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Effects of Fe Deficiency Chlorosis on Yield and Fruit Quality in Peach (*Prunus persica* L. Batsch)

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The effects of iron (Fe) deficiency on fruit yield and quality were measured in two peach cultivars, Carson (yellow-skin fruit) and Babygold (red-skin fruit). In both cultivars, Fe deficiency caused major decreases in fruit fresh weight per tree and number of fruits per tree. Fruits from Fe-deficient peach trees had a smaller size, resulting in a large decrease in the percentage of commercially acceptable fruits, whereas fruit firmness was unaffected. In cv. Babygold, Fe deficiency greatly decreased the red color of the fruit skin. Part of these results was likely associated with a delay in fruit ripening. When fruits with similar appearance were compared, taking into account fruit size, color, and firmness, Fe deficiency generally led to higher concentrations of organic anions (especially succinate and quinate), vitamin C, and phenolic compounds and to lower total sugar/total organic acid ratios. This could lead to decreased fruit eating quality and to a slight improvement in fruit nutritional value.

KEYWORDS: Iron chlorosis; fruit quality; yield; peach; *Prunus persica*

INTRODUCTION

Iron deficiency chlorosis is a worldwide problem in crop production on calcareous soils (*1*, *2*). The most important commercial crops affected are fruit crops such as citrus, deciduous fruit trees, and grapevines (*2*). In these woody species Fe chlorosis is a phenomenon more complex than in annual crops (*3*). Many European fruit orchards are located on calcareous and alkaline soils, which favor the occurrence of Fe chlorosis. In the Ebro river basin area, a large agricultural area in northeastern Spain, ~45000 ha of orchards is affected, of which 23400 ha grow peach trees (*4*), which are among the most chlorosis-susceptible fruit tree crops (*3*). In the Mediterranean area alone, treatments to correct Fe chlorosis cost growers approximately $100 million U.S. every year.

Low chlorophyll concentration (chlorosis) in young leaves is a visible symptom of Fe deficiency (*1*, *2*, *5*−*9*). Other symptoms are root, stem, branch, and leaf growth restriction and in some cases a complete inhibition of the formation of new leaves (*2*, *6*, *10*−*13*).

It has been commonly accepted that Fe deficiency induced chlorosis depresses fruit yield and quality. Severe reductions of yield per tree were associated with a decrease in leaf chlorophyll concentration in peach, kiwifruit, and pear trees growing in calcareous soils (*14*). Treatment of chlorosis-affected orchards with Fe generally results in yield increases. This has been shown in peach (*15*−*19*) and in other fruit crops such as citrus (*17*, *20*−*23*), olive (*16*), and kiwifruit (*12*). Most studies on the effects of Fe chlorosis on fruit quality have focused mainly on fruit size, which is increased by Fe fertilization in peach (*17*−*19*), olive (*16*), and citrus trees (*23*). However, other fruit quality parameters, such as chemical composition or color, have been little studied. Fruits from tomato plants grown with a limited supply of Fe contained 30% more ascorbic acid than Fe-sufficient controls (*10*). Iron applications to chlorotic orange trees increased soluble solids and juice volume per fruit, improving fruit color and decreasing titratable acid in juice (*20*). A delay in fruit ripening has been reported to occur with Fe deficiency in tomato (*10*), peach (*18*), and citrus (*24*). The effects of Fe deficiency on the mineral composition of fruits have not yet been reported, whereas the effects of Fe deficiency on the mineral composition of bark (*25*, *26*), wood (*25*, *26*), leaves (*13*, *15*, *27*−*30*), flowers (*29*−*31*), and xylem sap (*32*) have been described in detail.

Our objective was to evaluate the effects of Fe deficiency induced chlorosis on the fruits of two field-grown peach cultivars. Fruit yield and quality, as well as fruit mineral composition, were assessed in Fe-deficient and control trees.

