

Studies on the Manganese Excess of Muskmelon

V. The Manganese, Calcium and Iron Concentrations in Nutrient Solution

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Summary

Muskmelon plants were grown in sand culture and fertilized with a complete nutrient solution containing 0.5, 30 and 60 ppm Mn, and 1.33, 4.00 and 12.00 mM Ca; 30 and 60 ppm Mn, and 1,30 and 60 ppm Fe, in addition to control, 0.5 ppm Mn and 1 ppm Fe. In the experiment of Mn and Ca: Mn in the plant parts was significantly increased as Mn concentrations were raised from 0.5 to 60 ppm, while inversely decreased as Ca concentrations were raised from 1.33 to 12.00 mM. The fresh weight, soluble solids and scores of external fruit appearance were significantly decreased with increasing Mn levels, and were not improved with increasing Ca levels. No fruits with cracking at the blossom-end were found at 0.5 and 30 ppm Mn regardless of Ca levels, while they were found at 60 ppm Mn with 1.33 and 4.00 mM Ca. In the experiment of Mn and Fe: Mn in the plant was significantly increased with increasing Mn levels, while inversely decreased with increasing Fe levels. The fresh weight, soluble solids and scores of external appearance of the fruits decreased at 60 ppm Mn, and those receiving 30 ppm Mn were significantly decreased with increasing Fe levels. No cracked fruits were found at 30 ppm Mn, while they were found at 60 ppm Mn regardless of Fe levels.

Introduction

The physiological disorder of muskmelon with necrosis in the leaves and brownish spots on the stems and veins has been identified as caused by excess manganese (Mn) uptake from a survey of the plant tissues and soils (8). Cooper and Thompson (1) have reported that increased calcium (Ca) in the solution was effective in correcting the interveinal bark necrosis (IBN) of 'Delicious' apple trees which is associated with localized accumulation of Mn as a significant causal factor. Fukatsu et al. (6) have reported that brown leaf blight of cucumber caused by excess Mn uptake was alleviated with increasing iron (Fe) concentration in the nutrient solution. Therefore this study was made to determine whether differential Ca and Fe concentrations in the nutrient solution containing higher Mn are effective in alleviating the excess Mn toxicity of muskmelon.

I. Experiment of Mn and Ca Materials and Methods

Sixty-three uniform seedlings of 'Spring' No. 3, strain of 'Earl's Favourite' grafted on 'Barnett Hill Favourite' rootstock were used. Treatments were applied in a factorial arrangement involving 3 Mn levels (0.5, 30 and 60 ppm) of nutrient solution, and 3 Ca levels (1.33, 4.00 and 12.00 mM). Thus there were 9 treatments, each having 7 replications, with a total of 63 single plant plots. On April 15, 1972, 4-leaf seedlings were planted in 40×40×20 cm boxes filled with Tenryu River sand. This sand was washed with 0.2 N HCl and 0.2 N NaOH, and rinsed with deionized water. The differential Mn and Ca treatments were initiated when planted. The composition of nutrient solution used in this experiment is shown in Table 1. Solutions were adjusted to a pH of 5.0 using either 0.5 N HCl or 0.5 N NaOH. Throughout the experimental period, solutions were applied 1 or 3 times a day in equal quantities of 0.67 liters to each

Table 1. Mn and Ca concentrations, and nutrient solution used in excess Mn studies with muskmelon.

Treatments			Composition of nutrient solution
No.	Mn concn (ppm)	Ca concn (mM)	
1	0.5	1.33	Mn from MnSO ₄ (designated concn), Ca from CaCl ₂ ·2H ₂ O (designated concn), Na ₂ HPO ₄ ·12H ₂ O (0.5 mM), K ₂ SO ₄ (3 mM), MgSO ₄ ·7H ₂ O (2 mM), NaNO ₃ (15 mM), Fe from FeC ₆ H ₅ O ₇ ·5H ₂ O (1 ppm), B from H ₃ BO ₃ (0.5 ppm), Zn from ZnSO ₄ ·7H ₂ O (0.05 ppm), Cu from CuSO ₄ ·5H ₂ O (0.02 ppm), and Mo from Na ₂ MoO ₄ ·2H ₂ O (0.05 ppm)
2	0.5	4.00	
3	0.5	12.00	
4	30	1.33	
5	30	4.00	
6	30	12.00	
7	60	1.33	
8	60	4.00	
9	60	12.00	

box whether it was cloudy or sunny, and applications were applied up to 4 days prior to the end of the experiment. No solutions were applied in rainy weather. All plants received uniform care except for the experimental treatments. At the conclusion of the experiment records were taken of Mn toxicity symptoms in the plant parts, top and root growth, fruit weight, and fruit soluble solids. All plant analyses were by methods described in a previous paper (8).

