ACTA MYCOLOGICA
Vol. XXVII (1): 49-61
1991-1992

Studies on pine ectendomycorrhizae in nurseries

ROMAN PACHLEWSKI, JADWIGA KERMEN, ELŻBIETA CHRUŚCIAK and MARIA TRZCIŃSKA

Forest Research Institute, 05-090 Raszyn, Poland

Pachlewski R., Kermen J., Chruściak E. and Trzcińska M.: *Studies on Pine ectendomycorrhizae in nursieries.* Acta Mycol. XXVII (1): 49-61, 1991-1992.

Fungus isolates from ectendomycorrhizae of *Pinus sylvestris* seedlings showed uniform morphological, physiological and mycorrhizal features. Studies on hyphal ultrastucture suggest that the fungus is an *Ascomycetes*. Growth of cultures in vitro and formation of ectendomycorrhizae in the nursery were stimulated both by Ca compounds and urea. Strains of the fungus showed considerable ability to synthesize nitrate reductase. The studies provided evidence of a permanent occurrence of N_2-fixing *Bacillus polymyxa* in the ectendomycorrhizosphere.

INTRODUCTION

Since the first reports on ectendomycorrhizae by Melin (1922, 1923) numerous studies have been conducted on this kind of mycorrhizal symbiosis (Björkman, 1942; Goss, 1960; Mikola, 1965; Laiho, 1965; Palenzona, Fontana, 1970; Wilcox, 1971; Pachlewski, Pachlewska, 1971; Pachlewski, 1983; Yang, Wilcox, 1984; Wang, Wilcox, 1985; Piché et al., 1986). In forestry interest in ectendomycorrhizae is linked with their occurrence as the main or, frequently, unique kind of mycorrhizal symbiosis in pine seedlings in nurseries (Mikola, 1965; Laiho, 1965; Palenzona, Fontana, 1970; Wilcox, 1971; Pachlewski, 1983). In contrast, pine seedlings in the natural forest communities are characterized by ectomycorrhizae; they lack ectendomycorrhizae entirely or it occurs infrequently (Pachlewski, Pachlewska, 1960; Mikola, 1965; Laiho, 1965). Pachlewski (1983) indicates that ectendomycorrhizal fungi dominating in the soil of older forest nurseries often constitute a biological barrier in the process of adaptation of ectomycorrhizal inocula by inhibiting or even reducing their development.

Our research, following that of Mikola (1965) and Laiho (1965), involved

the biology of ectendomycorrhizae and their fungal component as well as the influence of mineral fertilization upon the dynamics of ectendomycorrhizae. We also analysed some components of microflora such as fungi and bacteria inhabiting the ectendomycorrhizosphere of pine in nurseries and attempted to define the systematic position of the ectendomycorrhizal fungi.

MATERIAL AND METHODS

Forest nursery. Experiments designed to assess the influence of mineral fertilization with nitrogen and phosphorus upon the dynamics of ectendomycorrhizae and the mycorrhizosphere of *Pinus sylvestris* seedlings were conducted in a many--year-old forest nursery located at the Forest Research Institute in Raszyn. The nursery was situated in a pine stand on podzol soil (slightly-loamy sand). Experimental plots were installed either on soil which had been cultivated for several years and having a pH of 4,7 or on soil previously treated for years with agromeliorative treatment and having a pH ranging from 5,7 to 6,7. The following fertilizers were applied on separate plots (1,2 x 3,0 m): a) nitrogen in the form of $(NH_4)_2SO_4$, $CO(NH_2)_2$ and $Ca(NO_3)_2$ at 80 kg N/ha of each form; $CO(NH_2)_2$ being additionally applied in double dose; b) phosphorous in the form of $AlPO_4$, ground phosphate rock and superphosphate at 50 kg P_2O_5/ha of each form, the latter being also applied in a double dose. In addition, the artificial inculation with *Rhizopogon luteolus* Fr. was made on the plot fertilized with $CO(NH_2)_2$ as well as on the unfertilized control, while *Hebeloma mesophaeum* (Pers.: Fr.) Quél. was applied on the $AlPO_4$-fertilized and on the control plots. Inoculations were made using the water suspension of fungi (Pachlewski, 1983). The experiments with nitrogen fertilization were carried out from April 1982 to October 1984, while those with phosphorus from May 1984 to June 1985.

