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Sporulation of Symbiotic Arbuscular Mycorrhizal Fungi Inside Dead Seeds of a Non-Host Plant

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Abstract

Abundant spores of arbuscular mycorrhizal fungi (AMF) were found in the cavities of dead seeds of the non-host plant species *Chenopodium album*, one of the first colonizers of polluted soils on waste deposits from the pyrite smelter and of power station fly ash. The aim of this study was to verify this phenomenon and to find out whether the cavity inside the seeds or the organic matter released from decomposing seeds is crucial for sporulation. Amendment of whole seeds into four polluted soils significantly increased sporulation of native fungi, namely indigenous *Glomus* sp. BEG140, a cluster-forming isolate preferably sporulating within seed cavities. No effect of seed amendment was found for non-indigenous *Glomus mosseae* BEG25, suggesting a possible adaptation of sporulation strategy of the AMF isolate indigenous in polluted soils. Whereas soil amendment with whole seeds stimulated sporulation many fold, as compared to soil without seeds, amendment with crushed seeds had no significant effect on sporulation; therefore increased sporulation was probably caused by the presence of seed cavities rather than by organic matter. There was no significant effect of seed amendment on root colonisation of lettuce as a host plant, however, seed amendment influenced total length of extraradical mycelium and its NADH-diaphorase activity. Biotest conducted with maize as a host plant grown in the soils from the experiment showed no effect of previous seed amendment on inoculum viability.

Keywords: *Chenopodium album* seeds, extraradical mycelium, *Glomus*, indigenous AMF, polluted soils, sporulation

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1. Introduction

Successful survival and growth of plants in toxic soils of the ecosystems degraded by industrial activities is greatly dependent not only upon the abiotic properties of the soil but also on the activity of microbial populations (Visser, 1985). The presence of arbuscular mycorrhizal fungi (AMF) may reduce negative effects of stresses caused by lack of nutrients or organic matter, by adverse soil structure, extreme pH, or high concentration of pollutants (Sylvia and Williams, 1992). AMF can also enhance the resistance of plants to drought stress and high salinity mainly due to increased absorption capacity of the mycorrhizal roots (Hardie, 1985). An important feature of the AMF might be a reduction of stress induced by high concentrations of heavy metals (Galli et al., 1994; Schuepp et al., 1987).

Many studies have reported that AMF are sensitive to environmental conditions, especially to edaphic factors such as soil disturbance (Waaland and Allen, 1987) high heavy metal concentration (Killham and Firestone, 1983), or low pH (Hayman and Tavares, 1983). However, the indigenous populations of AMF may became adapted to local environmental conditions (Stahl and Christensen, 1991) and they can display significant variation among populations in their tolerance to given environmental stresses (Gildon and Tinker, 1981; Haselwandter et al., 1994). Indigenous isolates of AMF can also show higher tolerance to heavy metals (Leyval et al., 1991; Weissenhorn et al., 1993). The ecology and physiology of AMF populations under severe environmental stress may change and this can implicate substantial variation of their life strategy.

First invaders during plant succession on the sedimentation ponds of pyrite waste or power station fly ash are mostly species from the family Chenopodiaceae which are thought to be typically non-mycotrophic (Gerdemann, 1968). Successive colonisers, particularly grasses, are considered to be facultative mycotrophs (Hetrick et al., 1989), but for their successful colonisation they need high numbers of mycorrhizal propagules able to initiate mycorrhizal symbiosis, or alternatively they need the presence of highly mycotrophic plant species in their vicinity (Ocampo et al., 1980).

The soils on both types of sedimentation ponds in the Czech Republic show high content of toxic heavy metals (e.g. manganese, iron or zinc), fluctuations of pH, and adverse physical properties. Therefore, the AMF symbionts that are present on these sites must have undergone strong selection pressure and have shown the capacity to resist long-term chronic stress (Batkhuugyin et al., 2000). Sampling on these sites showed significant presence of AMF spores within hollow seed coats of *Chenopodium album*. Such observation has not been reported previously and members of the Chenopodiaceae as a non-mycorrhizal family were supposed to even reduce the indigenous populations of AMF

