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Pigment Accumulation and Micronutrient Concentration of Iron-deficient Chile Peppers in Hydroponics

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Abstract. **Pigment and micronutrient concentrations of New Mexico 6-4 and NuMex R Naky chile pepper (*Capsicum annuum* L.) cultivars as affected by low Fe levels were studied under soilless culture. A custom-designed, balanced nutrient solution (total concentration <2 mM) was continuously recirculated to the plants potted in acid- washed sand (pot volume 15.6 L). Each set of plants from each cultivar received iron concentrations at 1, 3, 10, and 30 μM Fe as Fe-EDDHA. The pigments of leaves, green fruit, and red fruit were extracted with acetone and measured with a spectrophotometer. Surface color of green and red fruit was measured with a chromameter. Total concentrations of Fe, Cu, Zn, Mn, P, and K of leaf blades and red fruit were measured by inductively coupled plasma emission spectroscopy (ICP). Ferrous iron in leaf blades, and NO_3-N in petioles also were determined. Iron nutrition level affected total leaf chlorophyll and carotenoid content at early season, and the level of these pigments in green fruit at second harvest. No differences in extractable or surface color of red fruit were found among iron treatments in the nutrient solution, despite variations in red fruit iron content, total foliar iron, and foliar ferrous iron. Higher levels of iron in the nutrient solution increased both ferrous and total iron of the leaves, but depressed foliar Cu and P. High iron supply also increased fruit iron, and decreased fruit Cu content. High iron levels in the nutrient solution were associated with higher concentrations of leaf pigments at early season and higher pigment concentration in green fruit.**

Iron deficiency decreases chlorophyll and carotenoid accumulation in leaves (Abadía and Abadía, 1993). This plant disorder affects the total and relative concentration of photosynthetic pigments, namely chlorophylls and carotenoids. Carotenoids are responsible for the yellow color of leaves and are less affected than chlorophylls by iron deficiency, thus leaves lacking iron have a typical yellowish-green, chlorotic appearance along the interveinal region (Abadía et al., 1991; Abadía and Abadía, 1993).

Chlorophyll and carotenoid concentrations correlate strongly with color, quality, and nutritional parameters of horticultural commodities, such as green and red peppers. The green color of chile pepper (*Capsicum annuum* L.) fruit is due to high levels of chlorophylls, while red color is a result of carotenoids (Camara and Brangeon, 1981; Davies et al., 1970).

Some carotenoids used as natural colorings are found almost exclusively in pepper fruit (Almela and López-Roca, 1990). Fruit production is significant economically for New Mexico, the main producer of chile peppers in the United States (Bosland et al., 1993). Chile fruit color is important because nonpungent red chile is used principally as a source of pigments for food and other products. The iron status of chiles may influence the color attributes of both green and ripe fruit, which constitutes an important quality parameter for chile pepper marketing.

Martinez-Sanchez et al. (1989) found a highly significant correlation between leaf and stem iron and red color of field grown chile fruit, but not between fruit iron and color. More studies are needed to determine the specific contribution of iron to fruit color. The objective of this study was to evaluate the response in fruit color, leaf pigments, ferrous iron and micronutrient concentration of chile pepper under varying iron nutrition levels.

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Materials and Methods

Plant material and root substrate. Seeds of 'New Mexico 6-4' and 'NuMex R Naky' chile pepper (*C. annuum* L.) were germinated at room temperature in paper towels periodically moistened with a 0.1 mol·m^{-3} $CaSO_4$ solution to preserve membrane function (Gutschick, 1993). After 9 d, germinated seeds were transferred to plastic trays filled with 40-mesh white silica sand that had been washed with hydrochloric acid (HCl) to remove iron and other micronutrients.

The trays were watered as needed in the greenhouse during the 34 d of initial growth with the same nutrient solution used later in the hydroponic system described below. From these trays, 24 seedlings (43 d old) from each cultivar were randomly selected and individually transplanted into 15.7-L plastic pots filled with the acid-washed silica sand. The pots were painted white to reduce heat loading.

