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Effectiveness of indigenous arbuscular mycorrhizal fungi (AMF) isolated from hydrocarbon polluted soils

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Five different species of arbuscular mycorrhizal fungi (AMF), three of which were isolated from hydrocarbon polluted soils (*Glomus deserticola, G. geosporum* and *G. intraradices)* and two laboratory strains *(G. fasciculatum* and *G. mosseae)*, were screened for symbiotic response with *Medicago sativa* L. (alfalfa) under greenhouse conditions in a hydrocarbon polluted substrate. Four of the 5 treatments were found to improve plant height and shoot biomass: *G. deserticola* isolated from gas-oil polluted soil from Mar de Ajó, *G. geosporum* isolated from fuel-oil polluted soil from Berisso, *G. intraradices* isolated from crude-oil polluted soil from Ensenada (Argentina), and *Glomus fasciculatum* (laboratory culture). A significant increase of phosporous and zinc content was only found in shoots and roots after treatment with AMF isolated from polluted areas.

The mutual association between higher plants and arbuscular mycorrhizal fungi (AMF) improves not only plant growth, particularly under unfavourable growth conditions (DEHNE 1987, SIEVERDING 1981, WEISSENHORN *et al.* 1993, 1995) by supplementing the nutrient absorbing capacity of the plant root system (HAYMAN 1983), but also seedling survival and growth. Plants and their associated soil microorganisms are important components in revegetation of disturbed and toxic environments because they control formation of soil structure and contribute to nutrient availability (ELLIOT and Coleman 1988, JASTROW and MILLER 1991).

KHAN (1981), LINDSEY *et al.* (1977) and STAHL *et al.* (1988) showed that indigenous AMF used as inoculants could be useful to reclaim mine spoils as they have a positive effect on plant growth. However, little attention has been paid to the role of AMF in oil polluted soils (CALL and MCKELL 1982, 1984, CABELLO 1995, 1997). Indigenous AMF communities generally contain several fungal species. The propagule number in hydrocarbon polluted soils is high (CABELLO 1997) suggesting that the status of plants growing in those soils is dependent on the level of infective propagules. The number of indigenous AMF propagules is not related to the effectiveness of indigenous AMF populations, which are dependent on multiple factors such as soil nutritional status, host plant, AMF propagule density, effectiveness of AMF species, and competition between them and other soil microorganisms.

The aim of the present work was to study the effectiveness of different arbuscular mycorrhizal fungi isolated from hydrocarbon polluted areas, and that of two species of *Glomus* taken as laboratory controls, on the growth and phosphorous and zinc contents of shoots and roots of *Medicago sativa* L. (alfalfa) to assess the potential of strains for field inoculation.

Materials and methods

The soil used in the experiment was sampled in Estancia Chica, in the surroundings of La Plata city (Argentina) with no record of heavy oil pollution and a balanced nutrient status (organic matter: 59%; C: 3.24%; N: 0.252%; P: 8.1 ppm; K: 1.15 meq 100 g^{-1}; Ca: 15.1 meq 100 g^{-1}; Mg: 1.08 meq 100 g^{-1}; Na: 0.36 meq 100 g^{-1}).

The soil was air-dried (approx. 1 week), powdered and sieved through a 2-mm-mesh sieve and then subjected to steam-sterilization (1-h at 120 °C repeated three times after a 24-h interval). The bulk soil was then contaminated with 5% crude-oil obtained from La Plata YPF oil refinery and thoroughly homogeneized. This soil was used as substrate for plant growth.

As in a previous study (CABELLO 1997) alfalfa *(Medicago sativa)* was used as experimental plant. Two-day-old surface-sterilized seedlings of alfalfa, were transplanted onto 6 separated nurseries which contained 5 different kinds of AMF inocula and a non-inoculated control.

The following treatments were carried out:

a) control, without AMF inoculum, b) *Glomus deserticola* TRAPPE, BLOSS & MENGE isolated from gas-oil polluted soil from Mar de Ajó (Argentina), c) *Glomus fasciculatum* (THAXTER) GERDEMANN & TRAPPE emend. WALKER & KOSKE, d) *Glomus geosporum* (NICOLSON & GERDEMANN) WALKER isolated from fuel-oil polluted soil from Berisso (Argentina), e) *Glomus intraradices* SCHENCK & SMITH isolated from crude-oil polluted soils from Ensenada (Argentina) and f) *Glomus mosseae* (NICOLSON & GERDEMANN) GERDEMANN & TRAPPE.

AMF strains used in treatments b, d and e were isolated using the trap culture method from soils with a long record of pollution (12, 10 and 9 years, respectively).

