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T. A. Chatzistathis[a]; I. E. Papadakis[a]; I. N. Therios[a]; A. Giannakoula[b]; K. Dimassi[a]
[a] Laboratory of Pomology, Aristotle University, Thessaloniki, Greece [b] Department of Botany, Aristotle University, Thessaloniki, Greece

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IS CHLOROPHYLL FLUORESCENCE TECHNIQUE A USEFUL TOOL TO ASSESS MANGANESE DEFICIENCY AND TOXICITY STRESS IN OLIVE PLANTS?

T. A. Chatzistathis,[1] I. E. Papadakis,[1] I. N. Therios,[1] A. Giannakoula,[2] and K. Dimassi[1]

[1]*Laboratory of Pomology, Aristotle University, Thessaloniki, Greece*
[2]*Department of Botany, Aristotle University, Thessaloniki, Greece*

□ *A 130-day hydroponic experiment was carried out in a glasshouse to examine whether manganese (Mn) concentration in the nutrient solution affects the nutritional status of olive plants and to find out whether the chlorophyll fluorescence technique is suitable to assess Mn toxicity and/or deficiency stress in olive plants prior to the appearance of these two nutritional disorders. For this purpose, chlorophyll fluorescence parameters (F_v/F_m and F_v/F_0 ratios) were recorded every 40 days in the leaves of 'Kothreiki' and 'FS-17' olive cultivars, which were irrigated with Hoagland's nutrient solutions containing various Mn concentrations. In parallel the elongation of the main shoot of all experimental plants, as well as the concentrations of Mn, iron (Fe), zinc (Zn), boron (B), phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K) in their leaves were recorded. The following Mn treatments were applied: 0 μM Mn (to induce Mn deficiency), 40 μM Mn (to promote normal growth), and 640 μM Mn (to induce Mn toxicity). Our results indicated that not only the rate of shoot elongation but also the fluctuation with time of the leaf concentrations of all determined mineral elements (except for Mn) was not significantly affected by the Mn concentration in the nutrient solution, irrespectively of the cultivar. This was not observed with regard to the time variation of the F_v/F_m and F_v/F_0 ratios, where the values of these parameters were significantly reduced in the 640 μM Mn treatment at the 80th and 130th day of the experiment in both olive cultivars, compared to the relevant previous ones (those of the days 0 and 40th), something which did not happen in the other two Mn treatments (0 and 40 μM). However, in none of the two cultivars tested and in any of the three Mn treatments (0, 40 and 640μM) the F_v/F_m and F_v/F_0 ratios did not drop below the critical values of 0.8 and 4, respectively, even at the end of the experiment, where high Mn concentrations were found in the leaves of both cultivars treated with 640 μM Mn (616 $\mu g\ g^{-1}$ d.w. in 'FS-17' and 734 $\mu g\ g^{-1}$ d.w. in 'Kothreiki'). Symptoms of Mn toxicity (curling and brown speckles) were observed in the top leaves of both cultivars, after the 90th day of the experiment. At the same time, the final leaf Mn concentrations (those of the 130th day of the experiment) in plants grown under 0 μM Mn were 23 $\mu g\ g^{-1}$ d.w. in 'FS-17' and 20 $\mu g\ g^{-1}$ d.w. in 'Kothreiki', i.e., a little above of the deficiency range (<20 $\mu g\ g^{-1}$ d.w.). At the 130th day, Mn concentration in nutrient solution, as well as Mn concentration in the leaves of*

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Address correspondence to T. A. Chatzistathis, Laboratory of Pomology, School of Agriculture, Aristotle University, 54124 Thessaloniki, Greece. E-mail: ChChatzi@in.gr

both olive cultivars was negatively correlated with the leaf concentration of Fe and the values of the F_v/F_m and F_v/F_0 ratios, and positively with the concentrations of Zn and P in the leaves. Finally, the periodical measurement of the F_v/F_m and F_v/F_0 ratios was proved to be a non-reliable means to predict the appearance of the visible symptoms of Mn toxicity in olive leaves (although their values declined significantly at the 80th and 130th day of the experiment in both olive cultivars).

Keywords: chlorophyll fluorescence, manganese, photosystem II, olive cultivars, deficiency, toxicity

INTRODUCTION

Manganese (Mn) toxicity is a nutritional disorder affecting growth and yield of many plant species, mostly in acid soils (pH < 5), in soils rich in Mn parent material and/or poor in oxygen, and in glasshouses after soil sterilization. Furthermore, Mn toxicity conditions are common in areas where the crops are over-fertilized with ammonium sulfate $[(NH_4)_2SO_4]$ and/or Mn containing fertilizers, and in agricultural areas located nearby to highly polluted areas (El-Jaoual and Cox, 1998; Qing-Ren et al., 2002). On the contrary, when Mn concentration in leaves of different plant species is lower than 10–20 mg kg^{-1} d.w., vegetative growth is suppressed due to Mn deficiency (Hannam and Ohki, 1988; Marschner, 1995; Misra, 1997). Manganese deficiency is common in organic, calcareous or calciferous soils, in highly acid-leached soils and/or in soils with fluctuating moisture content. Chlorophyll fluorescence has been used to make a rapid quantification of the response of different species and varieties to certain stresses, such as drought (Faraloni et al., 2006), salinity (Wang et al., 2004), UVA or UVB stress (Adebooye et al., 2008), macro or micronutrient deficiency (Balakrishnan et al., 2000), heavy metal toxicity (Hajiboland and Hasani, 2007; Liu et al., 2008), and damage caused by pesticides (Chagas et al., 2008). Recently, chlorophyll fluorescence technique has been used in various plant species to assess the function of PSII of photosynthesis under Mn deficiency (Henriques, 2003, 2004; Hebbern et al., 2005) and toxicity (Hajiboland and Hasani, 2007) stress. The cultivation of olive tree is very important for many countries, especially those of the Mediterranean basin, for historical, ecological, and economical reasons. There is a lack of papers where both the effects of Mn deficiency and toxicity on olive plants' growth, nutritional status, and PSII activity have been investigated together, under the same experimental conditions. Therefore, one of the aims of the present study was to find out whether the availability of Mn in rhizosphere of olive plants affects the concentrations of various nutrient elements in leaves. The other aim was to study whether the values of various chlorophyll fluorescence parameters could be used as a fast and reliable indicator in assessing Mn deficiency and toxicity stress in olive plants. For these purposes, the time variation of

