ELSEVIER

FEMS Microbiology Ecology 22 (1997) 233–236

FEMS MICROBIOLOGY ECOLOGY

Hydrocarbon pollution: its effect on native arbuscular mycorrhizal fungi (AMF)

Marta Noemí Cabello *

Instituto Spegazzini, 53 # 477, Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, 1900 La Plata, Argentina

Received 30 August 1996; revised 24 November 1996; accepted 28 November 1996

Abstract

Communities of arbuscular mycorrhizal fungi (AMF) present in two hydrocarbon polluted soils from Argentina and Germany were analyzed by examining stained pieces of plant roots for degree of colonization and fungal structures. The numbers of AMF infective propagules were also analyzed and trap cultures were established from rhizospheric soils. Colonization by AMF was higher in plants grown in non-polluted soils than in both polluted areas. In addition proportion of arbuscles to vesicles was higher in plants grown in non-polluted soil than in those grown in hydrocarbon-polluted soils. This was consistent with larger populations of AMF propagules with a potential to colonize roots in non-polluted soils. Nevertheless, AMF propagules from polluted soils also had a high colonization capacity. *Glomus aggregatum* and *G. mosseae* were isolated and identified from both polluted and non-polluted soils.

Keywords: Arbuscular mycorrhizal fungi; Hydrocarbon pollution; Colonization; Propagule

1. Introduction

Recent studies have suggested that vegetation may play an important role in the bioremediation of surface soils contaminated with toxic organic chemicals [1,2] by stimulating the activity of microorganisms with a capacity for biodegradation. In the long-term (3–4 years), can pollutant biodegradation be increased only through biological technologies.

Arbuscular mycorrhizal (AM) fungi can be considered as a soil microbiological resource for bioremediation since they are ubiquitous and most plant species are hosts. These fungi are considered essential for the survival of many plants in natural competitive situations. Knowledge of the relationships between mycorrhizal fungi and their hosts in polluted habitats is limited, however, AMF can be important under adverse soil chemical and physical conditions because mycorrhizal plants may be more tolerant due to a more balanced mineral nutrition. Theoretically, the mycelium of AMF in the soil could compensate for the reduced root growth caused by pollutant toxicity. Further, some fungal species are well-adapted to adverse soil conditions [3]. Resting spores of the AM fungus, hyphae in the soil and root fragments with fungal structures are sources of propagules from which the colonization can be initiated.

One of the methods used to measure the mycorrhizal community is the Most Probable Number (MPN) technique in which host plants are established in serial dilutions of soil and assessed for level

* Corresponding author. Fax: +54 (21) 530 189; e-mail: anmabarr@isis.unlp.edu.ar

PII S0168-6496(96)00094-3

of mycorrhiza establishment [4]. The MPN test is the only method which quantifies all active AMF propagules and estimates their population, giving numbers of propagules per unit soil volume or weight. The method also results in an estimate of confidence limits.

In 1992, a 'Decontamination of oil-polluted soils by fungi' project was initiated at Spegazzini Institute, La Plata National University, Argentina in collaboration with the Friedrich-Schiller-Universität Jena, Germany. One of the aims of this agreement was to evaluate the status of mycorrhiza in plants grown in hydrocarbon-polluted soils from Argentina and Germany; to compare the number of effective propagules present in polluted and similar non-polluted soils, and to culture indigenous AMF populations in trap plants.

2. Materials and methods

Ten randomly-collected soil samples from the upper 15 cm and 10 plants complete with root systems from polluted and non-polluted locations in Ensenada (Argentina) and Rositz (Germany) were obtained in May and August, 1993, respectively. The plants were: *Cynodon dactylon* (L.) Pers. from polluted areas and *C. dactylon* and *Bromus brevis* Nees, apud Stend in non-polluted areas from Ensenada; *Solidago* sp. and *Dactylis glomerata* L. from polluted soils and *Melilotus* sp. from non-polluted soils from Rositz.

Soil from Ensenada was characterized as follow: pH 7.4; total C: 8%; organic matter: 12.17%; total N: 0.162%; C/N ratio: 38.48; P: 6.5 ppm. Soil from Rositz was characterized by pH 7.5; total C: 19%; total organic carbon 15.9%; total N: 0.209%; C/N ratio: 91 (Wolter, M. pers. comm.).

The hydrocarbon pollutant in soils from Ensenada was crude oil (total aliphatic 18.5% and aromatics such as phenanthrene 857.46 mg kg^{-1} soil and crysene 517 mg kg^{-1} soil) whereas in Rositz the main fraction was polycyclic aromatic hydrocarbons (PAH) at 700 mg kg^{-1} soil [5].

To determine AM colonization, the roots were cleared and stained [6], and the proportion of colonization of total root length was measured by Giovannetti and Mosse's [7] gridline intersection method (200 gridline intersections per samples). The percentage of arbuscles, number of entry points and number of vesicles were measured as described by Ocampo et al. [8].

