2.1	23.9 ± 4.8
P (g kg^{-1} DW)	2.8 ± 0.3	2.5 ± 0.4	2.6 ± 0.8	2.6 ± 0.5
K (g kg^{-1} DW)	24.0 ± 2.1	18.5 ± 2.1	14.8 ± 4.2	18.8 ± 4.4
Mg (g kg^{-1} DW)	2.2 ± 0.3	2.3 ± 0.2	1.7 ± 0.2	2.0 ± 0.4
Ca (g kg^{-1} DW)	3.0 ± 0.9	7.2 ± 1.1	4.9 ± 1.7	5.0 ± 2.2
Fe (mg kg^{-1} DW)	21.6 ± 3.4	61.8 ± 9.2	41.5 ± 7.6	41.7 ± 18.2
Cu (mg kg^{-1} DW)	6.6 ± 1.0	19.8 ± 4.1	49.3 ± 12.9	25.2 ± 19.4
Zn (mg kg^{-1} DW)	11.3 ± 1.1	20.2 ± 2.7	18.7 ± 6.4	16.7 ± 5.7
Mn (mg kg^{-1} DW)	12.3 ± 1.5	12.8 ± 1.6	11.4 ± 2.0	12.2 ± 1.8
Chlorophyll (μmol m^{-2})				
April (FB)	367 ± 90	333 ± 23	337 ± 17	345.1 ± 7.3
May (30 DAFB)	455 ± 56	382 ± 56	400 ± 30	412.1 ± 7.4
June (60 DAFB)	453 ± 64	419 ± 47	437 ± 39	436.7 ± 6.6
July (90 DAFB)	509 ± 54	422 ± 56	388 ± 49	440.0 ± 9.4
August (120 DAFB)	530 ± 63	480 ± 91	452 ± 60	488.4 ± 10.1

DW – dry weight

small coefficient of determination. Therefore, we also tested models based on nutrient ratios. The best model depended only on the ratio Mg:Zn:

$$y = (283.6 \pm 23.1) + (1.2 \pm 0.2)\ \text{Mg:Zn}, \quad (3)$$

where y is the chlorophyll concentration (μmol m^{-2}) in July and Mg:Zn is the ratio of Mg and Zn concentrations in flowers ($P < 0.00001$, $n = 55$, $R^2 = 0.49$). This equation shows that for an increase of one unit in the Mg:Zn ratio, the chlorophyll content of leaves in July increased 1.2 ± 0.2 μmol m^{-2}.

The leaves of 'Valencia late' show symptoms of iron chlorosis when their chlorophyll content is smaller than 400 μmol m^{-2} (Pestana, 2000). According to equation 3, this corresponds to a ratio of Mg:Zn in flowers below 100. In contrast, trees that have concentrations of Mg and Zn in flowers that result in a ratio Mg:Zn over 200 will have a chlorophyll content greater than 500 μmol m^{-2} in July and should not develop iron chlorosis.

To confirm this hypothesis, flowers and leaves from 20 trees in three different orchards were analysed for Mg:Zn and chlorophyll content. The equation relating the Mg:Zn and chlorophyll 90 days after full bloom for these plants was:

$$y = (281.6 \pm 50.1) + (1.2 \pm 0.3)\ \text{Mg:Zn}, \quad (4)$$

where y is the chlorophyll concentration (μmol m^{-2}) and Mg:Zn is the ratio of Mg and Zn concentrations in flowers ($P < 0.01$, $n = 20$, $R^2 = 0.56$). This equation is not significantly different from equation 3. Furthermore, when the results were compared with the critical values proposed for the Mg:Zn ratio, the prognosis of iron chlorosis was correct in all cases (Table 2). The difference between the calculated and the observed chlorophyll contents varied between −28 and +29%.

Discussion

The nutritional status of fruit trees is traditionally evaluated by foliar analysis. However, the iron concentration in leaves is usually not correlated with the degree of iron chlorosis when trees are grown in calcareous soils, as was the case in the present experiment (data not shown). It would be possible to search for alternative indicators of nutritional status by analysing the results of leaf analysis with the statistical tools available. However, foliar analysis of fruit trees takes place in mid-summer, because this is the period of greatest stability for leaf nutrient concentrations (Spiegel-Roy and Goldschmidt, 1996). At this point, it is too late to prevent losses in yield and fruit quality. It is therefore

Table 2. Ratios of Mg:Zn in flowers of citrus, SPAD readings in leaves later in the season, chlorophyll content calculated and observed, and colour of the leaf expected and observed.

