



## SHORT COMMUNICATION

SPREADING OF *GLOMUS MOSSEAE*,  
A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS,  
ACROSS THE RHIZOSPHERE OF HOST  
AND NON-HOST PLANTSH. VIERHEILIG,<sup>1</sup>\* M. ALT,<sup>1</sup> P. MÄDER,<sup>2</sup> T. BOLLER<sup>1</sup> and A. WIEMKEN<sup>1</sup><sup>1</sup>Department of Botany, University of Basel, Hebelstr. 1, 4056 Basel, Switzerland and <sup>2</sup>Research Institute of Biological Agriculture, Bernhardsberg, 4104 Oberwil, Switzerland

(Accepted 17 January 1995)

Incompatibility is observed between the generalistic, obligately symbiotic vesicular-arbuscular mycorrhizal (VAM) fungi and certain plant species (non-host plants). It has been hypothesized that non-host species fail to produce the necessary signals required by VAM fungi for successful invasion or that they are resistant to the invasion by VAM fungi because of the presence or production of inhibitory compounds (Koide and Schreiner, 1992).

In the Brassicaceae, a non-mycorrhizal family, substances released by roots have been shown to reduce VAM spore germination (El-Atrach *et al.*, 1989; Schreiner and Koide, 1993b). Gianinazzi-Pearson and Gianinazzi (1992) reported that intergeneric grafting between the mycorrhizal non-host plant lupin and the host plant pea induced the failure of a VAM symbiosis in pea roots, suggesting the presence of an inhibitory factor produced in the lupin shoot. However, studies on spore germination and hyphal growth of the VAM fungus *Glomus mosseae* in the presence of lupin root exudates showed neither stimulating nor inhibitory substances (Avio *et al.*, 1990; Giovannetti *et al.*, 1993). Little information is available about the causes for the incompatibility of the Chenopodiaceae with VAM fungi: substances released by root cultures of sugarbeet neither promoted nor inhibited hyphal growth (Bécard and Piché, 1990). However, root extracts of spinach inhibited spore germination of VAM fungi (Vierheilig and Ocampo, 1990a).

Our purpose was to investigate the hyphal growth and spreading of VAM fungi through the rhizosphere of mycorrhizal host or non-host plants.

The following plant material was used: soybean (*Glycine max* L. cv. Maple Arrow); tomato (*Lycopersicon esculentum* L. cv. Supermarmande); rape (*Brassica napus* L. cv. Jet Neuf, a rape 0 cultivar with low contents of erucic acid but normal amounts of glucosinolates; and cv. Arabella, a rape 00 cultivar with low amounts of both erucic acid and glucosinolates); spinach (Chenopodiaceae; *Spinacea oleracea* L. cv. Butterfly); and lupin [*Lupinus albus* cv. Blanca (bitter)]. All plants were grown in the greenhouse (day-night cycle: 14 h, 27°C/10 h, 20°C) in a steam-sterilized (40 min, 121°C) mixture of sand and loam (1:1 v/v). Seeds of plants were surface sterilized by soaking in 0.75% sodium

hypochlorite for 5 min, rinsed with tap water and germinated in vermiculite.

A three-compartment container system similar to the one developed by Wyss *et al.* (1991) was used. The shallow compartments (2 cm wide) were separated by vertical nylon screens (mesh size 60 µm) that could be penetrated by hyphae but not by roots [see Wyss *et al.* (1991) for details]. The three compartments containing a 1:1 (v/v) mixture of sand and loam autoclaved prior to the experiment, were joined at the beginning of the experiment (time 0) (Fig. 1). At the beginning of the experiment the first compartment (A = inoculum compartment), which served as a donor of the VAM fungus, contained a soybean plant (4 weeks old) that had been colonized by *G. mosseae* for 3 weeks. The second compartment (B = test compartment) contained soil free of VAM fungi, either without any plants (control) or with a test plant (tomato, rape, spinach or lupin). The test plants had been grown for 4 weeks at the beginning of the experiment; for controls, soil had been kept under the same conditions without plants. The third compartment (C = acceptor plant compartment) contained a soybean as an "acceptor plant". This acceptor plant had been planted at an age of 1 week and had been grown for a week while its compartment was tilted by an angle of 45°, so that the nylon screen joining the second compartment was covered with roots at the beginning of the experiment. These roots could be immediately colonized by the hyphae traversing the test compartment. Under the experimental conditions, the hyphae of *G. mosseae* had to traverse the second compartment (2 cm wide) with the rhizosphere of host or non-host plants in order to reach the root system of the acceptor plant (Fig. 1). Thus, formation of mycorrhizal structures in the root system of the acceptor plants in the third compartment was assessed as an indication of the spread of the VAM fungus through the second compartment.

