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Sporulation of Four Arbuscular Mycorrhizal Fungi Isolates inside Dead Seed Cavities and Glass Capillaries

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Abstract

A hypothesis was tested if intensive sporulation of arbuscular mycorrhizal fungi (AMF) in dead empty seeds of *Chenopodium album* L. could be an adaptation of AMF from polluted soils to adverse soil conditions, or if it can occur also in a substrate without pollution stress and with isolates from non-polluted soils. The effect of dead seeds on sporulation was compared for two indigenous and two non-indigenous AMF isolates and for two substrates: the soil from the sedimentation pond of a pyrite smelter and zeolite as a reference substrate without pollution stress. The results suggest that sporulation inside the seed cavities is not an adaptation of indigenous AMF isolates to avoid adverse conditions in polluted soil, but probably represents an isolate-specific ecological feature, which can be affected further by soil conditions.

Keywords: *Chenopodium album* seeds, *Glomus*, indigenous AMF isolates, polluted soil, sporulation

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1. Introduction

During field sampling on waste deposits from the pyrite smelter and those of power station fly ash, abundant spores of AMF were found in the cavities of dead seeds of the non-host plant species *Chenopodium album*, one of the first colonizers on such sites. Sporulation in dead seeds or root fragments can probably be advantageous for AMF because they would be more hidden from parasitic microorganisms or predatory larvae and these sites provide a good physical shelter for spores (Daniels-Hetrick, 1984). In literature, several reports can be found on sporulation of AMF at other specific places, such as in nematode cysts (Daniels-Hetrick, 1984), in *Rhizobium* nodules of clover and alfalfa (Vidal-Dominguez et al., 1994) or within dead spores of other AMF (Muthukumar and Udaiyan, 1999). Taber (1985) suggested that sporulation in various seeds might play a specific role in the life strategy of the AMF. The seeds can contain nutrients or provide more favourable environment for spores than bulk soil, and they can create a specific so-called mycospermal ecological niche. True reasons for this phenomenon have not been fully clarified yet. Stimulation effects of organic matter amendment on the development of AMF, especially the extraradical mycelium (ERM), could be explained by the improvement of physical properties of the growth substrate, especially the soil porosity (Joner and Jakobsen, 1995a).

In addition, AMF are probably able to exploit nutrients released by mineralisation of organic matter due to activities of mineralising microorganisms, as suggested by Joner and Jakobsen (1995b). Sporulation inside the seeds also could be directly stimulated by bacteria inhabiting the seed cavity. Some bacteria were reported to stimulate development of AMF and their ERM (Azcón-Aguilar and Barea, 1985; Gryndler et al., 1995; Gryndler and Vosátka, 1996). However, results regarding specifically the effects of bacteria on AMF sporulation are very scarce (von Alten et al., 1993) and are restricted to observation of new spores formation on mycelia growing from germinating resting spores on water-agar media (Azcón, 1987; Tobar et al., 1996).

In a previous study (Rydlová and Vosátka, 2000) it was shown that an amendment of the growth substrate with dead seeds of *Chenopodium album* significantly increased sporulation of indigenous AMF in the soil from the waste deposits of a pyrite smelter. Mechanism of the enhancement was an intensive sporulation of AMF inside the dead seed cavities. Following the study, two isolates of indigenous AMF have been isolated from soil used in the experiment mentioned above: *Glomus* "cluster-forming" sp. BEG140 and *G. claroideum* BEG96. Since sporulation of a non-indigenous AMF *Glomus mosseae* BEG25 was not affected by the seed amendment and there was no substantial increase of AMF sporulation due to amendment of crushed seeds, a hypothesis was postulated that the sporulation inside the seeds was an adaptation of

indigenous AMF to avoid adverse conditions in the polluted soil, and that it is probably not attributed only to increased AMF proliferation in the presence of organic matter as shown previously (St. John et al., 1983; Hepper and Warner, 1983; Warner, 1984; Joner and Jakobsen, 1995a). Specific strategies of AMF from zinc waste deposits were observed by Turnau (1998). She found more intensive intraradical sporulation of AMF on waste deposits as compared to control non-contaminated sites. In addition, intraradical spores did not usually contain heavy metals, and the mycelium emerging from such spores was often growing intraradically towards freshly formed roots, using dead roots as tunnels of much lower toxicity as compared to the surrounding soil.

