Mycorrhiza (2000) 9:331–336 

ORIGINAL PAPER

**A. Liu · C. Hamel · R.I. Hamilton · B.L. Ma**
**D.L. Smith**

# Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels

Accepted: 27 December 1999

**Abstract** Sustainability of soil-plant systems requires, among other things, good development and function of mycorrhizal symbioses. The effects of P and micronutrient levels on development of an arbuscular mycorrhizal fungus (AMF) and uptake of Zn, Cu, Mn and Fe by maize (*Zea mays* L.) were studied. A pot experiment with maize either inoculated or not with *Glomus intraradices* was conducted in a sand:soil (3:1) mix (pH 6.5) in a greenhouse. Our goal was to evaluate the contribution of mycorrhizae to uptake of Cu, Zn, Mn and Fe by maize as influenced by soil P and micronutrient levels. Two levels of P (10 and 40 mg $kg^{-1}$ soil) and three levels of a micronutrient mixture: 0, 1X and 2X (1X contained, in mg $kg^{-1}$ soil, 4.2 Fe, 1.2 Mn, 0.24 Zn, 0.06 Cu, 0.78 B and 0.036 Mo), were applied to pots. There were more extraradical hyphae at the low P level than at the high P level when no micronutrients were added to the soil. Root inoculation with mycorrhiza and application of micronutrients increased shoot biomass. Total Zn content in shoots was higher in mycorrhizal than non-mycorrhizal plants grown in soils with low P and low or no micronutrient addition. Total Cu content in shoots was increased by mycorrhizal colonization when no micronutrients were added. Mycorrhizal plants had lower Mn contents than non-mycorrhizal plants only at the highest soil micronutrient level. AMF increased total shoot Fe content when no micronutrients were added, but decreased shoot Fe when plants were grown at the high level of micronutrient addition. The effects of *G. intraradices* on Zn, Cu, Mn, and Fe uptake varied with micronutrient and P levels added to soil.

**Key words** Extraradical hyphae · Micronutrients · Nutrient uptake · Root colonization

A. Liu · C. Hamel (✉)
Department of Natural Resource Sciences, McGill University,
21111 Lakeshore Road,
Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada
Tel.: +1-514-398-7759
Fax: +1-514-398-7990
e-mail: hamel@nrs.mcgill.ca

R.I. Hamilton · B.L. Ma
Central Experimental Farm, Agriculture Canada,
Ottawa, K1A 0C6, Canada

D.L. Smith
Department of Plant Science, McGill University,
21111 Lakeshore Road,
Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada

## Introduction

Arbuscular mycorrhizal fungi (AMF) increase the volume of soil exploited by plants (Bolan 1991). As a result, root colonization by AMF often results in enhanced uptake of relatively immobile metal micronutrients, such as Cu, Zn and Fe (Faber et al. 1990; Kothari et al. 1990; Kucey and Janzen 1987; Li et al. 1991). On the other hand, under conditions of high soil Zn, Cu and Fe levels, the concentrations of these nutrients in shoots were reported to be lower in mycorrhizal plants (Dueck et al. 1986; El-Kherbawy et al. 1989; Leyval et al. 1991; Pacovsky 1986; Weissenhorn et al. 1995). In several studies, the Mn concentration was lower in mycorrhizal than in non-mycorrhizal plants (Kothari et al. 1991; Posta et al. 1994). Reduced concentrations of metal micronutrients in mycorrhizal plants are sometimes attributable to a dilution effect linked to increase in dry weight. However, experimental results can not always be explained by a dilution effect (Nielsen and Jensen 1983). The effects of AMF on acquisition of immobile metal nutrients by the host plant are still unclear, and factors responsible for the variable results reported by researchers in this field need to be understood.

Inconsistent responses of mycorrhizal plants in micronutrient uptake may be related to highly variable soil conditions, which influence AMF root colonization and extraradical hyphal development; the AMF in turn influences uptake of these metals. One such

variable soil condition is the level of available micronutrients. There are very few published results on the effects of micronutrients on mycorrhizal development. A negative correlation between Zn or Cu concentration and AMF root colonization was found for plants grown in soil to which sludge had been applied (Boyle and Paul 1988; Gildon and Tinker 1983). Reduced root colonization was also observed for mycorrhizal plants grown close to an old copper mine (Griffioen et al. 1994).