(Harley, 1989; Call and McKell, 1982). Taber (1985) found the spores of *Glomus fasciculatum* in partially decayed seeds of *Portulaca oleracea* in soil depth of 5 to 8 cm. Spores were also found in old seeds of *Amaranthus retroflexus, Stellaria media, Mollugo verticilata, Trianthema portulacastrum* and of three other unidentified plant species. Interestingly, the first four plant species are regarded as non-mycorrhizal or belong to non-mycorrhizal families. Daniels-Hetrick (1984) listed observations of other authors who found sporulations of *G. fasciculatum* in old seed coats and nematode cysts and *G. deserticola* sporulated even in the lid of a centrifuge minitube. Vidal-Dominguez et al. (1994) described very intensive sporulation of *G. fasciculatum* in *Rhizobium* nodules of clover and alfalfa. Some AMF sporulate also within dead spores of other AMF (Muthukumar and Udaiyan, 1999), inside dead root fragments, or even within growing roots as *G. intraradices, G. mosseae* or *G. manihotis*. However, it is not known whether the existence of cavities inside the seeds or a gradient of either organic matter or some microbes is crucial for the observed phenomenon of sporulation within the seeds or other objects. The role of *C. album* seeds on distribution of AMF symbionts in studied localities can be hypothesised either as an ecological adaptation of these organisms to adverse conditions in toxic soils or as a specific feature of certain AMF isolates. Behaviour and ecology of these adapted AMF and their relations with plants should be studied as they may alter not only the performance of individual plants but also the plant community structure as a whole.

The first aim of this study was to evaluate the effects of the presence of *C. album* seeds on the sporulation of indigenous and non-indigenous AMF and to study whether this sporulation strategy is related to the toxicity of soil or to the specific behaviour of AMF isolates. Secondly, the study addressed whether this sporulation is due to the cavity of dead seeds or to the stimulatory effects of organic matter added as dead seeds on development of AMF and consequently their sporulation.

2. Materials and Methods

Site and soil characteristics

Both sedimentation ponds Chvaletice and Opatovice are located in the eastern part of the Labe river basin, altitude 204 m and 208 m above sea level, respectively. Waste from the smelter factory processing pyrite raw materials has been stored in the sedimentation pond Chvaletice, while the Opatovice sedimentation pond contains fly ash from a power station burning brown coal. Waste materials from Chvaletice have a high content of sulphur in the forms of sulphides and therefore lower pH. Weathering of these materials causes

Table 1. Total contents of selected elements in the substrates from the Chvaletice and Opatovice sedimentation ponds (Rauch, 1996)

	Ca	Mg	K	Fe	Al	Mn	Zn
				(mg kg^{-1})			
Chvaletice	22,691	7,573	4,340	43,396	32,523	43,396	150
Opatovice	20,876	4,587	2,372	18,328	29,011	301	87

Table 2. Soil characteristics of four plots sampled on the Chvaletice sedimentation pond (CH1–CH3) and on the Opatovice sedimentation pond (OP) (average values from 4 measurements within one year)

	pH_{H2O}	P	Organic C	Conductivity
		(mg kg^{-1})	(%)	(μS cm^{-1})
CH1	6.9	2.3	0.9	1,039
CH2	6.1	7.7	1.5	238
CH3	5.7	8.0	1.6	353
OP	8.1	14.3	1.8	101

strong acidification followed by increase of salinity. The substrate has also high concentrations of heavy metals as Mn, Zn, Fe and Al (Table 1). Fly ash mixtures from the Opatovice sedimentation pond have alkaline reaction and are rich in soluble elements such as Ca, Mg and K, which influence availability of other elements and compounds. Both substrates are very vulnerable to erosion and drought and they are often heated in summer up to the temperature of 60°C.

Both sedimentation ponds were abandoned in the 1980s. On both sedimentation ponds, spontaneous primary succession begun with invasion of non-mycotrophic ruderal annuals, namely from the family Chenopodiaceae. On the Chvaletice site this was followed by development of three distinct types of vegetation according to the chemistry and water supply of given plots: CH1 – the driest plot with scarce vegetation of *Calamagrostis epigejos*; CH2 – *C. epigejos*, reeds, scarce occurrence of birch, poplar and willow; CH3 – the wettest site with *C. epigejos*, reeds and fully developed hardwood cover. Vegetation of the Opatovice sedimentation pond developed with much less diversity and *C. epigejos* gradually dominated a sowed mixture of grass species. Hardwoods, particularly willows, are rather scattered on the locality.

Pre-experiment soil sampling was conducted on all study plots during one year. In April, June, August and December 10 soil cores (15 × 15 × 15 cm) were taken from each plot (Table 2). Soil from each core was mixed by hand, air-dried, sieved through 2 mm sieve and a subsample was taken for the assessment of pH, extractable P (Mehlich, 1984), organic C (Nelson and Sommers, 1982) and electrical conductivity.

