Manganese toxicity in tomato plants: effects on cation uptake and distribution

Jacques Le Bot, E.A. Kirkby, M.L. van Beusichem

JOURNAL OF PLANT NUTRITION, 13(5), 513-525 (1990)

MANGANESE TOXICITY IN TOMATO PLANTS: EFFECTS ON CATION UPTAKE AND DISTRIBUTION

J. Le Bot[1], E. A. Kirkby[1] and M. L. van Beusichem[2]

1. Department of Pure and Applied Biology
The University of Leeds
Leeds LS2 9JT
U. K.

2. Department of Soil Science and Plant Nutrition
Wageningen Agricultural University
P.O. Box 8005 NL
6700 EC Wageningen
The Netherlands.

ABSTRACT: Two experiments are described in which tomato plants (*Lycopersicon esculentum* L. var Ailsa Craig) were grown in water culture supplied with 10-300 μM Mn. Toxicity symptoms associated with a yield reduction were observed only in treatments in excess of 50 μM Mn indicating that this species is relatively tolerant of high Mn supply. Dark brown/black spots appeared first in the cotyledons. Similar symptoms were observed in the leaves, progressively from the oldest leaf. Manganese concentration in the shoot tissues ranged from 286 to 4240 μg. g^{-1} dry weight. The high Mn concentration values found in the shoot tissues of the toxic plants indicate that Mn was highly mobile in the xylem as confirmed by xylem sap analysis.

The concentrations of both Ca and Mg were lower in the smaller Mn toxic plants. Not only was uptake of Ca and Mg retarded but so also was the distribution of Ca and Mg to the younger tissues as illustrated by measurements of Ca and Mg concentrations along a leaf age sequence. This is in accord with the cation-anion balance of the xylem exudates collected from decapitated plants.

Higher cation exchange capacity (CEC) was found in the leaf tissues of toxic plants particularly in the older leaves but similar values of C.E.C were recorded for the younger leaf tissues of both control and toxic plants.

INTRODUCTION

Manganese toxicity occurs frequently both on acid and waterlogged soils. Under such conditions large and toxic amounts of Mn can become available to plant roots and this can be detrimental to both agricultural and horticultural production. The toxicity is well known to affect glasshouse crops following steam sterilization. In the tomato crop Mn toxicity is characterized by a depression in yield and the appearance of dark brown/black spots and dark brown deposits, along the leaf veins of older leaves which become desiccated (16, 4)

In the present paper we report the effect of increasing Mn concentrations in the nutrient medium on yield and cation uptake by tomato plants. Since there is some evidence that not only cation uptake but also cation distribution within the plant can be affected by Mn toxicity (9, 4), we present results showing how Mn toxicity affects cation composition of an ageing sequence of leaves from the same plant. Further information on distribution is provided from cation exchange capacity data and from xylem sap analyses of control and Mn toxic plants.

MATERIAL AND METHODS

Two experiments were carried out to study the effect of increasing Mn concentrations in the nutrient medium. The first one was to investigate the appearance of toxicity symptoms, dry matter yield production and cation uptake. The second one to address the question of cation distribution within the plant.

In the first experiment, 70 similar sized 4 week old tomato plants (*Lycopersicon esculentum* var. Ailsa craig) were transplanted from a peat-perlite mixture to 50 litres of a preculture nutrient solution. This solution provided nutrients at the following concentrations: $Ca(NO_3)_2$ 2 mM, $MgSO_4$ 0.75 mM, K_2SO_4 0.65 mM, KH_2PO_4 0.15 mM. The micronutrient solution contained FeNaEDTA 50 μM, $MnSO_4$ 10 μM, $CuSO_4$ 0.95 μM, $ZnSO_4$ 0.65 μM, H_3BO_3 29.6 μM and

Na_2MoO_4 0.52 µM. The pH of the medium was adjusted to 5.5 using $Ca(OH)_2$ and the plants remained in this solution for a period of 6 days.

After the 6 day preculture, 40 similar sized plants were selected and divided into 4 treatments each of 10 plants supplied with the same nutrient solution but with a range of Mn concentrations (10, 50, 100 and 300 µM Mn). Nutrient solutions were completely replenished every 4 days. The plants were grown for 17 days in a greenhouse. After 9 days of the experiment, 5 plants were harvested in each treatment and the remaining plants were grown for a further period of 8 days when the second harvest was taken. On both occasions shoot and roots were harvested. Leaves, petioles and stem were separated into old (1st to 7th organ) and young (above the 7th) tissues. Oven dried weights (95 °C) were recorded. Dried plant material was prepared for mineral analysis by ashing at 500 °C followed by digestion with HCl. Calcium, magnesium and manganese were determined on the extract by atomic absorption and potassium by flame photometry.