Results

Growth of plant and fruit

The effects of differential Mn and Ca con-

centrations on the growth of plant and fruit of muskmelon are shown in Table 2. The growth of plants as expressed by the dry weight of leaves and stems was not influenced by the Mn levels, while the fresh weight, soluble solids and scores of external fruit appearance were significantly decreased as the Mn concentrations were increased from 0.5 to 60 ppm. No fruits with cracking at the blossom-end were found at the 0.5 and 30 ppm Mn regardless of the Ca levels, while 5 cracked fruits were found both in the 60 ppm Mn-1.33 mM Ca, and 60 ppm Mn-4.00 mM Ca. No marked differences in the plant growth were found among the Ca levels, while the fruit weight

Table 2. Effects of Mn and Ca concentrations in nutrient solution on the growth and fruit quality of muskmelon.

Mn concn	Ca concn	Plant ht	Leaves	Stems	Roots	Fruit			No. of cracked fruit
						Fresh wt	Soluble solids	External appearance ^a	
ppm	mM	May 11	dry wt	dry wt	dry wt	g	%		
0.5	1.33	103	28.2	13.3	2.1	718	15.0	4.74	0
0.5	4.00	96	28.6	13.0	2.0	698	14.7	4.66	0
0.5	12.00	90	29.2	13.6	2.4	655	15.0	4.56	0
30	1.33	100	32.8	16.6	2.3	630	14.1	3.01	0
30	4.00	94	31.8	15.2	2.0	645	14.2	2.87	0
30	12.00	91	32.4	15.8	2.2	555	14.0	2.50	0
60	1.33	94	32.1	15.7	2.0	450	12.9	0.49	5
60	4.00	95	31.0	15.6	1.6	445	12.8	0.51	5
60	12.00	94	28.7	13.0	1.7	469	13.2	0.57	0
Mean Mn concn	0.5 ^{ppm}	97	28.7	13.3	2.2	690	14.9	4.65	0
	30	95	32.3	15.9	2.2	610	14.1	2.80	0
	60	95	30.6	14.8	1.8	454	13.0	0.52	3.3
Mean Ca concn	1.33 ^{mM}	99	31.0	15.2	2.1	600	14.0	2.75	1.7
	4.00	95	30.5	14.6	1.9	596	13.9	2.68	1.7
	12.00	92	30.1	14.1	2.1	559	14.1	2.54	0
L. S. D. 5%	Mn, Ca Treatment	4.0	N. S.	N. S.	0.33	29	0.34	0.23	b
		6.9	N. S.	N. S.	0.58	50	0.60	0.40	b

a : Full score=5 b : Not subjected to statistical analysis

was slightly decreased at the 12.00 mM Ca.

Toxicity symptoms and Mn content in plant tissues

The effects of differential Mn and Ca concentrations on the Mn toxicity symptoms in the leaf blades, stems and petioles, and the Mn content of the leaves, stems, roots and fruit of muskmelon are presented in Table 3. No necrotic spots or lesions were found on the leaf blades at 0.5 ppm Mn. However these symptoms were significantly intensified as the Mn concentrations were raised from 30 to 60 ppm. Toxicity symptoms in the stems and petioles consisting of light to dark purple spots at the base of hairs showed a similar tendency to those on the leaf blades. The Mn in the leaves, stems, roots and fruit was significantly increased as the Mn concentrations were increased from 0.5 to 60 ppm, and the Mn in the leaves, stems and fruit was inversely decreased as the Ca concentrations were increased from 1.33 to 12.00 mM. That is, the Mn in the leaves, stems, roots and fruit growing at the 60 ppm Mn was 25.8, 18.9, 9.5 and 31.3 times as great, respectively, as those at the 0.5 ppm Mn, and the Mn in the leaves, stems and fruit growing at the 12.00 mM Ca was

0.59, 0.44 and 0.43 times as small, respectively, as those growing at the 1.33 mM Ca.

