

Susceptibility of ectomycorrhizal and ectendomycorrhizal fungi to pH of the environment

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The present study should yield some information on the reaction of different species and strains of mycorrhizal fungi to acidification or alkalization of substrate. The effect of acidity of medium on development of mycorrhizal fungi was tested at the following ranges of pH: 2.5, 3.0, 3.9, 4.9, 5.4, 6.6 and 7.3. At pH 2.5 and 3.0 almost half of the fungal cultures tested did not resume their growth during an entire period of the experiment. For some fungi pH 7.3 turned out to be such as much unfavourable as a high acidification of medium. The results of the study indicate that excessive acidity of substrate may be more unfavourable for the development of mycorrhizal fungi than its alkalization. The pH range tolerated by mycorrhizal fungi in the second case seems to be wider.

Key words: ectomycorrhizal and ectendomycorrhizal fungi, industrial emissions, pH requirements.

INTRODUCTION

The most ectomycorrhizal fungi are acidophilic although they differ much with regard to pH requirements (Hung, Trappe, 1983). The acidity of substrate has an effect on the growth of mycorrhizal fungi (Kampert, 1990; Pachlewski, 1993), their uptake of nutrients (Jongbloed, Borst-Pauwels, 1992; Bledsoe, Rygielwicz, 1995), and enzymatic activity (Antibus, Kroehler, Linkins, 1986; Antibus, Linkins, 1992). It may also influence the synthesis of growth hormones of plants (Strzelczyk, Kampert, Pachlewski, 1994).

Industrial pollution may result in an excessive acidification or alkalization of the environment. Both these situations are highly unfavourable for forest trees and affect their susceptibility to diseases. Strong acidification of the soil may activate aluminium, which is toxic to forest trees, and to many species of mycorrhizal fungi. Some fungi, however, e.g. *Laccaria bicolor*, tolerate very high concentration of this

element (J o n g b l o e d, B o r s t - P a u w e l s, 1992). Remedy for the soil acidity is its liming. This treatment also aims at the improvement of the total microbiological balance of the soil, which results in a faster decomposition of organic substances. Liming of forest soils should however be a careful operation because of the mycotrophic nutrition of forest trees. According to L e h t o (1994 a, b) liming in spruce stands affects the quantitative ration between mycorrhizal types and increases mortality of short roots. On the other hand, liming in *Pinus resinosa* stand resulted in the increase of the number of mycorrhizas, although the time of sampling had an effect on the results obtained (A n t i b u s, L i n k i n s, 1992).

Different reaction of mycorrhizal fungi and mycorrhizas of forest trees to variable pH of the environment should be one of the more important criteria for the selection of these fungi from the point of view of choice of proper strains best suited for the preparation of inocula with mycorrhizal fungi. The present study should yield some information on the reaction of different species and strains of mycorrhizal fungi to acidification or alkalization of substrate. This problem is important from a practical point of view in connection with investigations on the preparation of a mycorrhizal biopreparation which be used for the mycorrhization of forest tree seedlings intended for soil recultivation in regions degraded by industry.

MATERIALS AND METHODS

Fructifications of mycorrhizal fungi were collected in a permanent study area, which was strongly polluted (zone II) with industrial emissions, situated in Brynica. The collections were also made in areas, which were moderately polluted (zone II), in Pniowiec and the Niepolomice Forest. Moreover, the fructification were collected in an area free of excessive pollution situated in the Herby forest district (K o w a l s k i et al., 1989). Pure cultures of these fungi were isolated from their fructifications on the medium of M e l i n and R a m a D a s (1954) modified by addition of vitamine B₁, H, inositol, aureomycin and soil extract in various quantities. After isolation the pure cultures of mycorrhizal fungi were inoculated to test-tubes and identified.

Pure cultures of ectendomycorrhizal fungi were isolated from mycorrhizas of Scotch pine seedlings in four forest nurseries: Bobrek, Barwałd Górny, Janów Lubelski and Zabrzeg. The mycorrhizas, after their cleaning and superficial disinfection, were placed on the medium of M e l i n and R a m a D a s (l.c.), and the isolated fungi were identified.

