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Growth of mycorrhizal fungi of pine (Pinus sylvestris L.) in associative cultures with Actinomycetes at different pH values

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The effect of 5 actinomycete strains on growth of four mycorrhizal fungi of pine at different pH values was tested in laboratory media. Both inhibition and stimulation of growth was distinctly dependent upon the pH of the medium. In general the actinomycetes introduced to 7-days old fungal cultures more often stimulated than inhibited the fungi. This was not observed when the media were inoculated with both organisms simultaneously.

INTRODUCTION

It is obvious that different ecological factors affect growth of mycorrhizal fungi of scoth pine, mycorrhiza formation and function (Davey 1971; Rambelli 1973). Among these factors the direct involvement of saprophytic soil microorganisms in preventing infection of roots by soil pathogens has been recognized long ago (Eaton, Righre 1946). The same refers undoubtedly to mycorrhizal fungi. The role of ectomycorrhizae as biological deterrents to root infection by soil pathogens has been presented in comprehemsive reviews by Marx (1972) and Rambelli (1973).

Competition and antagonism are suggested as mechanisms responsible for depression of fungi in soil and rhizosphere (Wright 1956; Bowen, Theodorou 1979). Root and seed exudates as well as metabolites of saprophytic microorganisms may inhibit or stimulate plant pathogenic and mycorrhizal fungi (Erwin, Katznelson 1961; Bowen, Theodorou 1979; Fries, Biraux 1980; Strzelczyk, Pokojska-Burdziej 1984; Strzelczyk, Leniarska 1985).

Harley (1948), Moser (1956) suggested that mycorrhizal studies should be done in conjuction with studies on the root zone microorganisms. With few exception this however was not a common practice (Bowen, Theodorou 1979; Dahm 1985).

Despite the fact that *Actinomycetes* form a substantial portion of the microbial population of forest soils and forest trees root zone (Szabó 1974; Johansson, Marklund 1980; Różycki 1984). Little work on their effects on mycorrhizal fungi has been done. Therefore this research was undertaken.

MATERIALS AND METHODS

The following ectomycorrhizal fungi of pine: *Amanita muscaria* (L.: Fr.) O. Kuntze, *Suillus bovinus* (L.: Fr.) O. Kuntze, *Suillus luteus* (L.: Fr.) S.F. Gray, *Rhizopogon luteolus* Fr. et Nordh. and 5 *Actinomycete* strains isolated from the mycorrhizosphere of pine were used in this study. The *Actinomycetes* belonged to the genus *Streptomyces* sp.

The fungi were grown at 26°C in liquid medium according to Melin and Rama Das (1954) adjusted to pH 4.0, 5.0 or 6.0. Erlenmeyer flasks of 300 ml capacity with 100 ml of the above medium were inoculated with two discs (1 cm in diameter) of a 14-days old fungal culture grown on the same (but containing agar) medium and 0.5 ml of actinomycete supension obtained by washing off 7-days old slants prepared from Conn's medium (1921). The media were inoculated with the *Actinomycetes* and fungi either simultaneously (A) or 7-days old cultures of the fungi were seeded with the *Actinomycetes* (B). The cultures were grown for 21 days.

The findings are expressed in the terms of increase or decrease in mycelial dry mass compared with those of controls where fungi were grown without *Actinomycetes* (accepted as 100%). Dry weights of the *Actinomycetes* grown without the fungus were substracted from the dry mass of associated cultures. The experiments were run in triplicate.

RESULTS

Mycorrhizal Fungi inoculated simultaneously with the *Actinomycetes* (A).
Amanita muscaria. Growth of this fungus was inhibited at pH 4.0 by all the *Actinomycetes* used (by 41-79%). At pH 5.0 an increase in mycelium weight was noted in 4 isolates (101-109%). At pH 6.0 the *Actinomycetes* retarded growth of this fungus (33-47%) — (Fig. 1A).
Suillus bovinus. This fungus was inhibited by three isolates of *Actinomycetes* (at pH 4.0) and two stimulated. The isolate No 60 was most stimulatory. At pH 5.0 all *Actinomycetes* depressed of the fungus by 21-54%. Similar in-

hibition was noted in media of pH 6.0 (46-77%). Only the isolate No 9 stimulated considerable the growth of this fungus (Fig. 2A).

Suillus luteus. At pH 4.0 this fungus was inhibited by all the *Actinomycetes* Most inhibitory was the isolate No 4. At pH 5.0 three isolates retarded the growth of this fungus. However two were stimulatory (122-142%). At pH 6.0 all the *Actinomycetes* retarded the growth of this fungus (Fig. 1A).

Rhizopogon luteolus. The above fungus was enhanced at pH 4.0 by four *Actinomycetes* isolates (Fig. 2A). At pH 5.0 all *Actinomycetes* inhibited growth of this fungus by 127% and even 272% (Fig. 2A).

Actinomycetes introduced to 7-days old cultures of fungi (B).

Amanita muscaria. Growth of this fungus was stimulated at pH 4.0 by three n range of 106-121% and the remaining two exhibited inhibitory effect (44-77)%. A considerable inhibition was noted with the isolate No 60.

