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Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil

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Abstract

An investigation was carried out to test whether the mechanism of increased zinc (Zn) uptake by mycorrhizal plants is similar to that of increased phosphorus (P) acquisition. Maize (*Zea mays* L.) was grown in pots containing sterilised calcareous soil either inoculated with a mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe or with a mixture of mycorrhizal fungi, or remaining non-inoculated as non-mycorrhizal control. The pots had three compartments, a central one for root growth and two outer ones for hyphal growth. The compartmentalization was done using a 30-μm nylon net. The root compartment received low or high levels of P (50 or 100 mg kg^{-1} soil) in combination with low or high levels of P and micronutrients (2 or 10 mg kg^{-1} Fe, Zn and Cu) in the hyphal compartments.

Mycorrhizal fungus inoculation did not influence shoot dry weight, but reduced root dry weight when low P levels were supplied to the root compartment. Irrespective of the P levels in the root compartment, shoots and roots of mycorrhizal plants had on average 95 and 115% higher P concentrations, and 164 and 22% higher Zn concentrations, respectively, compared to non-mycorrhizal plants. These higher concentrations could be attributed to a substantial translocation of P and Zn from hyphal compartments to the plant via the mycorrhizal hyphae. Mycorrhizal inoculation also enhanced copper concentration in roots (135%) but not in shoots. In contrast, manganese (Mn) concentrations in shoots and roots of mycorrhizal plants were distinctly lower, especially in plants inoculated with the mixture of mycorrhizal fungi.

The results demonstrate that VA mycorrhizal hyphae uptake and translocation to the host is an important component of increased acquisition of P and Zn by mycorrhizal plants. The minimal hyphae contribution (delivery by the hyphae from the outer compartments) to the total plant acquisition ranged from 13 to 20% for P and from 16 to 25% for Zn.

Introduction

Vesicular-arbuscular (VA) mycorrhizae are widespread in soils, and often growth of VA-mycorrhizal plants is increased in comparison to non-mycorrhizal plants. This beneficial effect on plant growth has largely been attributed to higher phosphorus (P) uptake and consequently better P nutrition of mycorrhizal plants. The increased uptake of P by mycorrhizal plants is mainly due to absorption and translocation of P from distant areas which are otherwise inaccessible to plant roots (Sanders and Tinker, 1971; 1973; Viebrock, 1988). Rhodes and Gerdemann

(1975) showed that mycorrhizal hyphae absorb P even 7 cm away from the root surface and transport it into the host.

In addition to P, VA mycorrhizal fungi often also enhance acquisition of relatively immobile micronutrient cations, particularly Zn (Gnekow and Marschner, 1989b; Swaminathan and Verma, 1979) and Cu (Gildon and Tinker, 1983; Pacovsky, 1986). It is not clear whether this is due to enhanced uptake of Zn and Cu by fungal hyphae (mechanism analogous to that of P), due to alteration in root morphology (Price *et al.*, 1989) and/or root physiology (MacLeod *et al.*, 1986), or due to higher mobilization of micronutrient cations in rhizosphere through VAM exudates (*e.g.* siderophores; Römheld, 1987). In a short-term study with white clover, Cooper and Tinker (1978) demonstrated the ability of mycorrhizal hyphae to translocate Zn from an agar medium containing labelled ^{65}Zn but could not draw conclusions about uptake from soils, as they used a high concentration of Zn in the agar medium (2×10^{-6} M Zn), which seldom occurs in arable soils.

The mobility in soils of metals such as Cu and Zn is very low because of their strong adsorption to soil colloids. As shown by Elgawhary *et al.* (1970), of the total Zn absorbed by maize plants, 95% is supplied to the root surface by diffusion. Therefore, for Zn the concentration gradients and depletion zones in the rhizosphere may be similar to those of P. Mycorrhizal infection could thus improve nutrition of plants with micronutrient cations by a mechanism analogous to that for P, if metals are absorbed and translocated in the mycelium.

The present investigation was undertaken to test whether hyphal translocation is an important component in enhanced micronutrient uptake by mycorrhizal plants and, if so, what is its contribution to plant acquisition in a calcareous soil.

Materials and methods

Cultivation of plants

Pots were designed to permit spatial separation of root and hyphae growing zones in the soil. Each pot had three compartments (size of each compartment was $40 \times 25 \times 3$ cm), the central one for root growth and the two outer ones for hyphal growth (Fig. 1). For compartmentalization a 30 μm nylon net was used which allows hyphae to pass through, but not roots. The dry soil weight in each compartment was 3.33 kg.

