Effects of different sources of iron, hormones and *Agrobacterium tumefaciens* on the chlorophyll and iron concentration in the leaves of peach trees

C. TSIPOURIDIS, D. ALMALIOTIS, T. THOMIDIS, A. ISAAKIDIS

Pomology Institute (NAGREF), Naoussa, Greece

ABSTRACT: The aim of this study was to investigate the effect of different sources of iron, hormones and *Agrobacterium tumefaciens* in the chlorophyll and iron concentration of the peach cultivars Katerina and Fire Blight. The results showed that the concentration of Fe in the leaves was significantly increased after spraying with FeSO $_4$.7H $_2$ O (alone or in combination with KNO $_3$, urea, citric acid or ascorbic acid) or with GA $_3$ + Kinetin. Soil applications with Fe-EDDHA also significantly increased the iron concentration in leaves. Chlorophyll (a/b) increased only in trees sprayed with citric acid or H $_2$ SO $_4$ 60 days after application. SPAD chlorophyll measurements showed that Fe-EDDHA and FeSO $_4$.7H $_2$ O + organic matter, applied as soil drench, significantly increased the chlorophyll concentration in leaves of Katerina even up to 120 days after application. Foliar treatments had no significant effect on the SPAD index. SPAD measurements also showed that the concentrations of chlorophyll in leaves of the rootstocks St. Julien 655/2 and wild seedling rootstocks were significantly lower than in GF677 and Antafuel. The rootstocks inoculated with the *A. tume-faciens* strain Ag-28 alone showed a significantly lower chlorophyll concentration than the uninoculated ones.

Keywords: acidic compounds; hormones; iron chlorosis; peach cultivars; rootstocks

Iron chlorosis is a major problem of peach trees (SANZ et al. 1992), especially when they are grown in alkaline and calcareous soils (BASAR 2000; TAGLIA-VINI, MARANGONI 2002; Toselli et al. 1997), common in the fruit growing regions of Greece. It results in crop losses; in severe cases it can cause branch dieback or even entire tree killing. In order to overcome iron chlorosis, the best strategy lies in grafting trees on resistant rootstock such as GF677 (EL-GHARBI et al. 1994; STYLIANIDES et al. 1988). However, in high calcareous soils even peach trees grafted on the GF677 rootstock can show the symptoms of iron chlorosis. Agronomic practices should aim to increase the soil organic matter concentration, avoid excess water and nitrogen supply (more than recommended doses for each field) (STYLIANIDES, SYRGIANNIDIS 1995). Promising results in preventing chlorosis were obtained by sowing a mixture of graminaceous species along the tree row and supplying them with Fe sulphate (TAG-LIAVINI et al. 2000). If these practices are insufficient then iron fertilizers should be applied.

Soil and foliar application of iron containing chelates and acidic fertilizers have been recommended as remedies for iron-deficiency chlorosis (ALMALIOTIS, HOLEVAS 1991; KAUNDAL et al. 1994; TAGLIAVINI et al. 2000; TOSELLI et al. 1997). To be

effective, soil applications of Fe require either synthetic chelates or large amounts of inorganic iron fertilizers that lead to high production costs. Most of commercial chelate products contain Fe-EDDHA or Fe-EDDHMA, but their efficacy can differ (Hernandez et al. 1995). Furthermore, when Fe-EDDHA is applied excessively to peach and apple trees, it induces Mn deficiency.

A great deal of researches have been conducted over the past 40 years to determine the most effective and economical methods of correcting iron chlorosis in commercial crops (Mortvedt 1991; Sanz et al. 1992). Many Fe sources and methods of application have been tested during this time (Mortvedt 1991). However, effective treatments have not been found, yet. Foliar application of water soluble iron fertilizers appears to be one of the most cost-effective remedies to control Fe deficiency.

The level of total chlorophyll in a unit of fresh weight showed a progressive decrease with increasing deficient conditions (Abadia et al. 1999; Belkhodja et al. 1998; Morales et al. 1998; Nedunchezhian et al. 1997; Perez et al. 1995). El-Gharbi et al. (1994) suggested that the measurement of chlorophyll concentration is the best method for assessing iron chlorosis.

It is a well known fact that crown gall caused by *Agrobacterium tumefaciens* is malignant, i.e. once the plant cells have been stimulated by the bacteria to divide and enlarge, they continue to divide as long as they can obtain nutrients; such cells do not obey hormonal controls of the parent plant that regulate growth and differentiation (Agrios 1988). In addition, this pathogen affects the integrity or function of xylem vessels interfering with uptake of water and inorganic nutrients (Agrios 1988). A possible effect of *A. tumefaciens* in the chlorophyll concentration of leaves as an index of iron concentration has not been sufficiently investigated so far.

