Relationships between nutrient composition of flowers and fruit quality in orange trees grown in calcareous soil

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Summary   To determine if flower nutrient composition can be used to predict fruit quality, a field experiment was conducted over three seasons (1996–1999) in a commercial orange orchard (*Citrus sinensis* (L.) Osbeck cv. 'Valencia Late', budded on Troyer citrange rootstock) established on a calcareous soil in southern Portugal. Flowers were collected from 20 trees during full bloom in April and their nutrient composition determined, and fruits were harvested the following March and their quality evaluated. Patterns of covariation in flower nutrient concentrations and in fruit quality variables were evaluated by principal component analysis. Regression models relating fruit quality variables to flower nutrient composition were developed by stepwise selection procedures. The predictive power of the regression models was evaluated with an independent data set. Nutrient composition of flowers at full bloom could be used to predict the fruit quality variables fresh fruit mass and maturation index in the following year. Magnesium, Ca and Zn concentrations measured in flowers were related to fruit fresh mass estimations and N, P, Mg and Fe concentrations were related to fruit maturation index. We also established reference values for the nutrient composition of flowers based on measurements made in trees that produced large (> 76 mm in diameter) fruit.

Keywords: Citrus, floral analysis, iron chlorosis, models, nutrients, principal component analysis.

Introduction

Iron chlorosis in commercial fruit plants grown on calcareous soils is a problem that still has no simple solution (Démétriadrès et al. 1964, Chandra 1966) because the extent of the correction required is related to yield, fruit size and quality. Tagliavini et al. (2000) summarized the economical impact of iron chlorosis in kiwifruit (*Actinidea deliciosa* L.), peach (*Prunus persica* L.) and pear (*Pyrus communis* (L.) Batsch) orchards established on calcareous soils in Italy, Spain and Greece, and concluded that yield losses were directly related to the severity of iron chlorosis.

El-Kassas (1984) reported that the negative effect of iron chlorosis on gross yield and fruit quality of Balady lime (*Citrus aurantifolia* L.) resulted in smaller fruit that were more acidic and had lower ascorbic acid content. Iron chlorosis may also lead to a delay in fruit ripening in orange (*Citrus sinensis* (L.) Osbeck) and peach (Sanz et al. 1997*b*, Pestana 2000, Pestana et al. 2001*a*, 2002, Álvarez-Fernández et al. 2003). Correction of iron chlorosis with foliar sprays containing Fe resulted in larger oranges, with a gain of more than 35% in the gross income of the grower (Pestana et al. 2001*a*).

It is estimated that 20 to 50% of fruit trees in the Mediterranean basin suffer from iron chlorosis (Jaegger et al. 2000). Calcareous soils are strongly buffered, with a pH between 7.5 and 8.5. This strong buffering capacity combined with low annual precipitation, which is typically < 500 mm year^{-1} in arid and semi-arid Mediterranean climates, enhances iron chlorosis (Loeppert 1986).

Until rootstocks tolerant to iron chlorosis and with other favorable agronomic characteristics become available, the prevention or correction of iron chlorosis is of paramount importance to fruit growers (see Pestana et al. 2003 for a review). Sanz et al. (1993) proposed that floral analysis could be used to determine the nutritional status of crops at an early stage, because the nutrient composition of flowers at full bloom is often related to the concentrations of the same nutrients in leaves taken 120 days later, and they further suggested that the nutrient composition of flowers could be used to predict iron deficiency in pear trees. Floral nutrient analysis has now been developed for several fruit trees including pear (Sanz et al. 1993, 1994, Sanz and Montañés 1995), peach (Sanz and Montañés 1995, Sanz et al. 1997*a*, Belkhodja et al. 1998, Igartua et al. 2000), nectarine (*Prunus persica* L. var. nectarine) (Toselli et al. 2000), apple (*Mallus domestica* L.) (Morales et al. 1998, Sanz et al. 1998), walnut (*Juglans regia* L.) (Drossopoulos et al. 1996), olive (*Olea europea* L.) (Bouranis

et al. 1999), pistachio (*Pistacia lentiscus* L.) (Vemmos 1999), almond (*Prunus amygdalus* L.) (Bouranis et al. 2001), coffee (Martinez et al. 2003) and citrus (Pestana et al. 2001*b*, 2004).