Propagule populations were determinated by MPN bioassay based on Porter [4]. Ten serials dilutions of each soil were made by mixing the original soil with sand sterilized for 1 h at 100°C on 3 consecutive days in a proportion of 3 parts sand and one part of the previous dilution. Fifty grams of soil : sand dilution mixture was placed in 250 ml capacity plastic pots. Each pot was seeded with 4–5 alfalfa (*Medicago sativa* L.) seeds. *Medicago sativa* was selected as the test plant because of its ability to serve as a host to many AM fungi and its good response to mycorrhizal inoculation [9,10]. Five replicate pots were prepared for each dilution; 5 additional pots were prepared as control using sterilized sand without soil. Plants were watered from below using a capillary system; fed with the following nutrient solution: $MgSO_4 \cdot 7H_2O$, 0.75 mM; $NaNO_3$, 1 mM; K_2SO_4, 1 mM; $CaCl_2 \cdot 2H_2O$, 2 mM; $Na_2HPO_4 \cdot 12H_2O$, 3.2 μM; FeNa-EDTA, 0.025 mM; $MnSO_4 \cdot 4H_2O$, 5 μM; $CuSO_4 \cdot 5H_2O$, 0.25 μM; $ZnSO_4 \cdot 7H_2O$, 0.5 μM; H_3BO_3, 0.025 mM, $NaMoO_4 \cdot 2H_2O$, 0.1 μM. Plants were grown for 8 weeks under greenhouse conditions. MPN values were calculated from the number of pots with colonized plants by an approximation of the maximum likelihood method.

Trap cultures were established by mixing field-collected soils and roots with steam-sterilized coarse-grained sand. This mixture was placed in 15 cm plastic pots (1350 cm^3) and sown with *Medicago sativa* L. Pot cultures were harvested after 8 months. To extract spores, soils from pot cultures were wet sieved and decanted [11].

Original taxonomic papers and the INVAM Identification Manual [12] were used for identification of isolated species.

3. Results and discussion

Microscopic observations of stained roots showed the presence of AMF fungi in both polluted and non-polluted soils from both sities. Percentages of colonization were higher in plants grown in non-pol-

Table 1
Percentage VA root length colonization, arbuscles, vesicles and entry points in plants sampled from Ensenada (Argentina) and Rositz (Germany) polluted and non-polluted soils.

Sample	Fungal structures			
	% colonized root	% arbuscles	Vesicle number	Entry point number
Polluted soil, Ensenada				
C. dactylon	59.70[b]	10[b]	105[b]	0.75[c]
Non-polluted soil, Ensenada				
C. dactylon	94[a]	32.02[a]	26[a]	3.6[a]
B. brevis	96[a]	28.04[a]	28[a]	3.4[a]
Polluted soil, Rositz				
Solidago sp.	61.11[b]	8[b]	80[b]	0.55[c]
D. glomerata	59.15[b]	10.33[b]	83[b]	0.55[c]
Non-polluted soil, Rositz				
Melilotus sp.	93[a]	34.82[a]	29.7[a]	2.2[b]

The data are the means of 10 replicates. Means in the same column followed by the same letter are not significantly different (ANOVA, $P = 0.05$).

luted soils and decreased in both polluted sites (Table 1 1). When percentage of arbuscles and number of vesicles cm^{-1} root are compared in polluted and non-polluted areas, the proportion of arbuscles was higher in non-polluted than in polluted areas. The number of vesicles was higher when plants were stressed by pollutants.

The arbuscule is a significant structure in the AM complex, being the site for fungus and plant metabolite exchange [13,14]. On the other hand, vesicles increase in number in old or dead roots or in plants growing in stressful conditions and are said to be mainly resting structures [15]. In this research it was found that the increase in the vesicles number is correlated with a decrease in the percentage of arbuscles; during stress, the lipid reserves from the vesicles are utilized by the fungus and vesicles degenerate.

The number of entry points also is higher in roots of plants growing in non-polluted soils. The entry points connect internal and external fungal structures. In experimental conditions, there can be up to 10 entry points per cm of root [8], but connecting points can be much less numerous in the field especially if plants are growing in stressful situations, perhaps resulting in reduced external mycelium in polluted areas. Microscopic observations of stained roots from control pots showed no presence of fungi (data not shown).

Propagule population densities differed among polluted and non-polluted areas but not among the Argentinean and German polluted or non-polluted soils (Table 2). The highest densities were in non-polluted soils. Overall, densities in non-polluted areas were respectively seven and a half-fold and five-fold higher than those in polluted soils in Argentina and Germany.

There are no differences between populations of AMF infective propagules in non-polluted soils from Rositz and Ensenada. However the propagule number in polluted soil is also high, suggesting that the present mycorrhizal status of plants growing in those soils is dependent on the level of infective propagules.