The first aim of our study was to find whether sporulation in dead seeds could be an adaptation of indigenous AMF from polluted soils to adverse soil conditions or if it can occur also in inert substrate without pollution stress and in isolates from non-polluted soils. Secondly, the study addressed the question whether only the cavity or other specific components of the seed interior determine sporulation inside the seeds.

2. Materials and Methods

The experiment was conducted in sterilised soil (121°C and 100 kPa for 25 min in two consecutive days) taken from plot CH2 of the Chvaletice sedimentation pond containing waste material from the pyrite smelter factory (for site description see Rydlová and Vosátka, 2000) and a reference substrate zeolite (clinoptilolite). Characteristics of the substrates are given in Table 1.

Substrates were filled into 1 l pots with four 100 ml lateral hyphal compartments (hyphoboxes) separated from the central pot by a nylon mesh (opening size 42 μm) allowing the growth of ERM but not the roots into the hyphoboxes. Hyphal compartments were separated from the roots in order to reduce enrichment of the substrate with organic compounds from root exudates and decaying roots of the host plant. Substrate in each pot was inoculated with one of the following isolates: *Glomus* "cluster-forming" sp. BEG140, *G. claroideum* BEG96 (both isolated from the Chvaletice sedimentation pond), *G. intraradices* BEG75 and *G. claroideum* BEG23 (both from non-polluted soils). *G. intraradices* was chosen as a reference species for *G.* sp. BEG140 because of similar morphology and ecology of both AMF. Every pot received 2 g of inoculum, which consisted of the mixture of colonised root fragments, the ERM and spores. There were 5 replicates in each treatment. Microflora from the original non-sterile soil was reintroduced to CH2 soil by addition of the filtrate from 100 g of soil shaken for 30 min in 1 l of deionised water. Surface sterilised seeds of the host plant *Agrostis capillaris* L. (10% NaOCl, 10 min) were sowed to each pot.

Table 1. Chemical characteristics of the substrates. Available concentrations are given for macroelements and metals. nd – not determined.

	Chvaletice soil	Zeolite
pH (H_2O)	6.6	5.3
pH (KCl)	6.2	5.3
C (%)	1.3	0.1
N (%)	0.07	<0.01
C/N	18.4	nd
[1]P (mg.kg^{-1})	14.0	0.5
[2]Ca (mg.kg^{-1})	2,560	13,330
[2]Mg (mg.kg^{-1})	83.6	178.0
[2]K (mg.kg^{-1})	84.1	9,747
[2]Na (mg.kg^{-1})	36.5	361.6
[3]Fe (mg.kg^{-1})	15.1	5.7
[3]Mn (mg.kg^{-1})	38.4	0.1
[2]Zn (mg.kg^{-1})	27.5	0.0

[1]Extracted by sodium hydrogen-carbonate. [2]Extracted by ammonium acetate at pH 7.0. [3]Extracted by ammonium acetate at pH 4.8.

Two weeks after seedling emergence, the plants were thinned to 20 per pot. After six months of plant growth in the greenhouse, hyphoboxes were filled with the same substrate as the central pots. Substrate in the first hyphobox (S) was thoroughly mixed with dead empty seeds (i.e. without endoderm) of *Chenopodium album* L. (30 seeds per 1 ml of soil; for preparation of the seeds see Rydlová and Vosátka, 2000). Substrate in the second hyphobox (C) was mixed with glass capillaries imitating the seed cavity (1.2 pcs of capillaries per 1 ml of soil – outer diameter 1.6 mm, inner diameter 1.00 mm, average length 7.4 mm). It was not possible to keep the same numbers of seeds and capillaries per 1 ml of soil due to larger size of the latter (although inner diameters were approximately the same). The length of the capillary was chosen to preserve a free inner space despite partial filling of both capillary endings with soil. In the third hyphobox (S+C), substrate was amended with the mixture of seeds and capillaries (15 seeds and 0.6 pcs of capillaries per 1 ml). Substrate in the fourth hyphobox (U) was left unamended.

