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Influence of mycorrhiza vs. soluble phosphate on growth, nodulation, and N_2 fixation (^{15}N) in alfalfa under different levels of water potential

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Summary. The legume *Medicago sativa* (+*Rhizobium meliloti*) was grown under controlled conditions to study the interactions between soluble P in soil (four levels), or a mycorrhizal inoculum, and the degree of water potential (four levels) in relation to plant development and N_2 fixation. ^{15}N-labelled ammonium sulphate was added to each pot for a qualitative estimate of N_2 fixation, in order to rank the effects of the different treatments.

Dry-matter yield, nutrient content and nodulation increased with the amount of plant-available P in the soil, and decreased as the water stress increased, for each P-level. The mycorrhizal effect on dry matter, N yield, and on nodulation was little affected by the water potential. Since P uptake was affected by the water content in mycorrhizal plants, additional mechanisms, other than those mediated by P, must be involved in the mycorrhizal activity.

There was a positive correlation between N yield and nodulation for the different P levels and the mycorrhizal treatment at all water levels. A high correlation between plant unlabelled N content and atom% ^{15}N excess was also found for all levels of P. In mycorrhizal plants, however, the correlation between unlabelled N yield and ^{15}N was lower. This suggests that mycorrhiza supply plants with other N sources in addition to those derived from the improvement on N_2 fixation.

Key words: Vesicular-arbuscular mycorrhiza – N_2 fixation – ^{15}N-labelled fertilizers – *Rhizobium* – Legume symbiosis – Drought stress

Mycorrhiza, phosphate and water are three ecosystem components that strongly affect biological N_2 fixation by legume-*Rhizobium* symbioses. Nodulation and N_2 fixation require an adequate P level within the plant and mycorrhizal fungi improve phosphate transport from soil solution to cortical root cells (Barea and Azcón-Aguilar 1983; Hayman 1986). Soil moisture affects the movement of rhizobia in soil, and also nodule formation and function (Sprent 1986). The effect of vesicular-arbuscular mycorrhiza in improving P transport, there by enhancing plant growth, is mainly exerted through the ability of the extramatrical fungal hyphae to increase the root surface area for the uptake of low-diffusing nutrients, the absorption of which is also affected by soil moisture. Mycorrhizal hyphae are known to penetrate the soil beyond the water depletion zones that develop around the root. This can be critical to plant health when the diffusion of water in soils is low under dry conditions (Cooper 1984). In fact, there are many indications that vesicular-arbuscular mycorrhiza may help plant development under drought stress (Safir et al. 1971; Ellis et al. 1985; Hardie 1985; Dakessian et al. 1986; Augé et al. 1987; Daniels-Hetrick et al. 1987). However, it is not clear whether vesicular-arbuscular mycorrhiza improve water relations in the plant directly or merely facilitate P uptake when the diffusion rate of P in the soil is reduced under a low level of moisture (Cooper 1984).

Methodology using ^{15}N-labelled fertilizer is being increasingly used to determine the relative contribution to N_2-fixing plants of the three N sources (soil, fertilizer and atmosphere), and to assess whether any treatment affecting plant N nutrition acts directly on N_2 fixation (Danso 1986). The procedure requires a non-N_2-fixing reference crop, which must be carefully selected in order to avoid possible errors in a quantitative determination. However, when only qualitative differences the method does not need a reference crop; the effect of several treatments can be ranked simply by comparing the ^{15}N enrichment of a test legume

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growing in soil amended with a small amount of ^{15}N-labelled fertilizer. The lower the atom% ^{15}N excess in a sample of plant tissue, the better the treatment is at improving N_2 fixation (Hardarson et al. 1984; Danso 1986).

This procedure was used in the present study to assess the effects of vesicular-arbuscular mycorrhizae or different amounts of a soluble phosphate fertilizer, at four levels of soil water content, on N_2 fixation by alfalfa grown under controlled conditions.