Experiment 1: Sporulation of the indigenous AMF within cavities of Chenopodium album seeds

Field soil from each of four sampling plots was air-dried and sieved through a 4 mm sieve. Two soil treatments were used: undiluted soil and soil diluted with sand 1:1 (v:v) to reduce the toxicity and organic matter content of the soil. Sand was sterilised in an oven at 120°C for 24 hr. Each treatment had 10 pots as replicates. The pot volume of 180 ml was divided by nylon mesh with pore size of 0.5 mm into upper and lower compartments, each containing 90 ml of soil. Dead empty seeds of *C. album* mixed into the soil in the upper compartment (32 seeds per 1 ml of the soil). The soil in the bottom compartment was left without seeds. The seeds were boiled for 2 hr in 5 changes of 10% KOH and thoroughly washed in hot water to get intact but empty seeds without endoderm. Each pot was sown with ten seeds of lettuce (*Lactuca sativa* var. Winter Altenburg) which were treated with the fungicide Dithane (seeds mixed for 5 min in a powder, 1% of total seed weight). Two weeks after seedling emergence, plants were thinned to 4 per pot. The experiment was conducted in a growth room with the day light intensity of 330 $mmol.m^{-2}.s^{-1}$ for 14 hr and day/night temperature of 26°C and 21°C, respectively. Each pot was self-moistened separately by a fiberglass strip to which water from a dish bellow the pot was soaked. Plants were harvested after 18 weeks of growth. Chemical analyses of the soils were conducted before and after sterilisation and before and after seed amendment. The total content of carbon and nitrogen was analysed by the Elemental Analyzer CHN-Rapid Heraus (Monar, 1972). Bicarbonate extractable phosphorus (Olsen, 1954) was determined using UV-Vis Spectrometer Unicam UV4-200 after mineralization in the automatic mineralizer (Appion). Mn (extracted by ammonium acetate) was analysed using the AAS Spectrometer Unicam 9200X (Chapman and Moore, 1986).

At the harvest the soils from upper and bottom compartments were homogenised separately, and 15 ml of the soil was wet-sieved through three sieves (0.5 mm, 0.25 mm and 0.036 mm). Thirty-two seeds (equal to the number of seeds originally amended per 1 ml of soil) were randomly collected on the upper most sieve. The seeds were placed on wet filter paper in a Petri dish. Each seed was dissected and the spores inside the seed were counted at 25× magnification.

Average numbers of spores per seed were calculated. Roots were removed from the largest sieve as well, stained with Trypan blue in lactoglycerol (modified from Kormanik and McGraw, 1982). The root length colonised by AMF was evaluated microscopically by the segment method (Giovannetti and Mosse, 1980) at 100× magnification. Soil particles on the top sieve were passed through carefully not to break the remaining seeds. Material from the central sieve was homogenised in a blender for 20 s to release the spores from extraradical mycelium (ERM). Spores were extracted from the bottom sieve by centrifugation at 3,000 rpgm for 5 min in a 50% sucrose solution. The 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 a 1 cm square grid. The number of spores was evaluated on the filter in a Petri dish under a stereomicroscope at 40× magnification.

Test of inoculum vitality was conducted in the soils from the experiment using corn as a trap host plant. Soil from the upper (with seeds) and the lower (without seeds) compartment of each pot was mixed with sterile sand (1:3, v:v) and filled into 160 pots, volume of 180 ml. Each pot was sown by one seed of *Zea mays* (variety C 240) that had been surface sterilised in 1% H_2O_2 for 5 min. After 6 weeks cultivation in a growth room under the same conditions as the previous experiment, the roots were cleared and stained and colonisation was evaluated under a binocular microscope by the gridline-intersect method (Giovannetti and Mosse, 1980).

Experiment 2: Effects of amendment of Chenopodium album seeds on the development and sporulation of indigenous and exotic AMF

A factorial experiment (5×2×3) involved 5 substrates (CH1, CH2, CH3, OP) and inert substrate Terragreen (TER – attapulgite clay manufactured by Oil Dri, USA). Soils were either left unsterilised (i.e. with indigenous AMF populations) or were sterilised by γ-irradiation of 5 Mrad and inoculated with a non-indigenous AMF fungus *Glomus mosseae* (for TER only a sterilised and inoculated treatment was established). The last factor was the seed amendment: the soils were amended with either whole seeds or crushed seeds, or left without seeds. Host plants (*Lactuca sativa* L., variety Winter Altenburg) were cultivated in 180 ml plastic pots; each treatment had 6 replicates. Each pot in the inoculated treatments was amended with 100 spores of *G. mosseae* (Nicol. and Gerdemann), isolate BEG25. The inoculum was prepared on maize growing for 4 months in a mixture of sand and vermiculite. Bacterial populations from the original unsterilised soil were reintroduced by addition of filtrate from 300 g of soil shaken for 30 min in 3 l of deionised water. Seeds of *C. album* were prepared as in Experiment 1. Half of the seeds were

crushed and the same weight of seeds, either whole or crushed (0.84 g represents 31 seeds per 1 ml of soil), was thoroughly blended into the soil in the pot. Pots were sown with 8 seeds of lettuce. Two weeks after seedling emergence the plants were thinned to 5 per pot. Plants were cultivated in a temperate greenhouse without additional light for 19 weeks.

