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The effect of ectomycorrhizal fungi and bacteria on pine seedlings

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The effect of ectomycorrhizal fungi (*Hebeloma crustuliniforme* (Bull.: Fr.) Quél. 5392 and *Pisolithus tinctorius* (Pers.) Coker et Couch 5335) and bacteria (*Bacillus polymyxa* and *Azospirillum brasilense*) associated with mycorrhizas on the growth of pine seedlings was investigated. In addition the influence of bacteria on fungal biomass production and the relationship between ectomycorrhizal fungi and fungi pathogenic to root of pine seedlings were determined.

In general, the shoot/root ratio was higher in plants inoculated with *Hebeloma crustuliniforme* and bacteria than in the control seedlings (grown only under sterile conditions). In non-sterile substrate the root/shoot ratio of the mycorrhizal seedlings was lower as compared to the control. Similar phenomenon was noted in plants inoculated with the mycorrhizal fungus *Pisolithus tinctorius.* The bacteria used as well as the time of introduction of these organisms into the cultures of mycorrhizal fungi affected the production of fungal biomass. *Hebeloma crustuliniforme* and *Pisolithus tinctorius* inhibited the growth of *Rhizoctonia solani* and *Fusarium oxysporum* fungi pathogenic to pine seedlings.

Key words: ectomycorrhizal fungi, bacteria, pathogenic fungi.

INTRODUCTION

Mycorrhizal associations in higher plants are among the most widely known microbial interactions in nature. Under natural conditions this symbiosis is formed spontaneously and contributes to the plant growth. Nevertheless investigations have shown that natural processes could be improved by manipulating the symbionts to give more beneficent associations yielding more economic tree growth.

The most important step in any nursery inoculation program is the selection of the fungal inoculate (T r a p p e 1977; C h a l o t et al. 1988). Bacteria and other microorganisms inhabiting the soil and the rhizosphere interact with ectomycorrhizal fungi at different stages of the establishment of symbiosis, i.e., at growth of the fungus in the soil, at growth along the roots and at interaction of short roots (O l i v e i r a and G a r b a y e 1989). Some of these interactions may be negative but a large proportion of the bacteria associated with the rhizosphere of ectomycorrhizas stimulates the growth of the fungus and mycorrhizal infection. These so-called helper bacteria (G a r b a y e and B o w e n 1988) could be used in order to improve the efficiency of ectomycorrhizal inoculation in forest nurseries. G a r b a y e (1994) indicated that certain strains of *Pseudomonas* and *Bacillus* isolated from the mantle of Douglas-fir *Laccaria laccata* ectomycorrhizae could be strikingly fungus-specific in their ability to enhance mycorrhizae root tip formation.

Little is known, however, about the mechanisms involved in these stimulation's. D u p o n n o i s and G a r b a y e (1990) considered two hypotheses: direct stimulation by the bacterium providing substrates or growth factors to the fungus and indirect effect by the bacterium breaking down and/or using metabolites released by the fungus and toxic to the fungus itself (detoxification). Many authors have indicated the suppression of root diseases in trees by ectomycorrhizal fungi (M a r x 1971; D u c h e s n e 1994). These include their barrier effect provided by the mantle, competitive exclusion, plant-produced antimicrobials induced by ectomycorrhizae colonization and antibiotics produced by ectomycorrhizal fungi (M a r x 1973). However, recent evidence suggests that inhibition of the growth of pathogenic fungi by ectomycorrhizal fungi may be due to acidification (R a s a n a y a g a m and J e f f r i e s 1992).

Helper bacteria are also producers of antibiotics. Moreover the suppression of pathogenic fungi by bacteria has been demonstrated (M a l a j c z u k 1988). Mycorrhizal fungi may act as a chemical (competition, exudation of toxic metabolites) and/or physical barrier (M a r x 1971). Thus they are found to protect the plant against soil borne pathogens (M a r x 1971; A l l e n 1991). As the plant-fungus-bacteria interactions are different, the present studies were aimed at evaluation of some of them.

