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The effect of vesicular-arbuscular mycorrhizae in decreasing Ca acquisition by alfalfa plants in calcareous soils

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Summary. The legume *Medicago sativa* L. was grown in three calcareous soils supplied with increasing amounts of soluble phosphate, or a vesicular-arbuscular mycorrhizal (VAM) inoculum. The three test soils had high concentrations of extractable Ca. Analyses of dry-matter production and of the concentrations and content of the nutrients N, P, K, Ca, and Mg in plant tissues showed that, for each soil, a particular level of P application was able to match the VAM effects on N, P, and K levels. The Ca concentration and content in the VAM inoculated plants were, however, significantly lower than those in the P-supplied non-mycorrhizal treatments that matched the VAM effects. The N : P and the K : P ratios were about the same for mycorrhizal and non-mycorrhizal P-supplied control plants in all the three soils, but VAM inoculation lowered the Ca : P ratio in all soils. The mycorrhizae decreased Mg uptake in one of the soils, where non-mycorrhizal plants had high Mg concentrations in tissues. It is concluded that VAM depress the excessive acquisition of Ca by plants in calcareous soils.

Key words: Vesicular-arbuscular mycorrhiza – Ca uptake – *Rhizobium* – Legume symbiosis – Calcareous soils – VAM inoculum

Inoculation with VAM increases the plant uptake of P and other nutrients (N, Zn, Cu) from soil (Barea 1991). VAM may also decrease the acquisition by the plant of certain elements when these are in superoptimal, nearly toxic, concentrations in the soil. This is the case with heavy metals in polluted environments (Mosse 1986) or with Mn in acid soils (Arines and Vilariño 1989; Arines et al. 1989). Preliminary observation (Azcón et al. 1991) suggested that VAM have a buffering effect in the presence of nutrient (Ca and Mg) excess in a calcareous soil.

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Since P availability in calcareous soils is constrained by Ca mineral sinks (Lindsay 1979; Bolan et al. 1984), the decreased Ca uptake found by Azcón et al. (1981) might have been a consequence of the VAM effect in enhancing the P uptake in these soils. Mechanisms of mycorrhizal-enhanced P uptake in calcareous soils have been suggested previously depending either on the production of CO_2, which controls the solubility of Ca-phosphate minerals (Knight et al. 1989), or on increased production of oxalate in the mycorrhizosphere, which is able to scavenge Ca^{2+} ions from the soil solution (Jurinak et al. 1986).

In general, there have been few studies specifically designed to distinguish between a direct VAM effect and an indirect, P-mediated one on the uptake of nutrients other than P.

According to Abbott and Robson (1984), inclusion of the plant-growth response to added phosphate can be useful in experiments designed to compare the nutrient uptake of mycorrhizal and non-mycorrhizal plants. The idea is to compare matched mycorrhizal and P-supplied non-mycorrhizal plants for both plant growth and P status (Pacovsky 1986). Another, complementary approach is to use nutrient : P ratios to assess the effect of VAM on the uptake of nutrients other than P (Arines and Vilariño 1989).

Recent findings by our group (Azcón et al. 1991) on Ca uptake required confirmation in appropriate and specifically designed experiments. Since calcareous soils are very common in our region and contain high concentrations of assimilable Ca, we investigated the effect of VAM inoculation on nutrient acquisition by plants growing in these soils. We therefore compared VAM-inoculated plants with P-supplied non-mycorrhizal controls. The nutrient : P ratio approach was also used.

Materials and methods

Experimental design

The experiment consisted of three soils, each subjected to five treatments, comprising four doses of soluble P, as H_2KPO_4, and VAM. These 15 treatments were replicated five times for a total of 75 pots.

Table 1. Characteristics of experimental soils

Parameter	Soil I	Soil II	Soil III
pH (H_2O)	7.79	8.00	7.45
Clay (%)	40.70	38.76	28.00
Silt (%)	31.80	37.41	49.00
Sand (%)	27.50	23.83	22.50
Organic matter (%)	1.40	2.50	1.16
Total N (%)	0.31	0.15	0.10
Extractable P (mg kg^{-1})[a]	4.50	3.80	11.00
Extractable K (mEq 100 g^{-1})	0.52	0.61	0.63
Extractable Ca (mEq 100 g^{-1})	43.30	37.80	41.50
Extractable Mg (mEq 100 g^{-1})	3.82	4.10	4.90

[a] 0.5 *M* $NaHCO_3$ soluble P

Test soils and host plant

The test soils were collected from Granada Province, Spain. The main characteristics of these calcareous soils are summarized in Table 1. The soils were sieved (2 mm), diluted with sand (5:2, v:v), steam-sterilized (100 °C for 1 h on 3 consecutive days), and then re-inoculated with a soil filtrate containing the specific soil microbial population except for propagules of Endogonaceae. The soil filtrates were obtained by suspending 100 g of the experimental soil in 500 ml sterile water. After shaking and decanting, the suspension was filtered twice (Whatman no. 1). Each pot was given 2 ml of the filtrate. Alfalfa (*Medicago sativa* L cv. Aragón) was the test plant. Five-day-old seedlings were transplanted (four plants pot^{-1}) into pots containing 500 g of the experimental soil thoroughly mixed with the corresponding amount of soluble phosphate or VAM inoculum. At transplanting, a standard inoculum of *Rhizobium meliloti* was applied to all plants (Azcón et al. 1988).

