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The effect of two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus*, and of *Bacillus subtilis* on *Fusarium* damping-off in jack pine seedlings

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Article abstract

Two species of ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus*, and a bacterial strain of *Bacillus subtilis*, were tested against *Fusarium moniliforme*, the causal agent of damping-off in jack pine (*Pinus banksiana*) seedlings. Both *P. involutus* and *B. subtilis* inhibited in vitro growth of *F. moniliforme*. The culture filtrates of *P. involutus* and *B. subtilis* were toxic to *F. moniliforme*, but chlamydospore formation of *F. moniliforme* was observed. Greater jack pine seedling survival was observed when co-inoculated with *P. involutus* and *B. subtilis* than with *F. moniliforme* alone. *S. tomentosus* neither inhibited in vitro growth of *F. moniliforme* nor increased survival of jack pine seedlings against *F. moniliforme*. *F. moniliforme* reduced ectomycorrhiza formation on jack pine seedlings by *P. involutus* and *S. tomentosus*. The number of colony forming units of *F. moniliforme* was significantly reduced when seedlings were inoculated with *P. involutus* and *B. subtilis* alone or in combination. *S. tomentosus*, on the other hand, did not reduce the number of colony forming units of *F. moniliforme*. The suppression of *F. moniliforme* growth by *P. involutus* and *B. subtilis* involved production of antifungal compounds.

The effect of two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus*, and of *Bacillus subtilis* on *Fusarium* damping-off in jack pine seedlings

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Two species of ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus*, and a bacterial strain of *Bacillus subtilis*, were tested against *Fusarium moniliforme*, the causal agent of damping-off in jack pine (*Pinus banksiana*) seedlings. Both *P. involutus* and *B. subtilis* inhibited *in vitro* growth of *F. moniliforme*. The culture filtrates of *P. involutus* and *B. subtilis* were toxic to *F. moniliforme*, but chlamydospore formation of *F. moniliforme* was observed. Greater jack pine seedling survival was observed when co-inoculated with *P. involutus* and *B. subtilis* than with *F. moniliforme* alone. *S. tomentosus* neither inhibited *in vitro* growth of *F. moniliforme* nor increased survival of jack pine seedlings against *F. moniliforme*. *F. moniliforme* reduced ectomycorrhiza formation on jack pine seedlings by *P. involutus* and *S. tomentosus*. The number of colony forming units of *F. moniliforme* was significantly reduced when seedlings were inoculated with *P. involutus* and *B. subtilis* alone or in combination. *S. tomentosus*, on the other hand, did not reduce the number of colony forming units of *F. moniliforme*. The suppression of *F. moniliforme* growth by *P. involutus* and *B. subtilis* involved production of antifungal compounds.

Hwang, S.-F., P. Chakravarty et K.-F. Chang. 1995. Effet de deux champignons ectomycorhiziens, le *Paxillus involutus* et le *Suillus tomentosus*, et du *Bacillus subtilis* sur la fonte des semis du pin gris. PHYTOPROTECTION 76 : 57-66.

On a testé l'effet de deux espèces de champignons ectomycorhiziens, le *Paxillus involutus* et le *Suillus tomentosus*, et une lignée de la bactérie *Bacillus subtilis* sur le *Fusarium moniliforme*, agent pathogène de la fonte des semis du pin gris (*Pinus banksiana*). Le *P. involutus* et *B. subtilis* ont tous les deux inhibé la croissance *in vitro* du *F. moniliforme*. Les filtrats de culture de *P. involutus* et de *B. subtilis* ont été toxiques pour le *F. moniliforme*, mais la formation de chlamydospores par le *F. moniliforme* a été observée. Une meilleure survie des plantules a été observée lorsqu'elles étaient co-inoculées avec le *P. involutus* et le *B. subtilis* plutôt qu'avec le *F. moniliforme* seulement. Le *S. tomentosus* n'a pas inhibé la croissance *in vitro* du *F. moniliforme* ni accru la survie des plantules de pin gris en présence de *F. moniliforme*. Ce dernier a réduit la formation d'ectomycorhizes sur le pin gris par le *P. involutus* et le

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S. tomentosus. Le nombre d'unités formatrices de colonies du *F. moniliforme* a été significativement réduit quand les plantules ont été inoculées avec le *P. involutus* ou le *B. subtilis* seul ou en combinaison. D'autre part, le *S. tomentosus* n'a pas réduit le nombre d'unités formatrices de colonies du *F. moniliforme*. La suppression de la croissance du *F. moniliforme* par le *P. involutus* et le *B. subtilis* a entraîné la production de composés antifongiques.