This study was conducted to determine the effect of the most suitable sources of iron application, hormones and *Agrobacterium tumefaciens* on the chlorophyll and iron concentration in the leaves of peach trees.

MATERIAL AND METHODS

All experiments were conducted in the experimental fields of Pomology Institute, Naoussa during two consecutive years. Choice of material used in this study was based on preliminary studies that were conducted within a EU joint research project.

Experiment 1

Peach trees (11-year-old cv. Katerina grafted on GF677 peach rootstock), grown in calcareous soil (Table 1, experimental field 1), with the same chlorotic condition of the foliage (determined visually) were used. The experimental design was a completely randomized block: 14 treatments comprising of iron salts and/or acidic compounds (Table 2) and a surfactant, were applied to all trees. Six trees were replicated per treatment. Four foliar applications took place at monthly intervals starting on 23rd March 1998.

For each treatment, fifty randomly selected leaves per each replicated tree were collected around the tree canopy at the height of approximately 1.5 m. Only fully expanded mature healthy leaves were collected from the middle to top of the portion of shoots in the morning, placed in plastic bags and transferred to the laboratory.

The recovery from iron chlorosis has been carried out by the pigment measurement technique SPAD-502 (total index), and the measurements of minerals with atomic absorption spectroscopy for Fe, Mn, Zn, Cu, molybdophosphoric blue colour method for P, flame photometring for K and E.D.T.A. titration for Ca and Mg. In addition, chlorophyll (*a* and *b*) was

Table 1. Soil analysis of the experimental orchards of Naoussa Pomology Institute, before iron and hormone applications

2 :: 4 Fr. 2	11"	Free CaCO3		Conductivity	Ь	\bowtie		Microelements (ppm)	ents (ppm)	
ош туре	рн	(%)		(mmhos/cm)	(mdd)	(mdd)	Zn	Mn	Fe	Cu
Experimental field 1										
SCL ^a	7.8	11.4 ^b	06:0	0.347	22.5	230	5.12	29.9	8.44	10.5
Experimental field 2										
SC-SL ^a	7.6	37.7 ^b	1.31	0.530	32.75	51	5.73	30.1	7.15	9.88
Experimental field 3										
SCLa	7.4	9.7 ^b	1.30	1.014	22.4	270	6.26	29.9	9.00	14.9

S – silt, C – clay, L – loam; all soil are calcareous and alkaline, conditions which cause iron chlorosis

Table 2. Effect of different sources of iron spray application on peach leaf chlorophyll and mineral concentration measured at 60, 90 and 120 DAT on cultivar Katerina (these are the results for experiment 1)

