Occurrence and correction of lime-induced chlorosis in petunia plants

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ABSTRACT

The effect of various concentrations and ratios of iron (Fe) and manganese (Mn) chelates on growth, micronutrient uptake and chlorophyll content was investigated in a glasshouse experiment using potted petunia plants. The plants were cultivated in a peat substrate amended with dolomitic limestone at rate of 3.0 g/L (control substrate) or 12.0 g/L. The higher rate was established both to restrict the uptake of Fe, Mn and other nutrients and also to test the effectiveness of various Fe and Mn treatments. The plants in all the treatments were fertigated at weekly intervals with a nutrient solution containing macronutrients and micronutrients. Various chelate forms of Fe and Mn were used with the exception of two treatments where these two elements were omitted. The effects of different substrate pH levels (derived from different limestone contents) had a large effect on plant growth, chlorophyll content and content of Fe and Mn accumulated in the plant leaves. The plants in the high-limestone substrate devoid of Fe and Mn had reduced growth and lower chlorophyll content. They also had lower leaf Fe and Mn content than the control plants in the R3 substrate. Regular fertigation with a nutrient solution containing Mn and Fe improved plant growth rate and also increased chlorophyll content. However, its efficiency depended on the chelate form and concentration used. The effect of chelate application on the Fe and Mn leaf content was unclear as it only marginally increased leaf Fe absorption in some treatments. No effect of the various Fe/Mn ratios was observed.

Keywords: *Petunia* × *atkinsiana*; iron; manganese; iron chelates; Fe-EDTA; Fe-DTPA; Fe-EDDHA; Mn-EDTA

Fe-inefficient plants such as *Petunia* show growth depressions and chlorosis when cultivated in a high alkaline growing medium. Growth depression is associated with Fe uptake disorder (Fisher et al. 2003, Smith et al. 2004a,b, Wik et al. 2006, Šrámek and Dubský 2008). The phenomenon is also observed in plants fertilized with standard or recommended amounts of Fe and other trace elements such as in a preplant fertilizer or liquid fertilizer nutrition. As a corrective measure, additional Fe is applied to the plants, mainly in the form of chelates. There are three application methods: foliar sprays, regular liquid fertilization or drenches. Foliar sprays are usually efficient method, but repeated application is necessary and they can cause leaf burn in some plants (Fisher et al. 2003). Regular liquid fertilization (Wik et al. 2006) or substrate drenches using very high Fe concentrations (Fisher et al. 2003, Šrámek and Dubský 2008) are more reliable.

The efficiency of the treatment depends on the choice of Fe-chelate since various compounds differ in their stability under high pH conditions. The ferric salt of *N,N'*-ethylenediamine-di-(*o*-hydroxyphenylacetic) acid (Fe-EDDHA) is the most stable and can be successfully used in heavy calcareous soils (Tills 1987, Reed 1996). Tills (1987) and Reed (1996) both reported that the stability of the ferric salts of diethylenetriaminepentaacetic acid (Fe-DTPA) and ethylenediaminetetraacetic acid (Fe-EDTA) is lower at high pH values. Hence, their effect is usually weaker (Fisher et al. 2003, Wik et al. 2006).

Manganese deficiency symptoms are similar to Fe. Its uptake is depressed by high soil pH levels (Marschner 1995, Mengel and Kirkby 2001) and this can mask lime-induced chlorosis arising from iron deficiency. In addition, there is competition between Fe and Mn uptake and treatment with

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Fe-chelate (mainly Fe-EDDHA) can depress the rate of Mn uptake. This was reported in petunia (Smith et al. 2004b), *Calibrachoa* (Wik et al. 2006) and in other plant species (Roomizadeh and Karimian 1996, Ghasemi-Fasaei et al. 2003, Ylivainio et al. 2004a,b, Voogt and Sonneveld 2009). It was reported that slower Mn uptake results in growth depression (Roomizadeh and Karimian 1996, Ghasemi-Fasaei et al. 2003, Voogt and Sonneveld 2009).

The objective of the work was to compare the effect of various concentrations of Fe and Mn from dissimilar chelate compounds at various Fe/Mn ratios applied in liquid fertilizer on mineral uptake, chlorosis and growth of petunia plants growing in high pH substrates and standard mineral nutrition.