MATERIALS AND METHODS

Plant Materials. Fruits were sampled from two peach (*Prunus persica* L. Batsch) cultivars, Carson and Babygold 7, growing in calcareous soils. The Babygold orchard was located in El Temple, in the province of Huesca, Spain. Babygold trees were 21 years old, grafted on peach seedlings, with a frame of 4 × 5 m, and grown on a calcareous soil (clay-loamy texture with 32% total calcium carbonate, 12.6% active lime, 1.89% organic matter, pH in water of 8.4). The Carson orchard was located in La Almunia, Zaragoza, Spain. Carson trees were 19 years old, grafted on peach seedlings, with a frame of 3 × 4 m, and

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grown on a calcareous soil (23.8% total calcium carbonate, 13.9% active lime, 1.26% organic matter, pH in water of 8.6). Fruit thinning intensity was moderate in Carson trees and practically nil in Babygold. Calcareous soils are known to induce Fe chlorosis in peach and other fruit crops. Both orchards were well-fertilized, except for Fe, and irrigated as needed to prevent other nutrient disorders and water stress. Neither orchard had been treated with Fe fertilizers for 3 years.

Leaf chlorophyll concentration is an accepted tool to monitor Fe status in fruit trees provided other nutrient deficiencies are excluded, given that leaf Fe concentrations cannot be used for this purpose (*3*, *9*, *31*). At harvest time, some trees had marked chlorosis symptoms (average leaf chlorophyll of 155 ± 14 and 98 ± 15 μmol m^{-2} in Carson and Babygold, respectively), whereas other trees did not have chlorosis symptoms (average leaf chlorophyll of 270 ± 9 and 239 ± 10 μmol m^{-2} in Carson and Babygold, respectively). Chlorosis could only be due to Fe deficiency because (a) trees had leaf concentrations of other nutrients in the sufficiency range, (b) leaves had characteristics typical of Fe deficiency, such as increases in K and organic acid concentrations, displacements of the xanthophyll cycle pigments toward zeaxanthin, and increases in non-photochemical quenching and energy dissipation, and (c) chlorotic leaves became green when treated with $FeSO_4$ (data not shown) (see references cited in ref *9*, *13*, and *31*). For both cultivars, Fe-deficient trees showed a marked reduction of vegetative growth, affecting flowering, leaf area, and fruit set. As indicated by the chlorophyll leaf concentrations, iron chlorosis was severe in both cultivars.

Eight trees of each cultivar were used, four with Fe deficiency symptoms and four without symptoms. All fruits present on trees were harvested by hand at commercial maturity dates in the orchards in the study in that year (July 23 and August 1, 2001, for Carson and Babygold, respectively). Yield and number of fruits per tree were measured, and fruits were analyzed within a few hours.

Physical Analysis. Peaches were measured for *L*, *a*, and *b* color values, weight, size, and firmness. These measurements were made in a subsample of 100 peaches per tree (in all of the Babygold trees and the Fe-sufficient Carson trees) or in the whole fruit population (in Fe-deficient Carson trees, where total fruit number was <100). Color was measured on both faces of each fruit using a tristimulus colorimeter (CR200, Minolta Co., Ltd., Osaka, Japan), with an 8 mm aperture (only values for the greenest face are presented). Measurements were made using the *L*, *a*, and *b* color space coordinates (*33*). In this system, *L* represents color brightness, low for dark colors and high for bright colors; *a* is negative for green and positive for red; and *b* is negative for blue and positive for yellow. Individual peach size (maximum and minimum width diameter and length) was measured with a digital CD 15DC Absolute Digimatic Caliper meter (Mitutoyo Ltd.). Firmness was measured on opposite fruit faces using a Durofell pressure tester (Copa-Technologie S.A., Tarascon, France) with a 0.10 cm^2 tip.

Chemical Analysis. Twelve fruits per tree were selected with comparable size (71 ± 5 mm of maximum diameter for both cultivars), color (5 ± 2 and 14 ± 3 *a* values for Carson and Babygold, respectively), and firmness (0.67 ± 0.01 N for both cultivars). Peaches were peeled, and then a portion of the mesocarp was removed from each opposite face and diced into ~1 cm^3 pieces. A composite sample was built by mixing all of the small pieces from 12 peaches selected from each tree. Each composite sample was then divided into three aliquots to permit all chemical analyses. The material needed for each analysis was weighed and immediately frozen in liquid N_2. Soluble solids, pH, and titratable acidity measurements were made at harvest. For the rest of the assays, samples were stored at −80 °C until analysis.

