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MANGANESE TOXICITY ALLEVIATED BY MYCORRHIZAE IN SOYBEAN

Gabor J. Bethlenfalvay and Raymond L. Franson

USDA-ARS, Western Regional Research Center
Albany, California 94710

ABSTRACT: Soybean (Glycine max (L.) Merr.) plants nodulated with Bradyrhizobium japonicum, Nitragin strain 61A118, were grown with or without the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus mosseae (Nicol. Gerd.) Gerd. and Trappe in pot cultures in soil high (40.4 µg/g) in available Mn. Leaves of the nonVAM plants showed severe symptoms of Mn toxicity and had toxic (314 µg/g) concentrations of Mn in the foliage. NonVAM plants had significantly lower dry weights and nodule mass than VAM plants. Concentrations of Mn in the VAM plants were significantly ($P<0.05$) lower than in the nonVAM plants, and there were no symptoms of Mn toxicity. Both VAM and nonVAM plants had a significant negative correlation between shoot dry mass and leaf Mn concentration. Since levels of Mn increased with increasing VAM-fungal colonization, we conclude that it was not the VAM condition per se which alleviated Mn toxicity.

We suggest that the significantly higher levels of Mn in the leaves ($P<0.05$) and the roots ($P<0.001$) of nonVAM plants was due to increased uptake of Mn by the nonVAM plants. This exudation, which are generally observed in nonVAM plants, and to the role of such exudates in solubilizing MnO_2 and chelating the resulting Mn^{2+} for facilitated absorption.

INTRODUCTION

Reports of heavy-metal effects on plants are legion. Reviews have been recently listed by Baker (1987), summarizing the concensus view that plants resist heavy metal stress (22) by mechanisms of avoidance or tolerance (19). In either case, it is said to be some property of the plant itself which either confers tolerance, or whose lack lets the plant succumb to stress. Although crop plants are vesicular-arbuscular mycorrhizal (VAM) as a rule, the literature implies that most experiments and observations on metal tolerance have been done on sterilized (nonVAM) growth media or solution cultures, if under (VAM) field conditions, then without considering the endophyte as a contributing factor.

We have reported both the enhancement and inhibition of micronutrient uptake by VAM fungi in the past (28), and continue to observe that the endophytes modify transient irregularities in leaf and root-nodule development in plants grown in soil sterilized by autoclaving. Having diagnosed some

of the symptoms as Mn toxicity, and noting that large differences in tissue Mn between VAM and nonVAM plants have been reported by others (7), we compared nutrient uptake by VAM and nonVAM plants to determine toxic effects introduced by the sterilization process. The purpose of our paper is to report the findings, to put them into context with norms for adequate and excess levels of nutrition found in the literature, and to discuss mechanisms for the alleviation of Mn toxicity attributable to VAM fungi.

MATERIALS AND METHODS

Experimental design and growth conditions: Plants (10 VAM and 10 nonVAM) were grown in a walk-in type growth chamber using a completely random design, under uniform conditions of light intensity (800 μmol of photosynthetically active radiation $m^{-2}s^{-1}$ at soil level). Pots (1.5 L) were filled with a 2:1 (v:v) mix of a heavy silt-loam soil (Typic Xerorthent, Balcom Series, Yolo County, California) and fine sand. This mix had a pH of 8.2 and was deficient in plant-available (NH_4HCO_3-extractable) P (3.3 μg g^{-1}) and total N (0.7 μg g^{-1}). It was steamed for 12 h and autoclaved for 4 h. Soil (plant-available) Mn concentrations (extracted with NH_2HCO_3-diethyltriamine penta acetic acid) were 24.1 μg g^{-1} before and 40.4 μg g^{-1} after sterilizing. Total soil Mn was 650 μg g^{-1}.

Low levels of N and P were added to favor nodulation without inhibiting VAM colonization. Plants were watered at planting and on the 7th and 14th days after planting with a nutrient solution containing 1.0 mM $Ca(NO_3)_2$, 0.2 mM KH_2PO_4, 0.25 mM $MgSO_4$ and 0.5 mM K_2SO_4 and micronutrients (without Mn) at one-quarter strength Johnson's solution (16). Iron was added as the pH-stable chelate of FeEDDHA (0.02 mM). NonVAM plants received 0.4 mM KH_2PO_4 with the basal nutrient solution at day 21, at the end of the rapid-growth phase of fungal development in the root, when P input by the endophyte becomes significant (5). Plants were watered again on day 25 and harvested 30 d after planting. After 21 d of growth, N supplementation was discontinued for all plants and P supplementation for the VAM plants, thus adjusting the P regimes of VAM and nonVAM plants to make growth similar.