At harvest, roots were extracted from a 15 ml core sampled from the central part of the pot, washed in tap water, cleared and stained, and root colonisation was evaluated as in Experiment 1. For evaluation of AMF sporulation, a 15-ml soil sample was taken by core from each pot and wet-sieved through three sieves (0.5, 0.2 and 0.036 mm) in treatments with whole seeds, or two sieves (0.20 and 0.036 mm) in treatments with crushed seeds or without seeds. Thirty-one seeds (equal to the number of seeds originally used per 1 ml of soil) were randomly collected on the 0.5 mm sieve and placed on wet filter paper in a Petri dish. The seeds were carefully broken using two dissecting needles and the spores inside the seed cavity were counted under a stereomicroscope at 16–50× magnification. Soil particles on the sieve were carefully passed through in order not to break the remaining seeds. For treatments inoculated with *G. mosseae* BEG25, material from the 0.2 mm sieve was transferred to a Petri dish where sporocarps were collected and number of sporocarps and spores inside the sporocarps were counted. For all treatments, material from the 0.2 mm sieve was transferred to a household blender and further procedures of spore extraction and counting were the same as in Experiment 1. In treatments with whole seeds, the total number of spores was calculated as the sum of spores in the bulk soil and the spores within the seed cavities.

For evaluation of ERM length and NADH-diaphorase activity, a 15 ml core was sampled from 5 pots in each treatment and homogenised by hand mixing. A 5-g subsample was put into a household blender with 500 ml of H_2O and blended for 30 s. One ml of supernatant was pipetted onto Whatman membrane filter (25 mm diameter, 0.45 μm pore size) 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 the Olympus BX60 microscope at 200× magnification according to Brundrett et al. (1994). The length of the ERM was expressed in meters of total hyphae per 1 gram of dry substrate. Remaining supernatant was poured through a 0.036 mm sieve and clusters of ERM were transferred to Eppendorf tubes with 300 μl of the NADH diaphorase (NADH-d) staining solution (Sylvia, 1988). The Eppendorf tubes were incubated for 14 hours in the dark at 28°C. After incubation the mycelium was counter-stained with 0.05% Trypan blue solution in lactoglycerol. The percentage of ERM length, which contained purple precipitate (NADH-d activity), was estimated in thirty microscope fields of view at 400× magnification.

Table 3. Effects of seed amendment and dilution of soil by sand on sporulation in soil from four sites (CH1–CH3 different plots of the Chvaletice sedimentation pond, OP the Opatovice sedimentation pond). Values marked by the same letter within one soil treatment are not significantly different at the level $P \leq 0.05$ according to Kruskal-Wallis test, ns = non-significant difference (Experiment 1)

Soil	Seed amendment	Dilution	Spore no. per ml of soil	Spore no. per seed
CH1	0	0	0.3	–
	0	1	0.2 ns	–
	1	0	5.3	0.2 a
	1	1	10.7	0.3 a
CH2	0	0	1.5 c	–
	0	1	1.8 bc	–
	1	0	452.9 a	14.1 a
	1	1	171.7 ab	5.3 b
CH3	0	0	0.8 b	–
	0	1	1.1 b	–
	1	0	146.3 a	4.5 a
	1	1	329.4 a	10.2 a
OP	0	0	0.9 b	–
	0	1	1.4 b	–
	1	0	140.9 a	4.4 a
	1	1	225.1 a	7.0 a
Significance				
seed amendment			***	–
soil			***	***
dilution			ns	ns

Statistical analysis

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 test was used to calculate Chi-square values for evaluating main effects.

3. Results

Experiment 1

Analysis of all soils used in the experiment showed negligible effects of both

Table 4. Effect of different AM fungi, presence of seeds in soil, and soils from different plots of sedimentation ponds on mycorrhizal parameters. F-values of ANOVA, significance level: *P*0.001, *P*0.01, *P*0.05, ns - non-significant (Experiment 2)

Factor		AMF (A)	Seeds (B)	Soil (C)	A×B	A×C	B×C	A×B×C
Mycorrhizal colonization	F	127.5	4.7	20.3	2.7	12.3	5.0	3.2
	Significance	***	*	***	ns	***	***	**
Spore no. inside seeds	F	12.4	–	15.1	–	4.3	–	–
	Significance	**	–	***	–	***	–	–
Spore no. in bulk soil	F	24.9	8.8	44.5	1.5	22.7	3.1	3.8
	Significance	***	***	***	ns	***	**	**
Total spore no.	F	1.8	24.4	25.9	14.8	12.9	10.6	4.7
	Significance	ns	***	***	***	***	***	***
ERM length	F	0.04	5.6	10.6	15.5	1.8	2.0	4.1
	Significance	ns	**	***	***	ns	ns	*
NADH-diaph. activity	F	222.3	7.5	28.2	5.6	11.1	4.5	4.3
	Significance	***	***	***	**	***	***	***

sterilisation and seed amendment on chemical properties measured (data not presented). An increase of available Mn due to sterilisation was found in Chvaletice soils (CH1 from 2.7 to 13.6; CH2 from 3.5 to 16.7; CH3 from 3.5 to 62.2 mg kg^{-1} of soil).