Roots were carefully washed, cleared and stained according to the method of Phillips and Hayman (1970). VAM root colonization was estimated by the gridline intersect method (Giovannetti and Mosse, 1980) with a binocular microscope at a magnification between ×40 and ×50.

All experiments were repeated 3 times using 3–4 replicates per treatment. Means and standard error of means (SEMs) of a typical experiment are shown in Fig. 2. In the three-compartment system employed, the root system of an acceptor soybean plant could be colonized by *G. mosseae*

\*Author for correspondence, presently at: Faculté de Forêt et de Géomatique, Université Laval, Pavillon Marchand, Québec, Canada G1K-7P4.

spreading from an inoculum plant across a 2 cm wide soil compartment free of roots (Fig. 2, control). Since after initial colonization the acceptor plant can be further colonized by spreading within the plant, finally in all treatments the same degree of colonization will be reached. Therefore, the first harvesting dates are of special interest. After 13 days, <5% of the root system contained mycorrhizal structures; when the mycorrhizal inoculum plant was adjacent to the acceptor plant, a similar degree of colonization was reached after 3–4 days (Vierheilig *et al.*, 1994), indicating that it took the fungus about 9–10 days to cross the root-free soil compartment. Thereafter, the percentage of the root system colonized increased throughout the experiment, probably by spreading within the acceptor plant as well as by new colonization across the root-free compartment. When tomato, a host plant for VAM fungi, was grown in the compartment between the inoculum and the acceptor plant, the roots of the acceptor plants showed a much higher colonization than the controls after 13 days [Fig. 2(a)]. This indicates that the root system of a host plant promotes hyphal spreading [colonization of the tomato root system in the second container was assessed in separate experiments; at least 10% were colonized within 13 days (data not shown)]. Surprisingly, with tomato in the second compartment, the percentage of mycorrhizal colonization of the soybean acceptor plants in the third compartment increased less quickly than in controls at a later stage of the experiment, so that the degree of mycorrhizal formation

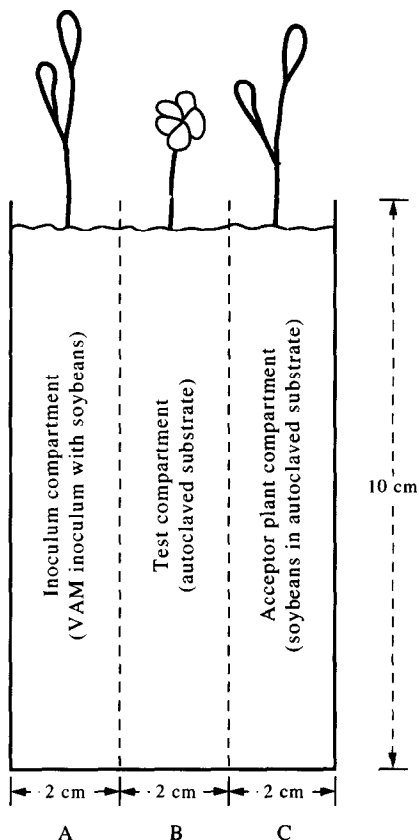


Fig. 1. Experimental design and container system used: (A) VAM inoculum compartment with soybean plants; (B) test compartment with different VAM fungi host and non-host plants or without plant; (C) acceptor plant compartment with soybean plants which were harvested sequentially and checked for VAM colonization. The compartments were separated by a 60  $\mu$ m mesh nylon screen (----) which can be penetrated by hyphae, but not by roots.