The sand was rinsed several times with reverse osmosis (R.O.) water mixed with 4 L concentrated HCl (commercial grade) and shaken within a manual, plastic cement tumbler for 20 min. After standing 2–3 d, the mix of acid and sand was drained, rinsed several times with R.O. water, neutralized with KOH, and rinsed again. Control of the washing process was achieved by sampling, extraction with diethylene triamine pentaacetic acid (DTPA), and analysis of the sand batches for iron in a JY70 inductively coupled plasma (ICP) spectrophotometer (Jovin Yvon, Edison, N.J.).

Hydroponic system and nutrient solution. In 1996–97, the recirculating hydroponic system was established in a greenhouse at the Fabian García Agricultural Science Center of the New Mexico State Univ. at Las Cruces. The greenhouse had natural light with a transmission factor near 70% for photosynthetically active radiation (PAR). Temperature was controlled within a range of 18 to 37 °C (12 to 35 °C in early April, to favor fruit set) by evaporative cooling and natural gas heating. The latter raised CO_2 mixing ratios to as much as 450 μmol·mol^{-1} episodically.

Four, 200-L plastic drums contained the nutrient solution (described below) for the 48 potted plants of the experiment. Each drum served a set of 12 randomly placed pots, half of which were planted to each cultivar. Each 15.7-L pot received a nutrient solution flow of 75 mL·min^{-1}. The nutrient solution was recirculated between the drums and the pots by 1/40 HP submersible pumps (Little Giant, Oklahoma City) until red fruit were harvested (231 d after germination). Every drum contained the same basic nutrient solution, plus one of four different iron concentrations [1, 3, 10, and 30 μmol Fe- EDDHA, ferric ethylenediamine di-(o-hydroxyphenyl-acetate)].

The composition of the nutrient solution was custom-designed according to the elemental composition of chile pepper (Winsor and

Adams, 1987). The concentration was determined on the basis of the expected growth rate of the crop and the replacement schedule of the nutrient solution, trying also to resemble the diluted concentrations of macronutrients often found in natural, high-fertility soils (Clarkson, 1985; Gutschick, 1987).

The nutrient solution was prepared with R.O. water and contained 1 mM KNO_3, 125 μM $Ca(H_2PO_4)_2$, 250 μM $MgSO_4$, 250 μM $CaSO_4$, 20 μM KCl, 25 μM H_3BO_3, 10 μM $MnSO_4$, 3 μM $ZnSO_4$, 1 μM $CuSO_4$, 0.5 μM Na_2MoO_4, and four varying levels of iron (1, 3, 10, and 30 μM Fe-EDDHA) that represented the four treatments of the experiment.

The pH of the nutrient solution was kept at 6.5 ± 0.3 by addition of HNO_3. Both N and K, as well as pH, were adjusted daily after monitoring with ion selective electrodes and combination electrodes, respectively. Iron levels in the nutrient solution were not adjusted between replenishments, because the solution volume was large enough to account for depletion. The nutrient solution was discarded and replaced with fresh solution every 2 weeks at the beginning of the season, and at shorter intervals when plants were larger, to avoid depletion of elements other than Fe that could not be measured practically for replenishment.

Iron was suppressed intentionally to treatments 1 and 3 (μM Fe-EDDHA) from days 96 to 106, to test the system after no visible symptoms of iron deficiency had appeared. Iron was suppressed accidentally to treatments 10 and 30 μM Fe from days 96 to 100 after a failure in the submersible pumps occurred. The supply of water and all other nutrients was unaffected during the iron shortage.

Experimental design. Every 15.7-L pot, containing one chile plant, represented an experimental unit. Twenty-four pots were planted with each of two cultivars, 'New Mexico 6-4' and 'NuMex R Naky'. Each cultivar received four iron treatments (1, 3, 10, and 30 μM Fe-EDDHA, the last being the control), in a 2×4 factorial arrangement. There were six replications, blocked in two directions to account for both light and temperature across the greenhouse.

Variables measured. Two young, fully expanded leaves from each experimental unit were harvested five times during the season, placed in plastic bags, cooled in an ice chest, and transported to the lab for determination of chlorophyll *a*, chlorophyll *b*, carotenoids and ferrous iron. For chlorophyll and carotenoid contents, ten leaf discs (6.5 mm diameter) were weighed, ground in a mortar with acetone plus $CaCO_3$ (Abadía and Abadía, 1993), brought to 10 mL volume with acetone neutralized with $CaCO_3$ and centrifuged at 5900 rpm for 5 min. Absorbance of the supernatant was measured at 645, 662, 470, and 730 nm in a spectrophotometer (Sequoia Turner model 690). The absorbance at 730 nm accounted for scattering by fine suspended debris. Chlorophylls *a* and *b*, as well as total carotenoids, were computed from equations based on extinction coefficients (Abadía and Abadía, 1993; Lichtenthaler, 1987). For specific leaf area, another 10-disc subsample was taken and weighed before and after drying at 65 °C. Dry weight data were used to express ferrous iron on a per gram basis.

