Folia Microbiol. 43 (6), 635–643 (1998)

Effect of Glucose on the Development of *Glomus fistulosum* Colonization and Extraradical Mycelium on Maize Roots

M. GRYNDLER[a], M. VOSÁTKA[b], H. HRŠELOVÁ[a], I. CHVÁTALOVÁ[a] and V. ŠKRDLETA[a]

[a]*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4*
[b]*Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic*

Received February 10, 1998
Revised version April 1, 1998

ABSTRACT. Various doses of glucose were added weekly to pots with maize growing in a mixture of soil and Perlite inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus fistulosum* to define the effects of an additional carbon source on plant growth, mycorrhizae and other microbial features of the cultivation substratum. Higher doses of glucose (100 and 300 mg per pot) decreased plant growth and abundance of root hairs after 6 weeks of cultivation. Lower doses of glucose (10 and 30 mg per pot) had a positive effect on some characteristics of the development of the arbuscular fungus, *e.g.* root colonization, abundance of arbuscules, the length of extraradical mycelium associated with root surface, length of mycelium in the substratum as well as the length of both mycelia showing dehydrogenase activity as compared with variants not supplied with glucose solution. Glucose did not affect the number of spores of AM fungus. No effect of glucose was found on substratum respiration but glucose amendment increased microbial biomass and particularly the occurrence of saprophytic fungi. In a subsequent experiment focused on nonsymbiotic phase of fungal life cycle, the mycorrhizal root segments were incubated for 6 weeks in Petri dishes on membranes covered with a soil layer and supplied weekly with four glucose concentrations from 0.3 to 10 mg. Highest total length of hyphae associated with the root surface and the length of hyphae showing dehydrogenase activity was found when the lowest dose of 0.3 mg glucose was added to the soil weekly, whereas a 10 mg dose increased the length and activity of hyphae associated with a membrane. The possible mechanisms of the effects of additional labile carbon pool on the development of mycorrhizal fungus are discussed.

Arbuscular mycorrhizal fungi (AMF), obligately biotrophic organisms, persist in the soil as resting spores or mycelium. Hepper and Warner (1983) and StJohn *et al.* (1983) showed that the development of arbuscular mycorrhizal fungi is stimulated by soil organic matter. The limited asymbiotic growth of detached germ tubes stimulated by some organics was also observed by Hepper and Warner (1983) *in vitro*. Proliferation of intraradical distributive hyphae was found on surface-disinfected mycorrhizae in the presence of sucrose (Strullu and Romand 1986) or bovine albumin (Williams 1990). There is still no direct evidence of the uptake of organic compounds by the mycelium of arbuscular mycorrhizal fungi but some kind of transport probably exists, as is supported by observations on the uptake of some pesticides (Nelson and Khan 1990, 1992).

By contrast, the inhibition of mycorrhizal colonization was observed when various organic materials (Calvet *et al.* 1992) or cellulose were applied to the substratum (Avio and Giovannetti 1988). The effect of addition of such organics might be attributable to usable carbon and energy content. Mycorrhizal fungi may be also affected by physico-mechanical features of additives, such as providing more space in the cavities and pores for mycelium growth or spore germination. Also the enhancement of soil aeration or the improvement of soil water regime might be important aspects of addition of organic substances. However, such effects cannot be suspected if a labile, readily soluble additional carbon source is applied.

The aim of our study was to estimate the effect of an additional labile carbon source on the development of mycorrhizal symbiosis, mycelial growth as well as on the metabolic activity and proliferation of hyphae of the symbiotic fungus from mycorrhizal root fragments and on the subsequent development of soil saprophytes in non-sterile conditions of cultivation. Glucose was chosen for this purpose as a general source of carbon and energy with neglected mechanical effects on the physical soil properties. The effects of glucose were studied in two experiments where the mycorrhizal fungi grew in the symbiotic or the nonsymbiotic phase of their life cycle.

MATERIALS AND METHODS

Experiment 1. Symbiotic growth of the mycorrhizal fungus

Plant cultivation system. Maize plants (*Zea mays* L., cv. CE 240) were grown in 80 × 80 mm plastic pots filled with substratum consisting of Perlite–soil (3:1, *V/V*). Sandy loam soil with P content of 10 ppm according to Olsen and pH 6.8 was twice autoclaved for 25 min prior to use and then reinoculated with soil filtrate. 100 g of soil was suspended in 1 L of distilled H_2O and then filtered through Whatman filter paper no. 1. 0.5 L of filtrate was added to 10 kg of soil. Reinoculated soil was then incubated in vented plastic bags for 6 weeks at 28 °C to ensure microbial recolonization.