Main elemental content of leaves

The effects of differential Mn and Ca concentrations on the elements, N, P, K, Ca, Mg and Fe, and the Fe/Mn ratio in the leaves of muskmelon are given in Table 4. Generally, as the Mn concentrations were raised from 0.5 to 60 ppm the Ca, Mg and Fe, and the Fe/Mn ratio in the leaves decreased, while the P and K increased. As the Ca concentrations were raised from 1.33 to 12.00 mM the Ca in the leaves increased, while the K and Mg decreased.

II. Experiment of Mn and Fe Materials and Methods

Forty-nine uniform seedlings of 'Spring No. 3' strain of 'Earl's Favourite' grafted on 'Barnett Hill Favourite' rootstock were used. Treatments were applied in a factorial arrangement involving 2 Mn levels (30 and 60 ppm) of nutrient solution and 3 Fe levels (1, 30 and 60 ppm), in addition to control (0.5 ppm Mn-1 ppm Fe). Thus, there were 7 treatments, each having 7 replications, with a total of 49 single plant plots. On April 27, 1972, 4-leaf

Table 3. Effects of Mn and Ca concentrations in nutrient solution on the intensity of Mn toxicity symptoms, and Mn content in the various parts of muskmelon.

Mn concn	Ca concn	Toxicity symptoms ^a		Mn (ppm in dry wt basis)			
		Leaf blades	Stems and petioles	Leaves	Stems	Roots	Fruit
0.5 ppm	1.33 mM	0	0	613	292	431	3i
0.5	4.00	0	0	583	217	404	22
0.5	12.00	0	0	608	167	414	20
30	1.33	1.6	2.4	9973	2739	2727	726
30	4.00	1.7	2.6	8311	2250	2680	319
30	12.00	1.1	1.6	6124	1147	2026	314
60	1.33	3.4	4.9	20181	5446	3846	1045
60	4.00	3.5	4.4	15574	4860	3815	1350
60	12.00	3.1	3.5	11590	2448	4132	444
Mean Mn concn	0.5 ppm	0	0	612	225	417	25
	30	1.5	2.2	8136	2046	2478	453
	60	3.3	4.2	15782	4252	3931	783
Mean Ca concn	1.33 mM	1.7	2.4	10266	2826	2335	601
	4.00	1.7	2.3	8156	2443	2300	400
	12.00	1.4	1.7	6108	1254	2191	259
L. S. D. 5%	Mn, Ca	0.43	0.38	595	602	154	93
	Treatment	0.75	0.67	1036	1048	267	163

a : 0=None, 5=Very severe

Table 4. Effects of Mn and Ca concentrations in nutrient solution on the major elemental content, and ratios of Fe : Mn in the leaves of muskmelon. (Dry wt basis)

Mn concn	Ca concn	N	P	K	Ca	Mg	Fe	Fe : Mn
ppm	mM	%	%	%	%	%	ppm	
0.5	1.33	3.10	0.21	1.90	6.73	2.49	621	0.97
0.5	4.00	3.06	0.23	1.75	7.19	1.86	595	1.10
0.5	12.00	2.92	0.20	1.73	9.61	1.49	646	1.09
30	1.33	3.10	0.24	2.62	5.62	2.12	492	0.05
30	4.00	3.11	0.27	2.20	6.32	1.76	423	0.05
30	12.00	3.04	0.25	1.89	8.64	1.26	424	0.07
60	1.33	2.93	0.28	3.33	4.89	1.94	487	0.02
60	4.00	2.85	0.28	2.61	6.30	1.73	357	0.02
60	12.00	2.71	0.31	2.21	8.19	1.25	391	0.02
Mean Mn concn	0.5	3.03	0.21	1.79	7.85	1.95	620	1.06
	30	3.08	0.26	2.24	6.86	1.71	446	0.06
	60	2.83	0.29	2.72	6.46	1.64	412	0.03
Mean Ca concn	1.33	3.04	0.24	2.61	5.75	2.18	533	0.35
	4.00	3.01	0.26	2.19	6.60	1.78	458	0.39
	12.00	2.89	0.26	1.94	8.81	1.34	487	0.40
L. S. D. 5%	Mn, Ca	N. S.	0.02	0.23	0.41	0.08	61	0.03
	Treatment	N. S.	0.03	0.40	0.72	0.14	107	0.05

Table 5. Mn and Fe concentrations, and nutrient solution used in excess Mn studies with muskmelon.