INTRODUCTION

Fusarium damping-off, caused by several species of *Fusarium*, is responsible for considerable losses in both container and bare root nurseries in North America (Filer and Peterson 1975; Hiratsuka 1987; Sutherland and Van Eerden 1980). The inoculum of the fungus can be carried on seed, contaminated growing media or in water. Various fungicides are used to control this disease. However, many of these fungicides are not very effective and do not protect the seedlings during their stay in the nurseries (Sinclair *et al.* 1975). In recent years, several root pathogens have been found to be resistant to many fungicides (Chakravarty and Kean, unpublished data). In addition to fungicides, proper fertility management may also reduce the incidence of damping-off. Alternative non-chemical strategies for protecting nursery seedlings from various soil pathogens have received considerable attention in the last few decades (Adams 1990; Baker and Cook 1982). Reducing residual toxicity from chemicals in the soil is also essential for maintaining the operation of forest tree nurseries environmentally acceptable.

Bacillus subtilis (Ehrenberg) Cohn, a soil-inhabiting bacterium, has been used to control diseases of field crops. The bacterium produces antifungal substances which are toxic to several plant pathogens (Hall and Davis 1990; Jordon and Tarr 1987; Krause *et al.* 1987; Krezel and Leszczynska 1978; Podile *et al.* 1985; Pusey and Wilson 1984; Pusey *et al.* 1986; Singh and Deverall 1984; Utkhede 1984; Utkhede and Sholberg 1986). Although *B. subtilis* has potential for biological control in agricultural and horticultural crops, our knowledge on the effect of *B. subtilis* on forest tree seedlings is limited.

Paxillus involutus (Batsch.) Fr. and *Suillus tomentosus* (Kauffman) Singer, Snell, & Dick, two species of ectomycor-

rhizal fungi, form abundant ectomycorrhizae with pine (*Pinus* spp.) seedlings. *P. involutus* is known to protect seedlings of red pine (*Pinus resinosa* Ait.) from damping-off pathogens by producing antifungal substances (Chakravarty *et al.* 1990, 1991; Duchesne *et al.* 1987a,b, 1988, 1989a,b). The effect of *S. tomentosus* on root pathogens of pines is not known. Marx (1972, 1973) and Zak (1964) hypothesized that root protection by ectomycorrhizal fungi may be the result of a protective barrier effect caused by the presence of a fungal mantle around the roots, nutrient competition in the rhizosphere, or production of antimicrobial substances either by the mycosymbiont or by the host plant. The effectiveness of the fungal symbiont for protecting against root diseases varies with the mycorrhizal species or isolate, host species, and soil conditions (Chakravarty and Unestam 1986, 1987a,b; Sampangi and Perrin 1985).

The objectives of this study were to investigate the interactions between *P. involutus*, *S. tomentosus*, *B. subtilis*, and *F. moniliforme* alone or in combination in the rhizosphere of jack pine (*Pinus banksiana* Lamb.) seedlings and to evaluate the mechanism of protection as hypothesized by Marx (1972, 1973) and Zak (1964).

MATERIALS AND METHODS

Organisms

Jack pine seedlings were used in this study. The root pathogen *F. moniliforme*, isolated from diseased jack pine seedlings in a greenhouse at the Northern Forestry Centre (Edmonton, Alberta, lat. 53°30' N long. 114°30' W) was used throughout the experiment. Two species of ectomycorrhizal fungi, *P. involutus* and *S. tomentosus* (isolated from fruiting bodies collected from Edmonton, and a soil-inhabiting bacterium, *B. subtilis* (isolated

from forest soil collected near Hinton, Alberta, lat. 53°23' N long. 117°32' W) were evaluated. The culture of *F. moniliforme* was maintained on potato dextrose agar (PDA), *B. subtilis* on nutrient agar (NA), and *P. involutus* and *S. tomentosus* on modified Melin Norkrans' (MMN) medium (Marx 1969).

***In vitro* antagonism**

Antagonism between *F. moniliforme* and *B. subtilis* was studied on PDA in 90-mm diam plastic Petri plates. Agar plugs (5-mm diam) with *F. moniliforme* were placed at the margin of the plate and allowed to grow at 20°C in the dark. After 3 d, *B. subtilis* was streaked on the plate opposite the *F. moniliforme* colony (4 cm apart). For the control, sterile distilled water was streaked on the plate opposite to the *F. moniliforme* colony. The plates were incubated as described above. The inhibition zone was measured from the edge of the *F. moniliforme* colony to the edge of the *B. subtilis* colony after 3 d.