F	Dose		SPAD		Chk	Chlorophyll a:b	a:b	P (%)	(%	K (%)	(%	Ca (%)	(%)	Mg (%)	(%)	Mn (ppm)	(mdc	Zn (ppm)	(mdı	Cn (Cu (ppm)	Fe (F	Fe (ppm)
Treatment	(mg/l)	p 09	p 06	120 d	p 09	p 06	120 d	P 06	120 d	p 06	120 d	p 06	120 d	p 06	120 d	P 06	120 d	P 06	120 d	p 06	120 d	p 06	120 d
Control		32.07	36.7	34.43	2.638	1.92	2.617	0.15	0.1	2.23	1.61	2.71	3.76	0.91	0.82	72.38	72.38	40.83	30.45	66.17	24.99	68.33	72.38
Fe-EDDHA	120	30.5^{a}	34.77	35.82	2.303	2.546	2.144	0.18	0.14	2.48	1.72	2.82	3.46	0.82	1	57.08	57.08	37.75	27.33	32.25	13.17	67.08	57.08
Citric acid	2,000	29.5	36.45	38.05	3.615	1.868	2.391	0.17	0.14	2.28	1.59	2.66	3.64	0.93	0.87	63.5	63.5	37.75	28.98	31.92	16.17	76.17	63.5
$FeSO_4$.7 H_2O	200	28.7	38.2	37.58	2.468	2.335	2.479	0.18	0.12	2.12	1.62	2.53	2.62	0.81	0.81	119.5	119.5	38.08	29.45	33.42	19.74	119	119.5
FeSO ₄ .7H ₂ O + citric acid	500 + 2,000	30.4	36.85	36.98	2.796	1.894	2.484	0.17	0.14	2.09	1.6	2.67	3.65	6:0	0.82	140	140	38.67	32.33	45.77	20.36	156.3	140
H_2SO_4	100	31.6	37.5	38.2	3.71	1.928	2.471	0.17	0.12	2.03	1.67	2.79	3.91	0.89	0.85	76.73	76.73	40.75	28.58	33.58	22.86	68.25	76.73
Mn-EDDHA	120	31.45	38.92	34.28	2.739	1.838	2.457	0.16	0.15	2.09	1.59	2.76	3.45	86.0	0.98	124.4	124.4	39.92	31.08	60.92	28.61	79.83	104.4
FeSO ₄ .7H ₂ O + Mn-EDTA	500 + 120	32.22	35.13	34.6	2.457	2.106	2.364	0.16	0.25	1.97	1.5	2.67	3.45	0.91	1.13	164.4	164.4	39.58	34.58	39.3	32.36	73.08	114.4
Ascorbic acid	1,000	30.88	35.9	34.65	2.329	1.83	2.008	0.17	0.12	2.03	1.59	2.5	3.92	0.95	0.92	71.5	71.5	41.42	28.2	39.47	16.99	70.08	71.5
$FeSO_4$.7 H_2O + citric acid + ascorbic acid	500 + 2,000 + 1,000	31.62	34.25	34.67	1.955	1.887	2.396	0.17	0.11	1.92	1.74	2.74	3.53	86.0	6.0	101	101	40.17	29.95	33.2	19.36	192.5	101
FeSO ₄ .7H ₂ O + ascorbic acid	500 + 1,000	30.9	36.77	35.55	2.414	1.889	2.441	0.17	0.15	2.1	1.73	2.72	3.87	1.02	0.98	93.58	93.58	40	28.33	49.6	24.75	144.3	93.58
Citric acid + ascorbic acid	2,000 + 1,000 ¹	30.72	36.22	35.92	2.305	1.882	2.452	0.2	0.12	2.01	1.59	2.52	3.84	0.94	1	68.58	68.58	39.83	32	35.6	19.75	94.17	68.58
${\rm FeSO_4}$.7 ${\rm H_2O}$ + urea	$500 + 1,000^{1}$	31.5	36.88	36.2	2.291	2.142	2.515	0.16	0.21	2.04	1.66	2.56	3.59	0.95	1.04	163	163	42.92	28.33	52.47	18.67	184.8	163
FeSO ₄ .7H ₂ O + KNO ₃	500 + 1,000	32.15	38.07	37.83	2.044	1.959	2.345	0.15	0.12	1.97	1.62	2.75	3.73	0.92	1.05	222.5	222.5	38.92	21.08	25.09	15.67	194.8	222.5
${}^{\rm b}{\rm LSD}_{0.05}$		3.58	4.39	4.31	0.7	0.78	89.0	0.03	80.0	0.5	0.1	0.4	0.4	0.2	0.1	50.2	50.2	4.2	5.3	18.4	6.5	44.9	50.2

Data are the mean of two experiments (one each year); analysis of variance (ANOVA) was used to analyze the data and treatment means were separated using the least significant difference test (< 0.05)

measured by using a spectrophotometer and was estimated by the equations: Chlorophyll (a) = 13.7 × A(665) – 576 × A(649)mg/g; Chlorophyll (b) = 25.8 × A(649) – 7.60 × A(665) mg/g.

Experiment 2

Peach trees (11-year-old cv. Fire Blight grafted on GF677 peach rootstock) cultivated on calcareous soil (Table 1, experimental field 2) and exhibiting uniform chlorotic conditions of their foliage were used. From each tree, eight annual shoots of about 50 cm in length and uniform chlorotic conditions of the foliage were selected to apply the treatments. Treatments and doses are presented in Table 3.

The experimental design was a completely randomized block: 8 treatments and 6 replicated trees per treatment.

Two foliar sprays were applied on 30th May and one month later.

Leaf sample collection and measurements were similar to those described above in experiment 1.

Experiment 3

Eleven-year-old Katerina peach trees, grafted on GF677 peach rootstock and cultivated in calcareous soil (Table 1, experimental field 3) were used. All trees showed uniform symptoms of chlorosis. The experimental design was a completely randomized block with 8 treatments and 6 replicated trees per treatment. Treatments and doses are presented in Table 4. Applications were made in 10 cylindrical holes (about 30 cm deep, 15 cm in diameter around each trunk and about 1 m away from each tree trunk).