G. fasciculatum and *G. mosseae* had been tested in a previous screening of hydrocarbon polluted soils (CABELLO 1995), and they were used as laboratory culture control.

Inocula consisted of rhizospheric soil from alfalfa plant pot culture which contained spores, mycelia and colonized root fragments. These 5 inocula were spread on the nursery beds at a rate of 3,000 infective propagules per 100 g dry soil, based on the most probable number estimation (PORTER 1979).

After 45 days, healthy seedlings of uniform size were transplanted from each treatment in pots containing 1.2 kg of hydrocarbon polluted substrate. There were 10 replicate pots per treatment where 1 plant per pot was grown in a greenhouse at 24 ± 1 °C day/20 ± 1 °C night, and 16-h photoperiod provided by halogen lamps for 60 days.

During growth experiments, plants were watered from below using a capillary system and fed with nutrient solution (CABELLO 1997).

After 60 days the plants were harvested. Growth of alfalfa plants belonging to different treatments was evaluated using the following parameters: plant height (cm); shoot dry weight (g), which were used to estimate the mycorrhizal effect on plant growth; tiller number $plant^{-1}$; root length (cm); root dry weight (g); shoot and root P and Zn content.

To determine AM colonization, roots were cleared and stained (PHILLIPS & HAYMAN 1970), and the proportion of colonization of total root length was measured by GIOVANNETTI and MOSSE's grindline intersection method (GIOVANNETTI & MOSSE 1980) (200 grindline intersections per sample). The percentage of arbuscles, number of vesicles and number of entry points were measured as described by OCAMPO *et al.* (1980).

Statistical analysis of data was performed by using the Least Significant Difference (LSD) test.

Results and discussion

Microscopic observations of stained roots are shown in Table 1. Stained roots from control pots showed no presence of fungi. The percentage of colonized root and arbuscles, and the number of vesicles and entry points were not significantly different among the 5 AMF treatments.

After 60 days, only alfalfa inoculated with AMF showed a significant increase in all the parametres analysed (Table 2) except for root length.

Alfalfa varied its response to inoculation with different arbuscular mycorrhizal fungi. The influence of indigenous AMF isolated from hydrocarbon polluted areas, *G. fasciculatum* and *G. mosseae* on alfalfa growth is presented in Figures 1 and 2. The AMF isolated from soils with different pollutants and *G. fasciculatum* had a significant effect on the plant height (Fig. 1) while shoot dry mass was significantly increased by *G. geosporum* isolated from fuel oil polluted soil and in a lesser degree by *G. deserticola* isolated from gas oil polluted soil and *G. fasciculatum*, an AMF laboratory culture (Fig. 2).

Table 1
Effect of different AMF on the percentage root length colonization, arbuscles, vesicles and entry points. Data are the means of 10 replicates.

	% colonized root	% arbuscles	Vesicle number	Entry point number
Uninoculated	–	–	–	–
Glomus deserticola	24.66 a	8.42 a	31 a	0.28 a
Glomus fasciculatum	36.62 a	11.92 a	68 a	0.42 a
Glomus geosporum	37.46 a	4.07 a	90.33 a	0.62 a
Glomus intraradices	31.43 a	2.34 a	50.33 a	0.59 a
Glomus mosseae	44.46 a	7.13 a	35.33 a	0.79 a

Means in the same column followed by the same letter are not significantly different (LSD, P = 0.01)

Table 2
Effect of different AMF on the different parametres evaluated in alfalfa. Data are the means of 10 replicates

	Plant height (cm)	Tiller number plant^{-1}	leaf number plant^{-1}	Dry weight shoot (g) plant^{-1}	Total length root (cm)	Dry weight root (g) plant^{-1}
Unicoculated	10.31 a	0.66 a	4.25 a	0.031 a	13.62 a	0.029 a
Glomus deserticola	19.75 b	2.55 b	25.62 b	0.145 ab	17 a	0.21 b
Relation M:NM*	1.9	3.9	6	4.7	1.2	7.2
Glomus fasciculatum	17 ab	2.33 ab	20.5 ab	0.191 ab	20.37 a	0.132 ab
Relation M:NM	1.6	3.5	4.8	6.16	1.5	4.5
Glomus geosporum	22.37 b	2.22 ab	20.25 ab	0.25 b	18.12 a	0.173 b
Relation M:NM	2.16	3.4	4.8	8	1.3	6
Glomus intraradices	17.5 ab	2.66 b	25.62 b	0.096 ab	18.50 a	0.140 ab
Relation M:NM	1.7	4	6	3	1.4	4.8
Glomus mosseae	14 ab	2.66 b	17.37 ab	0.133 ab	15.75 a	0.115 ab
Relation M:NM	1.4	4	4	4.3	1.2	4

Means in the same column followed by the same letter are not significantly different (LSD, P = 0.01).
* M, mycorrhizal; NM, non-mycorrhizal (Uninoculated)

These results are in agreement with those reported by CALL and MCKELL (1984) who transplanted container-grown fourwing saltbush plants inoculated with indigenous AMF into processed oil shale and disturbed native soil. They found that inoculated plants had a greater biomass, percent cover and height than non- inoculated controls.