For antagonism of *P. involutus* and *S. tomentosus* against *F. moniliforme*, 5-mm-diam agar plugs of *P. involutus* and *S. tomentosus* (1-wk-old cultures) were placed separately at the margin of plates containing PDA, and allowed to grow at 20°C in the dark. After 7 d, a 5-mm mycelial disk was removed from the PDA culture of *F. moniliforme* and placed on the plate opposite *P. involutus* and *S. tomentosus* (4 cm apart). For the control, 5-mm-diam sterile agar plugs were placed opposite to the *F. moniliforme* colony. The plates were incubated at 20°C in the dark. The inhibition zone was measured after 3-5 d and the mycelia of *P. involutus* and *S. tomentosus* were observed under a microscope at a contact point with *F. moniliforme*.

Effect of culture filtrates of antagonists on spore germination of *F. moniliforme*

Paxillus involutus and *S. tomentosus* were grown in liquid MMN medium at 20°C in the dark in a shaker. After 10 d, the mycelium was harvested on a Whatman No.1 filter paper and ethyl acetate was added to the filtrate to extract polar metabolites. The resulting solution was stored at 5°C in the dark. Similarly, culture filtrate of *B. subtilis* was prepared by

growing it in 50 mL nutrient broth at 20°C in the dark in a shaker. After 36 h, the broth was filtered through a filter paper (5.5 µm pore size) and the filtrate was extracted with ethyl acetate. A spore suspension of *F. moniliforme* was prepared in saline solution (0.08% NaCl). A drop of the spore suspension of *F. moniliforme* was placed on a cavity slide and 20 µL of the culture filtrate of *P. involutus*, *S. tomentosus* or *B. subtilis* was added separately on the cavity slide containing spore suspension of *F. moniliforme*. The slides were incubated in a sterile moist chamber at 20°C for 6, 12, and 24 h. Spore germination and formation of chlamydospores of *F. moniliforme* were determined by examination under a microscope. For each treatment, a total of 500 spores were counted.

Interactions of antagonists and *F. moniliforme* in the rhizosphere of jack pine seedlings

For this experiment, two systems were used to grow *P. banksiana* seedlings : a flask system and a tray system.

Flask system

Jack pine seeds were surface sterilized using 30% H₂O₂ for 30 min. Seeds were then washed 10 times with sterile distilled water and germinated in the dark on water agar. Erlenmeyer flasks (250 mL) were prepared by adding 100 mL vermiculite (pH adjusted to 5.0) and moistened with MMN liquid medium. The flasks were stoppered with a foam plug, wrapped with aluminium foil, and autoclaved for 45 min at 121°C. Ten-day-old jack pine seedlings were transferred into the flasks (one seedling per flask) under aseptic conditions. Seedlings were inoculated with *P. involutus*, *S. tomentosus*, *B. subtilis*, and *F. moniliforme*. The control treatment was not inoculated with any fungal or bacterial inoculum. The following treatments resulted : i) *P. involutus*, ii) *S. tomentosus*, iii) *B. subtilis*, iv) *F. moniliforme*, v) *P. involutus* + *F. moniliforme*, vi) *S. tomentosus* + *F. moniliforme*, vii) *B. subtilis* + *F. moniliforme*, viii) *P. involutus* + *B. subtilis* + *F. moniliforme*, ix) *S. tomentosus* + *B. subtilis* + *F. moniliforme*, and x) control. A 5-mL spore suspension (10⁵ spores mL⁻¹) of *F. moniliforme* was added using a sterile pipette to each flask except the control,

in the rhizosphere of jack pine seedlings. Control seedlings were inoculated with three 5-mm sterile MMN agar plugs and 2 mL of sterile distilled water. The flasks were kept in a growth chamber under a photoperiod of 16 h ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, provided by fluorescent lamps) at 20°C and randomized every 5 d. No fertilizer or water was added to the flasks. There were 25 seedlings per treatment. Seedlings were harvested after 10 wk. Seedling mortality, shoot and root length, total biomass, and number of mycorrhizal short roots were determined for each seedling.

Tray system

Jack pine seeds were surface sterilized using 30% H_2O_2 . Seeds were sown in a tray containing sterile vermiculite (pH 5.5) and inoculated with 5 g inoculum of *P. involutus* and *S. tomentosus* (grown in vermiculite). For the control, seeds were grown in trays containing sterile vermiculite without *P. involutus* and *S. tomentosus* inocula. The trays were kept in a growth chamber as described above. Ten-day-old seedlings were transferred to plastic trays ($18 \text{ cm} \times 45 \text{ cm} \times 15 \text{ cm}$) containing sterile moistened vermiculite. Twenty-five seedlings were planted in each tray. Two days later, seedlings were inoculated with a 24-h-old culture of *B. subtilis* by adding 1 mL cell suspension ($2 \times 10^6 \text{ cells mL}^{-1}$) to the rhizosphere of each seedling. Two days after inoculation with *B. subtilis*, seedlings were inoculated by adding 1 mL spore suspension ($10^6 \text{ spores mL}^{-1}$) of *F. moniliforme* in the rhizosphere of each seedling. The same 10 treatments as for the flask system were applied. There were 3 trays per treatment. Trays were returned to the growth chamber and randomized every 5 d. Ten weeks later, seedlings were harvested. Seedling mortality, shoot and root length, total biomass, and number of mycorrhizal short roots were determined for each seedling.

