

Physiological and genetical aspects of mycorrhizae

Aspects physiologiques et génétiques des mycorrhizes

**Proceedings of the 1st European Symposium
on Mycorrhizae,
Dijon, 1-5 July 1985.**

***Actes du 1er Symposium Européen
sur les Mycorrhizes,
Dijon, 1-5 juillet 1985.***

Editors/Editeurs
V. GIANINAZZI-PEARSON
S. GIANINAZZI
CNRS - INRA, Dijon

Editors/Editeurs

V. GIANINAZZI-PEARSON, S. GIANINAZZI
CNRS - INRA, Station d'Amélioration des Plantes
BV 1540, 21034 Dijon

For Sale/En vente

INRA, Service des Publications
Route de St. Cyr, 78000 Versailles

© INRA, Paris, 1986
ISBN 2-85340-774-8

Effect of root extracts of non host plants on VA mycorrhizal infection and spore germination

J.A. OCAMPO*, F.L. CARDONA** and F. EL-ATRACH*

* U.E.I. Microbiología, Estación Experimental Zaidín,
CSIC, Profesor Albareda 1, Granada, Spain

** Departamento de Microbiología, Universidad Colombia, Medellín, Colombia

INTRODUCTION

Plants belonging to *Cruciferae* and *Chenopodiaceae* families cannot develop vesicular-arbuscular (VA) infection in their roots (GERDEMANN, 1968). In some instances failure to infect these plants appreciably has been attributed to root exudates which inhibited the VA infection in a plant that is normally mycorrhizal (HAYMAN *et al.*, 1975; IQBAL and QURESHI, 1976). However, some infection in non-host plants when grown together with host plants have been observed (HIRRELL *et al.*, 1978; OCAMPO *et al.*, 1980) and non-host pre-crop did not depress root infection of host plants (OCAMPO and HAYMAN, 1981). On the other hand, root exudates of non-host plants did not affect the percentage of germination of *Glomus geosporum* spores (OCAMPO and ARCON, 1980). These findings indicate that the lack of VA infection in non-host plants does not involve exudation of toxic substance (HAYMAN, 1983).

The presence of many aborted entry points suggests that the barriers to mycorrhizal infection in non-host plants are intrinsic and more probably related to physiological characteristics for the root cortex or epidermis than to any compounds that might be released in root exudates (OCAMPO *et al.*, 1980). Because of that, the effect of root extracts of *Cruciferae* on VA mycorrhizal infection and spore germination has been studied.

MATERIAL AND METHODS

Experiment 1

The experiment was carried out in open pots of sterilized sand-vermiculite (1:1 v/v) or steamed soil (BAREA *et al.*, 1980). Alfalfa (*Medicago sativa* cv. Aragón) was the test plant. The soil was mixed with sterile sand (1:1 v/v), and inoculated with soil from stock plant cultures of *Glomus mosseae*, which contained spores, mycelium and infected root fragments, at the rate of 5 g soil inoculum mixed with 5 g sand per 75 ml pots. The sand-vermiculite pots were inoculated with 10 sporocarps of the same *G. mosseae* isotype. The alfalfa seedlings were also inoculated with the strain 203 of *Rhizobium meliloti* isolated in this laboratory. Plants were grown in a glasshouse at 19-25° C. Plants were watered from below and given Long Ashton nutrient solution (HEWITT, 1952) lacking nitrogen and phosphate for the

soil pots. The sand-vermiculite pots were given 1/2 strength plus 50 g/l of phosphate of the same nutrient solution.