The experiment on the effect of pH of substrate on the growth of cultures ectomycorrhizal and ectendomycorrhizal fungi was carried out on the medium mentioned above with the addition of chlortetracycline in an amount of 250 µg/100 ml and thiamine in an amount of 20 µg/100 ml. The pH of the medium thus prepared was 4.9. Further acidification was accomplished with 0.1 n H₂SO₄, while 0.1 n KOH added was determined on the basis of repeated measurements of pH of liquid

medium. On the basis of these measurements a curve representing a relationship between pH of the medium and an amount of a chemical compound added was drafted. Subsequently these values were checked on a medium with agar, and corrections were made as needed. Both sterile acid and sterile base were added to the initial medium after sterilization and cooling, shortly before placing it in Petri dishes. The initial cultures were incubated for 4 weeks at 22°C on the medium of M e l i n and R a m a D a s (l.c.) with the addition of 50 µm of thiamine per 1 l of medium. Inocula of equal size, 5 mm in diameter, were cut out from a marginal zone of these cultures and placed on a surface of medium of proper pH, in the center of a Petri dish. Incubation of each strain was repeated twice for each pH range. All inoculated dishes were incubated at 22°C. An effect of the acidity of medium on development of mycorrhizal fungi was tested at the following ranges of pH: 2.5, 3.0, 3.9, 4.9, 5.4, 6.6, and 7.3. The experiment was evaluated after 10, 20 and 30 days of incubation, and the results are presented in Table 1 and Figs. 1-4.

RESULTS

High acidity of the medium (pH 2.5, 3.0 and 3.9) limited or simply prevented the growth of a considerable number of mycorrhizal fungi tested. At pH 2.5 and 3.0 almost half of the fungal cultures tested did not resume growth from inocula 5 mm in diameter during the entire period of the experiment (Tab. 1, Figs. 1-4). These were as follows: *Amanita citrina* (W 48), *A. muscaria* (W 62), *Hebeloma crustuliniforme* (W 33 and W 40), *H. musophaeum* (W 120), *Paxillus involutus* (N 11), *Suillus granulatus* (W 66), *S. luteus* (2273), and strain of ectendomycorrhizal fungus JL30086. Among the tested fungi *A. citrina* (W 48) and three strains from the genus *Hebeloma* (W 33, W 40, and N 120) were particularly susceptible to the acidity of substrate since they did not grow at pH 3.9 as well (Figs. 1 and 2, Tab. 1). Under laboratory conditions *Lactarius rufus* (3673), *Suillus bovinus* (W 5), *S. luteus* (W 9) and the strain of ectendomycorrhizal fungus (B 30216) tolerated a higher acidity of substrate better than the other fungi. In case of *L. rufus*, the optimum growth of its colony occurred at pH 5.4, while at higher pH its growth was slowed down. On the other hand, fungi from the genus *Suillus* showed optimum growth at much wider range of pH, i.e. between 4.9 and 6.6 (Tab. 1). The diameter of cultures of ectendomycorrhizal fungi tested increased along with the pH of medium (Tab. 1).

The increase in the diameter of a colony between 10-th and 30-th day of incubation under laboratory conditions may better characterize a given strain than the actual diameter of colony at the end of the incubation period. It is established that mycorrhizal fungi differ with regard to the period of growth resuming even under best conditions. Taking this factor into account it may be concluded that cultures of fungi from the genus *Suillus* grew relatively well, and their growth was quite similar at a wide range of pH from 3.9 to 7.3, while some strains tolerated even lower pH (Fig. 3).

Table 1

Effect of pH of medium on the growth of cultures of fungi tested after 30 days of incubation at 22°C