Isolations from ectendomycorrhizae. Fungi and bacteria were isolated from the ectendomycorrhizae of pine seedlings growing on the described experimental plots which were either fertilized or unfertilized. Ten to twenty whole seedlings were lifted up from the soil and immediately carried to the laboratory. The seedling roots were then cut off and carefully washed under tap water to remove soil particles. The rootlets were then (cut) off by scalpel and the ectendomycorrhizae were selected by means of binocular. From each plot 100 ectendomycorrhizae were selected and subjected to the following surface sterilization: the mycorrhizae suspended in water were shaken in a microshaker in 20 successive volumes of sterile water – once for 1 min. and 19 times for 30 sec. The supernatant water was removed with a Pasteur pipette linked to a water pump. The mycorrhizae were then irrigated with 5 ml of 70 % ethanol for 1 min. and, after removing the alkohol with a pipette, 5 ml of 0,1 % $HgCl_2$ solution were added and removed after 15 sec. Subsequently, mycorrhizae were rinsed with sterile water – once for 1 min. and 29 times for 30 sec..

The result of sterilization was checked by sieving 5 x 0,5 ml of the last volume of rinsing water onto BR agar (see below) in petri plates. Usually from 0 to 3 colonies of fungi and/or bacteria were found on a plate. Analogous sieving from the 20th rinse before applying the disinfectants resulted in 300-700 colonies on a plate.

For isolation of fungi two parallel media were used in tubes, wherein single mycorrhizae were placed, i.e. the Pp basic medium (P a c h l e w s k i, P a c h l e w s k a, 1974) with the addition of 166 mg/l CaO, and the Melin-Nilsson medium (according to P a c h l e w s k a, 1968). The pH of both media was adjusted to 6,2-6,4. Then, 40 mg/l of gentamicin were added in order to inhibit the growth of bacteria. Isolation were made many times, always from at least 50 mycorrhizae. Incubation proceeded for 8 weeks at 25°C. At 7 to 10-day intervals, observations were made of the growth of mycelium and subcultures developed on the agar slants of Pp medium.

Colonies of bacteria isolated from single mycorrhizae were transfered to tubes containing the following media: bouillon, Clark medium, de Barjac medium for denitrificators (de B a r j a c, 1952), Bunt and Rovira (BR) medium (according to J o h n s o n et al., 1959), Norris medium for *Azotobacter* (N o r r i s, C h a p m a n, 1968) and a medium with the combined C source – CC medium (R e n n i e, 1981). These cultures were replicated 10 times and maintained in an incubator at 28°C. Bacteria which were able to grow on the nitrogenfree media were checked for N_2-fixation capacity with an acetylene method using a JEOL-type gas chromatograph. Their systematic position was determined according to B e r g e y (1974). The isolates of *Bacillus polymyxa* were additionally checked for cellulose--disintegration capacity on a Dubos medium supplemented with 10 µg/l of p-amino-benzoic acid and 5µg/l of biotin.

C u l t u r e s o f M r g X [1] e c t e n d o m y c o r r h i z a l f u n g u s. In order to determine the taxonomic position of the fungi their hyphae were examiined by electron microscopy. The hyphae of two strains were analyzed: MrgX-I isolated from the mycorrhizae of pine seedlings from a nursery in Wielkopolski National Park near Poznań, in 1975, and MrgX-II isolated from mycorrhizae of pine from a nursery in Sękocin near Warsaw, in 1982. The aerial mycelium were subjected to preparation and analyzed (P a c h l e w s k i, K o c o ń, 1985); the examination and photographs were made with the aid of JEOL TEM-100 transmission electron microscope. The magnification used range from 5 000 to 1 000 000 x (K o c o ń, 1980).

In order to assess the influence of nitrogen form upon the mycorrhizal properties of MrgX fungus the in vitro mycorrhizal test on agar was applied (P a c h l e w s k a, 1968). The experiments were carried out with common pine and two strains – MrgX-I and MrgX-II. The agar substrate was supplemented with a modified Melin-Norkrans (MMN) medium (M a r x, 1969) where $(NH_4)_2HPO_4$ was replaced with $CO(NH_2)_2$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ (urea was sterilized by filtering).The acidity of the substrate was adjusted to 6,5. The experiments were conducted in two series: a) from April 1982 to February 1984, and b) from August 1984 to March 1985. In order to

[1] Symbol of isolates of ectendomycorrhizal fungus (P a c h l e w s k i, 1983).
I, II, 5, 7, 32 – symbols of strains of ectendomicorrhizal fungus.

assess the growth response of MrgX ectendomycorrhizal fungus to different nitrogen forms the in vitro culture was maintained of 4 strains, i.e. MrgX-I, MrgX-5, MrgX-7 and MrgX-32. (The last three strains were isolated from the nursery in Sękocin, in 1983-1984.) The cultures were grown on both Pp liquid and Pp liquid modified media; in the latter ammonium tartrate was replaced with $CO(NH_2)_2$, NH_4NO_3, $Ca(NO_3)_2$ or with KNO_3. The media were inoculated with the MrgX cultures on Pp medium. The inoculated substrates were incubated in a shaker at 25-29°C for 5-7 days and, subsequently, stationary incubation was continued for 1 month at the same temperature. After 1 month mycelium was filtered, rinsed with water and dried at 80°C for 18-20 hours and, finally, dry weight was determined. The lose of $N - NO_3$ and $N - NH_4$ in the medium during the period of culture growth was assessed using a distillation method of Kjeldahl.

