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Effect of native and introduced arbuscular mycorrhizal fungi on growth and nutrient uptake of *Lygeum spartum* and *Anthyllis cytisoides*

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Abstract

The interaction between native and introduced fungi and their effect on plant growth and mineral uptake were studied. The host plants were *Lygeum spartum* and *Anthyllis cytisoides*, the introduced fungus was *Glomus fasciculatum*. The four soils used were selected from disturbed and contaminated by mining activities areas. Inoculated and uninoculated plants were grown in the unsterilized and sterilized soils (with and withouth native microflora, respectively). Plants inoculated with *G. fasciculatum* were higher and had higher tissue P concentration than uninoculated plants, especially in *A. cytisoides*. However, this inoculation was not effective in unsterilized substrates, suggesting a competition between introduced and native fungi. Concentration of mineral elements other than P varied depending on the host plant and soil. Decrease in Fe, Cu, Mn, Zn and Pb was observed in mycorrhizal *A. cytiosides* plants and a slight increase in Zn concentration was noted in mycorrhizal *L. spartum* plants. The study showed that the type of soil and their populations of native endophytes have a considerable effect on plant response to mycorrhizal symbiosis, especially in disturbed soils.

Key words: copper, *Glomus fasciculatum*, iron, lead, manganese, phosphorus, zinc

Introduction

Many studies have pointed to the importance of arbuscular mycorrhizal fungi in plant growth enhancement and stimulation of P uptake (Hayman 1983), specially in nutrient deficient or degraded soils. The effect of mycorrhizae on other mineral elements is not so clear and the differences mentioned in the literature can be explained by the differences in plant species, fungi species and soils, sterilized or not. Most studies which asses the beneficial effects of mycorrhizae on plant growth have been carried out in previously sterilized soil which is therefore bereft of microflora. However, the benefits to be obtained from inoculations of selected fungi

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depend also on microflora interaction. Our objectives were *1*) to study the interaction between a selected fungus and the natives ones in soils from degraded areas and *2*) to study their effect on plant growth and mineral absorption.

Materials and methods

The experiment described herein used four soils, two treatments of sterilization, two treatments of inoculation with arbuscular fungi and two plant species.

Table 1. Physical and chemical characteristics of the soils.

Parameter	Soil 1	2	3	4
pH 7.75	7.75	7.48	7.62	7.79
Organic matter [%]	3.86	1.69	0.53	0.53
Electrical conductivity [ds m^{-1}]	0.39	0.28	2.86	6.54
Available P [mg kg^{-1}]	5.89	4.03	0.62	2.09
Available K [mg kg^{-1}]	641.20	281.50	43.10	238.50
Total N [mg kg^{-1}]	2 500.00	680.00	320.00	470.00
Na [mg kg^{-1}]	80.50	59.80	69.00	3243.00
Cl [mol kg^{-1}]	0.0035	0.0025	0.0033	0.1750
SO_4 [mol kg^{-1}]	0.0047	0.0064	0.0756	0.0715
Available Fe [mg kg^{-1}]	1.95	5.24	5.60	4.26
Available Cu [mg kg^{-1}]	4.50	2.70	1.60	1.40
Available Mn [mg kg^{-1}]	7.40	45.80	5.70	2.10
DTPA extractable Zn [mg kg^{-1}]	57.30	142.70	62.70	236.70
DTPA extractable Pb [mg kg^{-1}]	233.00	264.00	311.00	368.00

Four soils were selected from La Unión, Murcia, Spain, where mining activities have led to vast areas of disturbed and contaminated soil. Physical and chemical characteristics of the soils were determined (Table 1). Large samples of soil were collected and passed through a 4 mm mesh sieve. Soil infectivity was previously determined by a bioassay (Díaz and Honrubia 1990, 1993a). Half of each soil sample was sterilized (100 °C for 1 h on three consecutive days) to kill the native endophytes, while the other half remained unsterilized. The soil was then mixed with autoclaved sand in the proportion 1 : 1 (v/v). Pots were filled with approximately 600 cm^3 of sterilized or unsterilized soil. Natural soil sievings without AM propagules were added to pots with sterilized soil to provide the natural microflora.

Half of the pots were inoculated with 10 g of an inoculum (mycorrhizal roots and rizospheric soil) of *Glomus fasciculatum* (Thaxter ss Gerdemann) Gerdemann and Trappe, provided by Estación Experimental del Zaidín, Granada, Spain and multiplied on *Medicago sativa* pot culture. This fungus was selected because of its high infectivity and the efficiency demonstrated in previous experiments (Díaz *et al.* 1992, Díaz and Honrubia 1993b). The other half of the pots remained uninoculated.