MATERIALS AND METHODS

Petunia × *atkinsiana* Karma was chosen as an experimental plant because chlorosis was often observed in this variety. Cuttings were rooted in 70:30 v/v peat:perlite substrate amended with 2.0 g/L dolomitic limestone (85% $CaCO_3$, 15% $MgCO_3$) and transplanted into 0.1 m plastic pots (400 ml) filled with peat substrate on March 21, 2008. The substrate was fertilized with 1.0 g/L of a soluble fertilizer PG Mix containing 14% N, 16% P_2O_5, 18% K_2O, 0.7% MgO, 0.09% Fe (as Fe-EDTA), 0.16% Mn, 0.04% Zn, 0.12% Cu, 0.03% B, 0.2% Mo. Dolomitic limestone was added at 3.0 g/L (control substrate P3) or 12 g/L (substrate P12). The latter was used to both limit the uptake of Fe, Mn and other micronutrients and also to make it possible to test the effectiveness of various Fe and Mn treatments. The plants were pinched and cultivated in a greenhouse at 17°C with a 2°C night set back. They were watered manually according to their requirements. Irrigation water contained (in mg/L) 20 Mg, 60 Ca, 260 HCO_3^-, 0.005 Fe, and 0.035 Mn, its pH was 7, EC 0.7 mS/cm. All plants were fertilized at weekly intervals with a nutrient solution between March 26 and April 21. The plants in the treatments W3 and W12 were fertilized with nutrient solution containing macronutrients and micronutrients except iron and manganese (in mg/L): 380 N, 53 P, 332 K, 36 Mg, 0.5 Zn (Zn-EDTA), 0.2 Cu (Cu-EDTA), 0.5 B (H_3BO_3), 0.08 Mo (Na_2MoO_4). The plants in the other treatments were fertilized with the same nutrient solution but in addition contained Fe and Mn at various rates and chelate forms. Four combinations were established: Fe-EDTA + Mn-EDTA, Fe-DTPA + Mn-EDTA, Fe-DTPA + Mn-DTPA, and Fe-EDDHA + Mn-EDTA with 20 different treatments (Table 1). There were four replications with eight plants per each replicate.

The substrates were analysed for chemical properties according to the European Standards. Electrical conductivity (Anon 1999b), pH value (Anon 1999a) and available calcium content (Anon 2001b) were determined in water extract 1:5 v:v, content of other available nutrients (Anonymous 2001a) in CAT extract (0.01 mol/L $CaCl_2$ and 0.002 mol/L DTPA) with extract ratio 1:5 v:v.

The plants were evaluated on April 28 and fresh weight was determined. The plants were oven dried and dry weights were measured. Samples for determination of leaf chlorophyll and nutrient content were taken. Frozen leaves (0.5–1 g fresh weight) were homogenized in liquid nitrogen and repeatedly extracted with hot (90°C) 80% ethanol according to Anonymous (1990). The extract absorbance at 661 nm (A_{661}) and 643.5 nm ($A_{643.5}$) were measured on a Beckman DU 530 spectrometer. The chlorophyll content was calculated according to the equations:

$$\text{Chlorophyll a (mg/g FW)} = 9.93\ A_{661} - 0.777\ A_{643.5}$$
$$\text{Chlorophyll b (mg/g FW)} = 17.6\ A_{643.5} - 2.81\ A_{661}$$
$$\text{Chlorophyll a + b (mg/g FW)} = 7.12\ A_{661} + 16.8\ A_{643.5}$$

Leaf macronutrient and micronutrient content was determined following milling in ball mill MM 301 (Retsch). Milled samples were mineralized in a microwave digestion appliance (Mileston model MLS 1200) according to their recommended procedure. The concentration of P, K, Ca, Mg, and micronutrients were determined using inductively coupled plasma spectrometer ICP – OES Trace Scan (Thermo Jarrell Ash). Total nitrogen samples were mineralized (Kjeldahl) in H_2SO_4 with selenium and determination was performed spectrometrically using the SAN Plus System analyzer (Skalar) according to their recommended procedure.

All the data sets were tested for normality and analysed by one-way ANOVA (Unistat 5.2). The significance level $P = 0.05$ was used and significant differences between means were evaluated by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

At the beginning of the experiment there were significant differences between substrates P3 and P12 in respect of pH, available calcium content and magnesium (Table 2) which resulted from different rates of dolomitic limestone at mixing.