The lack of general correlations between growth parametres and percentage root length colonization with the effectiveness of different AMF indicates that the latter could be high although the root internal fungus biomass is small. This result agrees with that of SIEVERDING (1991) who studied the efficiency of 23 AM fungal isolates on cassava growth. Both results contradict the commonly held belief in literature that a positive relationship exists between infected root length and plant growth (SANDERS *et al.* 1977). It is evident that effectiveness for yield is the result of the physiological interaction between the symbionts: host and endophyte under the stress produced by the hydrocarbon pollutant in the growth substrate.

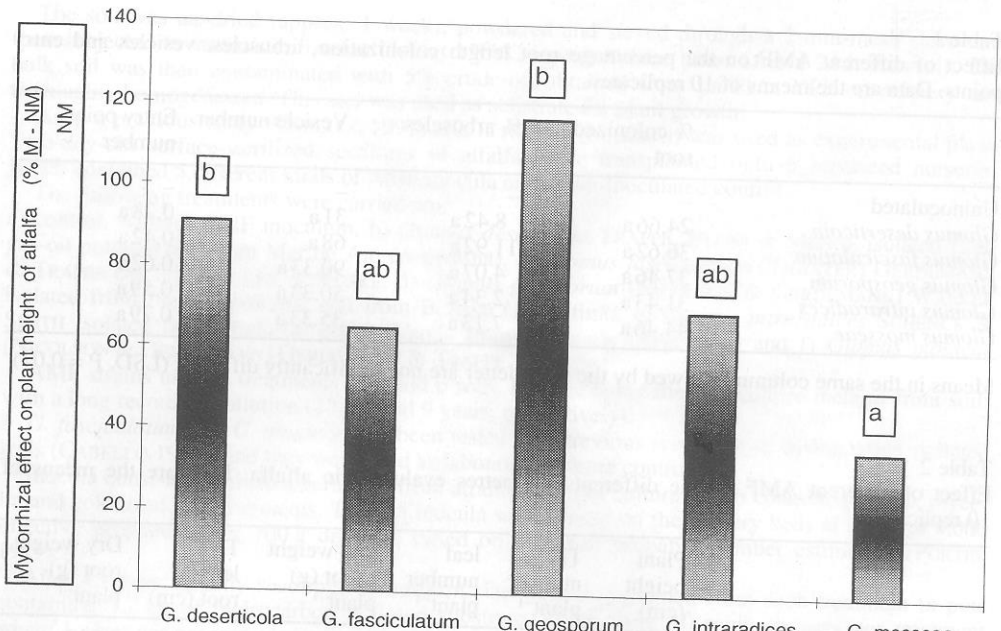

Fig. 1
Influence of mycorrhizal infection on plant height of alfalfa after 60 days growth in 5% of crude oil polluted soil.
M, mycorrhizal; NM, non-mycorrhizal.
Bars with the same letter are not significantly different (LSD, P = 0.01)

Inoculation with AMF isolated from hydrocarbon polluted soils significantly increased P content of alfalfa (Table 3). The shoot P content was higher in plants inoculated with the 3 AMF strains whereas root P content was significantly increased by *G. deserticola* from gas-oil and *G. geosporum* from fuel-oil polluted soils. On the other hand, plants inoculated with *G. fasciculatum*, *G. intraradices* and *G. mosseae* evidenced the lowest P content in roots. CALL and MCKELL (1984) found that phosphorous and water uptake was greater in plants inoculated with mycorrhizal fungi than in non-inoculated controls. These plants had been previously transplanted into processed oil shale and disturbed native soils.

Shoot and root Zn content was higher in plants inoculated with AMF strains isolated from polluted areas. In this regard, the highest value was shown in plants inoculated with *G. geosporum* isolated from fuel-oil polluted soil.