Sporulation of the AMF was significantly influenced by presence of the *C. album* seeds and by the soil used for cultivation, while the effect of soil dilution by sand was not significant (Table 3). In the soils from sites CH2, CH3 and OP, sporulation was significantly higher in the treatments with seed amendment compared to treatments without seeds (Table 3). In the soil from site CH1, addition of seeds did not influence sporulation. Spore numbers inside the seed cavities were significantly higher on the sites CH2, CH3 and OP, whereas remained low in the soil from site CH1 (Table 3). In the soils amended with seeds, the proportion of spores in the seed cavities was 95–99% from the total spore number, except soil CH1 where the spores within seed cavity represented 81% of the total number. Mycorrhizal colonisation of roots was higher than 90% in all pots of the experiment regardless of soil type, seed presence or soil dilution (data not presented). No significant effect of presence of seeds with spores on the soil infectivity was observed in the test with maize (data not presented).

Table 5. Effect of soil source and seed amendment on spore numbers of native AMF and *Glomus mosseae* BEG25 in bulk soil and total spore numbers (including spores in seeds) (CH1, CH2, CH3 different plots of the Chvaletice sedimentation pond, OP the Opatovice sedimentation pond, TER Terragreen). Values marked by the same letter are not significantly different at the level $P \leq 0.05$ according to Duncan Multiple Range test. Italic letters express the effect of soil, normal letters express the effect of seed amendment for each AMF treatment, ns = non-significant difference (Experiment 2)

	Native AMF			*G. mosseae* BEG25		
	None	Whole seeds	Crushed seeds	None	Whole seeds	Crushed seeds
Spore no. per 1 ml of bulk soil						
CH1	49 a *a*	14 b *ns*	40 a *a*	103 a *a*	66 b *a*	126 a *a*
CH2	13 a *b*	19 a *ns*	20 a *bc*	96 ab *a*	62 b *a*	128 a *a*
CH3	11 b *b*	18 b *ns*	27 a *ab*	37 a *b*	22 a *b*	18 a *c*
OP	17 a *a*	13 a *ns*	14 a *c*	13 a *c*	3 b *b*	17 a *c*
TER		nd		35 b *bc*	21 b *b*	54 a *b*
Total spore no. per 1 ml of soil						
CH1	49 a *a*	27 a *b*	40 a *a*	103 a *a*	83 a *a*	126 a *a*
CH2	13 b *b*	656 a *a*	20 b *bc*	96 a *a*	103 a *a*	128 a *a*
CH3	11 b *b*	629 a *a*	27 b *ab*	37 a *b*	52 a *a*	18 a *c*
OP	17 a *b*	36 a *b*	14 a *c*	13 a *b*	35 a *b*	17 a *c*
TER		nd		35 a *ab*	37 a *ab*	54 a *b*

Experiment 2

Root colonisation of the host plant was affected by AMF, by presence of seeds, and by the soil used (Table 4). Colonisation by indigenous AMF was about 20% higher than colonisation by the non-indigenous *G. mosseae* BEG25 regardless of the presence of seeds or soil type. There was no effect of seed amendment on root colonisation by indigenous AMF (Table 5). For *G. mosseae* BEG25, presence of seeds increased colonisation in soils from the Chvaletice site. In the soil CH1 the highest colonisation was found in the treatments with whole seeds and in the soils CH2 and CH3 in treatments with crushed seeds. Overall highest colonisation was found in the soil OP (average 70%), whereas in Terragreen the colonisation was the lowest (average 26%).

Sporulation within the seeds was influenced by the AMF treatment as well as by soil (Table 4). Although it occurred for both indigenous AMF and *G. mosseae* BEG25, intensive sporulation was found only for indigenous AMF in soils CH2 and CH3 (Fig. 1). Sporulation of *G. mosseae* BEG25 within seeds was substantially lower and it did not significantly differ between soils.