Mg:Zn ratio	SPAD reading	Chlorophyll (μmol m^{-2})			Colour of leaf	
		Value observed	Value calculated using Equation 3	% Differences	Observed	Expected
220	61	734	539	+27	green	green
238	60	714	560	+22	green	green
147	56	637	455	+29	green	?
262	57	663	588	+11	green	green
199	54	603	514	+15	green	green
197	57	659	513	+22	green	green
320	49	512	654	−28	green	green
251	47	479	575	−20	green	green
329	52	561	665	−18	green	green
358	57	661	698	−6	green	green
257	55	614	582	+5	green	green
91	35	317	390	−23	yellow	yellow
92	37	343	391	−14	yellow	yellow
93	34	307	393	−28	yellow	yellow
80	35	325	377	−16	yellow	yellow
78	40	388	375	+4	yellow	yellow
79	36	331	376	−14	yellow	yellow
72	42	415	368	+11	yellow	yellow
74	39	371	370	+0.3	yellow	yellow
75	37	349	371	−6	yellow	yellow

? - unable to predict

more rewarding to develop indicators based on floral analysis.

Data on the mineral composition of orange flowers had not been published previously. Compared with leaves, the concentrations of P and K were greater in flowers, while the opposite was true for Ca and Fe. With the exception of iron, similar results were obtained in apple (Sanz et al., 1998). The concentration of N in flowers was similar to that of other fruit trees, such as peach, apple and pear (Sanz et al., 1997a, 1998; Belkhodja et al., 1998; Igartua et al., 2000; Toselli et al., 2000), but the concentration of Ca was greater and the concentrations of P, K, Mg, Fe, Mn and Zn were smaller in orange flowers than in other fruit trees. These differences may be due to the life cycle of these fruit trees. In deciduous species (peach, pear and apple), flowering occurs before vegetative growth, while in orange full bloom is concomitant with new vegetative growth. Young leaves are thus likely to act as strong sinks for nutrients in citrus, and compete with translocation towards flowers. As a result of these differences, the concentrations or ratios of nutrients that can be used as indicators of nutritional status are likely to vary between species and have to be investigated in each case.

The interpretation of floral analysis requires powerful tools to identify potential indicators of iron chlorosis. To study the variation in nutrient contents, we used Principal Component Analysis. The most consistent pattern was the inverse variation of N, P and K with Ca, Fe and Zn. These results are in part similar to those of Igartua et al. (2000) in peach flowers, where Fe, Ca, P and Zn varied in opposition with K, Mg and Mn.

To use floral analysis as a tool to diagnose iron chorosis, some or all of the changes identified by Principal Component Analysis would have to be related with the degree of iron chlorosis later in the season. The iron content of flowers, unlike the iron concentration in leaves, was negatively correlated with chlorophyll content (data not shown), though it was a poorer indicator than the content of Zn. Zinc may

share with iron the acquisition and translocation mechanisms in plants (Grusak et al., 1999). In agreement with this, Fe and Zn were represented on the same side of the main component PC1 (Figure 3). The variation of Mg in flowers was also correlated with the degree of chlorosis later in the season, and the two nutrients could be included in a model that explained 44% of the variation in the chlorophyll concentration 90 days after full bloom (Equation 2). Magnesium was represented in the second principal component, and therefore contained information that was not redundant with the first principal component where Zn was located. Iron was excluded from this model because its variation in flowers was co-linear with that of Zn. Rather than the use of singular concentrations of nutrients, nutritional balances are frequently used to characterize the nutritional status of plants. Igartua et al. (2000) proposed the use of the K:Zn ratio to predict iron chlorosis in peach. In our work, the model that better predicted iron chlorosis in orange trees was based on the Mg:Zn ratio (Equation 3). This ratio accounted for near 50% of the variation in leaf chlorophyll concentration 90 days after full bloom, and was shown to have general applicability by studying data for three citrus species grown at three different locations in southern Portugal. Based on this model, we propose critical values for the Mg:Zn ratio in flowers of citrus: a ratio below 100 indicates that trees will develop iron chlorosis, while with flowers having a ratio above 200, the leaves will remain fully green.

The use of floral rather than foliar analysis can bring forward the diagnosis of iron chlorosis from July to April. An early prognosis of iron chlorosis can benefit growers, since it allows them to detect and correct any deficiencies before fruit set, thus giving sufficient time for nutrient applications to improve yield and fruit quality.

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