It is well known that high P levels in soil inhibit mycorrhizal development and root colonization (Abbott and Robson 1984). If uptake of micronutrients by mycorrhizal plants is related to internal and external development of AMF, nutrient acquisition will also be influenced by levels of P. Therefore, uptake of micronutrients by mycorrhizal plants may be related to mycorrhizal development and function as influenced by availabilities of both P and micronutrients in soils.

We investigated the effect of P and micronutrient levels on uptake of micronutrients by maize in a greenhouse experiment. The objectives of this study were (1) to determine the effects of micronutrient and P levels and their interaction on AMF root colonization and extraradical hyphal production, and (2) to assess the role of AMF in acquisition of Cu, Zn, Mn and Fe in shoots of maize hybrids grown under different P and micronutrient levels.

## Materials and methods

### Experimental conditions

The experiment included three factors: 2 mycorrhizal treatments (mycorrhizal or not), 3 rates of micronutrient solution (0, 1X and 2X levels, where the 1X level contained 4.2 Fe, 1.2 Mn, 0.24 Zn, 0.06 Cu, 0.78 B and 0.036 Mo mg $kg^{-1}$), and 2 P levels (10 and 60 mg $kg^{-1}$ soil). Treatment combinations were replicated four times.

The medium was made of three volumes of sand and one volume of sandy loam soil and is referred to as "soil" throughout the paper. The soil was steam pasteurized at 80 °C for 3 h. Maize was grown in 10-l pots (25 cm diameter × 22 cm deep pots) filled with the pasteurized soil, which had a pH of 6.5. Before application of the nutrient treatments, total and available Zn, Cu, Mn, and Fe were analysed (Table 1). Total Zn, Cu, Mn, and Fe were measured by atomic absorption spectroscopy (Perkin-Elmer 5380, Norwalk, Conn.) after digestion of the soil with nitric and perchloric acid at 150 °C (Baker and Amacher 1982). Available Zn, Cu, Mn, and Fe were determined by extraction with 5 mM diethylenetriaminepentaacetic acid, 10 mM $CaCl_2$ and 0.1 M $(HOCH_2CH_2)_3N$, adjusted to pH 7.3 (Lindsay and Norvell 1978). To create target P and micronutrient levels, the required amount of $KH_2PO_4$ and of a micronutrient mix (chelated micronutrient mix, Plant Products Co. Ltd., Brampton, Ontario) were dissolved in distilled water and applied to the soil before maize was planted. Other plant nutrients (N, Ca, Mg, S) were applied as a solution to the soil of all pots in equal amounts (80 mg $NH_4NO_3$, 30 mg $CaCl_2$ and 30 mg $MgSO_4$ per kg soil). A new maize hybrid, LNS, possessing the Leafy trait but being of normal stature, and developed at the Plant Research Centre, Agri-Food Canada (Ottawa, Ontario), was used in this experiment. This hybrid was selected for its high response to mycorrhiza. Maize seeds were surface sterilized in 30% $H_2O_2$ for 10 min and washed several times with distilled water. Thirty grams of a commercial inoculant containing *Glomus intraradices* Schenck & Smith (Mycorise, Premier Tech. Inc., Rivière-du-Loup, Quebec, Canada) was fully mixed with the medium of each pot as the mycorrhizal treatments. Control pots received the same amount of inoculant sterilized in an autoclave for 30 min at 121 °C. Three seeds were sown in each pot on April 25. Five days after seedling emergence, the plants were thinned to one seedling per pot. The plants grew in a greenhouse under controlled environment conditions with a 16/8 h day/night regime, 300 μmol $m^{-2}$ $s^{-1}$ photon flux density, 75% relative humidity and a day/night temperature of 26/22 °C. Pots were watered to field capacity on a mass basis three times per week.

