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Changes in Leaf Mn Concentrations in Mature 'Washington Navel' Orange Trees with Different Mn Status after a Single Foliar Application of $MnSO_4$

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Summary

Mn deficiency is a common nutritional disorder affecting the growth, yield and fruit quality of citrus plants. Twenty-three-year-old 'Washington Navel' orange (*Citrus sinensis* L.) trees budded onto sour orange (*C. aurantium* L.) rootstock and grown in two sites (I and II) of an orchard were used. Based on the leaf Mn concentrations, 20 Mn deficient and 10 non Mn deficient trees were selected on 26 January 2000. The trees were separated in 6 groups; each one consisted of 5 trees with similar Mn status. The mean Mn concentrations in the leaves of the groups 1, 2, 3, 4, 5, and 6 were 8.8, 10.5, 11.2, 13.8, 18.3, and 19.8 mg kg^{-1} dry weight (d.w.), respectively. The trees of the groups 1–4 were grown in the site I, while those of the groups 5 and 6 in the site II of the orchard. One tree group in each orchard site (site I: group 3; site II: group 6) was not sprayed with Mn and used as a control, while the trees of the other groups (1, 2, 4 and 5) were sprayed on 06 February 2000 with $MnSO_4 \cdot H_2O$ (800 mg l^{-1} Mn). From 18 February 2000 until 19 January 2001, 11 leaf samplings were conducted to monitor the Mn status of the trees. The concentrations of Mn in the leaves of the groups 3 and 6 (controls) were almost constant and presented no significant differences among the samplings. With regard to the Mn treated trees, at the first post-treatment sampling (18 February 2000), the leaf Mn levels were higher by 16.5–25.1 mg kg^{-1} d.w., respectively, in relation to their pre-treatment status. At the last sampling (19 January 2001), the Mn status of the trees was also improved by 4.5–7.5 mg kg^{-1} (d.w.), compared to their pre-treatment status. Overall, when the concentration of Mn in the leaves is equal or higher than 14 mg kg^{-1} d.w., before the spray Mn treatment, only one application of $MnSO_4 \cdot H_2O$ (800 mg l^{-1} Mn) on February is sufficient to cover the Mn needs of the trees for a whole year. In other cases (leaf Mn levels equal or lower than 13 mg kg^{-1} d.w.), the trees should be re-sprayed on July or August.

Key words. citrus – deficiency – fertilizer – manganese sulfate – spray

Introduction

Citrus plants are very sensitive to Mn deficiency (Bergmann 1992; Marschner 1995; Kabata-Pendias and Pendias 2001). Mn deficiency is a very common nutritional disorder affecting the physiology, the anatomy, the growth and the yield of citrus trees all over the world including many regions of Greece. Indeed, citrus plants grown under Mn deficiency conditions are characterised by: a) a low photosynthetic performance, especially due to disturbances in photosystem II activity, b) a high sensitivity to the photoinhibition of photosynthesis, c) a reduction of the leaf chlorophyll concentration, d) a decrease of the chloroplast size as well as of their number per leaf cell area, e) swelling of the thylakoid membranes (mild deficiency) or structural damages in both the peripheral membranes and thylakoids of the chloroplasts (severe deficiency), f) a decreased plant growth, g) a decreased tree yield and h) a poor coloration of fruits (Papadakis 2004).

According to Bergmann (1992), the most efficient way to prevent and correct Mn deficiency is the foliar application of solutions containing Mn sulphate ($MnSO_4$) or Mn chelate (MnEDTA). However, in a previous experiment where Mn deficient orange plants were sprayed at a rate of 800 or 1200 mg l^{-1} Mn in the form of $MnSO_4 \cdot H_2O$ or MnEDTA, $MnSO_4 \cdot H_2O$ was more efficient than MnEDTA (Papadakis et al. 2005). Similar results have been reported for some other fruit tree species such as apple (Thalheimer and Paoli 2002).