Ten replicate pots of each treatment were prepared. When alfalfa test plants were two-weeks-old the root extract from maize (*Zea mays*), alfalfa (*Medicago sativa* cv. Aragon), radish (*Raphanus raphanistrum* var. *Aplastada* de Egipito) and cabbage (*Brassica oleraceae* cv. Brunswick) were added. These plants were grown in pots of the same steamed soil and inoculated with *G. mosseae* endophyte as before. When plants were four-weeks-old, roots were extracted by grinding in a Waring Blender with 5 g of roots/100 ml of Tris-HCL buffer (pH = 7, 0.1 M) and filtering the liquid through filter paper (Whatman n°1) and afterwards through a Millipore filter (0.45 μ). Ten ml of root extract were added to each pot every fortnight during eight weeks. Plants receiving 10 ml of buffer were used as control. After ten weeks, root samples of each replicate pot were cleared and stained (PHILLIPS and HAYMAN, 1970), the root infection evaluated by the gridline intersect method (GIOVANNETTI and MOSSE, 1980) and the shoot dry matter recorded.

Experiment 2

Alfalfa and cabbage plants were grown in soil-sand pots inoculated with *G. mosseae* as in Experiment 1. When plants were eight-weeks-old, roots were extracted by grinding in a Waring Blender with 6 g of roots/20 ml of Tris-HCL buffer and sterilized by passing the liquid through a Millipore filter (0.45 μ). Twenty five surface-sterilized spores (MOSSE, 1962) were placed in a sterilized Petri dish with water-agar (1% Difco Bacto Agar) plus 1 ml of extracts solution or 1 ml of buffer for the controls. Ten replicates per treatment were used. Petri dishes were incubated at 25° C and the percentage of spore germination was examined after one and two weeks.

RESULTS

As Table 1 shows, the root extracts of the non-host plants radish and cabbage inhibit the VA infection in alfalfa plants grown in sand-vermiculite pots but not in soil pots. However, no effect on plant shoot dry weights was observed in either case.

Table 1. Effect of plant root extracts on VA mycorrhizal infection and shoot dry weights of alfalfa grown in sand-vermiculite and in soil pots.

| Root extract from | % root length infection | | shoot dry weights (mg) | |
|----------------------|-------------------------|------|------------------------|-------|
| | Sand-vermiculite | Soil | Sand-vermiculite | Soil |
| None | 22.5 | 40.5 | 52.2 | 164.1 |
| Alfalfa | 21.3 | 44.5 | 52.3 | 175.2 |
| Maize | 20.6 | 42.5 | 57.5 | 170.3 |
| Radish | 1.4 | 43.4 | 56.4 | 153.8 |
| Cabbage | 0 | 34.3 | 60.5 | 168.6 |
| L.S.D. 5% | 5.8 | 12 | 25 | 40 |

Each figure is the mean of 10 replicates.

On the other hand, the percentage of spore germination was also decreased significantly by the root extract of cabbage (Table 2).

Table 2. Effect of plant root extracts on the percentage of germination of *Glomus mosseae* spores

| Root extracts from | % spore germination after weeks | |
|-----------------------|---------------------------------|----|
| | 1 | 2 |
| None | 30 | 53 |
| Alfalfa | 47 | 72 |
| Cabbage | 9.9 | 31 |
| L.S.D. 5% | 8.5 | 16 |

Each figure is the mean for 10 replicates.

DISCUSSION

Different results explaining the possible cause of the lack of VA infection in non-host plants have been obtained. On the one hand, the non-host plants inhibited the VA infection of host plants (HAYASHI *et al.*, 1975; IQBAL and QURESHI, 1976) indicating that the root exudates of the non-host plants were implicated in the non-infection of these plants and in the decrease of VA infection in the host plants when they were grown together. On the other hand, other authors did not find any inhibitory substances produced by the non-host plants (OCAMPO *et al.*, 1980; GLENN *et al.*, 1985) suggesting that the barrier of the non-host plants to VA infection was more related to physical characteristics of the root or that it was due to absence of necessary stimuli for penetration and development of the fungus.

