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Germination, Early Mycelial Growth and Infectivity of a Vesicular-Arbuscular Mycorrhizal Fungus in Organic Substrates

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Abstract

The germination of *Glomus mosseae* and the infectivity of vesicular-arbuscular mycorrhizal (VAM) mycelium developed from resting spores were tested in three organic substrates, a sphagnum peat (SP), a composted pine bark (CPB) and a composted olive pumice (COP).

The percentage of germination was unaffected by the substrate after 2 weeks of incubation at 25°C. The production of vegetative spores per germinated resting spore was not different from the control treatment, sterilized sandy soil (SSS), although the number of vegetative spores produced was significantly higher in both composts than in peat (SP).

When *G. mosseae* spores were incubated for 4 weeks in the substrates, VAM colonization in the roots of marigold (*Tagetes erecta* L.) and onion (*Allium cepa* L.) seedlings was only apparent in SSS and in CPB after 3 weeks of growth. In pasteurized organic substrates, all seedlings became mycorrhizal. When the incubation period for spores was reduced to 2 weeks, seedlings were also not infected in unsterilized COP and SP. The inoculation of pasteurized COP with *Aspergillus fumigatus*, a saprophytic fungus isolated from COP, had a detrimental effect on the infectivity of VAM mycelium.

Keywords: vesicular-arbuscular mycorrhizae, soiless media

1. Introduction

Resting spores of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae* germinate readily in the soil under adequate conditions (moisture and temperature). When organic substrates are used as growing medium for plants, especially ornamentals, horticultural and fruit tree species, a negative effect has been reported on the development of VAM symbiosis (Biermann and Linderman, 1983). The presence of inhibitors, chemical or biological, in composts (Hoitink and Fahy, 1986) and the high nutrient content in peat (Graham, 1984) have been reported as possible causes for the lack of VAM infection in the roots of container-grown plants in organic media.

In this study, the germination *in vivo* of the VAM fungus *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe in 3 organic substrates was tested, and the potential infectivity of the VAM mycelium developed from resting spores was measured after a period of incubation in the substrates in the absence of a host plant.

2. Materials and Methods

Spores of *Glomus mosseae* obtained from Rothamsted Experimental Station, UK, were excised from sporocarps collected by wet-sieving the soil from heavily infected *Trifolium repens* cultures. The spores were then rinsed thoroughly in sterile distilled water several times and kept at 4°C on moistened filter paper for 4 days.

The germination, early mycelial growth and infectivity of the fungus were assessed in three different organic substrates: a sphagnum peat (SP, Floratorf-500) and two composts, composted pine bark (CPB, IMC-2 Bark products Ltd.) and composted olive pumice (COP) from raw olive pumice (Calvet et al., 1985). Both composts were suppressive for fusarium wilt of carnation (Pera and Calvet, 1989). The pH of SP was adjusted to 7 with $CaCO_3$. The pH of CPB and COP were 7.6 and 7.4 respectively and were not modified. In the control treatments, a sterilized sandy soil of neutral pH, moderate P (12 mg/kg) and low organic matter content (below 1%) was used.

Experiment 1

After cold storage, spores were placed between 2 Millipore membrane filters (8 μm pore size and 45 mm diam.) which were held together by a photographic slide binder (Hepper, 1979). The slide binders were buried 10 mm deep in the moistened organic substrates or sandy soil in a glass petri dish (90 mm diam.,

19 mm depth) covered with aluminum foil and kept for 15 days at 25°C. Then they were washed free of substrate and submerged in 0.05% Trypan blue in lactic acid for 5 min, to stain VAM mycelium grown from germinated resting spores. Slide binders were then opened and Millipore membranes were separated. Spore germination and the production of vegetative spores per germinated resting spore were assessed under the dissecting microscope. There were 3 replicates per substrate, with 30 spores each. The experiment was repeated three times and data were analyzed by a one-way ANOVA and Tukey's multiple range test ($p = 0.01$).