The plants used were *Lygeum spartum* L. (Poaceae) and *Anthyllis cytisoides* L. (Leguminosae), which are both highly susceptible to AM colonization and grow naturally in the soils selected (López-Sanchez *et al.* 1992, Díaz and Honrubia 1994). Before sowing, the *L. spartum* seeds were sterilized (H_2O_2 10 vol. for 30 min.) and kept in imbibition for 12 h. The seeds of *A. cytisoides* were scarified by a mechanical process which took off all the protecting covers. Several seeds per pot were sown and thinned to 2 per pot after germination.

Five replicates per treatment (total 80 pots for each plant species) were established and placed in a randomized complete block design in the greenhouse. After 16 weeks, the plants were harvested, and height, shoot and root dry mass (80 °C, 16 h) determined. Mycorrhizal colonization was estimated by the gridline intersect method (Giovannetti and Mosse 1980) on stained root samples (Phillips and Hayman 1970). All the shoots in a treatment were mixed and a subsample was digested in nitric-perchloric acid mixture (5:3) for 6 h. Phosphorus was determined by spectrophotometry (660 nm; malachite green reagent; Fernandez *et al.* 1985) and other elements by atomic absorption spectrophotometry (*Perkin-Elmer 1100 B*). Some *A. cytisoides* plants in unsterilized treatments were lost during the experiments, so there was not enough plant material for mineral determinations. Data on height, dry mass and colonization were subjected to analysis of variance, followed by a Duncan's multiple range test for means separation.

Results

Responses to mycorrhization differed according to soil, sterilization treatment and plant species (Table 2). In sterilized soil (without native endophytes) inoculation with *G. fasciculatum* stimulated significantly plant growth except in *L. spartum* grown in soil 4. Growth enhancement was higher in *A. cytisoides*: The aerial biomass of inoculated plants was 3 - 4 times higher than that of uninoculated ones, showing the substantial benefits gained with this fungus and the dependence of this plant on mycorrhizae. In unsterilized control treatments, native fungi were totally ineffective in promoting plant growth, although they showed capacity of infection. However, in unsterilized inoculated soils, no response to inoculation with the introduced fungus was observed, except in soil 2 for *L. spartum* and soil 3.

Inoculation led to an increase in P concentrations (Table 3), specially in sterilized soils. In *L. spartum*, Fe, Mn and Pb levels found in plants seem to depend on their concentration in the soil, with a slight diminution in Mn being induced by inoculation. On the other hand, inoculation increased the tissue concentration of Zn although the effect was less noticeable in soils where the effect on growth was also less (soil 4). In *A. cytisoides*, the concentration of other elements fell in general due to the formation of mycorrhizae. The levels found in the soils and plants were also related.

Table 2. Effect of native and introduced mycorrhizal fungi on plant growth and mycorrhizal colonization of *Lygeum spartum* and *Anthyllis cytisoides* grown in four different soils.

Soil	Treatment		*Lygeum spartum*				*Anthyllis cytisoides*			
			Height [cm]	Dry mass [mg plant^{-1}]		AM colonization [%]	Height [cm]	Dry mass [mg plant^{-1}]		AM colonization [%]
				shoot	root			shoot	root	
1	sterilized	C	17.8 a	66 a	79 a	0 a	3.1 a	17 a	6 a	0 a
		G.f.	34.8 b	171 b	162 b	34 b	7.3 b	68 b	15 b	65 c
	unsterilized	C	20.4 a	50 a	54 a	29 b	3.7 a	18 a	6 a	45 bc
		G.f.	21.8 a	58 a	55 a	39 b	3.2 a	20 a	6 a	25 b
2	sterilized	C	17.6 a	86 a	109 a	0 a	3.8 a	19 a	8 a	0 a
		G.f	34.2 b	126 b	131 a	37 b	7.3 b	85 b	33 b	53 c
	unsterilized	C	21.4 a	109 ab	140 a	23 b	4.1 a	21 a	9 a	39 b
		G.f.	27.8 ab	142 b	169 a	29 b	3.4 a	22 a	9 a	30 b
3	sterilized	C	18.0 a	68 a	103 a	0 a	5.0 a	22 a	11 a	0 a
		G.f.	25.3 b	131 b	137 a	45 b	5.5 a	63 b	32 b	55 b
	unsterilized	C	26.4 b	123 ab	156 ab	44 b	4.7 a	29 a	8 a	41 b
		G.f.	30.1 b	148 b	218 b	40 b	6.4 a	62 b	22 ab	52 b
4	sterilized	C	18.6 a	114 a	180 a	0 a	2.8 a	15 a	7 a	0 a
		G.f.	31.2 b	170 a	155 a	31 b	5.1 b	49 b	26 b	57 c
	unsterilized	C	33.4 b	165 a	191 a	49 b	3.4 a	14 a	4 a	25 b
		G.f.	29.2 b	153 a	212 a	30 b	4.2 b	16 a	7 a	12 b