Our results indicate that non-host plants have intrinsic factor/s which could be implicated in the absence of the development of VA infection in their roots. Inhibiting factors have been found in the seed coat of the weak host lupins (MORLEY and MOSSE, 1976). However, the production of toxic exudates by the root of non-host plants cannot be discarded. Because, as could happen with the root exudates, the absence of effect of root extracts of the non-host plants on VA infection of alfalfa grown in soil can be related to the more effective degradation of inhibiting substances by the soil rhizosphere microorganisms and/or by absorption site on soil particles and this effect may be different in different soils. In fact POWELL (1982) observed that kale pre-cropping decreased clover yield in two soils but not in another.

The different species of spores and type of inoculum may have also been implicated in the different results obtained by different authors. For example, no *Gigaspora margarita* entry points, aborted or not, were observed on cabbage roots in presence of lettuce (OCAMPO *et al.*, 1980) and *Gigaspora gigantea* did not penetrate brassica roots (GLENN *et al.*, 1985). By contrast, *Glomus fasciculatus* "E₃" (OCAMPO *et al.*, 1980), *G. mosseae* (OCAMPO, 1980; GLENN *et al.*, 1985) penetrated the roots of several *Cruciferae* tested. In our experiment, sand-vermiculite pots were inoculated with sporocarps but soil pots were inoculated with soil containing spores, mycelium and infected root fragments.

Experiments are carried out in order to see if non-host plants produce toxic exudates and if their effect on VA infection of host plants depends on soil type or the type of inoculum. Such substances would be of great interest in studies on the development of VA mycorrhizas. Future investigations are needed to elucidate the nature of such inhibitory substances and their

possible existence in non-host and in weak host plants such as lupins (TRINICK, 1977) and some wheat cultivars (AZCON and OCAIPIO, 1980) as well as the possible relationships with the spread of mycorrhizal infection in plant roots.

Financial support for this study has been obtained from CAICYT Project N° 1759.

BIBLIOGRAPHY

- AZCON R. and OCAIPIO J.A., 1981. *New Phytol.*, 87, 677-685.
- BAREA J.H., ESCUDERO J.L. and AZCON-AGUILAR C., 1980. *Plant Soil*, 54, 283-296.
- GERDEHANN J.W., 1968. *Ann.Rev. Phytopathol.*, 6, 397-413.
- GIOVANNETTI H. and HOSSE B., 1980. *New Phytol.*, 84, 489-500.
- GLENN M.G., CHEN F.S. and WILLIAMS P.H., 1985. *New Phytol.*, 99, 463-472.
- HAYMAN D.S., 1983. *Can. J. Bot.*, 61, 944-963.
- HAYMAN D.S., JOHNSON A.H. and RUDDLESSEIN I., 1975. *Plant Soil*, 43, 489-495.
- HEWITT E.J., 1952. *C.A.B. Tech. Co. n° 22*.
- HIRELL H C., MEHRAVARAN H. and GERDEHANN J.W., 1978. *Can. J. Bot.*, 56, 2813-2817.
- IQBAL S.H. and QURESHI K.S., 1976. *Biologia*, 22, 287-298.
- HOSSE B., 1962. *J. Gen. Microbiol.*, 27, 509-520.
- MORLEY C.D. and HOSSE B., 1976. *Trans Br. Mycol. Soc.*, 67, 510-513.
- OCAIPIO J.A., 1980. *Plant Soil*, 56, 283-291.
- OCAIPIO J.A. and AZCON R., 1980. *2nd Int. Symp. Microbiol. Ecol.*, Warwick, 128-129.
- OCAIPIO J.A. and HAYMAN D.S., 1981. *New Phytol.*, 87, 333-343.
- OCAIPIO J.A., MARTIN J. and HAYMAN D.S., 1980. *New Phytol.*, 84, 27-35.
- PHILLIPS J. and HAYMAN D.S., 1970. *Trans. Br. Mycol. Soc.*, 56, 158-161.
- POWELL C.L.L., 1982. *N.Z.J. Agricol. Res.*, 25, 461-464.
- TOFFERUP I.C., 1984. *New Phytol.*, 90, 489-495.
- TRINICK H.J., 1977. *New Phytol.*, 78, 297-304.