the concentrations of seven nutrient elements [iron (Fe), zinc (Zn), boron (B), calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P)] as well as of the chlorophyll fluorescence parameters (F_v/F_m and F_v/F_0) in the leaves of the olive cultivars 'Kothreiki' and 'FS-17' was determined, at regular time intervals, during the whole experimental period. The cultivars 'Kothreiki' and 'FS-17' were chosen for this investigation because the first one is an important dual purpose (oil producing and for table olives) Greek cultivar, while the second one is a quickly-expanding one and suitable for mechanical harvesting.

MATERIALS AND METHODS

Plant Material and Mn Treatments

Thirty well-grown (about 30 cm in height) olive plants (*Olea europea* L., cvs. 'Kothreiki' and 'FS-17') coming from a commercial nursery were transplanted into 5 L black plastic bags containing perlite. After transplanting, the plants of each cultivar were divided into three groups each one consisted of five plants (replicates). The total number of the experimental plants was 30 (three treatments × two cultivars × five replicates). The experiment was carried out during the period between the 9 March and the 17 July. The plants were grown in a glasshouse, in the experimental farm of the Aristotle University of Thessaloniki, northern Greece, under natural light conditions, with minimum, average and maximum temperature of 17, 29, and 36°C, respectively; the relative humidity was ranged from 54 to 82%. During the experimental period (130 days), all the plants were irrigated every second day, based on their needs, with a 50% modified Hoagland's nutrient solution (macronutrients were supplied at half strength and micronutrients, except for Mn, at full strength). As far as the Mn treatments are concerned, they were the following: 0 μM Mn (to induce Mn deficiency), 40 μM Mn (to promote normal growth), and 640 μM Mn (to induce Mn toxicity). The concentration of 40 μM Mn was chosen as the normal one, based on the results of preliminary experiments, which were carried out with these two olive cultivars under similar experimental conditions.

Plant Growth Parameters

Leaf samples were collected from all the experimental plants every about 40 days (at the day 0, as well as at the 40th, 80th and 130th day-last day of the experiment). Each one of the samples was weighed (fresh weight, f.w.), washed initially with tap and afterwards twice with distilled water, oven-dried at 75°C for 48 hours and weighed again (dry weight, d.w.). Furthermore, the elongation of the main shoot of each one experimental plant was recorded every 10 days.

Determination of Mn and Other Mineral Elements

In order to determine the concentrations of mineral elements, a portion of 0.5 g of the fine powder of each dried and milled leaf sample was dry-ashed in a muffle furnace at 515°C for 5h. Then, the ash was dissolved in 3 mL of 6 N hydrochloric acid (HCl) and diluted with deionized water up to 50 mL. The concentrations of Mn, Fe, Zn, Ca, Mg, and K were determined by atomic absorption spectroscopy (Perkin-Elmer 2340, Waltham, MA, USA). Then, the concentrations of P and B were assayed colorimetrically; P in 470 nm, using the vanado-molybdo-phosphate yellow method (Page et al., 1982) and B in 420 nm by the azomethine-H method (Wolf, 1971).

Chlorophyll Fluorescence Parameters

The chlorophyll fluorescence parameters (F_v/F_m and F_v/F_0; F_0: initial fluorescence, F_m: maximum fluorescence, $F_v = F_m$-F_0: variable fluorescence) were recorded every about 40 days (at the day 0, as well as at the 40th, 80th and 130th day-last day of the experiment) by the PAM-2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany), after preconditioning of leaves in the dark for 20 min.

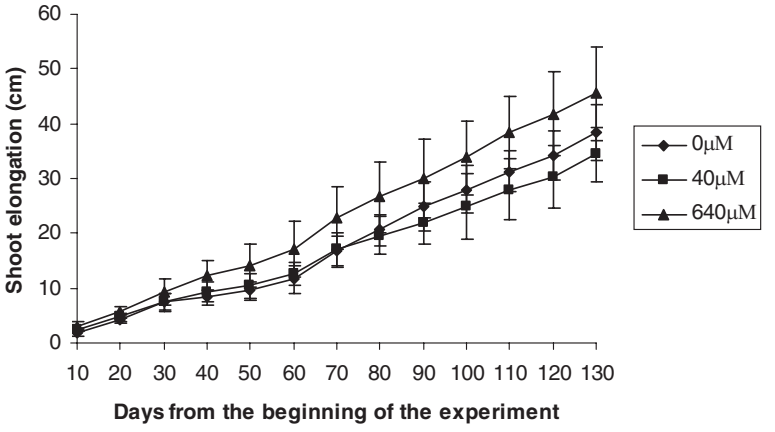
Statistics

The experimental layout was a 3 × 2 factorial with three Mn treatments (0, 40, and 640 μM Mn), two olive cultivars ('Kothreiki' and 'FS-17') and five replicates (n = 5) per treatment. The data were analyzed using the SPSS statistical package (SPSS, Inc., Chicago, IL, USA). For comparison of the means, the Duncan's multiple range test was used. Furthermore, the correlation coefficients between some of the parameters determined in the present experiment were also calculated using the same statistical package.