Recently, Pestana et al. (2004) established a model based on the Mg:Zn ratio in flowers of orange trees that can predict the development of iron chlorosis in April. Based on this finding and the approach developed by Sanz et al. (1993), our working hypothesis was that the chemical analysis of flowers can be used to predict fruit quality in the following season, thus allowing a predictive quantitative assessment of fruit variables. To test our hypothesis, we applied principal component analysis to determine the relationships between the nutrient composition of orange flowers and fruit quality. We also established reference values for the nutrient composition of flowers based on the values obtained in trees that produced large fruit.

Materials and methods

The study site was a commercial orchard located in southern Portugal (37°03′ N, 8°23′ W; Algarve). At the start of the experiment, the orange trees (*Citrus sinensis* (L.) Osbeck cv. 'Valencia Late' budded on Troyer citrange rootstock (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck) were 12 years old.

Soil samples were taken at a depth of 30 cm from between rows of trees at the beginning of the experiment (April 1996), oven-dried for 48 h at 30 °C, and then passed through a 2-mm sieve. Soil pH was determined in soil–water suspensions (1:2.5 v/v). Soil potassium (K) and phosphorus (P) were extracted with solutions of ammonium acetate (Riehm 1958) and sodium bicarbonate (Olsen and Sommers 1982), respectively. The P content in extracts was quantified colorimetrically, and K was measured by flame photometry (Isaac and Kerber 1971). Organic carbon was determined by oxidation with dichromate (Walkley and Black 1934). Total carbonates were measured by quantifying the CO_2 produced from the reaction of soil with HCl (Allison and Moodie 1965) and active lime by the Drouineau method (Drouineau 1942). Soil characteristics are presented in Table 1. The soil is characterized as a calcareous, sandy clay–loam (Calcaric Fluvisol).

Table 1. Chemical and physical variables of the soil at the beginning of the experiment (April 1996).

Parameters	Values
Potassium (mg K kg^{-1})	398
Phosphorus (mg P kg^{-1})	166
Organic carbon (%)	1.2
Total lime (%)	17
Active lime (%)	9
pH (H_2O)	7.7
Sand (%)	60
Silt (%)	11
Clay (%)	29
Texture	Sandy clay–loam

The climate of the region is typically Mediterranean. Total precipitation during 1996 was about 1030 mm, whereas during 1997 and 1998, only 594 and 298 mm, was recorded, respectively. Compared with the 20-year-period mean value of 514 mm (INMG 1991), 1996 was atypical, with more rain in January, March and December than normal. In contrast, seasonal variation in temperature was similar during the 3 years of the experiment. Maximum and minimum monthly temperatures during the 3 years were 23 ± 5.5 °C and 12 ± 3.8 °C, respectively.

Nutrient composition of flowers

Twenty orange trees were randomly selected and labeled in spring of 1996. The experiment was conducted over three growing seasons, from April 1996 to March 1999. Each year at full bloom (more than 75% of flowers open), 30 flowers were randomly collected per tree, irrespective of branch type, in all canopy orientations. Flowers were taken from the distal part of the branches.

Flowers, including petals, sepals, reproductive parts, bracts and peduncles, were washed with distilled water and oven-dried at 60 °C for 48 h. Plant material was ground, ashed at 450 °C, and digested in 10 ml of 1 M HCl. Nutrient concentrations were determined according to standard procedures (AOAC 1990). Nitrogen was analyzed by the Kjeldahl method, P was determined colorimetrically by the molybdovanadate method, K was measured by flame photometry, and Mg, Ca, Fe, Mn and Zn were measured by atomic absorption spectrometry.

Fruit quality variables

In March of each year (1997, 1998, 1999), 60 to 190 oranges at the same stage of maturity were collected from around the canopy of the selected trees. The fruit were transported to the laboratory and stored at 4 °C. Fruit fresh mass and diameter were determined for each fruit. The juice of each fruit was extracted with a mechanical juicer and separately analyzed for total soluble solids (expressed as ° Brix), and citric acid concentration (expressed as a percentage of total juice content). All analyses were performed according to standard methods (AOAC 1990). The maturation index of citrus fruit was determined as the ratio between total soluble solids concentration and citric acid concentration.