Xylem sap was collected at the middle of the day by detopping the plants about 1 cm above the cotyledons with a sharp blade and fitting the stumps with a soft PVC tubing of appropriate diameter. The bleeding sap accumulated in the tubing and was regularly removed with a syringe over the 2 hours of the sap collection. The samples were then stored in a deep freeze prior to analysis. Inorganic ions in the xylem sap samples were determined after wet digestion of subsamples in a H_2SO_4-Se-Salicilic acid mixture as described by van Beusichem *et al.* (15) except for SO_4^{2-} which was measured turbidimetrically after reaction with $BaCl_2$.

For the second harvest of the experiment for which data are presented, dry matter estimations and chemical analyses of the dried samples were made on 5 separate plants for each treatment. The data recorded are therefore the mean of 5 replicates.

In the second experiment 6 week old tomato plants were precultured as previously described and then divided into 2 sets of 5 plants each and subjected for 9 days to the two extreme Mn treatments (i.e. 10 and 300 µM Mn in the medium). At the end of that period, after the Mn toxicity symptoms had appeared in the 300 µM Mn treatment, the five plants of each treatment were harvested and the plant material separated into individual organs (leaf, petiole and stem) of different age except for the very young plant parts (above the 7th leaf) which were kept together

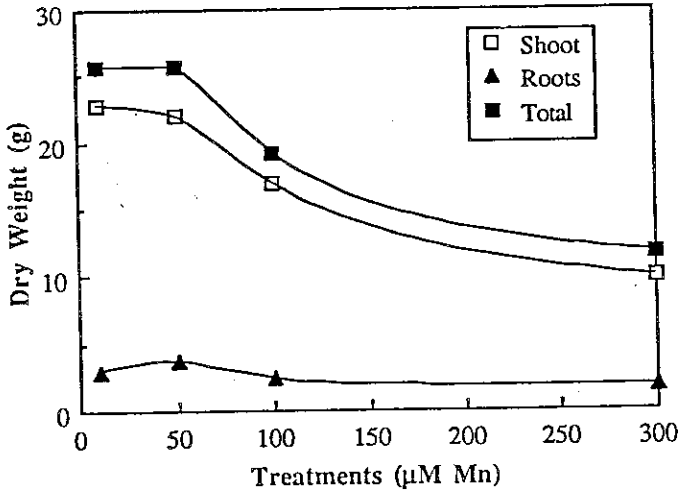

FIGURE 1.

Effect of Increasing Mn Concentrations in the Medium on the Dry Matter Yield of the Plants

TABLE 1

Dry Matter Yield (in grams) of Different Plant Parts in Relation to Extreme Mn Supply. (Data from Exp. 2)

Treatment	Leaves		Petioles	
	Old	Young	Old	Young
10 µM	6.49	4.14	1.67	1.07
300 µM	6.45	2.54	1.48	0.55

as "Top parts". Oven dried weight of plant parts including roots were recorded and mineral analyses carried out as described previously. The Cation Exchange Capacity (CEC in the text) of the leaves of different age were determined according to Crooke (2).

RESULTS

Mn toxicity symptoms were observed in only the two higher Mn treatments (i.e. in excess of 50 µM Mn in the nutrient medium). The symptoms appeared 7 days after the treatments began, typical brown/black spots, presumably of manganese oxides (8), being noted first on the cotyledons. Similar coloured markings were later observed along the veins of the older leaves which gradually became desiccated. These symptoms occurred progressively from older to younger plant parts. Roots of the plants showing toxicity were brown in colour.

Increasing Mn concentration in the nutrient medium in excess of 50 µM Mn, along with the appearance of toxicity symptoms, significantly depressed total dry matter yield (Figure 1). The decline in plant growth was not linear in relation to concentration but fell off rapidly between the 50 µM and 100 µM treatments then declined more slowly. No significant difference in dry matter yield was noted between the 10 µM and 50 µM treatments except for the roots which were significantly greater in weight in the 50 µM treatment.