The substratum under each plant in mycorrhizal treatments was inoculated with approximately 2000 surface-disinfected spores of AMF *Glomus fistulosum* SKOU and JAKOBSEN (isolate BEG 23). Soil from pot cultures of *G. fistulosum,* maintained in the same soil as used for the experiment, was wet-sieved (Gerdemann and Nicolson 1963) and centrifuged in 2.5 mol/L sucrose overlayered with 20 mm distilled H_2O for 5 min at 58 Hz (centrifuge Janetzki T20, rotor diameter 200 mm). The fungal spores were removed from the sucrose-water interface using a pipette and rinsed thoroughly in running tap water. The spores were surface-disinfected for 5 min in 2 % solution of chloramine T (*Sigma*) with a trace of Tween 80 and then, after multiple washings with sterile water, used for the inoculation.

The plants were watered as needed and supplied weekly with 10 mL of P2N3 nutrient solution (Gryndler *et al.* 1992), containing (mg/L): $MgSO_4 \cdot 7H_2O$ 720, KH_2PO_4 12.2, $Ca(NO_3)_2 \cdot 4H_2O$ 295, KNO_3 240, KCl 13.5, $MnCl_2 \cdot 4H_2O$ 0.75, KI 0.75, $ZnSO_4 \cdot 7H_2O$ 0.75, H_3BO_3 1.5, $CuSO_4 \cdot 5H_2O$ 0.001, FeNaEDTA 4.3, $Na_2MoO_4 \cdot 2H_2O$ 0.00017.

Glucose dissolved in the nutrient solution was added at five levels: 0, 10, 30, 100 and 300 mg per plant weekly, except the first two weeks of cultivation, so that the total doses over the whole cultivation period were 0, 60, 180, 600 and 1800 mg per plant, respectively. Sets of five control plants and five mycorrhizal plants were exposed to each level of glucose. Plants were cultivated in the Conviron growth chamber at constant environmental conditions (16 h photoperiod, 330 μmol photons per m^2 per s, 20 °C/70 % relative humidity during the day and 18 °C/75 % at night).

Harvest and mineral analysis. After 8 weeks (6 d after the last glucose application), the plants, root systems and growth substratum were removed and analyzed. The shoot dry matter was digested in a 1:2 mixture of concentrated sulfuric acid–30 % hydrogen peroxide at 360 °C and analyzed for phosphate content by the phosphomolybdate complex spectrophotometry (Watanabe and Olsen 1965). The pH of the substrate was measured in a 1:1 (*V/V*) mixture of substratum–distilled water.

Analysis of basal respiration and microbial biomass of the substratum. Immediately after harvest and removal of the roots, three 20-g, water-saturated samples of substratum were prepared from each variant. Each sample was divided into two equal subsamples in Petri dishes, each of them being incubated separately in two hermetically sealed, evacuated (50 kPa) 5 L glass desiccators for 2 d at 28 °C. The first desiccator was saturated only with water vapor, the second contained 50 mL of chloroform for fumigation of the soil microbial biomass (modified from Jenkinsen and Powlson 1976). After exhausting the fumigant under vacuum and multiple flushing the desiccator with air, the samples were transferred to 100-mL calibrated flasks. The flasks were then supplied with 0.2 mL of soil suspension (10 g nonsterile soil in 100 mL of water), sealed with rubber plugs and incubated at 28 °C in the dark. Production of CO_2 was measured after 10 d of incubation in 0.5 mL samples, using a Perkin–Elmer 8500 gas chromatograph with 2-m column Chromosorb 102/80–100, at 150 °C, with N_2 as carrier gas at a flux of 19 mL/min, using a thermal conductivity detector (350 °C). The substratum basal respiration was measured as nmol CO_2 produced per g dry, nonfumigated substratum per day. The microbial biomass was expressed in nmol CO_2 per g dry substratum and was calculated as the difference between the total amount of CO_2 produced in fumigated and nonfumigated samples.

Microbiological analysis of the substratum. Abundance of colony forming units (CFU) of bacteria and saprophytic fungi was determined in the substratum. Microbial counts were estimated using a dilution plate technique. Two grams of freshly harvested substratum from each of five pots of the treatment were mixed and shaken for 10 min with 100 mL of sterile water. After the shaking, the suspension was diluted from $1:10^2$ to $1:10^7$ for the determination of microbial counts. The CFU of saprophytic micromycetes were counted on a modified Smith and Dawson medium (Smith and Dawson 1944) with soil extract replaced with yeast extract (2 g/L). The CFU of bacteria were estimated using T3 medium (Taylor 1951).