There were 3 harvests of the experiment (8, 20 and 45 weeks after the hyphoboxes establishment). At every harvest, a 15-ml soil core was taken from central pot for evaluation of mycorrhizal colonisation. The roots were washed free from soil and stained with 0.05% solution of Trypan blue in lactoglycerol (modified from Kormanik and McGraw, 1982). Percentage of colonised root

length was evaluated by gridline intersect method (Giovannetti and Mosse, 1980) under a stereomicroscope at 40× magnification. At every harvest, 20-ml soil samples were taken from the hyphoboxes S, C and S+C. The soil samples were carefully wet-sieved through a 0.5 mm sieve and 30 randomly chosen seeds and/or all capillaries were collected. The seeds were placed on wet filter paper inserted in a Petri dish. Each seed was dissected using two preparation needles and the spores inside the seed were counted under a stereomicroscope at 25× magnification. The capillaries were placed in water drops in a Petri dish. The content of every capillary was carefully removed by a dissecting needle and number of spores was counted. Average numbers of spores per one seed and one capillary were calculated.

Besides the above-mentioned procedures, number of spores in bulk soil and length of the ERM were evaluated in all hyphoboxes (S, C, S+C, U) at the third harvest. The remaining substrate in each hyphobox was mixed by hand. For evaluation of AMF sporulation in bulk soil, a 5-ml subsample of soil was wet-sieved through 3 sieves (0.5, 0.2 and 0.036 mm) for hyphoboxes S, C, and S+C or through 2 sieves (0.2 and 0.036 mm) for hyphobox U. Soil particles on the 0.5 mm sieve were carefully passed through in order not to break the seeds or capillaries. Material from the 0.2 mm sieve was homogenised in a blender for 20 s to release the spores from the ERM. The spores were extracted from the bottom sieve by centrifugation at 3,000 rpm for 5 min in a 50% sucrose solution. Supernatant from the centrifugation tube was washed on the 0.036 mm sieve under tap water and filtered through Whatman filter paper No 1 with 1 cm square grid. The number of spores was evaluated under a stereomicroscope at 40× magnification. No quantification of intraradical spores of *Glomus* sp. BEG140 and *G. intraradices* BEG75 was done, so that it was not possible to compare total spore numbers of these isolates with that of both *G. claroideum* isolates.

For evaluation of the ERM length, a 5-g sub-sample was put into a household blender with 500 ml of H_2O and blended for 30 s. One ml of the supernatant was pipetted onto Whatman membrane filter (25 mm diameter, pore size 0.45 m) and vacuum filtered. The ERM on the filter was stained with 0.05% solution of Trypan blue in lactoglycerol. The whole filter was scanned and total length of ERM was evaluated under a microscope at 200x magnification according to Brundrett et al. (1994). The length of the ERM was expressed in meters of total hyphae per 1 g of dry substrate.

Data were analysed by ANOVA (SOLO 4.0/BMDP Statistical Software), checked for normality and significant differences calculated where F-values of treatments were significant using Duncan Multiple Range test. Where data were found to be non-normally distributed, non-parametric Kruskall-Wallis and Connover tests were used to calculate Chi-squared values for evaluating main effects.

3. Results

Sporulation within the cavities

Both average spore number per one seed or capillary and frequency of seeds or capillaries containing spores increased significantly from harvest 1 to harvest 2 (P=0.001) and they remained almost unchanged at harvest 3. Spore formation within the cavities was significantly higher in the soil than in zeolite (P=0.001). The most intensive sporulation within the seeds was found for indigenous *G.* sp. BEG140 (Fig. 1) where the highest spore numbers per one seed and the highest frequency of seeds containing spores were found, although non-indigenous *G. intraradices* BEG75 reached comparable values in some soil treatments (Tables 2 and 3). Spore numbers of indigenous *G. claroideum* BEG96 inside the seed cavities were negligible in either substrate. *G.* sp. BEG140 and *G. claroideum* BEG23 sporulated also inside the capillary cavities in the soil (Fig. 2). Although their spore numbers formed per one capillary were similar (Table 2), only for *G. claroideum* BEG23 were most of the capillaries (about 80%) occupied by spores at harvests 2 and 3 (Table 3). Almost no spores were found inside the capillaries in zeolite irrespectively of the AMF isolate.