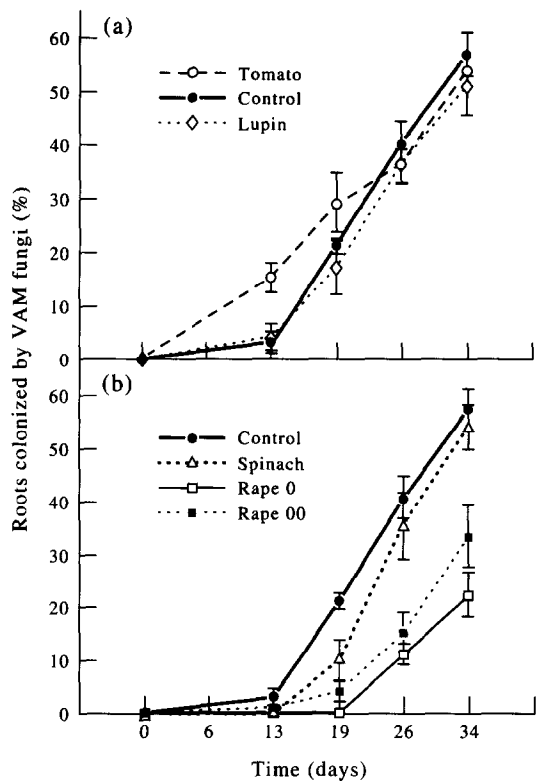


Fig. 2. Time course of colonization of the soybean acceptor plants by hyphae of *G. mosseae* spreading across a rhizosphere soil compartment containing (a) tomato (host plant), lupin (non-host plant) or no plants (control); or (b) spinach and rape (non-host plants) or no plants (control). Data are means  $\pm$  SEMs.

became similar after 26 days under both conditions [Fig. 2(a)].

Different results were obtained when different non-host plants were growing in the compartment between the inoculum plants and the acceptor plant. It has been reported before that the non-host plants lupin and spinach produced some mycorrhizal structures in their root system when inoculated with *G. mosseae* and there was evidence for a root response in all the 3 non-host plants, as assessed by measuring biosynthesis of ethylene and the activities of chitinase and glucanase in inoculated roots (Vierheilig *et al.*, 1994). However, in our experiments, mycorrhizal structures were not found in any of the non-host plants studied.

In the presence of a lupin plant, there was no difference in colonization of the acceptor plant compared to controls [Fig. 2(a)], indicating that the rhizosphere of lupins does not interfere with spreading of VAM fungi. It has already been reported that lupin roots neither elicit nor inhibit the germination and hyphal elongation of *G. mosseae* (Avio *et al.*, 1990; Giovannetti *et al.*, 1993) and it was assumed that they lack factors promoting or inhibiting hyphal growth in the rhizosphere. However, root exudates of lupin have been reported to hinder hyphal attachment and fungal recognition of roots (Giovannetti *et al.*, 1993).

When a spinach plant was growing in the compartment between the donor and the acceptor plant, there was an initial delay of colonization, with no mycorrhizal structures observed after 13 days [Fig. 2(b)]. However, colonization occurred later with a similar rate as in controls, indicating that spinach inhibited only spreading but did not affect

colonization once the hyphae had reached the acceptor plants. It remains to be seen what factors in the spinach rhizosphere are inhibitory to hyphal spreading. In reports of the effect of spinach root cultures on spore germination (Schreiner and Koide, 1993b) and of sugarbeet root cultures on hyphal growth (Bécard and Piché, 1990) no inhibitory effect could be observed. However, root exudates might have a different effect in the soil. In *Salsola kali* (Allen *et al.*, 1989) roots turned brown after inoculation with VAM fungi, indicating an accumulation of phenolic compounds with a possible inhibitory effect on the mycorrhizal fungus. Spinach root extracts have been shown to have an inhibitory effect on spore germination (Vierheilig and Ocampo, 1990a).