**Table 1** Initial concentration of total and diethylenetriaminepentaacetic acid extractable Fe, Mn, Zn and Cu in soil

| | Trace metal concentration (mg $kg^{-1}$) | | | |
|---|---|---|---|---|
| | Fe | Mn | Zn | Cu |
| Total | 9800 | 240 | 54 | 25 |
| DTPA | 4.5 | 1.13 | 0.18 | 0.06 |

### Harvest and analyses

A preliminary experiment indicated that maximal root colonization was reached 8 weeks after seeding. Thus 8 weeks after seeding, shoots and roots were harvested separately. Roots were collected by sieving pot contents on a 1-mm mesh screen and washed with tap water to remove adhering substrate. A 3-g fresh subsample was randomly taken from each maize root system for measurement of root colonization. Shoot and root biomasses were determined after drying at 70 °C for 48 h.

Root subsamples were cleared in a 10% KOH solution in the autoclave for 30 min (121 °C) and stained with 0.02% acid fuchsin dissolved in lactoglycerol solution (equal volumes of water, glycerine and 85% lactic acid) overnight. The percentage of root length colonized by AMF was estimated under a dissecting microscope using the grid line intersect method (Giovannetti and Mosse 1980).

The hyphal extraction and staining methods used were modifications of the procedures of Miller and Jastrow (1992). Soils were sampled after harvest. Soil moisture content was measured after oven-drying approximately 25 g of soil, sampled from each pot, for 24 h at 105 °C. Moist 20-g soil samples from each pot were placed in 50-ml centrifuge tubes with 40 ml of 2 M sucrose solution containing 2% sodium hexametaphosphate. Tubes were shaken for 30 min on a shaker and centrifuged for 15 min at 450 g. The upper part of the supernatant was collected with a pipette after each centrifugation. Soil samples were extracted consecutively three times. Hyphae were recovered on a filter paper and stained with a 0.02% acid fuchsin in lactoglycerol solution for 12 h. The line intersect method (Tennant 1975) was used to estimate the length of hyphae recovered on the filter. To express hyphal length on a dry soil basis, soil moisture content was measured after oven-drying a soil sample from each pot for 24 h at 105 °C.

Dried and ground (to pass a 1-mm sieve), plant shoot tissues were digested in concentrated $HNO_3$ and 30% $H_2O_2$ according to the procedure of Thomas et al. (1967). Concentrations of Cu, Zn, Mn and Fe in the digests were determined by atomic absorption spectroscopy.

### Statistical analyses

The data were subjected to analysis of variance using the ANOVA procedures of the SAS program (SAS Institute 1990).

Statistical significance was determined at $P = 0.05$. Means were compared by the Duncan's multiple-range test following a significant F test. When interactions between factors were significant, the means of combinations of each level of these factors were compared.

## Results

### Mycorrhiza development and root colonization

There was an interaction between P and micronutrient levels for the length of extraradical hyphae (Table 2). The extraradical hyphal length was higher at low P than at high P but only when no micronutrients were added (Table 3). The highest amount of extraradical hyphae was produced in the soil at low P without micronutrient addition, while the lowest amount was at high P with high micronutrient addition. Root colonization was lowest for plants grown in soil with either high P or high micronutrient treatments (Table 4).

### Shoot and root biomass

Shoot biomass production was increased by mycorrhizal colonization and when plants were grown at the high P level or with micronutrient application (Table 4). Root biomass was not influenced by AMF (Table 4). Addition of micronutrients enhanced root biomass production as did culture at high P.

### Total nutrient content in shoots

In a three-way interaction, at low application rates of both P and micronutrients or when no micronutrients

**Table 2** Summary of F significance from analysis of variance *DF* degree of freedom, *RCP* root colonization percentage, *EHL* extraradical hyphal length, *SDW* shoot dry weight, *RDW* root dry weight, *ns* not significant

| Sources | DF | RCP | EHL | SDW | RDW | Shoot nutrient content | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Zn | Cu | Mn | Fe |
| Phosphorus (P) | 2 | ** | * | ** | * | ns | ns | * | ns |
| Micronutrients (M) | 2 | ** | ** | * | ** | ** | ** | ** | ** |
| AM inoculation (I) | 1 | ** | ** | ** | ns | * | ** | * | ns |
| P*M | 4 | ns | * | ns | ns | * | * | * | * |
| P*I | 2 | ns | ns | ns | ns | ns | ** | ns | * |
| M*I | 2 | ns | ns | ns | ns | * | * | * | ** |
| P*M*I | 4 | ns | ns | ns | ns | * | ns | ns | ns |

