ELSEVIER

Applied Soil Ecology 3 (1996) 183–186

Applied Soil Ecology

Short communication

Viability and infectivity of mycorrhizal spores after long term storage in soils with different water potentials

J.M. Ruiz-Lozano, R. Azcón *

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, Profesor Albareda 1, 18008 Granada, Spain

Accepted 1 June 1995

Abstract

Spores of three *Glomus* species were examined to compare the effect of long term storage under three different soil water conditions (−0.04 MPa, −0.8 MPa or dried) on their infective capacity. After 6 months of incubation, the infectivity tests showed that spores were affected in their potential as inoculum by soil water potential during storage. Spores from *G. mosseae* and *G. deserticola* were more infective after storage under −0.04 MPa soil water potential, while in *G. fasciculatum* the highest infectivity was obtained at −0.8 MPa soil water potential. These results can not be ascribed to drought adaptation by the fungi during their production, since all spores used for this study were obtained from lettuce plants grown under the same conditions. The results appear to be related to the genetically determined ability of each fungus to tolerate drought. When the substrate was completely dried, the infective capacity of spores from the three fungi decreased considerably.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous in soils and play an important role in plant growth and development (Singh and Subba Rao, 1987; Harley, 1989). This is attributed to increased nutrient uptake, production of growth promoting substances, tolerance to drought, salinity and transplant shock. Other mechanisms to be considered are synergistic interactions with beneficial soil microorganisms such as N-fixers and P-solubilizers (Azcón et al., 1988).

AM fungi have a variety of viable propagules (spores, hyphal fragments and hyphae within senesced and living roots). Spores are generally regarded as the most durable form of propagule of AM fungi and are required for the spread, dispersal and persistence of mycorrhizal fungi. The relative importance and survival of these propagules differs among fungal species and are affected by environmental conditions (Gazey et al., 1993). In addition, inoculum viability may be affected by storage of AM fungal propagules in soil (Daft et al., 1987). However, little data are available on the survival of spores over prolonged periods of storage under various soil moisture conditions.

The objective of this work was to study how spores from three *Glomus* species were affected in their viability and infective capacity after long term storage under three different soil water conditions.

2. Materials and methods

2.1. Experimental design

Spores of three *Glomus* species were stored for 6 months in soil under three different water regimes

* Corresponding author.

SSDI 0929-1393(95)00076-3

(−0.04 MPa, −0.8 MPa or dry). After this time, the inocula were diluted with sterilized soil (1/4, 1/16 or 1/64) following the most probable number (MPN) method (Porter, 1979) to assay for AM-fungal colonization. Three replications were done for each treatment for a total of 81 pots.

Data were subjected to analysis of variance (ANOVA). When the main effect was significant ($P < 0.05$) differences among means were determined by Duncan's multiple range test (Duncan, 1955).

2.2. Soil and biological materials

Loamy soil collected from Granada city (a field around the Estación Experimental del Zaidín) was sieved (2 mm pore size), diluted with quartz sand (1/1 v/v) and sterilized (100°C, 1 h, 3 consecutive days). The characteristics of the soil before the sand dilution were: pH 8.1, 6.2 mg of available P (Olsen and Sommers, 1982) per kg, 2.5 mg of N (Bremner and Mulvaney, 1982) per kg, 132 mg K (Knudsen et al., 1982) per kg, 1.81% organic matter, 36% sand, 46% silt, and 20% clay. Pots (0.3 l) were filled with 300 g of the sterilized soil/sand mixture. The AM species used were: *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe; *Glomus fasciculatum* (Thax. sensu Gerd.) Gerd. and Trappe and *Glomus deserticola* (Trappe Bloss and Menge). Fifteen hundred spores of each of the above fungi were collected after 70 days of plant growth in a sand/soil mixture (1/9 v/v) and a nutrient solution (Table 1) as source of nutrients, by the water stream sieving method (Vilariño and Arines, 1990) and cleared of organic detritus and soil particles by sucrose density gradient centrifugation and washing (Mertz et al., 1979). The method of spore production ensures that no drought adaptation occurred. After cleaning, they were divided in three 500 spores sets. Each set was mixed with 100 g of soil/sand (1/1 v/v) mixture that was completely dried (60°C for 48 h) or had a soil water potential of −0.04 MPa or −0.8 MPa. Soil water potential was determined with a 15×10^5-Pa pressure plate apparatus (model 1500 ceramic plate extractor, Soilmoisture Equipment Corp.), and soil water content was determined by weighing samples before and after drying at 110°C for 24 h (Bethlenfalvay et al., 1990). The soil/sand/spores mixture was kept in black plastic bags and stored for 6 months at greenhouse temperature (a mean and range temperature of 28–32°C) into a polysprene box that shielded bags from direct sunlight and where the temperature range was 28–29°C. After this incubation time, at mentioned water regimes, the infectivity test started. Each inoculum type was serially diluted in a sterile soil–sand mixture. Three 3-fold serial dilutions from 1/4 to 1/64 (125, 32 or 8 spores per pot respectively, determined by the MPN method) were prepared, each placed in a small pot of 70 ml capacity. *Lactuca sativa* was used as the test plant.