Statistical analysis

The experiment had a completely randomized design, and the values obtained for each tree and each variable were considered independent replications. The number of trees sampled decreased from 20 to 18 in the last year because the grower removed two trees. All statistical analyses, including principal component and multiple regressions, were carried out using STATISTICA software (Statsoft 1995). Means were compared by analysis of variance (ANOVA) and by using the Duncan Multiple Range Test at $P < 0.05$.

The main patterns of covariation in the parameters that described the nutritional composition of flowers and fruit quality

variables were evaluated by principal component analysis (PCA). This is an exploratory multivariate statistical method that reduces many parameters to a small number of newly derived parameters. Principal components contain the same information as the original parameters, but have the advantage of being mutually uncorrelated so that there is no redundant information between them. This procedure reveals associations in the data that cannot be found by analyzing the parameters separately.

Each extracted component or factor accounts for part of the variation in all data sets and is associated with an eigenvalue. The eigenvalue associated with each eigenvector is a measure of the variance within parameters of the corresponding principal component (Legendre and Legendre 1998). For interpretation of the data, only the components with eigenvalues greater than one were kept, in agreement with the Kaiser criteria (Legendre and Legendre 1998). To obtain a better representation of gradients in flower nutrients associated with fruit quality variables, a varimax (normalized) rotation was applied to the PCA results. This approach simplifies interpretation of the patterns associated with any given parameter of interest, because the rotation maximizes the loading of each parameter in a single factorial axis.

To test if flower characteristics can be used to assess fruit quality in orange trees, fruit variables (fruit fresh mass and maturation index) were related to nutrient concentrations in flowers measured at full bloom in the previous season, based on multiple regression models of the form: $Y = b_0 + b_1X_1 + b_2X_2 + \ldots + b_nX_n$. For each coefficient ($b_1, b_2, \ldots, b_n$), the standard error was also calculated. The incorporation of parameters ($X_1 \ldots X_n$) in the models was done by stepwise backward selection to produce regression models without serious multicolinearity. In each case, the presence of quadratic effects (X_i^2) and interactions (X_iX_j) were tested for the variables included in the best stepwise model, to evaluate whether they could significantly improve its predictive power and interpretability. Similar regression approaches have already been used to estimate relationships between yield and leaf nutrient composition in carob (*Ceratonia siliqua* L.) trees (Correia et al. 2002), and between nutrient concentrations in citrus flowers and chlorophyll content later in the season (Pestana et al. 2004).

To test the regression models obtained, we used independent data from a different orchard. The variables were determined in 14 'Valencia Late' orange trees using the procedures previously described, and a new coefficient of determination (R^2) was calculated based on the independent data. The equation used was:

$$R^2 = \frac{\Sigma(y' - y_{\text{mean}})^2}{\Sigma(y - y_{\text{mean}})^2}$$

where y is the value of each observation, y_{mean} is the mean value of all observations and y' is the value estimated by the original model. When a model is applied to an independent data set, different from that used for the establishment of the original model, the difference between the two R^2 values expresses the loss of predictive power of the model (Glantz and Slinker 1990).

Reference values were estimated by calculating the mean concentration of each nutrient in flowers collected from trees that produced fruit with diameters greater than 76 mm.

Results

During the 3 years (1996–1998) of the experiment, mean N concentration in flowers ranged from 20.5 to 29.7 g kg_{DM}^{-1}. Phosphorus concentration ranged from 2.5 to 2.8 g kg_{DM}^{-1}, and K flower mean concentrations were between 14.8 and 24.0 g kg_{DM}^{-1}. Calcium and Mg concentration ranges were 3.0–7.2 and 1.7–2.3 g kg_{DM}^{-1}, respectively. Micronutrient concentrations were in the ranges of 22.0–62.0 mg kg_{DM}^{-1} for Fe, 11.3–20.2 mg kg_{DM}^{-1} for Zn and 11.4–12.8 mg kg_{DM}^{-1} for Mn.

Fruit fresh mass was higher in 1997 than in 1998, with an intermediate value in 1999 (Table 2). Mean fruit diameter was similar in all years. The concentration of total soluble solids was higher in 1998 than in 1997 and 1999. Citric acid concentration was highest in 1999.

