Research Reports

Fruit Analysis as an Indicator of the Iron Status of Nectarine and Kiwi Plant

Bruno Razeto and Graciela Valdés

ADDITIONAL INDEX WORDS. *Prunus persica* var. *nucipersica*, *Actinidia deliciosa*, iron chlorosis, fruit pulp, total Fe analysis, leaf color, chlorophyll

SUMMARY. Tissue analysis research for the diagnosis of iron (Fe) deficiency was carried out in nectarine (*Prunus persica* var. *nucipersica*) and kiwi (*Actinidia deliciosa*), two species susceptible to Fe deficiency. An experiment was performed during the 2003–04 growing season at Talagante County, Metropolitan Region, Chile, in 8-year-old ‘August Red’ nectarine trees growing on a calcareous soil. Eighty midshoot leaves and 20 fruit per tree were collected from four randomly selected trees each with severe, moderate, or no symptoms of Fe deficiency on 7 Jan., 1 month before fruit harvest. Leaf color and chlorophyll varied among trees according to their severity of chlorosis, as did fruit pulp Fe but not leaf Fe. Fruit pulp Fe was highly correlated with fruit yield ($R^2 = 0.73$), while leaf Fe was unrelated to yield. A second experiment was carried out in the 2004–05 growing season in two ‘Hayward’ kiwi vineyards located in Chile. At each site, 10 midshoot leaves and eight fruit were collected on 15 Mar., 1 month before harvest from three plants either with or without Fe deficiency symptoms. As for nectarine, fruit pulp Fe and leaf color but not leaf Fe were indicators of plant chlorosis. Also, fruit pulp Fe was correlated with leaf color and fruit weight. Consequently, fruit pulp appears a promising tissue, probably better than leaf, for diagnosing the Fe status of nectarine and kiwi plants.

Iron deficiency is a common problem in fruit trees. However the total Fe concentration in the leaves (leaf analysis) usually does not indicate the status of Fe in the plant. Several authors, including Abadía et al. (2000), Hurley et al. (1986), Koseoglu (1995), Palacios (2003), Razeto (1982), Razeto and Rojas (1977), and Ruiz et al. (1980), did not find a relationship between Fe chlorosis and the total Fe concentration in the leaves of various fruit trees. In fact, leaf symptoms are commonly used as an indicator of inadequate Fe in trees (Bould, 1966; Razeto and Uriu, 1971), and many

leaf analysis laboratories do not include Fe determination in their diagnostic work (Beutel et al., 1978; Cook and Wheeler, 1978). However, diagnosing symptoms requires experience and is not always reliable, since Fe deficiency symptoms may be confused with those of other nutritional problems, for instance, manganese deficiency (Razeto,

1993). Such difficulty increases when Fe deficiency develops simultaneously with the deficiency or excess of other nutrients. In addition, when symptoms become visible it is already too late to overcome the negative effects of the Fe deficiency.

Leaf chlorophyll analysis has been reported as a method for diagnosis of Fe status in fruit trees (Lucena, 1997; Peryea and Kammereck, 1997; Ruiz et al., 1980; Yadava, 1986), but several other nutritional problems also cause a reduction in chlorophyll level in the leaf (Porro et al., 2001; Yadava, 1986), which restricts its use as a commercial service tool. Fruit chemical analysis has also been proposed as an indicator of the status of some nutrients in trees, including boron in almond (*Prunus dulcis*) husk (Nyomara and Brown, 1997) and nitrogen in the fruit peduncle of avocado (*Persea americana*) (Razeto and Salgado, 2004). Research on avocado trees (Palacios, 2003; Razeto and Palacios, 2005) has been conducted in an attempt to relate total Fe concentration in leaves, inflorescences, and fruit to Fe chlorosis symptoms for avocado trees affected by different degrees of Fe chlorosis. Avocado is a species particularly susceptible to Fe deficiency. In these studies, dry fruit pulp was the only tissue whose Fe concentration was highly correlated ($R^2 = 0.84$) with Fe chlorosis measured as chlorophyll concentration in the leaves. This suggested that fruit Fe concentration is a promising indicator for diagnosing Fe deficiency in avocado trees.