The reduction in shoot dry matter yield resulting from Mn toxicity was mainly the result of a decrease in the yield of the young tissues (Table 1).

Although the Mn concentrations in the nutrient media were set at fixed levels, it was not possible to maintain constant concentrations during the experiment because of depletion resulting from plant uptake. Table 2 shows the uptake data figures in relation to the total Mn in the solutions as calculated from the number of time the solutions were replenished during the experiment. A mean percentage depletion value is also calculated for each treatment.

Highest Mn concentrations were observed in the roots (Table 3) although some of this Mn may have been precipitated on root surfaces rather than taken up as indicated by the brown colour of the roots in the two toxic treatments. Of the Mn in the shoot highest concentrations were found in the older leaves. As expected Mn

TABLE 2

Mn Uptake by the Plants vs Total Mn in Solution During the Course of the Experiment

Trt (µM)	Total Mn supplied (mg)	Mn uptake	% depletion
10	137.3	95.2	69.3%
50	686.7	239.0	34.8%
100	1373.4	289.6	21.1%
300	4120.3	402.4	9.8%

TABLE 3

Manganese Concentration (µg Mn . g^{-1} dry matter) and Percentage Distribution of Mn in Different Plant Parts of the Four Mn Treatments.

	Concentration of Mn in the nutrient medium			
Plant part	10 µM	50 µM	100 µM	300 µM
Old leaves	710	2085	3835	7900
Shoot	286 (47%)	916 (58%)	1800 (74%)	4240 (79%)
Root	2551 (53%)	3990 (42%)	4666 (26%)	6662 (21%)

TABLE 4

Concentration of Cations in the Shoot of Plants Subjected to the Two Extreme Mn Treatments (Exp. 1).

Treatment	Shoot DW (g)	Mn^{2+}	Ca^{2+}	Mg^{2+}	K^{+}
		(mg . g^{-1} dry matter)			
10 µM Mn	22.77	0.286	28.90	6.75	53.59
300 µM Mn	9.83	4.240	22.38	4.93	64.71

concentrations in all tissues increased with increasing Mn supply. The distribution of Mn between shoot and root shows that as Mn supply is increased so also is total uptake and the proportion of Mn reaching the shoots (from 47% to 79% of the uptake).

High Mn supply depressed the uptake of Ca and Mg but had relatively little affect on K uptake. The smaller Mn toxic plants were therefore lower in Ca and Mg but higher in K concentration (Table 4).

The influence of Mn toxicity on the distribution of the cations and the values for CEC between leaves of different ages but from the same plants is shown in Table 5. Leaf age had a considerable influence on both CEC and the cation composition. Calcium and magnesium concentrations were higher in the older leaves but the reverse was the case for CEC. The concentration of K was little affected by leaf age except in the youngest leaves in which like Ca and Mg it was depressed. In the Mn toxic plants, Ca and Mg concentrations were lower than in comparative control leaf tissues. CEC values on the other hand were increased until leaf 6. Similar CEC values were observed in the young tissues for both the control and the Mn toxic treatments. For Mn concentrations in the control plants, higher values were found in the older leaves which decreased in concentrations with age. For the much higher concentrations that occurred in the Mn toxic plants, the 5th leaf was highest in Mn.

As is illustrated in figure 2, for the Mn toxic plants considerably higher proportions of both the total shoot Ca and Mg were associated with the older leaves when expressed on a percentage content basis, indicating restriction of these elements to the older tissues under conditions of Mn toxicity.

Analyses of xylem sap (Table 6) revealed lower concentrations of Ca and Mg and much higher concentrations of Mn in the Mn toxic treatment. Additionally the exudation rate was almost halved. A close balance between cations and anions was recorded indicating that most likely all the charges had been accounted for.

DISCUSSION

Manganese toxicity occurred only in the two higher Mn treatments as shown by the fall off in the dry matter yields (Figure 1) and the appearance of Mn toxic symptoms. Plants supplied with 50 µM Mn but not 100 µM remained healthy and

TABLE 5

Cation Exchange Capacity and Cation Composition of a Leaf Age Sequence from Plants Exposed to the Two Extreme Mn Treatments (10 µM and 300 µM Mn in the Nutrient Medium). (Leaf 1= Old to Top leaves = Young). Results from Exp. 2.

