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The significance of the magnesium to manganese ratio in plant tissues for growth and alleviation of manganese toxicity in tomato (*Lycopersicon esculentum*) and wheat (*Triticum aestivum*) plants

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Abstract

Results are reported for tomato (*Lycopersicon esculentum* L. var. Ailsa craig) and wheat (*Triticum aestivum* L. cv. Mara) which demonstrate that increasing concentrations of Mg in the plant raises plant tolerance to Mn toxicity.

Water culture experiments with tomato show that under conditions of high Mn supply (200 μM, Mn), not only does increasing Mg application (0.75 mM to 15 mM) depress Mn uptake, but the higher Mg concentrations in the shoot counteract the onset of Mn toxicity when the concentrations of Mn in the shoot are also high. The ratio of Mg:Mn in the tissues is a better indicator of the appearance of toxicity symptoms than Mn concentration alone. Toxicity symptoms were observed when the Mg:Mn ratio in the shoot tissue was from 1.13 to a value between 3.53 and 6.54. The corresponding Mg:Mn ratio in the older leaves was from 0.82 to between 2.27 and 3.51.

For wheat grown in soil, analyses of leaves revealed that growth could be expressed by the following relationship: $Y = A + B\exp(-kX)$, where Y = growth, X = Mg:Mn ratio, A, B and k = constants. Growth was significantly reduced when the Mg:Mn ratio fell below 20:1. From a measurement of this ratio it is therefore possible to predict the appearance of Mn toxicity and its influence on growth.

Introduction

Manganese toxicity has long been recognized as an important factor limiting plant growth on acid and waterlogged soils. Different plant species and cultivars of the same species differ in tolerance to Mn. As well as these genetic differences, environmental factors including nutrition can also be important in conferring tolerance. In particular other nutrients such as Fe, Ca and Mg in the growth medium can modify the uptake of Mn from solution (Chinnery and Harding, 1980; Maas *et al.*, 1969).

In the older literature especially there are frequent references to the interaction between Mn and Mg as plant nutrients. The beneficial effects of Mg in the nutrient medium in depressing Mn toxicity was reported by Löhnis (1960) and more recently Hecht-Buchholtz *et al.* (1987) have observed that in Norway spruce seedlings excess Mn is taken up when Mg is in short supply. Interrelationships between Mn toxicity

and Mg deficiency have also been studied in melon (Elamin and Wilcox, 1986a; 1986b; Simon *et al.*, 1986).

The beneficial influence of Mg appears to result in part from depressing Mn uptake, though there are also indications that a higher concentration of Mg in plant tissues confers tolerance to high concentrations of Mn. In this paper we compare these factors in relation to the effects of Mg in raising the tolerance of tomato and wheat plants to Mn.

Materials and methods

Two sets of data are reported, one for tomato in experiments at the University of Leeds UK, and the other for wheat grown in pots of soil obtained from Portugal and known to produce symptoms of manganese toxicity in wheat (Goss and Carvalho, 1989).

Two water culture experiments used tomato plants (*Lycopersicon esculentum* L. var. Ailsa craig). The first was to study concentrations of Mn of 10 μM, 50 μM, 100 μM and 300 μM in nutrient solutions each provided with 0.75 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 0.65 mM K_2SO_4 and 0.15 mM KH_2PO_4. The micronutrient solution contained FeNa-EDTA 50 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 nutrient solution was adjusted to 5.5 with a saturated solution of $Ca(OH)_2$. Ten plants were grown from the 4th leaf stage for each of the 4 Mn treatments in 50 litre containers and the aerated nutrient solutions were renewed every 4 days. Two harvests were taken one of 5 plants after 9 days and the other of the remaining 5 plants after 17 days. Each plant was separated into leaves, petioles, stems and roots and the shoot organs were divided into old and young tissues. The samples were oven dried (95°C) and weighed.

The second experiment investigated increasing concentrations of Mg in the nutrient medium (0.75 mM, 1.5 mM, 7.5 mM and 15 mM as $MgSO_4$) at a Mn concentration of 200 μM which the result of the previous experiment indicated would normally be toxic. The basic nutrient solution was the same as in the first experiment except that the K_2SO_4 and KH_2PO_4 concentrations were slightly increased to 0.75 mM K_2SO_4 and 0.25 μM KH_2PO_4 respectively. Two harvests were taken, one of 5 plants after 11 days and the remaining 5 plants after 20 days. The same harvesting procedure was adopted as in the first experiment. In both experiments results of the second harvest are reported.