Two to four green fruit from each plant at two harvest dates (days 188 and 231, respectively) were analyzed for surface color (lightness, chroma, and hue) using a Minolta chromameter. Chlorophyll *a*, chlorophyll *b* and total carotenoids were extracted by grinding 24 fruit discs (6.5 mm diameter) in 25 mL of acetone neutralized with $CaCO_3$ in a homogenizer. The rest of the extraction procedure followed the method used for leaves (Abadía and Abadía, 1993).

At red harvest time (day 231), three to four red fruit from the third to the fifth node positions were harvested, washed in 0.1 N HCl plus liquid soap (to remove dust that may carry iron), dried at 65 °C, destemmed, deseeded, and divided in two subsamples for analysis of micronutrients (middle section of each fruit) and color (remaining portions of the fruit). The processing of the sample for micronutrients is described below. The subsample for color analysis was ground in a stainless steel mill, 40-mesh, for analysis of both surface and extractable color. Surface color of the powder was measured with a Minolta chromameter. Extractable color (total pigment content, ASTA method 20.1; American Spice Trade Association, 1985) was determined by placing 80 mg of powder into 100 mL of acetone in a 100-mL volumetric flask. Flasks were sealed with stoppers and held 16 h in the dark. A 6-mL aliquot was taken, and absorbance at 460 nm was measured with a spectrophotometer. ASTA units were calculated after multiplying the absorbance reading by the instrument correction factor (0.99) and a constant value of 16.4, and then dividing by the sample weight in grams (Wall, 1994).

The leaves used to measure pigment accumulation also were used to determine ferrous iron at 93, 106, 127, and 181 d. Ferrous iron was extracted with 10 mL 1.5% *o*-phenanthroline pH 3.0 from a 200 mg chopped leaf sample. The extracts were held in the dark for 24 h and filtered through Whatman No. 1 paper. Transmittance was measured at 510 nm (Katyal and Sharma, 1980) and absorbance at 730 nm (to check for fine suspended debris) with a spectrophotometer. The absorbance and transmittance of both extracts and standards were read before and after the addition of the reductant hydroxylamine, to account for the eventual extraction of ferric iron. Because the ferric iron was apparently not extracted, only the iron concentrations without hydroxylamine addition are reported.

On day 141, a 0.8-g sample of petioles from each plant was ground in a mortar with 25 mL of deioinized water, shaken at 120 rpm for 15 min, filtered through a Whatman 2V filter, and analyzed for nitrate concentration in an autoanalyzer. The remaining blades of the leaves were washed with 0.1 N HCl plus soap, rinsed, dried at 65 °C and ground in a glass mortar for tissue analysis. The mortar was washed and acid-rinsed between subsamples. Dried, ground leaf samples (0.5 g) were microwave digested with 5 mL concentrated nitric acid and 2 mL 30% hydrogen peroxide, diluted and analyzed for Fe, Mn, Cu, Zn, P, and K in an ICP spectrophotometer (Jobin Yvon, Edison, N.J.).

At red harvest time, a sample of red fruit from all remaining plants was cleaned and subsampled for micronutrient analysis. This subsample was taken from the middle section (axially) of each fruit to account for eventual gradients in nutrient accumulation along the fruit. The glass mortar used for sample grinding was washed and acid-rinsed between grindings. A 0.5 g sample of the powder was microwave digested and analyzed for Fe, Cu, Zn, Mn, P, and K as described above for leaves.

Statistical Analysis. Analysis of variance (ANOVA) of all variables was performed using the general linear models procedure (PROC GLM) of the SAS® system. Mean separation for those variables that had significant differences among treatments also was performed in SAS® and followed a cluster-based method (Bautista et al., 1997). This method produces a clearer, unambiguous separation of treatment means, without placing a given treatment mean in two or more different groups.