Biological Materials: Soybean (Glycine max (L.) Merr. cv. Hobbit) seeds were surface sterilized with 70% (v:v) ethyl alcohol, germinated for 3 d on moist paper, selected for uniformity and planted in the soil-sand mix soaked from below with deionized water. Soil inoculum (60 mL) of the VAM fungus Glomus mosseae (Nicol. & Gerd.) Gerd. and Trappe consisting of approximately 1000 sporocarps and many colonized root segments was mixed into the soil of half of the plants. The fungus (isolate number WRRC #1) was collected from an arid site in

TABLE 1

Plant growth parameters. Soybean plants were grown with or without a VAM fungus in a soil high in available Mn. Numbers are the means of 10 replications, ± standard error of the mean for VAM colonization. Plant parameters [VAM (+); nonVAM (-)] were evaluated by Student's t test (***, 0.1%; **, 1%; *, 5%).

VAM	Dry mass (g)				Colonization
	Plant	Shoot	Root	Nodule	(%)
+	1.7***	1.2***	0.47*	0.020***	26 ± 2
-	1.3	0.9	0.42	0.004	

California (6). All plants were inoculated with 10 mL of a culture medium containing 10^9 cells mL^{-1} of the diazotrophic bacterium Bradyrhizobium japonicum strain 61A118 (Nitragin Co.), and watered at planting with a wash of the VAM inoculum sieved (43 µm) to free it from VAM-fungal propagules.

Assays: Roots were carefully washed. Plant parts were dried at 70°C for 1 d. Roots and leaves were ground by mortar and pestle. The powder passing a 43 µm screen was analyzed for elemental composition utilizing standard methods by the Research Extension Analytical Laboratory, Ohio State University, Wooster, Ohio. Data were analyzed by Student's t-test for the VAM-nonVAM comparisons (10 replications each) and

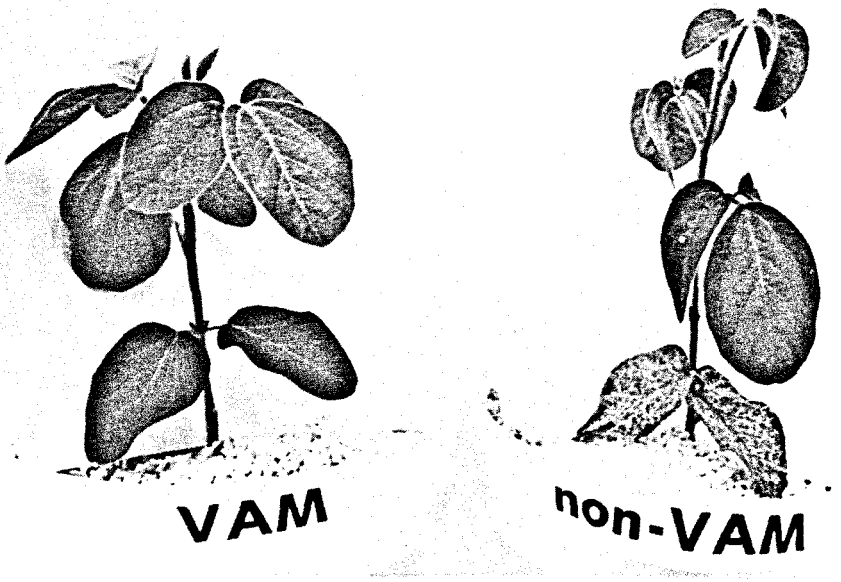

Fig. 1. Toxicity symptoms in soybean plants grown with or without a VAM fungus in soil high in available Mn.

by regression analysis for pertinent dependent-independent variable relationships.

RESULTS

Plant and nodule weights of VAM plants were significantly greater than those of nonVAM plants at harvest (Table 1). Comparisons with plants grown previously under similar conditions (except for steaming the soil) indicated growth retardation of both VAM and nonVAM plants and an inhibition of

VAM colonization in the current experiment. At the same time the nonVAM plants showed acute symptoms of Mn toxicity (Fig. 1) and an almost complete inhibition of nodulation, which has been associated by others with Mn toxicity and P deficiency.

