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Mycorrhizal dependency of a representative plant species in mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas

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Abstract

Lavender plants (*Lavandula* spp.) are small woody shrubs which belong to the natural succession in certain plant communities of semiarid mediterranean ecosystems in the southeast of Spain and they thrive in areas which are threatened by desertification. Lavender plants form arbuscular mycorrhizas and have been described as mycorrhizal-dependent species, but they are also known to display a stimulated root phosphatase activity in conditions of low-nutrient supply. Consequently, a series of microcosm experiments were carried out in order to identify the relative importance of the mycotrophic habit in comparison with phosphatase production as mechanisms which account for plant establishment and nutrient uptake, using four soils typical of the degraded area of study. It was found that lavender plants were obligatorily mycorrhizal species under the prevailing conditions of their natural habitat. The specific activity of root-associated acid phosphatase in lavender was enhanced in P-deficient, non-mycorrhizal, plants, but apparently this stimulated phosphatase activity did not result in an increased ability of the plant to take up P. Low organic phosphate content in the test soils may account for the absence of plant response to acid phosphatase activity. However, it is much more likely that this ineffectiveness is due to the fact that P-deficiency is concomitant, in the degraded test habitats, with deficiencies in other nutrients. Thus plant growth is being limited by an overall lack of nutrient supply. Mycorrhizal inoculation largely improved P, N and K uptake thereby restoring the biochemical cycling of plant nutrients. In conclusion, lavender plants must be mycorrhizal in order to thrive in the degraded soils from desertification threatened areas in typical mediaterranean ecosystems. © 1997 Elsevier Science B.V.

Keywords: *Arbuscular mycorrhizas*; *Lavandula* spp.; Acid phosphatase; Desertification; Revegetation

1. Introduction

Lavender plants (*Lavandula* spp.) are small woody shrubs that, together with certain N_2-fixing legumes, belong to the natural succession in certain plant communities of semiarid mediterranean ecosystems in the southeast of Spain (Barea et al., 1992). Particular climatic conditions, involving scarce and irregular rainfall and a long dry period in summer, in addition to man-mediated degradative activities, can cause or accelerate degeneration (desertification) in

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such ecosystems (Francis and Thornes, 1990; Morgan et al., 1990). It is well known that desertification is the result of a progressive degradation of both soil quality (nutrient availability, microbial activity, soil structure, etc.) and vegetation cover (structure, pattern, species diversity, etc.) (Barea and Jeffries, 1995). In summary, desertification implies loss or disturbance of existing vegetation and an increase in soil erosion (Francis and Thornes, 1990; Morgan et al., 1990). This usually produces the loss or reduction of the microbial activity, and particularly that of mycorrhizal fungi, a key ecological factor because mycorrhizal symbiosis plays an essential role in sustaining a vegetation cover in natural habitats. Establishing a network of AM mycelium seems critical for ecosystem sustainability (Bethlenfalvay and Schüepp, 1994; Gianinazzi and Schüepp, 1994; Degens et al., 1996), like those in semiarid mediterranean regions (Requena et al., 1996).

In addition to the N inputs derived from N_2 fixation, the supply of available P is critical for plant development in degraded habitats (Barea et al., 1992). In this concern, some plant inherent factors are recognized to account for P uptake from soil deficient in available P, two of them are relevant to the aims of this study: (i) the degree of mycorrhizal dependency and (ii) the root-associated phosphatase activity (Dodd et al., 1987).

Mycorrhizal dependency, is a plant property which refers to the degree of its responsiveness to mycorrhizal colonization. It can be measured by quantifying the growth improvement owing to the mycorrhizal performance, such as the relative contribution of root compared with mycorrhizal-mediated nutrient uptake (Gianinazzi-Pearson, 1984; Brundrett, 1991). Mycorrhizal dependency is the result of morphological and physiological plant traits, which are modulated by both the nutrient availability of the soil, particularly of P, and the effectiveness of the mycorrhizal fungus involved (Khalil et al., 1994). It can vary greatly from one plant species to another and even between cultivars or ecotypes within a single species (Azcón and Ocampo, 1981). Some plant species can be obligatorily mycorrhizal for P uptake (Janos, 1980a,b; Merryweather and Fitter, 1996).