Two treatments of mycorrhizal inoculations (*Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (M_1) and a mixture of mycorrhizal fungi (M_2) isolated from a field-grown barley crop (ICARDA, Syria) and a non-mycorrhizal control (−M) were combined with 4 treatments of P and micronutrient supply (Table 1). Each treatment had three replications. The mixture of mycorrhizal fungi (M_2) was identified according to Schenck and Perez (Manual for the Identification of VA Mycorrhizal Fungi, INVAM, University of Florida) and was found to contain *Glomus* sp. only, mainly from the *Glomus fasciculatum* complex and *Glomus occultum*. The soil used for the experiment was the subsoil of a Luvisol, very low in organic matter (0.2%); pH 7.3 (in $CaCl_2$) and a $CaCO_3$ content of 23.3%. The soil content of calcium acetate lactate (CAL)-extractable P and K (Schüller, 1969) was 13.1 and 58.1 $\mu g\, g^{-1}$, respectively, and DTPA-extractable Fe, Mn, Zn and Cu 1.43, 1.75, 0.10 and 0.16 $\mu g\, g^{-1}$, respectively. The soil was sieved (4 mm) prior to sterilisation (air-dry soil heated at 120°C for 48 h in an oven). Both mycorrhizal inocula were propagated on maize grown in a growth chamber (16 h daylight) for eight weeks. The inoculum containing dried colonized roots and adhering soil was stored at 5°C in a cold chamber. The two mycorrhizal inocula used for propagation are likely to contain similar microflora other than VA-mycorrhizal fungi as they were multiplied earlier using sterilised spores and filtered extract of the soil used for this experiment. For mycorrhizal treatments 50 g inoculum (containing about 1,250 and 1,100 infective propagules (most probable number; Powell, 1980) in the inoculum of M_1 and M_2 respectively) was mixed with the whole soil of the root compartment, while for non-mycorrhizal treatments filtered extract (Blue ribbon filter paper, No. 589[3], Schleicher and Schüll, 3354 Dassel, FRG) of the inoculum was added to the soil of the root compartment to introduce comparable microflora other than mycorrhizal fungi at the

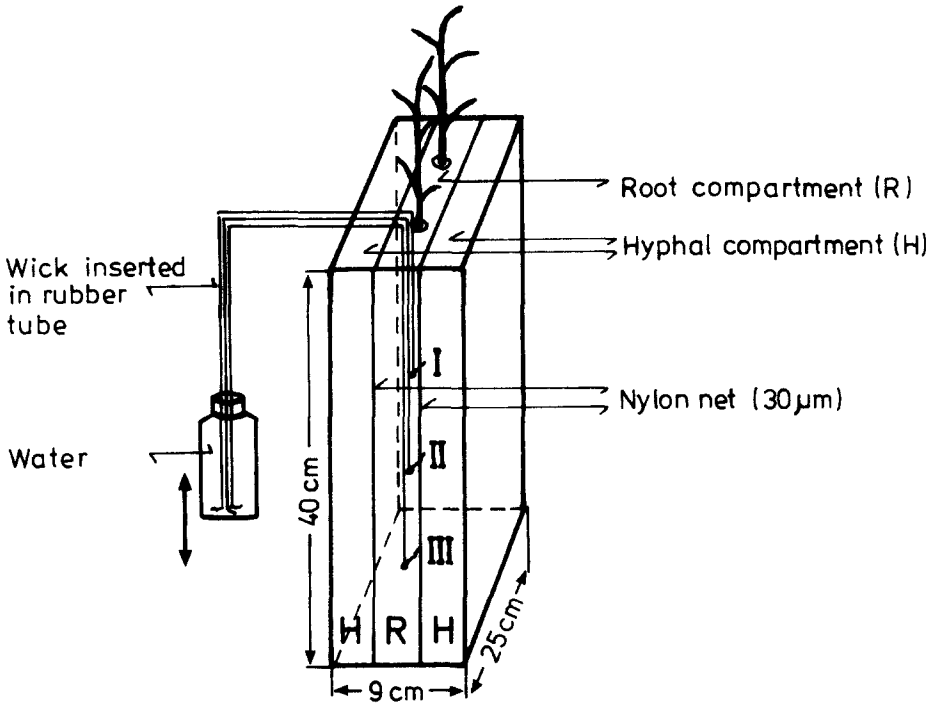

Fig. 1. Details of the arrangement for separating soil zones for root (inner compartment) and hyphal (outer compartments) growth; I, II and III are the positions of string wicks for water supply at different soil depths (10, 20 and 30 cm).