Rape plants growing between the donor and the acceptor plants had the strongest effect on the colonization of the acceptor plants [Fig. 2(b)]. Colonization was considerably delayed, with no mycorrhizal structures in the roots after 13 days for rape 00 or even after 19 days for rape 0. Progress of colonization appeared to be slightly inhibited even after 26 and 34 days (see the slopes of colonization curves). The rape 0 cultivar, known to have a high content of glucosinolates, compounds which can be metabolized to fungitoxic substances (isothiocyanates) known to inhibit VAM spore germination (Vierheilig and Ocampo, 1990b; Schreiner and Koide, 1993a), had a somewhat stronger inhibitory effect than the rape 00 cultivar with a low glucosinolate content. Vierheilig and Ocampo (1990a) have shown that extracts of roots of the rape 0 with a high glucosinolate content had a stronger inhibitory effect on spore germination than those from rape 00 with a lower glucosinolate content. This indicates that glucosinolates (or the isothiocyanates released from them) contribute to the inhibitory effect of the rape rhizosphere on mycorrhizal spreading. However, in alfalfa (host plant) grown in soil together with cabbage (non-host plant), VAM colonization in the alfalfa plant was not depressed, although spore germination was reduced in presence of cabbage roots (El-Atrach *et al.*, 1989). Apparently the inhibitory effect on hyphal growth and spore germination of the Brassicaceae does not affect the degree of colonization of a near by grown host plant.

In conclusion, our three-compartment system, in the configuration presented here, provides a functional assay for growth and spreading of mycorrhizal fungi in root-free soil and in the rhizosphere. The results demonstrate that the rhizospheres of some but not all non-host plants interfere with spreading of mycorrhizal fungi from a donor to an acceptor plant indicating the presence of inhibitory compounds in the rhizosphere.

*Acknowledgements*—Dr P. Römer (Süddeutsche Saat-zucht, Rastatt, Germany) kindly donated seeds of lupin. We thank Dr J. A. Ocampo (Estacion Experimental del Zaidin, Granada, Spain) and E. Wilson for helpful discussion and M. Schneider (Department of Botany, Basel) for constructing the inoculation containers. This work was supported by a grant from the Ciba-Geigy Foundation to H. Vierheilig and by the Swiss National Foundation (to T. Boller and A. Wiemken).

## REFERENCES

- Allen M. F., Allen E. B. and Friese C. F. (1989) Responses of the nonmycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **111**, 45–49.
- Avio L., Sbrana C. and Giovannetti M. (1990) The response of different species of *Lupinus* to VAM endophytes. *Symbiosis* **9**, 321–323.
- Bécard G. and Piché Y. (1990) Physiological factors determining vesicular-arbuscular mycorrhizal formation in host and nonhost Ri T-DNA transformed roots. *Canadian Journal of Botany* **68**, 1260–1264.
- El-Atrach F., Vierheilig H. and Ocampo J. A. (1989) Influence of non-host plants on vesicular-arbuscular mycorrhizal infection of host plants and on spore germination. *Soil Biology & Biochemistry* **21**, 161–163.
- Gianinazzi-Pearson V. and Gianinazzi S. (1992) Influence of intergeneric grafts between host and non-host legumes on formation of vesicular-arbuscular mycorrhiza. *New Phytologist* **120**, 505–508.
- Giovannetti M. and Mosse B. (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.
- Giovannetti M., Avio L., Sbrana C. and Citernesi A. S. (1993) Factors affecting appressorium development in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd & Trappe. *New Phytologist* **123**, 115–122.
- Koide R. T. and Schreiner R. P. (1992) Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 557–581.
- Phillips J. M. and Hayman D. S. (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.
- Schreiner R. P. and Koide R. T. (1993a) Antifungal compounds from the roots of mycotrophic and non-mycotrophic plant species. *New Phytologist* **123**, 99–105.
- Schreiner R. P. and Koide R. T. (1993b) Mustards, mustard oils and mycorrhizas. *New Phytologist* **123**, 107–113.
- Vierheilig H. and Ocampo J. A. (1990a) Role of root extract and volatile substances of non-host plants on vesicular-arbuscular mycorrhizal spore germination. *Symbiosis* **9**, 199–202.
- Vierheilig H. and Ocampo J. A. (1990b) Effect of isothiocyanates on germination of spores of *G. mosseae*. *Soil Biology & Biochemistry* **22**, 1161–1162.
- Vierheilig H., Alt M., Mohr U., Boller T. and Wiemken A. (1994) Ethylene biosynthesis and activities of chitinase and  $\beta$ -1,3-glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. *Journal of Plant Physiology* **143**, 337–343.
- Wyss P., Boller T. and Wiemken A. (1991) Phytoalexin in response is elicited by a pathogen (*Rhizoctonia solani*) but not by a mycorrhizal fungus (*Glomus mosseae*) in soybean roots. *Experientia* **47**, 395–399.