Experiment 2

The infectivity of *G. mosseae* after a period of incubation in the organic substrates was assessed. Following cold storage, spores were placed on 1 cm^2 portions of the fine filter paper and then buried 2 cm deep in moistened substrate in 100 ml pots. Forty spores per pot (2 paper portions with 20 spores each) were used as an inoculum source. Pots were then covered with tissue to avoid external contamination and kept at 25°C in the greenhouse. The substrate was moistened when needed with distilled water. After an incubation period (see below), a one week old seedling germinated in sterile quartz sand was transplanted to each pot. After 3 weeks growth, plants were removed from the soil, and roots were cut and stained (Phillips and Hayman, 1970) to assess VAM infection. There were 3 replicates per treatment (substrate) for each plant species.

Initially the incubation period of spores in the substrate was 4 weeks and 2 VA-mycorrhizal species were used as host plants: marigold (*Tagetes erecta* L.) and onion (*Allium cepa* L.). Later the organic substrates were pasteurized by heating them to 100°C for 30 min (on 2 consecutive days) before introducing the VAM spores. Marigold seedlings were transplanted as test plants. In order to check the effect of the incubation period on VAM infectivity, spores were incubated in the substrates for 2 weeks. Only two substrates, COP and SP, were used when the incubation period was reduced to 2 weeks because plants had not become infected by the VAM fungus in these substrates after 4 weeks incubation of resting spores (Table 2).

Pasteurized COP and SP also were inoculated with saprophytic fungi isolated by plate dilution tests from the same substrates, *Aspergillus fumigatus* and *Penicillium decumbens* respectively, to check their direct effect on the infectivity of *G. mosseae* in dual inoculation. Inocula were prepared as sterile conidial suspensions from PDA tube cultures for each fungus. Dilution was adjusted with sterile distilled water to obtain the density found in the

Table 1. Germination of *Glomus mosseae* resting spores in organic substrates

Substrate	No. of spores assessed	No. of germinated spores	germination %
Sterilized sandy soil (SSS)	230	216	93.91
Composted olive pumice (COP)	216	185	85.65
Composted pine bark (CPB)	255	235	92.16
Sphagnum peat	227	206	90.75

original substrates and measured by a plate dilution test: 5.74×10^6c.f.u./ml for *A. fumigatus* and 1×10^5c.f.u./ml for *P. decumbens*. Pots inoculated with the saprophytes were incubated for 1 week at 25°C. Then *G. mosseae* spores in filter papers were buried in the substrates and 4 weeks later, one week old marigold seedlings were transplanted. VAM infection was recorded in the roots after 3 weeks growth.

3. Results

Experiment 1

G. mosseae spore germination was unaffected by the substrate used as the percentage recorded was around 90% after 2 weeks incubation at 25°C in all cases (Table 1). Organic substrates did not stimulate nor inhibit the production of vegetative spores on the VAM mycelium developed from resting spores compared with the control treatment, SSS, although the production of vegetative spores in both composts, COP and CPB, was significantly higher than in peat (Fig. 1).

Experiment 2

VAM infection in the roots of marigold and onion was only apparent after 3 weeks growth in SSS and CPB (Table 2). No infection was detected in the roots of plants grown in SP and COP. When the organic substrates had been previously pasteurized, plants grown in SP and COP became mycorrhizal also.

When *G. mosseae* spores were incubated for only 2 weeks in unsterilised substrates, marigold plantlets did not become infected by the VA fungus in COP. Entry points plus a slight arbuscular infection were observed in the roots of plants grown in SP (Table 2).

No VAM colonization was detected when sterilized COP was inoculated with *A. fumigatus* whilst *P. decumbens* did not affect the VAM infection of pasteurized SP compared with the same uninoculated substrate (Table 2).