Population density of *F. moniliforme* in the rhizosphere of jack pine seedlings

The dilution plate technique was used to determine the population density of *F. moniliforme* in the rhizospheric vermiculite of jack pine seedlings. The vermiculite was diluted with sterile distilled

water and spread evenly on PDA medium. There were 25 replicates for each treatment. Plates were incubated at 20°C in the dark. Colonies of *F. moniliforme* were counted and identified after incubation for 4 d. The number of colonies per plate was multiplied by the dilution factor to obtain total concentration (no. g^{-1}) in vermiculite.

Statistical analysis

Data from all the experiments were analyzed by one-way ANOVA using SAS software (SAS Institute Inc. 1990). The individual means were compared using Scheffé's test for multiple comparison after arcsine transformation (Zar 1984).

RESULTS

In vitro antagonism

The growth of *F. moniliforme* was significantly inhibited when grown in dual culture either with *B. subtilis* or *P. involutus*. *B. subtilis* showed a greater inhibitory effect than *P. involutus* on agar plate studies (Fig. 1). The growth of *F. moniliforme* was not affected when grown in dual culture with *S. tomentosus*. In addition, inhibition zones were observed with *B. subtilis* and *P. involutus* but not with *S. tomentosus*. When observed under a microscope, mycelia of *P. involutus* and

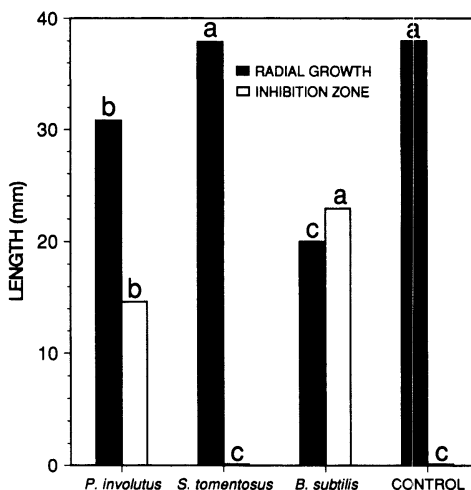


Figure 1. Inhibitory effect of *Paxillus involutus*, *Suillus tomentosus*, and *Bacillus subtilis* on radial growth and inhibition zone of *Fusarium moniliforme* on agar plates after 5 d incubation.

S. tomentosus or cells of *B. subtilis* did not penetrate the mycelia or spores of *F. moniliforme*.

Effect of culture filtrates of antagonists on spore germination and chlamydospore formation of *F. moniliforme*

The culture filtrates of *P. involutus* and *B. subtilis* significantly inhibited spore germination of *F. moniliforme*. The spore germination of *F. moniliforme* was 75% for controls whereas it was 30% when treated with culture filtrate of *P. involutus* and 41.4% when treated with culture filtrate of *B. subtilis* (Fig. 2). The spore

germination of *F. moniliforme* was not significantly reduced when treated with culture filtrate of *S. tomentosus*. The formation of chlamydospores of *F. moniliforme* was 10.6% when treated with culture filtrate of *B. subtilis* and only 4.3% when treated with culture filtrate of *P. involutus*. No chlamydospore formation was observed when treated with culture filtrate of *S. tomentosus* and non-treated controls (Fig. 2).

Effect of antagonists on jack pine seedling mortality and disease development caused by *F. moniliforme*

Flask system

Both *P. involutus* and *B. subtilis* significantly increased survival of jack pine seedlings infected with *F. moniliforme*. *F. moniliforme* alone caused 84% seedling mortality (Table 1). When co-inoculated with *P. involutus* and *B. subtilis*, mortality of the seedlings was 20 and 28%, respectively (Table 1). When both *P. involutus* and *B. subtilis* were concomitantly inoculated with *F. moniliforme*, seedling mortality was 16% (Table 1). *S. tomentosus* did not increase survival of seedlings when co-inoculated with *F. moniliforme*. Shoot height, root length, and total biomass of the seedlings were similar to the control when inoculated with *P. involutus*, *S. tomentosus* or *B. subtilis* (Table 1). Mycorrhizal formation by *P. involutus* and *S. tomentosus*