Principal component analysis of flower nutrients (N, P, K, Ca, Mg, Fe, Zn and Mn) in 1996, 1997 and 1998, and quality variables of fruit sampled in 1997, 1998 and 1999, extracted four axes with eigenvalues > 1, representing about 80% of the overall variability in the data. After the rotation of the PC axis with the varimax (normalized) approach, two axes represented almost exclusively the interrelationships between flower nutrients (not shown), whereas the other two axes showed patterns associated with fruit quality variables (Figure 1).

The first axis, which reflects fruit quality, represented variation in fruit size expressed as diameter and fresh mass (PC3). Fruit size was positively associated with increases in P concentration in flowers (and less strongly with increases in concentrations of K, Mg and Zn) and decreases in Ca and Mn concentrations, and to a lesser extent in total soluble solids

Table 2. Mean value and standard deviation (SD) for some fruit quality variables measured in March (1997, 1998 and 1999). In each row, means followed by different letters are significantly different ($P < 0.05$; Duncan test).

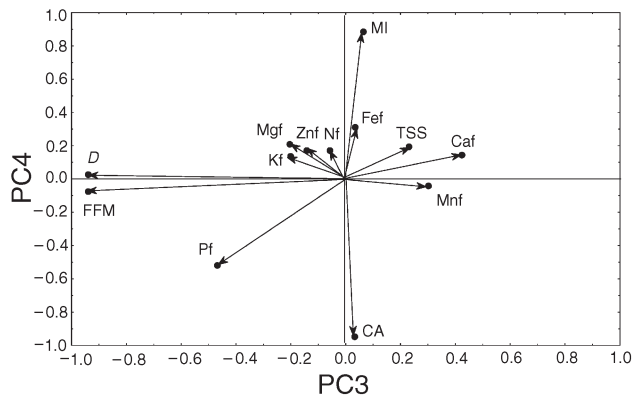| Variable	1997 ($n = 60$)	1998 ($n = 190$)	1999 ($n = 126$)
Fruit fresh mass (g)	208.7 ± 11.6 a	176.2 ± 6.5 b	194.1 ± 10.0 ab
Diameter (mm)	73.2 ± 1.5 a	71.7 ± 1.3 a	72.5 ± 1.2 a
Total soluble solids (° Brix)	10.0 ± 0.2 b	11.5 ± 0.1 a	10.0 ± 0.2 b
Citric acid concentration (%)	1 .1 ± 0.04 b	1.0 ± 0.03 c	1.4 ± 0.02 a

Figure 1. Rotated axis (varimax normalized) of a principal component analysis of nutrients in flowers during 3 consecutive years (1996, 1997 and 1998) and quality variables of fruit sampled the following year (1997, 1998 and 1999). Abbreviations: FFM = fruit fresh mass; D = diameter; TSS = total soluble solids; MI = maturation index; CA = citric acid concentration; Nf, Kf, Pf, Mgf, Caf, Fef, Znf, Mnf = concentration of the respective nutrients in flowers. Vectors represent the parameter (nutrients and fruit quality variables) loadings in each principal component, which are proportional to the relative contribution of each parameter to that component. The axes shown here are those reflecting variation in fruit quality variables (PC3 and PC4).

concentration (Figure 1).

Fruit diameter (D) and fresh mass (y axis) were strongly correlated as shown in Figure 1, ($R^2 = 0.94$; $F_{(1,53)} = 806.2$; $P < 0.0001$) and the relationship can be described by the equation (regression coefficients are given ± SE):

$$y = (7.9 \pm 0.3)D - (374.7 \pm 20.1) \qquad (1)$$

The variables of fruit maturation, such as maturation index and citric acid concentration, were represented in a separate axis (PC4) and were totally unrelated to the fruit size variables. Increases in maturation index were associated with decreases in citric acid concentration. The variations in fruit maturation variables along the PC4 axis were associated with a nutritional gradient in flowers contrasting increases in P concentration with increases in Fe concentration, and less strongly with increases in N, Zn and Mg concentrations (Figure 1).