The dried and ground plant material was analyzed for K by flame photometry and for Ca, Mg and Mn by atomic absorption spectrometry.

In the experiments with wheat (*Triticum aestivum* L. cv. Mara), a coarse-textured Portuguese soil derived from quartzdiorite was amended or not with $CaCO_3$ (limed or unlimed soil). Manganese and magnesium concentrations in the soil solution were modified by the addition of $CaCO_3$ and of increasing quantities of $MgSO_4$. These modified soils were then packed in columns (65 mm diameter and 250 mm height) and fertilized with NH_4NO_3 (31.6 mg N kg^{-1} of air-dry soil) and KH_2PO_4 (16.6 mg P and 20.8 mg K kg^{-1} of air-dry soil). The Mg:Mn ratio in the soil solution of these soils ranged between 1.6 (no $CaCO_3$ nor $MgSO_4$ added to the soil) and 347.4 (550 mg $CaCO_3$ kg^{-1} and 336 mg $MgSO_4$ kg^{-1} of air-dry soil). Four pre-germinated seeds were planted into each pot which was maintained at 17°C in a controlled environment chamber. After 3 weeks growth, shoots were cut off and weighed after oven drying at 60°C for 24 hours. Shoot material was digested in nitric acid and acidified to produce solutions with 5% v/v HCl which matched the calibration standards for the Inductively Coupled Plasma-Optical Emission Spectrometer (ARL 3400) used for measuring the Mg and Mn contents.

Results

Increasing Mg in the nutrient medium under conditions of high Mn supply (200 μM Mn) increased total dry matter yields of tomato plants and alleviated Mn toxicity symptoms in the two higher Mg treatments (Table 1). Both total Mn uptake per plant and the Mn concentration expressed on a whole plant dry matter basis were depressed by Mg. Magnesium uptake was markedly increased but that of Ca was depressed and

Table 1. The influence of increasing Mg supply (as $MgSO_4$) on the uptake of cations by tomato plants growing in a nutrient solution containing 200 μM Mn. (results expressed as $mg.g^{-1}$ dry weight and mg uptake per plant)

Treatment[a] (m*M* Mg)	Relative dry matter yields[b] (g)	K	Ca	Mg	Mn	Mn uptake[b] (mg plant^{-1})
		(mg g^{-1})[b]				
0.75*	72 c	63.4 b	22.2 a	5.6 d	3.5 a	65.5 a
1.5*	70 c	68.9 a	20.0 b	7.6 c	3.2 b	57.9 ab
7.5	87 b	62.4 b	13.0 c	15.4 b	2.2 c	49.2 bc
15	100 a	54.2 c	7.8 d	19.2 a	1.8 d	45.3 c

[a]*denotes the presence of Mn toxicity symptoms.
[b]Values followed by different letters within a column are significantly different ($P = 0.05$).

K was little affected except in the highest Mg treatment in which it was somewhat decreased.

That the influence of Mg in preventing the onset of Mn toxicity symptoms and increasing dry matter yield was not simply the result of a decrease in Mn uptake, and hence also in Mn tissue concentration, can be seen from Table 2. Raising the Mn supply to 100 μM whilst retaining Mg concentration in the nutrient medium constant, depressed shoot yields by about 25% and markedly increased Mn concentration in the shoot, where toxicity symptoms were first observed in the older leaves. However, even when the Mn concentration in the nutrient medium was doubled to 200 μM Mn by also raising the Mg supply tenfold (to 7.5 m*M* Mg) the plants remained healthy despite having a somewhat higher concentration of Mn in the shoots than in the plants suffering from Mn toxicity.

