FOLIA FORESTALIA POLONICA
Series A — Forestry, Number 36, 1994

Effect of Different Soil Bacteria on Mycorrhizae Formation in Scots Pine (*Pinus sylvestris* L.) — *in vitro* Studies

*Henryk Różycki, Maria Kampert, Edmund Strzelczyk, Ching-Yan Li, David A. Perry**

Laboratory of Microbiology,
Institute of Biology,
Nicolaus Copernicus University,
9 Gagarina, 87–100 Toruń
and
USDA, Forest Service,
Pacific Northwest Research Station,
Forestry Sciences Laboratory,
3200 S.W. Jefferson Way, Corvallis,
Oregon 97331, USA
and
Department of Forest Science,
Oregon State University, Corvallis,
Oregon 97331–5705, USA

*H. Różycki, M. Kampert and E. Strzelczyk, Laboratory of Microbiology, Institute of Biology, Nicolaus Copernicus University, ul. Gagarina 9, 87–100 Toruń, Poland; C.Y. Li, U. S.D.A., Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 S.W. Jefferson Way, Corvallis, Oregon 97331, USA; David A. Perry, Department of Forest Science, Oregon State University, Corvallis, Oregon 97331-5705, USA

Abstract

Studies were carried out on the effect of soil bacteria (*Arthrobacter* sp., *Bacillus subtilis* and *Pseudomonas fluorescens*) and the actinomycete *Frankia* on mycorrhizae formation by *Laccaria laccata* and *Rhizopogon vinicolor* in pine seedlings (*Pinus sylvestris* L.) Action of bacteria both on mycorrhizae formation and on some morphological parameters of the seedlings depended on: the fugal symbiont, the bacterium and on the parameter. Morphological parameters of pine seedlings were most strongly affected by *Bacillus subtilis* together with *Rhizopogon vinicolor*, but these effects differed depending on the parameters. *Bacillus* stimulated the total length of lateral roots, total number of needles, but inhibited the stem and main root lenght in seedlings inoculated both with *Laccaria laccata* and with *Rhizopogon vinicolor*. A stimulatory action of *Arthrobacter* sp. on the number of mycorrhizal roots was observed. Also a stimulatory influence of bacteria on the number of feeder roots was noted.

Key words

Mycorrhizal fungi, soil bacteria, Scots pine, mycorrhiza synthesis on agar (in vitro)

Introduction

It is well known that ectomycorrhizae formation and functioning depends upon many ecological factors (Slankis, 1974; Bowen and Theodorou, 1979; Gogala, 1991; Garbaye, 1991). It particularly depends upon the action of associated soil microflora (Bowen and Theodorou, 1979; Garbaye, 1991). There can be following mechanisms of the action of associated soil microorganisms on fungal symbionts and/or on the host plant: a) production of hydrolytic enzymes (cellulases, pectinases, proteases and chitinases), which may enable the penetration of symbiont's hyphae into the tissues of the host-plant root (Strzelczyk and Szpotański, 1989; Strzelczyk et al., 1990); b) production of B-vitamins, amino acids, plant growth hormones and other biologically active substances — affecting both the fungal symiont and the host-plant (Kampert and Strzelczyk, 1984; Strzelczyk and Pokojska-Burdziej, 1984; Strzelczyk and Leniarska, 1985; Strzelczyk and Różycki, 1985; Różycki and Strzelczyk, 1986); c) N_2-fixation by some ectomycorrhizae-associated bacteria — may provide both mycorrhizae partners with nitrogen in poor forest soils (Chanway and Holl, 1991; Li et al., 1992); d) production of antibiotics and phytotoxins — can affect negatively both the fungus and the host-plant (Friedman et al., 1989; Richter et al., 1989). Despite of many studies on the mechanisms of the effect of accompanying soil microbes on ectomycorrhizal fungi — the knowledge about possible effects of minroorganisms on mycorrhizae formation and functioning is very scarce (Bowen and Theodorou, 1979; Garbaye, 1991). Therefore this research was undertaken.