Materials and methods

Experimental design. We used a randomized complete block factorial with two factors: (1) water potential, four levels of water content in soil, and (2) P level, i.e. four doses of soluble P and the inoculation of a selected vesicular-arbuscular mycorrhizal fungus (*Glomus mosseae*). The 20 treatments were replicated five times for a total of 100 pots.

Host plant and test soil. Alfalfa, *Medicago sativa* L cv. Aragón was the test plant. Five-day-old seedlings were transplanted (two plants/pot) into pots containing 300 g of the experimental soil thoroughly mixed with the corresponding amount of soluble phosphate or a mycorrhizal inoculum. At transplanting, all the plants received a standard inoculum of *Rhizobium meliloti* strain 203, obtained as previously described (Barea et al. 1980). The test soil was collected from Granada Province, Spain. It was a "reddish-brown calcareous" type (42.0% clay, 39.8% loam, 18.2% sand), with 1.23% organic matter, pH 7.4 and 4.5 mg P/kg soil extracted with 0.5 *M* $NaHCO_3$ (Olsen P). The soil was sieved (2 mm), diluted with sand (5:2, v:v), steam-sterilized (100 °C for 1 h during 3 consecutive days) and then reinoculated with a soil filtrate containing its own microbial population except propagules of Endogonaceae. The soil filtrate was 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 filtrate. The water holding capacity of the experimental mixture was estimated at 17%.

Mycorrhizal inoculation and phosphate treatments. The experimental soil was divided into five batches: P_0 (untreated control), P_{150}, P_{200}, P_{250} (levels of H_2KPO in mg/kg soil), and M (mycorrhizal inoculum, applied 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 (in mg P/kg soil) were: $P_0 = 4.5$; $P_{150} = 10.3$; $P_{200} = 15.6$; $P_{250} = 21.8$; M = 4.6, indicating a high P-fixing capacity. For the mycorrhizal treatments, inoculum at 3 g/pot was applied to the planting hole having been obtained from a thoroughly homogenized rhizosphere sample (stock culture) of vesicular-arbuscular fungus *Glomus mosseae* with *Allium cepa*.

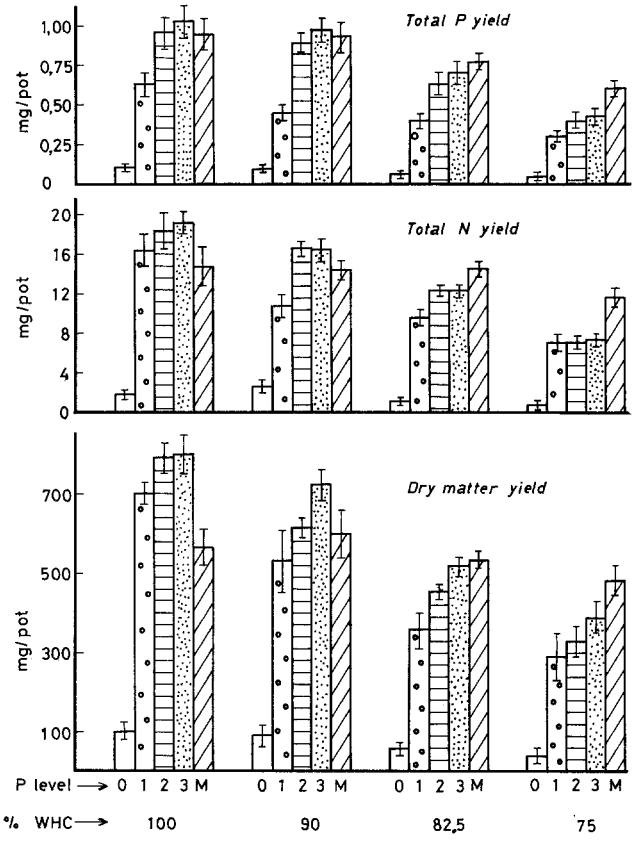

Fig. 1. Dry-matter, N and P yields of alfalfa shoots under different treatments. WHC, water-holding capacity. Treatments: PO, control; P1, 2, 3, represent H_2KPO applied at 100, 200, and 250 mg/kg soil, respectively; M, mycorrhiza