beginning. Besides P (as $Ca(H_2PO_4)_2$) and micronutrients (Fe, Zn and Cu as ferric-ammonium citrate, $ZnSO_4$ and $CuSO_4$, respectively, in the hyphal compartments only) supply as indicated in Table 1, each pot received 150 mg N and K kg^{-1} soil (1.5 g each of N and K per pot as $Ca(NO_3)_2$ and K_2SO_4, respectively) and 50 mg Mg kg^{-1} soil (0.5 g Mg per pot as $MgSO_4$). Maize (*Zea mays* L cv. Tau) seeds were surface-sterilised in 30% H_2O_2 for 10 minutes and were subsequently washed several times with distilled (sterile) water. After germination on moistened filter paper, four seeds were planted in each pot and all pots were transferred to a growth chamber (27/24°C and 16/8 h day/night regime, 250 μmol m^{-2} s^{-1} photon flux density). After seedling emergence, the number of plants was reduced to two per pot.

Water was supplied daily, directly into the root compartment at 10, 20 and 30 cm soil depth using PVC tubes with string wicks (Fig. 1), to maintain soil moisture content close to field capacity (about 20% w/w) during the period of growth. This method of watering was used to

Table 1. Details of phosphorus (P) and micronutrient supply

Treatments[a]	Nutrient supply (mg kg^{-1} dry soil) to	
	Root compartment (RC)	Hyphae compartments (HC)
$P_{L,L}$	*Low:* 50 mg P	*Low:* 50 mg P + 2 mg Zn, Cu and Fe
$P_{L,H}$	*Low:* 50 mg P	*High:* 100 mg P + 10 mg Zn, Cu and Fe
$P_{H,L}$	*High:* 100 mg P	*Low:* 50 mg P + 2 mg Zn, Cu and Fe
$P_{H,H}$	*High:* 100 mg P	*High:* 100 mg P + 10 mg Zn, Cu and Fe

[a]L, Low; H, High.

supply water uniformly at various soil depths of the root compartment and to prevent mass flow of nutrients from hyphal compartments to the root compartment.

Plant harvest and analysis

The plants were harvested after six weeks of growth (earing stage). Roots were carefully washed free from soil under a stream of cold tap water. The roots were cut into small pieces (approximately 2–3 cm length) using a ceramic scissor, mixed uniformly and fresh root weights were recorded. An aliquot of washed roots (10 g fresh weight) was collected from each pot for determination of root length and mycorrhizal infection. The roots were cut into approximately 1-cm length, cleared in 10% KOH and stained with lactophenol-trypan blue (Phillips and Hayman, 1970). The per cent root length infected was evaluated by the grid line intersect method (Giovannetti and Mosse, 1980).

Dry weight of roots and shoots were determined separately after drying at 70°C for 48 hours. The moisture content of the roots was calculated and the root dry weights were corrected for the amount of dry matter in the aliquot. Ground plant samples of roots and shoots were dry-ashed separately at 500°C and digested with 4.7 *N* nitric acid. The concentration of P was determined colorimetrically according to Gericke and Kurmies (1952), while micronutrient concentrations were determined by atomic absorption spectrophotometry (PHILIPS PU 9400X).

The lengths of external hyphae in the soil of the hyphal compartments were determined by the agar-film method (Bååth and Söderström, 1979). A grid intersection method (Newman, 1966) at 100 × magnification was used to estimate the length of hyphae. For each pot, the hyphal length was measured in six soil samples (1 g each) representing various soil depths (top, middle and bottom) of the hyphal compartments on both sides. The values were pooled and corrected for the background values in the corresponding non-mycorrhizal treatment before statistical analysis.