The concentration of macronutrients in plant tissues, except for P (Table 2), was within or near the ranges reported as 'sufficient' for soybeans (24). Unfortunately, there was not enough root or shoot dry matter available to assay for N in addition to the other analyses. Fixation of N_2 by the VAM plants may have partially accounted for their better growth. Of the micronutrients (Table 2) leaf B, Cu, Fe, and Zn concentrations were 'sufficient' (24) and well below toxic or excess levels (30). Leaf Al in all plants (28.8 μg g^{-1}, VAM; 18.5 μg g^{-1}, nonVAM) was within estimates of normal Al concentrations (11, 21).

Adams (1) reported toxicity symptoms and growth retardation above 300 μg Mn (g leaf dry matter)$^{-1}$, a level which was exceeded in our nonVAM plants. It was the only micronutrient at toxic levels (Table 2). The Mn concentration of the nonVAM roots was higher than that of the VAM roots by 68%. While soil contamination cannot be excluded, the very small variation in the values (see standard errors) indicates that this factor was minimized by the careful washing of the roots. Concentrations of Mn in VAM plant leaves (281 μg g^{-1}) were also excessive (> 200 μg g^{-1}, see reference 30). This was

TABLE 2

Mineral nutrient levels in plant organs. Growth conditions and statistics are as in Table 1.

Elemental	Leaf				Root	
composition	VAM	nonVAM	Sufficient[a]	Excess/Toxic[b]	VAM	nonVAM
Macronutrients (mg g dry mass^{-1})						
Ca	24.3 ± 0.5	24.7 ± 0.5	3.6 - 20.0	30.0	8.7 ± 0.3	9.6 ± 0.3
K	16.3*** ± 0.4	12.8 ± 0.4	17.1 - 25.0	27.5	21.7** ± 0.5	18.7 ± 0.4
Mg	4.0* ± 0.2	3.7 ± 0.2	2.6 - 10.0	15.1	16.5 ± 0.4	17.5 ± 0.4
P	1.3 ± 0.1	1.2 ± 0.1	2.6 - 5.0	8.0	1.3** ± 0.1	1.1 ± 0.1
Micronutrients (ug g dry mass^{-1})						
B	36.0*** ± 1.9	45.0 ± 2.1	21 - 55	80	28.1*** ± 1.7	23.8 ± 1.5
Cu	18.9 ± 1.4	21.0 ± 1.4	10 - 30	50	49.9*** ± 2.2	63.6 ± 2.5
Fe	100.8 ± 3.2	103.3 ± 3.2	51 - 350	500	3768* ± 19	3263 ± 18
Mn	281.4* ± 5.6	314.2 ± 5.9	21 - 100	300[c]	358*** ± 9	1432 ± 12
Zn	73.3* ± 2.7	30.2 ± 1.7	21 - 50	450[d]	180*** ± 4	51 ± 2

[a]Sufficiency levels were adapted from Mengel *et al.* (1981). [b]Excess levels are from Scott and Aldrich (1970). Toxic levels, as available are from: [c]Adams (1984) and [d]Macniol and Beckett (1985).

reflected by the linear decline of shoot dry mass with increasing leaf Mn concentration, paralleling a similar pattern in the nonVAM plants (Fig. 2, Table 3). In the VAM plants, both root and shoot Mn increased significantly ($P < 0.05$) with increasing VAM-fungal colonization (Fig. 3). Thus, the most-colonized VAM plants were the smallest, apparently as a result of their Mn concentrations. The relationship between root and shoot Mn and nodulation was not significant in VAM ($P > 0.2$) or nonVAM ($P > 0.9$) plants (Table 3). Nodule formation increased significantly ($P = 0.028$) with VAM colonization (Table 3).

Available soil Mn increased from 24.1 to 40.4 $\mu g\ g^{-1}$ as a result of steaming and autoclaving, and declined during the growth period in the soils of the VAM and nonVAM plants to 28.2 and 36.2 $\mu g\ g^{-1}$, respectively. The difference in available Mn in the soils of the two treatments was highly significant ($P < 0.001$). NonVAM soil pH (8.2) was higher ($P < 0.01$) than that of the VAM soil (7.8). Root, leaf, nodule and VAM-fungal parameters were not correlated significantly with soil Mn (Table 3). Soil pH, however, was significantly correlated with the development of both microsymbionts in the VAM plants, while it was not related to changes in nodule mass in the nonVAM plants (Fig. 4, Table 3).