Table 2
Population densities of infective propagules 100 g dry $soil^{-1}$

	Propagule number	Confidence limits	
		Lower	Upper
Polluted soil, Ensenada	226	105	482
Non-polluted soil	714	802	3663
Polluted soil, Rositz	340	159	727
Non-polluted soil	1795	839	3825

The spores extracted from pot cultures showed a very limited fungal spectrum. In the Ensenada non-polluted soil, *Glomus deserticola* Trappe, Bloss and Menge and *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe were found; in polluted soil from the same localities, *G. aggregatum* Schenck and Smith emend. Koske and *Gigaspora* sp. were identified. The Rositz non-polluted soil, contained *G. aggregatum* and *G. mosseae*, whereas only *G. mosseae* was found from the polluted soil from this site.

The data obtained suggest that hydrocarbon contamination affects the mycorrhizal fungal population associated with plants, and agrees with previous reports [16]. Pollution effects were evident at two distinct sites. However, the occurrence of AMF fungi in the contaminated sites indicates the possession of at least some hydrocarbon tolerance. The contribution of *Glomus aggregatum*, *G. mosseae* and other AMF to the hydrocarbon tolerance of their hosts is still under investigation. More information is needed on mycorrhizal fungi occurring in hydrocarbon polluted soils, their tolerance limits, the effects of nutrient addition, species efficience in promoting plant growth and survival, and assessment of the role of various propagule types in those soils.

Acknowledgments

I am grateful to CIC and CONICET for providing grants towards this study. I also wish to thank Lic M. Dabadie (CIC) and Mr J. Chayle (CONICET) for their technical assistance. Much appreciation goes to Dr C. Walker (Forestry Commission, Northern Research Station, Roslin, Midlothian, UK) and Dr A. Arambarri for their critical review of this manuscript and help with English. M.N.C. is researcher of the CIC, Buenos Aires, Argentina.

References

[1] Walton, B.T., Guthrie, E.A. and Hoylman, A.M. (1994) Toxicant degradation in the rhizosphere. In: Bioremediation through Rhizosphere Technology (Anderson, T.A. and Coats, J.R., Eds.), pp. 11–26. ACS Symposium series 563, American Chemical Society, Washington, D.C.

[2] Davis, L.C., Erickson, L.E., Lee, E., Shimp, J.F. and Tracy, J.C. (1993) Modeling the effects of plants on the bioremediation of contaminated soil and ground water. Environm.Prog. 12, 67–75.

[3] Sieverding, E. (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems, 371 pp. Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ No. 224. Eschborn.

[4] Porter, W.M. (1979). The "Most Probable Number" method for enumerating infective propagules of VAM fungi in soil. Austral. J. Soil Res. 17, 515–519.

[5] Sack, U., Rieg, M., Metzner, H. and Fritsche, W. (1992) Degradation of polycyclic aromatic hydrocarbons (PAH) by *Aspergillus versicolor* and autochthonous soil fungi. In: Soil Decontamination Using Biological Processes. (Preprints of the International Symposium Karlsruhe/Germany) pp. 342–350. DECHEMA, Frankfurt/M.

[6] Phillips, J.M. and Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and VA mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55(1), 158–161.

[7] Giovannetti, M. and Mosse, B. (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–499.

[8] Ocampo, J.A., Martin, J. and Hayman, D.S. (1980) Influence of plant interactions on vesicular-arbuscular mycorrhizal infection. I. Host and non host plants grown together. New Phytol. 84, 27–35.

[9] Barea, J.M., Azcon-Aguilar, C. and Azcon, R. (1983). Effect of interactions between a soluble fertilizer and mycorrhizal fungi on nodulation, mycorrhiza formation, growth and nutrition of alfalfa (*Medicago sativa* L). Ciencia del Suelo 1(1), 39–43. In Spanish.

[10] Diaz, G. and Honrubia, M. (1993) Infectivity of mine soils from Southeast Spain II: Mycorrhizal population levels in spoilt sites. Mycorrhiza 4 (2), 85–88.

[11] Gerdemann J.W. and Nicolson, T.H. (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46 (2), 235–244.

[12] Schenk, N.C. and Perez, Y. (1988) Manual for the identification of VA mycorrhizal fungi. INVAM. 241 pp. University of Florida. Gainesville, FLA.

[13] Cox, G., Sanders, F.E., Tinker, P.B. and Wild, J.A. (1975) Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. In: Endomycorrhizas (Sanders, F.E., Mosse, B. and Tinker, P.B., Eds.), 297 pp. Academic Press, London.

[14] Scannerini, S. and Bonfante-Fasolo, P. (1983) Comparative ultrastructural analysis of mycorrhizal associations. Can. J. Bot. 61, 917.

[15] Cooper, K.M. (1984) Physiology of VA mycorrhizal association. In: VA Mycorrhiza. (Powell, C.L. and Bagyaraj, D.J., Eds.), pp. 155– 186. CRC Press, Inc. Boca Raton, Florida.

[16] Cabello, M.N. (1995) Effect of hydrocarbon pollution on vesicular-arbuscular mycorrhizal fungi (VAM). Bol. Micol. (Chile) 10(1), 77–83. In Spanish.