Table 1. Survey of treatments

Treatment	Substrate	Fe form[c]	Mn form[c]	Fe concentration[d]	Mn concentration[d]
EEA	P12[a]	Fe-EDTA	Mn-EDTA	1.5	0.8
EEB	P12	Fe-EDTA	Mn-EDTA	3.0	1.6
EEC	P12	Fe-EDTA	Mn-EDTA	6.0	3.2
EEX	P12	Fe-EDTA	Mn-EDTA	3.0	0.8
EEY	P12	Fe-EDTA	Mn-EDTA	6.0	0.8
DEA	P12	Fe-DTPA	Mn-EDTA	1.5	0.8
DEB	P12	Fe-DTPA	Mn-EDTA	3.0	1.6
DEC	P12	Fe-DTPA	Mn-EDTA	6.0	3.2
DEX	P12	Fe-DTPA	Mn-EDTA	3.0	0.8
DEY	P12	Fe-DTPA	Mn-EDTA	6.0	0.8
DDA	P12	Fe-DTPA	Mn-DTPA	1.5	0.8
DDB	P12	Fe-DTPA	Mn-DTPA	3.0	1.6
DDC	P12	Fe-DTPA	Mn-DTPA	6.0	3.2
HEA	P12	Fe-EDDHA	Mn-EDTA	1.5	0.8
HEB	P12	Fe-EDDHA	Mn-EDTA	3.0	1.6
HEC	P12	Fe-EDDHA	Mn-EDTA	6.0	3.2
HEX	P12	Fe-EDDHA	Mn-EDTA	3.0	0.8
HEY	P12	Fe-EDDHA	Mn-EDTA	6.0	0.8
W12	P12	–	–	–	–
W3	P3[b]	–	–	–	–

[a]peat substrate amended with 12 g/L of dolomitic limestone; [b]peat substrate amended with 3 g/L of dolomitic limestone; [c]form of Fe and Mn in the nutrient solution; [d]concentration in the nutrient solution in mg/L

However, with the exception of phosphorus there were no significant differences in other macronutrient and micronutrient values between the P3 and P12 substrates. The content of Fe and Mn was relatively low (Table 2).

At the end of the experiment the differences in pH values between P3 and P12 substrates were similar to those at the beginning (Table 2). Available Fe content was higher than at the beginning. Available Mn and Zn were slightly higher than at the beginning whilst Cu, B, and Mo were similar. A substantial decrease was recorded in available N and P, in case of P content there was a significant difference between P3 and P12. Only a minor decrease in available K occurred. Similarly, available Mg was not substantially changed whilst the values of available Ca were approximately two-times higher than at the beginning (Table 2). It could be caused by irrigation water relatively higher in Ca bicarbonate (260 mg/L HCO_3^-). Ca content was measured in the water extract (other nutrients in CAT) and according to our experience such values are rather unreliable and their interpretation is difficult in some cases.

As for the nutrient content in the substrate P12 at the end of the experiment data concerning treatment W12 are only shown in Table 2 because various treatments did not have any substantial and significant effect. Manganese content was an exception. The lowest Mn values occurred in both W3 and W12 treatments without Mn application (Table 2) and similar values were found in treatments EEA, DDA, and HEA (1.9, 2.0, and 2.0 mg/L, respectively) where low Mn concentration was used. The highest values were recorded in EEC and HEC treatments (3.9 and 3.8, respectively).

The substrate pH resulted from different limestone application rates significantly influenced plant growth, chlorophyll content and the Fe and Mn contents in the leaves (Table 3). The plants grown in the low-limestone substrate (treatment W3) produced symptom-free healthy green leaves with high fresh and dry matter contents. On the other hand, the plants in the high-limestone sub-

Table 2. Electric conductivity (mS/cm), pH values and nutrient content (mg/L) in the substrates at the beginning and at the end of the experiment

Substrate (treatment)	pH	EC	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu	B	Mo
Beginning of the experiment													
P12	6.5[a]	0.32	219	40[b]	120	226[a]	36[a]	8.4	0.7	2.60	0.77	0.07	0.062
P3	5.0[b]	0.32	203	58[a]	129	173[b]	18[b]	8.0	0.9	2.27	0.79	0.12	0.048
End of the experiment													
P12 (W12)	6.5[a]	0.21	31	17[b]	106	204	70	22.6	2.0	3.51	1.04	0.07	0.066
P3 (W3)	4.8[b]	0.26	30	29[a]	108	211	71	23.1	1.7	3.06	0.84	0.10	0.091