From this evidence we conclude that a high concentration of Mg in plant tissues can alleviate Mn toxicity even though Mn tissue concentration is also high. If this is so, the Mg : Mn ratio should be a better indicator of plant Mn status in relation to Mn toxicity than Mn concentration alone. This is illustrated by comparing Figures 1 and 2. In Figure 1, Mn concentrations of 40 samples of old tomato leaves obtained from 5 replicate plants from 8 different treatments of Mg and Mn supply are evaluated in relation to the appearance of Mn toxicity symptoms. These older leaves which are the first plant tissues to show toxicity symptoms, have concentrations ranging from 658 μg Mn g^{-1} to 8579 μg Mn g^{-1} and the concentration is positively related to the extent to which the leaves show toxicity. However in the intermediate range there is no correlation, as leaves of 3758 μg Mn g^{-1} show toxicity whereas those with 5270 μg Mn g^{-1} are healthy. When these data are considered in terms of the Mg : Mn ratio (Fig. 2), the healthy leaves always have higher ratios than the leaves showing toxicity. The critical Mg : Mn ratio for the appearance of toxicity symptoms in old tomato leaves lies between 2.27 and 3.51. Corresponding values for the shoot are 3.53 and 6.54.

Measurements of Mn in the shoots of wheat plants grown on an unlimed Mn toxic soil showed that shoot growth declined as Mn concentration increased above 100 $\mu g\, g^{-1}$ (Fig. 3).

Table 2. The influence of magnesium and manganese supply in the nutrient medium at given ratios Mg : Mn on the relative shoot yields, Mg and Mn concentrations in shoots and on the ratios of Mg : Mn in the shoot of tomato plants. (Ratios calculated in terms of $\mu g\, g^{-1}$ dry weight)

Treatment[a]	Relative dry matter yield of shoots (g)	Mg concentration in shoot[a] ($\mu g\, g^{-1}$)	Mn	Mg : Mn ratio in shoot (in terms of $\mu g\, g^{-1}$)	Mg : Mn ratio in solution (in terms of $\mu g\, ml^{-1}$)
10 μM Mn, 0.75 m*M* Mg	100	6746	286	23.6	33.2
100 μM Mn, 0.75 m*M* Mg*	74.7	6061	1800*	3.4	3.3
200 μM Mn, 7.5 m*M* Mg	—	16190	1905	8.5	16.6

[a]* denotes the presence of Mn toxicity symptoms.

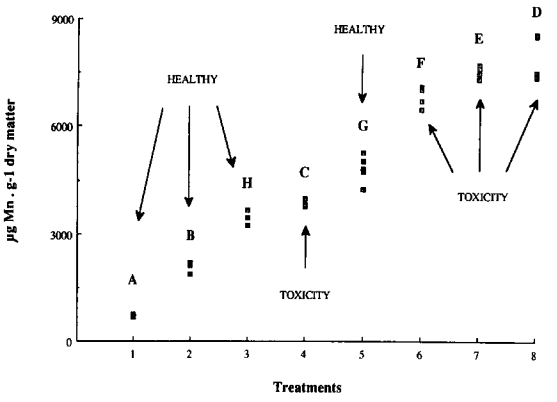

Fig. 1. Manganese concentrations in the old leaves obtained from 5 replicate tomato plants from 8 different nutritional regimes of magnesium and manganese. Treatments: A,0.75 m*M* Mg, 10 μM Mn; B, 0.75 m*M* Mg, 50 μM Mn; C, 0.75 m*M* Mg, 100 μM Mn; D, 0.75 m*M* Mg, 300 μM Mn; E, 0.75 m*M* Mg, 200 μM Mn; F, 1.5 m*M* Mg, 200 μM Mn; G, 7.5 m*M* Mg, 200 μM Mn; H, 15 m*M* Mg, 200 μM Mn

When the soil was limed, however, the Mn concentrations were decreased and fairly constant despite considerable differences in shoot growth. There was thus no satisfactory relationship between growth and the concentration of Mn (or Mg) in the shoots. By expressing the growth as a function of the ratio Mg:Mn concentration in shoot tissues obtained from plants grown on two different soils (limed and unlimed soil) (Fig. 4), an exponential equation of the form $Y = A + B \exp(-kX)$ was obtained in which: Y = shoot growth, X = Mg:Mn ratio in