Species	Symbol of strain of fungus and its origin	Mean diameter of colony in mm						
		pH						
		2.5	3.0	3.9	4.9	5.4	6.6	7.3
<i>Amanita citrina</i> (Schaeff.) Pers	W 48 * II : So **	5.0	5.0	5.0	15.5	44.0	43.8	30.0
<i>Amanita muscaria</i> (L.: Fr.) Pers.	W 21 II : Socz	8.0	9.0	11.5	15.0	14.5	17.0	17.3
	W 62 III : Socz	5.0	5.0	17.0	20.5	24.5	40.0	31.0
<i>Hebeloma crustuliniforme</i> (Bull.) Quéf.	W 33 II : Brz	5.0	5.0	5.0	13.8	17.8	28.8	40.0
	W 40 II : So, Db	5.0	5.0	5.0	19.5	23.0	35.5	46.8
	W 53 II : Sow	5.0	8.0	10.0	13.8	20.0	25.0	34.0
<i>Hebeloma mesophaeum</i> (Pers.) Quéf.	W120 II : So	5.0	5.0	5.0	12.3	18.0	22.0	17.0
<i>Lactarius rufus</i> (Scop.: Fr.) Fr.	3673 O : So	12.5	14.0	18.0	22.3	26.0	12.0	5.0
<i>Paxillus involutus</i> (Btsch: Fr.) Fr.	N 11 II : Brz	5.0	5.0	9.0	20.0	22.8	19.0	19.0
<i>Scleroderma citrinum</i> Pers.	N 158 II : So	5.0	8.0	10.0	13.8	29.0	28.0	25.0
<i>Suillus bovinus</i> (L.: Fr.) O. K.	W 5 II : Socz	42.5	z.u.	66.3	90.0	87.8	80.0	z.u.
<i>Suillus granulatus</i> (L.: Fr.) O. K.	W 66 III : Socz	5.0	5.0	68.5	82.3	80.0	77.5	79.0
<i>Suillus luteus</i> (L.: Fr.) S. F. Gray	W 9 II : So	10.3	40.0	50.3	59.5	66.8	68.3	30.5
	2273 III : So	5.0	5.0	41.0	45.8	59.0	60.0	53.5
non sporulating ectendomycorrhizal fungi ***	B 30216	8.5	9.0	15.0	25.5	28.3	46.3	44.3
	BG 30335	5.0	10.5	25.3	41.0	46.5	54.0	71.3
	JL 30086	5.0	5.0	33.0	63.8	66.3	84.0	83.8
	Z 30362	5.0	10.5	21.0	33.3	39.3	80.0	81.5

* — symbol of isolated culture of fungus

** — before colon — forest industrial damage zone: () — no damage, II — moderate damage, III — strong damage; — after colon — symbol of tree species under which the fructification were collected: So — *Pinus sylvestris*, Socz — *Pinus nigra*, Sow — *Pinus strobus*, Brz — *Betula verrucosa*, Db — *Quercus robur*

*** — fungi isolated from ectendotrophic mycorrhiza of Scotch pine seedlings in 4 different forest nurseries

Likewise, the strains of ectendomycorrhizal fungi tolerated a very wide range of pH of substrate. In this case, however, the diameter of colonies increased with the pH of the substrate and was distinctly higher at pH of 6.6-7.3 (Fig. 4). The requirements of fungi from the genus *Amanita* regarding the acidity of substrates differed considerably. It may be concluded that colonies of strains tested grew relatively well at pH of 5.4 and 6.6, while lower and higher pH clearly inhibited the growth of mycellium, although the growth of strain W 21 was similar at very low and high pH (Fig. 1). The cultures of *Lactarius rufus* and *Paxillus involutus* grew best at pH 4.9 and 5.4 but the former fungus tolerated much better lower pH while the latter one tolerated better higher pH (Fig. 1). *Scleroderma citrinum* (N 158) and the cultures of fungi from the genus *Hebeloma* grew better at higher than at lower pH range (Figs. 1 and 2). In the second case a distinct strain differentiation of *H. crustuliniforme* was observed, since the strain *H. crustuliniforme* (W 53) showed a mycelial increase beginning with pH of 3.0, while the remaining two strains started to grow on medium with pH of 4.9 (Fig. 2). Moreover, increment of *H. crustuliniforme* (W 40) strain was distinctly better under the experimental conditions than that of the remaining two (Fig. 2).

For some fungi the pH of 7.3 turned out to be as much unfavourable as the high acidification of medium. Under such conditions they reacted with inhibited growth (Tab. 1). This was especially true for following fungi: *A. citrina* (W 48), *A. muscaria* (W 62), *H. mesophaeum* (N 120), *L. rufus* (3673), and *Suillus luteus* (W 9).