MATERIAL AND METHODS

G r o w t h o f p i n e s e e d l i n g s. Seeds of *Pinus sylvestris* were surface sterilized by shaking for 20 min in 30% of H_2O_2 and washed several times in sterile distilled water. Dry seeds were placed in Petri dishes containing the following medium: peptone (Peptobak, POCH, Poland) 10.0 g, agar 15.0 g, H_2O dist. 1000 ml. The germinated seeds with equal lengths of the root were aseptically transferred to plastic cups (0.3 l). Each cup contained four seeds.

The growth medium was soil from under the pine, vermiculite and peat (1:1:1). Experiments were carried out with sterile and nonsterile substrate. Sterilization was performed in steam for 30 min. for seven consecutive days. The seedlings were grown in a plant growth chamber having the following photoperiod: 16 hours of light (2500 lux) at 20–24°C and 8 hours of darkness at 18–20°C. The seedlings were watered with a diluted (1:10) mineral Ingestad's (1960) medium free of phosphorus compounds.

Inoculation of seedlings with ectomycorrhizal fungi and associated bacteria. Fungi were grown on potato dextrose agar (PDA, Difco) slants. Bacteria were grown on the Rennie (1981) medium, respectively, for 7 days. Subsequently the microorganisms were washed off with 5 ml of Ingestad's medium enriched with glucose (1 g/l).

Each seedling (10 days old) was inoculated with 2 ml of the fungal and 400 µl of the bacterial suspension.

The seedlings were inoculated with the ectomycorrhizal fungus only or with the fungus and bacteria simultaneously or the bacteria were inoculated 7 days after the fungal inoculation. Uninoculated seedlings were used as the control. For these studies two ectomycorrhizal fungi – *Hebeloma crustuliniforme* (5392) and *Pisolithus tinctorius* (5335) and two bacteria – *Bacillus polymyxa* (isolated from ectomycorrhiza formed on roots of *Pinus sylvestris* L. and by a closer unidentified ectendomycorrhizal fungus MrgX (Pachlewski et al. 1991/92) and *Azospirillum brasilense* Cd (ATCC 27910) associated with ectomycorrhiza of *Pseudotsuga menziesii* formed by *Rhizopogon vinicolor* were used. The seedlings were grown for 3 months after the inoculation.

The influence of bacteria on fungal biomass production in liquid media. The studies were performed in the modified Melin-Norkrans (MMN) medium (Marx 1969). 100 ml aliquots of the medium were inoculated simultaneously with 1 ml of the fungal and 200 µl of bacterial suspension or the bacteria were introduced into 7-day-old fungal culture. The cultures were grown for 14 days at 26°C. Subsequently the mycelium was separated from the medium by filtration on filter paper, thoroughly washed and dried at 85°C subsequently the dry mass was determined. Cultures of the fungus grown without bacteria were used as the control.

Relationship between ectomycorrhizal fungi and fungi pathogenic to roots of *Pinus sylvestris*. The Petri dishes with MMN medium were inoculated with the mycorrhizal fungus and incubated for 4–7 days at 26°C. Subsequently the cultures were inoculated with the pathogenic fungus. After 7 days of growth of the ectomycorrhizal fungus with the pathogenic one the zones of growth of both fungi were measured. The above studies were performed with the following pathogenic fungi: *Fusarium oxysporum* Schlecht. (K) and *Rhizoctonia solani* Kühn (MB). These species were isolated from the roots of diseased pine seedlings with symptoms of damping-off. The results were evaluated statistically using Student's t-test for independent samples ($p \leq 0.05$) and the 3 factor ANOVA.

RESULTS

The results of the present study on biomass production by pine seedlings inoculated with *Hebeloma crustuliniforme* and with bacteria associated with this mycorrhizal fungus are shown in Table 1. From the above data it is clear that the seedlings inoculated with *H. crustuliniforme* and bacteria had lower root and higher shoot biomass than the plants, which were not, inoculated (control plants).