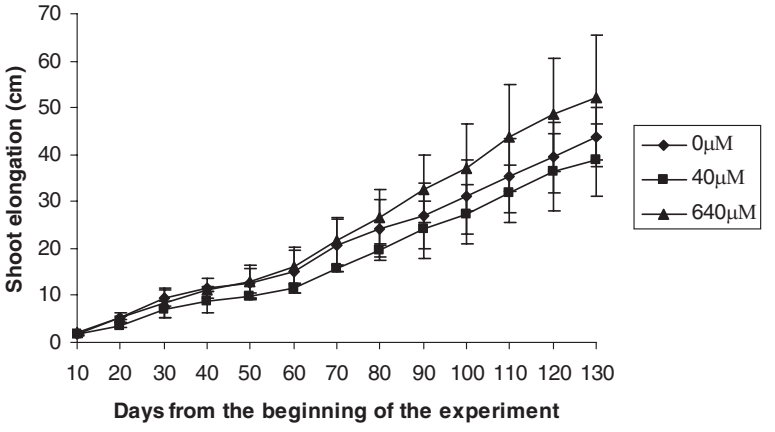
RESULTS

Plant Growth

For both studied cultivars, the elongation of the main shoot of the experimental plants during the whole experimental period, at 10-days intervals, in relation to Mn treatment is presented in Figure 1. As it is obvious, the influence of Mn treatment on the total elongation (final length) of the main shoot was non-significant for both cultivars. In both cultivars, the time variation of the elongation rate of the main shoot was similar for all Mn treatments and was not affected by Mn concentration in nutrient solution (Figure 1). In general, the minimum elongation rate of the main shoot was recorded between the 40th and the 50th day of the experiment, whereas the maximum one between the 60th and the 110th day (Figure 1), depending



(A)



(B)

FIGURE 1 Shoot elongation (Mean ± SE) of the olive cultivars (A) 'Kothreiki' and (B) 'FS-17' during the whole experimental period (at 10-days intervals) as affected by the Mn concentration in the nutrient solution.

also on the olive cultivar and Mn treatment. Of significance is also the fact that Mn toxicity symptoms (necrotic spots and leaf curling) were observed after the 90th day of the experiment in the top leaves of the plants treated with 640 μM Mn, irrespectively of the cultivar.

Time Variation of the Concentrations of Eight Nutrient Elements in Leaves

The time variation of the concentrations of Mn, Fe, Zn, B, P, K, Ca, and Mg in the leaves of the cultivars 'Kothreiki' and 'FS-17' as affected by Mn concentration in the nutrient solution is shown in the Tables 1 and 2,

TABLE 1 Time variation of the concentrations of Mn, Fe, Zn, B, P, K, Ca and Mg in the leaves of the olive cultivar 'Kothreiki' as affected by Mn concentration in the nutrient solution

Nutrient element	Mn treatment	Day 0	40th day	80th day	130th day
Mn (μg/g. d.w.)	0 μM	30a	26a	23b	20c
	40 μM	30a	28a	25b	72b
	640 μM	30a	30a	55a	734a
Fe (μg/g. d.w.)	0 μM	68a	88a	63a	85ab
	40 μM	68a	78a	68a	98a
	640 μM	68a	79a	58a	62b
Zn (μg/g. d.w.)	0 μM	22a	26a	16b	12b
	40 μM	22a	19a	24a	15ab
	640 μM	22a	19a	19ab	21a
B (μg/g. d.w.)	0 μM	10a	17a	15a	44a
	40 μM	10a	14a	14a	41a
	640 μM	10a	16a	15a	47a
P (% d.w.)	0 μM	0,18a	0,14a	0,17a	0,23a
	40 μM	0,18a	0,11a	0,20a	0,28a
	640 μM	0,18a	0,07b	0,20a	0,30a
K (% d.w.)	0 μM	1,02a	1,23a	1,10b	1,90a
	40 μM	1,02a	1,13a	1,12ab	1,88a
	640 μM	1,02a	1,09a	1,36a	1,88a
Ca (% d.w.)	0 μM	1,03a	1,38a	1,29a	1,11a
	40 μM	1,03a	1,36a	1,21a	1,17a
	640 μM	1,03a	1,45a	1,23a	1,04a
Mg (% d.w.)	0 μM	0,08a	0,14a	0,14a	0,12a
	40 μM	0,08a	0,12a	0,17a	0,12a
	640 μM	0,08a	0,13a	0,16a	0,13a

The different letters signify statistically significant differences between the three Mn treatments, in each sampling date (Duncan's multiple range test).

respectively. In general, the concentration of Mn in nutrient solution did not cause any remarkable change in the pattern of fluctuation of the concentrations of these nutrient elements (except Mn) in the leaves of both studied cultivars.