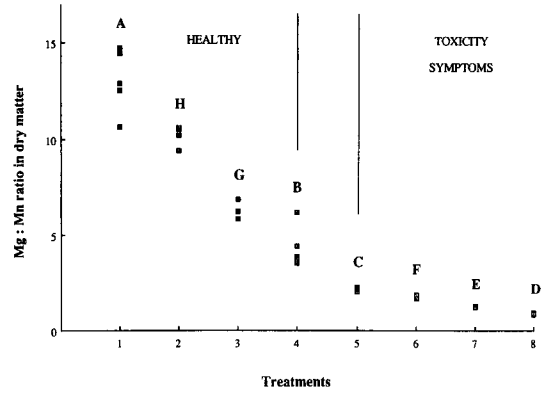

Fig. 2. Magnesium:manganese ratios [expressed in terms of $mg.g^{-1}$ dry weight] in the old leaves obtained from 5 replicate tomato plants from 8 different nutritional regimes of magnesium and manganese. (Treatments as for Fig. 1).

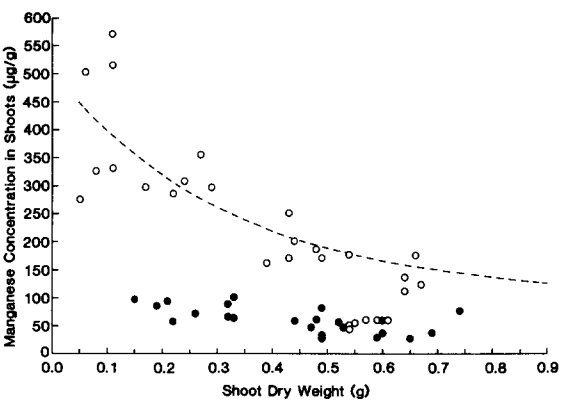

Fig. 3. Relationship between shoot dry weights and manganese concentration in the leaves of wheat. Closed symbols denote plants grown in soil with calcium lime added.

the shoot and A, B and k = constants. Using this relationship it was possible to predict shoot growth from Mg:Mn ratios in the leaves. Growth was significantly reduced when the ratio fell below 20:1.

A similar curve between Mg:Mn ratio and dry matter yield may also be derived from the data of Elamin and Wilcox (1986a; b) (Fig. 5), who investigated Mg/Mn interactions in melons. Again a shoot ratio of about 20:1 is required for optimum yield.

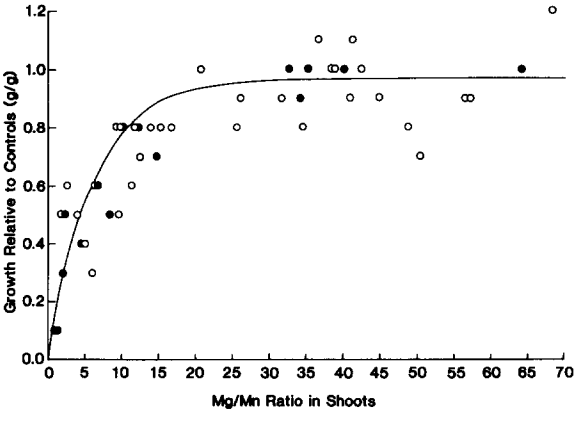

Fig. 4. Relationship between shoot growth and the magnesium : manganese ratio in wheat from limed (closed symbols) and unlimed soils (open symbols). The fitted curve is for the combined data obtained from both soils; values for constants: A = 0.97 B = −A (0.028) k = 0.167 (0.019) (numbers in brackets denote standard errors)

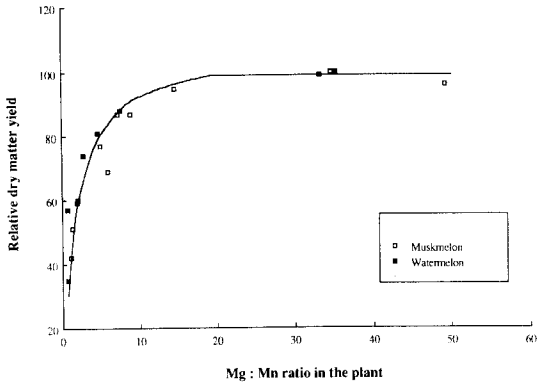

Fig. 5. Relationship between relative yields of muskmelon and watermelon and the magnesium : manganese ratio in the plants. (recalculated from the data of Elamin and Wilcox, 1986a, b).