Mean values labelled with the different letters were significantly different according to Duncan's Multiple Range test, $P < 0.05$. Non labelled values were not significantly different

strate without Fe and Mn fertigation (W12) showed growth depression and had a lower chlorophyll content (a, b, total) which visually expressed itself as severe chlorosis. The leaf Fe and Mn contents were significantly higher in the W3 treatment than the W12 one (Table 3). Regular fertilization with a nutrient solution containing Fe and Mn significantly improved the growth rate (fresh and dry weight) and chlorophyll content of the plants. Only the lowest concentration of Fe-EDTA + Mn-EDTA (EEA) was an exception. This chelate combination was only expressed when higher Fe and Mn concentrations were used. The effect of the other chelate combinations was not influenced by concentration (Table 3). In agreement with previous findings (Šrámek and Dubský 2009), regular fertilization with 1.4 mg Fe/L and 0.8 mg Mn/L from EDTA-chelates did not prevent leaf chlorosis. This finding is consistent with those of Smith et al. (2004a) who reported that 2.0 mg Fe/L from Fe-EDTA was insufficient to produce chlorosis free petunia grown in high-pH substrates.

These experiments reconfirmed earlier work concerning the efficacy of Fe-EDDHA in high pH substrates. This compares with the findings of Wik et al. (2006) who reported that for regular fertilization of *Calibrachoa*, 4.0 mg Fe/L from the Fe-EDTA source was necessary whereas 2.0 mg Fe/L in EDDHA form was equally effective. Similarly, when Fe-EDDHA was applied as a single drench, a rate of 20 mg/L was adequate, but if the Fe-EDTA form was used, a rate of 80 mg Fe/L was required (Fisher et al. 2003).

The effect of chelate application on the content of leaf Fe and Mn was unclear. It only marginally increased leaf Fe in some treatments (Table 3). Mills and Jones (1991) recorded a range of 84–168 μg Fe/g and 44–177 μg Mn/g in the dry matter of healthy petunia leaves. In this work, leaf Mn was also within this range in all treatments but at the lower end of the interval. Similarly, leaf Fe was mostly in the lower part of the range or slightly below it (Table 3). Smith et al. (2004b) obtained 159 μg Fe/g in the leaves of *Petunia* grown at pH 4.6 and 91 μg Fe/g at pH 7.0. A decrease in chlorophyll content commenced when leaf Fe content was less than 100 μg/g. In this work, there was no clear connection between chlorophyll content and the Fe content in the leaves. Low chlorophyll content occurred in the W12 treatment and corresponded to low leaf Fe whilst high chlorophyll content in W3 plants corresponded to high leaf Fe levels. There was no correlation between chlorophyll content and leaf Fe for the plants treated with Fe-chelates.

In this work, the effect of substrate pH was very evident on leaf Mn. This contrasts with the work of Smith et al. (2004b) who reported that leaf Mn was affected to the lesser extent than Fe in substrates with pH values ranging between 4.6 and 6.1. Additionally, they recorded a substantial increase in leaf Mn at pH 7.

The application of Mn-chelates together with Fe-chelates did not increase leaf Mn of plants cultivated in high-limestone substrate (Table 3). Several authors reported that the application of Fe-chelates decreased Mn uptake in some cases (Roomizadeh and Karimian 1996, Ghasemi-Fasaei et al. 2003, Smith et al. 2004b, Ylivainio et al. 2004a,b, Wik et al. 2006, Voogt and Sonneveld 2009). Hence, the reason for combining Fe-and Mn-chelates to prevent this. Manganese and iron were applied in various ratios but this had no effect on leaf Mn. In previous work, (Šrámek and Dubský 2009) regular fertilization with a solution containing 0.8 mg Mn/L and 1.4 mg Fe/L from EDTA-chelate decreased leaf Mn of petunia plants

Table 3. Fresh weight (FW) and dry weight (DW) of one plant, chlorophyll, Fe, and Mn content in the leaves