After the 80th day from the beginning of the experiment, Mn concentration in the leaves of both olive cultivars decreased in the plants irrigated with nutrient solution without Mn (0 μM Mn), remained about at the same levels ('FS-17') or slightly increased ('Kothreiki') at the 40 μM Mn treatment, while in the treatment of 640 μM Mn it was steeply increased. The concentration of Fe in the leaves of both cultivars was also fluctuated with time, with minimum values in most cases taking place at the 80th day of the experiment, depending also on olive cultivar and Mn treatment. Zinc concentration in leaves, in most cases, was slightly decreased with time (Tables 1 and 2).

The concentration of P in the leaves of both olive cultivars was fluctuated with time. Its minimum concentrations were determined at the 40th or at the 80th day of the experiment, depending on cultivar and Mn treatment, while the maximum ones at the 130th day (end of the experiment) (Tables 1

TABLE 2 Time variation of the concentrations of Mn, Fe, Zn, B, P, K, Ca and Mg in the leaves of the olive cultivar 'FS-17' as affected by Mn concentration in the nutrient solution

Nutrient element	Mn treatment	Day 0	40th day	80th day	130th day
Mn (μg/g. d.w.)	0 μM	52a	42b	53b	23c
	40 μM	52a	64ab	49b	55b
	640 μM	52a	71a	101a	616a
Fe (μg/g. d.w.)	0 μM	60a	102a	53b	82a
	40 μM	60a	53b	74a	70a
	640 μM	60a	48b	43b	66a
Zn (μg/g. d.w.)	0 μM	25a	17a	21a	18b
	40 μM	25a	20a	21a	20b
	640 μM	25a	17a	16a	35a
B (μg/g. d.w.)	0 μM	18a	17a	12a	37a
	40 μM	18a	18a	13a	35a
	640 μM	18a	18a	12a	36a
P (% d.w.)	0 μM	0,20a	0,12a	0,10b	0,18b
	40 μM	0,20a	0,16a	0,11b	0,35a
	640 μM	0,20a	0,14a	0,14a	0,33a
K (% d.w.)	0 μM	1,03a	1,26a	1,33a	2,08a
	40 μM	1,03a	1,41a	1,09a	2,02a
	640 μM	1,03a	1,35a	1,15a	1,82a
Ca (% d.w.)	0 μM	1,05a	1,65a	1,38a	2,59a
	40 μM	1,05a	1,53a	1,32a	2,63a
	640 μM	1,05a	1,55a	1,43a	2,71a
Mg (% d.w.)	0 μM	0,11a	0,17a	0,14a	0,11a
	40 μM	0,11a	0,17a	0,17a	0,07b
	640 μM	0,11a	0,16a	0,16a	0,11a

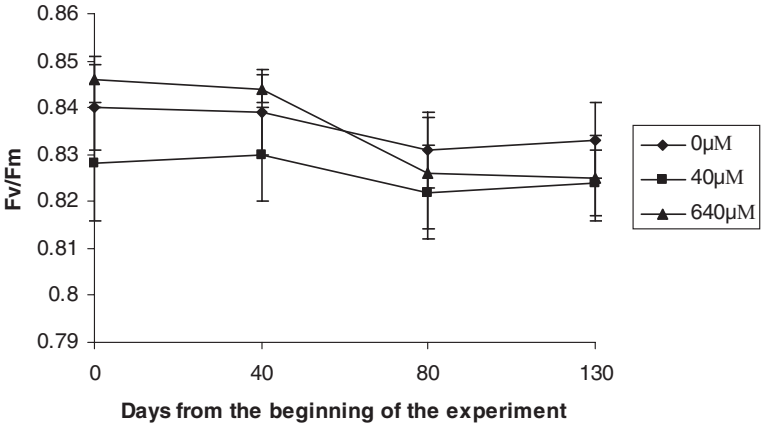
The different letters signify statistically significant differences between the three Mn treatments in each sampling date (Duncan's multiple range test).

and 2). Potassium concentrations in the leaves of both 'Kothreiki' and 'FS-17', in almost all treatments, was continuously increasing from the beginning until the end of the experiment (130th day), while those of Ca and Mg were higher at the middle of the experiment (40th and 80th day), than at its beginning (day 0) and end (130th day) (Tables 1 and 2).

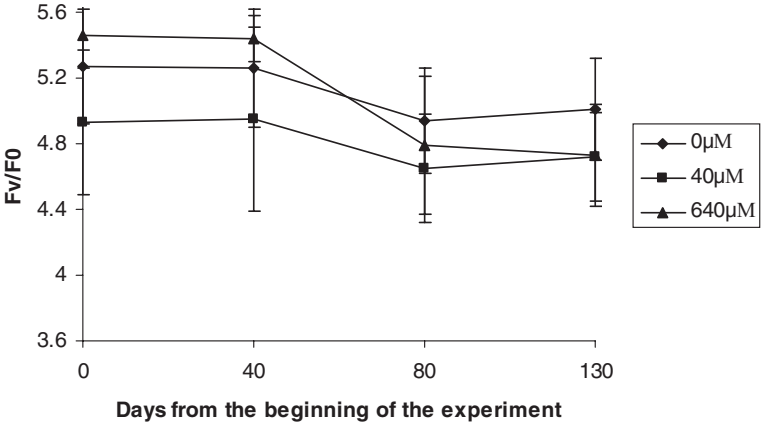
It should also be pointed out that, with the exception of: a) the Mn concentrations in the leaves of the plants grown at the 0 μM Mn treatment and b) the leaf Zn concentrations in the 0 and 40 μM Mn treatments, the final concentrations (determined at the 130th day of experiment) of all other nutrient elements were either greater or at about the same levels, than the corresponding initial ones (day 0). Furthermore, the final concentrations of Mn in the leaves of the 0 μM Mn-treated plants were lower by 33 and 56% for 'Kothreiki' and 'FS-17', respectively, compared to the corresponding initial concentrations (Tables 1 and 2).