Leaf sample collection and measurements were carried out as described in experiment 1.

Experiment 4

The roots of one-year-old peach rootstocks GF677, Antafuel (J1), St. Julien 655/2 (SJ) and wild seedling (S) were washed with tap water to remove soil residues. The roots were then wounded (cut with a knife) and dipped in a solution containing cell suspension (at concentrations about 6×10^{-6}) of *Agrobacterium tumefaciens* for 30 min. Two *A. tumefaciens* strains were used in this experiment. Both strains had been isolated from the roots of infected peach trees. Trees were inoculated with strains Ag-20, Ag-28 and with their combination. The inoculated trees were planted in sterile pots containing 5 litres of sterilized peat and perlite at a ratio 3:1, respectively. SPAD chloro-

able 3. Effect of different sources of hormone spray application on peach leaf chlorophyll and mineral concentration on cultivar Fire Blight (these are the results for experiment 2)

		•	, , ,						,)		•	
Treatment	Dose (mg/l)	SPAD	Chlorophyll a:b	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
IBA	100	29ª	2.151	60.0	2.2	4.5	1.08	35.5	63.3	63.5	18.5	18.2
KIN	100	27.4	1.956	0.10	2.4	4.4	1.11	37.6	64.2	63.8	20.4	17.7
GA_3	100	27.6	1.886	0.11	2.4	4.1	1.12	44.0	71.1	6.89	23.7	13.0
Kinetin + GA_3	100 + 100	28.5	2.003	60.0	2.6	4.0	1.12	41.8	81.2	46.6	20.5	18.9
$IBA + GA_3$	100 + 100	27.1	1.962	0.10	2.7	4.2	1.16	44.2	8.79	67.2	19.4	14.3
IBA + Kinetin	100 + 100	29.2	2.012	0.10	2.9	4.2	1.15	43.4	71.9	67.2	18.0	14.6
$IBA + KIN + GA_3$	100 + 100 + 100	29.2	2.115	06.0	2.7	4.1	1.11	46.2	74.9	57.2	19.5	18.1
Control	I	26.0	1.914	0.10	2.3	3.9	1.16	45.4	62.0	62.6	18.2	17.0
$^{ m b}{ m LSD}_{0.05}$		7.2	0.330	0.015	0.5	0.55	0.10	7.5	18.8	19.7	5.1	6.0

Data are the mean of two experiments (one each year); analysis of variance (ANOVA) was used to analyze the data and treatment means were separated using the least significant dif-

143

Table 4. Effect of different sources of iron soil applications on peach leaf chlorophyll and mineral concentration measured at 60 and 90 DAT on cultivar Katerina (these are the results for experiment 3)

Control Fe-FDDTA			SPAD		Chle	Chlorophyll $a:b$	a:b	P (%)	(%	K (%)	(%	Ca(%)	(%	Mg (%)	(%)	Mn (ppm)	pm)	Zn (ppm)	pm)	Cu (ppm)	(mdo	Fe (ppm)	pm)
	Sason	p 09	p 06	90 d 120 d	p 09	90 d 120 d	120 d	p 09	p 06	p 09	p 06	p 09	p 06	p 09	p 06	p 09	p 06	p 09	p 06	p 09	p 06	p 09	p 06
		29.3	24.6 ^a	26.45	1.751	26.45 1.751 1.942	2.201	0.24	0.18	2.1	1.8	1.8	2.8	0.97	0.89	44.4	38	49.4	40.6	17.9	26.7	63.03	65.42
	50 g/tree	36.87	33.76	38.34	1.802	1.802 1.982	2.136	0.24	0.18	2	1.8	1.8	2.5	0.61	0.78	36.1	34.5	53.2	34.4	17.2	24.5	154.8	64.6
FeSO ₄ .7H ₂ O	5 kg/tree	28.03	21.82	24.58	1.852	1.660	2.198	0.23	0.18	2.1	2	1.9	2.3	0.65	0.79	49.2	39.3	45.9	40.1	16.5	32.7	63.92	50.4
$FeSO_4.7H_2O_5$ + K_2SO_4	5 kg/tree + 5 kg/tree	30.3	27.45	30.74	1.950	30.74 1.950 2.010	2.248	0.23	0.17	2.1	1.9	1.9	2.6	0.64	0.94	37.9	43.1	46	39.2	16	27.2	58.08	60.5
$FeSO4.7H_2O$ + wrea	5 kg/tree + 2 kg/tree	29.7	23.78	24.29	1.999	1.999 1.988	2.182	0.23	0.17	7	1.8	1.9	2.7	0.64	96.0	58.1	46.2	44.9	40	15.5	31.4	78.83	64.2
$FeSO_4$.7 H_2O 5 kg/tree + S + 5 kg/tre	5 kg/tree + 5 kg/tree	28.53	28.53 26.66	29.93	2.006 2.000	2.000	1.943	0.23	0.18	2	1.8	1.9	2.5	0.61	0.9	38.4	39.7	47.6	41.9	17.2	36.4	72.83	60.33
$FeSO_4$.7H ₂ O 5 kg/tree + org. mat. + 50 kg/tr	5 kg/tree + 50 kg/tree	29.85	29.98	35.7	1.972	2.009	2.313	0.24	0.17	7	1.8	1.9	2.5	9.0	0.91	43.8	42.9	45.2	42.6	16.4	25.6	75.58	59.27
FeSO ₄ $.7H_2O + org.$ mat.+ K_2SO_4	5 kg/tree + 50 kg/tree + 5 kg/tree	30.02	28.5	39.44	1.880	1.880 1.975 2.265	2.265	0.22	0.17	7	2.1	2	2.5	0.56	0.82	52.6	44.4	44.5	39	15.5	26.8	62.75	67.5
$^{\mathrm{b}}\mathrm{LSD}_{0.05}$		2.5	3.7	4.8	0.35	0.45 0.38	0.38	0.03	0.02	0.1	0.2	0.2	0.5	0.09	0.1	16.8	7.4	7.9	10.3	0.9	15.5	66.5	10.5