General chemical fruit parameters were measured following official methods (*34*). The pH of the mesocarp juice was measured with a pH meter (Metrohm Ltd., Herisau, Switzerland). Titratable acidity (expressed as percentage of malic acid) was measured with NaOH up to pH 8.1. Soluble solids, reported as degrees Brix, were determined with a digital refractometer (Palette PR-101, Atago Co., Ltd., Tokyo, Japan). Vitamin C was extracted with metaphosphoric acid and determined by the 2,6-dichloroindophenol titrimetric method (*34*).

Total phenolic compounds were made according to the Folin−Ciocalteu method, using gallic acid as a standard (*35*). Ten grams of fresh mesocarp was homogenized with 50 mL of methanol/formic

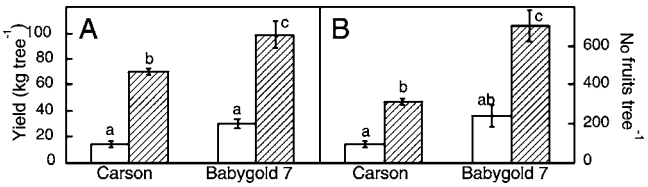

Figure 1. Effects of Fe deficiency on yield (A) and number of fruits per tree (B) in cv. Carson and Babygold peach trees. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE (n = 4).

(1:0.05, v/v) and maintained under gentle shaking for 24 h (Bühler SM orbital shaker, speed 1.5). Then the supernatant was recovered by centrifugation and stored at −20 °C, whereas the pellet was resuspended in fresh extractant and stirred again for 24 h. The process was repeated the third day, and the three supernatant fractions were pooled, evaporated under vacuum, diluted with fresh extractant to a total volume of 100 mL, and analyzed.

For the extraction of organic acids and soluble sugars, 10 g of fresh mesocarp was ground in a blender with 40 mL of 80% methanol. The slurry was centrifuged at 5000 rpm for 5 min, and the resulting supernatant was evaporated under vacuum to remove methanol, brought to a volume of 50 mL with water, and then frozen at −80 °C until analysis. For organic acid analysis, samples were thawed, and a 3 mL aliquot of sample was passed through a C18 Sep-Pak cartridge (Waters Corp., Milford, MA) to remove phenolic compounds and then filtered through a 0.45 μm PVDF filter (Waters Corp.). Organic acids were analyzed by HPLC using a 300 mm × 7.8 mm Organic Acid Analysis column (Bio-Rad, Hercules, CA) containing Aminex HPX-87H, a strong cation-exchange resin (8% cross-linked and 9 μm diameter), which separates organic acids by ion exclusion and partition chromatography. The HPLC Waters system used included a 515 pump, a 996 photodiode array detector, a column oven (Gecko 2000), and Millennium^{32} software. Analyses were performed isocratically at a flow rate of 0.6 mL min^{-1} and a temperature of 65 °C. The mobile phase used was 0.5 mM sulfuric acid. Samples were injected with a Rheodyne injector (20 μL loop). Peaks were identified by comparison of their retention times and UV−vis spectra with those of known standards. Quantification was based on peak area measurements at 210 nm.

For sugar analyses, samples were thawed, and a 1.5 mL aliquot was passed through two ion-exchange cartridges (Acell Plus QMA Sep-Pak and Accell plus CM Sep-Pak, Waters Corp.) and then filtered through a 0.45 μm PVDF filter (Waters Corp.). The HPLC system was that described for organic acids using as detector a differential refractometer (Waters 2410). Sugars were separated using a 300 × 7.8 mm Aminex HPX-87C column (Bio-Rad) maintained at 85 °C. The solvent was MilliQ water at a flow rate of 0.6 mL min^{-1}. The injected volume was 20 μL. Sucrose, glucose, fructose, and sorbitol were identified and quantified by comparison with peaks produced by known standard solutions.

For mineral analyses, lyophilized, milled 2 g mesocarp samples were dry-ashed in a muffle furnace at 550 °C, and the residue was dissolved in HNO_3 and HCl following the AOAC procedure (*34*). Ca (after La addition), Mg, Fe, Mn, Cu, and Zn were determined by atomic absorption, K was determined by emission spectrometry, and P was determined spectrophotometrically. Nitrogen concentrations were measured directly in lyophilized samples using an N analyzer (NA 2100, Thermoquest S.p.A., Milan, Italy).