DISCUSSION

Manganese toxicity in soybean varies with cultivars (14), and within cultivars symptoms differ with the ontogenic

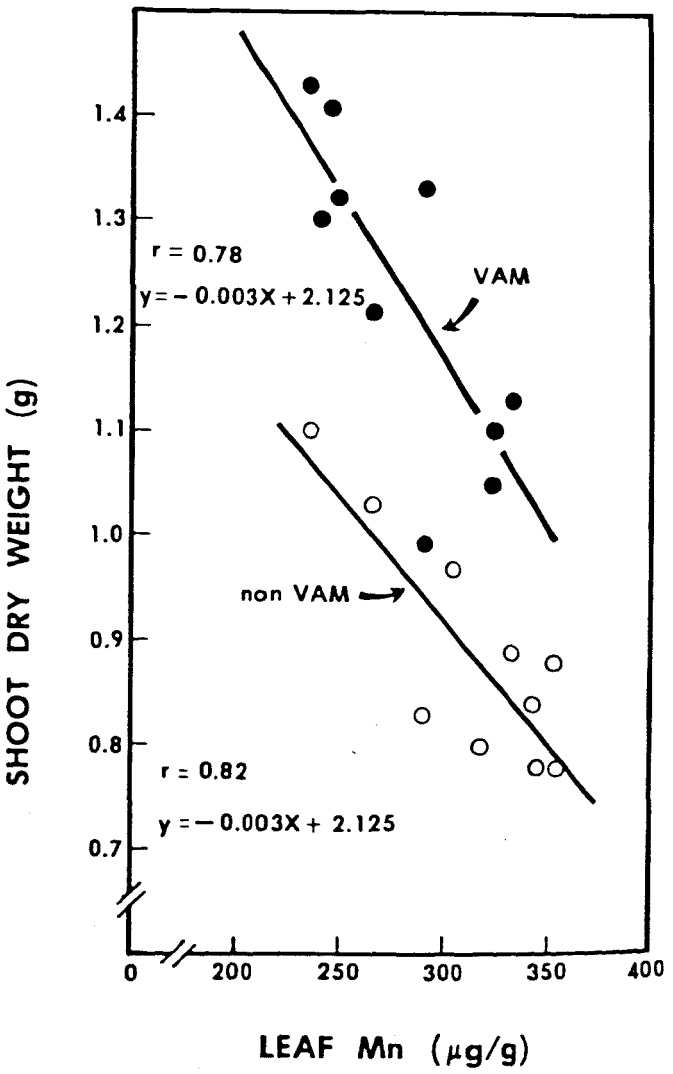

Fig. 2. Shoot dry mass vs. leaf manganese concentration. Regression analysis shows significant ($P < 0.01$) correlation in soybean plants grown with or without vesicular-arbuscular (VAM) fungi.

TABLE 3

Relationships between plant, endophyte, and soil parameters. Growth conditions were as in Table 1. Mn concentration effects were evaluated by regression analysis. Actual significance levels (P value) are shown to permit individual estimates.

Variable		r		P value	
Independent	Dependent	VAM	nonVAM	VAM	nonVAM
Plant					
Leaf Mn	Shoot mass	-0.783	-0.822	0.008	0.004
Root Mn	Root mass	-0.822	-0.206	0.004	0.568
Root Mn	Leaf Mn	0.371	0.017	0.291	0.963
Plant/endophyte					
Root Mn	Nodule mass	0.422	0.003	0.224	0.94
Leaf Mn	Nodule mass	0.434	0.002	0.210	0.96
% VAM	Leaf Mn	0.732	-----	0.061	----
% VAM	Root Mn	0.653	-----	0.041	----
Endophyte/endophyte					
% VAM	Nodule mass	0.688	-----	0.028	-----
Soil/plant					
Soil Mn	Root Mn	0.069	0.130	0.850	0.720
Soil Mn	Leaf Mn	0.062	0.472	0.865	0.167
Soil pH	Root Mn	-0.575	0.082	0.114	0.754
Soil pH	Leaf Mn	-0.529	0.116	0.128	0.934
Soil/endophyte					
Soil Mn	% VAM	-0.258	-----	0.462	-----
Soil Mn	Nodule mass	-0.009	-0.393	0.900	0.278
% VAM	Soil pH	-0.628	-----	0.052	-----
nodule mass	Soil pH	-0.653	0.342	0.041	0.326