Analysis of root length, root hairs, colonization by AM fungi, length of aseptate hyphae and their metabolic activity on the roots and in the substratum. Root systems were cut to approximately

30–40 mm segments and thoroughly mixed. The roots from aliquots (40–60 g) of the mixture were washed and used for determination of the total root length per plant. The percentage of root length colonized was evaluated by grid-line intersect method according to Giovannetti and Mosse (1980) after clearing in 10 % KOH and staining with Trypan blue. Intensity of mycorrhizal colonization (M, %) and arbuscule content (A, %) were determined to characterize the mycorrhizal colonization in the roots according to Trouvelot *et al.* (1986). Abundance of root hairs was estimated as a percentage of root length bearing hairs.

Length and dihydrolipoamide dehydrogenase (EC 1.8.1.4; "diaphorase") activity of fungal hyphae attached to the root surface and in the substratum were determined using iodonitrotetrazolium (INT) vital staining (Sylvia 1988). Ten-mm-long root segments were incubated in buffered (0.2 mol/L Tris, pH 7.4) solution of INT (1 mg/mL) + NADH (3 mg/mL) for 14 h at 28 °C. The root segments were then stained with 0.05 % Trypan blue in lactoglycerol. Forty randomly selected microscopic fields were observed along the root segments mounted on a microscopic slide. The length of dehydrogenase-active (stained red and containing red granules of reduced INT stain) and dehydrogenase-inactive hyphae (counterstained blue with Trypan blue) per mm of root was measured using a grid-line intersect method (grid on a plate in the eye-piece) under the microscope at the magnification of 400×.

Five grams of substratum was thoroughly mixed in 500 mL of tap water and poured through a 200-μm sieve into a blender. The sample was blended for 20 s and a 10 mL subsample of the supernatant was pipetted onto a membrane filter (Synpor, 45 mm diameter, 0.6 μm pore size) and stained as above. The length of dehydrogenase-active and -inactive hyphae was measured on 40 randomly selected microscopic fields on each filter using the grid-line intersect method and expressed as the length of hyphae per gram dry substratum.

Experiment 2. Nonsymbiotic proliferation of hyphae from mycorrhizal root segments

Six-week-old maize plants cultivated in a hydroponic regime and colonized with *G. fistulosum* were harvested and the root systems were washed with 0.1 % $MgSO_4 \cdot 7H_2O$. Colonized (bright yellow) and noninfected (whitish) roots were carefully separated and cut into 2-mm segments. The root aliquots were then stained with Trypan blue to verify the mycorrhizal status. The yellow segments were heavily infected (82 % of the length) whereas the infection in whitish roots was less than 1 %.

The remaining nonstained root segments were incubated in the cultivation system supplied with glucose. The nonsterile cultivation system consisted of a 90-mm Petri dish containing a 5-mm layer of Perlite saturated with 15 mL of a nutrient solution identical with the solution used in Experiment 1. The layer was covered with a nitrocellulose membrane (Millipore HAWP 09000, pore size 0.4 μm, diameter 90 mm). The root segments (100 mg of either mycorrhizal or nonmycorrhizal fresh roots per dish) were randomly distributed on the upper surface of the membrane and superposed by a layer of 5 g of the soil sterilized 3 times for 6 h in a humid chamber at 80 °C. The soil was inoculated with the filtrate of the nonsterile soil sample (100 g of soil per 1 L distilled water) and left for 2 weeks at room temperature to ensure microbial recolonization.

The experiment was designed as a two-factorial. Dishes containing mycorrhizal or nonmycorrhizal roots were added weekly with a glucose solution (1 mL per dish) at 5 concentrations: 0, 0.3, 1, 3, and 10 mg per mL of distilled water. Each treatment had 3 replicates. After 6 weeks of incubation, the membranes were removed with the soil layer intact. The total length and proportion of the length with dihydrolipoamide-dehydrogenase-active coarse aseptate mycelium, considered as *G. fistulosum* extraradical hyphae, were measured for (*i*) mycelium associated with washed root segments, (*ii*) mycelium grown on the soil bearing membranes, and (*iii*) mycelium in the soil.

The length and activity of fungal hyphae were estimated on the root surface, in the soil and on a nitrocellulose membrane using INT vital staining (Sylvia 1988). Forty randomly selected microscopic fields were used to evaluate the hyphae attached to the root segments. The length of active hyphae and inactive hyphae per 1 mm of root was measured using a grid-line intersect method as in Experiment 1.