In order to estimate the effect of Ca on the growth of these fungi two strains (MrgX-I and MrgX-II) were grown on the Pp-agar medium containing either CaO, $CaCO_3$, $CaCl_2$ or $Ca(NO_3)_2$ at three different concentration (166, 332 and 664 mg/1). The pH of the media ranged from 5,6 to 5,8, except for the one containing CaO which ranged from 6,0-6,2. Mycelium discs 7 mm in diameter, obtained from 14-21-day-old cultures, were placed in the centre of each plate. After 2-3 weeks of incubation at 25°C the diameters of the colonies were measured. Each treatment combination was replicated 3 times. The growth response of MrgX fungus to the substrate reaction was established for 3 strains: MrgX-I, MrgX-5 and MrgX-7 on Pp medium having the pH ranging from 3,5 to 8,0. The reaction of the medium was adjusted using HCl or NaOH. The cultures were maintained at 25°C for 14-21 days and, subsequently, diameters of colonies were measured.

Chitin was assayed on 6-week-old stationary cultures of MrgX strain growing on modified liquid Pp medium without maltose. Ammonium tartrate in the mycelium was replaced with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$. The culture was incubated at 27°C. After incubation mycelium was collected on a plastic sieve, irrigated with the distilled water, and dried overnight at 27°C. After the dried samples were hydrolysed with HCl chitin was determined using a colorimetric method according to Elson and Morgan (T r z c i ń s k a, P a c h l e w s k i, 1986).

The biotic effect of MrgX-II ectendomycorrhizal fungus on the mycorrhizosphere fungi was investigated. Isolates of *Cylindrocarpon* sp., *Mycelium radicis atrovirens* Melin and *Phlebia gigantea* Fr., the latter known for its antagonistic properties were tested on petri plates filled with Pp medium. MrgX-II mycelial discs from 14-17-day-old culture were first placed on the agar and incubated at 25°C for 5-7 days. Then discs of one from the three above mentioned fungi were placed at the distance of 2 cm from the border of the MrgX culture. After 5-10 days of joint culture an assessment was made of biotic interactions between MrgX and each of the above fungi.

RESULTS

Forest nursery. The results obtained support our earlier conclusions (Pachlewski, 1983) concerning the occurence of ectendomycorrhizae in pine seedlings in nurseries in Poland.

The proportion of ectendomycorrhizae in 1 and 2 year-old pine seedlings ranged from 50 to 100 % of the total mycorrhizae. The intensity of the occurence of ectendomycorrhizae was to a great extent conditioned by the form of mineral fertilizers containing N and P. Ectendomycorrhizae constituted the only or positively dominating proportion when urea was applied as fertilizer and the young mycorrhizae prevailed (Table 1, Fig. 1 A). In the case of P fertilization, a stimulating effect of ground phosphate rock was obserwed on the quantity and vitality of mycorrhizae (Table 1).

The influence of the above mentioned N and P-forms of mineral fertilization was also noted as far as the morphology of ectendomycorrhizae is concerned and, particularly, the intracellular infection. The latter occurred, as a rule, with great intensity when urea or ground phosphate rock were applied. It was observed in various stages, from that of pelotons in the cortical cells, through that of initial digestion (plasmotic granules), to the stage of advanced lysis of hyphae (fine grains).

The results obtained (Table 1) indicate that under the experimental conditions ectendomycorrhizal symbiosis does not influence negatively the growth of 1 and 2 year-old pine seedlings in nurseries. This was also found earlier by Laiho (1965) and Pachlewski (1983). The role of ectendomycorrhizae, however, in the advanced developmental stages of pine seedlings planted on various sites, is so far unknown.

It has been found, while observing the occurence of mycorrhizae in pine seedlings in a nursary, that on the plots where neither cultivation nor fertilization took place for many years, the mycorrhizal spectrum of seedlings was composed exclusively of ectomycorrhizae. Even the single nitrogen treatment with $CO(NH_2)_2$ or $(NH_4)_2SO_4$ did not resulted in the occurence of ectendomycorrhizae.