Data are the mean of two experiments (one each year); analysis of variance (ANOVA) was used to analyze the data and treatment means were separated using the least significant dif-(< 0.05) phyll measurements were made 6 months after the initiation of the experiment in a similar way as in experiment 1.

The experimental design was a completely randomized block. There were 20 replicated trees for each treatment and 20 noninoculated trees of each rootstock were used as a control.

RESULTS AND DISCUSSION

Responses of chlorophyll concentration in leaves of Katerina and Fire Blight cultivars to different sources of iron and hormone foliar application were studied. Chlorophyll (a/b) ratio was significantly higher in leaves of Katerina trees sprayed with citric acid or $\rm H_2SO_4$ 60 days after application (Table 2). In contrast, no significant differences were observed 90 and 120 days after application. None of the hormones and soil application affected significantly the concentration of chlorophyll (a/b) in leaves of Fire Blight and Katerina peach trees (Tables 3 and 4).

The results of SPAD measurements showed that any of the foliar applications with iron compound or hormones did not affect significantly the concentration index of chlorophyll in leaves (Tables 2 and 3). However, the leaves of trees sprayed with FeSO₄.H₂O alone or in combination with KNO₃, urea, citric acid or ascorbic acid regreened. Fe-EDDHA, FeSO₄.7H₂O + organic matter and FeSO₄.7H₂O + organic matter + K₂SO₄, applied by soil drenching, increased significantly the chlorophyll concentration of leaves (Table 4). Similarly, TAGLIAVINI et al. (2000) reported that iron sulphate amended in the soil proved to be effective only if applied together with high amounts of organic matter. ABADIA et al. (1985, 1989) reported that there is a linear relationship between iron concentration of the plant tissue and the rate of chlorophyll formation. Guardia et al. (1995) measured the chlorophyll concentration of leaves in peach rootstocks to evaluate the degree of their tolerance on iron chlorosis. Val et al. (1995) used the measurement of chlorophyll fluorescence to quantify Fe and Mn deficiencies in the peach cultivar Baby Gold.

The concentration of chlorophyll in the leaves differed from rootstock to rootstock (GF677, St. Julien 655/2, Antafuel, and wild

Table 5. Effect of rootstocks and *Agrobacterium tumefaciens* strains on leaf chlorophyll concentration (SPAD)

Rootstocks	Ag-20	Ag-20 + Ag 28	Ag-28	Uninoculated	Mean	LSD _{0.05}
GF677	20.4°	28.2	26.2	26.2	25.3	
J1	29.5	29.6	31.1	25.1	28.8	
SJ	24	10.6	8.1	10.9	13.4	10.5
S	22.4	0_{p}	0	19.1	13.8	
Mean	24.1	22.8	16.4	20.3	11.0	
$\mathrm{LSD}_{0.05}$		3.8			11.3	

Analysis of variance (ANOVA) was used to analyze the data and treatment means were separated using the least significant difference test (< 0.05); trees were killed three months after inoculation; values are the mean of two experiments

seedling). The St. Julien 655/2 and wild seedling rootstocks had the lowest concentration of chlorophyll, while the rootstocks GF677 and Antafuel the highest (Table 5). Tumors were observed in the roots of all trees inoculated by either of *A. tumefaciens* strains. Rootstocks inoculated with the strain Ag-28 alone had lower SPAD index (chlorophyll concentration) than control, while no significant differences were observed between other treatments. No explanation can be given for the differences among the strains and their combination.