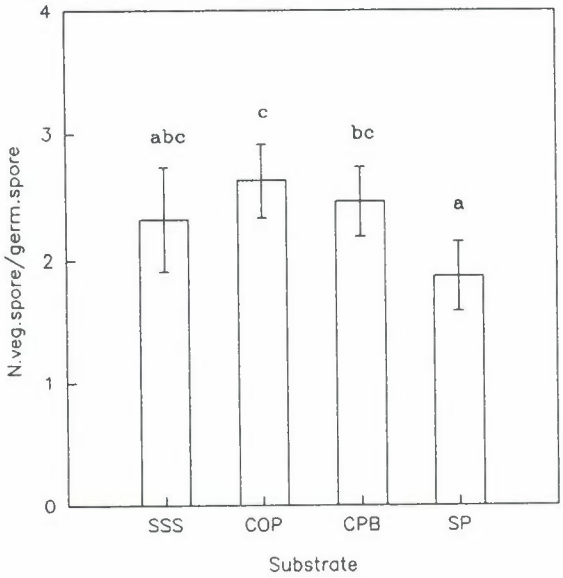

Figure 1. Production of vegetative spores per germinated resting spore of *Glomus mosseae* in organic substrates. SSS: sterilized sandy soil, COP: composted olive pumice, CPB: composted pine bark, SP: sphagnum peat. Bars with identical letters are not significantly different after Tukey's multiple range test ($p = 0.01$).

Table 2. VAM infectivity in organic substrates after incubation of *Glomus mosseae* resting spores

Host plant	Substrate	Incubation period (weeks)	Mycorrhizal infection
Onion (*Allium cepa* L.)	SSS	4	+
	CPB	4	+
	COP	4	–
	SP	4	–
Marigold (*Tagetes erecta* L.)	SSS	4	+
	CPB	4	+
	Pasteurized CPB	4	+
	COP	4	–
	COP	2	–
	Pasteurized COP	4	+
	Pasteurized COP + *Aspergillus fumigatus*	4	–
	SP	4	–
	SP	2	*
	Pasteurized SP	4	+
	Pasteurized SP + *Penicillium decumbens*	4	+

SSS: Sterilized sandy soil, CPB: composted pine bark, COP: composted olive pumice, SP: sphagnum peat. *Entry points

4. Discussion

The organic substrates studied in this work did not inhibit VAM fungus germination or early mycelial growth but some did have a negative effect on the establishment of VAM symbiosis. The experiment 1 set-up allowed a free interaction between the substrates and the *G. mosseae* spores, and no effects were found on spore germination nor the early mycelial growth when compared with the results obtained in sandy soil. The establishment of VAM colonization in both host plants was negatively affected by two of the substrates studied: COP and SP. When these substrates were pasteurized the inhibition factor was removed, suggesting a biological nature for this inhibition. The addition of a fungus previously isolated from COP, *Aspergillus fumigatus*, to the pasteurized COP reestablished the inhibition, proving that this fungus was one of the factors involved in the negative interaction between COP and VAM symbiosis establishment. The addition of *Penicillium decumbens*, one of the fungi isolated from SP, to pasteurized SP did not reestablish the inhibition, thus this fungus is not associated with the inhibition characteristics of the substrate. In CPB, the only organic substrate studied that did not affect VAM establishment, the fungus isolated was *Trichoderma harzianum*, an isolate that highly stimulated germination and mycelial growth of *G. mosseae in vitro* (Calvet et al., 1989). These results clarify some of the points raised by Wilson et al. (1988) when they found suppression of plant growth and root colonization in non-sterile soil. This suppression which Sylvia and Schenck (1983) ascribed to a group of saprophytic fungi of the genera *Fusarium, Penicillium* and *Trichoderma* on germination of *G. clarum*, has to occur at the later events of the pre-infection stage because neither the germination nor the early mycelial growth was affected by the substrates but there was a definite biological inhibition of SP and COP that prevented the VAM symbiosis establishment in these substrates.

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