Isolation from ectendomycorrhizae. The results obtained justify our choice of culture media used for isolation of the MrgX ectendomycorrhizal fungus. The addition of Ca to the substrate as well as the pH increase to 6,2-6,5 brought about the increase in successful isolations from 7 % (Pachlewski, 1983) to about 22 %. From the plots fertilized with different nitrogen compounds 32 strains were isolated while from those treated with phosphorus compounds — 22 strains. Most isolates were obtained from the plots fertilized with urea and ground phosphate rock.

The strains of MrgX ectendomycorrhizal fungus show considerable homogenity of cultural and morphological features corresponding to those of the isolates identified by Mikola (1985), Laiho (1965), Pachlewski, Pachlewska (1971), Pachlewska (1983).

Table 1

Results of mycorrhizal analysis of pine seedlings

	Fertilization	% of								Growth of seedlings (cm)	
		ectendomycorrhizae			ectomycorrhize			total	short roots (nonmycorrhizal)	stem	needles
		a	b	c	a	b	c				
1-year-old seedlings	**Phosphorus**										
	Unfertilized (control)	3	22	39	–	–	1	65	35	8,0	4,2
	Unfertilized + *H. mesophaeum*	–	5	38	2	–	–	45	55	9,6	4,3
	$AlPO_4$	–	30	29	–	–	1	60	40	4,8	3,7
	$AlPO_4$ + *H. mesophaeum*	5	35	10	28	2	–	80	20	5,6	3,4
	Superphosphate (single dose)	2	5	38	14	1	–	60	40	6,8	4,4
	Superphosphate (double does)	5	5	10	25	–	–	45	55	7,6	3,4
	Ground phosphate rock	64	20	–	1	–	–	85	15	8,2	3,9
2-year-old seedlings	**Nitrogen**										
	Unfertilized (control)	5	20	25	15	5	–	70	30	30,5	4,8
	Urea (single dose)	55	8	5	3	–	4	75	25	35,0	4,0
	Urea (double dose)	40	30	15	–	–	–	85	15	26,4	3,3
	Urea (single dose) + *R. luteolus*	10	15	15	15	5	–	60	40	26,6	4,1
	$(NH_4)_2SO_4$	–	17	26	2	–	–	45	55	21,9	4,5
	$Ca(NO_3)_2$	–	15	20	20	5	–	60	40	25,2	3,8

a – current year-young; b – last year showing renewed growth; c – last year without renewed growth