Mycorrhizal inoculation and phosphate treatments

Each of the experimental soils was divided into five batches: P_0 (untreated control), P_{150}, P_{200}, P_{250} (levels of H_2KPO_4, in mg kg^{-1} soil), and a mycorrhizal inoculum, to be applied to the control soil at transplanting. After incubation at 19–25 °C, with suitable watering, for 2 weeks, the soils were assessed for plant-available P (Olsen). The results after 2 weeks of incubation, and just before planting, are given in Figs. 1–3 and show that each of the test soils had a high P-fixing capacity. This was to be expected because of their high clay and Ca content (Hayman 1982). The mycorrhizal inoculum was applied at 3 g pot^{-1} having been obtained from a thoroughly homogenized rhizosphere sample (stock culture) of the vesicular-arbuscular fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe with *Allium cepa* L. The inoculum consisted of spores, mycelium, and mycorrhizal root fragments.

Growth conditions

The plants were grown in a controlled greenhouse under a 16-h light (21 °C) and 8-h dark (15 °C) cycle, with 50% relative humidity and a photosynthetic photon flux density of 700 μmol m^{-2} s^{-1} for the compensating photophase. During the assay the plants were fertilized (10 ml $week^{-1}$ pot^{-1}) with a micronutrient (macronutrient-free) solution (Hewitt 1952). Throughout the experiment, the pots were weighed every day and water loss was replaced by top watering.

Measurements

After a growth period of 10 weeks the plants were harvested. Shoot and root dry weights were recorded after drying the plant material at about 70 °C. The concentrations of P, N, K, Ca, and K were determined in plant tissues (Lachica et al. 1973).

Nodulation was assessed visually and the percentage of mycorrhizal root length was estimated by a microscopic examination of stained samples (Phillips and Hayman 1970), using the grid-line intersect method of Giovannetti and Mosse (1980). This method showed that 75%, 45%, and 50% of the total root length was colonized by mycorrhizae in soils I, II, and III, respectively.

Results and discussion

Table 2 shows the typical dry matter production in response to increasing additions of phosphate or to VAM inoculation. The nutrient content in plant tissues was taken as a representative effect of the P fertilizer or mycorrhizal treatments on plant growth and nutrient acquisition (Figs. 1–3). Previous research (Jarrel and Beverly 1981) has shown that these nutrient values give coordinated information about the effects of treatment on nutrient concentrations and biomass production. The values were calculated for each pot, combining data from 4 plants pot^{-1} to allow a statistical analysis. Figs. 1–3 also show the percentage of each nutrient in the shoot tissue.

These experimental results show clearly that mycorrhizal inoculation was as effective as the phosphate fertilizer in enhancing the N, P, and K content in plant shoots. The P treatments corresponded to Olsen P (mg kg^{-1}) values of 16, 15, and 27 for soils I, II, and III, respectively. However, comparing the phosphate treatments with the VAM treatment for each soil showed that the Ca concentration and content were significantly decreased in the mycorrhizal plants in all cases. The concentration and content of Mg were also significantly reduced by mycorrhizal inoculation in soil III (Fig. 3).

The average values reported by other studies for the concentration of Ca in plant tissues are in the range 0.5%–0.8% (Thompson and Troeh 1978; Hale and Orcutt 1987). However, the values for legumes in particular are higher, at 1.0%–2.5% (Azcón-Aguilar et al. 1986; Cardoso 1986). It is clear that the values obtained in the present study for non-mycorrhizal plants are far higher than those previously reported; a striking feature is that the presence of VAM fungi reduced the concentration of Ca in plant tissues to values that accorded well with those cited previously as being fairly optimal. For Mg, the commonest concentrations described in legumes are in the range 0.2%–0.5% (Azcón-Aguilar et al. 1986; Cardoso 1986). In the present study these were exceeded only in plants growing in soil III (Fig. 3), this being the only soil where VAM fungi reduced the concentration of Mg in plant tissues.