Mycorrhizal fungi contribution and minimal hyphal contribution to total P or Zn acquisition was calculated for both mycorrhizal types separately as given below:

$$\text{Mycorrhizal fungi contribution (\%)} = (A - B) \times 100/A \quad (1)$$

where, A = total P or Zn uptake by mycorrhizal plants, and B = total P or Zn uptake by non-mycorrhizal plants.

$$\text{Minimal hyphal contribution (\%)} = (A - A') \times 100/A \quad (2)$$

where, A = total P or Zn uptake by mycorrhizal plants at $P_{L,H}$ or $P_{H,H}$ level of nutrient supply, and A′ = total P or Zn uptake by mycorrhizal plants at $P_{L,L}$ or $P_{H,L}$ level of nutrient supply (Table 1).

Statistical analysis

Data were statistically analysed by the procedure of analysis of variance to test the treatment effects on different measured parameters (Sokal and Rohlf, 1981). LSD ($P = 0.05$) was used to separate treatment means.

Results

In the root compartment the root growth was dense and the roots were in firm contact with the nylon net separating the root and the hyphal compartments. Macroscopic observation revealed no root growth in the soil of the hyphal compartments. In both mycorrhizal fungi treatments the root colonization rates averaged 60–70%, while no colonization was observed in non-mycorrhizal control plants (Table 2). The colonization rate also did not differ appreciably among the various treatments of P and micronutrient supply. However, the hyphal length was significantly higher for both mycorrhizal inocula at low P supply compared to high P supply in the root compartment, whereas it was not influenced by low or high nutrient supply in the hyphal compartments only. On average, the hyphal length was 35% higher with *G. mosseae* than with mycorrhizal fungi mixture. Neither the P

Table 2. Shoot and root dry weights, per cent root infection and length of hyphae in the soil of the hyphal compartments as influenced by VAM fungi and P and micronutrient supply[a]

Treatments		Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Per cent root infection	Length of hyphae (m g^{-1} dry soil)
$P_{L,L}$	−M	23.8 a	7.8 c	00.0	—
	$+M_1$	25.2 a	5.3 a	68.7 a	6.70 d
	$+M_2$	26.8 a	5.8 ab	59.0 a	4.45 c
$P_{L,H}$	−M	25.4 a	7.1 c	00.0	—
	$+M_1$	26.3 a	5.2 a	65.4 a	5.95 d
	$+M_2$	25.4 a	5.6 ab	63.6 a	4.60 c
$P_{H,L}$	−M	26.9 a	6.2 b	00.0	—
	$+M_1$	26.6 a	5.5 ab	59.9 a	4.18 abc
	$+M_2$	25.1 a	5.4 ab	60.6 a	3.67 ab
$P_{H,H}$	−M	28.0 a	5.8ab	00.0	—
	$+M_1$	28.3 a	5.2 a	62.8 a	4.55 c
	$+M_2$	26.1 a	5.5 ab	61.9 a	3.42 a

[a] Mean values within a column followed by the same letter are not significantly different based on LSD ($P = 0.05$).

levels nor the mycorrhizal inoculation influenced shoot dry weight (Table 2). This was envisaged in order to allow direct comparisions to be made of the mycorrhizal fungi effects on the nutrient concentrations in the plant dry matter, without complications by dilution or concentration effects (Jarrel and Beverly, 1981). In contrast, root dry weights were significantly higher in non-mycorrhizal plants at low P supply to the root compartment ($P_{L,L}$ or $P_{L,H}$), compared to other treatments (Table 2). Mycorrhizal inoculation significantly decreased root dry weight only at low P supply to the root compartment.

Phosphorus concentrations in roots and shoots were increased by 1.4 to 3-fold due to VAM fungi inoculation (Table 3). In general, the concentration of P in the shoot was higher with *G. mosseae* than with mycorrhizal fungi mixture. In non-mycorrhizal plants, higher P supply to the root compartment ($P_{H,L}$ or $P_{H,H}$ compared to $P_{L,L}$ or $P_{L,H}$) significantly increased the P concentrations in the roots and shoots, but no effect

Table 3. Phosphorus and micronutrient concentrations in shoots and roots of maize as influenced by VAM fungi and P and micronutrient supply[a]