For these reasons two trials were conducted on nectarine and kiwi (two other Fe chlorosis susceptible species), with the objective of defining whether total Fe analysis of fruit pulp could be used as a tool for diagnosing the Fe status of the tree.

Materials and methods

NECTARINE TRIAL. Eight-year-old ‘August Red’ nectarine trees on NemaGuard (*P. persica* × *P. davidiana*) rootstock located at Talagante County,

Units

To convert U.S. to SI,
multiply by

U.S. unit

SI unit

To convert SI to U.S.,
multiply by

0.3048

ft

m

3.2808

2.54

inch(es)

cm

0.3937

0.4536

lb

kg

2.2046

28.3495

oz

g

0.0353

1

ppm

mg·kg⁻¹

1

(°F – 32) ÷ 1.8

°F

°C

(1.8 × °C) + 32

Faculty of Agricultural Sciences, University of Chile, Casilla (P.O. Box) 1004, Santiago, Chile.

Metropolitan Region of Chile (lat. 33°40'S, long. 70°50'W), were used. The orchard was planted at 7 × 3.5 m and furrow irrigated every 10 d during the growing season. The trees were quite homogeneous in size, but showing different degrees of Fe chlorosis symptoms, ranging from normal to severe, scattered around the orchard. Chlorotic trees were located on strips of calcareous soil erratically distributed throughout the orchard (Petrocalcic Calciaquolls, U.S. classification system, type of soil). Four trees with no symptoms, four with moderate chlorosis, and four with severe chlorosis were randomly selected. Composite soil samples (0–50 cm) were collected from the ground beneath normal (no symptoms) and severely chlorotic trees. Four subsamples were taken around each tree at 1 m from the trunk. Free calcium carbonate was determined by the calcimeter method [carbon dioxide (CO₂) evolution (Allison and Moodie, 1965)], while pH was potentiometrically measured in a 1:2.5 water solution (Peech, 1965).

Representative samples of 80 midshoot new year extension leaves and 20 fruit were collected throughout the canopy of the tree on 7 Jan. 2004 (i.e., early summer, 1 month before fruit harvest). Chlorophyll concentration was measured in the field with a chlorophyll meter (SPAD-CCM-200; Opti-Sciences Inc., Hudson, N.H.) on 15 additional representative leaves per tree on the same day samples were collected. The calibration curve for this method was obtained from 9-mm-diameter disks taken from each leaf sample and chlorophyll was determined using the modified Lichtenthaler and Wellburn (1983) method in a spectrophotometer (UV-1601; Shimadzu Corp., Kyoto, Japan). Color was measured on the same 15 leaves with a chroma meter (CR-300; Minolta Co., Ltd. Osaka, Japan) and was expressed as L*, a*, and b* values.

The leaf samples were washed with distilled and double-distilled water and dried at 70 °C for 36 h. Two peeled slices of pulp (from surface to stone) were longitudinally cut from opposite sides of each fruit, previously washed with distilled water, and dried at 70 °C for 60 h. All samples were ground in an electric steel mill. Total Fe concentration was determined via atomic absorption spectrophotometry, with previous calcination (520 °C for

15 h) and uptake in volume of 1 N hydrochloric acid.

Yield per tree was recorded at harvest (2 Feb. 2004). At harvest, soluble solids concentration was measured with a thermocompensated refractometer (ATC-IE; Atago Co., Ltd., Tokyo) for 12 fruit randomly collected throughout the canopy of each tree.

Trees were selected in a completely randomized design with three levels of chlorosis and four single-tree replications per level. The experimental unit was a whole tree. The results were analyzed by analysis of variance (ANOVA) using Duncan's multiple range test at the 5% level. A simple regression analysis was carried out between pairs of different variables.

In order to confirm that Fe chlorosis was the problem present in the orchard, a soil application of 500 g iron ethylenediamine di(o-hydroxyphenylacetate) (Fe-EDDHA) per tree was performed on 11 Nov. 2003 in three additional severely chlorotic trees, different from those included in the trial. The chelate was applied diluted in water (5%) at both sides of the tree on the closest irrigation furrow.

KIWI TRIAL. This trial was conducted the following year in two 'Hayward' kiwi vineyards growing on high pH soils (pH 8.1 and 8.0, respectively) in Calera and Paine counties, Metropolitan Region of Chile (lat. 33°35'S, long. 70°41'W and lat. 33°46'S, long. 70°20'W, respectively).