Time Variation of the Chlorophyll Fluorescence Parameters

The time variation of the chlorophyll fluorescence parameters F_v/F_m and F_v/F_0 of the cultivars 'Kothreiki' and 'FS-17', in relation to the Mn



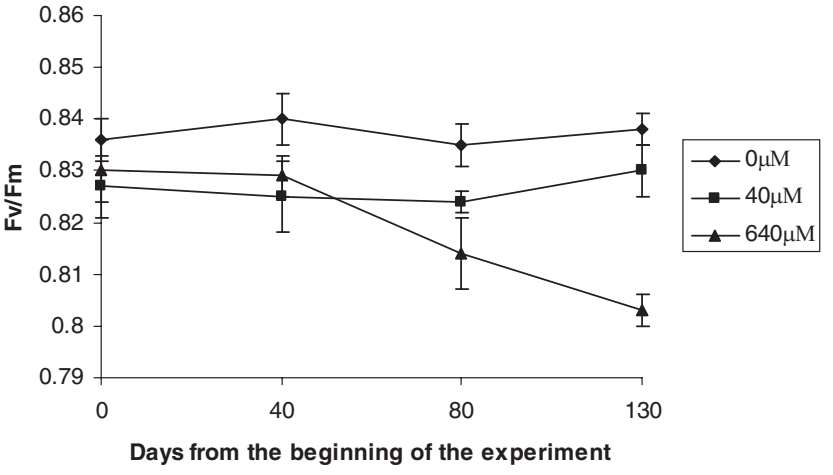
(A)



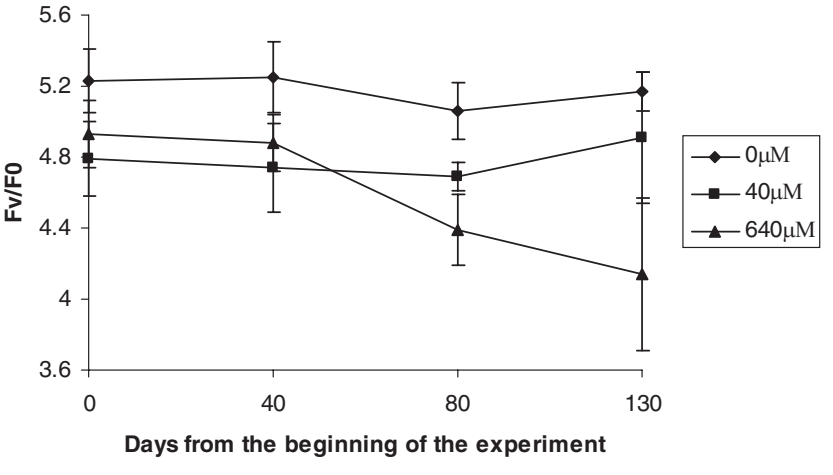
(B)

FIGURE 2 Time variation of the chlorophyll fluorescence parameters (A) F_v/F_m and (B) F_v/F_0 in the leaves of the olive cultivar 'Kothreiki' as affected by Mn concentration in the nutrient solution.

concentrations (0, 40 and 640 μM) in nutrient solution is presented in the Figures 2 and 3, respectively. The fluctuation of the values of both of these parameters was relatively limited. Furthermore, in none Mn treatment and/or cultivar, the intermediate values (those of the 40th and 80th day of the experiment), as well as the final ones (those of the 130th day of the experiment) of the ratios Fv/Fm and Fv/F0 dropped below 0.8 and 4, respectively (Figures 2 and 3). Nevertheless, a significant drop was observed in these ratios' values of both olive cultivars from the 80th day of the experiment when treated with 640 μM Mn, compared to the corresponding values at the days 0 and 40th (Figures 2 and 3). The lowest value of the ratio Fv/Fm was recorded at the 130th day of the experiment in the leaves of 'FS-17' plants treated with



(A)



(B)

FIGURE 3 Time variation of the chlorophyll fluorescence parameters (A) F_v/F_m and (B) F_v/F_0 in the leaves of the olive cultivar 'FS-17' as affected by Mn concentration in the nutrient solution.

640 μM Mn and the highest one at the 40th day of the experiment in the leaves of 'Kothreiki' plants grown under 640 μM Mn (Figures 2 and 3).

Linear Correlation Coefficients between Various Parameters

At the end of the experiment (130th day), the Mn concentration in nutrient solution, as well as the Mn concentration in the leaves of both olive cultivars was negatively correlated with the concentration of Fe and positively with the concentrations of Zn and P in the leaves, i.e., the increase of Mn

concentration in nutrient solution and in the leaves of both olive cultivars was accompanied by a relatively linear decrease of the Fe concentration and an increase of the Zn and P concentrations in the leaves of both cultivars (Table 3). As far as the chlorophyll fluorescence parameters are concerned, both of them were negatively correlated with the concentration of Mn both in the nutrient solution and in the leaves of both studied cultivars. The chlorophyll fluorescence parameters were also linearly reduced with the increase of Zn and P concentration in the leaves of both cultivars (Table 3).