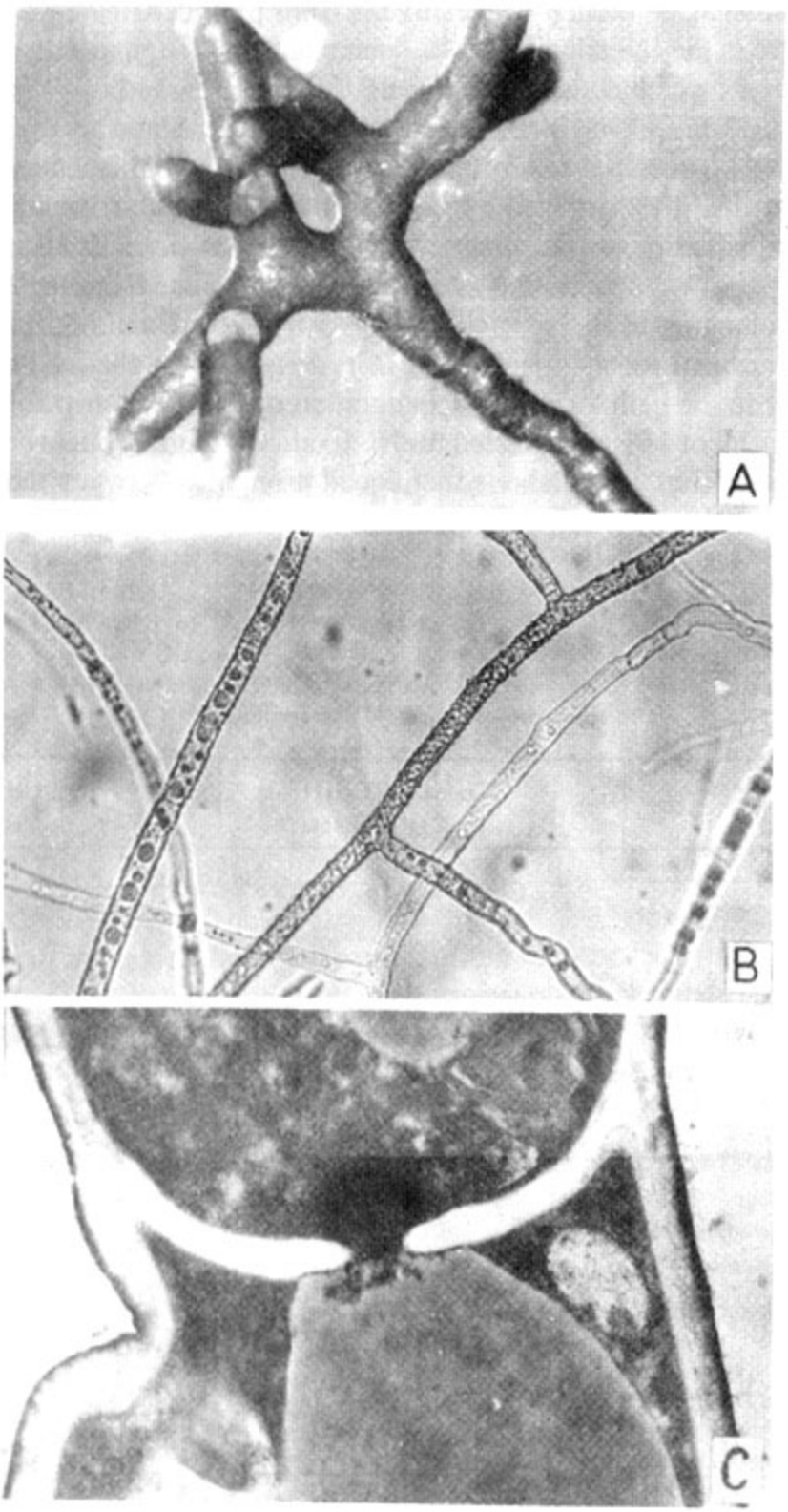

Fig. 1. A – ectendomycorrhizae of one-year-old seedling treated with urea in nursery; B – hyphae of aerial mycelium of MrgX-I ectendomycorrhizal fungus, 280 x; C – longitudinal section of the hyphae of ectendomycorrhizal fungus with simple septal pore. Strains MrgX-I 40000 x

Observations made during 4 vegetative seasons provided evidence of a permanent occurrence in the ectendomycorrhizosphere of some fungi, mostly of *Mycelium radicis atrovirens*, which constituted up to 46 % of isolates, and *Cylindrocarpon* sp. along with *Fusarium* sp. which comprised 2-22 % of the isolates.

The studies on bacterial microflora demonstrated that the ectendomycorrhizal roots are more densely colonized by bacteria that the nonmycorrhizal ones. The bacteria belonged mainly to the genera: *Bacillus, Arthrobacter* and *Pseudomonas*. 70 % of the isolates were identified as members of the genus *Bacillus*, 20-100 % of this amount belonging to the species *Bacillus polymyxa*. From both the ectendo--and non-mycorrhizal roots 44 isolates of *B. polymyxa* were obtained-of which 40 could decompose the cellulose. The deintegration of cellulose strip followed after about 2 weeks. Out of 13 isolates tested for N_2 fixation 12 turned out to be active. No differences were found in the above mentioned properties between the isolates of *B. polymyxa* from the ectendomycorrhizae and those from the nonmycorrhizal roots (Table 2).

Table 2

Acethylene reduction (N_2-fixing activity) and cellulose decomposition by *Bacillus polymyxa* isolates obtained from roots of 2-year-old pine seedlings

Soil treatment	Quantity of C_2H_4 (nM) per 1 ml of culture	Celulose decomposition
	Ectendomycorrhizae	
Unfertilized (control)	465	+
Unfertilized (control)	295	+
Unfertilized (control)	1140	+
Urea (single dose)	523	+
Urea (single dose)	389	+
Urea (double dose)	0	+
Ammonium sulphate	162	+
Ammonium sulphate	542	+
Superphosphate (single dose)	75	+
Superphosphate (single dose)	796	+
	Nonmycorrhizal roots	
Urea (single dose) + *R. luteolus*	430	+
Superphosphate (single dose)	571	+
Superphosphate (single dose)	906	+

Cultures of MrgX ectendomycorrhizal fungus. The electron microscopy study on hyphae (Fig. 1 B) revealed the presence of simple pores in the septa (Fig. 1 C). The septa usually slightly convex and having the thickness of about 0,15-0,30 mm, are dilated on the side of external cell walls and become narrower,

with softly sharpened edge on the side of the pore. Single pores located in the middle of each septum are 0,2-0,5 µm in diameter. These septa are similar to those of *Cenococcum graniforme* (Sow.) Ferd. et Winge described by T r a p p e (1971) and ranked among the imperfect *Ascomycetes*. Therefore the ultrastructural study of hyphae of the ectendomycorrhizal fungi (MrgX-I and -II strains) obtained from our nurseries provided some evidence that they probably belowed to the *Ascomycetes*.