Materials and methods

Two fungal isolates (isolated from ectomycorrhizae of Douglas-fir) were used in present studies:

- *Laccaria Laccata* (Scop. ex Fr.) Berk and Br. (S-238)
- *Rhizopogon vinicolor* Smith (7534).

Both fungal isolates were obtained from the Forestry Sciences Laboratory (USDA — Forst Service, Pacific Northwest Research Station, Corvallis, Oregon, USA).

Bacterial strains (*Arthrobacter* sp., *Pseudomonas fluorescens* and *Bacillus subtilis*) were from the culture collection of the laboratory of Microbiology, N. Copernicus University, Toruń, Poland (originally — from American Type Culture Collection [ATCC]). Isolate of *Frankia* sp. — isolated from actinorrhizae of red alder — was provided (similarly as in the case of mycorrhizal fungi) by Dr. C.Y. Li of Forestry Sciences Laboratory (USDA, Corvallis, Oregon, USA).

Seeds of Scots pine (*Pinus sylvestris* L.) (first class of quality, harvested in 1989, >95% or germination) were surface sterilized in 1% bromine water for 5 min., washed several times in sterile distilled water and germinated on glucose-peptone agar. After 4–7 days uncontaminated germlings at uniform stage of root emergence were aseptically transferred into large (35×3.5 cm) culture tubes, containing 100 ml of starvation agar medium (Pachlewski and Pachlewska, 1974) for mycorrhizae formation testing (0.7% water containing 1 mg thiamine/liter).

The plants were grown in a plant growth chamber (photoperiod: 16 hours — light of 4500 lux with temperature between 20–22°C and 8 hour dark period with temperature between 17–19°C). 2-week old seedlings were inoculated with plugs (diameter: 1 cm) mycelium-containing agar cut from the edge of actively growing colonies on PDA (Difco); non-mycorrhizal control was "inoculated" with sterile PDA plugs (at least 10 seedlings per each combination). After one more week (the third week) part of seedlings (except of the second — "mycorrhizal fungus only" control) was inoculated with bacterial wash-off (grown on "A" medium [Lochhead and Chase, 1943] agar slants) suspensions applied separately — and in one case in combination (*Pseudomonas* + *Arthrobacter*). Titre of the bacterial suspensions used for inoculation ranged between $5–7\times10^7$ cfu×ml^{-1}. In one combination (for both fungi) a homogenized (previously washed) suspension of actinomycete *Frankia* (grown in modified BAP medium by Murry et al., 1984) was used for inoculation. Seedlings were grown for 3 months (since inoculation with bacteria); periodic observations of stem — and root growth as well as those of lateral roots — and ectomycorrhizae formation were performed. At the termination of the experiment the following parameters for each seedling were estimated:

- length of stem (cm);
- length of main root (cm);
- total length of lateral roots (cm);
- number of brown needles;
- total number of needles;
- % of brown needles;
- total number of feeder roots (sum of non-mycorrhizal- and mycorrhizal feeder roots);

- number of mycorrhizal feeder roots (only in combinations with seedlings inoculated with fungi);
- % of mycorrhizal roots ("inoculated combinations").

Both fungi and bacteria were resolated from the roots of seedlings after completion of the experiment. Roots of seedlings after withdrawal from the agar medium were dipped into the liquid medium "A" (Lochhead and Chase, 1943) in culture tubes. After incubation at 26°C, bacterial growth was checked in medium "A". Selected roots — after serial washings with sterile distilled water were cut into 0.5 mm pices and placed on the surface of PDA (Difco) in Petri dishes — to grow the appropriate mycorrhizal fungus.

In order to evaluate the obtained results — the following statistical methods were used:

- Student's t-test for independent samples ($p \leq 0.05$);
- 1.factor analysis of variance (ANOVA) and Newman-Keuls multiple range test ($p \leq 0.05$);
- multiple correlation and regression.

Per cent data were transformed according to the function $y = \text{arc sin } \sqrt{x}$. All the calculations were performed using CSS:STATISTICA package (release 3 E, 1991, StatSoft, Tulsa, Oklahoma, USA) for IBM PC and compatibles.