The results show that peach trees growing on calcareous soil may benefit from leaf sprays of acidic and iron containing compounds to increase leaf Fe. The evidence provided in this study is well in accordance with the assumption that the pH of the leaf apoplast plays an important role in lime-induced iron chlorosis. The results indicated that 90 days after spraying with FeSO₄.H₂O alone or in combination with KNO₂, urea, citric acid or ascorbic acid, the concentration of Fe in the leaves significantly increased (Table 2). KAUNDAL et al. (1994) found that FeSO₄.H₂O and Fe-DTPA sprays at 0.2% concentration were better than Fe citrate and equally effective in influencing iron chlorosis in peach rootstock seedlings cv. Sharbati, plant growth characteristics and foliar iron and chlorophyll concentration.

The foliar applications affected the concentration of phosphorus, potassium, calcium, zinc, copper, magnesium and manganese in leaves as compared to the control trees (Table 2). Leaf Mn concentration was significantly higher in the trees treated with FeSO₄.H₂O + KNO₃ than in the control trees, which seems to have an antagonistic effect on Cu concentration (Table 2). The level of iron concentration in leaves of trees sprayed with FeSO₄.H₂O or its combination with ascorbic acid or ascorbic acid + citric acid was reduced 120 days after application. In contrast, a positive effect of spraying with sulphuric acid was found. Leaves, unlike the roots, do

not have acidifying mechanisms; therefore spraying with low pH solutions increases the mobility of iron compounds precipitated on the leaf surface.

Only the combination of the hormone treatment GA₃ + Kinetin significantly increased the concentration of iron in leaves of the variety Fire Blight (Table 4). Applications with IBA significantly increased the concentration of calcium in the leaves; IBA applied with Kinetin significantly increased the potassium; and IBA applied with Kinetin and GA₃ increased the concentration of phosphorus. An increased concentration of manganese was found in the leaves sprayed with GA₂ (Table 4). A positive effect of gibberellic acid on the mineral composition of plants has been reported (EL-QUESNI et al. 1989; HARB 1992). In contrast, ABDALLA et al. (1992) reported that GA₃ at 50 mg/l decreased the concentration of iron in the leaves of drought-stressed radish plants.

Soil applications of Fe-EDDHA significantly increased the iron concentration in Katerina leaves 60 days after application (Table 3); however, its effectiveness was reduced 90 days after application. Althoug Fe-EDDHA is widely used to control iron deficiency (Almaliotis, Holevas 1991; Kaundal et al. 1994), an application with Fe-EDDHA controls iron deficiency temporarily because its effectiveness is reduced with time (Table 3). Soil application with FeSO₄.7H₂O + organic matter or FeSO₄.7H₂O + organic matter + K₂SO₄ increased the chlorophyll concentration index of leaves in treated trees, but not the iron concentration. An explanation is given by Morales et al. (1998), who suggested that the iron might be immobilized in an unavailable form somewhere in the chlorotic leaves.

This work showed that foliar application of iron salts and/or acidic compounds and soil application of Fe-EDDHA can be used to increase leaf iron concentration in peach trees. Symptoms of iron deficiency can be observed in leaves of peach

trees that are infected by *A. tumefaciens*. A careful consideration is necessary when only chlorophyll concentration of leaves is used to evaluate iron chlorosis. More studies are required to confirm the overcoming effect to determine suitable concentrations, combinations and possible negative consequences of the applied treatments after many years of application.

Acknowledgement

We thank the Benaki Phytopathological Institute for supplying the two strains of *Agrobacterium tumefaciens* used in this study and Dr. Themis J. Michailides for reviewing this manuscript.