Table 2. Dry matter yield (mg pot^{-1}) of alfalfa plant (shoots) given increasing amounts of phosphate or a vesicular-arbuscular mycorrhizal (VAM) inoculum, and grown for 10 weeks in the three test soils

H_2KPO_4 (mg kg^{-1} soil)	Soil I	Soil II	Soil III
P_0	519a	501a	1054a
P_{150}	2474b	1461b	1464b
P_{200}	2685bc	1819c	1548c
P_{250}	2812c	2090d	1714d
VAM	2570b	1976d	1669d

For each soil, mean values (five replicates) not sharing a letter in common differ significantly ($P<0.05$)

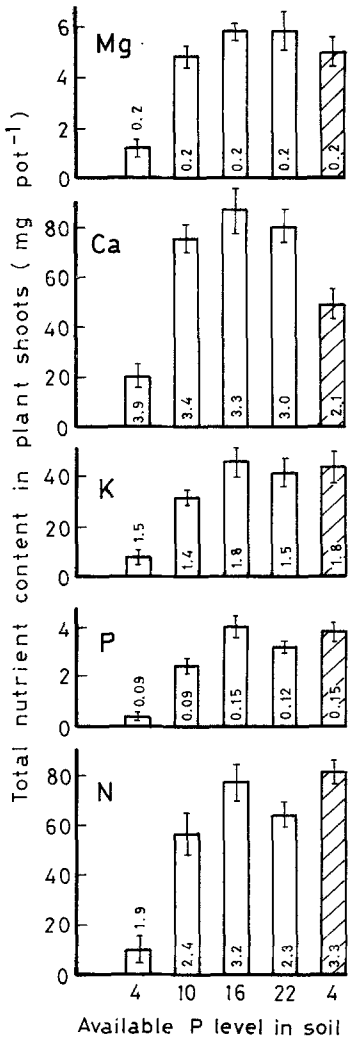

Fig. 1. Nutrient content in the shoots of alfalfa plants supplied with increasing levels of available P (mg kg^{-1} soil) compared with a mycorrhizal inoculum (▨) in soil I. The nutrient concentration as a percentage of the plant dry matter is given inside the *bars*. Confidence limits (5%) are shown

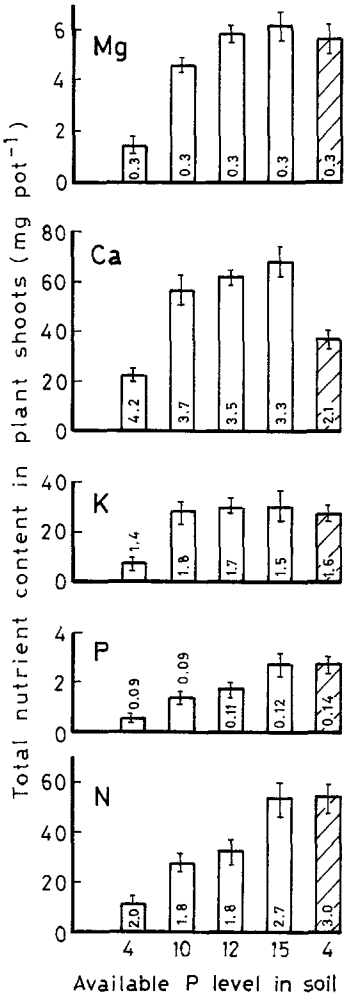

Fig. 2. Nutrient content in the shoots of alfalfa plants supplied with increasing levels of available P (mg kg^{-1} soil) compared with a mycorrhizal inoculum (▨) in soil II. For other explanations, see Fig. 1

Table 3. Number of nodules (per pot) on alfalfa plants grown for 10 weeks, supplied with increasing amounts of phosphate or a vesicular-arbuscular mycorrhizal (VAM) inoculum, in three test soils

H_2KPO_4 (mg kg^{-1} soil)	Soil I	Soil II	Soil III
P_0	25a	58a	83a
P_{150}	133b	77ab	95a
P_{200}	210c	98b	130b
P_{250}	275d	149c	175c
VAM	240cd	170c	189c

See footnotes to Table 2

Nutrient : P ratios were calculated in order to compare the effects of VAM and the P additions (Arines and Vilariño 1989). The phosphate treatment selected was the level that best matched the mycorrhizal effect on the yield of N, P, and K, for each of the soils (Fig.s 1 – 3). These data are shown in Fig. 4. In all three soils the mycorrhizal inoculation and the P treatment had important and significantly similar effects on the N : P and K : P ratios. Since the phosphate added was in the KH_2PO_4 form, these data indicate that the mycorrhizal inoculation behaved like a P fertilizer in improving N nutrition, presumably by affecting N_2 fixation (Azcón et al. 1988) because similar nodulation rates were observed in VAM-inoculated and P-fertilized plants (Table 3), and in facilitating a balanced K uptake. However, there was a significant decrease in the Ca : P ration in the VAM inoculated plants.