Treatments			Composition of nutrient solution
No.	Mn concn	Fe concn	
	ppm	ppm	
1	30	1	Mn from $MnSO_4$ (designated concn), Fe from $FeC_6H_5O_7 \cdot 5H_2O$ (designated concn), $Na_2HPO_4 \cdot 12H_2O$ (0.5 mM), K_2SO_4 (3 mM), $CaCl_2 \cdot 2H_2O$ (4 mM), $MgSO_4 \cdot 7H_2O$ (2 mM), $NaNO_3$ (15 mM), B from H_3BO_3 (0.5 ppm), Zn from $ZnSO_4 \cdot 7H_2O$ (0.05 ppm), Cu from $CuSO_4 \cdot 5H_2O$ (0.02 ppm), and Mo from $Na_2MoO_4 \cdot 2H_2O$ (0.05 ppm)
2	30	30	
3	30	60	
4	60	1	
5	60	30	
6	60	60	
Control	0.5	1	

seedlings were planted in $40 \times 40 \times 20$ cm boxes filled with Tenryu River sand. This sand was washed by the same methods described in the previous experiment of Mn and Ca, and rinsed with deionized water. The differential Mn and Fe treatments were initiated when planted. The composition of nutrient solutions used in this experiment is shown in Table 5. The pH of nutrient solutions was adjusted at 5.0, and application of solutions and records were accomplished with the same methods as described earlier.

Results

Growth of plant and fruit

The effects of differential Mn and Fe concentrations on the growth of plant and fruit

of muskmelon are shown in Table 6. The growth of plants as expressed by plant height, and dry weight of leaves, stems and roots was not influenced by the Mn levels, while the fresh weight, soluble solids and scores of external appearance of fruit decreased at the 60 ppm Mn. No fruits with cracking at the blossom-end were found at the 30 ppm Mn regardless of the Fe levels, while 4, 3 and 3 cracked fruits were found in the 60 ppm Mn with 1, 30 and 60 ppm Fe, respectively. The fresh weight, soluble solids, and scores of fruit decreased at the 30 ppm Mn as the Fe concentrations were raised from 1 to 60 ppm, while those at the 60 ppm Mn were not influenced by the Fe levels.

Toxicity symptoms, Mn and Fe content,

Table 6. Effects of Mn and Fe concentrations in nutrient solution on the growth and fruit quality of muskmelon.

Mn concn	Fe concn	Plant ht May 27	Leaves dry wt	Stems dry wt	Roots dry wt	Fruit			No. of cracked fruit
						Fresh wt	Soluble solids	External appearance ^a	
30 ppm	1 ppm	122 cm	32.0 g	16.0 g	3.6 g	637 g	15.1 %	3.9	0
30	30	111	29.6	15.0	3.1	615	14.5	3.4	0
30	60	101	27.2	13.6	3.0	535	14.0	2.4	0
60	1	118	34.4	17.2	3.0	512	13.8	1.9	4
60	30	103	30.0	15.5	2.5	500	12.9	1.6	3
60	60	105	29.7	15.9	2.8	522	13.1	2.0	3
Mean Mn concn	30 ppm	111	29.6	14.9	3.2	596	14.5	3.2	0
	60	109	31.4	16.2	2.8	511	13.3	1.8	3.3
Mean Fe concn	1 ppm	120	33.2	16.6	3.3	575	14.5	2.9	2
	30	107	29.8	15.3	2.8	558	13.7	2.5	1.5
	60	103	28.5	14.8	3.0	529	13.6	2.2	1.5
L. S. D. 5%	Mn	N. S.	N. S.	N. S.	N. S.	36	N. S.	0.4	b
	Fe	5.6	2.2	1.8	N. S.	N. S.	0.8	0.5	b
	Treatment	9.2	3.7	2.8	N. S.	72	1.4	0.8	b
Control		122	31.3	15.1	3.2	701	15.2	4.5	0

a : Full score=5 b : Not subjected to statistical analysis.