Both vineyards, pollenized by 'Matua' and 'Tomuri' males, were 10-years-old, planted at 6 × 4 m and trickle irrigated. They were successfully treated with Fe-EDDHA (fertigation) but the plants used in the trial were located in rows left without chelate application. Three untreated 'Hayward' plants with no symptoms and three with symptoms of Fe chlorosis were randomly selected in each vineyard.

Representative samples of 10

midshoot leaves and eight fruit were randomly taken from each plant on 15 Mar. 2005 (i.e., approximately 1 month before fruit harvest). Color was measured on every leaf by the same method employed in the nectarine trial. Each fruit of the sample was weighed. Total Fe concentration in the leaf and fruit samples was determined as for the nectarine samples.

Fruit production was not recorded in this trial.

A factorial design was used, where factors were the vineyard location and severity of Fe chlorosis. The experimental unit was a whole plant. The results were analyzed by ANOVA and least significant difference test at 1% level. A regression analysis was carried out to relate tissue Fe concentration to leaf color and fruit weight.

Results and discussion

NECTARINE TRIAL. Soil sampled from normal nectarine trees had 1.1% free calcium carbonate, while soil from severely chlorotic trees had 22.1% calcium carbonate, suggesting this high level was the probable cause of Fe chlorosis present in different portions of the orchard. Soil pH was 7.9 and 8.1, respectively. The three additional chlorotic trees treated with Fe-EDDHA application to the soil started to change foliage color 18 d after application, and they became completely green 10 d later, remaining so until fall. The rest of the chlorotic trees continued in their condition, verifying that Fe deficiency was the cause of the chlorosis present in the orchard.

Further, yield was higher in the normal trees (Table 1), confirming the negative effect of Fe chlorosis on fruit production found in peach (*P. persica*) trees (Abadía et al., 2000; Razeto, 1982; Sanz et al., 1997).

Soluble solids concentration in the fruit was higher in the normal trees, intermediate in the moderately chlorotic

Table 1. Leaf and fruit pulp average iron concentration, soluble solids in fruit pulp, and yield of nectarine trees affected by different degrees of iron chlorosis at Talagante, Chile.

Severity of chlorosis	Total iron concn [mg·kg ⁻¹ (ppm)]		Yield (kg/tree) ^z	Soluble solids concn (%)
	Leaf	Fruit pulp		
Severe	127.75 ^y a	10.75 b	50.9 b	12.5 b
Moderate	97.25 b	13.00 ab	66.1 ab	13.6 ab
Absent	95.00 b	17.25 a	90.2 a	14.6 a

^z1 kg = 2.2046 lb.

^yDry weight basis. Different letters in the same column indicate significant differences between chlorosis category means $P \leq 0.05$ (analysis of variance, Duncan's multiple range test).

trees, and lower in the severely chlorotic ones (Table 1). This result agrees with those obtained by Razeto and Rojas (1977) and Sanz et al. (1997).

L*, a*, and b* values of color in the leaf and chlorophyll concentration (SPAD) in the leaf showed differences among the nectarine trees according to their degree of chlorosis (Table 2). These results from nectarine support previous research for avocado showing these three parameters are good indicators of Fe chlorosis. (Palacios, 2003; Razeto and Palacios, 2005). Results are also similar to results obtained by Peryea and Kammereck (1997) for pears (*Pyrus communis*) using the SPAD meter. In addition, there was a high association ($R^2 = 0.92$, Fig. 1) between chlorophyll measured in the field on leaves by SPAD and that measured in the laboratory by the Lichtenthaler and Wellburn (1983) method, which supports the reliability of the SPAD system.

Total Fe concentration in the leaf was higher in the severely chlorotic trees (Table 1). However, this result agrees with previous research (Morales et al., 1998; Palacios, 2003; Römheld, 2000), confirming this parameter as not useful for diagnosing Fe status in the tree (Abadía et al., 2000; Hurley et al., 1986; Koseoglu, 1995).