C - control; *G.f.* - inoculated with *Glomus fasciculatum*.
Data are mean of five replicates. Values in a column within one soil and followed by the same letter are not significantly ($P < 0.05$) different as determined by Duncan's multiple range test.

Table 3. Effect of native and introduced mycorrhizal fungi on P, Fe, Cu, Mn, Zn and Pb concentrations [mg kg^{-1}] in *Lygeum spartum* and *Anthyllis cytisoides* grown in four different soils. In *Lygeum spartum* Cu was not detected at the level of precision used.

Soil	Treatment		*Lygeum spartum*					*Anthyllis cytisoides*					
			P	Fe	Mn	Zn	Pb	P	Fe	Cu	Mn	Zn	Pb
1	sterilized	C	231	188	69	80	0	261	194	26	178	234	80
		G.f.	509	118	56	205	0	580	35	16	70	68	14
	unsterilized	C	460	140	69	76	0	430	-	-	-	-	-
		G.f.	477	128	55	112	0	336	-	-	-	-	-
2	sterilized	C	259	190	87	188	0	341	105	15	279	230	65
		G.f.	553	160	90	269	0	462	30	10	88	138	17
	unsterilized	C	284	167	85	182	0	272	152	20	268	240	75
		G.f.	576	190	75	296	0	319	157	18	186	158	56
3	sterilized	C	265	390	56	63	22	318	205	15	88	163	125
		G.f.	626	382	53	110	25	670	58	9	55	130	18
	unsterilized	C	531	302	54	119	20	469	-	-	-	-	-
		G.f.	584	326	60	145	20	660	41	8	43	116	25
4	sterilized	C	274	164	37	142	11	310	259	30	75	538	116
		G.f.	481	183	32	177	12	694	30	8	53	437	10
	unsterilized	C	520	200	31	170	16	475	-	-	-	-	-
		G.f.	579	147	31	205	13	410	45	12	65	530	110

C - control; *G.f.* - inoculated with *Glomus fasciculatum*.

Discussion

The study reveals that the type of soil and their populations of native endophytes have a considerable effect on plant response to mycorrhizal symbioses.

A cytisoides, a mycorrhizae dependent-plant (Díaz *et al.* 1992, López-Sanchez *et al.* 1992), benefits from inoculation in all the sterilized soils assesed. However, the formation of efficient mycorrhizae by *L. spartum* depend very much on soil. For example, in soil 4, where it grows naturally and is well adapted to adverse conditions (salinity, seasonal flooding, *etc.*) its capacity for mycorrhizal symbiosis does not result in any increase in biomass production. It is well known that non mycorrhizal, facultative and not very dependent plants are usually the first to colonize degraded ecosystems (Trappe 1987, Allen and Allen 1990) and this might explain the abundance of this plant in the most degraded areas, where it is the predominant species. These phenomena of dependence and facultativeness are very important when considering changes in vegetation since they can affect plant succession patterns in disturbed ecosystems such as mine wastes or volcanic substrates (Gemma and Koske 1990).

The native population of fungi can also affect plant growth. In our study, the native endophytes, despite its infectivity, only showed a slight stimulation of *L. spartum* in some soils and were incapable of stimulating *A. cytisoides* growth. Therefore it would be advisable to introduce more effective AM fungi in the soils assesed by inoculation. Such an inoculation with *G. fasciculatum* was effective with the sterilized substrates, although this fungus, which was chosen for its high degree of infectivity and effectiveness in sterile substrates was unable to carry out its beneficial role in unsterilized soils.