These observations were made in plants grown in sterile vermiculite-peat-soil substrate. Simultaneous inoculation of the seedlings with the fungus and bacteria did not change the biomass of roots inoculated only with the ectomycorrhizal fungus. When the bacteria were introduced 7 days after the inoculation of seedlings with the fungus no impact on root growth was observed, but the biomass of the shoots increased (Tab. 1). A reverse phenomenon was noted in the non-sterile substrate. The decrease in the rate of growth of shoots and stimulation of the roots was observed but only when the seedlings were inoculated with *H. crustuliniforme* alone or with the above fungus and 7 days later with the bacterium *Bacillus polymyxa*. In general, the shoot/root ratio was higher in plants inoculated with *Hebeloma crustuliniforme* and bacteria than in the control seedlings but only when the plant was grown in sterile conditions. In non-sterile substrate the shoot/root ratio of the mycorrhizal seedlings was lower as compared to the control one. A similar phenomenon was noted in plants inoculated with the mycorrhizal fungus *Pisolithus tinctorius* (Tab. 2).

After inoculation of the pine seedlings with *Pisolithus tinctorius* only a weak development of mycorrhizae was observed. In sterile substrate, however, the dry mass of roots in plants inoculated with the fungus and associated bacteria was lower and that of shoots higher as compared to the control (Tab. 2). In non-sterile substrate the dry mass of roots of seedlings grown with *Pisolithus tinctorius* and bacteria was similar to that of the control roots. However the dry mass of shoots of seedlings inoculated either with the fungus or the bacteria was lower than that of the control shoots (Tab. 2).

Table 1

Biomass of mycorrhizal pine seedlings after three months in growth-chamber (n = 10).
Seedlings inoculated with ectomycorrhizal fungus *Hebeloma crustuliniforme* (5392) and bacteria *Azospirillum brasilense* or *Bacillus polymyxa*

	Time of inoculation with fungi and bacteria	Fungus and bacterium	Dry weight (g) ± SE		Shoot/root ratio
			Root	Shoot	
Growth medium sterile		Non-mycorrhizal plant (control)	0.050 ± 0.021	0.012 ± 0.011	0.591 ± 0.191
		Hebeloma crustuliniforme	0.007 ± 0.005(−)	0.029 ± 0.012(+)	6.624 ± 0.541
	Simultaneously	*H. crustuliniforme* + *Azospirillum brasilense*	0.005 ± 0.002(−)	0.026 ± 0.009	5.053 ± 0.171
		H. crustuliniforme + *Bacillus polymyxa*	0.006 ± 0.002(−)	0.023 ± 0.008	4.783 ± 0.474
	Bacteria introduced seven days after the fungus	*H.crustuliniforme* + *Azospirillum brasilense*	0.008 ± 0.004(−)	0.033 ± 0.016(+)	4.593 ± 0.209
		H. crustuliniforme + *Bacillus polymyxa*	0.012 ± 0.004(−)	0.047 ± 0.009(+)	4.044 ± 0.136
Growth medium nonsterile		Non-mycorrhizal plant (control)	0.008 ± 0.002	0.092 ± 0.033	11.827 ± 0.614
		Hebeloma crustuliniforme	0.016 ± 0.006(+)	0.063 ± 0.024	4.031 ± 0.138
	Simultaneously	*H. crustuliniforme* + *Azospirillum brasilense*	0.004 ± 0.002(+)	0.021 ± 0.005(−)	4.982 ± 0.232
		H. crustuliniforme + *Bacillus polymyxa*	0.006 ± 0.001	0.028 ± 0.004(−)	5.052 ± 0.381
	Bacteria introduced seven days after the fungus	*H. crustuliniforme* + *Azospirillum brasilense*	0.007 ± 0.003	0.033 ± 0.006(−)	5.991 ± 0.515
		H. crustuliniforme + *Bacillus polymyxa*	0.016 ± 0.009	0.059 ± 0.035	3.956 ± 0.272