Discussion

Our results with tomato show clearly that increasing Mg supply in the nutrient medium depressed Mn uptake, in accordance with earlier findings for other plant species (Elamin and Wilcox, 1986a; 1986b; Maas *et al.*, 1969). However this was not the only influence of Mg in alleviating Mn toxicity in tomato. Plants rich in Mg are better able to tolerate high Mn tissue concentrations and the Mg:Mn ratio in shoot tissue is a good indicator in predicting the presence or absence of Mn toxicity symptoms in older leaves (Table 2, Figs. 1 and 2). We therefore agree with the conclusion of Horst (1988) that for a given species or cultivar it is not possible to identify a Mn tissue concentration at which toxicity will occur.

In tomato we were not able to measure the effect of the Mg:Mn ratio on yield, as in each of the two experiments there were only 4 treatments. However for wheat when growth was expressed as a function of the ratio of Mg to Mn concentrations in the leaves, a relationship of the form $Y = A + B \exp(-kX)$ was obtained. This relationship indicates that growth was significantly reduced when the ratio of the shoot Mg:Mn fell below 20:1.From this evidence it is possible to predict that the limitation of growth depends on the ratio of the two ions in the shoot and not the absolute concentration of Mn.

The Mg:Mn leaf ratio of 20:1 for optimum growth of wheat is similar to values for melons derived from the results of Elamin and Wilcox (1986a, b) but is higher than the ratio which we found in healthy leaves of tomato. The lower ratio for tomato may indicate the much greater tolerance of this crop to Mn (Edwards and Asher, 1982; Le Bot *et al.*, 1990). Also the ratios for tomato relate to the appearance of toxicity symptoms rather than to optimum growth.

The effect of increasing Mg supply in the nutrient medium in depressing Mn uptake relates to the competition of the two divalent cations in membrane transport. It is of interest in this respect that competition for binding sites in the roots is more than a 1:1 competition (in μM terms) in favour of Mn. According to Marschner (1986) Mn^{2+} not only competes more effectively but also blocks binding sites for Mg^{2+} uptake. The competitive effect of Mg^{2+} in depressing Ca^{2+} and to a lesser extent K^{+} uptake has been reported by other workers, and may be interpreted as general cation competition for cellular anion charge (Mengel and Kirkby, 1987).

It is still largely a matter of speculation why increasing concentrations of Mg in the plant should raise plant tolerance to Mn. A similar effect of Si on Mn tolerance of bean plants has been reported by Horst and Marschner (1978). These workers suggest that increased tolerance results from the altered and more homogeneous microdistribution of Mn brought about by Si. In the case of Mg and Mn it is well established that both ions are similar and to a certain extent interchangeable in biochemical behaviour. The ratio of Mg:Mn in plant cells may therefore also affect intracellular Mn distribution thereby increasing tissue tolerance. When the ratio is high, even in the presence of high concentrations of Mn^{2+}, Mg^{2+} may replace Mn^{2+} from physiologically active sites in the cytoplasm and Mn^{2+} may be sequestered in cell walls and vacuoles and thus rendered harmless.

Recent evidence of Houtz *et al.* (1988) from experiments with tobacco also indicates that the ratio of Mg:Mn in leaf tissue can influence growth by a direct effect on the rate of net photosynthesis, as ribulose bisphosphate (RuBP) carboxylase/oxygenase is activated by either Mg^{2+} or Mn^{2+}. Under normal circumstances when Mg^{2+} is dominant, carboxylation is favoured but when Mn^{2+} is high relative to Mg^{2+}

the oxidative rather than the reductive photosynthetic cycle becomes operative. One of the earliest physiological symptoms of Mn toxicity is thus a fall in the net rate of photosynthesis. The alleviating effect of high Mg tissue concentration on Mn toxicity may therefore depend on this relationship, a high Mg:Mn ratio being required for RuBP carboxylase activity, and hence also net photosynthesis and growth. The presence of Mn oxides as a symptom of Mn toxicity in the plant tissues may also result from the shift towards oxidative processes when the Mg:Mn ratio is low.

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