The ineffectiveness of inoculation in unsterilized substrates has already been noted (Hetrick *et al.* 1986, Gianinazzi *et al.* 1989, Hetrick and Wilson 1991), some authors explaining this fact by the action of soil microbiota. The germination of fungi spores (Hetrick and Wilson 1989), sporulation (Kitt *et al.* 1987), P uptake (Kitt *et al.* 1988), root colonization (Hetrick *et al.* 1988b, 1990), and plant growth response (Kitt *et al.* 1988) have all been seen decrease in the presence of native soil microbiota, particularly in soils of low fertility. Hetrick *et al.* (1986, 1988a) point to the competition for nutrients between AM fungi and other soil microorganisms as a possible cause of supresssion of mycorrhizal response. Howewer, this does not entirely explain our findings, since the native microflora was reinoculated in the preparation of the substrates for the experiments although we cannot be sure that it was incorporated in the same amounts and with the same activity as would be present in natural soil.

Perhaps, too, during the sterilizing process the physico-chemical characterictics of the soil were affected, for example by the liberation of some nutrients which favour mycorrhizal action. It has also been suggested that the supression of mycorrhizal response might be related with soil fertility since the addition of P overcomes the suppression (Hetrick *et al.* 1988b). Other hypotheses suggest the involvement of other nutrients (Kitt *et al.* 1988), the reduction of mycorrhizal response being related

with high Al, Mn and NH_4 concentrations and low levels of Na, Mg and organic matter, although the mechanisms by wich this might occur are not clear.

It seems more likely that the ineffectiveness of the introduced fungus in unsterilized soil is due to competition with other mycorrhizal fungi. In natural soils, the growth of *A. cytisoides* was only stimulated by inoculation in soil 3. In this case, unlike in the others, neither the degree of infection nor the P concentration in the unsterilized inoculated treatment was less than that found in the sterilized inoculated one. This leads to the conclusion that the infection produced by the native fungi (albeit at a low level) in the other three soils interferes with *G. fasciculatum* root colonization and consequently with the translocation of P to the plant cells. Competition phenomena between native and introduced endophytes also seem to arise in the case of *L. spartum*. In soil 1, the native (ineffective) endophytes seem to have a stronger competitive capacity than the introduced (effective) endophytes, for which reason there is no response to inoculation in the unsterilized soil. In soils 2 and 3, on the other hand, the native endophytes (of average effectiveness) cooperate with the introduced (more effective) endophytes in the promotion of plant growth. Hastly, in soil 4, neither the native nor the introduced AM fungi seems to be very effective in promoting *L. spartum* growth.

Little is known about the mechanism involved in competition phenomena between different AM fungi and more research is needed in this subject. This problem could be overcome by the selection of plant mutants which only form mycorrhizae with certain specific fungi (Gianinazzi 1991) that avoid competition with other fungi.

As regards the uptake of nutrients and other mineral elements, the results also varied according to plant species and substrate.

A clear positive effect on P uptake by the mycorrhizae was seen, probably because this nutrient was deficient in all the soils studied. It has been widely demonstrated that AM stimulates P uptake by plants (Hayman 1983) mainly in deficience conditions and this seem to be the principal cause of plant growth increase.

Several studies have shown an increase in the tissue concentrations of nutrients other than P as a consequence of mycorrhizae formation (Pacovsky 1986, Kothari *et al.* 1990, Thomson 1990). Our results point to a variability between plant species, suggesting that an increase in the uptake of certain nutrients will not necessarly occur in all situations. As has been suggested by Plenchette *et al.* (1983), the increased absorption of a certain mineral might simply be related with the specific plant's requirement for this nutrient. *A. cytisoides* (Table 3) showed a clear decrease in the concentration of heavy metals (Fe, Cu, Mn, Zn and Pb) in the tissue of mycorrhized plants. This has been described in similar previous studies, some in deficiency conditions (Pacovsky 1986) but most in conditions of toxicity (Dehn and Schüepp 1989, Heggo *et al.* 1990), as occur in the soils studied for Zn and Pb due to mine contamination. The fall in the level of these elements in mycorrhized *A. cytisoides* plants might be due to a dilution effect caused by growth as Plenchette *et al.* (1983) suggested: When a plant increases in size, mineral concentration disminishes so that the total quantity of an element is similar in both infected and uninfected plants. In *L. spartum* the pattern is not so clear and the levels seems to vary according to the element in question and the soil more than to the mycorrhizal treatment, probably

due to the fact that *L. spartum* is less dependent on mycorrhizae than *A. cytisoides.* Only it is important to point out the general increase in Zn absorption.

The results of this study reveal the importance of experimenting with inoculations in unsterilized substrates and bearing in mind the possible interaction between native and introduced fungi, especially in disturbed soils where the fungus chosen might not be competitive against native endophytes.

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