Results

Obtained results are presented in Tables 1–5.

Inoculation with the fungus *Lacaria laccata* did not affect significantly the length of stem (except of inhibitory action of *Arthrobacter* sp. and *Bacillus subtilis*) and total length of laterial roots, total number of needles and per cent of brown needles in pine seedlings (Tab. 1 and 3). Similarly inoculation with *Rhizopogon vinicolor* did not affect the number of brown needles and their percentage (Tab. 2 and 4). Total number of feeder roots was significantly ($p<0.05$) higher in all experimental combinations inoculated both with *Laccaria laccata* and with *Rhizopogon vinicolor* (+ bacteria) as compared with the control (seedlings not inoculated) (Tab. 1–4). The highest stimulation was observed in the combination: *Rhizopogon* + *Arthrobacter* Sp. (Tab. 2 and 4).

Bacteria (except *Pseudomonas fluorescens* inhibited significantly the main root length in seedlings inoculated with *Laccaria laccata. Pseudomonas fluorescens* together with *Laccaria laccata* ($p \leq 0.05$) diminished significantly the number of feeder roots (Tab. 1 and 3). The same bacterium coinoculated with *Rhizopogon vinicolor* diminished the total length of lateral roots — as well as the length of stem in the pine seedlings studies (Tab. 2 and 4).

Bacillus subtilis increased of the total length of lateral roots, as well as — the total number of needles in seedlings inoculated with *Rhizopogon vinicolor* (Tab. 2 and 4). However the same organism considerably inhibited the growth of both of the main root and stem in seedlings seeded with both the ectomycorrhizal fungi (Tab. 1–4).

Both *Pseudomonas fluorescens* and *Bacillus subtilis* caused significant decrease of the number of mycorrhizal roots in seedlings inoculated with *Laccaria laccata.* In seedlings inoculated with the same fungus — *Arthrobacter* sp. exerted an inhibitory effect on the total number of needles (Tab. 1 and 3). We stated significant ($p<0.05$) stimulatory action of *Arthrobacter* sp. on the number of mycorrhizal roots in seedlings inoculated with both fungi (Tab. 1–4).

TABLE 1. Effect of bacteria on growth of pine seedlings and on ectomycorrhize formation

ings inocu-

mpletion of
were dipped
r incubation
after serial
n the surface

ls were used:

le range test

$\sqrt{x}$. All the
)91, StatSoft,

the length of
d total length
pine seedlings
ct the number
der roots was
oth with *Lac-*
th the control
l in the combi-

ain root length
together with
ots (Tab. 1 and
the total length
(Tab. 2 and 4),
as — the total
Tab. 2 and 4).
e main root and

decrease of the
ta. In seedlings
fect on the total
latory action of
with both fungi

TABLE 1. Effect of bacteria on growth of pine seedlings and on ectomycorrhize formation by *Laccaria laccata**

Experimental combinations (Inoculation)	Length of stem (cm)	Length of main root (cm)	Total length of lateral roots (cm)	Number of brown needles	Total number of needles	% ** of brown needles	Total number of feeder roots	Number of mycorrhizal roots	% of ** mycorrhizal roots
Control (not inoculated)	3.185a± 0.0632	11.07b± 0.3458	23.46a± 1.9035	3.55a± 0.2284	21.95a± 0.5716	16.30± 1.1869	48.9a± 4.4921	–	–
Laccaria (without helper)	3.80a± 0.1240	11.44b± 0.3961	24.01a± 2.5472	4.50a± 0.5095	23.62a± 1.3400	18.64a± 1.6011	111.12b± 6.3261	74.88a± 6.1224	66.53ab± 3.2393
Laccaria ± *Arthrobacter sp.*	3.28a± 0.1803	8.80a± 0.6287	23.77a± 2.5179	3.30a± 0.6327	18.90a± 1.3364	17.67a± 3.6213	108.40b± 9.4441	86.80b± 8.4695	79.35b± 2.1701
Laccaria ± *Pseudomonas fluorescens*	3.73a± 0.1248	10.50a± 0.7993	23.90a± 2.3360	2.70a± 0.6483	20.20a± 1.1286	13.21α± 3.0943	86.70b± 5.3732	53.10a± 5.9989	60.47α± 4.1405
Laccaria ± *Frankia sp.*	3.30a± 0.1057	9.78a± 0.3168	27.17a± 3.7445	4.00a± 0.5472	21.90a± 1.2435	17.53a± 2.1377	99.00b± 6.0808	71.00a± 4.3091	72.15b± 2.2177
Laccaria ± *Arthrobacter* ± *Pseudomonas*	3.55a± 0.1954	9.75a± 0.4646	25.89a± 2.3975	4.90a± 0.3589	21.10a± 0.9421	23.63a± 1.9717	89.93b± 5.7971	66.60a± 5.3679	73.72b± 2.8503
Laccaria ± *Bacillus subtilis*	3.15a± 0.1743	9.60a± 0.3063	35.02a± 4.8053	3.90a± 0.5731	22.00a± 1.1566	18.63a± 3.0535	99.30b± 6.5060	56.00a± 4.0338	56.50a± 2.1598