Plant part	CEC		Ca^{2+}		Mg^{2+}		K^{+}		Mn^{2+}	
	(meq per 100 g of dry matter)									
	10	300	10	300	10	300	10	300	10	300
Leaf 1	11.4	19.0	398	293	116	71	70	111	1.6	17.5
Leaf 2	11.5	20.7	382	255	86	67	74	112	1.5	18.8
Leaf 3	11.6	29.3	324	242	78	59	83	112	1.3	20.2
Leaf 4	16.8	36.2	289	230	79	56	80	110	1.2	22.2
Leaf 5	20.4	39.4	292	192	71	53	76	98	1.3	24.1
Leaf 6	24.8	42.2	241	176	65	52	80	102	1.2	23.2
Leaf 7	25.1	27.4	212	147	57	48	83	87	1.0	21.3
Top parts	32.5	32.0	128	99	46	41	78	77	0.7	15.4

dry matter yields were not affected. It is clear of course that because of depletion of Mn from the nutrient medium by the growing plants, fluctuating and lower concentrations of Mn must have occurred in the media during the growth period, particularly in the 10 and 50 µM treatments than the nominal concentrations would indicate (Table 2). Nevertheless it can still be concluded that tomato is relatively tolerant of Mn since the highest and not necessarily maximum dry matter yields of the 50 µM Mn treatment were associated with relatively high Mn concentrations in the shoot (916 µg $Mn.g^{-1}$ dry weight) and in the older leaves (2085 µg $Mn.g^{-1}$ dry weight) (Figure 1 and Table 1). In the elegant experiments of Edwards and Asher (3) in which 15 plant species, not including tomato, were grown in a nutrient solution with a wide range of Mn concentrations which were maintained constant throughout the experiment, in only 2 of the 15 species investigated, sunflower and centro were maximum yields recorded with Mn shoot concentrations in excess of those reported here for tomato.

It is of interest to note that a number of nutrient solutions, including the full strength Long Ashton solution (7), contain at least 10 µM Mn. From the finding of Edwards and Asher, this concentration of Mn, if maintained at the plant root

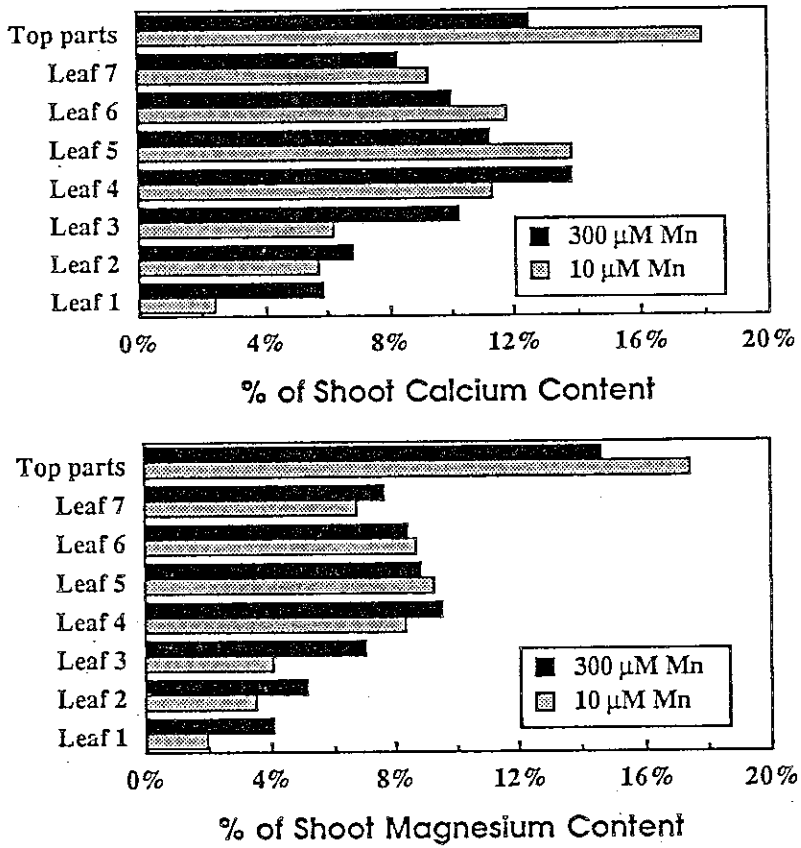

FIGURE 2.
Distribution of Calcium and Magnesium in a Leaf Age Sequence of Plants Subjected to the Two Extreme Mn Treatments. The Values are Expressed as Percentage of the Total Shoot Content in Ca and Mg.