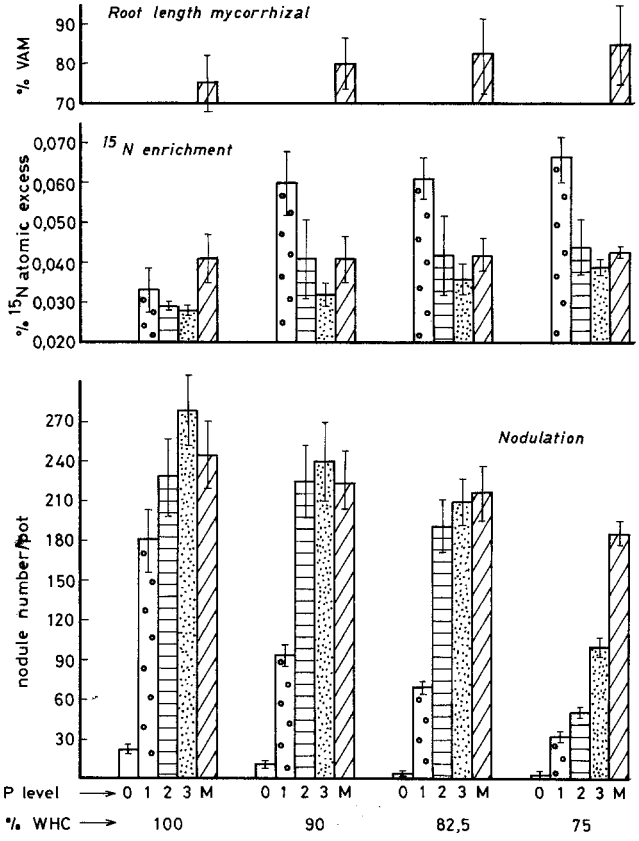

Fig. 2. Formation of microbe–root symbioses and N isotopic composition of alfalfa shoots under different treatments (see Fig. 1 for explanation of symbols)

The inoculum consisted of spores, mycelium, and mycorrhizal root fragments.

Growth conditions. The plants were grown for 1 week at field capacity before being exposed to four levels of irrigation: 100%, 90%, 82.5%, and 75% of the water-holding capacity of the test soil, the wilting point of the soil being taken as 70% water-holding capacity. Each water-stress regime was applied to five replicate pots in each treatments, by weighing each pot and adding water to reach the calculated weight for the desired water regime. The plants were grown in a controlled environmental chamber under a 16/8 h light/dark cycle, 21/15 °C day/night temperature, 50% relative humidity and a photosynthetic photon flux density of 400–700 μmol m^{-2} s^{-1}.

Throughout the experiment, the pots were weighed twice a day and water loss was replaced by top watering.

^{15}N-labelled fertilizer application. After 10 days of plant growth each pot was given a solution of $(NH_4)_2SO_4$ with 10% ^{15}N. Two mg N/kg soil was given, which is equivalent to 5 kg N/ha.

Measurements. After a growth period of 11 weeks the plants were harvested. Shoot dry weight was recorded after drying at about 70 °C. P and N concentrations were measured after Kjeldahl digestion (for N) or by the molybdenum blue (for P) procedures (Lachica et al. 1973). The N isotopic composition of plant shoots was determined by mass spectrometry, as described by Fiedler and Proksch (1975) at the FAO/IAEA Agricultural Biotechnology Laboratory, Seibersdorf, Austria.

The roots were carefully washed and the number of nodules was assessed visually. the percentage of mycorrhizal root length was estimated by microscopic examination of stained samples (Phillips and Hayman 1970), using the grid-line intersect method of Giovannetti and Mosse (1980).