Explanations:

* Average values from 10 replications ± standard error. Values in a given column marked with the same letter do not differ significantly ($p \leq 0.05$; Newman-Keuls multiple range test);

** Per cent data for analysis of variance were transformed according to the function: $y = \arcsin\sqrt{x}$

TABLE 2. Effect of bacteria on growth of pine seedlings and on mycorrhizae formation by *Rhizopogon vinicolor**

Experimental combinations (Inoculation) Lenght of stem (cm)	Length of main root (cm)	Total length of lateral-roots (cm)	Number of brown needles	Total number of needles	% ** of brown needles	Total number of feeder roots	Number of mycorrhizal roots	% of ** mycorrhizal roots
Control (not inoculated)								
3.185ab±0.0632	11.07b±0.3458	23.46ab±1.9035	3.55a±0.2284	21.95a±0.5716	16.30a±1.1869	48.9a±4.4921	–	–
Rhizopogon (without helper)								
3.70b±0.1148	11.83b±0.8239	27.15b±2.2691	3.22a±0.7580	22.88a±0.6231	14.06a±3.4071	84.29b±7.2943	42.62a±7.2751	49.95a±6.1994
Rhizopogon +Arthrobacter sp.								
3.91bc±0.1877	12.30b±0.4535	23.15ab±1.8461	3.40a±0.6951	23.30a±1.1396	15.54a±3.2570	115.15b±8.0317	73.60b±6.1481	63.33a±1.9561
Rhizopogon + Pseudomonas fluorescens								
3.33ab±0.1140	12.10b±0.3373	17.74a±3.1414	4.10a±0.6069	21.60a±0.6951	18.89a±2.5461	86.40b±8.4570	38.50a±4.0910	46.87a±4.7448
Rhizopogon + Frankia sp.								
3.50b±0.1232	8.11b±0.5733	21.17a±1.6601	2.00a±0.6776	21.60a±0.9810	9.94a±3.5170	77.70b±8.6540	23.90a±7.4198	40.40a±11.5512
Rhizopogon + Arthrobacter + Pseudomonas								
3.46b±0.0896	12.07b±0.3447	16.30a±2.7703	1.90a±0.6390	23.60a±1.0780	7.83a±2.6379	78.42b±8.7286	39.20a±7.2364	49.65a±7.2509
Rhizopogon + Bacillus subtilis								
2.92a±0.0596	8.15a±0.4367	42.50c±2.2395	4.40a±0.5509	26.80b±0.8914	16.57a±2.1469	98.90b±13.4231	48.50a±14.1330	40.84a±7.6189

Explanations: see Table 1

TABLE 3. Effect of bacteria on growth of pine seedlings and on mycorrhizae formation by *Laccaria laccata* (per cent of control)

Experimental combinations (I...