Soil samples of 2.5 g were thoroughly mixed in a 500 mL beaker of tap water and poured through a 200 μm sieve into the blender. The sample was blended for 20 s and a 5-mL subsample of supernatant was pipetted onto a membrane filter (45 mm diameter, 0.6 μm pore size). The length of active and inactive hyphae was measured on 40 randomly selected microscopic fields within each filter and expressed as the length per g of dry substratum. To estimate the length and activity of hyphae associated with the nitrocellulose membrane, the membrane was carefully washed and directly immersed in the INT solution, processed as indicated above, and 40 randomly selected microscopic fields were evaluated for the length and dehydrogenase activity of hyphae.

Statistical analysis. Data were analyzed by two-way ANOVA and Duncan's multiple range test was used to separate treatment means when the F values were significant. Homogeneity of variance of the data was tested (F-test) at different levels of experimental factors. If a significant nonhomogeneity between mycorrhizal and nonmycorrhizal treatments was observed, the data obtained in mycorrhizal treatments are evaluated using one-way ANOVA. Correlation coefficients and their significances were calculated for concentrations of bacterial and fungal CFU in the substratum for Experiment 1.

RESULTS

Experiment 1

The root length was not significantly influenced by glucose or inoculation (Table I). Plant shoot dry mass was significantly decreased by both inoculation with the mycorrhizal fungus and higher glucose inputs. The abundance of root hairs on the root system decreased with increasing doses of glucose. The effect of inoculation on the root hair abundance was not significant. Mycorrhizal inoculation significantly increased the concentration of phosphorus in the plant shoots. No correlation between the phosphorus content in shoot biomass and the glucose dose was found.

Table I. Effect of AMF and glucose on the dry mass of maize shoots, content of phosphorus in the shoots, total root length and percentage of root length bearing root hairs

Source of variability	Shoot dry mass g	Shoot P mg/g	Root length m	Root hairs %
AMF (*a*)				
noninoculated	2.34 a	1.07 b	43.7	52 a
inoculated	2.11 b	1.16 a	37.6	44 a
Glucose (*b*), mg				
0	2.42 a	1.13 ab	37.4	66 a
10	2.64 a	1.05 b	45.9	63 a
30	2.44 a	1.13 ab	42.9	49 b
100	1.95 b	1.24 a	41.2	33 c
300	1.62 b	1.02 b	35.9	27 c
Significance (two-way ANOVA)				
a	*	*	ns	ns
b	***	*	ns	***
a × *b*	ns	ns	ns	ns

Means followed by the same letter do not differ by Duncan's multiple range test at $p < 0.05$; *, *** — significant at $p < 0.05$ and 0.001, respectively.

Respiration of the substratum (Table II) showed that glucose was quickly utilized by the saprophytic microflora. Six days after the last glucose amendment, the respiration of the nonfumigated subsamples was almost uniform, and it was slightly increased only in the variants amended with 300 mg glucose. The substratum microbial biomass significantly increased with increasing glucose supply but there were no significant differences in the microbial biomass between nonmycorrhizal and mycorrhizal treatments supplied with the same amount of glucose. No significant effect of additions of glucose on the number of spores produced by the *G. fistulosum* was observed as compared to the mycorrhizal treatment without any glucose amendment.

The final pH of the substratum (Table III) was significantly increased only by the highest glusose doses, compared to the treatments without glucose amendment. The glucose application was significantly correlated with CFU counts of saprophytic fungi and bacteria in the substratum. The highest values of bacterial and fungal CFU were found in the non-inoculated treatment with maximum glucose input.

The highest values of the percentage of the root length colonized were observed in the treatment with 30 mg glucose added (Table IV). The highest dose of glucose resulted in the depression of

all parameters of mycorrhizal colonization. The arbuscules were also most abundant in the roots of plants amended with 30 mg glucose.

Table II. Effect of glucose amendment on the basal respiration of the substratum, microbial biomass at the end of cultivation and on sporulation of *G. fistulosum*

Source of variability	Basal respiration nmol CO_2 per g per d	Microbial biomass nmol CO_2 per g	Number of AM spores per g substratum
AMF (*a*)			
noninoculated	772 a	1264 a	19 b[1]
inoculated	803 a	1457 a	55 a
Glucose (*b*), mg			
0	737 b	396 b	31 a
10	718 b	849 b	40 a
30	727 b	1044 b	41 a
100	789 b	2346 a	42 a
300	966 a	2167 a	30 a
Significance (two-way ANOVA)			
a	ns	ns	***
b	***	***	ns
a × *b*	ns	ns	ns

Means followed by the same letter do not differ by Duncan's multiple range test at $p < 0.05$; *** — significant at $p < 0.001$.
[1]Nonliving residual spores from the original soil, killed by sterilization.