Statistical Analysis. Data were analyzed by analysis of variance (ANOVA), and means were compared using Duncan's test at $p < 0.05$ to determine the significance of differences found.

RESULTS

Effects of Fe Deficiency on Yield. Iron deficiency caused 79 and 70% decreases in fruit fresh weight per tree in cvs. Carson and Babygold, respectively, when compared to the Fe-sufficient controls (**Figure 1A**). The number of fruits per tree also decreased with Fe deficiency by 71 and 66% in Carson and Babygold, respectively (**Figure 1B**).

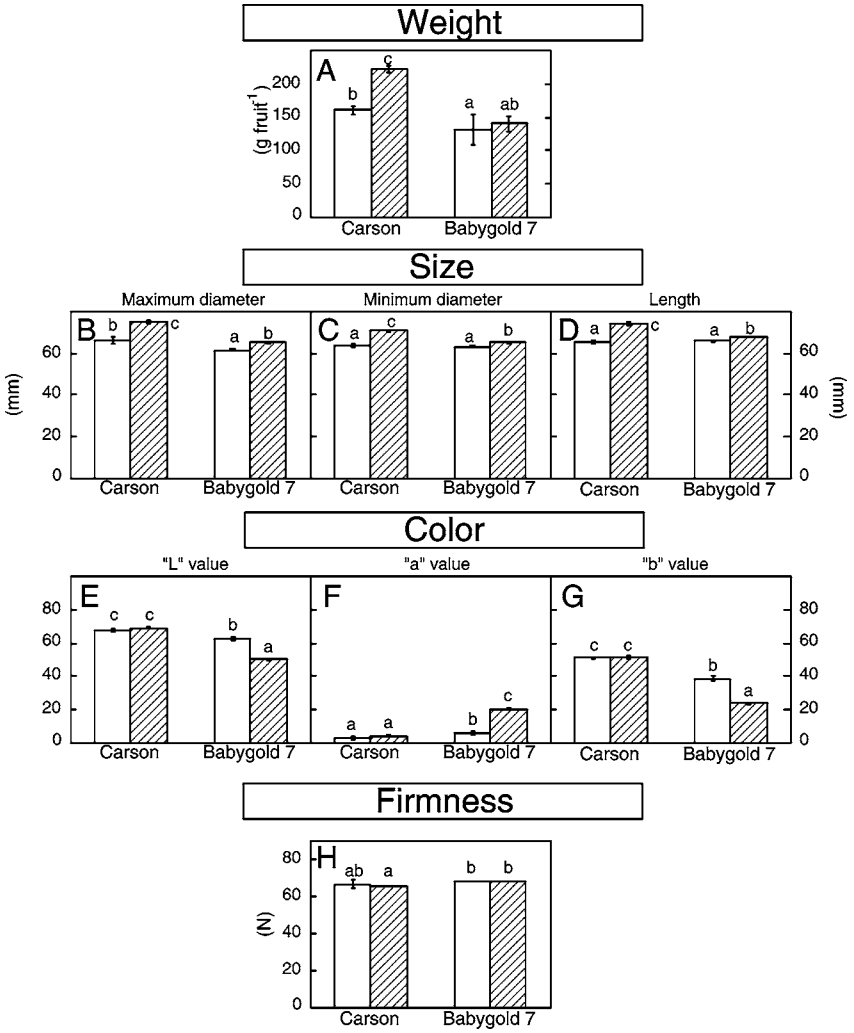

Figure 2. Effects of Fe deficiency on fruit weight (A), size (B–D), color (E–G), and firmness (H) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE (*n* ranged from 337 to 400 depending on the treatment and cultivar).

Effects of Fe Deficiency on Fruit Fresh Weight, Size, Color, and Firmness. The largest changes in fruit physical characteristics caused by Fe deficiency were for individual fruit fresh weight in Carson, for color in Babygold, and for fruit size in both cultivars (**Figure 2A−F**). Both maximum and minimum fruit diameter as well as fruit length decreased significantly with Fe deficiency in both cultivars (**Figure 2B−D**). Fruit size decreases were larger in Carson (between 12 and 9% depending on the parameter) than in Babygold (between 6 and 3%). No fruit color parameter was affected significantly by Fe deficiency in Carson (**Figure 2E−G**), but in Babygold Fe deficiency caused decreases in the *a* color coordinate and increases in the *L* and *b* color coordinates. In neither peach cultivar was fruit firmness affected by Fe deficiency (**Figure 2H**).