Results and Discussion

The cultivar × treatment interaction was nonsignificant for all variables considered, therefore, the iron treatment means across cultivars are reported. Also, the growth and yield depressing effects of iron deficiency induced in this study are reported elsewhere (Anchondo-Najera, 1999). Briefly, low iron treatments (1 and 3 μM Fe) produced lower relative growth rates and photosynthetic rates, and less total dry matter accumulation and red fruit yield than high iron treatments (10 and 30 μM Fe) (Anchondo-Najera, 1999).

Pigment accumulation in leaves. No plant, regardless of the iron treatment, showed chlorosis or any other visible symptom of deficiency during the first 100 d. Leaves from plants receiving Fe at 1 and 3 μM had less extractable chlorophylls and carotenoids at 93 d (Table 1), showing also a relative enrichment of carotenoids (as shown by the chlorophyll to carotenoid ratio). At the same date, the ratio of chlorophyll *a* to *b* in 1 μM Fe plants was higher than that of other treatments. The reduction of total chlorophylls, the relative enrichment in carotenoids, and the increased chlorophyll *a/b* ratio represent typical responses of plants to iron deficiency (Abadía et al., 1991).

Differences in leaf pigment concentration between low iron (1 and 3 μM Fe) and high iron (10 and 30 μM Fe) plants were more evident after the intentional, temporary, iron shortage initiated on day 96. When iron was resupplied to treatments 1 and 3 μM Fe, 10 d later (day 106), the total chlorophyll and carotenoid content of 3 μM Fe, and the ratio between the these pigments, appeared to drop drastically (Table 1). At 106 d, the low-iron treatments had not only the lowest chlorophyll and carotenoid accumulation, but also the lowest ferrous iron concentration (Table

Table 1. Leaf pigment content of chile peppers at three sampling times after germination. Plants were hydroponically-grown at four iron concentrations[z].

Iron treatment	Leaf pigment content (mg·cm^{-2})											
	93 days				106 days				181 days			
(μM Fe-EDDHA)	*a* + *b*[y]	Carot	*a/b*	(*a* + *b*)/Carot	*a* + *b*	Carot	*a/b*	(*a* + *b*)/Carot	*a* + *b*	Carot	*a/b*	(*a* + *b*)/Carot
1	24.8 b[x]	5.2 b	3.0 a	4.7 b	24.6 b	5.3 b	3.0 a	4.6 b	49.3	9.6	2.5	12.2
3	29.2 b	5.8 b	2.9 b	5.0 b	15.8 b	4.1 b	2.8 b	3.5 c	47.2	9.3	2.5	11.8
10	37.1 a	7.0 a	2.9 b	5.3 a	42.9 a	8.0 a	3.0 a	5.4 a	42.1	9.1	2.4	11.5
30	38.6 a	7.2 a	2.9 b	5.3 a	43.4 a	8.3 a	3.0 a	5.2 a	45.2	9.1	2.5	11.6
P > F	0.0001	0.0001	0.020	0.001	0.0001	0.0001	0.022	0.0001	0.12	0.67	0.54	0.61

[z]Data are the sample means of New Mexico 6-4 and NuMex R Naky cultivars. Iron was suppressed to treatments 1 and 3 μm Fe -EDDHA from days 96 to 106 and to treatments 10 and 30 μM Fe from days 96 to 100.
[y](Chlorophyll *a* + *b*, total carotenoids (carot), *a/b* ratio and chlorophyll/carotenoid ratio, in mg·cm^{-2}).
[x]Means within columns were separated by cluster approach, $P \leq 0.05$.

5). No differences in pigment concentration occurred during the subsequent sampling dates. For this reason, the pigment contents at only the last sampling date (day 181) are reported (Table 1).

Pigment content of green fruit. No differences in the surface or extractable color of green fruit at first harvest were found among iron treatments (data not shown). The green fruit from 1 μM Fe plants at second harvest (day 231) were more yellow than those of the other iron treatments, as indicated by the lowest hue angle. The chroma and lightness, however, were not different from those of green fruits that received 10 and 30 μM Fe (Table 2). Plants grown with 3 μM Fe produced fruit that were both paler (higher lightness) and more vivid (higher chroma). Green fruit from the 3 μM Fe plants also contained the lowest concentration of total chlorophyll and carotenoids. Pale green fruit, as a result of lower chlorophyll content, represent a marketing disadvantage for the green chile grower and processor. The concentrations of total chlorophyll, chlorophyll *a/b* ratios and total carotenoid values from the present study are in general agreement with the pigment concentration values determined for mature green fruit in a study following color development in two paprika cultivars (Madrid et al., 1999).