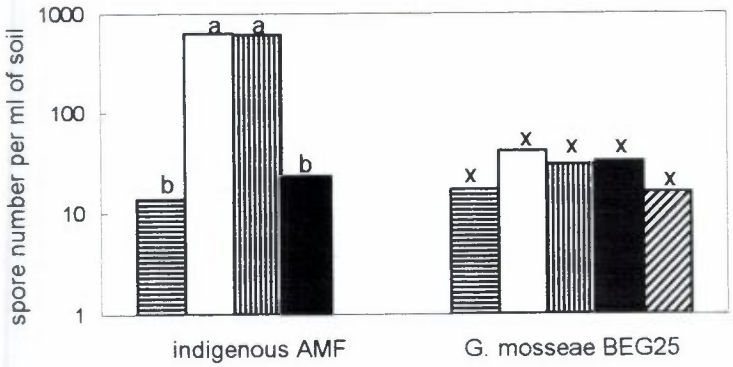

Figure 1. Spore numbers of native AMF and *Glomus mosseae* BEG25 formed inside seeds (per 1 ml of soil) in soils from different plots of the Chvaletice sedimentation pond (CH1 – horizontally hatched columns, CH2 – open columns, CH3 – vertically hatched columns), from the Opatovice sedimentation pond (black columns) and in Terragreen (obliquely hatched columns). Columns marked with the same letter are not significantly different at the level $P \leq 0.05$ according to Duncan Multiple Range test (Experiment 2).

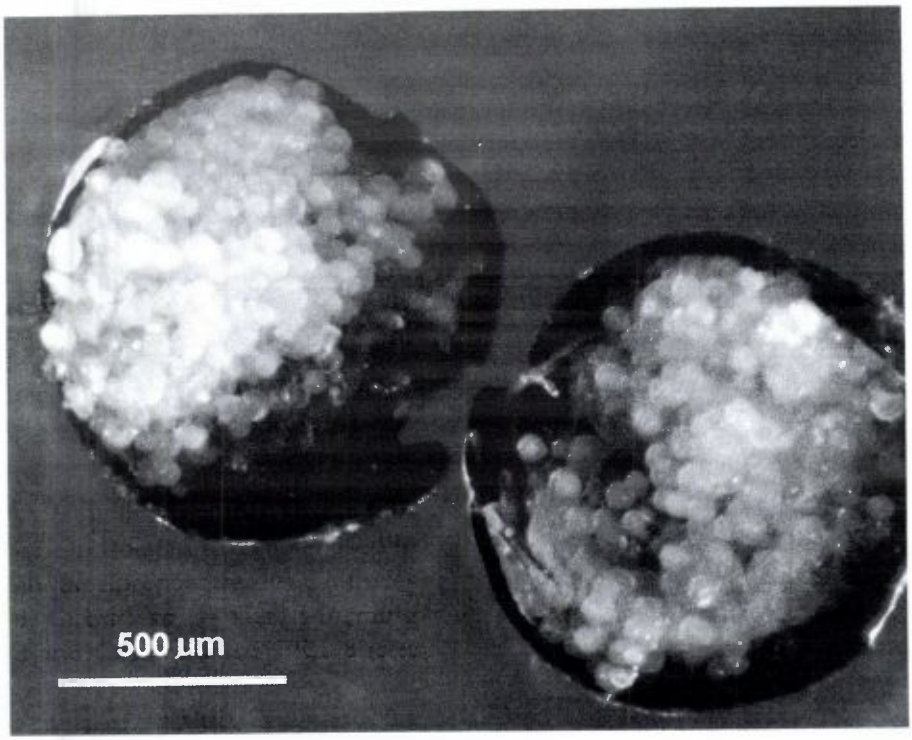

Figure 2. Spores of *Glomus* sp. BEG140 in the cavity of a broken dead seed of *Chenopodium album*.

Sporulation in bulk soil was significantly influenced by all factors – type of the AMF, presence of seeds and soil (Table 4). Number of spores of indigenous AMF in soil CH1 was significantly lower in the treatment with whole seeds compared to treatments with crushed seeds or without seeds (Table 5). In the soil CH3, amendment of crushed seeds significantly increased sporulation in bulk soil. In soils CH2 and OP amendment of whole or crushed seeds did not have any effect on spore numbers in bulk soil. The effect of soil on the sporulation was found particularly in treatments without seeds and with crushed seed amendment where indigenous AMF sporulated most intensively in CH1. *G. mosseae* BEG25 sporulated twice as much in bulk soil compared to native AMF from polluted soils. In all soils except for CH3, *G. mosseae* BEG25 sporulated significantly less in treatments with whole seeds. The effect of soil on the sporulation was similar in all seed treatments: sporulation of *G. mosseae* BEG25 was the highest in soils CH1 and CH2, the lowest in soil OP. The highest numbers of sporocarps and spores inside the sporocarps were found in soil CH1. Amendment with whole or crushed seeds significantly increased sporocarp production and spore numbers formed in sporocarps in soil OP and in Terragreen (data not presented). Total number of spores calculated from spore number in bulk soil and in the seeds was influenced by seed presence and by soil (Table 4). Due to very intensive sporulation inside the seeds, significant increase of total number of spores was found for indigenous AMF from plots CH2 and CH3 when the substrates were amended with whole seeds (Table 5). In the soil from plot CH1, addition of whole seeds influenced only the distribution of spores but not their total number (about 50% of spores were developed inside the seeds). Addition of crushed seeds slightly stimulated sporulation in the soil from the plot CH3, but this stimulation was much less than in the case of whole seeds. No effect of either whole or crushed seed amendment was found for indigenous AMF from the plot OP. Spore numbers of *G. mosseae* BEG25 in all soils were not influenced by any seed treatment, however, addition of whole seeds influenced the spore distribution similarly as for the indigenous AMF from the plot CH1.