Table 1
Composition of nutrient solution used to produce AM inocula

	Stock solution ($g\ l^{-1}$)	Solution ($ml\ l^{-1}$)
$CaSO_4$	13.0	10
$MgSO_4.7H_2O$	18.4	20
Fe-EDTA	2.3	10
NaH_2PO_4	2.6	10
$MnSO_4$	2.3	1
$ZnSO_4$	2.9	1
$CuSO_4$	2.4	1
H_3BO_3	16.6	1
$Mo_7(NH_4)_6O_2.4H_2O$	0.4	1
K_2SO_4	62.2	8
$Ca(NO_3)_2.4H_2O$	350.3	1
$(NH_4)_2SO_4$	188.6	1
H_2O (ml)	1000	935
pH	–	7.5

2.3. Growth conditions

Plants were cultivated for 30 days in a greenhouse with 16/8 h day/night cycle maintained at 80% relative humidity and a temperature of 28–32°C. Photosynthetically active radiation was 800 $\mu E\ m^{-2}\ s^{-1}$ as measured with a lightmeter (LICOR LI-188B). During this time, pots were watered to field capacity.

2.4. Determinations

Plants were harvested 30 days after sowing and the percentage mycorrhizal colonization of the roots was determined by staining 1 cm fragments of the central part of the roots with trypan blue (Phillips and Hayman, 1970) before examination at ×200 magnification following the gridline-intersect method of Giovannetti and Mosse (1980).

3. Results

Spores from *G. mosseae* were very sensitive to soil moisture treatments during storage, being more infective under a previous incubation at −0.04 MPa soil water potential (Table 2). At dilutions of 1/4 and 1/16 these spores reduced the infective capacity to 32–18%, when the soil water potential was −0.8 MPa, and to 12–13% respectively, when the soil was dried.

The behaviour of *G. fasciculatum* spores was different. The highest infectivity of the spores was obtained when the soil water potential during storage was −0.8 MPa (Table 2), reaching, at dilutions of 1/4 and 1/16, an increase in this parameter of 410% and 240% respectively.

G. deserticola spores showed the highest infectivity (23%) in the case of incubation in soil with a high water content and the lowest inoculum dilution.

For the three fungi, the lack of moisture in soil during the time of spore incubation (in dried soil) considerably decreased the infective capacity of such fungal propagules concerning −0.8 MPa for *G. fasciculatum* or −0.04 MPa for *G. mosseae* and *G. deserticola* (Table 2).

It is also clear, in the present study, that reduction in the number of spores, in all cases, resulted in a progressive drop in the intensity of mycorrhizal infection. A more probable number of 8 spores was insufficient to induce root colonization (except in *G. mosseae* at −0.04 MPa). A minimum of 32 spores produced a detectable infection and with a quantity of 125 spores as inoculum, the root colonization ranged from 8% to 23% depending on the water level during spore storage (Table 2).

Table 2
Percentage of vesicular-arbuscular mycorrhizal colonization reached in *Lactuca sativa* plants with spores of three *Glomus* species after 6 months storage in soil with different water potential or dried, and diluted at 1/4, 1/16 or 1/64 with soil

	−0.04MPa			−0.8MPa			Dry		
	1/4	1/16	1/64	1/4	1/16	1/64	1/4	1/16	1/64
G. mosseae	9.2b	6.8c	1de	2.9c	1.2de	0e	1.1b	0.9b	0c
G. fasciculatum	1.9d	1.1de	0e	7.8a	2.6cd	0e	1.3b	1.1b	0c
G. deserticola	23.3a	8.7b	0e	5.3b	2.3cd	0e	2.0a	1.1b	0c

Within each soil moisture level, means followed by the same letter are not significantly different ($P<0.05$) using Duncan's multiple range test ($n=3$).

4. Discussion

The test of spore infectivity on roots of *Lactuca sativa* showed that their potential as inoculum was affected by soil water content during storage. Results indicate that the soil moisture is a factor of importance in storage of propagules and may affect the viability of spores (Daft et al., 1987). However, the effect of soil moisture on the spores infective capacity depended on the fungus. Spores from *G. mosseae* and *G. deserticola* were more infective after storage in soil at −0.04 MPa, while those of *G. fasciculatum* showed the highest infectivity when the soil water potential during incubation was −0.8 MPa. This effect can not be ascribed to drought adaptation by the fungi during their production, since all spores used in this study were obtained from lettuce plants grown in a soil/sand mixture (1/9 v/v) and a nutrient solution as source of nutrients which is similar to a hydroponic system and can not induce drought adaptation. The found effect seems to be related to the different abilities of each fungus to tolerate drought (Ruiz-Lozano et al., 1995).