Pigments in red fruit. There were no differences in extractable (ASTA) or surface color (lightness, chroma and hue angle) of red fruit produced under different iron concentrations in the nutrient solution (Table 3). Although the 30 μM Fe plants had the highest concentration of iron in their fruit, and the 1 μM Fe the lowest, their extractable and surface color was not significantly different from the rest. Martinez-Sanchez et al. (1989), also found no significant relationship between whole-fruit iron content and red chile color (expressed as capsanthin content). However, these researchers found a highly significant correlation between leaf iron content and red fruit color, which was not evident in the present study.

Table 2. Surface color and extractable pigment content of green chile pepper fruit grown in hydroponics at four iron levels[z].

Iron treatment	Surface color[y]			Extractable pigment content (μg·cm^{-2})[y,x]			
(μM Fe-EDDHA)	Lightness	Chroma	Hue	*a*+*b*	Carotenoids	*a/b*	(*a*+*b*)/Carotenoids
1	45.3 b[v]	19.9 b	108.1 c	28.5 a	7.2 a	2.29	3.9
3	47.8 a	22.8 a	125.1 b	23.0 b	5.9 b	2.29	3.9
10	45.6 b	20.3 b	127.4 a	29.6 a	7.4 a	2.27	4.0
30	45.0 b	19.2 b	127.5 a	28.2 a	7.2 a	2.34	3.9
P> F	0.002	0.036	0.021	0.011	0.034	0.09	0.76

[z]Data are the sample means of New Mexico 6-4 and NuMex R Naky cultivars. Iron was suppressed to treatments 1 and 3 μM Fe EDDHA from days 96 to 106 and to treatments 10 and 30 μmol Fe from days 96 to 100.
[y]n = 4–5 plants from each of two cultivars, 2–4 green fruit/plant.
[x]Chlorophyll *a* + *b*, total carotenoids, *a/b* ratio and chlorophyll/carotenoid ratio of green fruit.
[v]Means within columns were separated by cluster approach, $P \leq 0.05$.

The lack of an iron effect on the extractable color of red fruits in our study may be due to the inherent high variability of this trait, both between and within plants. In the present experiment, it was difficult to sample fruits from exactly the same node. This problem was aggravated by the fruit abortion that occurred from high night temperatures, that affected the early bloom of the two high iron treatments at the beginning of fruit set.

Micronutrient concentration in leaves. Total iron in leaves (ICP analysis) at day 141 increased accordingly with the concentration of iron in the nutrient solution (Table 4). High leaf iron levels depressed copper and phosphorus concentration. Zinc remained almost unaffected, except for the lower zinc concentration of leaves at 10 μM Fe.

Iron and phosphorus are antagonistic to each other (Gutschick, 1987). The classical antagonism between leaf iron and manganese (Marschner, 1986) was not evident in this study. Moraghan (1980) alleviated manganese toxicity in flax plants with a soil application of 2 ppm Fe (as Fe-EDDHA), which not only reduced foliar manganese but also leaf zinc concentrations and slightly increased foliar phosphorus content.

All elemental nutrients in the leaf blades were within the sufficiency range reported for greenhouse chile crops (Winsor and Adams, 1987) for all treatments except the 1 μM Fe plants. However, the levels of foliar iron and copper in the present study were considerably lower than those reported by Guzman and Romero (1988) for commercial greenhouse-grown chile.

Nitrate-nitrogen from leaf petioles did not follow a clear, definite pattern (Table 4). With low iron, a lower nitrate reduction was expected. There was high variability among replicates. Lower levels of nitrate-N in the

Table 3. Extractable color, surface color and nutrient concentration of chile pepper fruit at red harvest time.