DISCUSSION

Although significant reduction of the growth of several plant species when they were grown under either Mn excess (Tracy, 1991; Foy et al., 1998; Alam et al., 2001; Quartin et al., 2001; Sarkar et al., 2004) or Mn deficiency conditions (Ohki, 1985; Hannam and Ohki, 1988; Sadana et al., 2002; Papadakis, 2004) was reported by many researchers, in our investigation the plant growth of none of the two studied olive cultivates was significantly influenced by anyone of the Mn treatments (Figure 1). This could be probably ascribed to the fact that the plants of both cultivars irrigated with nutrient solution without Mn (0 μM Mn) had, during all the experimental period, leaf Mn concentration higher than the least value (>20 μg g^{-1}. d.w.) required for normal plant growth (Tables 1 and 2). Indeed, Panagiotopoulos (2001) reported that when the Mn concentration in olive leaves, sampled from the middle of last vegetation flush of growth, is within the range 5 and 20 μg g^{-1}. d.w. olive trees suffer from a relative Mn deficiency. According to Marschner (1995), the critical leaf Mn level, below which symptoms of Mn deficiency are occurred in most plant species, is varied between 10 and 20 μg g^{-1}. d.w. The fact that the concentrations of Mn in leaves of both studied cultivars were greater than 20 μg g^{-1}. d.w., even in the 0 μM Mn treatment, could be probably ascribed to an initial (before the beginning of the experiment) 'pool' of Mn existed in roots plants (95 μg g^{-1}. d.w. for 'FS-17' and 102 μg g^{-1}. d.w. for 'Kothreiki'; data not shown) which was proved to be enough to cover Mn nutritional needs of plants (at least for the period of 130 days of the present experiment). Loneragan (1988) also reported that many plant species can accumulate a considerable quantity of Mn in their root system or in their shoot, when they are grown under Mn sufficient conditions, and so later, if Mn deficiency conditions are occurred, they are able to mobilize part of this Mn quantity from these tissues to leaves in order to cover their nutritional needs for Mn. On the other hand, when Mn concentration in olive leaves is greater than 150 μg g^{-1}. d.w., the element is considered to be in excess (Panagiotopoulos, 2001). In the current study, however, although the leaf Mn concentrations in the 640 μM Mn treatment were found to be high enough (up to 734 μg g^{-1} d.w.; Tables 1 and 2), the elongation of the

TABLE 3 Linear correlation coefficients between various parameters determined in the leaves of the olive cultivars 'Kothreiki' and 'FS-17' at the end of the experiment (130th day).

	Mn concentration in the nutrient solution	Nutrient concentration in the leaves									
		Mn	Fe	Zn	B	P	Ca	Mg	K	F_v/F_m	F_v/F_0
						'Kothreiki'					
Mn concentration in the nutrient solution		0.998	−0.911	0.947	0.830	0.749	−0.823	n.s.	n.s.	−0.442	−0.511
Nutrient concentration in the leaves											
Mn	0.998		−0.906	0.939	0.824	0.760	−0.828	n.s.	n.s.	−0.450	−0.522
Fe	−0.911	−0.906		−0.738	−0.941	n.s.	0.954		n.s.	n.s.	n.s.
Zn	0.947	0.939	−0.738		0.622	0.895	−0.602		−0.737	−0.654	−0.709
B	0.830	0.824	−0.941	0.622		n.s.	n.s.	0.853	n.s.	n.s.	n.s.
P	0.749	0.760	n.s.	0.895	n.s.		n.s.	0.707	−0.862	−0.876	−0.897
Ca	−0.823	−0.828	0.954	−0.602	−0.938	n.s.		−0.844	n.s.	n.s.	n.s.
Mg	n.s.	n.s.	−0.919	0.939	0.853	0.707	−0.847		n.s.	n.s.	n.s.
K	n.s.	n.s.	n.s.	−0.737	n.s.	−0.862	n.s.	n.s.		n.s.	n.s.
F_v/F_m	−0.442	−0.450	n.s.	−0.654	n.s.	−0.876	n.s.	n.s.	n.s.		0.993
F_v/F_0	−0.511	−0.522	n.s.	−0.709	n.s.	−0.897	n.s.	n.s.	n.s.	0.993	
						'FS-17'					
Mn concentration in the nutrient solution		0.992	−0.729	0.958	n.s.	0.487	n.s.	0.436	n.s.	−0.971	−0.957
Nutrient concentration in the leaves											
Mn	0.992		−0.714	0.936	n.s.	0.477	n.s.	0.434	n.s.	−0.962	−0.954
Fe	−0.729	−0.714		−0.663	n.s.	−0.921	−0.932	n.s.	0.775	0.798	0.805
Zn	0.958	0.936	−0.663		n.s.	0.428	0.818	0.457	−0.887	−0.907	−0.886
B	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P	0.487	0.477	−0.921	0.428	n.s.		0.823	−0.515	−0.621	−0.609	−0.635
Ca	n.s.	n.s.	−0.932	0.818	n.s.	0.823		n.s.	−0.911	−0.879	−0.892
Mg	0.436	0.434	n.s.	0.457	n.s.	−0.515	n.s.		n.s.	n.s.	n.s.
K	n.s.	n.s.	0.775	−0.887	n.s.	−0.621	−0.911	n.s.		n.s.	n.s.
F_v/F_m	−0.971	−0.962	0.798	−0.907	n.s.	−0.609	−0.879	n.s.	n.s.		0.995
F_v/F_0	−0.957	−0.954	0.805	−0.886	n.s.	−0.635	−0.892	n.s.	n.s.	n.s.	