Table III. Effect of glucose and AMF on pH of the substratum (one-way analysis of variance) and CFU of bacteria and saprophytic fungi (correlation) in control noninoculated pots and pots inoculated with *G. fistulosum* at the end of the cultivation

AMF	Glucose mg per pot	pH of substratum	Bacteria CFU × 10^8/g	Saprophytic fungi CFU × 10^3/g
Control	0	6.57 d	2.6	7.3
	10	6.64 cd	1.6	4.7
	30	6.63 cd	2.2	4.0
	100	6.62 d	2.2	13.3
	300	6.80 b	5.0	118.0
G. fistulosum	0	6.76 bc	2.1	19.3
	10	6.66 cd	1.1	13.3
	30	6.60 d	2.3	24.0
	100	6.85 ab	3.1	58.0
	300	6.95 a	2.8	67.0
R		nd	0.756*	0.885***
Significance (one-way ANOVA)		***	nd	nd

Means followed by the same letter do not differ by Duncan's multiple range test at $p < 0.05$; *, *** — significant at $p < 0.05$ and 0.001, respectively; R = coefficient of correlation between glucose dosis and CFU counts of saprophytes; pH values gave significant interaction between both factors when evaluated using two-way ANOVA; nd — not determined.

Total length of root-associated aseptate hyphae was found to be significantly greater in treatments receiving the lowest doses of glucose (Table V). The length of dehydrogenase-active root-associated hyphae was highest when 30 mg of glucose was added, but the activity decreased at higher doses. The highest total hyphal length in the substratum was produced when 30 mg of glucose was added,

whereas the highest dehydrogenase-active length (not significantly different from the control receiving no glucose) was found when 100 mg glucose was added to the pots.

Experiment 2

Active mycelial structures were observed in all of the mycorrhizal treatments (Table VI). The values of lengths of mycelium in nonmycorrhizal treatments were very low as compared to the mycorrhizal treatments. In the mycorrhizal treatments, the highest lengths of total root associated hyphae were found in a treatment supplied with 0.3 mg glucose dosis and these were significantly different from the treatment without glucose amendment. A significant increase in the length of active mycelium associated with the nitrocellulose membrane was observed in treatment supplied with 10 mg of glucose. No significant response to glucose amendment was observed for mycelium in the soil.

Table IV. Effect of glucose on the percentage of root length colonized by AMF, intensity of colonization (M, %) and on abundance of arbuscules (A, %) in the root cortex

Source of variability	Root length colonized, %	M	A
AMF (*a*)			
noninoculated	0	0	0
inoculated	20.3	9.9	6.0
Glucose (*b*), mg			
0	21.3 ab	10.2 a	3.7 bc
10	25.1 ab	11.7 a	8.0 ab
30	27.6 a	15.4 a	10.5 a
100	20.6 b	10.8 a	6.7 ab
300	7.3 c	1.6 b	0.9 c
Significance	***	**	**

Variance in all variables differs significantly between mycorrhizal and nonmycorrhizal treatments, therefore only values obtained in mycorrhizal treatments could be analyzed by one-way ANOVA and Duncan's multiple range test. The means at levels of factor *b* present data from mycorrhizal treatments only. Means followed by the same letter do not differ by Duncan's multiple range test at $p < 0.05$; **, *** — significant at $p < 0.01$ and 0.001, respectively.

Table V. Effect of glucose amendment on the total and dehydrogenase (DH)-active length of aseptate hyphae on the root surface and in the substratum in control uninoculated pots and pots inoculated with *G. fistulosum*

Source of variability	Length of aseptate hyphae			
	mm/mm of root		m/g of substratum	
	total	DH active	total	DH active
AMF (*a*)				
noninoculated	0.012	0.001	1.11	0.24
inoculated	0.412	0.110	13.65	4.36
Glucose (*b*), mg				
0	0.430 bc	0.082 b	9.72 c	2.03 a
10	0.687 a	0.171 a	15.48 b	5.55 a
30	0.529 ab	0.204 a	20.86 a	4.74 a
100	0.217 c	0.053 b	15.88 ab	7.78 a
300	0.199 c	0.039 b	6.31 c	1.73 a
Significance	***	***	***	ns

Variance in the variable differs significantly between mycorrhizal and nonmycorrhizal treatments, therefore only values obtained in mycorrhizal treatments were analyzed by one-way ANOVA. The means at levels of factor *b* present data from mycorrhizal treatments only. Means followed by the same letter do not differ by Duncan's multiple range test at $p < 0.05$; *** — significant at $p < 0.001$.