The presence and activity of root-associated acid phosphatases have been related to the acquisition of P by plants under conditions of low nutrient availability (McLachlan, 1980a,b; Tarafdar and Marschner, 1994). Such phosphatase activity, which produces the hydrolysis of organic P, may originate from plant roots, soil microorganisms or from mycorrhizal roots (Dinkelaker and Marschner, 1992; Tarafdar and Marschner, 1994; Tarafdar and Rao, 1996). The importance of these enzymes is not always obvious and their role is modulated by several factors such as P availability in soil, certain characteristics of the plant species (mainly concerning the threshold value for effective P uptake), and the microbial interactions in the rhizosphere/mycorrhizosphere (McLachlan, 1980a,b; Azcón et al., 1982; Dodd et al., 1987; Khalil et al., 1994; Tarafdar and Marschner, 1994). Particularly interesting is the relationship between mycorrhizal dependency and phosphatase activity, a topic on which contradictory results have been published (Azcón et al., 1982; Dodd et al., 1987; Khalil et al., 1994; Tarafdar and Marschner, 1994).

A reclamation strategy for the revegetation of desertification-threatened areas in mediterranean ecosystems has been proposed (Herrera et al., 1993), involving the artificial acceleration of natural succession. For such purposes, drought-tolerant, native species, able to cope with nutrient stress in the eroded soil, have been recommended to re-establish functional shrublands (Francis and Thornes, 1990). Pioneering studies (Herrera et al., 1993) demonstrated that woody legumes are useful for such nutrient-deficient, arid ecosystems because of their ability to fix N_2 in association with rhizobial species, and to form mycorrhizas. The phosphate supply by arbuscular mycorrhizal (AM) activities is thought to be critical for both plant growth and N_2-fixation, which, in turn, benefits other species growing in association with legumes, through N-transfer. This is the case for the small woody non-N_2 fixing members of the shrubland community, of which *Lavandula* spp. are an example (Barea et al., 1992). Lavender plants have been described as mycorrhizal dependent species forming arbuscular mycorrhizal (AM) associations (Azcón et al., 1976). However it has also been suggested that, in conditions of low nutrient supply, particularly phosphate, *Lavandula* spp. display a stimulated root phosphatase activity (Azcón et al., 1982), which was suggested as an alternative mechanism for P uptake under low fertility conditions

(Azcón et al., 1982; Dodd et al., 1987). In fact, lavender plants currently thrive in such desertified natural systems, but improvement of their performance is still needed, and this forms one of the objectives of a current revegetation programme (Barea et al., 1996).

Against this background a series of experiments was carried out to assess the relative importance of the mycorrhizal symbiosis in comparison with root phosphatase production with regard to improved nutrient uptake from degraded low-nutrient soils.

2. Materials and methods

2.1. Experimental ecosystem areas

A target experimental area was chosen within a desertification-threatened region in the southeast of Spain (Barea et al., 1996; Requena et al., 1996). This was situated in a sedimentary basin, about 600–800 m high, located in the Sierra de los Filabres, Almeria (Spain). The mean annual precipitation is 230 mm. A typical mediterranean vegetation cover (López-Bermúdez and Albaladejo, 1990) was formerly present. However, historic overgrazing and felling of woodlands, and further poor agricultural practices, lead to a desertification process. Re-establishment of the natural shrubland has been recommended for these areas (Francis and Thornes, 1990). Natural vegetation now includes shrub legume species, like *Anthyllis cytisoides* and *Retama spherocarpa*. Lavender species, which formerly grew as components of the natural succession, are now scarce or practically absent. The soil in this degraded area, described in this paper as Soil 1, is a Eutric Regosol. In a neighbouring area, where the climatic conditions are quite similar, lavender plants are actually growing as members of the natural succession of the shrub community, and Soil 2, a calcareous Cambisol, was collected from this site. In addition, Soil 3 (Cromic Luvisol) and Soil 4 (Calcareous Fluvisol) were collected from a marginal agroecosystem and an arable ecosystem, respectively, in areas that have been selected by local farmers for lavender cropping. The characteristics of the four test soils are given in Table 1.