**Table 3** Extraradical hyphal length and total contents of Zn, Cu, Mn and Fe in corn shoots as influenced by mycorrhiza, P and micronutrient levels. Means followed by the same letter within the same measurement are not significantly different at $P < 0.05$ by Duncan's multiple-range test *+AM* inoculation with *Glomus intraradices*, *–AM* not inoculated

| P level (mg kg$^{-1}$) | Micro-nutrients level | Extraradical hyphal length (m g$^{-1}$ dry soil) | Total content of micronutrients in shoots (mg plant$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Zn | | Cu | | Mn | | Fe | |
| | | +AM | +AM | –AM | +AM | –AM | +AM | –AM | +AM | –AM |
| 10 | 0 | 1.63 a | 2.21 c | 1.78 d | 0.47 b | 0.33 d | 1.17 d | 1.08 d | 4.64 bc | 3.86 d |
| 10 | 1 | 1.36 b | 2.51 b | 2.18 c | 0.50 ab | 0.45 bc | 1.29 cd | 1.32 cd | 4.90 b | 4.64 bc |
| 10 | 2 | 1.20 bc | 2.92 a | 2.65 ab | 0.51 ab | 0.52 ab | 1.41 c | 1.69 b | 4.78 b | 5.42 a |
| 60 | 0 | 1.33 b | 2.05 c | 1.81 d | 0.41 c | 0.34 d | 1.17 d | 1.08 d | 4.48 bc | 3.18 e |
| 60 | 1 | 1.41 b | 2.52 b | 2.40 bc | 0.47 b | 0.47 b | 1.42 c | 1.48 c | 4.71 bc | 4.51 c |
| 60 | 2 | 1.07 c | 3.00 a | 2.94 a | 0.56 a | 0.53 ab | 1.46 c | 1.89 a | 4.95 b | 5.43 a |

**Table 4** Root colonization percentage, shoot dry weight and root dry weight as influenced by mycorrhiza, P and micronutrient levels. Means followed by the same letter within the same factor are not significantly different at $P < 0.05$ by Duncan's multiple-range test *+AM* inoculation with *G. intraradices*, *–AM* not inoculated

| Factor | Level | Percentage of colonization (%) | Shoot dry weight (g plant$^{-1}$) | Root dry weight (g plant$^{-1}$) |
|---|---|---|---|---|
| Mycorrhizal status | +AM | – | 27.3 a | 8.31 a |
| | –AM | – | 23.9 b | 8.15 a |
| Phosphorus | 10 mg/kg | 70.4 a | 24.3 b | 7.50 b |
| | 60 mg/kg | 53.6 b | 26.9 a | 8.96 a |
| Micronutrient | 0 | 66.2 a | 23.9 b | 7.56 b |
| | 1 | 65.2 a | 26.3 a | 8.38 ab |
| | 2 | 54.6 b | 26.5 a | 8.75 a |

were added, shoot Zn content of mycorrhizal plants was higher than that of non-mycorrhizal plants (Table 3). Shoots of mycorrhizal plants had enhanced total Cu contents only when plants were grown without micronutrient application (Table 3). Interactions between mycorrhizae and micronutrient treatments were also found for Mn and Fe contents in shoots (Table 2). At the highest dose of micronutrients, mycorrhizal plants had lower Mn contents in shoots than non-mycorrhizal plants, while at the lower dose of micronutrients or with no micronutrient addition, mycorrhizal and non-mycorrhizal plants had similar Mn contents (Table 4). The difference in total shoot Fe content between mycorrhizal and non-mycorrhizal plants depended on micronutrient application rates. Total shoot Fe content was higher in mycorrhizal than in non-mycorrhizal plants grown with no added micronutrients, similar at the low micronutrient rates, and lower at the high rate of micronutrients (Table 4). Shoot Mn content was also influenced by soil P level. The high rate of P addition increased Mn uptake when micronutrients were added (Table 3).