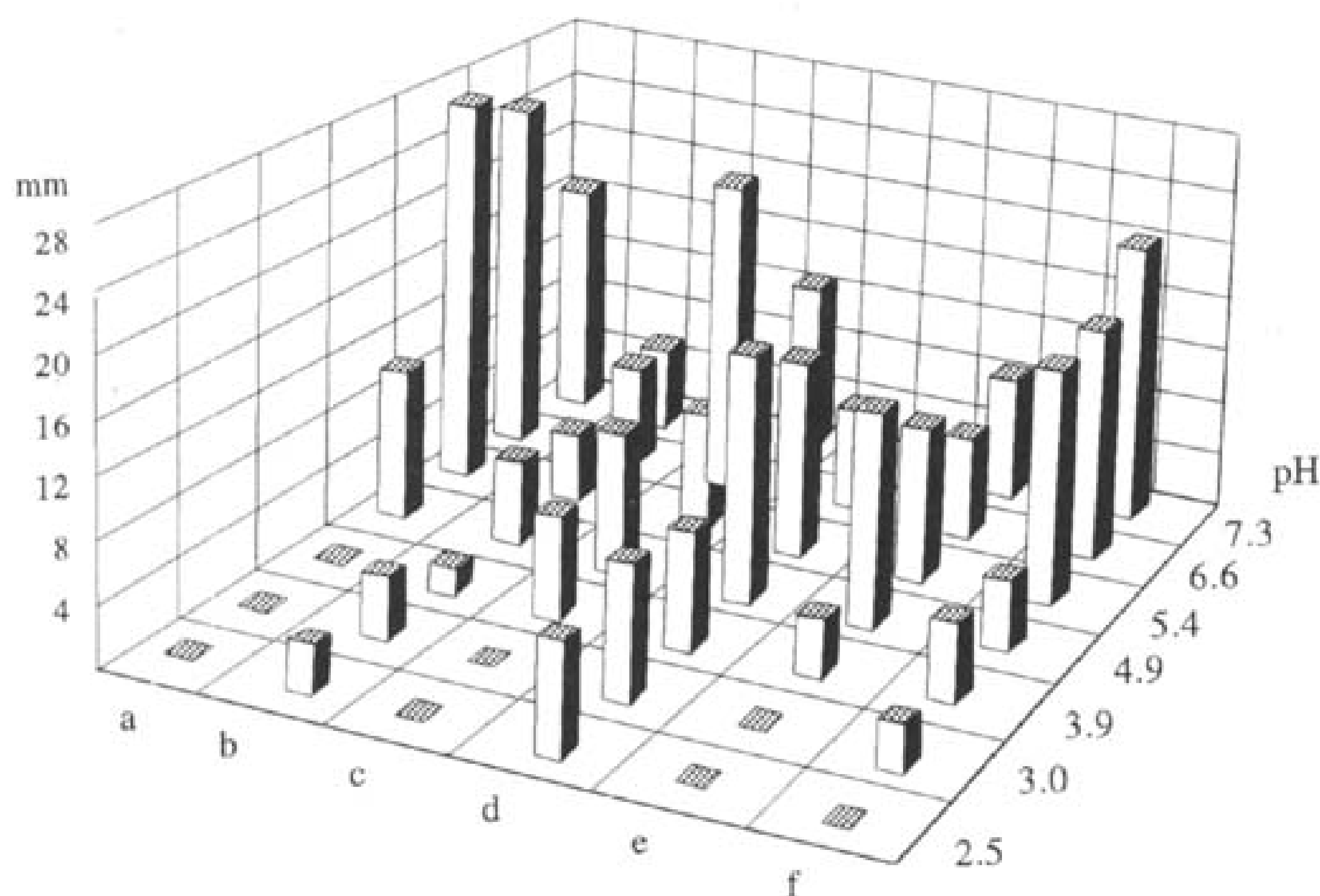


Fig. 1. Effect of pH of substrate on diameter increment (mm) of colonies of five species of mycorrhizal fungi between the 10th and 30th day of incubation
 a - *Amanita citrina* W 48, b - *A. muscaria* W 21, c - *A. muscaria* W 62, d - *Lactarius rufus* 3673,
 e - *Paxillus involutus* N 11, f - *Scleroderma citrinum* N 158