Treatments		P concentrations (mg g^{-1} dry weight)		Micronutrient concentrations (μg g^{-1} dry weight)						
				Zn		Cu		Mn		Fe[b]
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
$P_{L,L}$	−M	1.1 a	0.6 a	11 a	83 a	5.6 a	25 a	137 ef	234 d	83 b
	$+M_1$	2.6 de	1.4 c	31 c	104 cd	6.3 a	79 fg	98 c	99 b	60 a
	$+M_2$	2.1 c	1.3 c	19 b	95 bc	5.5 a	61 cd	79 b	77 a	54 a
$P_{L,H}$	−M	1.0 a	0.7 a	11 a	85 a	5.0 a	37 b	127 e	217 cd	61 a
	$+M_1$	3.0 f	1.7 de	42 e	113 de	6.1 a	89 h	113 d	113 b	62 a
	$+M_2$	2.5 d	1.6 d	30 c	97 bc	5.6 a	69 de	73 ab	75 a	56 a
$P_{H,L}$	−M	1.8 bc	0.9 b	11 a	91 ab	6.0 a	24 a	140 f	207 c	96 b
	$+M_1$	2.9 ef	1.8 ef	31 c	112 de	5.7 a	73 ef	113 d	114 b	60 a
	$+M_2$	2.5 d	1.8 ef	19 b	110 de	5.1 a	59 c	68 a	66 a	54 a
$P_{H,H}$	−M	1.7 b	0.9 b	12 a	98 bc	5.2 a	34 b	113 d	201 c	64 a
	$+M_1$	3.4 g	1.9 f	37 d	128 f	6.2 a	87 gh	99 c	101 b	56 a
	$+M_2$	2.8 def	1.8 ef	30 c	114 e	6.0 a	66 cde	69 ab	72 a	58 a

[a] Mean values within a column followed by the same letter are not significantly different based on LSD ($P = 0.05$).
[b] The concentration of Fe in the roots has been excluded because they were very high probably due to contamination with soil.

was observed when the P supply was increased in the hyphal compartments only ($P_{L,L}$ to $P_{L,H}$ or $P_{H,L}$ to $P_{H,H}$). On the other hand, higher P levels in the hyphal compartments substantially increased P concentration in the shoot of mycorrhizal plants.

Similar to P, the concentration of Zn in shoots was also increased by 1.7 to 3.8-fold due to mycorrhizal fungi inoculation (Table 3). The increase in Zn concentration in the roots was much less compared to that in the shoot. The Zn concentration in the shoots of mycorrhizal plants was also increased significantly when the supply of micronutrients was increased in the hyphal compartments only. Like P, the enhancement effect of VAM fungi inoculation on the Zn concentrations in shoots and roots was higher with *G. mosseae* (M_1) than with the mycorrhizal fungi mixture (M_2).

The Cu concentration in roots of mycorrhizal plants was also increased by a factor of 2 to 3 compared to non-mycorrhizal plants, irrespective of P supply to the root compartment (Table 3). Similar to P and Zn, when the supply of micronutrients was increased in the hyphal compartments, only in *G. mosseae*-inoculated plants Cu in the roots was increased significantly. Shoot Cu concentration was not influenced either by VAM fungi or by P and micronutrient supply treatments.

Interestingly, the concentrations of Mn in both shoots and roots was much lower in mycorrhizal plants, especially with the VAM fungi mixture (M_2) (Table 3). The concentrations of Mn declined more in the root than in the shoot. Compared to other treatments, significantly higher concentrations of Fe were found in non-mycorrhizal plants supplied with low P and micronutrients in the hyphal compartments (Table 3).

Mycorrhizal fungi contribution to total plant acquisition ranged from 26 to 66% for P and from 15 to 48% for Zn (Table 4). In general, the absolute or the relative contributions were higher with *G. mosseae* (M_1) than with the VAM fungi mixture (M_2). This higher contribution of M_1 occurred regardless of P supply in the root compartment.

In Table 5 data are presented for the minimal values of contribution of external mycelium in the hyphal compartments ('minimal hyphal contribution') to the total plant acquisition of P and Zn. This contribution ranged from 13 to 20% for P and from 16 to 25% for Zn (Table 5).

