

INFLUENCE OF NON-HOST PLANTS ON VESICULAR-ARBUSCULAR MYCORRHIZAL INFECTION OF HOST PLANTS AND ON SPORE GERMINATION

F. EL-ATRACH, H. VIERHEILIG and J. A. OCAMPO

Soil Microbiology Department, Estacion Experimental del Zaidin, Professor Albareda 1,
18008 Granada, Spain

(Accepted 5 May 1988)

Summary—Host (*Medicago sativa*) and non-host (*Brassica oleracea*) plants were grown in pots of sand-vermiculite or soil inoculated either with soil inoculum or with sporocarps of *Glomus mosseae*. Cabbage only decreased the vesicular-arbuscular (VA) mycorrhizal infection of alfalfa plants, when both plants were grown together in sand-vermiculite pots inoculated with sporocarps. The root exudates of the non-host plants did not affect the percentage spore germination. However, the percentage spore germination was decreased by volatile compounds of non-host plant roots.

The characteristics of the inhibiting factors of VA mycorrhizal infection in non-host plants are discussed.

INTRODUCTION

Several plants belonging to families such as Cruciferae and Chenopodiaceae do not develop VA mycorrhizal infection in their roots (Harley and Harley, 1987; Newman and Reddell, 1987). The lack of VA infection in these plants has been attributed to intrinsic characteristics of the cortex rather than to factors released in exudates (Ocampo *et al.*, 1980; Glenn *et al.*, 1985). Nevertheless, inhibition of the VA infection of host plants by some non-host plants has been observed (Hayman *et al.*, 1975; Iqbal and Qureshi, 1976). These results indicated that the root exudates of the non-host plants can be implicated in the lack of infection of these plants and in the decrease of VA infection in the host plants, when they were grown together. However, from these experiments the possible negative effect of volatile compounds cannot be excluded. The inhibitory effects of non-host plants seem to depend on the conditions under which they were grown (Powell, 1980). The importance of volatile compounds of plant roots on VA mycorrhizal infection has been suggested (Koske, 1982). Indications of the negative effect of volatile compounds from *Brassica napus* roots on spore germination and VA hyphal extension have been observed (Tommerup, 1984). Therefore the effect of root exudates and volatile compounds of Cruciferae and their effect on VA infection of host plants with different type of inoculum and plant growth medium was studied.

MATERIALS 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), mixed with sterilized sand (1:1 v/v). Alfalfa (*Medicago sativa* cv. Aragon) and cab-

bage (*Brassica oleracea* cv. Brunswick) were used as test plants. Seeds were grown in moistened sand, and 2-week-old seedlings of alfalfa alone or together with cabbage were transplanted to the pots and grown under greenhouse conditions [supplementary light (Silvania incandescent and cool-white lamps, 400 nmol m⁻² s⁻¹ 400–700 nm), 16–8 h light-dark cycle, 25–19°C and 50% R.H.].

The soil and sand-vermiculite pots were inoculated either with 30 sporocarps of *Glomus mosseae* or with 5 g of rhizosphere soil from maize plant pot cultures of the same *G. mosseae* isotype, which contained spores, mycelium and infected root fragments. Plants were also inoculated with the strain 203 of *Rhizobium meliloti* isolated in this laboratory. Plants were watered from below, using a capillary system. Every week 5 ml of Long Ashton nutrient solution (Hewitt, 1952), lacking nitrogen and phosphate, were added to the soil pots. The sand-vermiculite pots were given 1/2 strength plus 50 mg l⁻¹ of phosphate of the same nutrient solution. Ten replicates per treatment were used. After 10 weeks, root samples of each replicate pot were cleared and stained (Phillips and Hayman, 1970), and the root infection evaluated by the gridline intersect method (Giovannetti and Mosse, 1980). Shoot dry matter was recorded.

Experiment 2

Seeds of alfalfa and cabbage were surface-sterilized with HgCl₂ for 10 min and then thoroughly rinsed with sterile water. Seedlings were transferred to sterilized glass tubes, containing a strip of filter paper (Azcon-Aguilar and Barea, 1979) and 10 ml of 1/2 strength Long Ashton nutrient solution at pH = 7, were used. Tubes without plants were used as nutrient solution controls. Plants were grown in a growth cabinet under the same ambient conditions as described for the greenhouse. Root exudates from plants were those accumulated in the nutrient solu-

Table 1. VA infections and shoots dry weights of alfalfa grown alone or together with cabbage and inoculated with *G. mosseae*