The distribution of the fruit population for size (maximum diameter), color (*a* value), and firmness provided more information than mean parameter values (**Figure 3**). Ninety-five percent of the fruits in control Carson trees had a maximum diameter of >67 mm, which is considered as commercially acceptable (**Figure 3A**). In Fe-deficient trees, however, only 47% of the fruits reached that size. In Babygold, which had no fruit thinning, only 43 and 28% of fruits had maximum diameter of >67 mm in control and Fe-deficient trees, respectively (**Figure 3B**). In Carson, a yellow-skin peach cultivar, 54% of fruits in control trees had *a* values between 0 and 5, whereas in Fe-deficient trees only 38% of fruits were in this color range. In Babygold, a red-skin peach cultivar, 33% of the fruits in Fe-deficient trees were green (with negative *a* values) and only 13% of the fruits had a strong red color (*a* values >20; **Figure 3D**). Conversely, in Babygold Fe-sufficient trees did not have any green fruit, whereas 65% of fruits had a strong red color. Iron deficiency decreased the percentage of fruits with a firmness of 60−70 N in Carson from the control value of 53% to 35% (**Figure 3E**). In Babygold, however, Fe deficiency did not change the shape of the fruit firmness distribution for the fruit population (**Figure 3F**).

Effects of Fe Deficiency on Vitamin C and Total Phenolic Concentrations, Soluble Solids, and Acidity in Pulp. The concentrations of vitamin C and total phenolic compounds, soluble solids, and acidity were not significantly affected by Fe deficiency at $p < 0.05$ (**Figure 4**). However, differences between Fe-deficient and control fruits for vitamin C concentrations were significant at $p < 0.10$ in Carson.

Effects of Fe Deficiency on Organic Acids and Sugars in Pulp. Four organic anions (citrate, malate, succinate and quinate) and four sugars (sucrose, glucose, fructose, and sorbitol) were detected in peach fruit pulp. In both cultivars, Fe deficiency generally caused moderate increases in organic anion concentrations, whereas sugar concentrations were unaffected (**Figures 5** and **6**). The concentrations of succinate and quinate were more

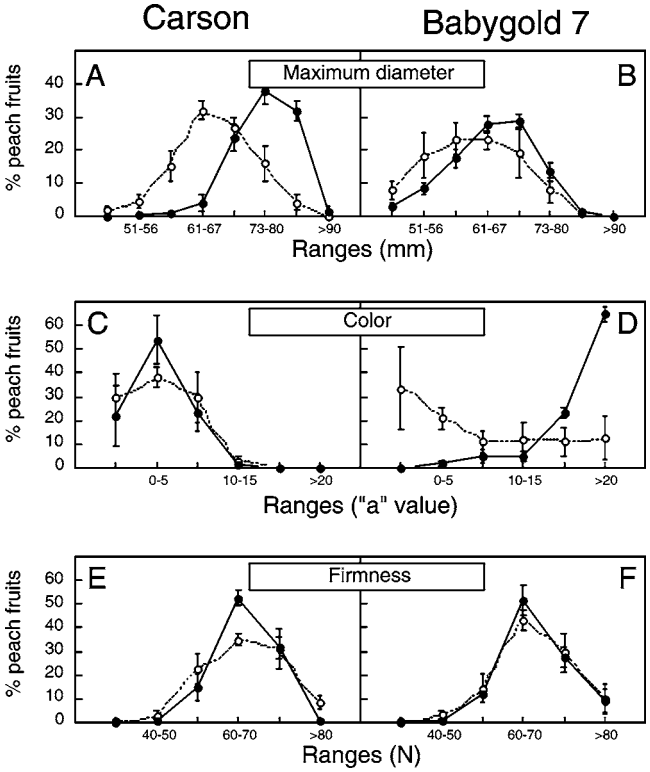

Figure 3. Distribution of fruit population for fruit maximum width diameter (A, B), "*a*" color coordinate (C, D), and firmness (E, F) of cv. Carson and Babygold peaches. Open circles represent Fe-deficient trees, and solid circles represent Fe-sufficient trees. Data are means ± SE ($n = 4$).