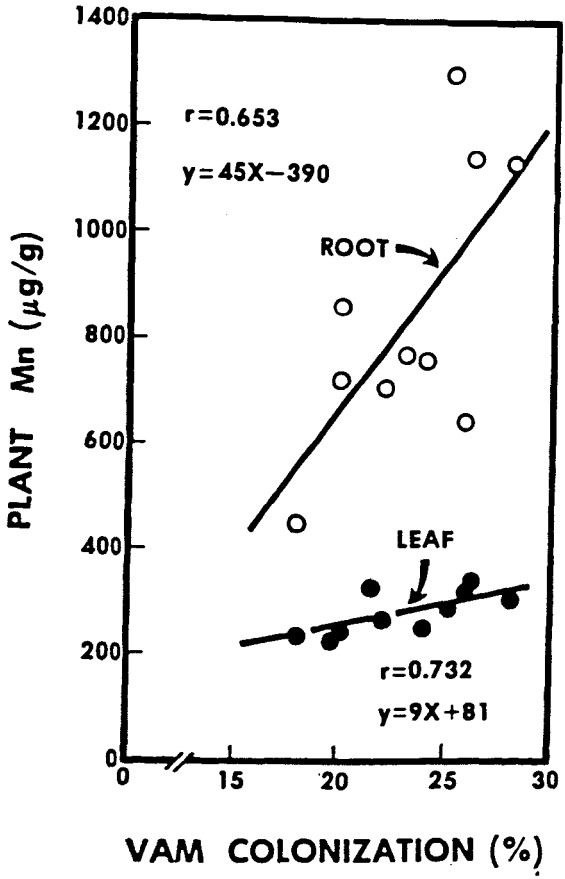

Fig. 3. Plant manganese concentration vs. VAM colonization. Regression shows significant correlations ($P < 0.05$) in soybean plants.

development of leaves along the stem (26). It usually occurs in soils of much lower pH (22) than that of the present experiment. Changes in the pH of the VAM soils are therefore of interest (31) but unlikely to be related to the toxic

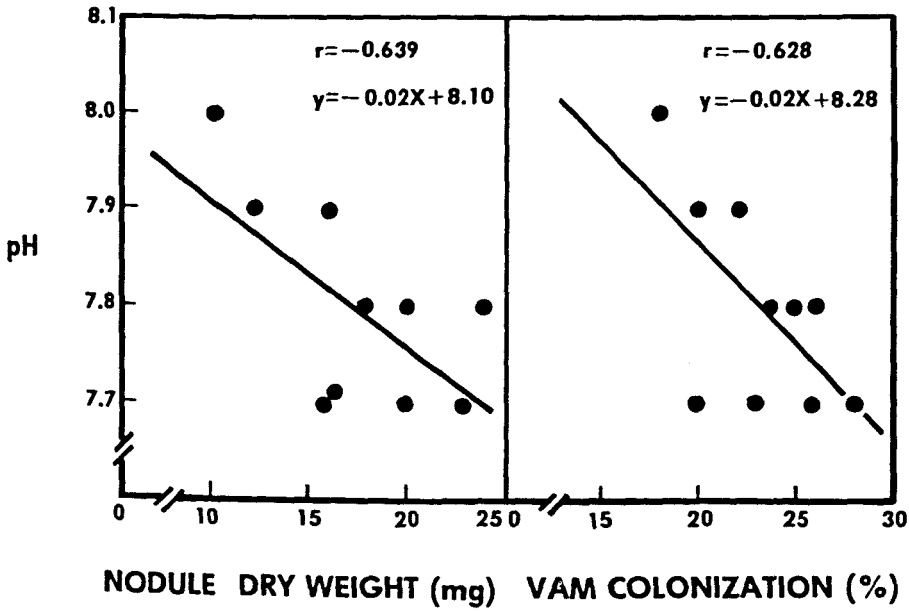

Fig. 4. Microsymbiont effects on soil pH. Regression analysis of soil pH vs. nodulation or colonization of soybean roots shows significant correlations of P = 0.041 and P = 0.052, respectively.

effects observed in the nonVAM plants. These relatively slight changes in pH observed here in the bulk soil may be explained as resulting from the release of H^+ by roots in response to N uptake through N_2 fixation (27) and the enhanced uptake of cations (24) by our VAM roots (Table 2). Greater cation uptake is generally observed in VAM plants (29). The pH change in the soil of our well-nodulated VAM plants may therefore be ascribed to a concerted effect of both microsymbionts (Fig. 4). It should be noted, however, that a slight pH change, as measured

in the bulk soil, may be far more pronounced in the rhizosphere (23). In that case, distinctly lower pH at the root-soil interface of our VAM plants should have increased the Mn toxicity effect, which did not occur.