Explanations:
(−) significant inhibition
(+) significant stimulation as compared to the control

T a b l e 2

Biomass of mycorrhizal pine seedlings after three months in growth-chamber (n = 10).
Seedlings inoculated with ectomycorrhizal fungus *Pisolithus tinctorius* (5335) and bacteria *Azospirillum brasilense* or *Bacillus polymyxa*

	Time of inoculation with fungi and bacteria	Fungus and bacterium	Dry weight (g) ± SE		Shoot/root ratio
			Root	Shoot	
Growth medium sterile		Non-mycorrhizal plant (control)	0.049 ± 0.021	0.012 ± 0.011	0.594 ± 0.193
		Pisolithus tinctorius	$0.010 \pm 0.002(-)$	$0.048 \pm 0.007(+)$	4.813 ± 0.200
	Simultaneously	*P. tinctorius* + *Azospirillum brasilense*	$0.004 \pm 0.002(-)$	0.012 ± 0.071	11.740 ± 0.889
		P. tinctorius + *Bacillus polymyxa*	$0.007 \pm 0.005(-)$	$0.052 \pm 0.004(+)$	10.771 ± 0.976
	Bacteria introduced seven days after the fungus	*P. tinctorius* + *Azospirillum brasilense*	$0.006 \pm 0.003(-)$	$0.061 \pm 0.025(+)$	12.496 ± 1.376
		P. tinctorius + *Bacillus polymyxa*	$0.006 \pm 0.003(-)$	$0.045 \pm 0.018(+)$	8.101 ± 0.260
Growth medium nonsterile		Non-mycorrhizal plant (control)	0.008 ± 0.002	0.093 ± 0.033	11.511 ± 0.373
		Pisolithus tinctorius	0.008 ± 0.002	$0.047 \pm 0.006(-)$	6.259 ± 0.148
	Simultaneously	*P. tinctorius* + *Azospirillum brasilense*	0.011 ± 0.005	$0.053 \pm 0.017(-)$	5.889 ± 0.750
		P. tinctorius + *Bacillus polymyxa*	0.005 ± 0.002	$0.035 \pm 0.003(-)$	8.023 ± 0.930
	Bacteria introduced seven days after the fungus	*P. tinctorius* + *Azospirillum brasilense*	0.005 ± 0.001	$0.032 \pm 0.005(-)$	7.063 ± 0.336
		P. tinctorius + *Bacillus polymyxa*	0.006 ± 0.002	$0.032 \pm 0.005(-)$	5.374 ± 0.295

Explanations:
(–) significant inhibition
(+) significant stimulation as compared to the control

The results of studies of the effect of bacteria on biomass production with the mycorrhizal fungi grown in liquid media are illustrated in Tabs 3, 4.

Bacillus polymyxa introduced to the medium simultaneously (but not after 7 days) with *Hebeloma crustuliniforme* retarded the growth of the fungus. *Azospirillum brasilense* had no considerable effect on the biomass of this fungus (Tab. 3). *Azospirillum brasilense* or *Bacillus polymyxa* introduced into 7 days old cultures of *Pisolithus tinctorius* stimulated the biomass increase of this fungus. Simultaneous introduction into the medium of the fungus and bacteria had no significant effect on the growth of *Pisolithus tinctorius* (Tab. 4).

Table 3

Biomass of ectomycorrhizae fungus — *Hebeloma crustuliniforme* (5392) as affected by bacteria associated with coniferous mycorrhizae in liquid media (n = 3) (Student's t-test)

Bacteria	Time of inoculation	Biomass [dry weight (g) ± SE]
Axenic culture of fungus (control)		0.124 ± 0.003
Azospirillum brasilense	Bacterium introduced simultaneously with the fungus	0.083 ± 0.028
	Bacterium introduced seven days after the fungus	0.132 ± 0.004
Bacillus polymyxa	Bacterium introduced simultaneously with the fungus	0.012 ± 0.004*
	Bacterium introduced seven days after the fungus	0.149 ± 0.012