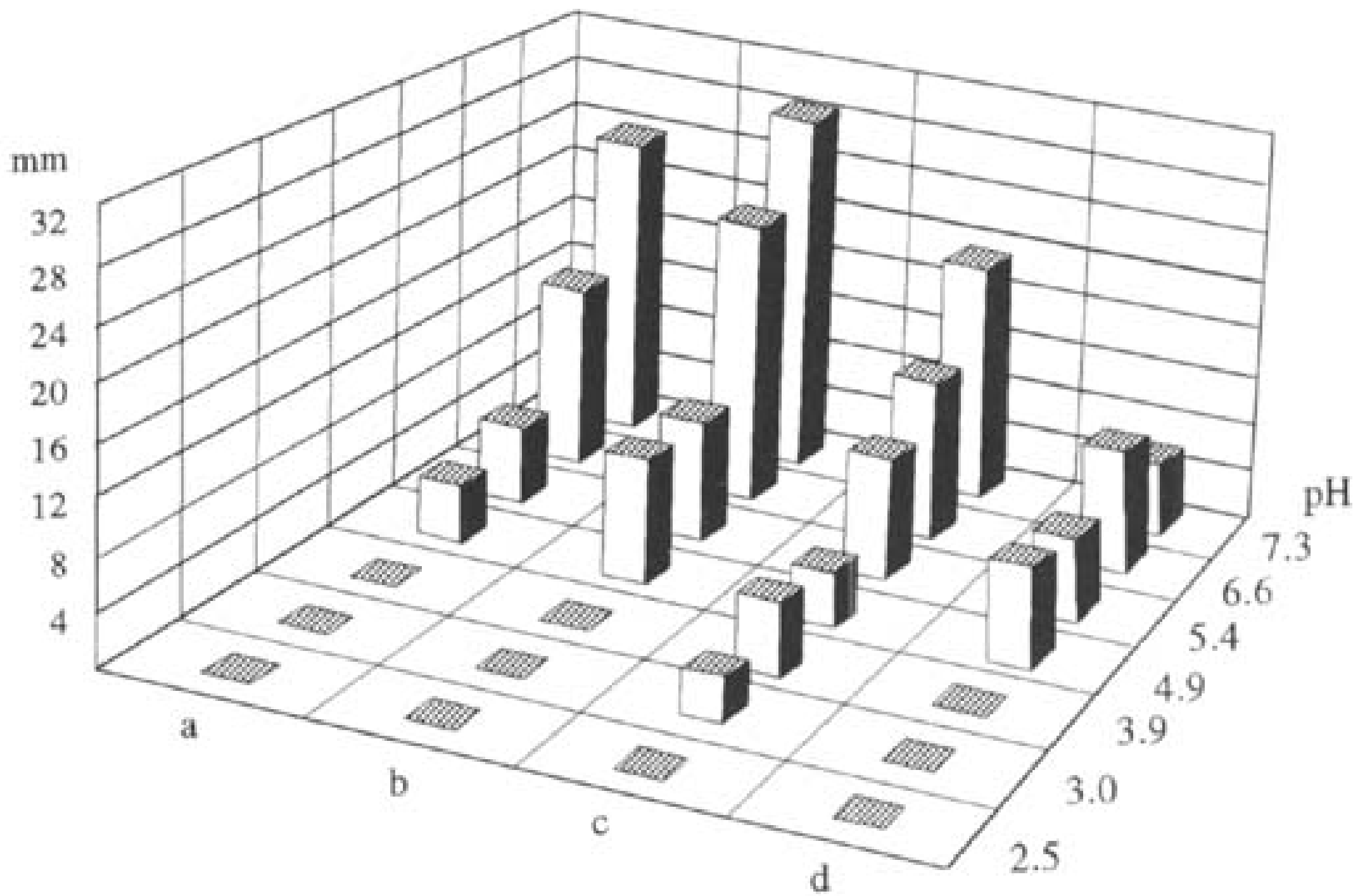


Fig. 2. Effect of pH of substrate on diameter increment (mm) of colonies of fungi from the genus *Hebeloma crustuliniforme* between the 10th and 30th day of incubation

a – *Hebeloma crustuliniforme* W 33, b – *H. crustuliniforme* W 40, c – *H. crustuliniforme* V
d – *H. mesophaeum* W 120