Since in the absence of other contributing factors host-plant and nodule development in the VAM plants were clearly related to the VAM condition, the increasing root and shoot Mn concentrations with increasing VAM-fungal colonization (Fig. 3) seem to be counteractive. As a possible contributing factor, Si must be considered, since it is known to counteract Mn toxicity in leaves by modifying the distribution of Mn (15). This effect could not be verified in this experiment due to limitations in plant materials available for analysis. Effects of VAM fungi on Si uptake have not been reported in the literature to our knowledge, but Si levels were found to be higher in VAM than in nonVAM squirrel tail (Sitanion hystrix J.G. Sm.) plants (R.M. Miller, unpublished data). If such raising of Si concentrations were applicable to VAM plants in general, it would corroborate the Mn effect reported here. The same holds for competitive inhibition on Mn uptake by P_i (J.J. Maddox, personal communication). We suggest that greater root exudation generally observed in nonVAM plants (2, 3, 13, 17) is causally connected to the toxicity responses observed here. Low molecular-weight exudates reduce MnO_2 to plant-available Mn^{2+} (12). Significantly higher levels of available Mn were

indeed found in the soils of our nonVAM plants. In addition to solubilization, chelation on Mn by such exudates can further enhance its uptake (20).

Our data suggest that VAM fungi can affect the alleviation of Mn toxicity in soybean indirectly by influencing plant functions, as compared to direct mechanisms proposed by others for Zn in VA mycorrhizae (10) and ectomycorrhizae (9), and for Zn and Cu in ericalean mycorrhizae (8). However, since mycorrhizal fungi may also increase heavy metal uptake resulting in growth reduction (18), their effects on cost-benefit relationships in the uptake-exclusion balance will vary with the state of excess or deficiency of the metals concerned under any given soil conditions. In view of the role of these fungi in root-soil exchange relationships, their effects may help to explain the very complex and confused state of the literature on trace metal uptake (32).

REFERENCES

1. Adams, F. 1984. Crop response to liming in the southern United States, pp. 211-265. In: F. Adams (ed.) Soil Acidity and Liming, 2nd Edition. Publication No. 12, Am. Soc. Agron. Madison, WI.

2. Azcón, R. and J.A. Ocampo. 1984. Effect of root exudation on VA mycorrhizal infection at early stages of plant growth. Plant Soil 82:133-138.

3. Azcón, R. and J.A. Ocampo. 1981. Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol. 87:677-685.

4. Baker, A.J.M. 1987. Metal Tolerance. New Phytol. 106:93-111.

5. Bethlenfalvay, G.J., M.S. Brown, R.N. Ames, and R.S. Thomas. 1988. Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. Physiol. Plant. 72:565-571.

6. Bethlenfalvay, G.J., S. Dakessian, and R.S. Pacovsky. 1984. Mycorrhizae in a California desert: ecological implications. Can. J. Bot. 62:519-524.

7. Bierman, B.J. and R.G. Linderman. 1983. Increased Geranium growth using pre-transplant inoculation with a mycorrhizal fungus. J. Am. Hort. Soc. 108:972-976.

8. Bradley, R., A.J. Burt, and D.J. Read. 1982. The biology of mycorrhiza in the Ericaceae. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytol. 91:197-209.

9. Brown, M.T. and D.A. Wilkins. 1985. Zinc tolerance of mycorrizal Betula. New Phytol. 99:101-106.

10. Dellavalle, C., A. Viera, and M.G. Glenn. 1987. Zinc tolerant VA mycorrhizae, pp. 149. In: D.M Sylvia, L.L. Hung and J.H. Graham (eds) Mycorrhizae in the Next Decade. Proc. 7th North Amer. Conf. on Mycorrhizae. Univ. of Florida, Gainesville, FL.