Investigations on 4 isolates of MrgX fungus in the in vitro mycorrhizal test provided clear evidence of the positive effect of urea as the nitrogen source on the development of ectendomycorrhizae (Table 3).

Table 3

Mycorrhizal test of MrgX ectendomycorrhizal fungus with pine on agar in vitro

N – source	MrgX strain	Number of positive mycorrhizal synthesis	Number of mycorrhizae on seedlings	Stem height of seedlings (cm)	
				inoculated	not inoculated
$(NH_4)_2HPO_4$	MrgX-I	0	–	8.5	11.2
$CO(NH_2)_2$	MrgX-I	2	10-12	6.8	11.0
	MgrX-II	1	45	6.5	11.0
$Ca(NO_3)_2$	MgrX-I	1	4	9.0	12.0
$(NH_4)_2SO_4$	MgrX-I	0	–	8.4	10.8

This is in agreement with the results of the nursery experiments. It should be stressed, however, that in contrast to the nursery conditions, under conditions of the in vitro mycorrhizal test, in all N treatments, the infection with ectendomycorrhizal fungus brought about reduction of seedlings growth. Urea as nitrogen source posityvely influenced the growth of mycelium of MrgX strains under laboratory conditions; colonies on the substrate with $CO(NH_2)_2$ have larger diameter and mycelium mass (Table 4).

Table 4

Dry weight of 4-week-old mycelium (in g) of MrgX strain on substrates containing various nitrogen sources (g/l)

MrgX strain No	Ammonium tartrate	$CO(NH_2)_2$	NH_4NO_3	$Ca(NO_3)_2$
1	0.3212	0.4246	0.2926	0.2377
5	0.0482	0.2815	0.1933	0.1828
7	0.1843	0.3710	0.3524	0.2742
32	0.2177	0.6301	0.3746	0.2207

The presence of urea in the substrate affected also the morphology of cultures so that they showned a dark brown pigmentation, thicker membranes in the hyphae and numerous swellings of the chlamydospore type.

When assessing the nitrogen demand, attention has been drawn to N as NO_3 consumption by MrgX fungus. The analysis demonstrated that the MrgX strain under study was growing better on the medium with nitrate than on that with ammonium. The 6-day-old cultures of MrgX strain on the medium with nitrate (KNO_3) yielded, on the average, 14 mg of mycelium dry weight, whereas on the medium with ammonium – only 5,9 mg d.w. The facility with which N as NO_3 is utilized by MrgX fungus testified to a high activity of nitrate reductase in the mycelium. The studies have shown that the quantity of nitrogen used for producing 1 mg of mycelium is similar regardless the form of nitrogen in the medium. The consumption was 39 ± 5 µm/mg dry weight of mycelium at nitrate and 41 ± 9 µm/mg dry weight of mycelium at ammonium form of nitrogen. For comparison, the ectomycorrhizal strain of *Suillus bovinus*, showing weaker growth on the medium with NO_3, utilized 35 ± 7 µg N at NO_3 as the nitrogen source and 47 ± 6 µg N on the medium with NH_4.

The studies on 3 MrgX strains showed that their optimal range of pH is 6,4-7,0. Within this range the best results of MrgX isolation from ectendomycorrhizae were obtained as well as the best growth of subcultures of the fungus (Fig. 2 A-E).