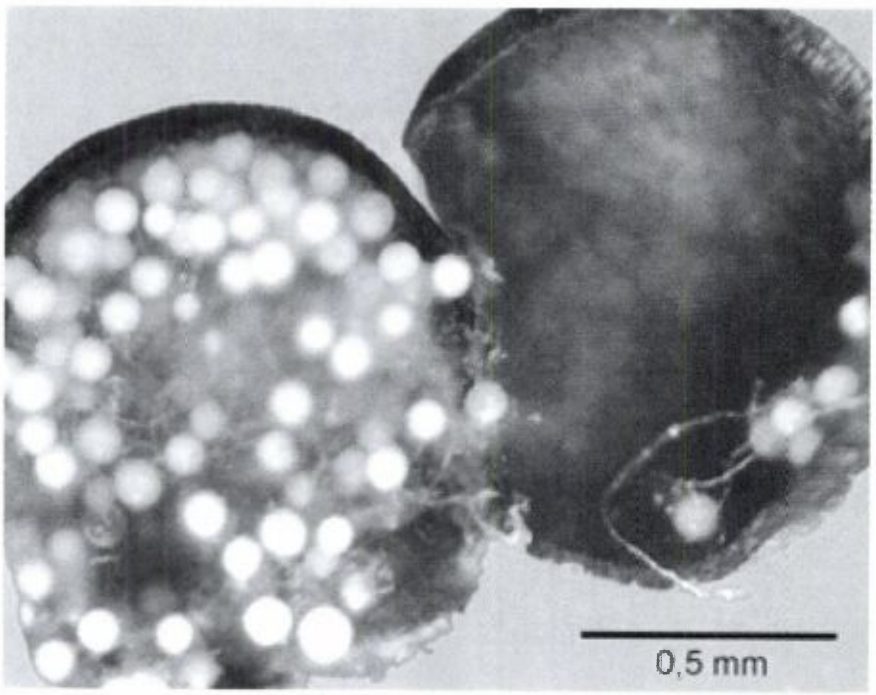


Figure 1. Broken seed of *Chenopodium album* with spores of *Glomus* sp. BEG140.

Table 2. Comparison of mean spore numbers per one seed or one capillary formed by 4 AMF isolates (*Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96 and *G. claroideum* BEG23) in soil or zeolite amended with seeds (S), capillaries (C) or seeds + capillaries (S+C) at 3 consecutive harvests. Values in columns marked by the same letter are not significantly different within one treatment at the level $P \leq 0.05$ according to Duncan's (for seeds) or Kruskal-Wallis (for capillaries) tests, ns – non-significant difference.

AMF	Seeds		Capillaries	
	S	S+C	C	S+C
	Harvest 1			
Soil				
BEG140	0 b	1 b	1	0
BEG75	7 a	4 a	0 ns	0 ns
BEG96	0 b	0 b	0	0
BEG23	0 b	0 b	2	1
Zeolite				
BEG140	7 a	10 a	1	0
BEG75	2 ab	6 ab	1 ns	0 ns
BEG96	0 b	0 b	0	0
BEG23	0 b	0 b	0	0
	Harvest 2			
Soil				
BEG140	33 a	66 a	16 a	12 a
BEG75	33 a	32 b	0 b	1 b
BEG96	0 c	0 d	1 b	1 b
BEG23	7 b	7 c	13 a	14 a
Zeolite				
BEG140	31 a	51 a	1	0
BEG75	6 b	20 b	0 ns	0 ns
BEG96	1 b	0 c	0	0
BEG23	0 b	0 c	0	0
	Harvest 3			
Soil				
BEG140	41 a	55 a	16 a	11 a
BEG75	21 ab	38 a	4 b	0 b
BEG96	1 c	1 c	2 b	1 b
BEG23	14 b	19 b	24 a	27 a
Zeolite				
BEG140	18 a	42 a	0	0
BEG75	7 b	17 b	0 ns	1 ns
BEG96	3 bc	1 c	0	0
BEG23	1 c	0 d	0	0

Table 3. Comparison of frequencies (%) of seeds or capillaries containing spores formed by 4 AMF isolates (*Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96 and *G. claroideum* BEG23) in soil or zeolite amended with seeds (S), capillaries (C) or seeds + capillaries (S+C) at 3 consecutive harvests. Values in columns marked by the same letter are not significantly different within one treatment at the level P≤0.05 according to Duncan's (for seeds) or Kruskal-Wallis and Connover (for capillaries) tests, ns – non-significant difference.