Growth medium	VA inoculum	Plants	Shoot dry wt (mg)	% Root length infection
Soil	Soil inoculum	Alfalfa	62 ± 10	60 + 8
		Alfalfa plus cabbage	74 + 12	58 + 7
	Sporocarps	Alfalfa	71 + 38	ND
		Alfalfa	57 ± 19	13 + 7
		Alfalfa plus cabbage	36 ± 5	15 + 2
		Alfalfa	161 + 24	ND
Sand/ vermiculite	Soil inoculum	Alfalfa	39 + 14	23 + 6
		Alfalfa plus cabbage	23 + 4	25 + 5
	Sporocarps	Alfalfa	159 + 31	ND
		Alfalfa	47 + 15	29 + 5
		Alfalfa plus cabbage	21 + 2	4 + 0.5
		Alfalfa	213 + 21	ND

Each figure is the mean for 10 pots. Standard errors of mean are given. ND = not determined.

tion over the whole period of plant growth. After 15 days plants were harvested and contaminated tubes were discarded.

Twenty five surface-sterilized spores (Mosse, 1982) of *G. mosseae* were placed in a sterile Petri dish and water-agar (1% Difco Bacto Agar) plus 1 ml of exudate solution or 1 ml of sterile distilled water as controls. Ten replicates per tube were used. Petri dishes were kept at 25°C. The percentage of spore germination was examined after 1 and 2 weeks.

Experiment 3

In order to see the effect on spore germination of gaseous substances from alfalfa and cabbage roots, a technique based on the method of Koske (1982) was used.

Seeds of alfalfa and cabbage were surface-sterilized and germinated in Petri dishes containing moistened filter paper. When roots of the seedlings were 2–3 cm long, plants were transferred to plastic Petri dishes with 3 pieces of filter paper moistened with 5 ml of Long Ashton nutrient solution. Surface sterile spores of *G. mosseae* were placed on an agar plug (2 cm dia × 4 mm) which was attached to the inside of the Petri dish lid directly above the roots. Ten plates were used, containing 10–15 spores per plate. Petri dishes without plants, with nutrient solution or with water, were used as controls.

Table 2. Effect of plant roots exudates on the percentage of germination of *Glomus mosseae* spores

Root exudates from	% Spore germination after weeks	
	1	2
None	20 + 3	52 + 8
Nutrient solution	26 + 9	58 + 9
Alfalfa	30 + 8	73 + 14
Cabbage	28 + 6	54 + 10

Each figure is the mean for 50 replicates. Standard errors of mean are given.

Then the Petri dishes were wrapped in aluminium foil covering spores and roots but not plant shoots and kept inclined on a tray with a 45° angle. Plants were grown in a growth cabinet for 15 days and spore germination percentage was determined after 1 and 2 weeks.

The three experiments were repeated twice under the experimental conditions described and similar results were obtained.

RESULTS

Table 1 shows that in sterilized soil alfalfa plants grown singly and inoculated with soil inoculum of *G. mosseae*, developed more VA infection than inoculated with sporocarps. VA infection was not decreased by the presence of the "non-host" cabbage. In contrast VA infection was reduced when two plants were grown together in sand-vermiculite pots, inoculated with sporocarps, though not when inoculated with soil inoculum.

Non-significant differences in the percentage spore germination (Table 2) in presence of root exudates from alfalfa or from cabbage were found. There was some indications that root exudates from alfalfa increased the percentage spore germination, but this was not statistically significant.

The production of volatile compounds by cabbage plants decreased the percentage of spore germination (Table 3).

DISCUSSION

The inhibitory effect of root extracts of non-host plants on VA mycorrhizal infection of host plants (Ocampo *et al.*, 1986), indicated that the barriers to mycorrhizal infection in non-host plants are intrinsic and more probably related to physiological characteristics of the root cortex or epidermis than to any compounds that might be released by root exudates (Ocampo *et al.*, 1980). From the results of our

Table 3. Effects of gaseous substances of roots on the percentage of germination of *Glomus mosseae* spores

Root gaseous substances from	% Spore germination after weeks	
	1	2
None	24 + 9	55 + 9
Nutrient solution	25 + 10	58 + 13
Alfalfa	26 + 8	60 + 10
Cabbage	2.8 + 0.5	27 + 5

Each figure is the mean for 10 replicates. Standard errors of mean are given.