TABLE 6

Volume of Sap and Concentrations of the Main Cations and Anions for the Two Extreme Mn Treatments.

Treatment (µM Mn)	Volume (ml)	K^+	Ca^{2+}	Mg^{2+}	Mn^{2+}	ΣC^+	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	ΣA^-
		($meq.L^{-1}$)								
10	9.78	7.72	7.48	2.21	0.08	**17.50**	14.67	0.80	2.21	**17.97**
300	4.90	10.47	5.76	1.99	1.13	**19.35**	14.36	1.21	3.04	**19.28**

surface, may well be toxic to a large number of plant species, although not to tomato.

A very high proportion of Mn taken up by tomato even under non toxic conditions (50 µM Mn) is translocated to the shoot (58%) and in the extreme treatment this makes up almost 80% of the uptake (Table 3). The high mobility of Mn in the xylem is also evident from the data of the Mn toxic treatments from the concentrations of Mn in leaves of different ages (Table 5) and from the xylem sap analyses (Table 6). The tomato plant therefore appears to tolerate high Mn concentrations in the nutrient medium by virtue of the fact that shoot tissues are able to withstand high concentrations of Mn. In this respect tomato is similar to the extremely Mn tolerant sunflower. By contrast other plants species retain high amounts of Mn in the roots and this can be an important factor in conferring tolerance (3).

Since Mn toxicity symptoms were observed in the leaves of plants supplied with 100 µM Mn but not in those supplied with 50 µM Mn it can be assumed that Mn becomes toxic in the concentration range found in the plants between these two treatments. Using analysis of the entire shoot is not a good guide to the Mn status of plants since Mn concentrations can vary widely between organs. This can be seen in the comparative data for shoot (1800 µg $Mn.g^{-1}$ dry weight) and for old leaves (3835 µg $Mn.g^{-1}$ dry weight) of plants grown in the 100 µM Mn toxic treatment (Table 3). The importance in selecting specific tissue of a given age in such experiments has been reported in numerous studies of Ohki (see 14).

That Mn depresses the uptake of other cations from the nutrient medium has been observed by a number of authors including Heenan and Campbell (6). Our results with tomato confirm their observations with soybean that Mn competes very strongly particularly with Mg for uptake and the uptake is a more than 1:1 competition for specific binding sites. Mn competes more effectively than its concentration in the nutrient medium would warrant on a 1:1 basis and it appears to block binding sites for Mg as discussed by Marschner (12). Our results in Table 4 are in accord with these views. Increasing the Mn concentration in the outer medium results in a greater than expected uptake of Mn in relation to the fall in Mg uptake if a 1:1 competition only were involved. The same to a lesser extent is true for Ca. Similar observations on cation competition can be noted from the xylem sap

composition data in Table 6. In agreement with Heenan and Campbell (6) we also found no competition effect between Mn^{2+} and K^+ for uptake, presumably because different binding sites are involved for these two cation species.

The influence of Mn toxicity in affecting cation distribution has been reported previously, the toxicity having been shown to induce Ca deficiency particularly in younger tissues both in cotton (5) and in bean (9). Our results also show that there is marked influence of Mn in restricting both Ca and Mg to the older leaves in the shoot of tomato (Figure 2). Not only were the concentrations of Ca and Mg reduced in the younger leaves of the Mn toxic plants (Table 5) but so also were dry matter yields. Whether this yield depression resulted from the higher Mn concentrations or the lower Ca and Mg concentrations or both is not clear. The transport of Ca - and to a lesser extent Mg - from root to shoot occurs mainly in the transpiration stream (see 11). In the Mn toxic plants there is evidence that transpiration was detrimentally affected from both the observation of the symptoms of the older leaves and the much slower rate of bleeding of the xylem sap from decapitated plants (Table 6). This lower rate of exudation of sap containing lower amounts of Ca and Mg in the Mn toxic treatment is in accord with the lower Ca and Mg concentrations recorded in the leaves of these plants (Table 5).

Exchange sites on the cell walls of the xylem may inhibit long distance transport of Ca (1, 10) and the higher values for CEC for the leaves of the Mn toxic plants (Table 5) may be considered in this context since the more numerous exchange sites in the cell walls of the Mn toxic leaves may offer a greater possibility for the retention of the lower concentrations of Ca and Mg.