AMF	Seeds		Capillaries	
	S	S+C	C	S+C
	Harvest 1			
Soil				
BEG140	2 b	6 b	13	7
BEG75	20 a	11 a	1 ns	3 ns
BEG96	0 b	0 b	0	3
BEG23	3 b	3 b	26	10
Zeolite				
BEG140	19 a	19 a	6	1
BEG75	11 ab	11 ab	1 ns	0 ns
BEG96	2 b	0 c	0	0
BEG23	3 b	2 bc	0	0
	Harvest 2			
Soil				
BEG140	64 a	77 a	43 ab	39 ab
BEG75	53 a	51 a	1 c	1 c
BEG96	21 b	16 b	25 bc	32 bc
BEG23	51 a	60 a	77 a	81 a
Zeolite				
BEG140	33 a	49 a	3	5
BEG75	5 b	24 a	0 ns	0 ns
BEG96	21 ab	17 a	0	0
BEG23	4 b	1 b	0	0
	Harvest 3			
Soil				
BEG140	78 a	87 a	43 a	36 bc
BEG75	38 b	49 b	5 b	1 c
BEG96	31 b	34 c	43 a	39 ab
BEG23	48 b	83 a	77 a	84 a
Zeolite				
BEG140	24 a	53 a	1	4
BEG75	6 b	22 b	1 ns	1 ns
BEG96	44 a	36 ab	0	1
BEG23	6 b	2 c	0	0

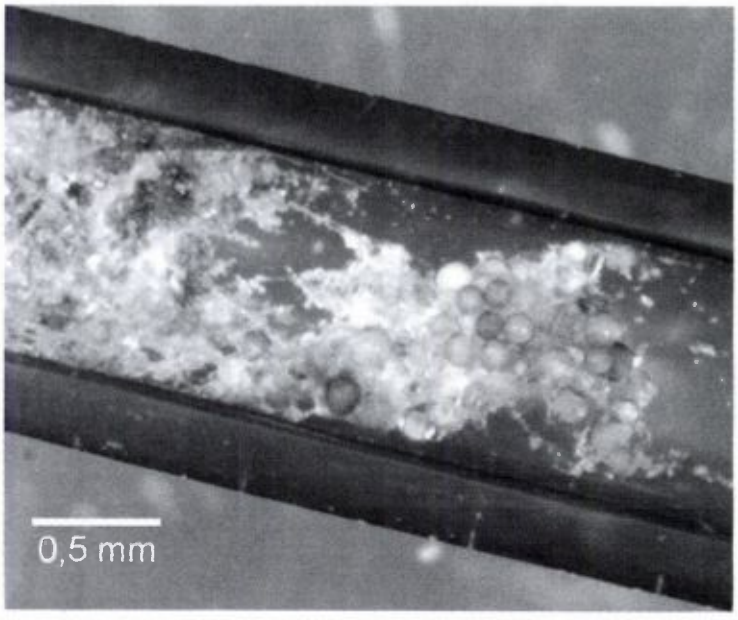


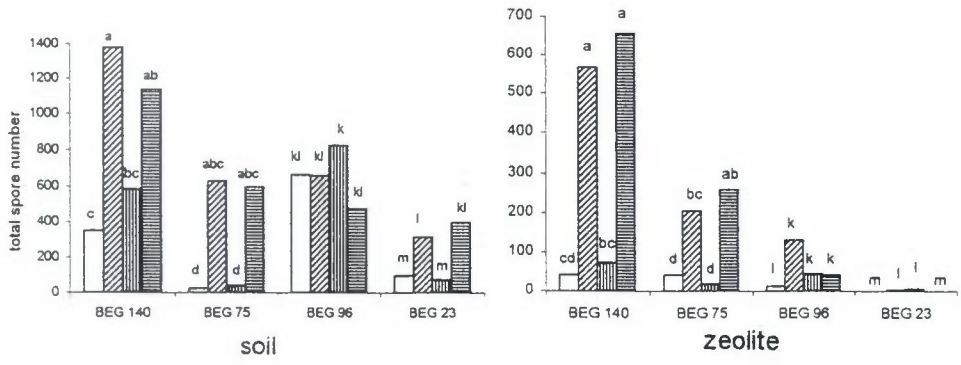
Figure 2. Spores of *Glomus claroideum* BEG23 inside the cavity of a glass capillary.