experiments the absence of inhibitory effect of soluble root exudates of cabbage cannot be discarded because we do not know whether the concentration of exudates was similar to that in the soil, nor whether exudates from older plants are different. However, in other experiments, germination and hyphal growth of VA spores were unchanged either by the age of non-host seedlings at transplanting (Tommerup, 1984) or by the age of non-host plants grown in soil or in sand pots (Azcon and Ocampo, 1984). Thus the reduced rate of spore germination and the decrease of root infection of alfalfa plants, when grown together with cabbage, indicates that inhibitory volatile compounds were produced by cabbage. This effect was influenced by the medium in which the plants were grown. As could happen with the root extracts (Ocampo *et al.*, 1986), the absence of effect of gaseous substances of 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 or by absorption sites on soil particles. The different species of spores and types of inoculum may also have been implicated in the different results obtained by different authors. 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); in contrast, *Glomus fasciculatum* "E3" (Ocampo *et al.*, 1980) and *G. mosseae* (Ocampo *et al.*, 1980; Glenn *et al.*, 1985) which penetrated the root of several Cruciferae tested. VA infection of host plants, grown in sand-vermiculite pots, were inhibited by non-host plants (Table 1), or by its root extracts (Ocampo *et al.*, 1986), only when were inoculated with sporocarps of *G. mosseae*.

Future investigations are needed to elucidate the nature of these volatile inhibitory substances and their possible relationships to the spread of mycorrhizal infection in plant roots.

Acknowledgement—Financial support for this study has been obtained from Comision Interministerial de Ciencia y Tecnologia.

REFERENCES

- Azcon-Aguilar C. and Barea J. M. (1979) An improved procedure for the study of axenic growth of the endomycorrhizal fungus *Glomus mosseae*. *Microbios Letters* **9**, 127–131.
- Azcon R. and Ocampo J. A. (1984) Effect of root exudation on VA mycorrhizal infection at early stages of plant growth. *Plant and Soil* **82**, 133–138.
- Barea J. M., Escudero J. L. and Azcon-Aguilar C. (1980) Effects of introduced and indigenous VA mycorrhizal fungi on nodulation, growth and nutrition of *Medicago sativa* in phosphate-fixing soils as affected by P. fertilizers. *Plant and Soil* **54**, 283–286.
- Giovannetti M. and Mosse B. (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots *New Phytologist* **84**, 489–500.
- Glenn M. G., Chew F. S. and Williams P. H. (1985) Hyphal penetration of *Brassica* (Cruciferae) roots by a vesicular-arbuscular mycorrhizal fungus. *New Phytologist* **99**, 403–472.
- Harley J. L. and Harley E. L. (1987) A check-list of mycorrhiza in the British flora. *New Phytologist* **105**, 1–102.
- Hayman D. S., Johnson A. M. and Ruddlesdin I. (1975) The influence of phosphate and crop species of *Endogone* spores and vesicular-arbuscular mycorrhiza under field conditions. *Plant and Soil* **43**, 489–495.
- Hewitt E. J. (1952) Sand water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureau Technical communication No. 22.
- Iqbal S. H. and Qureshi R. S. (1976) The influence of mixed sowing (cereals and crucifers) and crop rotation on the development of mycorrhiza and subsequent growth of crop under field conditions. *Biologia (Pakistan)* **22**, 287–298.
- Koske R. E. (1982) Evidence for a volatile attractant from plant roots affecting germ tubes of a VA mycorrhizal fungus. *Transaction of the British Mycological Society* **79**, 305–310.
- Mosse B. (1982) The establishment of vesicular-vesicular mycorrhiza under aseptic conditions. *Journal of General Microbiology* **27**, 509–520.
- Newman E. I. and Reddell P. (1987) The distribution of mycorrhizas in families of vascular plants. *New Phytologist* **106**, 745–751.
- Ocampo J. A., Cardona F. L. and El-Atrach F. (1986) Effect of root extracts of non host plants on VA mycorrhizal infection and spore germination. In *Mycorrhizae: Physiology and Genetics* (V. Gianinazzi-Pearson and S. Gianinazzi, Eds), pp. 720–724. INRA, Paris.
- Ocampo J. A., Martin J. and Hayman D. S. (1980) Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. I. Host and non-host plants grown together. *New Phytologist* **84**, 27–35.
- Phillips J. M. and Hayman D. S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizas fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.
- Powell C. Ll. (1980) Effects of kale and mustard crops on response of white clover to VA mycorrhizal inoculation in pot trials. *New Zealand Journal of Agricultural Research* **25**, 461–464.
- Tommerup I. C. (1984) Development of infection by a vesicular-arbuscular mycorrhizal fungus in *Brassica napus* L. and *Trifolium subterraneum* L. *New Phytologist* **98**, 487–495.