When Mn deficiency occurs in citrus and some other tree crops, foliar applications of Mn solutions are recommended every time a new vegetation flush grows, due to limited Mn mobility within plant tissues. However, a greenhouse experiment including two citrus genotypes revealed that a part of the foliar applied Mn could be transported from the sprayed shoots to the new flush of growth after the spray treatment (Papadakis et al. 2007). Similar results were recorded in a different study with

young 'Navelate' orange plants (PAPADAKIS 2004). From a practical point of view, growers would like to know how long a single Mn spray treatment covers the Mn needs of mature citrus trees suffering from Mn deficiency.

The objectives of this study were: 1) to monitor the variation of Mn concentrations in the leaves for a whole year after a single spray on mature 'Washington Navel' orange trees with 800 mg l^{-1} Mn; and 2) to evaluate whether a second foliar Mn treatment was required to maintain leaf Mn concentration above the deficiency range.

Materials and Methods

Plant material and growth conditions

Twenty-three-year-old orange trees (*Citrus sinensis* L. var. 'Washington Navel'), grafted on sour orange (*C. aurantium* L.) rootstock and cultivated in an orchard, which was located close to the Olive Tree and Subtropical Plants Institute of Chania (Crete, Greece), were used in this study. All the experimental trees were grown under uniform cultural and management conditions and planted at a spacing of 5 x 5 m.

Leaf tissue and soil samplings

The trees, which were grown in two sites of the orchard, were differing in their leaf nutrient status, e.g. visible symptoms of Mn deficiency (interveinal leaf chlorosis with dark green veins) were present only in the trees cultivated in site I of the orchard. On 26 January of 2000, leaf and soil samples were collected to evaluate the Mn status of each tree growing in the grove (site I, site II) and the physical and chemical properties of the soil (site I, site II). Twenty to thirty leaves (4- to 6-month-old) per tree, from the last flush of growth, were taken from non-fruiting terminals. Afterwards, the leaves were thoroughly washed initially with tap water in order to replace the Mn fixed in the negative charges of the leaf free space by the calcium and magnesium salts contained in the water (EPSTEIN 1972). The pH of washing water was acidic. Then, the leaves were also washed twice with deionized water, oven-dried at 75 °C for 3 days and milled to a fine powder to pass a thirty-mesh screen. Dried and milled samples (0.5 g) were dry-ashed for 6 h at 550 °C, dissolved in 3 ml of 6 N HCl and diluted with deionized water up to final volume of 50 ml. Leaf Mn concentration was determined by atomic absorption spectroscopy. For soil analysis, a composite sample (20 cores) was taken from each of the two sites of the orchard at a depth of 0.05 to 0.45 m. Each sample was air-dried, crushed, and tested for pH, electrical conductivity, free $CaCO_3$, organic matter, exchangeable Ca and Mg (EDTA). The available K was determined after extraction with ammonium acetate, and the micronutrients (Mn, Zn and Fe) after extraction with DTPA (Diethylenetriamine-N,N,N',N",N"-pentaacetic acid). Finally, the available P was extracted by the Olsen's procedure and analyzed by the vanado-molybdo-phosphate yellow color method.

Treatments

Based on the leaf Mn concentrations of trees (derived from the inorganic analysis of leaves sampled on 26 January 2000), thirty trees were selected, i.e., 20 Mn deficient trees (leaf Mn concentration <18 mg kg^{-1} d.w.; MILLS and JONES Jr. 1996) grown in the site I of the orchard, while the other 10 trees (non Mn deficient) in the site II of the orchard. The trees were separated in 6 groups. Each group consisted of 5 trees (replications) with similar Mn status. The mean Mn concentration in the leaves was 8.8, 10.5, 11.2, 13.8, 18.3, and 19.8 mg kg^{-1} d.w. for the trees of the groups 1, 2, 3, 4, 5, and 6, respectively. The trees of the groups 1–4 were grown in the site I of the orchard, while those of the groups 5 and 6 in the site II.