Treatment	Chlor. a	Chlor. b	Chlor. a + b	FW	DW	Fe	Mn
	(mg/g FW)			(g)		(μg/g DW)	
EEA	0.54^{bc}	0.22^{cd}	0.76^{cd}	73.0^{abc}	7.3^{a}	94^{abcd}	52^{b}
EEB	0.70^{a}	0.29^{ab}	0.99^{ab}	68.8^{abc}	7.0^{a}	90^{bcd}	56^{b}
EEC	0.70^{a}	0.28^{ab}	0.98^{ab}	71.2^{abc}	7.4^{a}	86^{bcd}	59^{b}
EEX	0.72^{a}	0.28^{ab}	1.00^{ab}	68.7^{abc}	7.3^{a}	104^{ab}	55^{b}
EEY	0.72^{a}	0.28^{ab}	0.98^{ab}	75.0^{ab}	7.6^{a}	78^{bcd}	52^{b}
DEA	0.65^{ab}	0.24^{bc}	0.89^{abc}	66.7^{bc}	7.0^{a}	100^{abc}	57^{b}
DEB	0.69^{a}	0.26^{ab}	0.95^{ab}	71.5^{abc}	7.1^{a}	83^{bcd}	59^{b}
DEC	0.76^{a}	0.29^{a}	1.05^{a}	70.5^{abc}	7.6^{a}	83^{bcd}	55^{b}
DEX	0.67^{ab}	0.26^{abc}	0.93^{abc}	65.4^{bc}	7.0^{a}	71^{cd}	56^{b}
DEY	0.68^{a}	0.26^{ab}	0.94^{ab}	74.3^{abc}	7.5^{a}	86^{bcd}	56^{b}
DDA	0.62^{ab}	0.24^{bc}	0.86^{bc}	77.3^{a}	7.8^{a}	80^{bcd}	50^{b}
DDB	0.75^{a}	0.30^{a}	1.05^{a}	66.3^{bc}	7.5^{a}	82^{bcd}	61^{b}
DDC	0.64^{ab}	0.25^{abc}	0.89^{abc}	73.7^{abc}	7.4^{a}	81^{bcd}	52^{b}
HEA	0.69^{a}	0.28^{ab}	0.97^{ab}	63.8^{cd}	7.2^{a}	75^{bcd}	59^{b}
HEB	0.75^{a}	0.30^{a}	1.05^{a}	75.2^{ab}	7.8^{a}	80^{bcd}	60^{b}
HEC	0.68^{a}	0.26^{ab}	0.94^{ab}	72.7^{abc}	7.3^{a}	82^{bcd}	62^{b}
HEX	0.74^{a}	0.29^{a}	1.03^{ab}	63.8^{cd}	6.8^{a}	80^{bcd}	60^{b}
HEY	0.72^{a}	0.29^{ab}	1.01^{ab}	72.6^{abc}	7.7^{a}	82^{bcd}	56^{b}
W12	0.50^{c}	0.20^{d}	0.70^{d}	54.8^{d}	5.8^{b}	68^{d}	55^{b}
W3	0.71^{a}	0.28^{ab}	0.99^{ab}	69.8^{abc}	7.2^{a}	125^{a}	85^{a}

Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range test, $P < 0.05$

grown in peat substrate irrespective of pH level, especially for plants grown in peat-bark substrates where available Mn was very high. On the contrary, leaf Mn increased after three drenches of 30 mg Mn/L from $MnSO_4$ and 90 mg Fe/L from Fe-EDTA, Fe-DTPA, or Fe-EDDHA (Šrámek and Dubský 2008).

There was no effect of substrate pH on leaf content of N (34.3 ± 1.6 mg/g), P (4.3 ± 0.2 mg/g), K (24.2 ± 1.9 mg/g), and Mg (2.2 ± 0.1 mg/g). A significantly higher calcium content obtained in the W12 treatment plants (7.7 mg/g) compared to those in the W3 treatment plants (5.5 mg/g) was an exception. Similarly, boron was higher in W3 plants (43 μg/g) than in W12 plants (29 μg/g). Copper and zinc were not affected (4.91 ± 2.35 and 38.8 ± 4.18 μg/g, respectively). According to previous reports (Tills 1987, Reed 1996, De Kreij 1998, Pestana et al. 2003) the Fe in Fe-EDTA could be replaced by Zn and Cu in high pH soils because these two ions create more stable chelates with EDTA than Fe. Therefore, in this experiment it was anticipated that the application of Fe chelates to the substrate could affect Zn and Cu uptake but this was not confirmed.