Total spore numbers

Total spore numbers (in the bulk soil plus cavities at harvest 3) of *G.* sp. BEG140 and *G. intraradices* BEG75 were substantially increased in treatments with seed amendment, both in soil and zeolite, while presence of capillaries had no effect on this parameter (Figs. 3a, b). In both substrates, these isolates sporulated preferentially inside the seed cavities as compared to the free substrate (Figs. 4a, b).

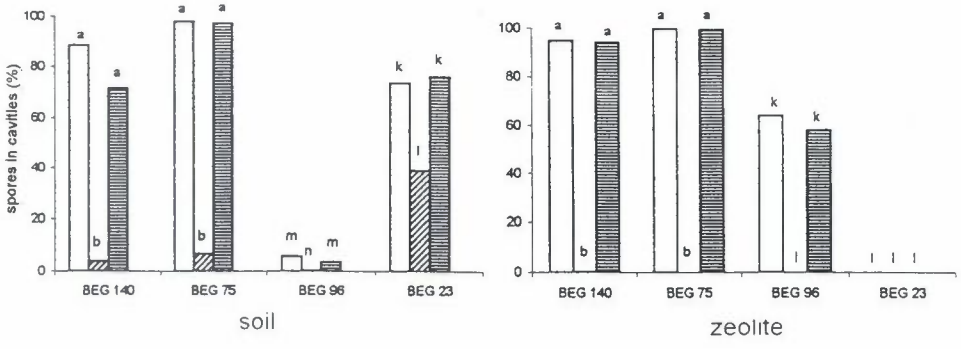
There was no significant effect of seed or capillary amendment on total spore numbers of *G. claroideum* BEG96 in the soil (Fig. 3a), where only a negligible part of the spores was formed within the cavities (Fig. 4a). In zeolite, amendment with seeds, capillaries or their combination significantly increased sporulation (Fig. 3b). While most spores in this substrate were formed inside the seeds, no spores inhabited the capillary cavities (Fig. 4b).

Total spore numbers of *G. claroideum* BEG23 in the soil were significantly increased in both treatments with seeds and most spores inhabited the seed cavities (Figs. 3a, 4a). In zeolite, some stimulation of sporulation was observed in treatments with seed or capillary amendment, but no spores of this isolate were found inside the seed or capillary cavities (Figs. 3b, 4b).



a) b)

Figure 3. Comparison of total (in the bulk soil plus cavities) spore numbers (per 1 ml of soil) formed by 4 AMF isolates (*Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96 and *G. claroideum* BEG23) in soil (a) or zeolite (b) unamended (open columns) or amended with seeds (obliquely hatched columns), capillaries (black columns), or seeds + capillaries (horizontally hatched columns) at the third harvest. Columns marked by the same letter are not significantly different at the level P≤0.05 according to Duncan's test.



a) b)

Figure 4. Comparison of percentage proportions of spores formed inside the cavities of seeds or capillaries by 4 AMF isolates (*Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96 and *G. claroideum* BEG23) in soil (a) or zeolite (b) amended with seeds (open columns), capillaries (black columns) or seeds + capillaries (hatched columns) at the third harvest. Columns marked by the same letter are not significantly different at the level P≤0.05 according to Duncan's test.

Mycorrhizal colonisation and length of the ERM

Except for *G. intraradices* BEG75, root colonisation of the host plant increased

from harvest 1 to harvest 2 and remained almost the same at harvest 3 (Table 4). In the soil, the colonisation did not differ between the isolates at harvests 2 and 3. In zeolite, *G.* sp. BEG140 showed the highest root colonisation at all harvests. With this isolate, no significant differences in mycorrhizal colonisation between the soil and zeolite were found at any harvest.

Amendment of seeds, capillaries or their combination positively affected ERM development (Table 5). However, in *G.* sp. BEG140 growing in both substrates and *G. intraradices* BEG75 growing in the soil (i.e. in treatments with the highest stimulation of sporulation) presence of seeds, capillaries or their combination had no effect on ERM length (Table 5). Almost in all treatments, more ERM hyphae were formed in the soil than in zeolite.