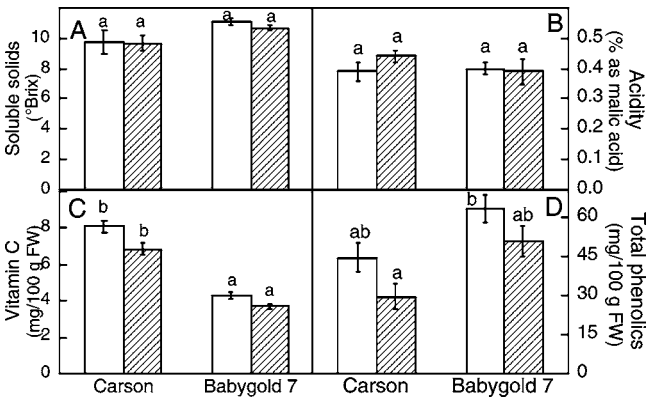

Figure 4. Effects of Fe deficiency on soluble solids (A), acidity (B), vitamin C (C), and total phenolics (D) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE ($n = 12$).

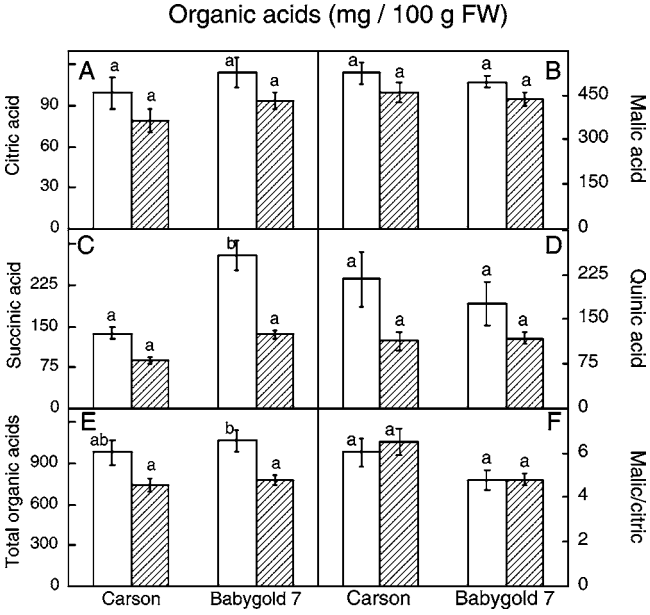

Figure 5. Effects of Fe deficiency on the concentrations of citric acid (A), malic acid (B), succinic acid (C), quinic acid (D), total organic acids (E), and malic/citric ratio (F) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE ($n = 12$).

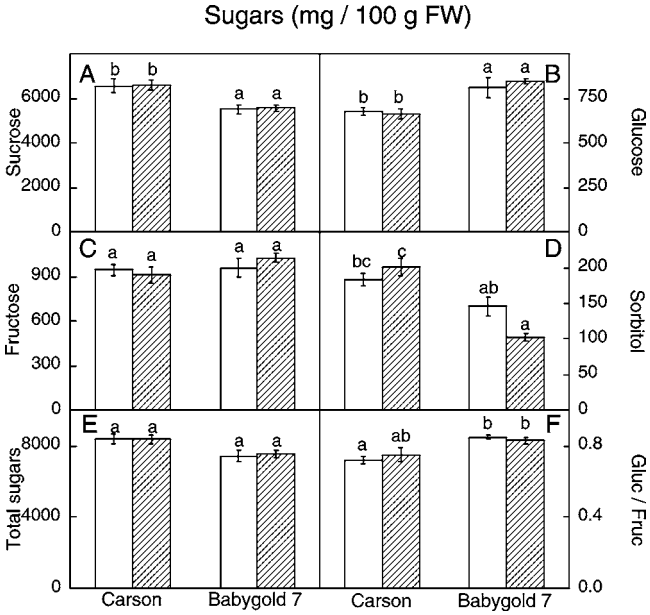

Figure 6. Effects of Fe deficiency on the concentrations of sucrose (A), glucose (B), fructose (C), sorbitol (D), total sugars (E), and glucose/fructose ratio (F) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE ($n = 12$).

affected than those of citrate and malate. Increases brought about by Fe deficiency were 17−20, 11−12, 35−52, and 33−48% for citrate, malate, succinate, and quinate, respectively. However, the only statistically significant organic anion concentration changes at $p < 0.05$ were increases for succinate and total organic acids in Babygold (in both cases, significances were $p < 0.10$ in Carson). The increases in total organic anions were not accompanied by significant changes in the relative organic acid composition of the pulp (not shown). Also, the pulp malate/citrate ratio did not change with Fe deficiency in either cultivar.