In a previous report (CABELLO 1995) *Glomus fasciculatum* and *G. mosseae* were used as inocula in field and laboratory experiments. *G. fasciculatum* was more efficient than *G. mosseae* in improving plant growth. Our present results agree with those ones. However, AM fungal strains isolated from hydrocarbon polluted soils were found to be more tolerant to pollution than laboratory reference strains. These results suggest an adaptation of indigenous AM fungal isolates to persistent toxicants in soils.

Many species of AMF occur over a broad range of habitats and environmental conditions. This fact strongly suggests that these fungi are capable of genetic differentiation at the intraspecific level (DANIELS and DUFF 1978, STAHL and SMITH 1984). Therefore, hydrocarbon-tolerant mycorrhizal species used as inocula should be considered in order to improve plants growing in field under such unfavourable conditions.

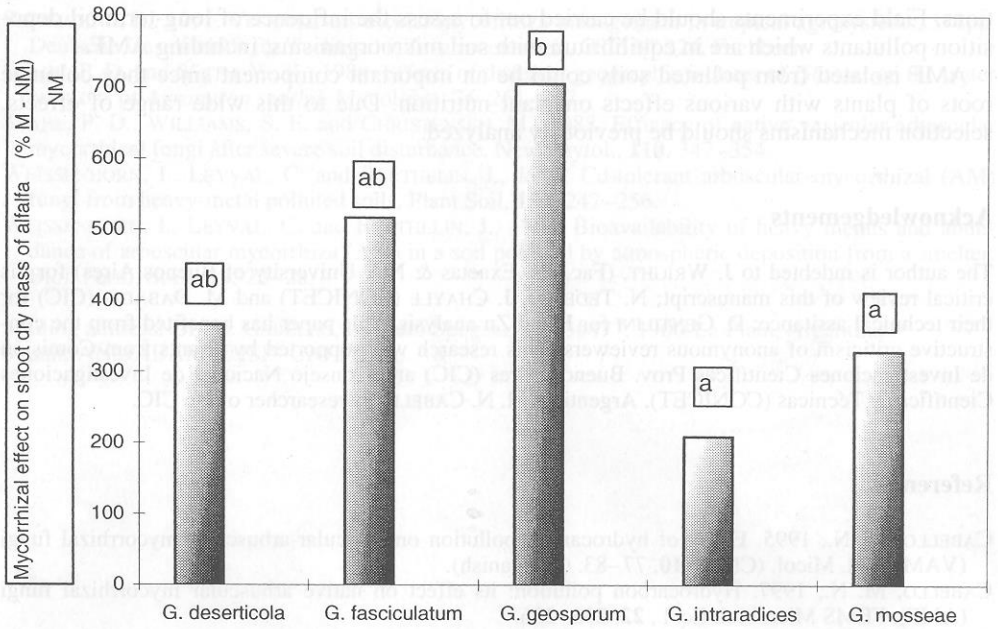

Fig. 2
Influence of mycorrhizal infection on shoot dry mass of alfalfa after 60 days growth in 5% of crude oil polluted soil.
M, mycorrhizal; NM, non-mycorrhizal.
Bars with the same letter are not significantly different (LSD, P = 0.01)

Phytoremediation as a natural process carried out by plants will give better results when plants are associated with efficient AMF isolates which can tolerate hydrocarbon pollution. Further information will be necesary to know the influence of mycorrhiza on the metabolite accumulation produced by natural degradation of hydrocarbons carried out either by soil microorganisms (biodegradation) or by plants growing in polluted soils (phytoremediation).

Further research is needed in order to achieve a more realistic approach about AMF strains isolated from hydrocarbon polluted soils and inoculated plants under field condi-

Table 3
Effect of different AMF on shoot and root P and Zn content on alfalfa

	P content mg plant^{-1}		Zn content µg plant^{-1}	
	shoot	root	shoot	root
Uninoculated	12.51 a	9.38 a	44.4 a	51 a
Glomus deserticola	17.9 b	14.11 b	60.3 b	106.7 b
Glomus fasciculatum	15 a	11.48 ab	50.9 a	58.1 ab
Glomus geosporum	18 b	17.76 b	65.2 b	158.8 c
Glomus intraradices	17.6 b	12.86 ab	54.6 ab	71.7 b
Glomus mosseae	14.27 a	11.30 ab	45.8 a	52.4 a

Values followed by the same letter are not significantly different (LSD, P = 0.01)

tions. Field experiments should be carried out to assess the influence of long-term oil deposition pollutants which are in equilibrium with soil microorganisms, including AMF.

AMF isolated from polluted soils could be an important component since they colonize roots of plants with various effects on plant nutrition. Due to this wide range of effects, selection mechanisms should be previously analyzed.

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