TABLE 3. Effect of bacteria on growth of pine seedlings and on mycorrhizae formation by *Laccaria laccata* (per cent of control)

Experimental combinations (Inoculation)	Length of stem	Length of main root	Total length of lateral--roots	Number of brown needles	Total number of needles	% of brown needles	Total number of feeder roots	Number of mycor-rhizal roots	% of mycorrhizal roots
Control (not inoculated)	100	100	100	100	100	100	100	–	–
Laccaria (without helper)	119.31*** 100	103.32 100	102.35 100	126.76 100	107.63 100	114.39 100	227.25*** 100	100	100
Laccaria + *Arthrobacter sp.*	#102.98 ##86.31*	79.49** 76.94**	101.32 98.99	92.96 73.33	86.10 80.00*	108.44 94.80	221.68*** 97.55	115.93	119.28**
Laccaria + *Pseudomonas fluorescens*	117.11** 98.16	94.85 91.80	101.87 99.53	76.06 60.00	92.03 85.50	81.08 70.88	177.30*** 78.02*	70.92*	90.89
Laccaria + *Frankia sp.*	103.61 86.84**	88.32* 85.49**	115.80 113.13	112.68 88.88	99.77 92.70	107.57 94.04	202.45*** 89.09	94.82	108.45
Laccaria + *Arthrobacter* + *Pseudomonas*	111.46 93.42	88.07* 85.24*	110.35 107.81	138.03** 108.88	96.13 89.31	145.00** 126.76	183.91*** 80.92*	88.95	110.80
Laccaria + *Bacillus subtilis*	98.90 82.89**	86.72** 83.93**	149.27* 145.84	109.85 86.66	100.23 93.12	114.30 99.92	203.07*** 89.36	74.79*	84.93*

Explanations:

Significance of differences as compared with control (t-test): * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$;

\# per cent of control I (not inoculated);

\#\# per cent of control II (inoculated with the fungus only)

TABLE 4. Effect of bacteria on growth of pine seedlings and on mycorrhizae formation by *Rhizopogon vinicolor* (per cent of control)

Experimental combinations (Inoculation)	Length of stem	Length of main root	Total length of lateral--roots	Number of brown needles	Total number of needles	% of brown needles	Total number of feeder roots	Number of mycorrhizal roots	% of mycorrhizal roots
Control (not inoculated)	100	100	100	100	100	100	100	–	–
Rhizopogon (without helper)	116.17**	106.95	115.73	90.77	104.28	86.30	172.37**		
	100	100	100	100	100	100	100	100	100
Rhizopogon +	#122.76**	111.11	98.70	95.77	106.15	95.33	235.49***		
Arthrobacter sp.	##105.67	103.94	85.29	105.52	101.80	110.46	136.61*	172.67**	126.79
Rhizopogon +	104.55	109.30	75.62	115.49	98.40	115.93	176.89**		
Pseudomonas fluorescens	90.00*	102.25	65.34*	127.24	94.37	134.33	102.50	90.32	93.83
Rhizopogon +	109.89*	73.26***	90.24	56.34	98.40	61.02	158.89*		
Frankia sp.	94.59	68.53**	77.97	62.07	94.37	70.70	92.18	56.07	80.88
Rhizopogon + *Arthrobacter* +	108.63*	109.03	69.48	53.52**	107.51	48.07*	160.37*		
Pseudomonas	93.51	102.00	60.04**	58.96	103.11	55.70	93.04	91.96	99.9
Rhizopogon +	91.68**	73.62***	181.16***	123.94	122.09***	101.65	202.25**		
Bacillus subtilis	78.92***	68.87***	156.54***	136.55	117.09**	117.79	117.33	113.78	81.75

Explanations: see Table 3

■ Dis

Micr
lishing o
(Rambell
nois and

Ta
hologi
of feed
were si
was ver
inocula
rhizal rc
signific
roots as

Explanat

Inocu
fungi

All
con

Ex

T
th
an
2)
wi

TABLE 5. The relationship between different parameters of pine seedlings (independent variables: x_1–x_5[#] and the number of feeder roots (y) in all the experimental combinations (including non-inoculated control) and between the above mentioned parameters (independent variables) and: 1) the number of mycorrhizal roots; 2) the total number of feeder roots; 3) % of mycorrhizal roots — in combinations with seedlings inoculated with mycorrhizal fungi