Twelve days after the first leaf sampling (i.e., 6 February 2000), the trees of the groups 1, 2, 4, and 5 were sprayed with 800 mg l^{-1} of Mn as $MnSO_4 \cdot H_2O$, while those of the groups 3 and 6 were not treated with Mn and served as controls for the sites I and II, respectively. The volume of the spray solution was 10 l per tree.

Twelve days after the foliar application of Mn (i.e., 18 February 2000), another leaf sampling was conducted to re-determine the Mn status of the trees. This procedure was repeated 10 times until the middle of January of 2001 (17 March 2000, 21 April 2000, 27 May 2000, 01 July 2000, 31 July 2000, 31 August 2000, 09 September 2000, 12 November 2000, 09 December 2000, and 19 January 2001) to monitor the annual variation of leaf Mn concentration of the trees.

Statistics

Each of the 6 groups of trees consisted of 5 trees (replications). Leaf Mn concentrations in all experimental trees were measured 11 times through the experimental period (26 January 2000 until 19 January 2001). The data were subjected to analysis of variance (ANOVA). The comparison of the mean leaf Mn concentrations of each group of trees among the different sampling dates was conducted with the Duncan's multiple range test for $P \leq 0.05$.

Results and Discussion

Pre-treatment status

Based on the relative percentages of the three major soil particles (sand, silt, and clay), the soils of the sites I and II were classified as SCL (sandy clay loam) and SiL (silty loam), respectively (Table 1). The soil of the site I contained more Mn, $CaCO_3$ and exchangeable Ca and K, and less Zn, Fe and exchangeable Mg compared to the soil of the site II. With regard to P concentration and electrical conductivity of the soil solution, no differences were found between the two sites.

The analysis of the leaf samples collected before the Mn foliar treatment (i.e., 26 January 2000) indicated that the trees of the site I had very low Mn concentrations (<14 mg kg^{-1} d.w.). However, leaf Mn concentrations of the trees grown in the site II were higher than 18 mg kg^{-1} d.w. (Fig. 1). Based on the most generally accepted standards of Mn critical levels in the orange leaves proposed by MILLS and JONES Jr. (1996), the leaf Mn concentrations of the trees grown in the sites I and II were deficient and just sufficient, respectively. The fact that the trees grown in the two sites of the grove differed from each other concerning their leaf Mn status seems to be as-

Table 1. Physical and chemical properties of the soil samples taken from the sites I and II of the orchard.

	Site I	Site II
Texture	SCL	SiL
pH (H_2O:Soil, 1:1)	7.6	6.9
EC (dS m^{-1})	1.8	1.8
Organic matter (%)	0.67	1.08
Free $CaCO_3$ (%)	5.3	0.0
P (mg kg^{-1})	27	24
K exchangeable (mg kg^{-1})	250	160
Ca exchangeable (mg kg^{-1})	620	452
Mg exchangeable (mg kg^{-1})	26	74
Mn (mg kg^{-1})	40	26
Zn (mg kg^{-1})	3	12
Fe (mg kg^{-1})	19	40

cribed to some soil factors and not to variation of the cultivation practices and/or climatic factors, since the experimental trees were grown under uniform cultural and management practices.