In contrast, total Fe concentration in fruit tissue increased as chlorosis diminished in the trees, normal trees having a concentration significantly higher than chlorotic trees (Table 1). The concentration in normal trees was 33% and 60% higher than in moderate and severely chlorotic trees, respectively. This result agrees with results from avocado (Palacios, 2003; Razeto and Palacios, 2005), and supports the use of total Fe analysis in fruit pulp as a possible tool for the diagnosis of Fe status of nectarine trees. Iron in fruit pulp was the only factor that showed a significant relationship with fruit yield ($R^2 = 0.73$, Fig. 2). The correlation between leaf chlorophyll and yield was less strong ($R^2 = 0.44$).

KIWI TRIAL. The location of kiwi vineyards did not significantly influence any measured variables. There was a significant difference in leaf color, fruit weight, and fruit Fe concentration between chlorotic and non-chlorotic plants, irrespective of location. Leaf Fe concentration was not different between chlorotic and non-chlorotic plants (Table 3).

Table 2. Color and chlorophyll average concentration in leaf of nectarine trees affected by different degrees of iron chlorosis at Talagante, Chile.

Chlorosis degree	Color			Chlorophyll concn ($\mu\text{g}\cdot\text{cm}^{-2}$)
	L*	a*	b*	
Severe	59.3 a ^z	-10.5 c	47.1 a	28.7 c
Moderate	51.5 b	-14.1 a	35.6 b	49.4 b
Absent	41.7 c	-12.5 b	20.3 c	163.7 a

^zDifferent letters in the same column indicate significant differences between chlorosis category means $P \leq 0.05$ (analysis of variance, Duncan's multiple range test).

Fig. 1. Relationship between SPAD readings and chlorophyll concentration of the leaf for nectarine at Talagante, Chile.

Fig. 2. Relationship between iron (Fe) concentration of the fruit pulp and fruit yield for nectarine trees at Talagante, Chile.

Table 3. Average leaf color, leaf and fruit pulp iron (Fe) concentration, and fruit weight in kiwi plants at Calera and Paine, Chile.

	Leaf color			Fe concn [$\text{mg}\cdot\text{kg}^{-1}$ (ppm)]		Fruit wt (g) ^z
	L*	a*	b*	Leaf	Fruit	
Location						
Calera	42.4 a	-10.9 a	19.4 a	115.2 ^y a	9.7 ^z a	96.6 a
Paine	40.3 a	-10.7 a	18.9 a	124.7 a	10.3 a	88.8 a
	NS	NS	NS	NS	NS	NS
Type of plant						
Chlorotic	49.3 b	-14.3 a	29.0 b	118.8 a	6.3 a	80.2 a
Non-chlorotic	33.4 a	-7.4 b	9.2 a	121.0 a	13.6 b	105.2 b
	**	**	**	NS	**	**

^z1 g = 0.0353 oz.

^yDry weight basis. Different letters in the same column indicate significant differences between factors means $P \leq 0.01$ (analysis of variance, least significant difference test).

The fruit Fe concentration in non-chlorotic plants was more than double the concentration in chlorotic plants. In addition, fruit Fe concentration was significantly correlated with leaf color ($R^2 = 0.75$; 0.84 , and 0.74 with L^* , a^* , and b^* values, respectively) and with fruit weight ($R^2 = 0.59$) (data not shown).

Conclusions

The collective results for nectarine and kiwi suggest total Fe analysis of dry fruit pulp is a promising tool, and probably better than leaf analysis, as an indicator of Fe status of the plant for these two species, and possibly in other horticultural species.