Table 1
Some physico-chemical characteristics of the experimental soils

Parameter	Soil			
	1	2	3	4
pH (H_2O)	7.7	7.0	7.6	7.4
Organic matter (%)	0.4	0.8	1.3	1.4
Available P (mg kg^{-1})	4.9	6.9	9.0	12.0
Total N (%)	0.1	0.3	0.1	0.3
Sand (%)	65.0	58.7	41.0	23.0
Silt (%)	24.5	26.4	19.0	49.0
Clay (%)	10.5	14.9	40.0	28.0

2.2. Microcosm experimental design, test plant and treatments

To test the relative significance of the mycorrhizal symbiosis in comparison with root phosphatase production in lavender plants, with regards to their re-establishment in the target degraded ecosystem, two microcosm experiments were carried out. Since the available P level in soil affects both mycorrhiza formation and root phosphatase activity (Dodd et al., 1987; Barea and Jeffries, 1995) a range of increasing amounts of a P fertilizer were tested.

The experimental soils were sieved (4 mm), steam-sterilized (100°C for 1 h three consecutive days) and then reinoculated with 2 ml per pot of a soil filtrate containing its natural microbial population except propagules of AM fungi. The soil filtrate was obtained by suspending 100 g of the experimental soil in 500 ml sterile water for 60 min. After shaking and decanting, the suspension was filtered twice (Whatman No. 1) which removed propagules of AM fungi.

Experiment 1 utilized the four test soils, and three levels of soluble P, with and without a mycorrhizal inoculum, therefore giving six treatments that were replicated five times for a total of 30 microcosm units per test soil. Experiment 2 utilized two test soils (Soils 3 and 4), six levels of soluble P, with and without a mycorrhizal inoculum, thus giving twelve treatments that were replicated five times for a total of 60 microcosm units per test soil. The two more-fertile soils were selected for this experiment because they are being used to grow lavender as a crop, thus P-fertilizer inputs are being applied by the farmers. A completely randomized arrangement was followed in both experiments.

For Experiment 1, each one of the four test soils was divided into three batches: P_0 (untreated control soil), P_1, P_2, which represent levels of H_2KPO_4 at, 0, 250 and 500 mg kg^{-1} soil, respectively. In Experiment 2, a wide range of P levels was studied including low, moderate, optimal and supraoptimal P doses. Each soil was divided into six batches: P_0, P_1, P_2, P_3, P_4, P_5, which represent increasing levels of H_2KPO_4 at, 0, 250, 500, 1200, 1380 and 1760 mg kg^{-1} soil, respectively. In all cases, and once phosphate-treated, the soils were left to equilibrate for three weeks and then analysed for plant available P (Olsen).

Lavandula spica L., one of the commonest lavender species in the area, was the host plant. Seeds collected from the field were germinated on moistened sand and five-day-old seedlings were individually transplanted to the microcosms, each one containing 500 ml of soil previously supplied with the appropriate amount of soluble P. Ten replicate pots were prepared for each P level and test soil, five to grow mycorrhiza-inoculated seedlings and the other five to grow uninoculated control plants. At transplanting, seedlings (one per pot) received the mycorrhizal treatments. The mycorrhizal inoculum was obtained from a stock-pot culture of *Glomus mosseae* (Azcón et al., 1991), using *Allium cepa* L. as a host

Table 2
Growth, phosphorus nutrition, phosphatase activity and mycorrhizal colonization of lavender plants growing for 16 weeks in the four experimental soils under different phosphate and mycorrhizal inoculation treatments

Soil no.	Treatment			Dry weight shoot (mg)	Plant phosphorus		Root[c] P-ase	AM[d] colonization (%)
	Phosphate fertilizer		Mycorrhizal inoculation		Conc. (%)	Content (mg per plant)		
	Applied[a]	Resultant[b]						
1	0	5	Control	60a	0.06a	36a	4.1a	—
			G. mosseae	510b	0.15b	765b	5.2b	61a
	250	25	Control	65a	0.06a	39a	3.8a	—
			G. mosseae	570b	0.18c	1026c	4.2a	57a
	500	32	Control	68a	0.06a	41a	3.3a	—
			G. mosseae	650c	0.21c	1365d	3.7d	53a
2	0	7	Control	43a	0.09a	39a	12.4a	—
			G. mosseae	223d	0.21c	468d	7.3b	69a
	250	18	Control	140b	0.14b	196b	7.2b	—
			G. mosseae	310e	0.29d	899e	6.4c	62ab
	500	28	Control	193c	0.20c	386c	5.8c	—
			G. mosseae	408f	0.35e	1428f	3.6d	59b
3	0	9	Control	28a	0.03a	8a	52a	—
			G. mosseae	382c	0.22d	840d	25b	69a
	250	22	Control	256b	0.08b	205b	28b	—
			G. mosseae	504cd	0.28e	1410e	30b	52b
	500	29	Control	424c	0.15c	636c	28b	—
			G. mosseae	512d	0.29e	1995f	26b	45c
4	0	12	Control	56d	0.04a	224a	13.7a	—
			G. mosseae	450c	0.41d	1845d	1.6b	75a
	250	26	Control	254b	0.15b	381b	3.6b	—
			G. mosseae	450c	0.42d	1890d	2.4b	69ab
	500	38	Control	217b	0.34c	734c	2.5b	—
			G. mosseae	450c	0.52d	2340e	2.8b	60b