These data, together with those on nutrient concentrations, can be interpreted as indicating that mycorrhizal fungi depressed the plant uptake of Ca in the calcareous soils tested. This effect could be considered a consequence of the mechanisms that have been proposed to explain the enhanced P uptake by VAM-inoculated plants in soils where P availability is limited (Jurinak et al. 1986; Knight et al. 1989). According to those authors, the oxalates produced by VAM fungi bind Ca to make P available in calcareous soils. In the present study, VAM fungi increased P concentrations and decreased Ca concentrations in plant tissues compared with non-mycorrhizal

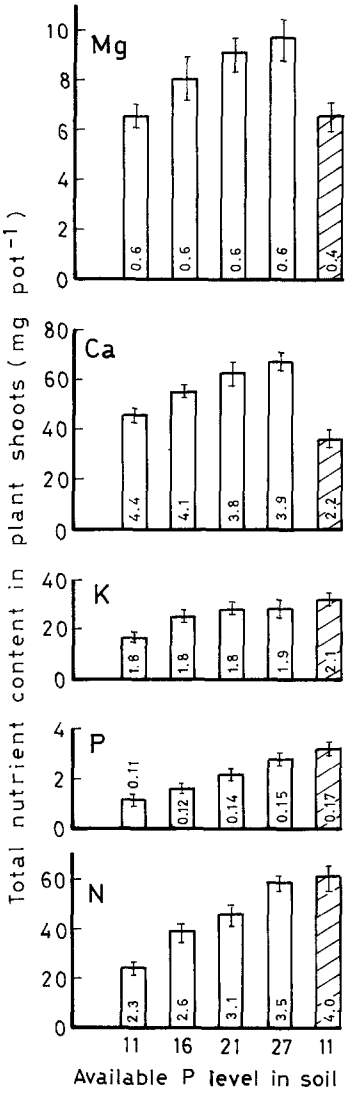

Fig. 3. Nutrient content in the shoots of alfalfa plants supplied with increasing levels of available P (mg kg^{-1} soil) compared with a mycorrhizal inoculum (▨) in soil III. For other explanations, see Fig. 1

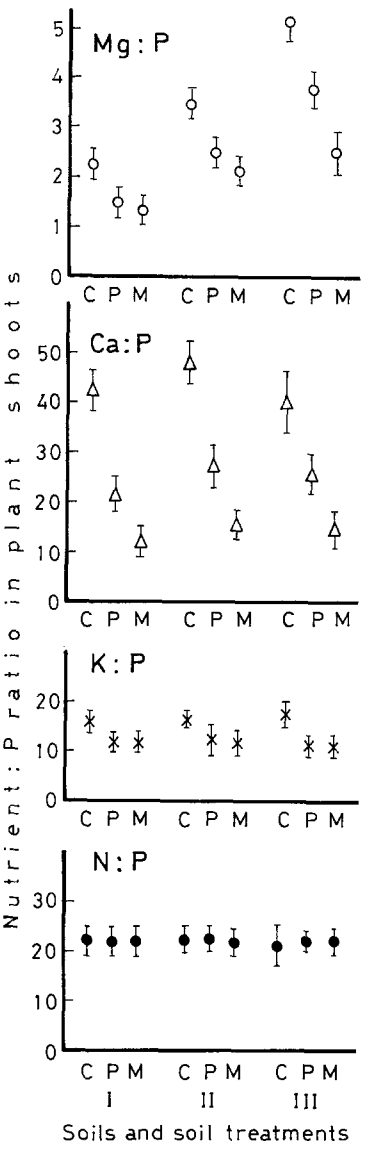

Fig. 4. Nutrient : P ratio in 10-week-old alfalfa plants. *C*, control; *M*, inoculated with vesicular-arbuscular mycorrhizae; *P*, fertilized with P. Confidence limits (5%) are shown

non-fertilized controls (Figs. 1–3). These results therefore suggest that VAM fungi have a buffering effect in the presence of a nutrient excess in the medium, as already described for heavy metals in polluted environments (Mosse 1986) or for Mn in acid soils (Arines and Vilariño 1989; Arines et al. 1989). In calcareous soils this effect is seen in relation to Ca (and, in some cases, Mg). This mycorrhizal effect of increasing the tolerance of the plant to high concentrations of Ca (as $CaCO_3$) has also been proposed for other types of mycorrhiza (Clement et al. 1977; Lapeyre and Chilvers 1985; Leake and Read 1989). If complexing is involved as a mechanism, the fungal substance responsible, perhaps oxalate, must be exuded into the mycorrhizosphere, where the cation in excess might be chelated, thereby preventing an excessive uptake while allowing balanced acquisition by the plant. Work is currently in progress to elucidate the possible mechanism(s) involved in the proposed buffering effect of VAM fungi and its implications for the ecosystem.

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