## Discussion

The results of this study clearly indicate a negative impact of high P and micronutrients on development of both internal mycorrhizal root colonization and extraradical hyphae. This effect appeared to be confined to *G. intraradices* because no visible hyphae were extracted from non-inoculated soil.

The highest rate of micronutrients added to soil in this experiment was inhibitory to *G. intraradices* but not to the host plant. The absence of growth reduction in response to the high micronutrient rate indicates that the latter was within the sufficiency range and was not toxic to the plants. In contrast, AMF colonization and extraradical hyphae growth were suppressed when plants were grown with the high level of micronutrients. Hence, AMF colonization and development were more sensitive to high levels of micronutrients than was maize plant growth.

Our results agree with those of Koomen et al. (1990), who reported that AMF spore germination and root colonization can be severely repressed by high concentrations of trace metals. Gildon and Tinker (1983) found that VA mycorrhizal infection was strongly reduced by Zn additions and could be completely inhibited by high application rates. A previous experiment from this laboratory (unpublished data) also showed that high micronutrient levels in soil reduced AMF extraradical development. In contrast to these previous studies involving very high levels of metals in soil, we have found that AMF inhibition can occur at relatively low metal levels.

The reduced root colonization and extraradical hyphal development at high soil P indicates that the soil P was inhibitory to AMF. In contrast, increased shoot and root growth with increasing soil P level indicates that the higher amount of soil P was beneficial for plants. This shows that the influences of soil P level on development and growth of AMF and plants were quite different.

The mobility of Cu, Zn, Mn and Fe in soils is low. As a result, uptake of these metal nutrients by roots is diffusion limited (Barea 1991; Oliver and Barber 1966; Tisdale et al. 1993). When no micronutrients were added to the soil, available Cu, Zn, Mn and Fe levels were low and "a depleted zone" of these metal nutrients would have formed around the roots. As a result, the uptake of these nutrients was limited in non-mycorrhizal plants. Mycorrhizal plants could take up more metal nutrients via extraradical hyphae, which provide larger surface areas than the roots alone and reduce the distance for diffusion, thereby enhancing the absorption of immobile metal nutrients (Jakobsen et al. 1992). Accordingly, Bürkert and Robson (1994) showed that uptake of Zn was influenced by the distribution and length of the extraradical hyphae of three AMF in soil. The higher the density of extraradical hyphae in soil, the higher the absorption surface, the shorter distance these metals have to diffuse, and the more effectively mycorrhizal plants will absorb these low mobility metal nutrients.

We found that the mycorrhizal contribution to Zn, Cu, Mn and Fe uptake by maize was significantly influenced by soil P and micronutrient levels. Whether this influence was positive or negative also depended not only on P and micronutrient levels but also on which metals were considered, Cu and Zn, or Mn and Fe. Extraradical hyphae can absorb and transport Cu and Zn to their host plants (Bürkert and Robson 1994; Li et al. 1991). When no micronutrients were added to soil, enlarging the root absorption area and reducing Cu and Zn diffusion distance by means of extraradical hyphae was crucial to uptake of these nutrients. The beneficial effect of mycorrhizal inoculation on Cu and Zn uptake was eliminated by micronutrient addition to soil. Under conditions of Cu and Zn abundance, maize roots appeared to depend less on mycorrhizal hyphae for uptake of these nutrients. Heggo and Angle (1990) also indicated that the effect of AMF on metal uptake was dependent on the soil metal concentration after a pot experiment with collected soils of various metal contents.

Suppressed extraradical hyphal development was observed in soil with high micronutrient levels. The potential for uptake by the mycorrhizal plant root system was reduced under these conditions. After fertilization with 1.1 mg Zn $kg^{-1}$, which is higher than the highest level used in our experiment, Abbott and Robson (1985) found lower concentrations of Zn in shoots of mycorrhizal plants than non-mycorrhizal plants. Our results indicated that mycorrhizal plants grown with high levels of micronutrients had neither higher nor lower Cu and Zn contents in shoots. The

high micronutrient level used in our experiment had a relatively mild negative impact on Cu and Zn uptake.