[a]As H_2KPO_4 in mg kg^{-1} soil.
[b]Actual concentration of available P in soil solution, as mg P per litre of soil.
[c]Phosphatase activity as μg *p*-nitrophenol per gramme fresh root tissue per hour.
[d]Percentage root length mycorrhizal.
Data are given on a per pot (per plant) basis. For each soil and parameter, mean values (five replicates) not sharing a letter differ significantly ($P < 0.05$).

plant to produce a soil containing five sporocarps per gramme (with six mature spores per sporocarp, on average), together with some single spores, mycelium and mycorrhizal root fragments. Ten grams of such mycorrhizal inoculum was added per pot and was thoroughly mixed with the soil.

2.3. Growth conditions

Plants were grown in a greenhouse under a day/night cycle of 16/8 h, 21/15°C, 50% relative humidity. Throughout the experiment, the pots were weighed every day and the water loss was compensated for by top watering.

2.4. Measurements

After a growth period of 70 days, plants were harvested. Shoot dry weight was recorded after drying at 70°C. Shoot nutrient concentrations were then measured. Colorimetry for N (after Kjeldahl digestion) and P (by the molybdenum blue procedure), flame photometry for K, and atomic absorption spectrometry techniques for Ca and Mg were followed.

The percentage of mycorrhizal root length was estimated by microscopical examination of stained samples (Phillips and Hayman, 1970), using a grid-line intersect method (Giovannetti and Mosse, 1980).

Acid phosphatase (E.C.3.1.3.2 orthophosphoric-monoester phosphohydrolase) associated with intact root segments was measured using adaptations of the method of Tabatabai and Bremner (1969). Samples (100 mg) of fresh root tissue were incubated with 1 ml of 50 mM *p*-nitrophenol phosphate and 4 ml of 0.1 M sodium acetate buffer, pH 5.2, for 1 h at 25°C. The reaction was terminated by the addition of 0.5 M NaOH and the samples were centrifuged at 2500 g for 10 min. The *p*-nitrophenol released was determined spectrophotometrically by measuring the optical density of the supernatant at 400 nm.

2.5. Statistical analysis

Data from the microcosm experiments were processed by ANOVA and Duncan's new multirange test ($P \leq 0.05$). Data given at percentage values were first subjected to arc-sine square root transformation.

3. Results

The four experimental, low fertility, soils have a neutral to neutral–alkaline reaction. Soils 3 and 4 have a higher organic matter content, available P and clay concentration than Soils 1 and 2 (Table 1).

Table 2 records data from plants growing in Soil 1. Mycorrhizal inoculation was a critical factor for plant growth and P uptake in this soil. In fact, lavender plants did not increase shoot dry weight at all in P applications, showing an absolute mycorrhizal dependency in this soil. Addition of a P fertilizer at doses which produce up to 32 ppm of available P in soil solution did not interfere significantly with AM formation. Increasing levels of soluble P and AM fungi resulted in improved plant growth and P acquisition. Root phosphatase activity slightly increased in AM-inoculated plants and also slightly decreased with increasing P additions.

In Soils 2, 3 and 4 (Table 2), lavender plants responded to soluble P, AM inoculation and the combined P and AM treatment. Doses of P which give soil solution P values in the common ranges found in natural soils (5–30 ppm Olsen P, Hayman, 1975) did not have adverse influence on AM colonization. The highest doses of P applied decreased the level of AM colonization. However, lavender plants showed a considerable degree of mycorrhizal dependency in these soils, since even the P doses which produced the highest effect on plant growth and P uptake were less effective than AM inoculation at P_0 level (no P addition). In Soil 2 (Table 2), both AM inoculation and P additions induced a slight decrease in root phosphatase activity. In Soils 3 and 4 (Table 2), absolute control plants (no P application, no AM inoculation) produced the highest levels of acid phosphatase activity. For each soil, the rest of the treatments, either P additions or AM inoculation, induced a similar root phosphatase activity, but it was not possible to establish any generalizable relationship between P concentration in shoot tissues and phosphatase activity (Table 2).