Iron treatment	Fruit color[z]				Fruit nutrient content[z]					
	Extractable	Surface[y]			Fe	Mn	Zn	Cu	P	K
(μM Fe-EDDHA)	(ASTA units)	L*	C	Hue	(mg·kg^{-1})				(%)	
1	145.8	39.7	29.0	33.6	33.39 c[x]	10.76 b	19.72	12.92 a	0.33	2.31
3	125.1	38.1	25.3	33.6	45.43 b	11.99 b	18.84	11.90 a	0.37	2.32
10	147.2	38.9	27.9	33.8	45.23 b	17.32 a	19.84	8.41 b	0.34	2.17
30	120.4	39.1	27.6	34.9	58.89 a	14.10 b	21.22	9.80 b	0.35	2.25
P > F	0.48	0.91	0.65	0.27	0.0001	0.004	0.56	0.0002	0.91	0.77

[z]Data shown are the average over the sample means of New Mexico 6-4 and NuMex R Naky cultivars. Iron was suppressed to treatments 1 and 3 μM Fe-EDDHA from days 96 to day 106 and to treatments 10 and 30 μM Fe from days 96 to100.
[y]Lightness (L*), chroma (C), and hue angle.
[x]Means within columns were separated by cluster approach, $P \leq 0.05$.

Table 4. Leaf micronutrient and petiole nitrate-N content of hydroponically grown chile peppers 141 d after germination.[z]

Iron treatment	Fe	Mn	Zn	Cu	P	K	NO_3^--N
(μM Fe-EDDHA)	(mg·kg^{-1})				(%)		(mg·kg^{-1} fresh wt)
1	65.37 c[y]	289.08	109.81 a	26.73 a	0.53 a	4.94	626.9
3	125.12 b	341.27	111.83 a	26.47 a	0.51 a	4.83	1176.4
10	138.13 b	273.65	88.82 b	17.07 b	0.41 b	5.09	663.0
30	188.95 a	326.57	102.73 a	17.39 b	0.42 b	5.26	427.4
$P > F$	0.0001	0.054	0.035	0.0001	0.0001	0.21	0.06

[z]Data shown are the sample averages of New Mexico 6-4 and NuMex R Naky cultivars. Iron was suppressed to treatments 1 and 3 μM Fe-EDDHA from days 96 to 106 and to treatments 10 and 30 μM Fe from days 96 to 100.
[y]Means within columns were separated by cluster approach, $P \leq 0.05$.

Table 5. Ferrous iron content of leaves of hydroponically grown chile peppers at four sampling times after germination as a function of iron in the nutrient solution.

Iron treatment	Days after germination			
(μM Fe)	93	106	127	181
	– – – – –	Ferrous iron (mg·kg^{-1} dry wt)[z]		– – – – –
1	19.39 a[y]	65.85 b	83.20 c	337.98 b
3	21.29 a	70.51 b	173.77 b	458.73 a
10	37.46 a	150.29 a	194.74 b	448.46 a
30	52.39 a	126.46 a	335.91 a	525.53 a
$P > F$	0.075	0.0001	0.0001	0.002

[z]Data shown are the sample averages of New Mexico 6-4 and NuMex R Naky cultivars. Iron was suppressed to treatments 1 and 3 μM Fe-EDDHA from days 96 to 106 and to treatments 10 and 30 μM Fe from days 96 to 100.
[y]Means within columns were separated by cluster approach, $P \leq 0.05$.

petioles of higher iron treatments could perhaps be explained by the higher depletion rate of nitrogen from the solution in the tanks. Plants grown under 3 μM Fe, however, showed an apparent trend towards nitrate accumulation in the petiole ($P = 0.06$). Like many cultivated *Solanaceous* species, chile is reported to reduce nitrates mainly in the shoot (González and Salas, 1999).

It is not clear if iron level had some effect on nitrate levels, via enzyme activity. Nenova and Stoyanov (1995) showed that iron-deficient maize plants had a reduced activity of the enzyme nitrate reductase, which reduces nitrate to nitrite.

Nitrate-N levels from petioles were, on the average, considerably below the standards (6,000 to 10,000 ppm N) followed for some commercial laboratories for field chile crops at the fruit set and development stage (Laboratory Consultants, Ltd.). However, they are closer to the standards (1,000 to 1,500 ppm NO_3-N) followed by the Univ. of California for field grown chiles at early fruit set (Lorenz and Tyler, 1983).