11. deMooy, C.J. J. Pesek, and E. Spaldon. 1973. Mineral nutrition. pp. 267-352. In B.E. Caldwell (ed.) Soybeans: Improvement, Production and Uses. Am. Soc. Agron. Publication No. 16. Madison, WI.

12. Godo, G.H. and H.M. Reisenauer. 1980. Plant effects on soil manganese activity. Soil Sci. Soc. Am. J. 44:993-995.

13. Graham, J.H. R.T. Leonard, and J.A. Menge. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68:548-552.

14. Heenan, D.P. and O.G. Carter. 1976. Tolerance of soybean cultivars to manganese toxicity. Crop Sci. 16:389-391.

15. Horst, W.J. and H. Marschner. 1987. Effect of silicon on manganese tolerance of bean plants (Phaseolus vulgaris L.) Plant Soil 50:287-303.

16. Johnson, C.M., P.R. Stout, T.C. Boyer, and A.B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant Soil 8:337-353.

17. Johnson, C.R., J.A. Menge, S. Schwab, and I.P. Ting. 1982. Interaction of photoperiod and vesicular-arbuscular mycorrhizae on growth and metabolism of sweet orange. New Phytol. 90:665-669.

18. Killham, K. and M.K. Firestone. 1983. Vesicular-arbuscular mycorrizal mediation of grass response to acidic and heavy metal depositions. Plant Soil 72:39-48.

19. Levitt, J. 1980. Stress concepts. pp. 3-21. In Responses of Plants to Environmental Stress, 2nd Edition, Vol. 2. Academic Press, New York, NY.

20. Lindsay, W.L. 1974. Role of chelation in micronutrient availability, pp. 507-522. In: E.D. Carson (ed.) The Plant Root and its Environment. Univ. Press of Virginia, Charlottesville, VA.

21. Macniol, R.D. and H.T. Beckett. 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil 85:107-129.

22. Marschner, H. 1986. Diagnosis of deficiency and toxicity of mineral nutrients. pp. 391-407. In Mineral Nutrients of Higher Plants. Academic Press, London.

23. Marschner, H., V. Romheld, W.J. Horst, and P. Martin. 1986. Root-induced changes in the rhizosphere: Importance for the mineral nutrition of plants. Z. Pflanzenernaehr. Bodenk. 149:441-456.

24. Mengel, D.B., W. Segars, and G.W. Rehm. 1987. Soil fertility and liming. pp. 461-496. In R. Wilcox (ed.) Soybeans: Improvement, Production, and Uses, 2nd Edition. Am. Soc. Agron. Publication No. 16. Madison, WI.

25. Munns, D.N. 1977. Mineral nutrition and the legume symbiosis. pp.353-391. In R.F.W. Hardy and A.H. Gibson (eds.) A Treatise on Dinitrogen Fixation, Section IV, Agronomy and Ecology. John Wiley & Sons, New York, NY.

26. Ohki, K. 1981. Manganese critical levels for soybean growth and physiological processes. J. Plant Nutr. 3:271-284.

27. Nyatsanja, T. and W.H. Pierre. 1973. Effects of nitrogen fixation in legumes on soil acidity. Agron. J. 65:936-940.

28. Pacovsky, R.S., G.J. Bethlenfalvay, and E.A. Paul. 1986. Comparisons between P-fertilized and mycorrhizal plants. Crop Sci. 26:151:156.

29. Saif, S.R. 1987. Growth responses of tropical forage plant species to vesicular-arbuscular mycorrhizae. I. Growth, mineral uptake and mycorrhizal dependency. Plant Soil 97:25-35.

30. Scott, W.O. and S.R. Aldrich. 1970. Modern Soybean Production. p. 94. S & A Publication, Champaign, Il.

31. Smith, S.E. and F.A. Smith. 1984. Could mycorrhizas be involved in changes in soil pH?, pp. 125-126. In: I.R. Kennedy and L. Copeland (eds.) Proc. 7th Australian Legume Nodulation Conference. Australian Inst. Agric. Sci. Occasional Publication No. 12, Adelaide.

32. Tinker, P.B. and A. Gildon. 1982. Mycorrhizal fungi and ion uptake, pp. 21-32. In: D.A. Robb and W.S. Pierpoint eds.) Metals and Micronutrients, Uptake and Utilization by Plants. Academic Press, London.