Results from Experiment 2 are shown in Figs. 1 and 2. It was found that mycorrhizal inoculation was a critical factor for dry matter production and P accumulation in shoot tissues. The effect on these parameters of increasing amounts of P, either with or without AM inoculation, reached a "plateau" but

following a different pattern in each soil. Thereby, at P levels far higher than those present in natural soils, AM inoculation and P application produced plants with similar biomass and P content in Soil 3, whereas in Soil 4, the most effective P treatment did not produce the level of response that the least effective AM treatment (AM inoculation in P_0 plants) did. Doses of phosphate which led to available P levels in soil up to 130 ppm produced no negative effects on either plant growth or P acquisition in both non-mycorrhizal and AM-inoculated plants. This is applicable to both shoots (Fig. 1) and roots (data not shown).

Fig. 1 also shows that even supraoptimal P concentrations, which, in fact, are not natural, did not interfere AM formation in Soil 4, whereas they did in Soil 3. The highest levels of phosphatase activity were found in non-mycorrhizal non-P-added, but P additions depressed such activity in both soils (Fig. 1).

Data from nutrient concentration analyses of shoot material from plant growing in Soils 3 and 4 are

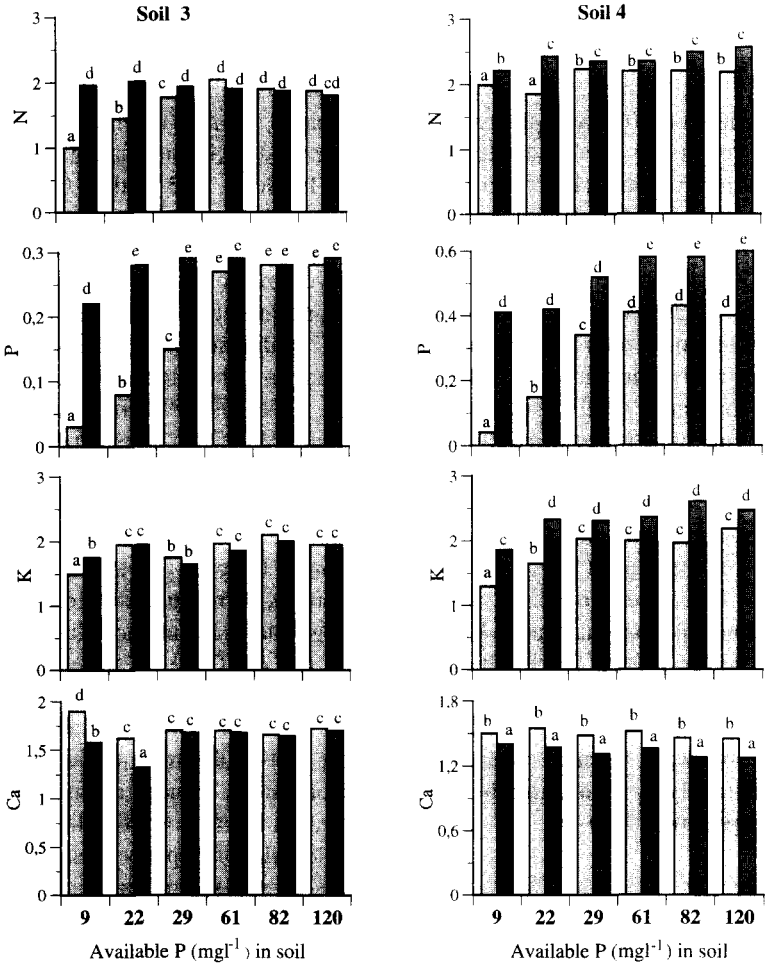

Fig. 1. Growth, phosphorus nutrition, phosphatase activity and mycorrhizal colonization of lavender plants growing, either nonmycorrhizal (□) or mycorrhizal (■), for 16 weeks in Soils 3 and 4 under different phosphate levels. Phosphatase activity as μg *p*-nitrophenol per gramme fresh root tissue per hour. AM colonization as percentage root length mycorrhizal. Data are given on a per pot (per plant) basis. For each parameter, mean values (five replicates) not sharing a letter differ significantly ($P < 0.05$).