Seed presence and soil source influenced significantly the total length of the ERM (Table 4). Amendment of whole seeds significantly increased ERM length of indigenous AMF only in OP soil (Table 6). For *G. mosseae* BEG25, the ERM length was increased by amendment of whole seeds in all three Chvaletice soils, but not in OP soil. In Terragreen, lower ERM length was found in treatment with crushed seeds compared to treatment without seeds or with whole seeds. Activity of NADH-diaphorase in ERM was significantly influenced by AMF, seed amendment and by soil source (Table 4). NADH-d activity in the ERM of *G. mosseae* BEG25 was, in most treatments, higher compared to indigenous AMF. In the soil CH1, amendment of whole seeds increased NADH-d activity of indigenous AMF compared to treatments without seeds or with crushed seeds

Table 6. Effect of soil source and seed amendment on mycorrhizal colonisation of lettuce, ERM length and NADH-diaphorase activity of native AMF and *Glomus mosseae* BEG25 (CH1, CH2, CH3 different plots of the Chvaletice sedimentation pond, OP the Opatovice sedimentation pond, TER Terragreen). Values marked by the same letter are not significantly different at the level $P \leq 0.05$ according to Duncan Multiple Range test. Italic letters express the effect of soil, normal letters express the effect of seed amendment for each AMF treatment, ns = non-significant difference (Experiment 2)

	Native AMF			*G. mosseae* BEG25		
	None	Whole seeds	Crushed seeds	None	Whole seeds	Crushed seeds
Mycorrhizal colonization (%)						
CH1	75 a *ns*	87 a *a*	83 a *ab*	49 b *bc*	76 a *a*	52 b *b*
CH2	83 a *ns*	83 a *a*	87 a *a*	53 ab *ab*	48 b *b*	71 a *a*
CH3	73 a *ns*	69 a *b*	64 a *c*	38 b *c*	33 b *c*	54 a *b*
OP	70 a *ns*	71 a *b*	73 a *bc*	66 a *a*	70 a *a*	74 a *a*
TER		nd		19 a *d*	27 a *c*	32 a *c*
ERM length (m g^{-1})						
CH1	4.6 a *a*	3.7 a *ns*	5.4 a *ab*	2.8 b *ns*	11.2 a *a*	3.9 b *a*
CH2	6.9 a *a*	2.7 b *ns*	10.2 a *a*	3.5 b *ns*	9.1 a *a*	4.7 b *a*
CH3	5.3 a *a*	6.3 a *ns*	5.9 a *a*	2.9 b *ns*	8.0 a *a*	3.3 b *a*
OP	1.7 b *b*	3.1 a *ns*	2.8 ab *b*	5.2 a *ns*	3.8 a *b*	2.1 a *b*
TER		nd		2.2 a *ns*	1.2 a *c*	0.4 b *c*
NADH-diaphorase activity (%)						
CH1	41 b *b*	72 a *a*	43 b *ns*	83 a *ns*	85 a *ns*	78 a *a*
CH2	47 a *ab*	46 a *b*	35 a *ns*	80 a *ns*	78 a *ns*	72 a *a*
CH3	43 a *b*	31 a *c*	38 a *ns*	72 a *ns*	81 a *ns*	46 b *b*
OP	53 a *a*	53 a *b*	40 b *ns*	83 a *ns*	71 b *ns*	67 b *a*
TER		nd		78 a *ns*	79 a *ns*	75 a *a*

(Table 6), while in OP soil there was reduced NADH-d activity in treatment with crushed seeds. For *G. mosseae* BEG25, amendment of crushed seeds to soil OP and amendment of both whole and crushed seeds to TER significantly reduced the activity of ERM.