Horst and Marschner (9) have suggested that, as cell wall expansion and synthesis are to some extent regulated by auxins, Ca translocation into growing leaves, where transpiration appears not to play a role, may be inhibited by auxin deficiency induced by Mn toxicity. There is evidence that Mn toxicity leads to an increase in IAA oxidase activity and thus caused auxin deficiency (13). Data of Horst and Marschner indicate a lowering of CEC in the primary leaves of Mn toxic bean plants. Our results for the CEC of young leaf tissue of tomato plants with and without Mn toxicity were in the same range as those obtained by Horst and Marschner but we were unable to detect the slight reduction in CEC (15%) which they reported in Mn toxic tissues. In our experiments relatively large amount of young plant tissue were bulked in the 'tops' samples and it may well be that effects

on the CEC in the youngest tissues may have been masked. There is still therefore a need for a critical biochemical study of causal relationships between auxin metabolism, Mn toxicity and Ca transport.

ACKNOWLEDGMENTS

The authors express their appreciation to Mr. J. A. Nelemans for his valuable technical assistance with the analytical work.

REFERENCES

1. BELL, C. W. and O. BIDDULPH 1963. Translocation of calcium, exchange versus mass flow. Plant Physiol. 38, 610-614.

2. CROOKE, W. M. 1964. The measurement of the Cation-Exchange Capacity of plant roots. Plant and Soil 21, 43-49.

3. EDWARDS, D. G. and C. J. ASHER 1982. Tolerance of crops and pasture species to manganese toxicity. pp 145-150. In A. Scaife (ed) Proceedings of the 9th International Plant Nutrition Colloquium, Warwick England. Commonwealth Agricultural Bureau, Farnham Royal Bucks, England.

4. FOY, C. D. 1984. Physiological effects of hydrogen, aluminium and manganese toxicities in acid soil. pp 57-97. In F. Adams (ed) Soil Acidity and Liming-Agronomy Monograph n°12 (2nd ed.) American Society of Agronomy, Madison, WI, USA.

5. FOY, C. D., H. W. WEBB and J. E. JONES 1981. Adaptation of cotton genotypes to an acid, manganese toxic soil. Agron. J., 73, 107-111.

6. HEENAN, D. P. and L. C. CAMPBELL 1981. Influence of potassium and manganese on growth and uptake of magnesium by soybeans (*Glycine max* (L.) Merr. cv. Bragg). Plant and Soil, 61, 447-456.

7. HEWITT, E. J. 1966. Sand and Water Culture Methods Used in the Study of Plant Nutrition, 2nd ed. Commonwealth Agricultural Bureau Technical Communication 22.

8. HORST, W. J. 1988. The physiology of manganese toxicity. pp 175-188. In Graham, R. D., R. J. Hannam and N. C. Uren (eds) Manganese in Soils and Plants, Kluwer Academic Publishers, Dordrecht, The Netherlands.

9. HORST, W. J. and H. MARSCHNER 1977. Effect of excessive manganese supply on uptake and translocation of calcium in bean plants (*Phaseolus vulgaris* L.). Z. Pflanzenphysiol. Bd., 87, 137-148.

10. ISERMANN, K. 1970. The effect of adsorption processes in the xylem on the calcium distribution in higher plants. Z. Planzenernähr. Bodenk. 126, 191-203.

11. KIRKBY, E. A. and D. J. PILBEAM 1984. Calcium as a plant nutrient. Plant Cell Environ. 7, 397-405.

12. MARSCHNER, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, London.

13. MORGAN, P. W., D. M. TAYLOR and H. E. JOHAM 1966. Effect of manganese toxicity on the indoleacetic acid oxidase system in cotton. Plant Physiol. 41, 718-724.

14. OHKI, K. 1977. Manganese and zinc status related to maximum growth for selected agronomic crops. pp 660-668 In Soc. Sci. Soil Manure, Japan. Proceedings of the International Seminar on Soil Environment and Fertility Management in Intensive Agriculture. Tokyo, Japan.

15. VAN BEUSICHEM, M. L., E. A. KIRKBY and R. BAAS 1988. Influence of nitrate and ammonium nutrition on the uptake, assimilation, and distribution of nutrients in *Ricinus communis*. Plant Physiol., 86, 914-921.

16. WARD, G. M. 1977. Manganese deficiency and toxicity in greenhouse tomatoes. Can. J. Plant Sci., 57, 107-115.