4. Discussion

Sporulation inside the cavities of dead seeds substantially increased total spore numbers of *G.* sp. BEG140 and *G. intraradices* BEG75, both in the contaminated soil from the sedimentation pond of a pyrite smelter and in an inert reference substrate (zeolite), and total spore numbers of *G. claroideum* BEG23 in the soil. As both *G. intraradices* BEG75 and *G. claroideum* BEG23 were isolated from non-polluted soils, it seems probable that the sporulation inside the seed cavities is not an adaptation of indigenous AMF to avoid adverse conditions in the contaminated soil, rather it represents a specific ecological feature of some AMF species or isolates. This is further supported by the fact that for indigenous *G. claroideum* BEG96 only low amounts of spores inhabited the seed cavities. In addition, for both indigenous *G.* sp. BEG140 and non-indigenous *G. intraradices* BEG75, a high tendency to preferential sporulation inside the seeds as compared to the free substrate was evident in both contaminated and reference substrates.

Some other AMF species that were found to sporulate at similarly specific places were e.g. *Glomus fasciculatum* sporulating inside dead seeds, nematode cysts or legume nodules (Taber, 1985; Daniels-Hetrick, 1984; Vidal-Dominguez et al., 1994) or *G. microaggregatum* sporulating in dead spores of other AMF (Koske et al., 1986; Muthukumar and Udaiyan, 1999). All these species form small spores, often in loose or compact clusters or sporocarps and they regularly sporulate within plant roots (Schenck and Pérez, 1988). On the contrary, *G. claroideum* preferably forms single spores in the soil. For both *G. claroideum* isolates, frequency of seeds containing spores was in some treatments comparable with the two highly sporulating isolates. However, the mean spore number of *G. claroideum* BEG96 per one seed was very low. Although ERM hyphae of this isolate entered the seed cavities, the fungus was not able to fill up the space inside the seeds by spores.

Table 4. Mycorrhizal colonisation (%) of *Agrostis capillaris* in central pots inoculated with *Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96, or *G. claroideum* BEG23 and growing in soil or zeolite at 3 consecutive harvests. Significances according to ANOVA (***P≤0.001, **P≤0.01, ns – non-significant). Values in columns marked by the same letter are not significantly different within one treatment at the level P≤0.05 according to Duncan's test.

	Soil Harvest			Zeolite Harvest		
AMF	1	2	3	1	2	3
BEG140	10 b	30	32	16 a	28 a	23 a
BEG75	32 a	24 ns	26 ns	10 b	20 b	15 b
BEG96	9 b	23	32	2 c	8 c	10 bc
BEG23	7 b	15	17	1 c	5 c	8 c

	Harvest (A)	AMF isolate (B)	Substrate (C)	AxB	AxC	BxC	AxBxC
F	26.7	33.0	63.2	4.5	2.2	6.0	3.5
Significance	***	***	***	***	ns	***	**

Table 5. Length of the extraradical mycelium (m.g^{-1} dry soil) of 4 AMF isolates (*Glomus* sp. BEG140, *G. intraradices* BEG75, *G. claroideum* BEG96 and *G. claroideum* BEG23) in soil or zeolite unamended (U) or amended with seeds (S), capillaries (C) or seeds + capillaries (S+C) at harvest 3. Significances according to ANOVA (***P≤0.001, **P≤0.01, ns – non-significant). Values in columns marked by the same letter are not significantly different within one treatment at the level P≤0.05 according to Duncan's test.

Amendment	*G.* sp. BEG140	*G. intr.* BEG75	*G. clar.* BEG96	*G. clar.* BEG23
	Soil			
U	1.36	1.25	1.49 ab	0.29 bc
S	1.61 ns	2.81 ns	2.78 a	0.59 ab
C	1.76	1.85	2.40 a	0.23 c
S+C	1.31	1.61	0.91 b	1.29 a
	Zeolite			
U	0.61	0.31 bc	0.09 b	0.06
S	0.66 ns	0.20 c	0.74 a	0.13 ns
C	0.68	0.49 ab	0.64 a	0.08
S+C	0.54	0.84 a	0.65 a	0.07

	AMF isolate (A)	S/C treatment (B)	Substrate (C)	AxB	BxC	AxC	AxBxC
F	59.9	7.6	180.0	2.9	4.8	2.3	4.1
Significance	***	***	***	**	**	ns	***

Interestingly, for *G. claroideum* BEG23 spore numbers inside the capillaries were as high as in *G.* sp. BEG140. However, sporulation of *G.* sp. BEG140 and *G. claroideum* BEG23 inside the capillaries in the soil did not contribute significantly to their total spore numbers. The reason could be among others a lower amount of capillaries added to the substrate as compared to the seeds due to their larger size. The reasons for intensive sporulation of some AMF isolates inside the cavities of dead seeds or capillaries are probably isolate-specific and dependent on the soil conditions.