Several regression models were tested to estimate fruit quality variables based on the nutrient composition of flowers at full bloom in the previous year. In all cases, the best stepwise models included linear effects only, and no significant improvement was obtained by the addition of quadratic or interaction terms. The best model obtained for fruit fresh mass was dependent on the concentrations of Mg, Ca and Zn (regression coefficients are given ± SE):

$$y = (31.6 \pm 13.6)\text{Mg} - (17.6 \pm 2.7)\text{Ca}$$
$$+ (4.7 \pm 1.2)\text{Zn} + (139.6 \pm 31.6) \qquad (2)$$

where y is fruit fresh mass (g), and Mg (g Mg kg_{DM}^{-1}), Ca (g Ca kg_{DM}^{-1}) and Zn (mg Zn kg_{DM}^{-1}) are the concentrations of these nutrients in flowers ($R^2 = 0.46$; $F_{(3,51)} = 14.8$; $P < 0.0001$).

When the concentration of P was incorporated in a regression model instead of Mg concentration, the equation had a marginally lower coefficient of determination ($R^2 = 0.44$; $F_{(3,50)} = 14.7$; $P < 0.0001$).

We also tested the possibility of using the nutrient composition of flowers to estimate the maturation index, because this index is normally used to determine the harvest date of citrus fruit. The best model included the concentrations of N, P, Mg and Fe in flowers ($R^2 = 0.55$; $F_{(4,50)} = 15.1$; $P < 0.0001$) according to the equation (regression coefficients are given ± SE):

$$y = (0.07 \pm 0.01)\text{Fe} + (0.16 \pm 0.05)\text{N} + (1.78 \pm 0.58)\text{Mg}$$
$$- (1.03 \pm 0.43)\text{P} + (1.8 \pm 0.1) \qquad (3)$$

where y is maturation index, and N (g N kg_{DM}^{-1}), P (g P kg_{DM}^{-1}), Mg (g Mg kg_{DM}^{-1}) and Fe (mg Fe kg_{DM}^{-1}) are the concentrations of these nutrients in flowers.

An independent set of 14 'Valencia Late' orange trees from a different orchard was used to evaluate the predictive power of the best-fit equations. Fresh fruit mass ranged between 139 and 203 g and the maturation index ranged from 5.4 to 8.4. The observed mean value for fruit fresh mass was 158 ± 33 g, whereas the corresponding estimated value was 162 ± 24 g. There was a loss of 6% in the predictive power of the model (Table 3) as estimated by comparing R^2 of the model (0.46) with the calculated R^2 when the independent data were used in the model (0.40).

For the maturation index, the observed mean value for the independent data set was 6.3 ± 1.5, whereas the mean estimated by the model was 7.4 ± 1.5. The model explained 55% of the variability of the original data set, but when the independent data set was used, the R^2 dropped to 0.28, indicating a 27% reduction in its predictive power (Table 4).

To establish reference values for the nutrient composition of flowers, we calculated mean values for the nutrient concentrations of flowers from trees that produced fruit with a diameter greater than 76 mm (class of commercial fruit with the highest price) (Table 5). The reference values for the macronutrients (g kg_{DM}^{-1} ± SD) were: 25.2 ± 3.8 N; 2.9 ± 0.4 P; 20.7 ± 5.0 K; 2.1 ± 0.4 Mg; and 3.7 ± 1.5 Ca. The reference values for the micronutrients (mg kg_{DM}^{-1} ± SD) were: 38.3 ± 18.5 Fe; 17.9 ± 5.7 Zn; and 12.1 ± 1.0 Mn.

Discussion

It was possible to estimate fruit quality parameters that are affected by iron chlorosis, namely fruit diameter, fresh mass and maturation index (Sanz et al. 1997b), based on analysis of the nutrient composition of flowers. The fresh mass of fruits was positively correlated with their diameter (cf. Fernández 1995). The variation in the two variables was associated with a nutritional gradient that contrasted increases in P, K, Mg and Zn concentrations with increases in Ca and Mn concentrations in the flowers.

In accordance with the established regression model, the concentrations of Mg, Ca and Zn in the flowers could be used

Table 3. Concentrations of Ca, Mg and Zn in flowers of citrus and the observed and calculated (based on Equation 2) values for fruit fresh mass (SD = standard deviation).