Experimental combinations	No. of seedlings (N)	Mycorrhizal fungus	Dependent variable (y)	Multiple correlation coefficient (R)[##*]
All (including non-inoculated control)	70	*Laccaria laccata*	Total number of feeder roots	0.4332*
		Rhizopogon vinicolor	Total number of feeder roots	0.5253 ***
Inoculated with mycorrhizal fungi (+ putative "helpers")	60	*Laccaria laccata*	Number of mycorrhizal roots	0.3251
			Total No. of feeder roots	0.3963
			% of mycorrhizal roots	0.3221
		Rhizopogon vinicolor	Number of mycorrhizal roots	0.3837
			Total No. of feeder roots	0.5342**
			% of mycorrhizal roots	0.1398

Explanations:
[#] Independent variables:
x_1 — length of stem,
x_2 — length of main root,
x_3 — total length of lateral roots,
x_4 — number of brown needles,
x_5 — total number of needles;
[##] Significance of correlation coefficient: * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$

Table 5 points out that multiple correlation coefficients between five different morphological parameters of pine seedlings taken as independent variables — and total number of feedder roots in all the experimental combinations (including non-mycorrhizal control) were significant both for *Laccaria laccata* and for *Rhizopogon vinicolor* (in the last case it was very high — $p<0.001$). Analogical coefficients were also calculated for combinations inoculated with fungi, taking for dependent the following variables: a) number of mycorrhizal roots; b) total number of feeder roots and c) per cent of mycorrhizal roots. In this case significant correlation was noted only for *Rhizopogon vinicolor* with total number of feeder roots as the dependent variable (Tab. 5).

■ Discussion

Microorganisms accompanying mycorrhizae are of great importance both for establishing of symbiosis and its functioning as well as for the survival of mycorrhizal fungi (Rambelli, 1973; Bowen and Theodorou, 1979; Garbaye and Bowen, 1987, 1989; Duponnois and Garbaye, 1990; Garbaye et al., 1990; Garbaye, 1991). Microbes promoting

mycorrhizae formation ("helpers") can be isolated not only directly from the mycorrhizal mantle, but they can be found also beyond the mycorrhiza (Garbaye and Bowen, 1989). For instance free-living, N_2-fixing bacteria of the genera *Azospirillum* and *Bacillus* (associated with ectomycorrhizae of Douglas-fir) can affect the formation and functioning of ectomycorrhizae (Li and Castellano, 1987; Li et al., 1992).

In the present work an influence of some soil bacteria (*Arthrobacter* sp., *Bacillus subtilis*, and *Pseudomonas fluorescens*) and the actinomycete *Frankia* sp. on ectomycorrhizae formation by *Laccaria laccata* and *Rhizopogon vinicolor* in pine seedlings was studied. Results obtained here — especially as related to particular bacteria studied are not unambiguous. Morphological parameters of pine seedlings were most strongly affected by *Bacillus subtilis* together with *Rhizopogon vinicolor*, but these effects differed depending on the parameters. *Bacillus* stimulated the total length of lateral roots, total number of needles, but inhibited the stem and main root length both in seedlings inoculated with *Laccaria laccata* and with *Rhizopogon vinicolor*. A stimulatory action of *Arthrobacter* sp. on the number of mycorrhizal roots was observed. Also stimulatory influence of bacteria on the number of feeder roots was noted. Similarly Garbaye and Bowem (1989) observed a promoting effect of helper bacteria (mainly *Pseudomonas* sp.) on the formation of feeder roots in *Pinus radiata* (with *Rhizopogon luteolus* as a symbiont) both under sterile and non-sterile conditions.

In our work different morphological parameters of seedlings inoculated with ectomycorrhizal fungi and with bacteria were estimated. Total number of feeder roots has turned out to be a parameter correlated better with other morphological characteristics of pine seedlings than number and percentage of mycorrhizal roots. Further studies on interrelationships between different morphological characteristics of tree seedlings tested on mycorrhizae formation are needed. Their results may facilitate the evaluation of effectiveness of mycorrhizal inoculation with a simultaneous limitation of difficult and timeconsuming work dealing directly with mycorrhizas.