Leaf ferrous iron, as determined by the *o*-phenanthroline method, tended to increase linearly following the concentration of the metal in the nutrient solution, although during the first sampling date there were no significant differences among treatments (Table 5). Ferrous iron was significantly lowest in leaves from 1 μM Fe plants at mid and late season. There was also a trend towards the increase of iron with age of the plants. This trend may not reflect a real enrichment of leaf iron with time, but some problems associated with the *o*-phenanthroline extraction method when the sample contains high levels of chlorophylls. The residues of chlorophyll degradation may interfere with the transmittance (Katyal and Sharma, 1984). As shown in Table 1, leaf total chlorophyll increased with plant age in this study. One alternative to reduce the ferrous iron overestimation of the method is to analyze the *o*-phenanthroline extracts for total iron by atomic absorption (AA) spectrophotometry (Katyal and Sharma, 1984). This type of analysis, however, was not done in the present study. Even without AA analysis, the capability of the *o*-phenanthroline method to discriminate between low and high ferrous iron has been demonstrated (Katyal and Sharma, 1984) and was apparently effective in this study.

Micronutrients in red fruit. High iron levels in the nutrient solution were associated with low copper levels in the red fruit (Table 3). The highest iron treatment also was related to a low manganese content, though this trend was not clear for the rest of the treatments. Fruit zinc, as well as phosphorus and potassium contents, remained unaffected by iron concentration in the nutrient solution.

Fruit nutrient concentrations (Table 3) were, in general, markedly lower than leaf nutrient concentrations (Table 4). Fruit iron, for example, was one-third to one-half the magnitude of leaf iron, while manganese was 25–30 times more concentrated in leaves than in fruits.

Different plant organs have different functions and different nutrient requirements. Vegetative organs have a higher concentration of nutrients than fruits, which helps to reduce the biomass construction costs (Gary et al., 1998). In drip-irrigated chile pepper, the iron concentration in stems (pooled with roots) was about twice as much as leaf iron and 10 times higher than fruit iron (Martínez-Sánchez et al., 1989). When the absorbed iron is partitioned to different parts of the plant, roots have preference (Baker, 1983). Nenova and Stoyanov (1999) found that hydroponically grown, iron-deficient maize seedlings (up to 1-month-old) decreased root iron concentration more severely than leaf iron. Roots of well iron-fed maize seedlings, on the other hand, concentrated up to 16 and 30 times more iron than leaves and stems, respectively. It is not clear, however, if Nenova and Stoyanov (1999) removed iron from the root free space before drying and grinding of maize roots.

There is little information available on the micronutrient concentration of red chile fruit to be used as reference, and almost all that information comes from field experiments. The average fruit iron (from the 1 μM Fe plants), zinc and copper concentrations of this study were, on the average, only half of those measured in Israel by Navrot and Levin (1976) in field grown *Capsicum annuum* cv. Vinedale. We are aware of no information on field fruit-manganese. Similar differences existed with regard to fruit iron from field cultivated chile in Spain (Martinez-Sanchez et al., 1989), although the differences for manganese and zinc were smaller.

Besides differences in fruit nutrient status likely introduced by cultivar, environment, culture medium, and crop management, there is also the issue of frequent differences in the protocols used for cleaning, grinding, digestion and analysis of the fruit samples.

Conclusions

Low iron levels (1 and 3 μM Fe) decreased the pigment accumulation of leaves (at early season only), while 3 μM Fe reduced total chlorophyll and carotenoids of green fruit at final harvest. However, the total extractable pigments of red fruits and their surface color remained unaffected by iron treatment.

Iron supply favorably impacted leaf Fe and Fe^{++}, while reducing copper, phosphorus and zinc leaf concentrations. Similarly, higher solution and fruit iron were associated with low copper.

Differences in leaf ferrous iron among the treatments were detected only after the iron shortage (106 d). At this date, ferrous iron, total chlorophyll and total carotenoids were all lower in the low-iron treatments (1 and 3 μM Fe) than in the high-iron ones (10 and 30 μM Fe).

The intentional suppression of iron to treatments 1 and 3 μM Fe during 10 d (days 96 to 106, to test the system for iron stress) induced clear short-term effects on leaf pigment accumulation. Absence of chlorosis indicates that plants acclimate new tissue growth rapidly to minimize tissue nutrient deficiencies.

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