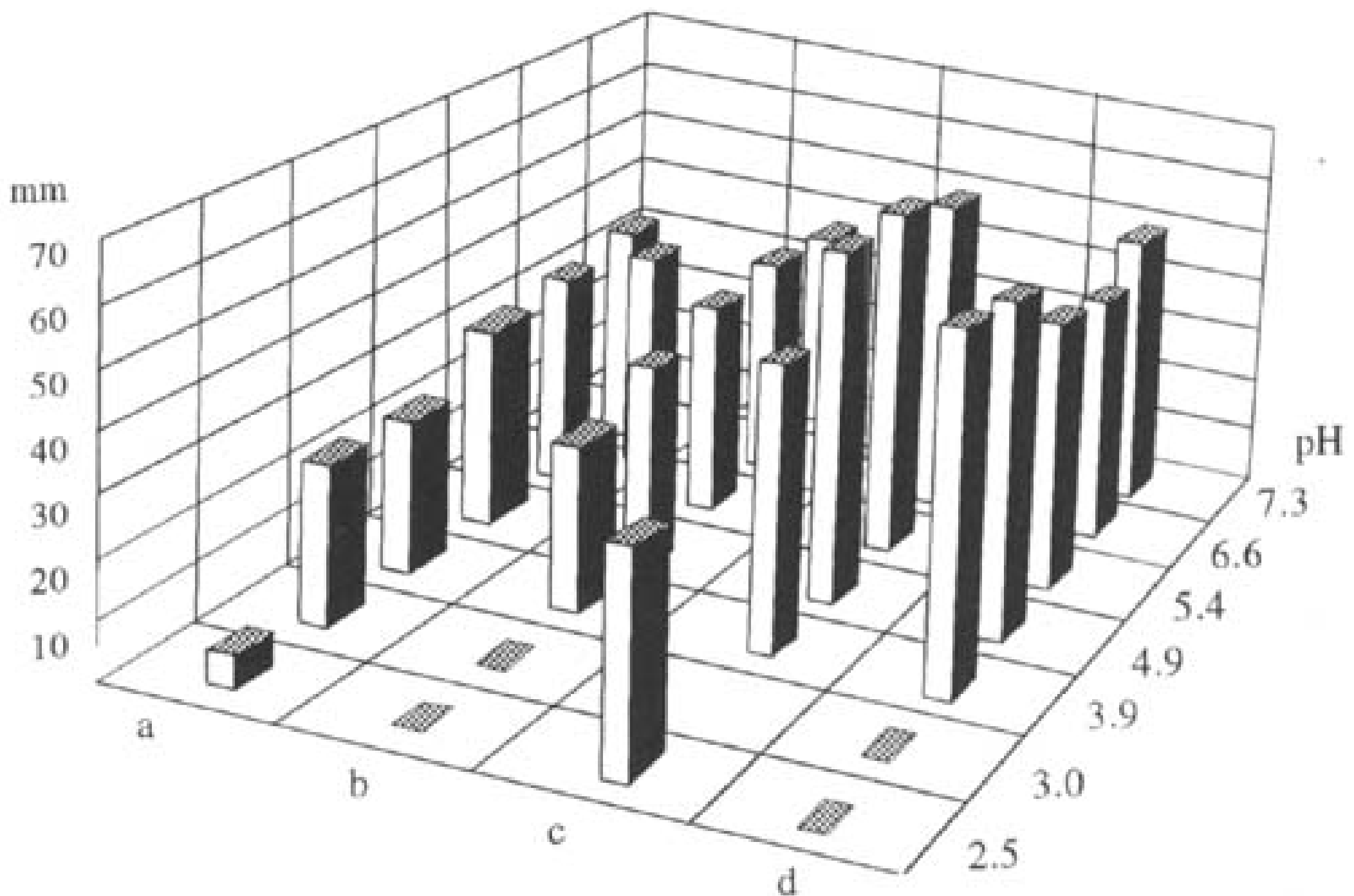


Fig. 3. Effect of pH of substrate on diameter increment (mm) of colonies of fungi from the genus *Suillus luteus* between the 10th and 30th day of incubation

a – *Suillus luteus* W 9, b – *S. luteus* 2273, c – *S. bovinus* W 5, d – *S. granulatus* W 66 *S. granul.*

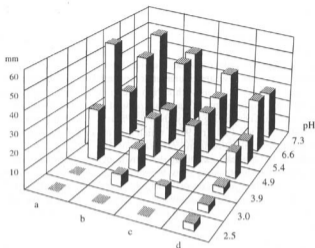


Fig. 4. Effect of pH of substrate on diameter increment (mm) of colonies of various strains of ectendomycorrhizal fungi between the 10th and 30th day of incubation
a - JL 30086, b - Z 30362, c - BG 30335, d - B 30216