The experimental results showed that regular liquid fertilization with Fe and Mn chelates prevented chlorosis and growth depression in petunia plants grown at high substrate pH. Comparing Fe-EDTA, Fe-DTPA, and Fe-EDDHA the results reconfirmed previous reports that Fe-EDTA is the least efficient Fe chelate compound. No effect of various Fe/Mn ratios in the nutrient solution was observed.

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REFERENCES

Anonymous (1990): Chlorophyll in plants. Spectrophotometric method for total chlorophyll and the a and b components. In: AOAC Official Methods of Analysis, No 942.04. AOAC. 62–63.

Anonymous (1999a): EN 13 037. Soils improvers and growing media – Determination of pH. CEN Brussels.

Anonymous (1999b): EN 13 038. Soils improvers and growing media – Determination of electrical conductivity. CEN Brussels.

Anonymous (2001a): EN 13 651. Soils improvers and growing media – Extraction of calcium chloride/DTPA (CAT) soluble nutrients. CEN Brussels.

Anonymous (2001b): EN 13 652. Soils improvers and growing media – Extraction of water soluble nutrients and elements. CEN Brussels.

De Kreij C. (1998): Exchange of iron from chelate in the fertilizer against copper, manganese, and zinc in peaty substrates. Communications in Soil Science and Plant Analysis, *29*: 897–1902.

Fisher P.R., Wik R.M., Smith B.R., Pasian C.C., Kmetz-González M., Argo W.R. (2003): Correcting iron deficiency in calibrachoa grown in a container medium at high pH. HortTechnology, *13*: 308–313.

Ghasemi-Fasaei R., Ronaghi A., Maftoun M., Karimian N., Soltanpour P.N. (2003): Influence of FeEDDHA on iron-manganese interaction in soybean genotypes in a calcareous soil. Journal of Plant Nutrition, *26*: 1815–1823.

Marschner H. (1995): Mineral Nutrition of Higher Plants. Academic Press, San Diego, 889.

Mengel K., Kirkby E.A. (2001): Principles of Plant Nutrition. 5th Edition. Kluwer Academic Publishers, Dordrecht/Boston/London, 849.

Mills H.A., Jones J.B. Jr. (1996): Plant Analysis Handbook II. MicroMacro Publishing Inc., Athens, Georgia, 422.

Pestana M., Varennes A., Araújo Faria E (2003): Diagnosis and correction of iron chlorosis in fruit trees: a review. Journal of Food Agriculture and Environment, *1*: 46–51.

Reed D.W. (1996): Micronutrient Nutrition. In: Reed D.W. (ed.): Water, Media, and Nutrition for Greenhouse Crops. Ball Publishing, Batavia, Illinois, 171–195.

Roomizadeh S., Karimian N. (1996): Manganese-iron relationship in soybean grown in calcareous soils. Journal of Plant Nutrition, *19*: 397–406.

Smith B.R., Fisher P.R., Argo W.R. (2004a): Growth and pigment content of container-grown impatiens and petunia in relation to root substrate pH and applied micronutrient concentration. HortScience, *39*: 1421–1425.

Smith B.R., Fisher P.R., Argo W.R. (2004b): Nutrient uptake in container-grown impatiens and petunia in response to root substrate pH and applied micronutrient concentration, HortScience, *39*: 1426–1431.

Šrámek F., Dubský M. (2008): Chlorosis in petunia mother plants and its elimination. Acta Pruhoniciana, *89*: 63–68. (In Czech)

Šrámek F., Dubský M. (2009): Occurrence and correction of chlorosis in young petunia plants. Horticultural Science, *36*: 147–153.

Tills A.E. (1987): Chelates in horticulture. Professional Horticulture, *1*: 120–125.

Voogt W., Sonneveld C. (2009): The effect of Fe-chelate type and pH on substrate grown roses. Acta Horticulturae, *819*: 411–418.

Wik R.M., Fisher P.R., Kopsell D.A., Argo W.R. (2006): Iron form and concentration affect nutrition of container-grown *Pelargonium* and *Calibrachoa*. HortScience, *41*: 244–251.

Ylivainio K., Jaakkola A., Aksela R. (2004a): Effect of Fe compounds on nutrient uptake by plants grown in sand media with different pH. Journal of Plant Nutrition and Soil Science, *167*: 602–608.

Ylivainio K., Jaakkola A., Aksela R. (2004b): Impact of liming on utilization of ^{59}Fe by lettuce (*Lactuca sativa* L.). Journal of Plant Nutrition and Soil Science, *167*: 523–528.

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