References

- ABADIA J., MORALES F., ABADIA A., 1999. Photosystem II efficiency in low chlorophyll, iron-deficient leaves. Plant and Soil, *215*: 183–192.
- ABADIA J., NISHIO J., MONGE E., MONTANES L., HERAS L., 1985. Mineral composition of peach leaves affected by iron chlorosis. Journal of Plant Nutrition, 8: 697–707.
- ABADIA A., SANZ M., RIVAS J., ABADIA J., 1989. Photosynthetic pigments and mineral composition of Fe deficient pear leaves. Journal of Plant Nutrition, *12*: 123–125.
- ABDALLA M., EL-TELWANY K., HASSANEIN R., 1992. Effect of 2,4-D, GA3 and BA on some metabolites and mineral composition of leaves of drought stressed red radish plants. Egyptian Journal of Physiological Sciences, *16*: 37–54.
- AGRIOS G., 1988. Plant Pathology. Third Edition. New York, Academic Press: 558.
- ALMALIOTIS D., HOLEVAS C., 1991. The effectiveness of iron chelate for the control of iron deficiency of peach trees. Annales de l'Institut Phytopathologique Benaki, *16*: 97–103.
- BASAR H., 2000. Factors affecting iron chlorosis observed in peach trees in the Bursa region. Turkish Journal of Agriculture and Forestry, *24*: 237–245.
- BELKHODJA R., MORALES F., SANZ M., ABADIA A., ABADIA J., 1998. Iron deficiency in peach trees: Effects on leaf chlorophyll and nutrient concentrations in flowers and leaves. Plant and Soil, 203: 257–268.
- EL-GHARBI A., JRAIDI B., BARBERA G., 1994. Performance of rootstocks of almond, peach and peach \times almond hybrids with regard to iron chlorosis. Acta Horticulturae, 373:91-97.
- EL-QUESNI F., ABO-LILA B., EL-DESOKY G., 1989. The effect of gibberellic acid on growth, oxalic acid and mineral contents of spinach plants. Bulletin of Faculty of Agriculture, University of Cairo, 40: 161–171.
- GUARDIA M., FELIPE A., ALCANTARA E., FOURNIER J., ROMERA F., ABADIA J., 1995. Evaluation of experimental

- peach rootstocks grown in nutrient solutions for tolerance to iron stress. Iron nutrition in soils and plants. In: Proceedings of the Seventh International Symposium on Iron Nutrition and Interactions in Plants. Zaragoza, Spain, 27 June–2 July 1993: 201–205.
- HARB E., 1992. Effect of foliar application with GA3 and micronutrients on growth and productivity of soyabean plants grown under different levels of fertilization. Bulletin of Faculty of Agriculture, University of Cairo, 43: 411–427.
- HERNANDEZ A., GARATE A., LUCENA J., 1995. Efficacy of commercial Fe (III)-EDDHA and Fe(III)-EDDHMA chelates to supply iron to sunflower and corn seedlings. Journal of Plant Nutrition, *18*: 1209–1223.
- KAUNDAL G., SINGH M., KAUR N., 1994. Correcting limeinduced iron chlorosis in peach rootstock seedlings. Indian Journal of Plant Physiology, *37*: 217–220.
- MORALES F., GRASA R., ABADIA A., ABADIA J., 1998. Iron chlorosis paradox in fruit trees. Journal of Plant Nutrition, *21*: 815–825.
- MORTVEDT J., 1991. Correcting iron deficiencies in annual and perennial plants: Present technologies and future prospects. Plant and Soil, *130*: 273–279.
- NEDUNCHEZHIAN N., MORALES F., ABADIA A., ABADIA J., 1997. Decline in photosynthetic electron transport activity and changes in thylakoid protein pattern in field grown iron deficient peach (*Prunus persica* L.). Plant Science Shannon, *129*: 29–38.
- PEREZ C., VAL J., MONGE E., ABADIA J., 1995. Effects of iron deficiency on photosynthetic structures in peach (*Prunus persica* L. Batsch) leaves. In: Proceedings of the Seventh International Symposium on Iron Nutrition and Interactions in Plants. Zaragoza, Spain, 27 June–2 July 1993: 183–189.
- SANZ M., CAVERO J., ABADIA J., 1992. Iron chlorosis in the Ebro river basin, Spain. Journal of Plant Nutrition, *15*: 1971–1981.
- STYLIANIDES D., SYRGIANNIDIS D., 1995. Fertilization of stone trees. Agricultural and Stockbreeding, 9: 191–197.
- STYLIANIDES D., TSIPOURIDIS C., MICHAILIDIS Z., 1988. Resistance to iron deficiency of five peach rootstocks. In: International Peach Symposium. Clemson, USA, 19–23 June 1988: 25–31.
- TAGLIAVINI M., MARANGONI B., 2002. Major nutritional issues in deciduous fruit orchards of Northern Italy. Hort-Technology, *12*: 26–31.
- TAGLIAVINI M., ABADIA J., ROMBOLA A., ABADIA A., TSIPOURIDIS C., MARANGONI B., 2000. Agronomic means for the control of iron deficiency chlorosis in deciduous fruit trees. Journal of Plant Nutrition, 23: 11–12.
- TOSELLI M., TAGLIAVINI M., MARANGONI B., SANSA-VINI S., 1997. Iron chlorosis of peaches: recognition,