Tree	Nutrient (g kg$^{-1}_{DM}$)			Fruit fresh mass (g)		
	Ca	Mg	Zn	Observed	Calculated	% Difference
1	4.3	1.2	10.5	146.1	151.7	+3.9
2	5.4	1.9	15.5	180.1	176.6	–2.0
3	3.8	1.6	13.5	139.0	187.3	+34.7
4	4.9	1.7	12.5	157.5	164.8	+4.7
5	6.8	1.7	14.0	162.7	139.0	–14.5
6	6.2	1.9	12.5	154.4	148.3	–4.0
7	5.4	1.8	14.0	203.2	168.3	–17.2
8	5.8	1.9	13.5	170.8	160.6	–6.0
9	4.5	1.7	12.5	144.8	173.9	+20.1
10	5.1	1.6	12.5	159.0	159.4	+0.2
11	6.2	1.7	14.5	158.7	153.5	–3.3
12	4.6	1.7	13.0	151.2	173.0	+14.4
13	6.7	1.8	14.5	122.1	146.6	+20.0
14	5.1	1.8	12.0	118.9	162.1	+36.3
Mean	5.3	1.7	13.2	157.7	161.8	+3.9
SD	0.3	0.03	0.3	33	24.1	26.9

Table 5. Values of fruit diameter (*D*) and nutrient composition of flowers used to calculate the reference values of nutrients in flowers (SD = standard deviation).

Tree	*D* (mm)	Nutrient (g kg$^{-1}_{DM}$)					Nutrient (mg kg$^{-1}_{DM}$)		
		N	P	K	Mg	Ca	Fe	Zn	Mn
	1997	*1996*							
1	83.7	30.0	2.3	25.4	2.1	3.1	29.8	26.2	13.2
3	80.2	25.5	2.4	26.3	2.1	1.7	22.7	11.9	12.5
7	77.7	32.7	3.0	25.5	2.3	2.0	19.7	10.8	13.6
15	80.7	27.7	3.0	25.4	2.5	2.5	17.6	10.1	11.8
16	78.6	26.7	3.2	26.4	3.0	3.7	17.3	12.6	12.0
	1998	*1997*							
7	90.5	22.8	2.5	18.1	2.2	6.4	79.5	23.5	12.5
11	78.2	26.4	2.6	17.6	2.1	6.3	52.0	26.5	12.0
	1999	*1998*							
13	76.9	21.3	2.8	14.7	1.6	3.4	42.0	17.5	10.5
14	78.8	20.4	3.4	16.0	1.8	4.0	41.5	17.5	10.5
15	79.8	25.3	3.4	15.8	1.7	3.4	43.0	18.5	12.5
18	80.9	21.4	3.2	14.1	1.7	3.8	56.0	18.5	13.0
19	78.8	22.3	3.4	22.7	2.0	4.4	38.5	21.0	11.5
Mean	80.4	25.2	2.9	20.7	2.1	3.7	38.3	17.9	12.1
SD	3.6	3.8	0.4	5.0	0.4	1.5	18.5	5.7	1.0

to predict fruit fresh mass in the following year. Although the concentrations of Zn and Mg in the flowers were positively related to fruit fresh mass, Ca concentration showed an inverse trend, indicating that high concentrations of Mg and Zn, and low concentrations of Ca in flowers in April were associated with large fruit formed on those trees.

Carranca et al. (1993) obtained positive relationships between the size of 'Valencia Late' orange and the concentration of Mg in the leaves of fruiting branches, which may express a behavior similar to that of flowers. The fact that the present

Table 4. Concentrations of Fe, N, Mg and P in flowers of citrus and observed and calculated (based on Equation 3) maturation index (SD = standard deviation).