Garbaye (1991) and Duponnois and Garbaye (1990) have pointed out the following possible mechanisms of effect of helper microorganisms both on ectomycorrhizal fungi and on ectomycorrhiza formation: direct trophic stimulation (production of organic compounds utilized as nutrients and growth factors, N_2-fixation), detoxication in the rhizosphere (decomposition or neutralization of toxic compounds), modification of the root exudates and production of cellulolytic, pectolytic and other enzymes. However effects of associative inoculation with ectomycorrhizal and potential helper are dependent not only on the kind of host, symbiont, helper and the mechanism(s) of helpers' action (Garbaye, 1991) but they depend also on temperature and pH (Garbaye and Bowen, 1989), density of helper's inoculum (Andrade et al., 1991), duration of the experiment (Pachlewski and Pachlewska, 1974; Rudawska, 1986) and other environmental factors (Garbaye, 1991).

It has to be stressed that potential helper microorganisms act not only on the ectomycorrhizal fungus, but also on the host plant itself. Therefore effects of soil microbes on ectomycorrhizal fungi *in vitro* and on mycorrhiza formation can be different. E. g. Bowen and Theodorou (1979) observed that bacteria inhibiting growth of a given ectomycorrhizal fungus in vitro stimulated ectomycorrhiza formation by this organism. According to Garbaye (1991) effect of soil microbes on ectomycorrhiza can be associated with the regulation of gene expression both in the symbiont and in host-plant. Therefore they can affect the symbiosis in a different way depending on the host-seedling.

Summarizing, it has to be pointed out that any experiments with mycorrhiza in vitro can only help to understand simple physiological relationships, but they cannot explain interactions that take place in natural ecosystems. The effect of potential helpers in natural environment can be different than in vitro due to the presence of other microorganisms — particularly antagonistic towards helpers. Besides changeable physical and chemical conditions in natural ecosystems can affect biochemical processes and thus they can also act on interrelationships between the host-plant, fungal symbiont and associated soil microflora. Thus beneficial action of helpers on mycorrhiza formation — very distinct both in sterile- and in glasshouse conditions can be hardly noticeable under field nursery conditions (Garbaye et al., 1990). For this reason selected of helpers suitable for the practical use should be based on the results of experiments performed under natural conditions (or at least ones very close to them). Therefore the results of our work are very preliminary and studies on the effect of bacteria on ectomycorrhiza formation should be continued towards confirming possible beneficial action of bacteria on ectomycorrhizae under nursery conditions.

Conclusions

- Total number of feeder roots was significantly higher in plants inoculated with *Laccaria laccata* and *Rhizopogon vinicolor* (+ bacteria) than in the non-inoculated seedlings.
- The highest stimulation of pine seedlings was noted with *Rhizopogon* + *Arthrobacter.*
- *Pseudomonas fluorescens* used together with *Laccaria laccata* and *Rhizopogon vinicolor* affected negatively the growth of roots of pine seedlings.
- *Bacillus subtilis* affected positively the growth of pine only when inoculated with *Rhizopogon vinicolor.*

Acknowledgements

This research was carried out under a joint programme performed in cooperation with the United States Department of Agriculture and the Polish Ministry of Agriculture and Food Production under the Second M. Skłodowska-Curie Fund (Project No. PL-AES-139).

References

Andrade G., Azcon-Aguilar C., Barea J.M., 1991. Effect of different soil bacteria on the axenic development of *Glomus mosseae:* Effect of the inoculum density. In Abstracts from the 3rd European Symposium on Mycorrhizae — Mycorrhizas in Ecosystems, Sheffield, August 19–23, 1991 (Abstr.).

Bowen G.D., Theodorou C., 1979: *Soil Biol. Biochem.* 11, 119.

Chanway C.P., Holl F.B., 1991: *Can. J. Bot.* 69, 507.