prevention and therapy. In: XXII Convegno Peschicolo, Cesena, Italy, 28–30 September 1995: 108–113. VAL J., SANZ M., MONTANES L., MONGE E., TAGLIAVINI M., NEILSEN G., MILLARD P., 1995. Application of chlorophyll fluorescence to study iron and manganese deficiencies in peach tree. Acta Horticulturae, *383*: 201–209.

Received for publication January 6, 2006 Accepted after corrections February 9, 2006

Vliv rozdílných zdrojů železa, hormonu a *Agrobacterium tumefaciens* na koncentraci chlorofylu a železa v listech broskvoní

ABSTRAKT: Experimenty byly prováděny v pokusných výsadbách ovocnářského ústavu v Řecku. Cílem bylo zjistit účinek foliární aplikace rozdílných zdrojů železa a hormonu na koncentraci chlorofylu a železa v listech broskvoňových odrůd Katerina a Fire Blight na podnoži GF 677. Zjišťoval se také vliv půdní aplikace rozdílných zdrojů železa na koncentraci chlorofylu a železa v listech. Výsledky ukázaly, že koncentrace železa v listech odrůdy Katerina se zvýšila 90 dnů po postřiku FeSO₄.7H₂O samostatně nebo v kombinaci s KNO₃, močovinou, kyselinou citronovou a askorbovou. Vzestup koncentrace Fe v listech pokračoval 120 dní po aplikaci FeSO₄ v kombinaci s kyselinou citronovou a KNO_3 . Pouze kombinace hormonálního přípravku GA_3 + Kinetin zvýšila koncentraci železa v listech odrůdy Fire Blight. Půdní aplikace Fe-EDDHA průkazně zvýšily koncentraci železa v listech odrůdy Katerina 60 dní po aplikaci. Koncentrace železa v listech 90 dní po aplikaci však nebyla průkazně rozdílná od kontroly. Obsah chlorofylu (a/b) byl průkazně vyšší v listech odrůdy Katerina po postřiku kyselinou citronovou nebo $CuSO_4$ 60 dní po aplikaci. Devadesát nebo 120 dnů po postřiku průkazné rozdíly zjištěny nebyly. Aplikace hormonu a půdní aplikace průkazně neovlivnily koncentraci chlorofylu (a/b) v listech odrůd Fire Blight a Katerina. SPAD měření chlorofylu ukázala, že FE-EDDHA a FeSO₄.7H₂O s organickou látkou, aplikované půdní závlahou, zvýšily průkazně koncentraci chlorofylu v listech odrůdy Katerina ještě po 120 dnech od aplikace, zatímco listová aplikace železa a hormonu neměla průkazný vliv na koncentraci železa v listech odrůd Katerina a Fire Blight. Byl zjišťován možný účinek Agrobacterium tumefaciens (kmeny Ag-20 a Ag-28 izolované ze stromů broskvoní) na obsah chlorofylu čtyř podnoží pro broskvoně (GF 677, St. Julien 655/2, Antafuel a broskvoňový semenáč) po naočkování namočením kořenů v roztoku bakterií. SPAD měření ukázala, že koncentrace chlorofylu v listech kolísala v závislosti na podnoži. Nejnižší koncentrace chlorofylu v listech byla u podnoží St. Julien 655/2 a broskvoňový semenáč a nejvyšší byla u podnoží GF 677 a Antafuel. Když byl inokulován pouze jeden kmen Agrobacterium tumefaciens Ag-28, měly očkované podnože koncentraci chlorofylu průkazně nižší než podnože neočkované.

Klíčová slova: kyselé sloučeniny; hormony; chlorózy z nedostatku železa; broskvoně; podnože

Corresponding author:

Dr. Thomas Thomidis, Pomology Institute, NAGREF, P.O. Box 122, 59200 Naoussa, Greece tel.: + 302 332 041 548, fax: + 302 332 041 178, e-mail: thomi-1@otenet.gr