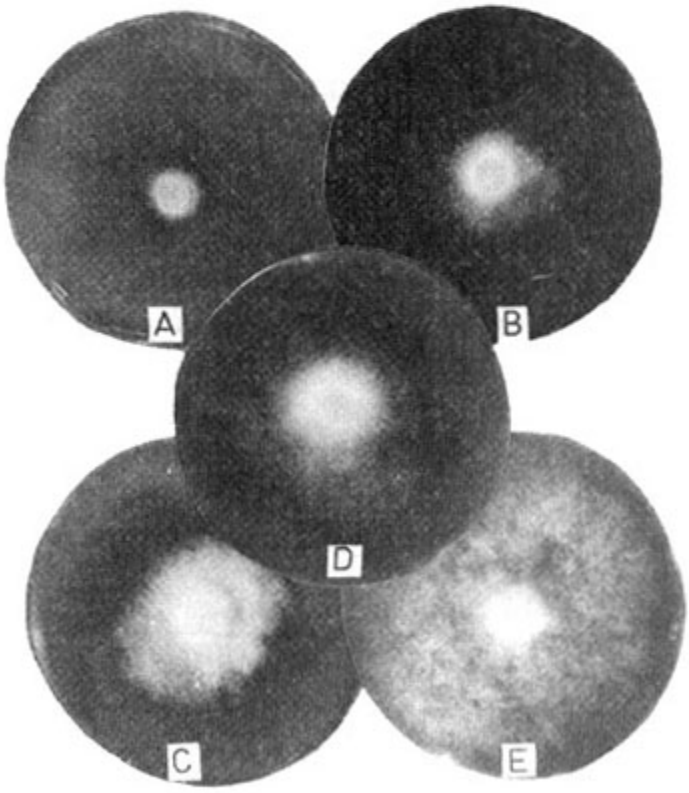

Fig. 2. Cultures of MrgX-II ectendomycorrhizal fungus on Pp agar medium with v ying pH: 3,5 (A), 5,5 (B), 6,5 (C) and 7,5 (D) and with CaO added in the amount of 166 mg/l (E), at 5°C after 16 days

While investigating the Ca effect on the growth of MrgX fungus, an attempt was made at explaining the role of Ca, i.e. if it was limited only to increasing the pH of the substrate or should it be treated as an important nutritional element. At this point the pH of the substrate was maintained at the level of 5,6-5,8. It was found that, in general, both MrgX strains responded positively to the addition of all calcium compounds in question, CaO and $CaCO_3$ being obviously prefered. The above results suggest that Ca take part in the metabolism of fungus.

Since competition may play an important role in the adaptation of microorganisms in the definite soil environment, pertinent studies were carried out on the MrgX fungus. The observation results testify to the lack of antagonistic interrelations between the MrgX-II strain and 3 isolates under study i.e. *Cylindrocarpon* sp., *Mycelium radicis atrovirens* and *Phlebia gigantea*; the cultures of the above fungi were contiguous or mutually overgrown without zones of inhibited growth. Thus, it may be assumed that the expansion of ectendomycorrhizal fungus is conditioned chiefly by its ecological and mycorrhizal properties.

In addition to the studies on the biology of ectendomycorrhizal fungus, an attempt also made was at the assessment of chitin level in the vegetative mycelium. MrgX-I strain was tested with the use of different nitrogen forms. Chitin content, expressed in µg of glucosamine/mg dry weight of mycelium, was 31 ± 10 in the hyphae of MrgX-I grown on nitrate for 6 weeks while on NH_4 it increased to 44 ± 10. Despite better growth on NO_3, the MrgX hyphae contained less chitin. The same was observed in other fungi including those showing weaker growth on nitrate than on NH_4, e.g. *Suillus bovinus*. P l a s s a r d et al. (1982) as well as M e n t i o n and P l a s s a r d (1983) also found lower level of chitin in mycelium growing on nitrate in a study on ectomycorrhizal fungi.

CONCLUSIONS

- In forest nursery soil subjected to permanent cultivation and mineral fertilization, ectendomycorrhizae constitute the main type of mycorrhizal symbiosis on pine.
- Ectendomycorrhizal symbiosis on pine was formed with MrgX fungus whose strains showed a high homogenity of morphological and mycorrihizal properties.
- The ultrastuctural analysis of the MrgX hyphae suggested that the fungus was an *Ascomycetes*.
- Optimal pH of culture medium for MrgX fungus isolation from the ectendomycorrhizae and for in vitro growth of the fungus culture was 6,5-7,0.
- Isolation from the ectendomycorrhizae and the in vitro tests showed that the calcium takes part in the metabolisms of the MrgX fungus.
- MgrX strains are capable of utilizing nitrates as a nitrogen source which is determined by a high activity of nitrate reductase in the mycelium.
- The MrgX fungus had the ability to utilize urea as a source of nitrogen. Nursery

experiments demonstrated the stimulatory effect of urea of the development of ectendomycorrhizae.

- The chitin level in the mycelium of fungus MrgX growing on medium with ammmonium salts was higher than in mycelium growing on medium with nitrate.
- Nursery experiments indicated that ground phosphate rock stimulates the occurrence and dynamics of ectendomycorrhizae.
- Reaction and calcium level in forest nursery soil favoured the development of MrgX fungus.
- Bacteriological studies indicated that *Bacillus polymyxa* was consistently found to inhibit the pine rhizosphere. The N_2 fixing capability of izolated strains of *B. polymyxa* suggests that these bacteria might supply nitrogen to the plant by mycorrhizal association.

REFERENCES

Barjac de H., 1952. La puissance dénitrifiante du sol mise au point d'une technique d'évaluation. Ann. Inst. Pasteur 83.

Bergey's manual of determinative bacteriology. 1974. Williams and Wilkins Co, Baltimore, VII ed.

Björkman E., 1942. Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. Symb. Bot. Upsalien 6.2: 1-190.