Deficient Mn levels in the leaves of citrus plants are more likely to occur when soil Mn (DTPA-extracted) ranges from 1 to 5 mg kg^{-1} (Kabata-Pendias and Pendias 2001). In the present study, both soils contained much more Mn than the above mentioned critical range. Moreover, the soil Mn concentration in the site I (40 mg kg^{-1}) was higher than that of site II (26 mg kg^{-1}), although leaf Mn deficiency was exclusively observed in the site I. The higher leaf Mn levels recorded in the site II, compared to the site I, was apparently due to higher availability of Mn^{2+} to the root systems of the trees growing in the site II. Soil pH is the key factor which influences the availability of Mn in soils. The two sites of the experimental grove differed from each other concerning the soil pH value (site I, pH 7.6; site II, pH 6.9). In general, an increase of soil pH value by one unit causes a diminution of Mn^{2+} availability by about 100 times (Lindsay 1972). In contrast to the site II, the presence of 5.3 % $CaCO_3$ as well as the higher concentration of exchangeable Ca^{+2} in the site I are two other factors contributing to the very low leaf Mn concentration found in the trees grown in the site I.

Post-treatment status

The concentrations of Mn in the leaves of unsprayed trees in both sites of the orchard (groups 3 and 6) presented no statistically significant variation during the studied period (Fig. 1). In a previous study with 'Encore' mandarin trees, Mn also showed least change (Papadakis et al. 2004).

The annual variation of Mn concentration in the leaves of the Mn sprayed trees (Fig. 1) showed that: (i) the spray application of Mn resulted initially in an increase of leaf Mn levels by 16.5 to 25.1 mg kg^{-1} d.w. (18 February 2000), (ii) afterward, a progressive decline of leaf Mn concentration was a fact, and (iii) leaf Mn levels were stabilised at the last 3–5 samplings. Comparing the results of the first sampling (26 January 2000) with those of the last one (19 January 2001), it is obvious that on 19 January of 2001 the concentrations of Mn were higher by 4.5 to 7.5 mg kg^{-1} d.w.. Furthermore, the leaf Mn levels of the groups 4 and 5 were continuously above the deficiency limit of 18 mg kg^{-1} d.w., irrespective of the sampling date. Finally, the leaf Mn concentrations of the group 5 trees were within the sufficiency range (>25 mg kg^{-1} d.w.) during the entire post-treatment period.

The increase of leaf Mn concentration in various plant species as an effect of the foliar application of $MnSO_4 \cdot H_2O$ has also been reported by many researchers (Broschat 1991; Swietlik and Laduke 1991; Smith et al. 2001; Papadakis et al. 2007). A nutrient element after its absorption by the leaves may move to other plant tissues and organs. However, the degree of an element translocation among various plant organs varies extremely and depends greatly on the mobility of the element as well as on plant organ and its age. Moreover, a part of an already absorbed nutrient element may leach out from the foliage by means of the rainfall. This leaching concerns not only the part of already absorbed quantity of the element located in the apoplast, but also that found in the surface of leaves. Of course, the degree of depletion of each element depends on the functional and metabolic role that the specific nutrient plays in the plant cells (Kabata-Pendias and Pendias 2001). Consequently, the progressive decrease of Mn concentrations in the leaves of groups 1, 2, 4 and 5 observed during the post-spraying period was more likely caused by: (i) Mn depletion from the foliage due to rainfalls and (ii) Mn translocation to other plant tissues and organs, mainly to the new vegetation, that was formed after the middle of April. In citrus plants, Mn translocation from the shoots sprayed with Mn to those grown after the Mn treatment was also reported in other experiments (Papadakis 2004; Papadakis et al. 2007). In apple trees, a part of Mn applied to the leaves was found in the roots (Wooldridge 2002).

An indirect indication about partial transport of Mn from the sprayed leaves to the new vegetation grown after the Mn treatment may be concluded from the data of this study. Given that in each sampling we collected 4- to 6-month-old leaves from the last flush of growth, the leaves of the last 4 samplings did not come in contact with the spraying solution, since they were developed after the middle of April (spring flush of vegetation). In fact, the leaf sampling that carried out on 31 August of 2000 was the last sampling in which Mn sprayed leaves were collected. In the following samplings, the collected leaves were grown after the Mn spray treatment. Consequently, the noteworthy increased leaf Mn concentrations measured in the last 4 samplings, in contrast to those recorded at the first sampling (26 January 2000), it is rather a result of partial transport of absorbed Mn from the sprayed leaves and stems to the new vegetation. Furthermore, since the variation of Mn in the leaves of groups 3 and 6 (control trees) is non-significant (Fig. 1), it is impossible the increased leaf concentrations of Mn recorded in the groups 1, 2, 4 and 5 at the last 4 samplings not to be ascribed to Mn transport from the sprayed tissues.