Non-significant (n.s.) or significant correlations at probability level $P \leq 0.05$.

main shoot of plants was not affected negatively, irrespectively of the cultivar (Figure 1). This maybe could be explained by the observation that Mn excess did not affect visibly the functional integrity of the apical meristem of olive shoots, a fact that permitted the uninhibited elongation of plant shoots. Instead, after the 90th day of the experiment, Mn excess resulted in the appearance of necrotic spots and curling in the top-fully expanded leaves of the plants treated with 640 μM Mn, irrespectively of the cultivar. As far as the elongation rate of the main shoot is concerned, both cultivars showed a minimum rate between the 40th and the 50th day of the experiment and a maximum one between the 60th and the 110th day, depending also on olive cultivar and Mn treatment (Figure 1). This was probably happened because the period between the 60th and the 110th day of the experiment coincided with that of May-early June, when the day length gets its optimum values for vegetative growth. Generally, the time variation of the elongation rate of the main shoot for each one of the two olive cultivars was similar for all Mn treatments (0, 40 and 640 μM) and, therefore, it was not affected by Mn concentration in the nutrient solution (Figure 1).

Our data indicated that Mn concentration in the leaves of both olive cultivars decreased in the 0 μM Mn treatment, remained about at the same levels ('FS-17') or increased ('Kothreiki') in the 40μM treatment, while in the treatment of 640 μM Mn it was steeply increased after the 80th day from the beginning of the experiment (Tables 1 and 2). This was probably ascribed to the great accumulation of Mn in the root system of both cultivars until 'deprivation' of its capacity to tolerate such high Mn concentrations (10500 μg/g. d.w. for cultivar 'Kothreiki' and 8033 μg g^{-1} d.w. for 'FS-17' at the 130th day of the experiment, data not shown) without any damage in its function. When the root system deprives of that capacity, the extra Mn, which is accumulated in plant roots, begins to move towards to stems and leaves, increasing steeply Mn concentrations in leaves. Such a phenomenon maybe took place in the present study, after the 80th day of the experiment, explaining thus the great increase of leaf Mn concentration in plants treated with 640 μM Mn.

In general, the concentrations of all nutrient elements determined in leaves of both studied olive cultivars were in absolutely sufficient levels during the whole experimental period, based on the reports of many other researchers (Razmjoo et al., 1997; Panagiotopoulos, 2001; Maier and Chvyl, 2003; Chatzissavvidis et al., 2004, 2005; Roca-Perez et al., 2006). The only exception concerned the final (that of the 130th day of the experiment) leaf Mn concentration in the treatment of 640 μM Mn, where the element was in excess, as it was already discussed above (Tables 1 and 2). In addition, at the end of this experiment (130th day), Mn concentration in leaves was negatively correlated with Fe concentration and positively with those of Zn and P in the leaves of both olive cultivars (Table 3). As far as the interaction of Mn with Fe in plants is concerned, many studies showed a negative correlation

between their concentrations in various plant organs, as well as between their accumulation in root and shoot of many plant species (El-Jaoual and Cox, 1998; Foy et al., 1998; Alam et al., 2001; Quartin et al., 2001). Tracy (1991) reported that leaf P concentration of avocado plants was increased with the increase of Mn in the nutrient solution. Furthermore, the concentration of P in the roots of four triticales cultivars remained unaffected, while in the shoots increased with the increase of Mn from 2.5 to 50 mg L^{-1} in the nutrient solution (Quartin et al., 2001).

Fe concentration in leaves fluctuated with the time from the beginning of the experiment, with minimum values taking place at the 40th or 80th day of the experiment (April-late May) (Tables 1 and 2). Roca-Perez et al. (2006) observed that the minimum concentrations of Fe in the leaves of *Digitalis obscura* took also place during the period of May. Furthermore, Chatzissavvidis et al. (2005) found lower concentrations of Fe in the leaves of olive cultivar 'Chondrolia Chalkidikis' during the period between the end of spring and the beginning of summer, in comparison to the relevant concentrations during the period autumn-winter. Zinc concentration in the leaves of cultivar 'Kothreiki' was reduced with the time, from the beginning (March) until the end of the present experiment (July) (Tables 1 and 2). However, Chatzissavvidis et al. (2005) found that the concentrations of Zn in the leaves of olive cultivar 'Chondrolia Chalkidikis' during the period March-July were about at the same levels, without valuable fluctuations.

Phosphorus concentrations in the leaves of both olive cultivars fluctuated with time, with the minimum values taking place at the 40th (April) or at the 80th (May) day of the experiment (depending also on cultivar and Mn treatment) and the maximum ones at the 130th day (July) (Tables 1 and 2). Other researchers also reported that P concentration in leaves of different plant species fluctuated during the period of year (Chatzissavvidis et al., 2005; Roca-Perez et al., 2006). Maier and Chvyl (2003) found that P concentration in the leaves of *Chamelaucium uncinatum* plants was reduced during the period from October to March and increased from March to August. With regard to K, its concentration in the leaves of both olive cultivars studied was continuously increasing until the 130th day (July) of the experiment, when reached its maximum values (Tables 1 and 2). According to Chatzissavvidis et al. (2005), the maximum K concentration in the leaves of olive cultivar 'Chondrolia Chalkidikis' recorded during the period June-July, while Roca-Perez et al. (2006) found that the maximum concentration of K in the leaves of *Tilia sp.* and *Digitalis obscura* took place in May. Ca and Mg concentrations in the leaves of 'Kothreiki' and 'FS-17' also fluctuated during the experiment and got in most cases their maximum values at the 40th (April) or at the 80th (late May) day of the experiment (Tables 1 and 2). According to Maier and Chvyl (2003) and Roca-Perez et al. (2006), Ca, which is a phloem immobile nutrient, presents its minimum concentrations in leaves during the period of maximum vegetative growth.