Table 7. Effects of Mn and Fe concentrations in nutrient solution on the intensity of Mn toxicity symptoms, Mn content in the various parts, Fe content in the leaves, and ratios of Fe : Mn in the leaves of muskmelon.

Mn concn	Fe concn	Toxicity symptoms ^a		Mn (ppm in dry wt basis)				Fe(ppm)	Fe : Mn
		Leaf blades	Stems and petioles	Leaves	Stems	Roots	Fruit	Leaves	Leaves
30 ppm	1 ppm	0.6	1.8	5542	1662	4846	127	474	0.09
30	30	0.3	1.2	4869	1351	3586	133	556	0.12
30	60	0.2	0.6	3789	801	3985	79	524	0.14
60	1	3.3	4.8	13689	2761	6432	326	499	0.04
60	30	2.5	3.7	10900	2347	6334	263	478	0.05
60	60	1.5	2.5	9382	1740	6711	222	452	0.05
Mean Mn concn	30 ppm	0.4	1.2	4733	1271	4139	113	518	0.12
	60	2.4	3.7	11324	2283	6492	270	476	0.05
Mean Fe concn	1 ppm	2.0	3.3	9615	2212	5639	227	486	0.07
	30	1.4	2.5	7884	1849	4960	198	517	0.09
	60	0.9	1.6	6585	1271	5348	151	488	0.10
L. S. D. 5%	Mn	0.39	0.35	677	453	749	41	N. S.	0.013
	Fe	0.48	0.42	828	554	N. S.	51	N. S.	0.015
	Treatment	0.79	0.70	1376	921	1521	84	N. S.	0.026
Control		0	0	686	383	466	36	604	0.93

a : 0=None, 5=Very severe

and Fe/Mn ratio in plant tissues

The effects of differential Mn and Fe concentrations on the Mn toxicity symptoms in the leaf blades, stems and petioles, Mn content of the plant parts, Fe content in the leaves,

and Fe/Mn ratio in the leaves of muskmelon are presented in Table 7. Toxicity symptoms of leaf blades, stems and petioles were significantly intensified as the Mn concentrations were raised from 30 to 60 ppm, and were

inversely decreased as the Fe concentrations were raised from 1 to 60 ppm. The Mn in the leaves, stems, roots and fruit was significantly increased as the Mn concentrations were raised from 30 to 60 ppm, while the Mn in those plant parts except for roots, and the Fe/Mn ratio in the leaves were significantly decreased as the Fe concentrations were raised from 1 to 60 ppm. The Fe in the leaves was not influenced by the Fe levels.

Discussion

It is well known that Ca aids in decreasing the toxic accumulation of ions(7) and depresses Mn uptake(5). Cooper and Thompson(1) reported that IBN of 'Delicious' apple trees caused by excess Mn uptake was corrected by an increase of Ca in the solution. In this experiment of Mn and Ca, the Mn in the leaves, stems and fruit was significantly decreased as the Ca concentrations were increased from 1.33 to 12.00 mM. However, plant growth and fruit quality of muskmelons receiving 30 or 60 ppm Mn were not influenced by the Ca levels. This seems to be associated with an excessively high level of Mn found in the plants. In a previous paper(9) the authors pointed out that critical Mn in the leaves for appearance of toxicity symptoms was nearly 2000 ppm. As compared with this critical level, the Mn in the leaves receiving 30 or 60 ppm Mn was as high as 8136 or 15782 ppm, respectively, so that the beneficial effects of Ca on plant growth and fruit quality seems to be counteracted. Thus increasing Ca in the presence of toxic levels of Mn was less effective in the plant growth and fruit quality, but the important implications of Ca application to soil should not be overlooked. As reported in the previous papers(10,11), Ca application resulting in increasing soil pH has a positive influence on the conversion of soil Mn from available to non-available forms, and offers one of the most promising approaches in preventing excess Mn injury.