4. Discussion

The cavity of the seeds amended to soil can provide a physical protection of the fungus and/or nutritional sources for hyphae. 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 mycospermal ecological niche. Sporulation in the seed cavities can correlate with reported increase of AMF development in the presence of organic matter (Joner and Jakobsen 1995a). Also specific bacteria associated with the dead seeds can be stimulatory for ERM development and probably also for AMF sporulation (Puppi et al., 1994). Daniels-Hetrick (1984) suggested that sporulation on such sites as in seeds or root fragments is probably advantageous for AMF because they are more hidden from parasitic microorganisms or predatory larvae and they just provide good physical shelter for spores. Hyphae and spores in the seed cavity can be protected particularly from nematodes or collembolans (Harley, 1989).

It seems probable that the observed ecological strategy of sporulation is related to certain AMF species or isolates. That can explain the differences in sporulation between soils from Chvaletice (CH1 vs. CH2 and CH3) where the AMF populations are composed mainly by two fungal symbionts *Glomus* sp. BEG140 and *G. claroideum* BEG96. The differences in sporulation inside seeds between the soils probably can be attributed to different composition of dominant symbionts within each soil. In further work we found that it was a cluster-forming *Glomus* sp. BEG140, which predominantly sporulated in the cavity of seeds (Rydlová, unpublished results). It is probable that on the site CH1 of the Chvaletice sedimentation pond the prevailing species was *G. claroideum*, however, these two species were not distinguishable when counting spores under a binocular microscope at low magnification. Different dominance of these AMF species could be induced by different chemical properties of soils (content of available P and organic matter or salinity).

Specific reasons for the difference in sporulation inside the seeds for indigenous AMF and *G. mosseae* BEG25 could be only speculated. Sporulation within the seed and similarly within the roots could be specific adaptation of indigenous AMF, which tend to sporulate in relatively stable environments protected from parasites and consuments. These particular isolates or species might belong to "stress tolerators" among AMF. Following this study four isolates of two species of indigenous AMF have been isolated from soils used in these experiments. They were registered in Banque Européenne des Glomales (Dodd et al., 1994): *Glomus claroideum* BEG96, *Glomus* sp. – cluster-forming BEG140 (site Chvaletice); *G. claroideum* BEG93 and *G. intraradices* BEG98 (site Opatovice).

True reasons for sporulation inside the seeds are still unclear. The phenomenon probably cannot be attributed only to higher concentration of nutrients and organic matter inside the seeds, since there was no substantial increase of sporulation due to amendment of crushed seeds. In another

experiment where the seeds were replaced by glass capillaries, similar sporulation of some isolates occurred within the cavities of glass capillaries (Rydlová, unpublished results). It might be possible that the AMF are able to exploit nutrients released outside the seeds by mineralization of organic matter due to activities of mineralizing microorganisms, the mechanism suggested by Joner and Jakobsen (1995b). Bécard and Piché (1989) reported better growth of ERM at increased concentration of CO_2 and similar stimulation due to the activity of bacteria mineralizing organic matter from the seeds may be suggested for the observed increase in sporulation. Sporulation inside the seeds could be stimulated by bacteria inhabiting the seed cavity, however, there are no specific results reported regarding the effects of bacteria on AMF sporulation. The AMF have a potential to change species composition of bacteria in rhizosphere soil (Linderman, 1992) and some bacteria can stimulate development of AMF and its ERM (Azcón-Aguilar and Barea, 1985; Gryndler et al., 1995; Gryndler and Vosátka, 1996). Joner and Jakobsen (1995a) suggested that stimulation effects of the organic matter they used (decomposed straw) on the growth of ERM can be explained also by improvement of physical properties of the growth substrate, e.g. higher soil porosity. That can be applied for sporulation as well since Giovannetti and Avio (1985) found that amendment of different materials increasing soil porosity supported development and sporulation of the AMF. Effects of seed amendment on other mycorrhizal parameters such as viability of inoculum in the soil, root colonisation or growth and activity of the ERM were not clear. Increase of mycorrhizal colonisation and ERM length for *G. mosseae* BEG25 found in Chvaletice soils after seed amendment is in agreement with the observation of Joner and Jakobsen (1995a) on positive effects of organic matter on ERM development.

Study of ecophysiological features of AMF isolates and their comparison needs further research focused on the reasons for sporulation of indigenous fungi inside the seeds and on finding whether they are purely physical or if there are any other aspects of this phenomenon. The whole process should be also studied in a time course to determine all stages of ERM hyphae penetrating into the seed cavity and forming a base for spore development. In general, it could be assumed from the fact that seeds of *C. album* facilitate sporulation that even the presence of non-mycotrophic plant species could positively influence the populations of symbiotic AMF in soil. Ecological aspects of this phenomenon may have thus specific implication for succession of vegetation on contaminated soils in degraded ecosystems.

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