DISCUSSION

The investigations on the effect of pH of substrate on the growth of mycorrhizal fungi showed their great variability with regard to this factor. This is very important from the point of view of selecting proper strains which may be used for an artificial mycorrhization of the forest tree seedlings designated for afforestation of degraded soils, sometimes of extreme values of pH. The results concerning the effect of pH on the diameter growth of colonies of various strains of ectendomycorrhizal fungi showed their considerable conformity with the characteristics of the isolate MrgX2 investigated by Pachlewski (1993). In both cases there was a rapid acceleration of growth of the colony at 4.9-7.3. Stimulation of growth of this fungus in a weak basic environment is undoubtedly one of the factors of expansion of ectendotrophic mycorrhiza in many forest nurseries. The strain of *Hebeloma mesophaeum* (W 120) seems to be more susceptible to low pH than the strain of this fungal species investigated by Pachlewski (1993), although in both cases maximum growth occurred at pH of about 6.6. On the other hand the pH requirements of the *Suillus bovinus* (W 5) strain tested by us were similar to those of the *S. bovinus* (1941) strain tested by Pachlewski (1993). In both of these cases the fungi grew well at pH of 2.5, and their maximum growth was observed at pH of about 4.9. In addition the present results were in agreement with those of Kamper

(1990), especially with regard to pH requirements of *Suillus luteus* and *S. bovinus*. In our case, however the strains of *Amanita muscaria* preferred a higher pH. The addition of colonies of *Actinomyces* to liquid cultures of mycorrhizal fungi could have a somewhat different effect on the results. Preference for lower pH by *Lactarius rufus* was also shown by Jongbloed and Borts-Pauwels (1992). The strain tested by these authors showed a rapid increment drop already at pH above 4, while in the case of the strain tested by us such a drop occurred not till pH of 6.6. Our results concerning pH requirements of various strains of *Amanita muscaria* are in agreement with the results concerning the synthesis of *Pinus sylvestris* and *A. muscaria* (Metzler and Oberwinkler, 1987), which may indicate usefulness of tests in pure cultures for their utilization in mycorrhizal syntheses.

Such factor as too low pH, inhabiting the growth of mycorrhizal fungi, may also restrict the development of mycorrhizas. Investigations on the effect of sulphuric acid (pH 3.0) on roots and mycorrhizas of *Pinus sylvestris* showed that the development of mycorrhizas of type B and F was inhibited. The decline of these mycorrhizal types the authors (Dighton et al., 1986) ascribe to the decreasing pH of the soil. The decrease in the pH of soil results in an increase in the concentration of soluble toxic microelements such as Al and Mn, while their presence hinders the growth of mycorrhizal fungi (Thompson and Madre after Dighton et al., 1986). However, Froidevaux (1985) in his work on afforestation of degraded soils concluded that there are fungi tolerating the low pH of the substrates. *Pisolithus tinctorius* may serve as an example since at pH below 4.0 it forms mycorrhizal connections and stimulates the growth of seedlings more strongly than *Suillus granulatus*. Moreover, the author gives examples of inoculation of seedlings with this fungus, which gave positive results. In our studies *S. granulatus* did not develop on the medium with pH lower than 3.9. Investigations of Fortin (1970) concerning the interaction of mycorrhizal fungi and pine roots in the presence of indole-3-acetic acid produced by mycelium showed that roots by accumulating the acid facilitate the growth of fungi protecting them against a disadvantageous action of this acid. This accumulation reaches the highest level at pH below 4.0 in the soil and decreases with the increase in pH, in a neutral environment being very low. These results are in agreement with the opinion of Björkman (1942), who maintains that the reaction too alkaline does not favour the formation of mycorrhizas. In our studies most of the isolates of mycorrhizal fungi tested developed better in a weak acid, neutral or even weak basic environment than at lower values of pH. Dominik (1961) who referred to the investigations of Smith, Björkman and his own concluded that well developed mycorrhizas occurred at pH 4.0-7.5, e.i. in weakly acid or even neutral environment. Our studies on the effect of pH of the medium on the growth of cultures of mycorrhizal fungi showed that pH optimum for the growth of fungi tested varied from 5.4 to 7.3, while a high acidity of medium (pH 2.5 and 3.0) eliminated many strains of mycorrhizal fungi tested. The results obtained may form basis for a careful liming of degraded soils which are strongly acid, because of not univocal results of field tests in this respect. They were rather positive in pine stand (Antibus, Linkins, 1992), and clearly negative in spruce stand (Lehto, 1994).