Fruits with cracking at the blossom-end were found at the 60 ppm Mn with low or medium Ca concentrations, 1.33 or 4.00 mM Ca, but they were not found at the 60 ppm Mn with high Ca concentration, 12.00 mM Ca. This seems to be associated with Ca and

Mn in the fruit. Generally, Ca is an important constituent of cell walls, the middle lamella being composed largely of colloidal pectic compounds. A large number of these pectic compounds contain Ca. Maynard et al.(13) reported that the blossom-end rot of tomato fruit is a manifestation of insufficient Ca within the fruit which causes the characteristic cellular breakdown at the blossom-end of the fruit. The cracked fruits of muskmelon may also be caused by a decrease of Ca in the fruit which results from the lower Ca and high Mn supplies in the solution. The Mn in the fruit may be responsible, in part, for the occurrence of the fruit cracking. The authors(12) detected large of Mn at the epidermal layers just under the nets in the blossom-end of muskmelon fruit by using a potassium periodate-tetrazole test under a microscope. Cellular breakdown in these tissues was observed. From this observation fruit cracking seemed to occur as a breakdown of tissues.

The relation of Mn and Fe in plant metabolism has been extensively investigated(2,3,14,16,17,18,19,20). Fukatsu et al.(6) reported that the brown leaf blight of cucumber caused by excess Mn uptake was alleviated with 20 ppm Fe from ferric citrate in the solution. In this experiment of Mn and Fe, the Mn in the leaves, stems and fruit receiving 30 or 60 ppm Mn was significantly decreased with increasing Fe levels. However, the Mn depression by Fe did not affect the plant growth and fruit quality. This probably is due to an excessively high level of Mn found in the plants as described in the previous experiment. The Fe in the leaves was not influenced by the Fe levels as indicated in Table 6. In this experiment ferric citrate was used as Fe sources. However, when iron chelate was used instead of ferric citrate, Fe would be largely absorbed through the roots, and this increased Fe would antagonize the Mn uptake as Osawa pointed out(15). No fruits with cracking were found at the 30 ppm Mn, while they were found at the 60 ppm Mn regardless of Fe levels. This seems to be associated with an increase of Mn and a decrease of Ca at the epidermal layers in the blossom-end of the fruit as described earlier. Finally, in the control receiving 0.5 ppm Mn and 1 ppm Fe the plant

growth and fruit quality were favorable, and the Fe/Mn ratio in the leaves was 0.93. In this case any excess or deficient Mn symptoms were not found in the plant parts. Therefore, 0.5 ppm Mn and 1 ppm Fe should be applied in the nutrient solution for the optimum growth of muskmelon.

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メロンのマンガン過剰症に関する研究 (第5報)

培養液のマンガン, 石灰, 鉄濃度

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摘 要

Mn 0.5, 30, 60 ppm, Ca 1.33, 4.00, 12.00 mM を含む培養液と, Mn 30, 60 ppm, Fe 1, 30, 60 ppm を含む培養液 (対照区は, 0.5 ppm Mn, 1 ppm Fe) とでメロンを砂耕栽培した。Mn と Ca の実験では, 植物体各部の Mn は, Mn 濃度が 0.5 から 60 ppm へと高くなるにつれて明らかに増加し, Ca 濃度が 1.33 から 12.00 mM へと高くなるにつれて逆に減少した。果実の新鮮重, 糖度, 外観の評点は, Mn レベルが高くなるにつれて明らかに減少したが, Ca レベルによつては影響されなかつた。果実のしり割れが, Mn 0.5, 30 ppm で

は, Ca レベルと無関係に発生しなかつたが, Mn 60 ppm では, Ca, 1.33, 4.00 mM で発生した。Mn と Fe の実験では, 植物体各部の Mn は, Mn レベルが高くなるにつれて明らかに増加し, Fe レベルが高くなるにつれて逆に減少した。果実の新鮮重, 糖度, 外観の評点は, Mn 60 ppm で減少し, また, Mn 30 ppm のそれらは, Fe レベルが高くなるにつれて明らかに減少した。しり割れ果実は, Mn 30 ppm ではみられなかつたが, Mn 60 ppm では, Fe レベルと無関係にみられた。