The main question remains if the free space inside the cavities or amendment of organic matter and nutrients potentially released during its mineralisation are relatively more important for the sporulation. The importance of free space inside the cavities can be documented by the fact that in treatments with the most intensive sporulation (*G.* sp. BEG140 in both substrates and *G. intraradices* BEG75 in the soil), no positive effect of seed amendment on the ERM growth was observed. Increased ERM proliferation is often reported as a response of AMF to amendment of the growth substrate with organic matter (Joner and Jacobsen, 1995a; Gryndler et al., 1998, 2002, 2003). In addition, in a previous experiment, amendment with crushed seeds of *Chenopodium album* to four soils did not affect spore numbers of the indigenous AMF and the non-indigenous *G. mosseae* BEG25 isolate (Rydlová and Vosátka, 2000).

On the other hand, much evidence exists on the positive effect of organic matter on sporulation and ERM development of the AMF. In a long-term experiment, Gryndler et al. (2002) found that while amendment of organic matter in the form of cellulose inhibited sporulation of a mixture of 3 *Glomus* species after 5 months, sporulation was substantially stimulated after 11 months, when a positive effect of cellulose on the length of the ERM was also observed. The authors concluded, that positive effects of cellulose on AMF sporulation probably could be attributed to release of nutrients (e.g. P) from slowly decomposing saprophytic microflora, the biomass of which contained nutrients accumulated during the first months of the experiment. In short-term experiments, the carbon sources, cellulose or starch, depressed ERM growth of *G. intraradices* BEG87 (Ravnskov et al., 1999) or that of three different *Glomus* species (Avio and Giovannetti, 1988). These negative effects of organic matter were probably related to stimulation of saprophytic microorganisms with adverse effects on the AMF.

On the contrary, in another short-term experiment Gryndler et al. (1998) found that lower doses of glucose had a positive effect on colonisation and ERM length of *G. claroideum* BEG23; spore numbers were, however, not affected. According to the authors, the explanation of the effect of glucose on the obligate biotrophic AMF in an artificial substrate was direct utilisation by the mycelium or indirect stimulation of beneficial associative saprophytic microflora supporting the AMF development. Also amendment of chitin to the

cultivation substrate stimulated substantially spore production and ERM growth of *G. claroideum* BEG23 (Gryndler et al., 2003). Joner and Jakobsen (1994, 1995b) found that mycorrhizal hyphae could absorb biologically and chemically immobilised nutrients released from organic matter.

In addition or alternatively, sporulation inside the seeds also could be stimulated directly by bacteria inhabiting the seed cavity. Von Alten et al. (1993) observed increased sporulation from the combination of the rhizosphere bacterium *Bacillus mycoides* and one AMF isolate. Tobar et al. (1996) reported that genetically modified and wild-type strains of *Rhizobium meliloti* increased hyphal growth from germinating spores and new spore formation in *Glomus mosseae* on a water-agar medium. Azcón (1987) observed that addition of complete bacterial cultures and cell-free supernatants significantly stimulated hyphal growth and the number of new vegetative vesicles formed per germinated resting spore of *Glomus mosseae* on water-agar medium under axenic conditions.

Inside the cavities, there also can be specific conditions such as enhanced aeration, improved moisture regime or, in the case of dead seeds, also increased CO_2 concentration. Carbon dioxide was reported to stimulate germ tube and extraradical hyphae growth (Bécard and Piché, 1989). Increased sporulation of both *G. claroideum* isolates growing in zeolite amended with the seeds or capillaries without spore formation inside the cavities probably could be attributed to improved physico-mechanical properties of the substrate as suggested by Giovanetti and Avio (1985) or Joner and Jacobsen (1995a). Lower sporulation of AMF in zeolite as compared to the soil may be related to lower root colonisation of the host plant in this substrate. It is also possible that in the soil there were more nutrients available for intensive sporulation.

It can be concluded that the sporulation in dead seed cavities is probably not an adaptation of indigenous AMF from polluted soils to adverse soil conditions as it can occur also in isolates from non-polluted soils and in inert substrate without pollution stress. Rather may represent an isolate-specific ecological feature of AMF species which form clusters of small spores and regularly sporulate within plant roots.

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