Sucrose, glucose, fructose, and sorbitol concentrations (**Figure 6A−D**) as well as total sugar concentration and the glucose/fructose ratio (**Figure 6E**,**F**) did not change with Fe deficiency. The sugar to organic acid (w/w) ratios, calculated from the total organic acid and sugar concentrations, decreased significantly with Fe deficiency, from 12 to 9 in Carson and from 10 to 7 in Babygold (**Figure 7**).

Effects of Fe Deficiency on the Fruit Mineral Composition. The influence of Fe deficiency on the mineral content was minimal (**Figure 8**). The only significant changes with Fe deficiency were increases in pulp Ca and Zn concentrations (27 and 26%, respectively) and decreases in Cu concentrations (34%) in Babygold (**Figure 8C**,**F**,**H**).

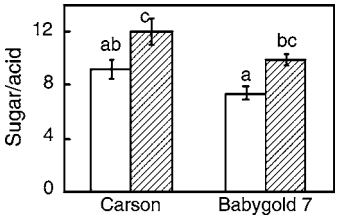

Figure 7. Effect of Fe deficiency on the sugar/organic acid (w/w) ratio (calculated from the total organic acid and sugar concentrations) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE ($n = 12$).

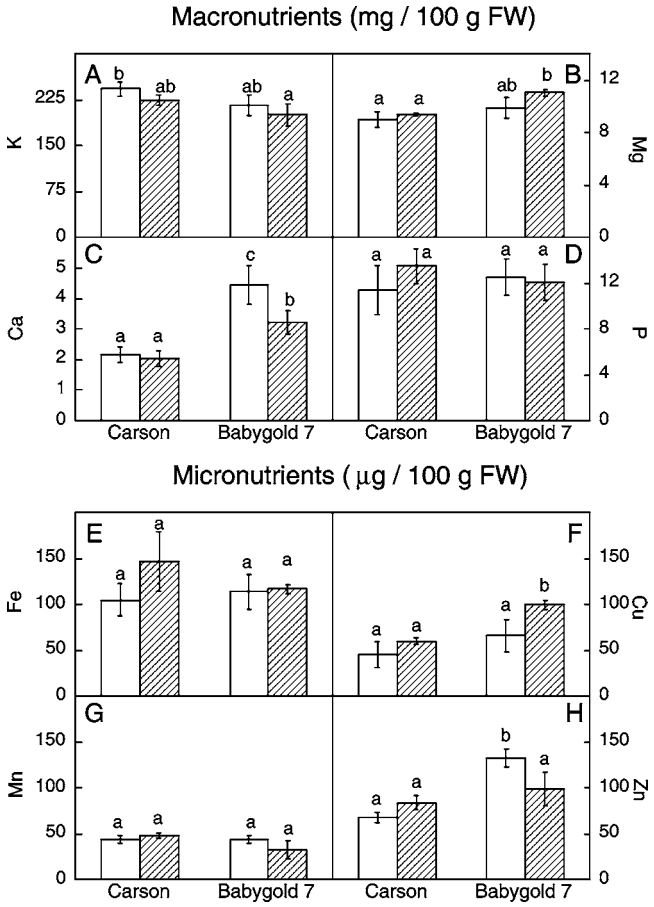

Figure 8. Effects of Fe deficiency on the concentrations of K (A), Mg (B), Ca (C), P (D), Fe (E), Cu (F), Mn (G), and Zn (H) in cv. Carson and Babygold peaches. Open columns represent Fe-deficient trees, and shaded columns represent Fe-sufficient trees. Data are means ± SE ($n = 12$).