recorded in Fig. 2. The most relevant information could be summarized as follows: (i) the concentration of N and P in shoot tissues was higher in AM plants than in the corresponding non-mycorrhizal controls at the lower three levels of P-addition in Soil 3 and at all levels of phosphate addition in Soil 4; (ii) for plants growing in Soil 4, the concentration of K was higher, and that of Ca was lower in AM plants than in their non-mycorrhizal controls, at all levels of P addition.

In Soil 3, however, these effects of AM inoculation in increasing the concentration of K and in decreasing that of Ca were evident only at the lower level of P addition (Fig. 2). The concentrations of Mg in shoot tissues (data not shown in Fig. 2) ranged from 0.42 to 0.60% in Soil 3, and from 0.32 to 0.41% in Soil 4, but there were no significant differences between the effects of any of the treatments.

4. Discussion

The category of "obligatorily mycorrhizal" for a given plant species implies that individuals from such species must be mycorrhizal for optimal growth,

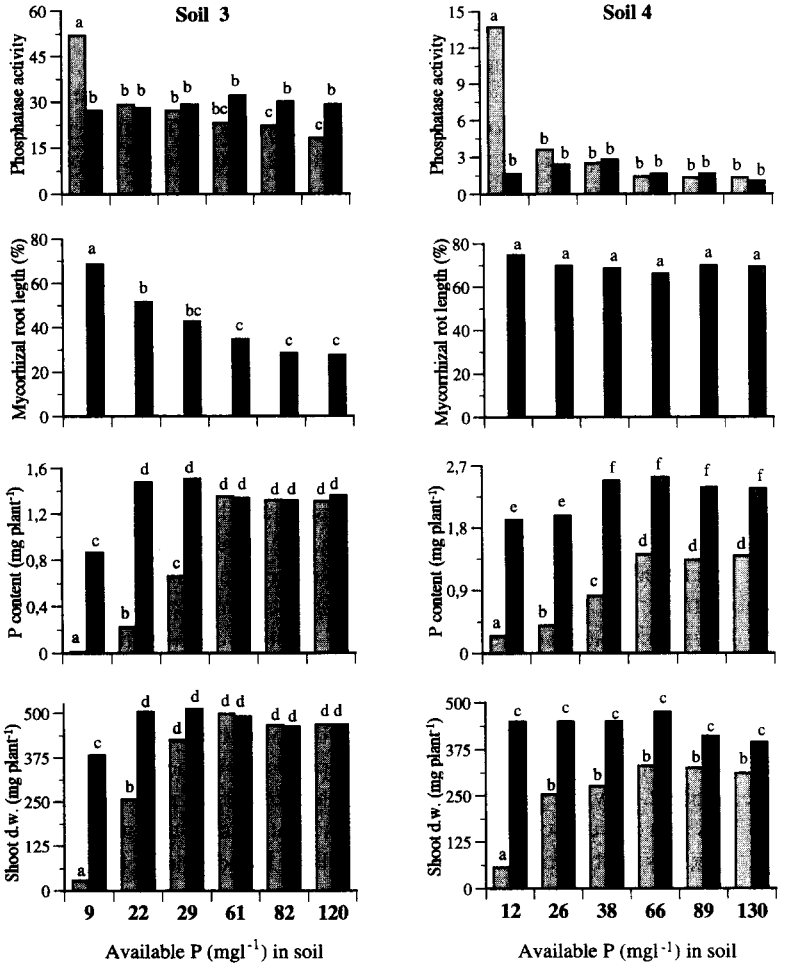

Fig. 2. Nutrient concentration in lavender plants growing, either non-mycorrhizal (□) or mycorrhizal (■), for 16 weeks in soils 3 and 4 under different phosphate levels. Data are given on a per pot (per plant) basis. For each parameter, mean values (five replicates) not sharing a letter differ significantly ($P < 0.05$).

reproduction or fitness under the prevailing fertility conditions of their natural habitats (Brundrett, 1991). Accordingly, our results support that lavender plants must be catalogued as obligatory mycorrhizal (Brundrett, 1991) or as "highly dependent on mycorhiza" (Habte and Manjunath, 1991). In fact, AM formation was critical for plant growth and P uptake at the range of available P concentrations usually found in soils from natural ecosystems (Hayman, 1975). Moreover, AM inoculation was even more effective than additions of P fertilizers which are commonly applied in agriculture to guarantee P supply from soil to crop plants, and which are far higher than those found in natural soil-plant systems (Hayman, 1975).