Duponnois R., Garbaye J., 1990: *Can. J. Bot.* 68, 2148.

Friedman J., Hutchins A., Li C.Y., Perry D.A., 1989: *Biol. Plantarum* (Praha) 31 (6), 487.

Garbaye J., 1991: *Experimentia* 47, 370.
Garbaye J., Bowen G.D., 1987: *Can. J. For. Res.* 17, 941.
Garbaye J., Bowen G.D., 1989: *New Phytol.* 112, 383.
Garbaye J., Dupponois R., Wahl J.L., 1990: *Symbiosis* 9, 267.
Gogala N., 1991: *Experientia* 47, 331.
Kampert M., Strzelczyk E., 1984: *Acta Microbiol. Polon.* 33, 77.
Li C.Y., Castellano M.A., 1987: *Trans. Br. Mycol. Soc.* 88, 563.
Li C.Y., Massicotte H.B., Moore L.V.H., 1992. *Plant and Soil* 140, 35.
Lochhead A.G., Chase F.E., 1943: *Soil Science* 55, 185.
Murry M.A., Fontaine M.S., Torrey J.G., 1984: *Plant and Soil* 78, 61.
Pachlewski R., Pachlewska J., 1974: Studies on symbiotic properties of mycorrhizal fungi of pine (*Pinus sylvestris* L.) with the aid the method of mycorrhizal synthesis in pure culture on agar. Publ. by Forest Res. Inst., Warsaw.
Rambelli A., 1973: The rhizosphere of mycorrhizae. In Ectomycorrhizae — Their Ecology and Physiuology. Edited by G.C. Marks and T.T. Kozlowski. Academic Press, New York. pp. 299–349.
Richter D.L., Zuellig T.R., Bagley S.T., Bruhn J.N., 1989: *Plant and Soil* 115, 109.
Różycki H., Strzelczyk E., 1986: *Zentralbl. Mikrobiol.* 141, 423.
Rudawska M., 1986: *Arboretum Kórnickie* 31, 269.
Slankis V., 1974: *Ann. Rev. Phytopath.* 12, 437.
Strzelczyk E., Kampert M., 1987: *Angew. Botanik* 61, 157.
Strzelczyk E., Leniarska U. 1985: *Plant and Soil* 86, 387.
Strzelczyk E., Pokojska-Burdziej A., 1984: *Plant and Soil* 81, 185.
Strzelczyk E., Różycki H., 1985: *Zentralbl. Mikrobiol.* 140, 293.
Strzelczyk E., Szpotański T., 1989: *Biol. Fertil. Soils* 7, 365.
Strzelczyk E., Gorlach K., Różycki H., 1990: *Biol. Fertil. Soils* 9, 268.

Streszczenie (Summary)

Wpływ bakterii glebowych na zawiązywanie się mikoryz u sosny zwyczajnej (*Pinus sylvestris* L.) — badania in vitro

Przeprowadzono badania nad wpływem bakterii glebowych (*Arthrobacter* sp., *Bacillus subtilis* i *Pseudomonas fluorescens*) oraz promieniowca *Frankia* na tworzenie mikoryz przez *Laccaria laccata* i *Rhizopogon vinicolor* u siewek sosny (*Pinus sylvestris* L.). Oddziaływanie bakterii, zarówno na zawiązywanie się mikoryz jak i na niektóre morfologiczne parametry siewek sosny, zależało od: grzybowego symbionta, bakterii i parametru. Na morfologiczne parametry siewek sosny najsilniej wpływał *Bacillus subtilis* wraz z *Rhizopogon vinicolor,* lecz ich działanie zależało od parametrów. *Bacillus* stymulował całkowitą długość korzeni bocznych, całkowitą liczbę szpilek, lecz hamował długość pędu i korzenia głównego, zarówno u siewek zaszczepionych *Laccaria laccata* jak i *Rhizopogon vinicolor.* Stwierdzono stymulujące działanie *Arthrobacter* sp. na liczbę korzeni mikoryzowych. Obserwowano także stymulujący wpływ bakterii na liczbę korzeni odżywczych.

Received on June 21, 1993