DISCUSSION

Iron deficiency chlorosis caused major decreases in fruit fresh weight per tree and number of fruits per tree in both peach cultivars. These data confirm that Fe chlorosis has a large impact on yield in tree fruit crops, as recently reviewed by Tagliavini and Rombolà (*3*) and Rombolà et al. (*36*). In our case, crop yield per hectare would decrease approximately from 50−60 to 12−15 tons.

Iron deficiency also caused deleterious effects in the fruit morphological characteristics in both peach cultivars at the commercial harvest date. In the yellow-skin cultivar Carson (where thinning was used, leading to fewer fruits per tree and larger fruit sizes), marked decreases in average fruit fresh weight and size were found with chlorosis. In this cultivar, Fe chlorosis increased fruit size variability and decreased markedly the percentage of commercially acceptable size fruits from control values of 95 to 47%. The average color of fruits was not affected significantly, although the percentage of fruits with *a* values between 0 and 5 decreased with Fe deficiency. Fruit firmness was unaffected by Fe deficiency in this cultivar.

In the red-skin peach cultivar Babygold (where fruit thinning was not used, leading to a large number of small fruits), Fe deficiency caused small decreases in average fruit size and major decreases in the percentage of fruits with a commercially acceptable size. Iron deficiency also caused major changes in color, including decreases both in the average *a* color coordinate, considered a good nondestructive index of maturity (*37*), and in the percentage of fruits with a strong red color, from control values of 65 to 13%. Most fruits from control trees were red, whereas fruits from Fe-deficient trees had large color variability, from green to red. Fruit weight and firmness were unaffected in this cultivar.

Part of the decreases in fruit quality found at the commercial harvest date are likely to result from a delay in maturity caused by Fe deficiency. Delays in fruit maturity with Fe deficiency have been previously found in tomato (*10*), peach (*18*), and citrus (*24*). Also, the differences in maturity level within the whole tree fruit population (very common in peach trees; *38*) were widened with Fe deficiency. Further experiments would be needed to ascertain the differences in quality between fruit from Fe-sufficient trees taken at commercial harvest time and those of Fe-deficient trees taken later in the season. Some of the deleterious effects caused in fruits by Fe deficiency, such as decreases in fruit number, would not be changed by harvest time. Other effects such as those found in size and color, however, could be reduced to some extent by delaying the harvest date in Fe-deficient trees, although fruits are likely to still be quite different in chlorotic and green trees (see below).

Changes in fruit quality with Fe chlorosis were found when fruits of similar appearance were compared, taking into account fruit size, color, and firmness. Fruits from Fe-deficient trees tended to have higher concentrations of vitamin C and phenolic compounds, especially in Carson. Fruit phenolic compounds affect color and flavor, whereas vitamin C content contributes to fruit nutritive value. Delays in maturity caused by Fe deficiency could explain the high phenolic compound concentrations found in peach fruits from Fe-deficient trees when compared to controls. High phenolic compound concentrations could cause an increase in astringency, therefore decreasing tasting quality (*39*, *40*). Also, Fe deficiency caused generally moderate increases in organic anion concentrations (especially succinate and quinate) in both cultivars, whereas sugar concentrations did not change. As a result, the total sugar/total organic acid ratio, a commonly used fruit quality index, decreased significantly with Fe deficiency. Increases in total organic acidity (sum of all organic acids present, free or combined with cations) with Fe deficiency were not accompanied by increases in titratable acidity and pH (data not shown). Therefore, the ratio H^+/titratable acidity, often used as an index of maturity, was not affected by Fe deficiency. Increases in total organic acids may be one of the causes of the increased vitamin C concentration, through the increased capacity to chelate metal ions (*41*). The malate/citrate ratio in fruit pulp did not change in either cultivar with Fe deficiency, conversely to what happens in other tree parts such as leaves, roots, xylem sap, and leaf apoplastic fluid (*42*).

In summary, Fe deficiency chlorosis decreased markedly not only fruit yield but also fruit quality in two cultivars of peach. Part of these results was likely associated with a delay in fruit ripeness. Furthermore, the chemical characteristics of fruits with similar appearance were different in Fe-deficient and Fe-sufficient trees. Fruits from chlorotic trees tended to have more organic acids, phenolic compounds, and ascorbate than fruits from control trees, whereas sugar levels and mineral contents were unaffected. These changes in chemical composition of fruits are likely to decrease to some extent their eating quality but to improve slightly their nutritional value.

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