* significant differences as compared to the control

Table 4

Biomass of ectomycorrhizae fungus — *Pisolithus tinctorius* (5335) as affected by bacteria associated with coniferous mycorrhizae in liquid media (n = 3) (Student's t-test)

Bacteria	Time of inoculation	Biomass [dry weight (g) ± SE]
Axenic culture of fungus (control)		0.029 ± 0.011
Azospirillum brasilense	Bacterium introduced simultaneously with the fungus	0.013 ± 0.001
	Bacterium introduced seven days after the fungus	0.169 ± 0.051*
Bacillus polymyxa	Bacterium introduced simultaneously with the fungus	0.035 ± 0.059
	Bacterium introduced seven days after the fungus	0.084 ± 0.025*

* significant differences as compared to the control

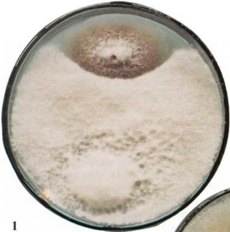
The interactions between the ectomycorrhizal and pathogenic fungi (*Rhizoctonia solani, Fusarium oxysporum*) are illustrated on Figs 1 – 3. Fig. 1 shows the inhibitory effect of *Pisolithus tinctorius* on the growth of *Rhizoctonia solani. Hebeloma crustuliniforme* also inhibited the growth of *Rhizoctonia solani* (Fig. 2) and *Fusarium oxysporum* (Fig. 3).

DISCUSSION

The ectomycorrhizae are of great importance in improving the physiological quality of plants destined for reforestation. Not all fungi species exert the same effect on the growth and survival of the plant since certain mycorrhizal fungi are more effective than others (P e r r y et al. 1987). Therefore, the selection of the ectomycorrhizal fungus for the tree is of great importance.

Mycorrhizal infection is often enhanced by mycorrhization helper bacteria commonly found in the rhizosphere, in different soils and plant-fungus associations. These bacteria can also be fungus-specific in that they are beneficial to the fungus from which they were isolated. However they may inhibit the growth of other species (D u p o n n o i s et al. 1993). G a r b a y e (1994) reported that certain strains of *Pseudomonas* and *Bacillus* isolated from the mantle of *Pseudotsuga menziesii – Laccaria laccata* ectomycorrhizae could be strikingly fungus specific in their ability to enhance mycorrhizal root tip formation. None of these mycorrhiza infection-stimulating bacterial strains were capable of promoting seedling growth directly, i.e. in the absence of the appropriate mycorrhizal fungus. S h i s h i d o et al. (1996) demonstrated that the inoculation of mycorrhizal pine seedlings with *Bacillus* strains had no significant effect on the mycorrhizal status of seedlings. Plant growth was however stimulated by these bacteria. These results suggest that *Bacillus* stimulated seedling growth through a mechanism that was unrelated to mycorrhizal fungi. Positive correlation between seedling biomass and the number of mycorrhizal root tips was insignificant in the presence of *Bacillus*. P a c h l e w s k i et al. (1991/92) found ectendomycorrhizal roots of pine seedlings to be more densely colonized by bacteria than the nonmycorrhizal ones. The bacteria belonged mainly to the species *Bacillus polymyxa*. Out of 13 isolates tested for N_2 fixation 12 were active. Strains of *Bacillus polymyxa* used in the present study were derived from experiments of P a c h l e w s k i et al. (1991/92). *Azospirillum brasilense* was isolated from the nitrogenase active cultures of tissue from within sporocarps of *Rhizopogon vinicolor* by L i and C a s t e l l a n o (1987).