Results and discussion

Effects on plant growth and nutrient content

Data sumarized in Fig. 1 indicate that the dry-matter yield and the N and P content in alfalfa increased with the amount of soluble P added to the growing medium, and that the effects of the P amendments on plant growth and nutrient uptake diminished as the water stress increased. These results were expected. The response to mycorrhizal inoculation was, however, surprising in that the effect on dry-matter production and N yield was little affected by the level of water stress. Mycorrhizal inoculation was the most effective treatment at the two highest levels of water stress studied. In contrast, the mycorrhizal effect on P uptake was influenced by the soil water content, suggesting that additional mechanisms in mycorrhizal plants, other than the known P-mediated effect, were responsible for the enhanced growth of stressed plants. The overall results confirm that vesicular-arbuscular mycorrhiza benefit plants under stress, as described earlier. Mycorrhizal development was little affected by the various water treatments (Fig. 2).

Table 1. Correlation coefficients for two parameters related to N nutrition for different phosphate additions or mycorrhizal inoculation

Correlations of total N yield with	P_{100}	P_{200}	P_{250}	M
Atom % ^{15}N excess	−0.795[a]	−0.832[a]	−0.864[a]	−0.460[b]
Nodule number	0.996[a]	0.937[a]	0.967[a]	0.945[a]

[a] $P = 0.001$

[b] $P = 0.05$; P_{100}, P_{200}, P_{250}, represent levels of H_2KPO in mg/kg soil

Effects on N_2 fixation

It is well known (Barea and Azcón-Aguilar 1983; Hayman 1986) that the increased level of N usually found in nodulated and mycorrhizal legumes is the result of an increased rate of N_2 fixation, as shown by studies using ^{15}N (Barea et al. 1987). Nodulation is usually stimulated by mycorrhiza. In the present study, the number of nodules was increased by P additions and decreased by lowering the water potential (Fig. 2), yet the effect of mycorrhizal inoculation on nodulation was significantly altered only at the lowest water level studied, and it was the best treatment for improving nodulation at this level. Irrespective of water potential, there was a positive correlation between N yield and nodule number for the different phosphate additions or mycorrhizal inoculation (Table 1).

The well-known effect of plant-available P on N_2 fixation has been supported in the present study by the use of ^{15}N methodology (Fig. 2). ^{15}N enrichment of alfalfa shoots has been shown to diminish, indicating an improvement of N_2 fixation rates (Danso 1986), as the P supply increases. With the phosphate treatments in the present study, the lower the soil water content the higher the ^{15}N per cent excess was in plant samples. This indicates a direct relationship between N_2 fixation and water stress. A high negative correlation between plant N content and ^{15}N per cent excess was found at all levels of phosphate addition (Table 1). However, the effect of mycorrhizal inoculation on the N isotopic composition of alfalfa shoots was not altered by the soil water content. Since the water level did affect P uptake by mycorrhizal plants (Fig. 1), it is possible that extramatrical hyphae increased the water uptake. While there was a high correlation between N yield and nodule number in mycorrhizal plants (Table 1), that between N yield and ^{15}N was lower. One possible explanation is that vesicular-arbuscular mycorrhiza supply plants with other sources of N in addition to those derived from the improvement of N_2 fixation (Barea et al. 1987). The ability of vesicular-arbuscular mycorrhiza hyphae to transport N from soil to plant has been demonstrated by using

^{15}N under both controlled and field conditions (Ames et al. 1983; Kessel et al. 1985; Barea et al. 1987).

Although soil water content has been reported to affect mycorrhiza formation (Reid and Bowen 1979; Mosse et al. 1981), the final colonization levels in the present study were little affected (Fig. 2). These results suggest that mycorrhiza enhance growth and N uptake in plant exposed to water stress. Since the present experimental design did not allow the elucidation of mechanisms involved, the advantages of vesicular-arbuscular mycorrhiza for legume development in arid and semiarid lands need further study.

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