Literature cited

- Abadía, J., M. Tagliavini, R. Grasa, R. Belkhdja, A. Abadía, M. Sanz, E. Araujo, C. Tspouridis, and B. Marangoni. 2000. Using the flower Fe concentration for estimating chlorosis status in fruit tree orchards: A summary report. *J. Plant Nutr.* 23: 2023–2033.
- Allison, L. and C. Moodie. 1965. Carbonate, p. 1379–1396. In: C. Black (ed.). *Methods of soil analysis. Part 2, Chemical and microbiological properties.* Agron. No. 9, Amer. Soc. Agron., Madison, Wis.
- Beutel, J., K. Uriu, and O. Lilleland. 1978. Leaf analysis for California deciduous fruits, p. 11–14. In: H. Reisenauer (ed.). *Soil and plant tissue testing in California.* Bul. 1879, Div. Agr. Sci., Univ. of California, Berkeley.
- Bould, C. 1966. Leaf analysis of deciduous fruits, p. 651–684. In: N. Childers (ed.). *Fruit nutrition.* Horticultural Publ., Rutgers Univ., New Brunswick, N.J.
- Cook, J. and D. Wheeler. 1978. Soil and plant-tissue testing in California, p. 14–16. In: H. Reisenauer (ed.). *Soil and plant tissue testing in California.* Bul. 1879, Div. Agr. Sci., Univ. of California.
- Hurley, A., R. Walser, T. Davis, and D. Barney. 1986. Net photosynthesis, chlorophyll, and foliar iron in apple trees after injection with ferrous sulfate. *HortScience* 21:1029–1031.
- Koseoglu, A. 1995. Effect of iron chlorosis on mineral composition of peach leaves. *J. Plant Nutr.* 18:765–776.
- Lichtenthaler, H. and A. Wellburn. 1983. Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. *Biochem. Soc. Trans.* 603:591–592.
- Lucena, J. 1997. Methods of diagnosis of mineral nutrition of plants: A critical review. *Acta Hort.* 448:179–192.
- Morales, F., R. Grasa, A. Abadía, and J. Abadía. 1998. Iron chlorosis paradox in fruit trees. *J. Plant Nutr.* 21:815–825.
- Nyomara, A. and P. Brown. 1997. Fall foliar-applied boron increased tissue boron concentration and nut set in almond. *J. Amer. Soc. Hort. Sci.* 122:405–410.
- Palacios, J. 2003. Clorosis férrica y su relación con el nivel de clorofila y hierro en diferentes órganos en palto (*Persea americana* Mill.). Memoria Ingeniero Agrónomo. Santiago. Universidad de Chile, Facultad de Ciencias Agronómicas. Santiago, Chile.
- Peech, M. 1965. Hydrogen-ion activity, p. 914–926. In: C. Black (ed.). *Methods of soil analysis. Part 2, Chemical and microbiological properties.* Agron. No. 9, Amer. Soc. Agron., Madison, Wis.
- Peryea, F. and R. Kammereck. 1997. Use of Minolta SPAD-502 chlorophyll meter to quantify the effectiveness of mid-summer trunk injection of iron on chlorotic pear trees. *J. Plant Nutr.* 20:1457–1463.
- Porro, D., C. Dorigatti, M. Stefanini, and A. Ceschini. 2001. Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. *Acta Hort.* 564:243–252.
- Razeto, B. 1982. Treatments for iron chlorosis in peach trees. *J. Plant Nutr.* 5(4–7):917–922.
- Razeto, B. 1993. La nutrición mineral de los frutales, deficiencias y excesos. Sociedad Química y Minera de Chile S.A. (Soquimich), Santiago, Chile.
- Razeto, B. and J. Palacios. 2005. Efecto de la clorosis férrica en el tamaño y la concentración de aceite en el fruto de palto (*Persea americana* Mill.). *Agricultura Técnica* 65:105–111.
- Razeto, B. and S. Rojas. 1977. Tratamientos correctivos de la clorosis férrica en duraznero. *Investigación Agrícola* 3(1):17–22.
- Razeto, B. and J. Salgado. 2004. The inflorescence and fruit peduncle as indicators of nitrogen status of the avocado tree. *HortScience* 39:1173–1174.
- Razeto, B. and K. Uriu. 1971. Estado de la nutrición mineral de frutales de hoja caduca en Chile. *Boletín Técnico* N°47. Central de Divulgación Técnica. Servicio Agrícola y Ganadero, Santiago, Chile.
- Römhild, V. 2000. The chlorosis paradox: Fe inactivation as a secondary event in chlorotic leaves of grapevine. *J. Plant Nutr.* 23:1629–1643.
- Ruiz, R., M. Helle, and R. Espinoza. 1980. Análisis de clorofila como índice indirecto de clorosis férrica en nectarinos. *Agricultura Técnica* 40(4):161–163.
- Sanz, M., J. Pascual, and J. Machín. 1997. Prognosis and correction of iron chlorosis in peach trees: Influence on fruit quality. *J. Plant Nutr.* 20:1567–1572.
- Yadava, U. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. *HortScience* 21:1449–1450.