The effect of P on Cu and Zn uptake appears to have two components. On the one hand, the roots of plants grown in the low P regime had more extraradical hyphae and, therefore, could potentially absorb more Cu and Zn than plants grown in a soil with less-developed extraradical hyphae at high P. After studying the effects of application of different sewage sludges and P levels on uptake of nutrients by mycorrhizal plants, Lambert and Weidensaul (1991) concluded that high P inhibition of mycorrhizal activity was the main reason for decreased Cu and Zn uptake. On the other hand, increased shoot P content in plants grown at high soil P levels can increase Cu and Zn sink size. This may induce uptake and translocation of Cu and Zn to plant shoots. Li et al. (1991) found that Cu in shoots increased from 12% to 58% with increases in P level in the hyphal compartment. The overall effect of soil P level on the contents of Cu and Zn in shoots was not significant.

The availability of Mn and Fe in soil depends on soil pH value and soil oxidation-reduction potential. The reduced forms of these elements are more available to plants (Marschner 1988). It has been reported that Mn acquisition was decreased in AM plants (Kothari et al. 1991). AMF were found to reduce the number of Mn-reducing bacteria (Posta et al. 1994) or increase the number of Mn-oxidizing bacteria in the rhizosphere (Arines et al. 1992), thereby indirectly reducing oxidation-reduction potential and availability of Mn and Fe in the mycorhizosphere. Uptake of nutrients by plants does not only depend on the availability of nutrients in soil solution, but also on the effectiveness of root systems for absorption. The effectiveness of mycorrhizal root absorption is increased by external hyphae (Read 1984; Bürkert and Robson 1994). Whether AMF increase or decrease uptake of Mn and Fe may depend on which of these two functions prevails under given soil conditions. We found that under conditions of low micronutrient level, AMF hyphae enhanced uptake of Fe, most likely by improved scavenging of this element. The results are in agreement with those of Clark and Zeto (1996), who found that mycorrhizae improved Fe uptake in maize grown in Fe-deficient soils.

At the high level of micronutrients, there was less Mn and Fe uptake by AMF-colonized plants than by non-mycorrhizal plants. These results agree with those of Kothari et al. (1990), who found that Mn and Fe levels were lower in mycorrhizal plants. It is possible that an AMF-induced decrease in Mn and Fe uptake was due more to reduced availability of these nutrients than to increased absorption efficiency by AMF at the high micronutrient level.

Other mechanisms may also have influenced the impact that AMF had on Mn and Fe uptake by maize. For example, it was proposed by Turnau et al. (1993) that polyphosphates in the fungal hyphae could sequester metals and minimize transfer to roots of the mycorrhizal plants.

**Acknowledgements** The authors thank Dr. X. M. Zhou for her assistance. This project was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant held by D. L. S.

## References

Abbott LK, Robson AD (1984) The effect of VA mycorrhizae on plant growth. In: Powell C-L, Bagyaraj DJ (eds) VA mycorrhiza. CRC, Boca Raton, Fla, pp 113–130

Abbott LK, Robson AD (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytol 99 : 245–255

Arines J, Porto ME, Vilarino A (1992) Effect of manganese on vesicular-arbuscular mycorrhizal development in red clover plants and on soil Mn-oxidizing bacteria. Mycorrhiza 1 : 127–131

Baker DE, Amacher MC (1982) Nickel, copper, zinc and cadmium. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis. Am Soc Agron, Madison, Wisc, pp 323–336

Barea J M (1991) Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. In: Stewart BA (ed) Advances in soil science, vol 15. Springer, New York, pp 8–18

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134 : 189–207

Boyle M, Paul EA (1988) Vesicular-arbuscular mycorrhizal associations with barley on sewage-amended plots. Soil Biol Biochem 20 : 945–948

Bürkert B, Robson AD (1994) $^{65}$Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. Soil Biol Biochem 26 : 1117–1124

Clark RB, Zeto SK (1996) Iron acquisition by mycorrhizal maize grown on alkaline soil. J Plant Nutr 19 : 247–264