The relative stability observed at the last 3–5 samplings with regard to the leaf Mn levels is more probably related with the reduction of ability of the sprayed leaves to supply the newer vegetation with Mn. In addition, it should not be ignored the need of fruits for Mn. In other

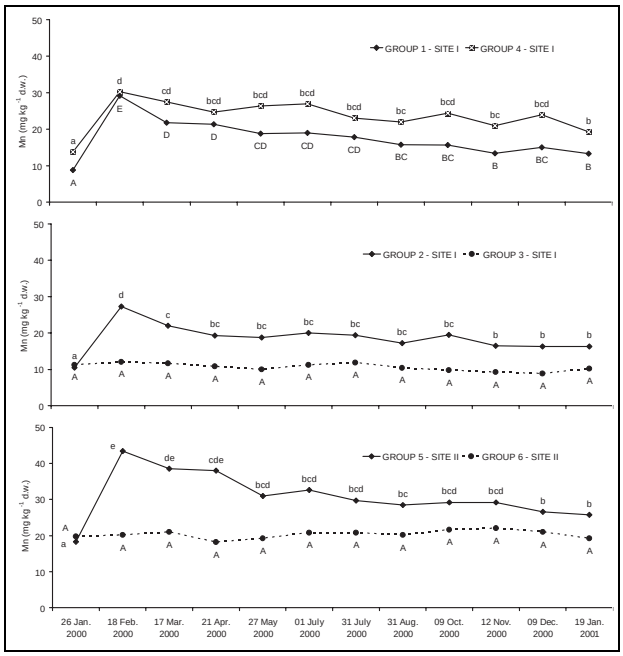


Fig. 1. The variation of Mn concentrations in the leaves of the trees grown in the sites I (groups 1, 2, 3, 4) and II (groups 5, 6) of the orchard. The trees of the groups 1, 2, 4 and 5 were sprayed with $MnSO_4 \cdot H_2O$ (800 mg l^{-1} Mn) on 6 February 2000. The trees of the groups 3 and 6 were not treated with Mn sprays and served as controls for the sites I and II, respectively. The means of each tree group marked with the same letter(s) do not differ significantly from each other (Duncan's multiple range test, $P \leq 0.05$).

words, the previously mentioned stability could be ascribed to the achievement of a relative balance between the Mn sources (treated-leaves and -stems) and those organs that function as main sinks of Mn such as the fruits and the post-treatment vegetation.

According to MILLS and JONES Jr. (1996), the limit of leaf Mn concentration in orange trees, under which Mn deficiency symptoms occur, is the value of 18 mg kg^{-1} d.w.. However, leaf Mn levels above 25 mg kg^{-1} d.w. are considered sufficient. In the present study, the average leaf Mn concentrations in the trees of groups 1 and 2 were under the limit of 18 mg kg^{-1} d.w. from the end of months July and August, respectively. Instead, the leaf Mn levels of groups 4 and 6 in all the post-treatment samplings were above the deficiency limit, while those of the group 5 were steadily above the 25 mg kg^{-1} d.w.. Consequently, in the cases where the concentration of Mn in the leaves is equal or higher than 14 mg kg^{-1} d.w., before the spray application of Mn, only one application of $MnSO_4 \cdot H_2O$ (800 mg l^{-1} Mn) on February is sufficient and covers the Mn needs of the trees for a whole year. In other cases (leaf Mn levels equal or lower than 13 mg kg^{-1} d.w.), the trees should be sprayed again on July or August.

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