CONCLUSIONS

The following conclusions were drawn from this study:

1. The range of pH for individual species of mycorrhizal fungi is an important factor which should be taken into account during their mass culture under laboratory conditions.
2. Mycorrhizal fungi are characterized by a great variability with regard to pH requirements. This should be one of the criteria in their selection for an artificial mycorrhization of forest tree seedling intended for afforestation of degraded soils.
3. The specific selection of mycorrhizal fungi intended for an artificial mycorrhization of forest tree seedlings should not be neglected. Special attention should be drawn to their strain variability. The studies showed that within a population of a given species there may be strains tolerating extreme values of pH of the environment.
4. Excessive acidity of substrate may be more unfavourable for the development of mycorrhizal fungi than its alkalization. The range of pH tolerated by mycorrhizal fungi in the second case seems to be wider. This may be an important factor when deciding on liming of strongly acidified soils. The laboratory investigations may be treated here only as a certain indication, while the field tests are a necessity.
5. The study points to the importance of biovariability of mycorrhizal fungi not only at specific level but also among strains.

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Wrażliwość grzybów ektomikoryzowych i ektendomikoryzowych na pH środowiska

Streszczenie

Podjęte badania miały dostarczyć informacji na temat reakcji różnych gatunków i szczepów grzybów mikoryzowanych na zakwaszenie lub alkalizację podłoża.

Czyste kultury grzybów ektomikoryzowanych izolowano z owocników, które zbierano i identyfikowano na powierzchniach badawczych w różnych strefach skażenia emisjami przemysłowymi. Grzyby ektendomikoryzowe izolowano z mikoryz siewek sosny pospolitej w 4 szkółkach leśnych.

Doświadczenie przeprowadzono na pożywce Melina i Rama Das (1954), o wyjściowej wartości pH 4,9. Do dalszego zakwaszenia pożywki używano 0,1 n H₂SO₄, a do podwyższenia pH – 0,1 n KOH. Przy wartościach pH 2,5 i 3,0 prawie połowa badanych kultur grzybów nie wznowiła wzrostu przez cały okres doświadczenia (tab. 1, ryc. 1-4). Były to: *Amanita citrina* (W 48), *A. muscaria* (W 62), *Hebeloma crustuliniforme* (W 33 i W 40), *H. mesophaeum* (W 120), *Paxillus involutus* (N 11), *Suillus granulatus* (W 66), *S. luteus* (2273) i szczep grzyba ektendomikoryzowego JL 30086. Do grzybów, które w warunkach doświadczenia stosunkowo lepiej, w porównaniu z innymi, znosiły większe zakwaszenie pożywki należał *Lactarius rufus* (3673), *Suillus bovinus* (W 5), *S. luteus* (W 9) i szczep grzyba ektendomikoryzowego (B 30216).

Dla niektórych grzybów wartość pH pożywki 7,3 okazała się równie niekorzystna jak silne jej zakwaszenie. Reagowały one wtedy bardzo wyraźnym zahamowaniem wzrostu (tab. 1). Należały tutaj: *A. citrina* (W 48), *A. muscaria* (W 62), *H. mesophaeum* (N 120), *L. rufus* (3673) i *Suillus luteus* (W 9).

Przeprowadzone badania pozwalają na stwierdzenie, że optymalne pH dla wzrostu badanych grzybów wahało się w granicach 5,4-7,3. Uzyskane wyniki badań mogłyby być podstawą do ostrożnego stosowania wapnowania gleb zdegradowanych, silnie kwaśnych. Ostrożnego, z uwagi na niejednoznaczne wyniki badań polowych w tym zakresie, raczej pozytywne w drzewostanie sosnowym (Antibus, Linkins, 1992), a wyraźnie negatywne w drzewostanie świerkowym (Lehto, 1994).