Dueck TA, Visser P, Ernst WHO, Schat H (1986) Vesicular-arbuscular mycorrhizae decrease zinc-toxicity to grasses growing in zinc-polluted soil. Soil Biol Biochem 18 : 331–333

El-Kherbawy M, Angle JS, Heggo A, Chaney RL (1989) Soil pH, rhizobia and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa *(Medicago sativa* L.). Biol Fertil Soils 8 : 61–65

Faber BA, Zasoski RJ, Burau RG, Uriu K (1990) Zinc uptake by corn affected by vesicular-arbuscular mycorrhizae. Plant Soil 129 : 121–131

Gildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. 1. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol 95 : 247–261

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytol 84 : 489–500

Griffioen WAJ, Jetswaart JH, Ernst WHO (1994) Mycorrhizal infection of an *Agrostis capillaris* population on a copper-contaminated soil. Plant Soil 158 : 83–89

Heggo A, Angle S (1990) Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22 : 865–869

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytol 120 : 371–380

Koomen I, Firestone MK, Giller I (1990) Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. Soil Biol Biochem 22 : 871–873

Kothari SK, Marschner H, Römheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. New Phytol 116 : 637–645

Kothari SK, Marschner H, Romheld V (1991) Effect of a vesicular-arbuscular mycorrhizal fungi and rhizosphere micro-organisms on manganese reduction in the rhizosphere and manganese concentrations in maize (*Zea mays* L.). New Phytol 117 : 649–655

Kucey RMN, Janzen HH (1987) Effects of reduced nutrient availability on growth and phosphorus and micronutrient uptake of wheat and field beans under greenhouse conditions. Plant Soil 104 : 71–78

Lambert DH, Weidensaul TC (1991) Element uptake by mycorrhizal soybean from sewage-sludge-treated soil. Soil Sci Am J 55 : 393–398

Leyval C, Berthelin J, Schontz D, Weissenhorn I, Morel JL (1991) Influence of endomycorrhizas on maize uptake of Pb, Cu, Zn and Cd applied as mineral salts or sewage sludges. In: Farmer JG (ed) Heavy metals in the environment. CEP, Edinburgh, pp 204–207

Li X-L, Marschner H, Römheld V (1991) Aquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil 136 : 49–57

Lindsay WL, Norvell WA (1978) Development of DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42 : 421–428

Marschner H (1988) Mechanisms of manganese acquisition by roots from soils. In: Graham RD, Hannam RJ, Uren NC (eds) Manganese in soils and plants. Kluwer, London, pp 191–204

Miller RM, Jastrow JD (1992) Extraradical hyphal development of vesicular-arbuscular mycorrhizal fungi in a chronosequence of prairie restoration. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB, Wallingford, England, pp 171–176

Nielsen Jd, Jensen A (1983) Influence of vesicular-arbuscular mycorrhiza fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medicago sativa*). Plant Soil 70 : 165–172

Oliver S, Barber SA (1966) Mechanisms for the movement of Mn, Fe, B, Zn, Al and Sr from one soil to the surface of soybean roots (*Glycine max*). Soil Sci Soc Am Proc 30 : 468–470

Pacovsky RS (1986) Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans. Plant Soil 95 : 379–388

Posta K, Marschner H, Romheld V (1994) Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5 : 119–124

Read DJ (1984) The structure and function of the vegetative mycelium of mycorrhizal roots. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 215–240

SAS Institute (1990) SAS User Guide, 6.08 edn. SAS Institute, Cary, N.C

Tennant D (1975) A test of a modified line intersection method of measuring root length. J Ecology 63 : 995–1001

Thomas RL, Sheard RW, Moyer JR (1967) Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. Agron J 59 : 240–243

Tisdale S L, Nelson W L, Beaton J D, Havlin J L (eds) (1993) Soil fertility and fertilizers. Macmillan, New York, pp 304–337

Turnau K, Kottke I, Oberwinkler F (1993) Element location in mycorrhizal roots of *Pteridium aquilinum* (L.) Kuhn collected from experimental plots treated with cadmium dust. New Phytol 123 : 313–324

Weissenhorn I, Leyval C, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza 5 : 245–251