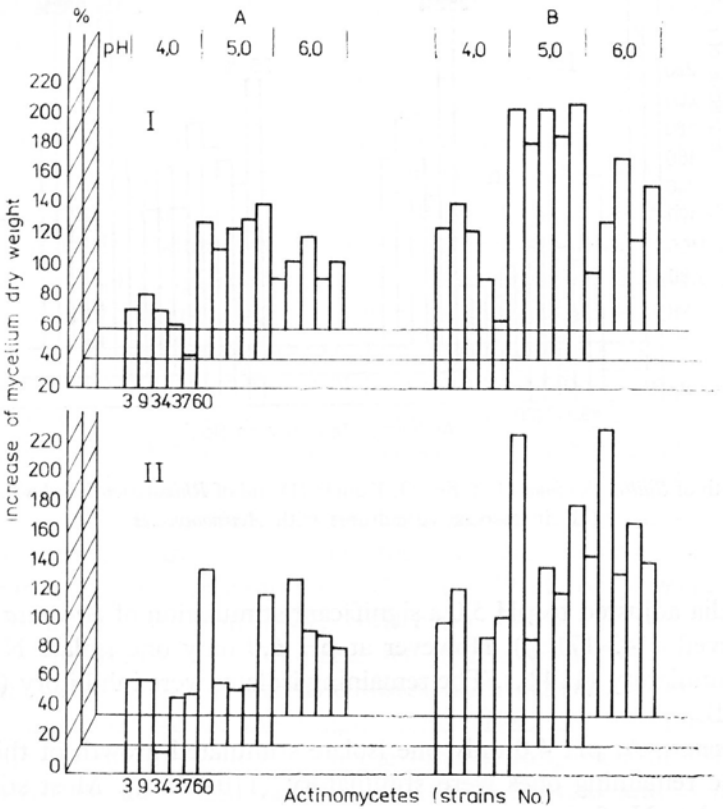

Fig. 1. Growth of *Amanita muscaria* (L.: Fr.) O. Kuntze (I); and of *Suillus luteus* (L.: Fr.) S.F. Gray (II) in associative cultures with *Actinomycetes*

A — Fungus and *Actinomycetes* inoculated simultaneously; *B* — *Actinomycetes* introduced to 7-days old cultures of the fungus

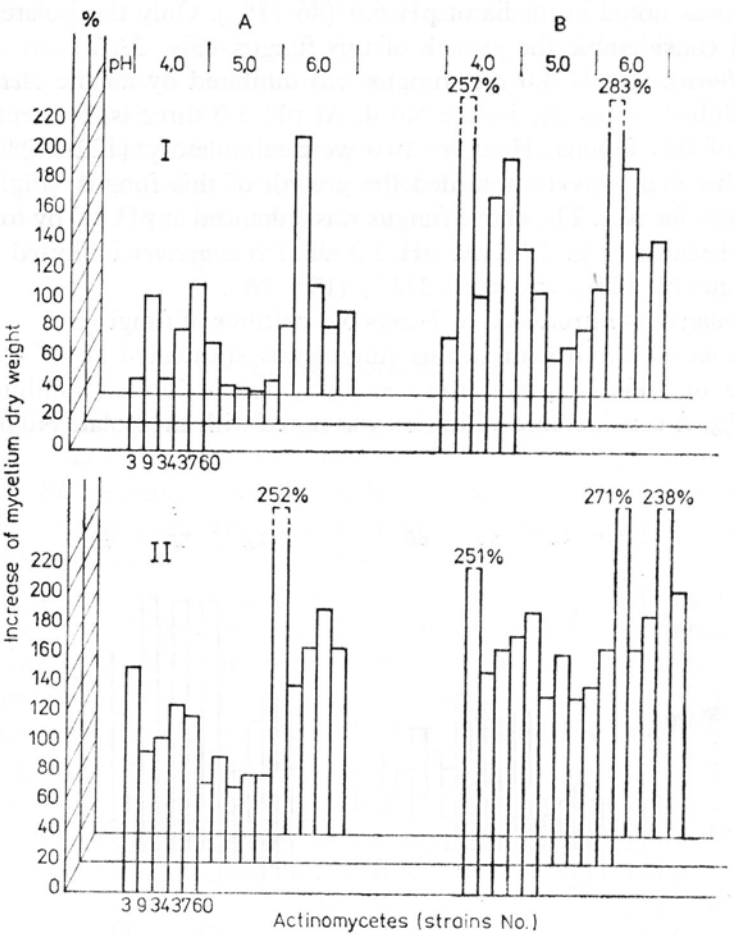

Fig. 2. Growth of *Suillus bovinus* (L.: Fr.) O. Kuntze (I) and of *Rhizopogon luteolus* Fr. et Nordh. (II) in associative cultures with *Actinomycetes*

In media adjusted to pH 5.0 a significant stimulation of *Amanita muscaria* was observed (143-171%). However at pH 6.0 only one isolate No 34 was slightly stimulatory (114%). The remaining isolates were inhibitory (33-97%) — Fig. 1B.