Discussion

Maize plants did not respond to VAM fungi inoculation in terms of shoot dry weight, indicating non-limiting nutrient (particularly P) supply

Table 4. Contribution of VAM fungi in acquisition of P and Zn by maize plants as influenced by VAM fungi and P and micronutrient supply[a]

Treatments		Phosphorus			Zinc		
		Uptake (mg plant^{-1})	VAM fungi contribution		Uptake (mg plant^{-1})	VAM fungi contribution	
			Absolute (mg plant^{-1})	Relative (%)		Absolute (mg plant^{-1})	Relative (%)
$P_{L,L}$	−M	31 a			0.90 a		
	$+M_1$	73 d	42 b	58	1.33 cd	0.43 b	32
	$+M_2$	63 c	32 b	51	1.06 b	0.16 a	15
$P_{L,H}$	−M	30 a			0.88 a		
	$+M_1$	88 e	58 c	66	1.69 e	0.81 d	48
	$+M_2$	72 d	42 b	58	1.32 c	0.44 b	33
$P_{H,L}$	−M	53 b			0.86 a		
	$+M_1$	86 e	33 b	38	1.44 d	0.58 c	40
	$+M_2$	72 d	19 a	26	1.07 b	0.21 a	20
$P_{H,H}$	−M	52 b			0.90 a		
	$+M_1$	107 f	55 c	51	1.72 e	0.82 d	48
	$+M_2$	84 e	32 b	38	1.42 cd	0.52 c	37

[a] Mean values within a column followed by the same letter are not significantly different based on LSD ($P = 0.05$).

Table 5. Minimal hyphal contribution in acquisition of P and Zn by maize plants as influenced by VAM fungi and P supply in root compartment[a]

Treatments		P uptake (mg plant^{-1})[b]		Hyphal contribution[d] of P		Zn uptake (mg plant^{-1})[b]		Hyphal contribution[d] of Zn	
Mycorrhizal strain	P supply in root compartment	Nutrient supply in hyphal compartments		Absolute[c] (mg plant^{-1})	Relative (%)	Nutrient supply in hyphal compartments		Absolute[c] (mg plant^{-1})	Relative (%)
		High	Low			High	Low		
M_1	Low	88	73	15 b	17	1.69	1.33	0.36 b	21
	High	107	86	21 c	20	1.72	1.44	0.28 a	16
M_2	Low	72	63	9 a	13	1.32	1.06	0.26 a	20
	High	84	72	12 a	14	1.42	1.07	0.35 b	25

[a] Mean values within a column followed by the same letter are not significantly different based on LSD ($P = 0.05$).
[b] Uptake data from Table 4.
[c] Difference in P (Zn) uptake by mycorrhizal plants due to high and low nutrient supply in hyphal compartments ($P_{L,H} - P_{L,L}$ or $P_{H,H} - P_{H,L}$).
[d] The values are designated as minimal because the hyphal contribution at low nutrient supply in hyphal compartments ($P_{L,L}$ or $P_{H,L}$) is taken as zero.

to non-mycorrhizal plants (Table 2). Lack of shoot growth response to VAM fungi inoculation particularly under high P supply is well documented (Harley and Smith, 1983; Smith *et al.*, 1986). Similarly, decreased root growth when plants are exposed to a high P supply or a low P supply in combination with mycorrhizal inoculation is a common response to high P nutritional status of the plant (Abbott and Robson, 1984; Gnekow and Marschner, 1989a).

The decreased length of extramatrical hyphae of both mycorrhizal types (M_1 and M_2) at high P supply in the root compartment (Table 2) is in agreement with the findings of Abbott *et al.* (1984). Although high P supply in the root compartment did not influence percentage root infection, it decreased the length of external hyphae, confirming in principle the results of Abbott *et al.* (1984).

Phosphorus concentration (as well as total P uptake) in the non-mycorrhizal plants was not influenced by increasing P supply in the hyphal compartments only, indicating little diffusion of P from the hyphal to the root compartment and thus, almost no access of the non-mycorrhizal plants to the P present in the hyphal compartments (compare $P_{L,L}$ and $P_{L,H}$ or $P_{H,L}$ and $P_{H,H}$ in tables 3 and 4). In contrast, the P concentration of mycorrhizal plants increased when the supply of P was increased in hyphal compartments. This finding of a capacity of the hyphae to translocate P from the hyphal compartments into plant roots is in agreement with earlier findings (Rhodes and Gerdemann, 1975; Sanders and Tinker, 1971; 1973; Viebrock, 1988).

Similar to P, the concentration of Zn in shoots of mycorrhizal plants also increased significantly when the supply of micronutrients was increased in the hyphal compartments (Table 3). These results clearly demonstrate the capacity of mycorrhizal hyphae to take up Zn from a calcareous soil and transport it into the host root, and confirms the earlier finding of Cooper and Tinker (1978) on the capacity of mycorrhizal hyphae to translocate ^{65}Zn from an agar medium into roots of white clover.