Microorganisms in mycorrhizae associations have been shown to possess different activities in relation to the mycorrhizal fungi. In some cases acted as liked antagonists, like "helper" bacteria by plant growth or by fixing N_2 (F i l i p p i et al. 1995). Z a a d y and P e r e v o l o t s k y (1995)



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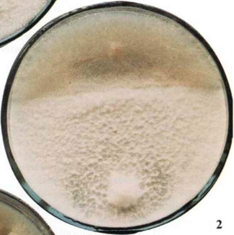

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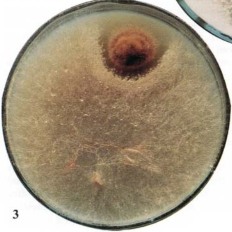

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Fig. 1-3. Interactions between the ectomycorrhizal and pathogenic fungi:
1. *Pisolithus tinctorius* and *Rhizoctonia solani*
2. *Hebeloma crustuliniforme* and *Rhizoctonia solani*
3. *Hebeloma crustuliniforme* and *Fusarium oxysporum*

investigated the effects of inoculation with *Azospirillum brasilense* on the growth of *Quercus ithaburensis* seedlings. *Azospirillum brasilense* caused a considerable increase in seedling root surface area, root dry weight, foliage and shoot dry weight. Chanway and Holl (1990) found that inoculating with beneficial *Bacillus* strain stimulated the growth of pine seedlings. Growth promotion did not result from increased formation of ectomycorrhizae but could have been attributed to nitrogen fixation. In the present study the bacteria were neither specific for ectomycorrhizal fungi nor derived from mycorrhizae formed by these fungi. In sterile medium simultaneous inoculation of the seedlings with the fungus and bacteria did not change the biomass of the root inoculated only with the fungus. When the bacteria were introduced 7 days after the inoculation of seedlings with the fungus, no impact on root growth was observed. However the biomass of the shoots increased. In non-sterile medium a reverse phenomenon was noted.

In general the shoot/root ratio was higher in plants inoculated with ectomycorrhizal fungi and bacteria than in the control seedlings grown in the sterile substrate and lower in the non-sterile one. Torres and Honrubia (1994) found the dry weight of mycorrhizal roots to be much higher in sterilized than in unsterilized substrate. The reduction of growth rate or plant biomass due to mycorrhizal infections have also been recorded (Nylund and Wallender 1989; Doskey et al. 1990). Colpaert et al. (1992) indicated mycorrhizal plants with nine highly compatible mycobionts. All of them reduced plant size. Differences in shoot/root ratio suggested that mycorrhizal fungi reduced root growth more than shoot growth. Chanway and Holl (1991) indicated that *Wilcoxina* inoculated alone caused the synthesis of ectomycorrhizae but decreased shoot biomass. *Bacillus* inoculation alone did not affect the seedling biomass. The inoculation of seedlings with the fungus and bacteria resulted in a similar degree of ectomycorrhizal infection as in the fungal inoculation alone. However the shoot biomass was greater than that of seedlings receiving the fungus alone. The root biomass and stem height was not altered by inoculation. In studies of the effect of soil bacteria (*Arthrobacter* sp., *Bacillus subtilis* and *Pseudomonas fluorescens*) on mycorrhizae formation by *Laccaria laccata* and *Rhizopogon vinicolor* in pine Różycki et al. (1994) found that the action of the bacteria both on mycorrhiza formation and on seedling growth depended on the fungal symbiont, the bacterium and on the parameters of the seedlings. The seedlings were most strongly affected by *Bacillus subtilis* together with *Rhizopogon vinicolor*. *Bacillus* stimulated the total length of lateral roots but inhibited the stem and main root length both in seedlings inoculated with *Laccaria laccata* and *Rhizopogon vinicolor*. A stimulatory action of *Arthrobacter* sp. on the number of mycorrhizal roots was also observed (Różycki et al. 1994).

This was supported by findings indicatory that bacteria provide substrates or growth factors to the fungus (direct stimulation). In addition they broke down and (or) used metabolites released by the fungus into the medium and toxic to the fungus itself (indirect effect). V a r e s e et al. (1996) suggested that some bacterial strains were highly fungus-specific. Some of the bacterial species stimulated the growth of ectomycorrhizal fungi by producing amino acids, vitamins and other growth factors and organic acids (G a r b a y e 1994) or by removing fungal catabolites such as polyphenols and inhibiting fungal growth by producting toxic metabolites or competiting for nutrients.