Suillus bovinus. At pH 4.0 only one isolate stimulated growth of this fungus (76%) the remaining ones were stimulatory (110-275%). Most stimulatory was the isolate No 9.

Growth of this fungus in media of pH 5.0 was inhibited by one isolate (No 3) the remaining were inhibitory (41-90%). In media with pH 6.0 a significant stimulatory effect was noted in isolate No 9 (283%). Two iso-

lates were less stimulatory and the remaining two were inhibitory. (Fig. 2B).
Suillus luteus. This fungus in media of pH 4.0 was retarded by two isolates. Two slightly enhanced growth of *S. luteus* but at pH 5.0 only one isolate No 9 inhibited, the remaining stimulated growth of this fungus (106-164%). At pH 6.0 a stimulation of growth of this fungus appeared (Fig. 1B).
Rhizopogon luteolus. Growth of this fungus was stimulated by all *Actinomycetes* used at all pH values applied (Fig. 2B).

DISCUSSION AND CONCLUSIONS

Saprophytic soil microorganisms are know to enhance or retard growth of mycorrhizal fungi and mycorrhiza formation (Rambelli 1973; Davey 1971; Slankis 1973; Bowen, Theodorou 1979; Strzelczyk, Kampert 1986). This effect are attributed to the metabolites they release into the environment.

Shemakhanova (1962) found that formation of mycorrhiza in pine was best supported by *Trichoderma lignorum*, *Azotobacter chroococcum* and fluorescent bacteria. Similar observation was reported earlier by Malyshkin (1955). According to Vedenyapina (1955) the value of azotobacter in mycorrhiza formation may be ascribed rather to vitamins synthesis than to nitrogen fixation. It is know that B-vitamins are essential for growth of ectotrophic mycorrhizal fungi. Growth of *Basidiomycetes* is stimulated by two vitamins, of these thiamine i required by more species than biotin (Norkans 1950; Davey 1971; Slankis 1973). The root zone of forest trees harbours great numbers of microbes capable of producing vitamins, plant growth regulators, free aminoacids, organic acids, sugars etc (Różycki, Strzelczyk 1985; Strzelczyk, Kampert, Michalski 1985; Strzelczyk, Różycki 1985; Strzelczyk, Leniarska 1985; Różycki, Strzelczyk 1985).

Certain aminoacids as well as some plant growth regulators are essential or stimulatory for growth of mycorrhizal fungi and for the formation and function of the fungus-host tree symbiosis (Melin 1959; Davey 1971; Slankis 1973; Krupa, Bränström 1974; Schinner 1976).

It is evident from the results presented in this work thar the growth of most of the test fungi in mixed cultures with *Actinomycetes* proved in most cases unfavourable for the fungus. This effect depended however on the pH value of the medium and on the time of fungal culture inoculation with the *Actinomycetes*. Simultaneous inoculation (fungus and *Actinomyces*) of the medium and low pH in most cases caused inhibition of the fungal growth.

The usual cause of the inhibition of the fungus in mixed cultures could well be the harmful action of the actinomycete metabolites or competition for food.

Actinomycetes are know to be producers of many antibiotics. The production of antibiotics and their stability depends on many enviroumental factors (Jefferys 1952; Strzelczyk, Strzelczyk 1961).

Stimulation of growth of the fungi by *Actinomycetes* could be caused by organic substances required by the fungi and released by the *Actinomycetes*. The concentration of both inhibitory and stimulatory substances has also to be taken into consideration. Also other mechanisms could be involved. For example post-culture liquids of *Actinomycetes* affected not only growth of mycorrhizal fungi but also production of cytokinin-like substances by these organisms (Strzelczyk, Kampert, Michalski 1985).

In a previous work from this laboratory it was reported that bacteria but seldom actinomycete stimulated growth of *Cylindrocarpon destructans* (Strzelczyk, Różycki, Michniewicz 1985). Bacteria stimulated also growth of mycorrhizal fungi, especially when 7-days old fungal cultures were inoculated by these organisms. This effect depended however upon the pH of the culture medium (Strzelczyk, Kampert 1986).

The precise mechanism of such an action as well as the effect of enviroumental conditions on microbial interrelation-ships requires further laboratory and field studies.

Much of the evidence as to interrelationships between microorganisms in soil and in the root zone of plants are circumstantial. However in order to learn the physiological as well as biochemical activites of foil microorganisms it is still necessary to isolate them and to study in the laboratory under controlled conditions.

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Rozwój grzybów mikoryzowych sosny w kulturach z *Actinomycetes* na pożywkach o różnej wartości pH

Streszczenie

Przebadano 4 gatunki grzybów mikoryzowych, współkomponentów sosny, w kulturach z promieniowcami, na pożywkach o różnej wartości pH; rozwój grzybów zależał od stopnia ich kwasowości. Promieniowce wprowadzone do 7-dniowych kultur grzybni częściej stymulowały niż hamowały rozwój grzybni, czego nie zaobserwowano po jednoczesnym wprowadzeniu pożywki inoculum grzyba i promieniowca.