That lavender plants are very highly mycorrhiza-dependent was particularly apparent when the mycorrhizal response of plants was compared over a very wide range of P concentrations in soil solution. The effect of AM inoculation was still significant at very high P concentrations in the soil solution (Soil 4) or, at least, the AM status did not induce negative effects on plant growth at such high levels of available P in soil solution (Soil 3) which agrees with the findings of Manjunath and Habte (1992).

As it was also found for an obligatorily mycorrhizal *Citrus* ecotype (Roldán, 1985), lavender plants accumulated high P concentrations in their tissues, and these did not have an adverse influence on AM colonization (Soil 4), or induce a decrease except at high P concentrations (Soil 3). The concepts of nutrient supply, nutrient demand and plant response to AM symbiosis, as discussed by Koide (1991), would gain new insights from the information obtained on highly AM-dependent plant species. Apparently, these plants can support concentrations of P in their tissues which do not produce negative effects on plant growth. Koide (1991) considered the case of P accumulation, and stated that when the P supply exceeds P demand, the nutrient may be stored to be subsequently utilized. This can have a considerable ecological importance, which is particularly relevant in the case of lavender. The experimental approaches followed in our study do not allow us, however, to distinguish the form(s) and systems(s) for such P storage in lavender plants.

The effect of AM fungi in improving N uptake from soil, and in decreasing Ca acquisition from the test calcareous soils, as found in this study corroborate previous findings (Azcón and Barea, 1992; Barea et al., 1992; Tobar et al., 1994a,b).

Acid phosphatase activity associated with the roots of lavender plants was clearly shown. This corroborated preliminary observations with this species (Azcón et al., 1982). The specific activity of root acid phosphatase in lavender was enhanced in P-deficient plants, being inversely related to extractable inorganic P from soil, which corroborates previous studies with other plant species (McArthur and Knowles, 1993; Khalil et al., 1994). It is obvious that the role of phosphatases in soil would depend on the concentration and chemical status of soil P (Dodd et al., 1987), particularly organic phosphates, which are the natural substrate for such an enzymatic activity (Tarafdar and Marschner, 1994). Clearly, the organic matter content in the test soils used in our study is very low (Soils 1 and 2), or moderately low (Soils 3 and 4). This could explain how lavender plants showing high values of phosphatase activity still grew poorly, and an apparent deficiency of available P supply was evident. This agrees with findings of Alexander and Hardy (1981) suggesting that a high phosphatase activity does not compensate for an inadequate supply of assimilable P to the plant.

Besides the lack of adequate amounts of the natural substrate for acid phosphatases, is difficult to expect an improvement of plant performance when soils are deficient in other nutrients as well as P. Consequently, the role of AM symbiosis in increasing the uptake of not only P but also of other nutrients, particular N, must be critical to guarantee plant performance in such nutrient-deficient habitats.

Another controversial topic is the relationship between root phosphatase and AM formation. Khalil et al. (1994) found a positive correlation between these two biological factors but the level of the correlation depended on the plant species tested. Mycorrhizal colonization decreased phosphatase activity in lavender roots, but it had no effect on wheat (Azcón et al., 1982). Dodd et al. (1987) found an increase in root phosphatase by AM inoculation with two out of the three fungal species tested, therefore they suggest that the isolate of fungus could determine the phosphatase response. A positive effect of AM on root phosphatase was also described as being evident only at low P levels (McArthur and Knowles, 1993).

The consequences of the mycorrhizal dependency of lavender plants with regards to a revegetation strategy are obvious. These plants must be mycorrhizal to thrive in degraded nutrient-poor soils. In a particular revegetation programme currently being developed (Barea et al., 1996), and because the AM inoculum potential is not sufficient in the experimental area (Requena et al., 1996), it must be advisable to transplant pre-inoculated seedlings. This is currently done and lavender plants are being managed, together with other small woody legumes of the natural succession, to try to restore a self-sustaining vegetation cover to improve soil quality, and to combat desertification.

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