The inhibition of growth of fungi by Gram-positive bacteria especially after the first 7 days, was probably due to the formation of secondary metabolites during sporulation. *Bacillus* spp. produced a large number of polypeptides with antibiotic activity when grown in a rich medium (V a r e s e et al. 1996).

In the present study *Bacillus polymyxa* introduced to the medium simultaneously with *Hebeloma crustuliniforme* retarded the growth of this fungus. The same bacteria introduced 7 days after the fungus did not affect the growth of this fungus. *Azospirillum brasilense* had no considerable effect on biomass production by *Hebeloma crustuliniforme*. *Azospirillum brasilense* and *Bacillus polymyxa* stimulated the growth of *Pisolithus tinctorius* but only when introduced into 7-day-old cultures of this fungus. Simultaneous introduction into the medium of the fungus and bacteria had no significant effect on the growth of *Pisolithus tinctorius*.

Root pathogens are a major factor in limiting plant production. The most widespread among the various crop plants and forest trees are fungi belonging to the genera *Phytophthora*, *Fusarium*, *Phytium* and *Rhizoctonia* (G a r b a y e 1991). Many studies have documented the suppression of root diseases in conifers by ectomycorrhizal fungi (D u c h e n e s e 1994).

In the present study studies, *Hebeloma crustuliniforme* and *Pisolithus tinctorius* had an inhibitory effect on the growth of *Rhizoctonia solani* and *Fusarium oxysporum*. Several mechanisms may be involved in disease suppression by ectomycorrhizal (EM) fungi. These include the barrier effect provided by the mantle, competition for nutrients, plant-produced antimicrobials induced by EM fungi colonization and antibiotic production by EM fungi. Recent evidence suggests that the inhibition of growth of pathogenic fungi by EM fungi in vitro is due to acidification of the medium rather than to antibiotic production (S c h e l k e and P e t e r s o n 1996).

Further studies are required to determine the effect of bacteria and mycorrhizal fungi. The mechanism of such an action is little understood. It is not known whether or not bacteria are fungi-specific. If so they may influence the inhibition and functioning of mycorrhiza, sporocarp formation etc. These questions however need to be answered. In our laboratory studies are directed in this field.

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Wpływ ektomikoryzowych grzybów i bakterii na siewki sosny

Streszczenie

Badano wpływ grzybów ektomikoryzowych (*Hebeloma crustuliniforme* (Bull.: Fr.) Quél. 5392 i *Pisolithus tinctorius* (Pers.) Coker et Couch 5335) oraz bakterii (*Bacillus polymyxa* i *Azospirillum brasilense*), związanych z mikoryzami, na rozwój siewek sosny (*Pinus sylvestris*). Zbadano również wpływ tych bakterii na przyrost biomasy grzybów ektomikoryzowych oraz zależności pomiędzy ektomikoryzowymi grzybami oraz grzybami patogenicznymi dla korzeni sosny (*Fusarium oxysporum* Schlecht. i *Rhizoctonia solani* Kühn).

Ogólnie, stosunek masy pędu do korzenia był wyższy u roślin zaszczepionych *H. crustuliniforme* i bakteriami niż u roślin kontrolnych (rosnących w warunkach sterylnych). W podłożu niesterylnym stosunek masy pędu do korzenia siewek mikoryzowych był niższy w prównaniu z kontrolą (siewki niezaszczepione). Podobne obserwacje dotyczyły roślin zaszczepionych *P. tinctorius*. Bakterie oraz czas wprowadzenia ich do płynnych hodowli grzybów ektomikoryzowych wpływały na wytwarzanie biomasy grzybów ektomikoryzowych. *H. crustuliniforme* oraz *P. tinctorius* hamowały wzrost grzybów patogenicznych (*R. solani* oraz *F. oxysporum*).