Tree	Nutrient (g kg$^{-1}_{DM}$)				Maturation index		
	Fe	N	Mg	P	Observed	Calculated	% Difference
1	50	1.5	1.2	1.7	8.5	6.0	–29.9
2	53	1.6	1.9	2.8	8.7	6.1	–29.3
3	42	1.5	1.6	2.4	9.0	5.4	–40.0
4	52	1.5	1.7	2.8	8.9	5.8	–34.3
5	93	1.7	1.7	3.0	6.8	8.4	+23.0
6	59	1.7	1.9	3.2	7.4	6.2	–16.7
7	60	1.6	1.8	3.2	6.8	6.3	–8.5
8	57	1.4	1.9	3.1	7.1	6.1	–13.7
9	51	1.5	1.7	3.0	6.9	5.6	–18.0
10	57	1.8	1.6	2.2	7.0	6.6	–5.7
11	78	1.5	1.7	2.8	7.8	7.6	–2.4
12	51	1.6	1.7	2.6	6.9	5.9	–14.6
13	50	1.5	1.8	2.3	6.3	6.4	+0.7
14	42	1.3	1.8	2.5	5.9	5.6	–6.3
Mean	57	1.5	1.7	2.7	7.4	6.3	–14.0
SD	14	0.1	0.1	0.4	1.0	0.8	16.3

model includes the concentrations of Mg and Zn in flowers strengthens the importance of these nutrients in citrus grown in calcareous soils. Previously, Pestana et al. (2004) developed a model based on Mg and Zn concentrations in citrus flowers to estimate the concentration of chlorophyll in leaves, 90 days after full bloom.

Crop load has a marked effect on the vegetative growth of *Citrus* (Spiegel-Roy and Goldschmidt 1996). During the "on" year of alternate-bearing cultivars, there is a depletion of nutrient reserves at the expense of leaf endogenous pools (Goldschmidt and Koch 1996). In our study, because of the absence of yield data, it was impossible to quantify the effect of crop load on nutrient retranslocation. There was no clear effect of year on fruit quality (Table 2). Therefore, we assumed that the effect of crop load on flower nutrient retranslocation was small.

The maturation index is used to evaluate the maturation of fruits, which is characterized by variations in the composition and concentration of several juice components (Spiegel-Roy and Goldschmidt 1996). Variation in maturation index was related to a nutritional gradient that contrasted the increase in P concentration with increases in the concentrations of Fe, N, Zn and Mg in the flowers. It was possible to estimate the maturation index of oranges based on the concentrations of Fe, N, Mg and P in the flowers. However, the predictive power of this model (Equation 3) decreased by 27% when tested with independent data, suggesting that the maturation index depends on factors other than the nutritional status of the trees, such as climatic conditions during fruit growth.

Increases in the concentrations of N, Mg and Fe in flowers led to a greater maturation index. The behavior of Fe is in ac-

cordance with the finding that fruit from trees suffering from iron chlorosis mature later than fruit borne on non-chlorotic trees (Pestana et al. 2001*a*, 2002). Iron affects the percentage of citric acid in the juice because of its role in aconitase activity (Sadka et al. 2000). The concentration of P in flowers had an inverse effect compared with the other nutrients. In citrus, P concentration is higher in flowers than in leaves (Pestana et al. 2004) and, based on PCA, it is possible to assume that P dynamics in fruit may be associated with citric acid, which is known to decrease during fruit maturation (Spiegel-Roy and Goldschmidt 1996). Magnesium appears to play an important role in fruit quality, because it is positively related to fresh mass and maturation index (Equations 2 and 3). Magnesium tends to accumulate at a uniform rate in fruit (Shear 1980) and can compete with Ca for uptake and translocation, which may explain both the positive relationship between Mg and fruit fresh mass and the inverse relationship between fruit fresh mass and Ca.

The use of flowers to diagnose iron chlorosis brings forward the evaluation of the nutritional status from October (the recommended date for leaf sampling in *Citrus*) to April (Pestana et al. 2004). However, the establishment of reference values for each species and variety is needed for this purpose. Reference values are normally determined, taking into account the yield of each tree (Sanz and Montañés 1995). Because it was not possible to obtain fruit production from each selected tree in our study, the nutrient concentrations that are proposed as reference values for flowers of orange trees were calculated based on flowers that developed into large fruit (> 76 mm in diameter) of commercial value. We believe that this approach can be used to evaluate the results of floral analysis because it is based on nutritional balances that lead to high quality.

In conclusion, nutrient composition of flowers during full bloom in a particular year may be used to estimate fruit quality variables (i.e., fruit fresh mass and maturation index) in the following year. These results were validated through the interpolation of external and independent data. Based on the proposed models, it is possible to correct the nutritional status of trees before yield is affected. Reference values for floral analysis were established based on trees that produce large fruit (> 76 mm in diameter).

Acknowledgments

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