Chatzissavvidis et al. (2005) observed that the maximum concentration of Mg in the leaves of olive cultivar 'Chondrolia Chalkidikis' measured during the summer (July–August), with a second maximum value in spring (March–May).

Chlorophyll fluorescence measurement is a rapid, extremely sensitive and non-destructive method, since it can be performed on intact, attached leaves. It has become an important tool in the study of photosynthesis, in particular the functioning of PSII (Schreiber et al., 1995). In general, fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000). Generally, the F_v/F_m ratio (maximum quantum yield of PSII) is the most frequently used chlorophyll fluorescence parameter. A reduction of the maximum quantum yield of PSII (F_v/F_m) (values lower than 0.8), as well as of the F_v/F_0 ratio (values lower than 4) in leaves, means that the plants are under stress conditions, where the structural and functional integrity of chloroplasts have been damaged extensively. It should be pointed out that under growth conditions chlorophyll fluorescence is affected by many factors and not only by one (Mn), as happened in our experiment. In the present study, the maximum quantum yield of PSII (F_v/F_m) was not severely affected ($F_v/F_m > 0.8$) by the change of Mn concentration in the nutrient solution, irrespectively of the date of measurement (day 0, 40th, 80th, or 130th day), although in the 640 μM Mn treatment, at the 80th day of the experiment and afterwards a statistically significant drop was observed in the ratio's values of both cultivars, compared to the corresponding values of the days 0 and 40th (Figures 2 and 3). That tendency was not observed in the 0 and 40 μM Mn treatments. The same was observed concerning the ratio Fv/F_0 (Figures 2 and 3). This drop in the values of F_v/F_m and F_v/F_0 at the 80th day and afterwards took place at least 10 days before the appearance of the first Mn toxicity symptoms in the top leaves. The fact that under Mn toxicity conditions the functionality of the PSII of photosynthesis was not notably affected in none of the two tested olive cultivars, is in agreement with the results of other researchers (Kitao et al., 1997; Subrahmanyan and Rathore, 2000). In citrus plants, it was also observed that excess Mn did not affect neither the maximum quantum yield of PSII (F_v/F_m), nor the structural integrity of chloroplasts (Papadakis et al., 2007a, 2007b). As previously mentioned, all the values (initial, intermediate or final) of the F_v/F_m and F_v/F_0 ratios did not drop below 0.8 and 4, respectively, irrespectively of the Mn treatment, the cultivar and/or the date of measurement (Figures 2 and 3). Therefore, it could be concluded that the decrease of these two chlorophyll fluorescence measurements observed under 640 μM Mn at the 80th day of the experiment and afterwards, but not in the 0 and 40 μM Mn treatments (Figures 2 and 3), was without any practical impact on the proper functionality of PSII of photosynthesis in the leaves of both olive cultivars studied. In contrast to that, the maximum

efficiency of PSII decreased significantly in Mn-starved maize leaves (Jiang et al., 2002). Furthermore, at the end of the present experiment, it was observed that the linear correlation coefficients between Mn concentration in leaves or in nutrient solution and the F_v/F_m and F_v/F_0 ratios were significant and negative, irrespectively of the cultivar (Table 3). This observation in combination with the significantly reduced values of F_v/F_m and F_v/F_0 ratios at the 80th and 130th day of the experiment, compared to the previously measured ones (those of the days 0 and 40th) in the leaves of both cultivars treated with 640 μM Mn (Figures 2 and 3), may support the idea that these chlorophyll fluorescence parameters may be good indicators in assessing Mn toxicity in olive plants, but only when their leaves have extremely high Mn concentrations. Indeed, at the end (at the 130th day) of the present study, although: i) the mean Mn concentration in the leaves of both cultivars treated with 640 μM Mn were high enough (616 $\mu g\ g^{-1}$ d.w. in 'FS-17' and 734 μg/g d.w. in 'Kothreiki') (Tables 1 and 2), ii) visible symptoms of Mn toxicity were present in top leaves of both cultivars, and (iii) the reductions in F_v/F_m and F_v/F_0 ratios in the leaves of both cultivars grown under 640 μM Mn, compared to the other two Mn treatments (Figures 2 and 3), it could be concluded that the periodical measurement of F_v/F_m and F_v/F_0 ratios was not a reliable index to predict the appearance of the visible symptoms of Mn toxicity in olive leaves. On the other hand, it could be further concluded that the appearance of Mn toxicity symptoms in olive leaves was not due to improper functionality of PSII of photosynthesis and thus due to damage caused by oxidative stress. In other words, the effects of Mn excess in olive leaf structure and function could be primary ascribed to other factors, such as the accumulation of Mn oxides which form brown speckles in leaves, or accumulation of large starch grains within chloroplasts which affect negatively the photosynthetic rate of leaves and cause malformations in leaves etc. In citrus plants, it was also observed that excess Mn did not affect neither the maximum quantum yield of PSII (F_v/F_m), nor the structural integrity of chloroplasts, but it significantly increased the starch accumulation in chloroplasts (Papadakis et al., 2007a, 2007b). In contrast to our results, the F_v/F_m and F_v/F_0 ratios have been proved to be good indicators in assessing other abiotic stresses in olive plants. According to Faraloni et al. (2006), chlorophyll fluorescence parameters decrements were observed prior to fresh weight decrements in olive trees subjected to drought stress.

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