Table VI. Effect of glucose amendment on the total length and dehydrogenase active length of aseptate hyphae associated with root segments, bearing membrane and hyphae present in soil in control uninoculated pots and pots inoculated with *G. fistulosum*

Source of variability	Length of hyphae associated with					
	root mm/mm		membrane m/20 cm^2		soil m/g	
	total	active	total	active	total	active
AM fungus (*a*)						
noninoculated	0.16	0.02	0.20	0.06	2.4	0.2
inoculated	2.55	1.23	2.42	0.79	9.1	5.6
Glucose (*b*), mg						
0	1.08 b	0.53	1.68	0.43 b	11.5	8.8
0.3	5.47 a	3.18	2.31	0.55 b	8.4	4.4
1	1.61 b	0.55	1.25	0.32 b	7.5	3.4
3	3.43 ab	1.60	2.17	0.59 b	10.3	6.2
10	1.18 b	0.30	4.70	2.08 a	7.9	5.1
Significance	*	ns	ns	*	ns	ns

Variance of all variables differs significantly between mycorrhizal and nonmycorrhizal treatments, therefore only values obtained in mycorrhizal treatments could be analyzed by one-way ANOVA and Duncan's multiple range test. The means at levels of factor *b* present data from mycorrhizal treatments only. Means of 3 replicates followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 0.05$; * — significant at $p < 0.05$.

DISCUSSION

Plant growth and phosphorus content in the first experiment were significantly changed by colonization with the mycorrhizal fungus, indicating the often observed changes in physiology, nutrition and growth of the mycorrhizal plants. Decreased shoot biomass was probably the result of the cultivation conditions in a growth chamber with relatively low light intensity. The increased content of phosphorus in plant biomass is a common result of inoculation with AMF (Smith *et al.* 1988).

Glucose has shown a significant effect on the physiology of the system. It was reflected in the decreased plant biomass and abundance of root hairs in treatments with high glucose doses. The pH variation due to glucose amendments did not exceed 0.26 pH unit at the end of the experiment, therefore the effects of glucose on the infection parameters and external mycelium are unlikely to be the result of change in the pH.

In the soil, there exist suppressive conditions for the growth of saprophytic micromycetes. This feature of soil environment is termed soil fungistasis and it can be strongly affected by an additional energy source (Watson and Ford 1972). The highest doses of glucose, used in the experiment, seemed to be sufficient to destroy the fungistasis in the substratum. It resulted in increased counts of CFU of saprophytic microfungi. An analogous, but much less remarkable effect for bacteria was also observed. Glucose was probably quickly utilized by the saprophytic microflora.

The respiration of the non-fumigated subsamples was almost uniform and was slightly increased only in the variants amended with 300 mg glucose. This is in agreement with the result of Huntjens *et al.* (1981) who reported that a major part of glucose added to the soil is converted to carbon dioxide during the first 3 d of cultivation.

As expected, the substratum microbial biomass significantly increased with increasing glucose supply. There was significant difference in microbial biomass between mycorrhizal and control treatments receiving the same glucose dose. This indicates that the biomass of mycelium of the symbiotic fungus represents only a smaller part of the total microbial biomass in the substratum. However, the evaluation of this mycelium biomass was probably below the resolution of the estimation and design used.

Many of the characteristics of the symbiotic fungus in the mycorrhizal treatments supplied with lower glucose doses were significantly higher when compared with the mycorrhizal treatment with-

out glucose amendment, indicating the general importance of the low concentration of carbon and energy source for the formation of mycorrhizae and particularly for the development of the extraradical mycelium of the fungus. The abundance of aseptate hyphae was negligible in control treatments. We can thus consider the hyphae found in mycorrhizal treatments to be mainly the mycelial structures of AMF.