Germination of extramatrical chlamydospores is known to be affected by several factors such as pH (Green et al., 1976) temperature (Daniels and Trappe, 1980) and water potential (Sylvia and Schenck, 1983). In this study we used mycorrhizal spores and the lack of moisture in soil during the time of spore incubation (soil dried) considerably decreased the infective capacity of such fungal propagules as Sylvia and Schenck (1983) pointed out in the case of chlamydospores.

In nature, mycorrhizal propagules must remain viable from one period of root growth to the next. However, under drought stress conditions, loss of viability may be a critical factor in the success or survival of AM fungi.

In our study none of the three fungi was able to colonize roots when the most probable number of spores was eight, indicating that a minimum of about 30 spores is needed to get a detectable infection level.

Acknowledgements

We thank T. Morris, University of Cardiff (School of Pure and Applied Biology), for correcting the English text.

References

Azcón, R., El-Atrach, F. and Barea, J.M., 1988. Influence of mycorrhiza vs. soluble phosphate on growth, nodulation and N_2 fixation ^{15}N in alfalfa under different levels of water potential. Biol. Fertil. Soils, 7: 28–31.

Bethlenfalvay, G.J., Brown, M.S. and Franson, R.L., 1990. The *Glycine–Glomus–Bradyrhizobium* symbiosis. X. Relationships between leaf gas exchange and plant and soil water status in nodulated, mycorrhizal soybean under drought stress. Plant Physiol., 94: 723–728.

Bremner, J.M. and Mulvaney, C.S., 1982. Nitrogen. In: A.L. Page, R.H. Miller, D.R. Keeney, D.E. Baker, J.D. Rhodes, R.C. Dinauer, K.E. Gates and D.R. Buxton (Editors). Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, WI, pp. 595–562.

Daft, M.J., Spencer, D. and Thomas, G.E., 1987. Infectivity of vesicular arbuscular mycorrhizal inocula after storage under various environmental conditions. Trans. Bri. Mycol. Soc., 88: 21–27.

Daniels, B.A. and Trappe, J.M., 1980. Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia, 72: 457–471.

Duncan, D.B., 1955. Multiple range and multiple *F* tests. Biometrics, 11: 1–42.

Gazey, C., Abbott, L.K. and Robson, A.D., 1993. VA mycorrhizal spores from three species of *Acaulospora*: germination, longevity and hyphal growth. Mycol. Res., 97: 785–790.

Giovannetti, M. and Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol., 84: 489–500.

Green, N.E., Graham, S.O. and Schenck, N.C., 1976. The influence of pH on the germination of vesicular-arbuscular spores. Mycologia, 68: 929–934.

Harley, J.L., 1989. The significance of mycorrhiza. Mycol. Res., 95: 129–139.

Knudsen, D., Petersen, G.A. and Pratt, P.F., 1982. Lithium, sodium and potassium. In: A.L. Page, R.H. Miller, D.R. Keeney, D.E. Baker, J.D. Rhodes, R.C. Dinauer, K.E. Gates and D.R. Buxton (Editors). Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, WI, pp. 225–245.

Mertz, S.M., Heithaus, J.J. Jr. and Bush, R.L., 1979. Mass production of axenic spores of the endomycorrhizal fungus *Gigaspora margarita*. Trans. Brit. Mycol. Soc., 72: 167–169.

Olsen, S.R. and Sommers, L.E., 1982. Phosphorus. In: A.L. Page, R.H. Miller, D.R. Keeney, D.E. Baker, J.D. Rhodes, R.C. Dinauer, K.E. Gates and D.R. Buxton (Editors). Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, WI, pp. 403–427.

Phillips, J.M. and Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc., 55: 158–161.

Porter, W.M., 1979. The most probable number method for enumerating infective propagules of Vesicular-arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res., 17: 515–519.

Ruiz-Lozano, J.M., Azcón, R. and Gómez, M., 1995. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: Physiological and nutritional plant responses. Appl. Environ. Microbiol., 61: 456–460.

Singh, C.S. and Subba Rao, N.S., 1987. Yield and phosphorus content of wheat (*Tritium aestivum*) as influenced by co-inoculation with *Azospirillum brasilense* and *Glomus fasciculatum*. In: Mycorrhiza Round Table-Proceeding of a national workshop help at J.N.U. Delhi, India, September 1986, pp. 335–346.

Sylvia, D.M. and Schenck, N.C., 1983. Germination of chlamydospores of three *Glomus* species as affected by soil matrice potential and fungal contamination. Mycologia, 67: 30–35.

Vilariño, A. and Arines, J., 1990. An instrumental modification of Gerdemann and Nicolson's method for extracting VAM fungal spores from soil samples. Plant Soil, 121: 211–215.