Goss R. W., 1960. Mycorrhizae of ponterosa pine in Nebraska grassland soil. Nebr. Agr. Exp. Sta. Res. Bull. 192: 47.

Johnson L. E., Curl E. A., Bond and J. F., Fribourg H. A., 1959. Methods for studying soil microflora-plant disease relationships. Burges Publ. Co, Minneapolis.

Kocoń J., 1980. Application of electron microscopy and X-ray structural analisis in the fundamental studies in agriculture. Podst. Probl. Współcz. Tech. 22: 57-102. (in Polish). PWN, Warszawa.

Laiho O., 1965. Further studies on the ectendotrophic mycorrhiza. Acta Forest. Fenn. 70 (3): 1-35.

Marx D. H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection. I. Antagonism of mycorrhizal fungi to root pathogrnic fungi and soil bacteria. Phytopathology 59: 153-163.

Melin E., 1922. Untersuchungen über die *Larix* – Mycorrhiza. I. Sven. Bot. Tidskr. 16: 161-196.

Melin E., 1923. Experimentelle Untersuchungen Über die Konstitution und Ökologie der Mykorrizen von *Pinus sylvestris* und *Picea abies*. Mycol. Unters. Ber. 2: 73-331.

Mention M., Plassard C., 1983. Comparision de la nutrition nitrique et ammoniacale de quatre éspeces de *Basidiomycetes* ectomycorrhiziens. C.R. Acad. Sc. Paris, S. III 297: 489-492.

Mikola P., 1965. Studies on the ectendotrophic mycorrhiza of pine. Acta Forest. Fenn. 79(2): 1-56.

Norris J. R., Chapman, 1982. Classification of Azotobacters. [In:] Identification methods for microbiologists. (Gibbs B. M., Shapton D. A., eds.), p. B, Acad. Press, London, New York.

Pachlewska J., 1968. Study on mycorrhizal synthesis in pine in pure cultures on agar. Prace IBL 345-350: 1-75.

Pachlewski R., 1985. Symbiotic fungi and pine mycorrhiza (*Pinus sylvestris* L.). Prace IBL 615: 4-132.

Pachlewski R., Kocoń J., 1985. Ultrastructure of the hyphae of ectodomycorrhizal fungus of Scotch pine. Acta Mycol. 21: 23-25 (in Polish).

Pachlewski R., Pachlewska J., 1960. Morphological-anatomic studies on mycorrhizae of pine (*Pinus sylvestris* L.) in the forest communities of Białowieski National Park. Prace IBL 205: 3-48.

Pachlewski R., Pachlewska J., 1971. Studies on mycorrhizal fungi of pine (*Pinus sylvestris* L.) in forest nurseries. Prace IBL 395: 3-66.

Pachlewski R., Pachlewska J., 1974. Studies on symbiotic properties of mycorrhizal fungi of

pine(*Pinus sylvestris* L.) with the aid of the method of mycorrhizal synthesis in pure cultures on agar. IBL 1-228. Warsaw.

Palenzona M., Fontana A., 1970. Influenza di tipi di suolo sutre forme micorrhiziche del Pino strobo. Allionia 16: 101-113.

Piché Y., Ackerley A., Peterson R. L., 1986. Structural characteristics of ectendomycorrhizas synthesized between roots of *Pinus resinosa* and E-strain fungus, *Wilcoxina mikolae* var. *mikolae*. New Phytologist 104: 447-452.

Plassard C., Moussain D. G., Salsac L. E., 1982. Estimation of mycelial growth of *Basidiomycetes* by means of chitin determination. Phytochemistry 21: 345-348.

Rennie R. J., 1981. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. Can. J. Microbiol. 27: 6-14.

Trappe J. M., 1971. Mycorrhiza-forming *Ascomycetes*. In: Mycorrhizae (E. Hacskaylo, ed.) USDA Forest Serv. Misc. Publ. 1189: 19-37.

Trzcińska M., Pachlewski R., 1986. Studies on chityn level in vegetative mycelium of some mycorrhizal fungi. Acta Mycol. 22: 89-93.

Wang C. J. K., Wilcox H. E., 1985. New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia, Chloridium paucisporum* and *Phialocephala fortini*. Mycologia 77: 951-958.

Wilcox H. E., 1971. Morphology of ectendomycorrhizae in *Pinus resinosa*. [In]: Mycorrhizae (E. Hackaylo, ed.) USDA Forest Serv. Misc. Publ. 1189: 54-68.

Yang C. S., Wilcox H. E., 1984. An E-strain ectendomycorrhiza formed by a new species *Tricharina micolae*. Mycologia 76: 675-684.