The explanation of the effect of glucose on the obligate biotrophic AMF in an artificial substratum may consist in direct utilization by the mycelium or indirectly in the stimulation of beneficial associative saprophytic microflora supporting the development of mycorrhizal fungus. Several studies indicate the stimulation and attraction of hyphal growth by soil organic matter (Hepper and Warner 1983; StJohn *et al.* 1983) but very little is known about the direct utilization of organic compounds by AMF. Hepper and Jakobsen (1983) reported the stimulation of spore germination and germ-tube growth by amino acids without any evidence of their uptake. Mycelium of AMF can absorb radioactive Atrazine (Nelson and Khan 1992) or Fonofos (Nelson and Khan 1990). If the AMF are able to absorb these unnatural compounds, they may be able to absorb some free organic molecules from the soil solution (Gryndler *et al.* 1997), even if they are not involved directly in the decomposition of soil organic matter (Cuenca *et al.* 1983). The decomposing organic matter in close proximity to the mycorrhizal mycelium definitely represents a rich source of available carbon and energy (Mary *et al.* 1993).

The effects of organic substrates on the infectivity of propagules (nonsymbotic or pre-symbiotic stage of the life cycle) of mycorrhizal fungi was observed in numerous trials. In some cases, the mycelium was associated with organic matter particles in the soil (StJohn *et al.* 1983; Warner 1984). It may be interpreted as a result of attraction or growth stimulation of the mycelium in the soil microsites with a high content of organic matter. In other cases, growth of the mycorrhizal fungus decreased due to the presence of organic matter (Calvet *et al.* 1992; Soedarjo and Habte 1993). A remarkable negative effect of additional cellulose was observed by Avio and Giovannetti (1988). The results probably indicate that various kinds of organic compounds differ in their effects on mycelium development and that the effects depend also on other experimental conditions. As we observed in our second experiment, the small amounts of glucose added to the cultivation system stimulate the growth of extraradical mycelium significantly. The mechanisms of such a stimulation may have different reasons as the AMF may directly utilize the sugar added. Nevertheless, such an explanation is less probable since glucose is quickly utilized by saprophytic microorganisms. Molecules of glucose might be either incorporated into microbial biomass or pass through glycolysis as a source of energy, being finally converted into carbon dioxide. Carbon dioxide was reported to stimulate germ tube and extraradical hyphal growth of *Gigaspora margarita* (Bécard and Piché 1989).

It was observed that the development of mycorrhizal fungi is influenced by some soil bacteria (Azcón 1987) and microfungi (McAllister *et al.* 1994) and thus the glucose added to nonsterile cultivation systems may be utilized by saprophytic microorganisms and consequently stimulate their growth. Calvet *et al.* (1992) observed that sterile water extracts of two composts and peat stimulated mycelial growth as well as some of the microfungi isolated from the substrata. Although the stimulation caused by substratum extracts could be attributed to a nutrient supply, the metabolites produced by the microflora present in the substrata could be also involved in the effect.

For further research it is necessary to separate the direct effect of a source of organic carbon from the indirect effects of carbon dioxide and saprophytic microflora. Nevertheless it may be concluded that the development of AMF *G. fistulosum* is stimulated by labile carbon and energy source which is quickly utilized in the cultivation system. The effect is thus not attributable to the physical changes of growth substratum but to the increase of energy or carbon supply.

Research was supported by the *Ministry of Education, Youth and Sports of the Czech Republic* in a frame of the EC COST Project (821.10) and by the *Grant Agency of the Czech Republic*, grant 526/97/0595 – experiment 2. The autors wish to express the thanks to Mrs. H. Máchová and Mrs. J. Roučková for technical assistence.

REFERENCES

Avio L., Giovannetti M.: Vesicular-arbuscular mycorrhizal infection of lucerne roots in a cellulose amended soil. *Plant & Soil* **112**, 99–104 (1988).

Azcón R.: Germination and hyphal growth of *Glomus mosseae in vitro*: effects of rhizosphere bacteria and cell-free culture media. *Soil Biol.Biochem.* **19**, 417–419 (1987).

Bécard G., Piché Y.: Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Appl.Environ.Microbiol.* **55**, 2320–2325 (1989).

CALVET C., BAREA J.M., PERA J.: *In vitro* interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. *Soil Biol.Biochem.* **24**, 775–780 (1992).

CALVET C., ESTAUN V., CAMPRUBI A.: Germination, early mycelial growth and infectivity of a vesicular-arbuscular mycorrhizal fungus in organic substrates. *Symbiosis* **14**, 405–411 (1992).

CUENCA G., ARANGUREN J., HERRERA R.: Root growth and litter decomposition in a coffee plantation under shade trees. *Plant & Soil* **71**, 477–486 (1983).

GERDEMANN J.W., NICOLSON T.H.: Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans.Brit.Mycol.Soc.* **46**, 235–244 (1963).

GIOVANNETTI M., MOSSE B.: An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84**, 489–500 (1980).