The mycorrhizal fungi contribution to total P and Zn acquisition by maize plants was remarkable (Table 4) and highlights the role of VAM fungi in Zn acquisition by plants grown in cal-

careous soils, where Zn deficiency is widespread. The higher VAM fungi contribution to both P and Zn in plants inoculated with *G. mosseae* than with VAM fungi mixture is at least partly related to the higher length of external hyphae of *G. mosseae* (Table 2).

The mycorrhizal fungi contribution as shown in Table 4 is based on the difference in amount of P or Zn acquisition by mycorrhizal and non-mycorrhizal plants at a particular level of nutrient supply in root and hyphal compartments. However, this calculation does not take into account any change in root morphology and/or physiology brought about by root infection with VA mycorrhizal fungi and may underestimate mycorrhizal contribution at higher P supply (Gnekow and Marschner, 1989a). In the present investigation mycorrhizal inoculation decreased not only total and specific root length, but also density and length of root hairs (data not shown). Thus, the changed root morphology of mycorrhizal plants is not expected to enhance but rather to depress P or Zn acquisition by roots only.

Calculations on minimal hyphal contribution (Table 5) are based on differences in amounts of P or Zn acquired by mycorrhizal plants at low and high supply of nutrients (P and micronutrients) in the hyphal compartments. Since the nutrients present in the hyphal compartments, particularly P and Zn, have not been accessible to the non-mycorrhizal plants, the increased amount of P or Zn acquired by mycorrhizal plants results from uptake from the hyphal compartments and translocation to the host. These values are designated as minimal because the mycorrhizal hyphae contribution is taken as zero at low nutrient supply in the hyphal compartments ($P_{L,L}$ or $P_{H,L}$). In addition, this calculation does not take into account the contribution of hyphae in the root compartment.

If polyphosphates are the main form of P translocation in mycorrhizal hyphae (Harley, 1989), Zn may be bound to them and translocated together with P into the plant roots. Thus, the amount of Zn translocated may be limited either by the amount of polyphosphate in the fungal hyphae or by the amount of Zn available to the hyphae. From the present study it is not clear which factor is most likely to limit Zn translocation in the hyphae.

Increased Cu acquisition by mycorrhizal plants has been reported by Gildon and Tinker (1983) and Pacovsky (1986). In the present study, however, mycorrhizal fungi inoculation did not affect the Cu concentration in the shoots but increased it in the roots (Table 3). Since estimation of micronutrient concentration in roots of plants grown in soil is always doubtful (contamination by soil), any conclusion drawn on the basis of only the concentration in roots may be misleading. Moreover, it is not clear whether the increased amount of Cu in roots of mycorrhizal plants is available to the plant as they may be bound to fungal polyphosphate granules, as has been shown for Ca, Fe and Mn by White and Brown (1979), or sequestered in fungal structures.

The concentration of Mn, both in shoots and roots, was much lower in mycorrhizal plants, especially with the mycorrhizal fungi mixture (Table 3). This depressing effect on the Mn concentration was more distinct in the roots than in the shoot indicating no translocation blockage from root to shoot. Lower Mn concentrations in mycorrhizal plants have been observed earlier (Arines *et al.*, 1989; Pacovsky, 1986) but the reasons are not known. The causes of such decreases in Mn concentrations were investigated in another study (in preparation). VAM fungi inoculation was found to significantly decrease the Mn^{+IV} reduction potential of rhizosphere soil because of lower numbers of Mn-reducing bacteria. Since VA mycorrhizal fungi affect the release of low molecular weight organic compounds by the roots (Bagyaraj, 1984; Bethlenfalvay and Franson, 1989), it may depress Mn acquisition indirectly via changes in the microbial activity in the rhizosphere.

As for Fe reliable data can only be given for the shoots (Table 3), interpretations of the treatment effects on uptake of Fe are difficult. The concentration of Fe in the shoot of non-mycorrhizal plants was distinctly higher in the treatments with low P and micronutrient supply in the hyphal compartments. This unexpected result is difficult to explain unless interactions with microbial siderophore production and corresponding increase in supply of ferrated siderophores to the roots is considered. It has been observed (Kothari *et al.*, in preparation) that in non-mycorrhizal plants at the interface between hy-

phal and root compartment, the soil was firmly aggregated indicating high microbial activity and perhaps, also production of siderophores.

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