GRYNDLER M., HRŠELOVÁ H., CHVÁTALOVÁ I.: An improved procedure for root surface disinfection suitable for observations of proliferation of intraradical hyphae of arbuscular mycorrhizal fungus *Glomus fistulosum*. *Folia Microbiol.* **42**, 489–494 (1997).

GRYNDLER M., VEJSADOVÁ H., VANČURA V.: The effect of magnesium ions on the vesicular-arbuscular mycorrhizal infection of maize roots. *New Phytol.* **122**, 455–460 (1992).

HEPPER C.M., JAKOBSEN I.: Hyphal growth from spores of the mycorrhizal fungus *Glomus caledonius*: effect of aminoacids. *Soil Biol.Biochem.* **15**, 55–58 (1983).

HEPPER C.M., WARNER A.: Role of organic matter in growth of a vesicular-arbuscular mycorrhizal fungus in soil. *Trans.Brit. Mycol.Soc.* **81**, 155–156 (1983).

HUNTJENS J.L.M., OOSTERVELD-VAN VLIET W.M., SAYED S.K.Y.: The decomposition of organic compounds in soil. *Plant & Soil* **61**, 227–242 (1981).

JENKINSEN D.S., POWLSON D.S.: The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol.Biochem.* **8**, 209–213 (1976).

MARY B., FRESNEAU C., MOREL J.L., MARIOTTI A.: C and N cycling during decomposition of root mucilage, roots and glucose in soil. *Soil Biol.Biochem.* **25**, 1005–1014 (1993).

MCALLISTER C.B., GARCIA-ROMERA I., GODEAS A., OCAMPO J.A.: *In vitro* interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*. *Soil Biol.Biochem.* **26**, 1369–1374 (1994).

NELSON S.D., KHAN S.U.: Effect of endomycorrhizae on the bioavailability of bound ^{14}C residues to onion plants from an organic soil treated with [^{14}C] Fonofos. *J.Agric.Food Chem.* **38**, 894–898 (1990).

NELSON S.D., KHAN S.U.: Uptake of Atrazine by hyphae of *Glomus* vesicular-arbuscular mycorrhizae and root systems of corn (*Zea mays* L.). *Weed Sci.* **40**, 161–170 (1992).

SMITH N.R., DAWSON V.T.: The bacteriostatic action of bengal rose in media used for plate counts of soil fungi. *Soil Sci.* **58**, 467–471 (1944).

SMITH S.E., GIANINAZZI-PEARSON V.: Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Ann.Rev.Plant Physiol.Plant Mol.Biol.* **39**, 221–224 (1988).

SOEDARJO M., HABTE M.: Vesicular-arbuscular mycorrhizal effectiveness in an acid soil amended with fresh organic matter. *Plant & Soil* **149**, 197–203 (1993).

STJOHN T.V., COLEMAN D.C., REID C.P.P.: Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. *Ecology* **64**, 957–959 (1983).

STRULLU D.G., ROMAND C.: Methode d'obtention d'endomycorrhizes a vesicules et arbuscules en conditions axeniques. *C.R.Acad.Sci.Paris* **303**, 245–250 (1986).

SYLVIA D.M.: Activity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *Soil Biol.Biochem.* **20**, 39–43 (1988).

TAYLOR C.B.: The nutritional requirements of predominant flora of the soil. *Proc.Soc.Appl.Bacteriol.* **14**, 101–111 (1951).

TISSERANT B., GIANINAZZI-PEARSON V., GIANINAZZI S., GOLLOTTE A.: *In-planta* histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. *Mycol.Res.* **97**, 245–250 (1993).

TROUVELOT A., KOUGH J., GIANINAZZI-PEARSON V.: Estimation of VA mycorrhizal infection levels. Research for methods having a functional significance, pp. 223–232 in V. Gianinazzi-Pearson, S. Gianinazzi (Eds): *Physiological and Genetical Aspects of Mycorrhizae*. INRA, Service des Publications, Versailles (France) 1986.

WARNER A.: Colonization of organic matter by vesicular-arbuscular mycorrhizal fungi. *Trans.Brit.Mycol.Soc.* **82**, 352–354 (1984).

WATANABE R.S., OLSEN S.R.: Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci.Soc.Am.Proc.* **29**, 677–768 (1965).

WATSON A.G., FORD E.J.: Soil fungistasis — a reappraisal. *Ann.Rev.Phytopathol.* **10**, 327–348 (1972).

WILLIAMS P.G.: Disinfecting vesicular-arbuscular mycorrhizae. *Mycol.Res.* **94**, 995–997 (1990).