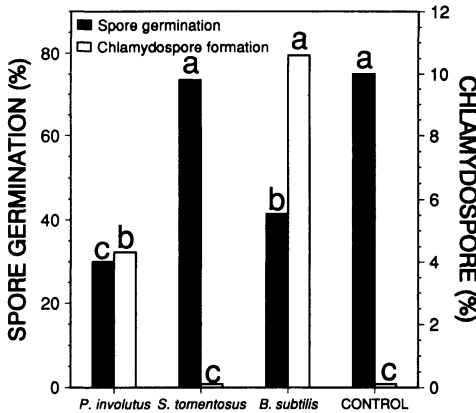


Figure 2. Effect of culture filtrates of *Paxillus involutus*, *Suillus tomentosus* and *Bacillus subtilis* on spore germination and chlamydospore formation of *Fusarium moniliforme*.

Table 1. Effect of *Paxillus involutus* (Pi), *Suillus tomentosus* (St), and *Bacillus subtilis* (Bs) on seedling mortality and disease development caused by *Fusarium moniliforme* (Fm) on jack pine grown in Erlenmeyer flasks

Treatment	Seedling mortality (%)	Shoot height (cm)	Root length (cm)	Total dry wt (mg)	No. of mycorrhizal short roots (%)
Control	0 d†	3.5 a	2.2 ab	74.0 b	0 d
Fm	84.0 a	1.0 c	0.6 c	10.3 e	0 d
Bs	0 d	3.1 ab	2.5 a	82.0 a	0 d
Pi	0 d	3.0 ab	2.4 a	85.0 a	52.2 a
St	0 d	3.2 ab	2.3 a	86.0 a	51.5 a
Bs + Fm	28.0 b	2.8 b	1.9 b	50.0 d	0 d
Pi + Fm	20.0 c	2.8 b	2.4 a	57.0 c	35.0 b
St + Fm	82.5 a	1.2 c	0.7 c	9.5 e	10.0 c
Bs + Pi + Fm	16.0 c	2.8 b	2.3 a	60.0 c	30.0 b
Bs + St + Fm	27.5 b	2.5 b	1.7 b	49.5 d	10.0 c

† Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Scheffé's test.

was significantly decreased when seedlings were co-inoculated with *F. moniliforme* (Table 1).

Tray system

As in the Erlenmeyer flasks, both *P. involutus* and *B. subtilis* significantly increased survival of seedlings infected with *F. moniliforme* (Table 2). *S. tomentosus* did not increase survival of seedlings when co-inoculated with *F. moniliforme*. Shoot height, root length, and total biomass of the seedlings were significantly higher when co-inoculated with *F. moniliforme* and *P. involutus* or *B. subtilis* than with *F. moniliforme* alone (Table 2). My-

corrhizal formation by *P. involutus* and *S. tomentosus* was significantly reduced when seedlings were co-inoculated with *F. moniliforme* (Table 2).

Population density of *F. moniliforme* in the rhizosphere of jack pine seedlings inoculated with antagonists

The colony forming units (cfu) of *F. moniliforme* were significantly reduced in both Erlenmeyer flasks and trays when inoculated with *P. involutus* or *B. subtilis* (Table 3). *S. tomentosus* did not reduce the numbers of cfu of *F. moniliforme*. However, no significant difference in

Table 2. Effect of *Paxillus involutus* (Pi), *Suillus tomentosus* (St), and *Bacillus subtilis* (Bs) on seedling mortality and disease development by *Fusarium moniliforme* (Fm) of jack pine seedlings grown in trays

Treatment	Seedling mortality (%)	Shoot height (cm)	Root length (cm)	Total dry wt (mg)	No. of mycorrhizal short roots (%)
Control	0 c†	3.5 bc	3.5 a	85.0 a	0 d
Fm	76.0 a	2.0 d	0.5 d	12.3 c	0 d
Bs	0 c	4.0 ab	2.0 c	80.0 a	0 d
Pi	0 c	4.5 a	2.6 b	86.0 a	61.0 a
St	0 c	4.0 ab	2.0 c	82.5 a	59.0 a
Bs + Fm	24.0 b	3.0 c	2.0 c	58.0 b	0 d
Pi + Fm	20.0 b	3.5 bc	2.6 b	60.0 b	30.0 b
St + Fm	75.0 a	2.2 d	0.6 d	13.0 c	10.5 c
Bs + Pi + Fm	20.0 b	3.8 bc	2.6 b	65.0 b	30.2 b
Bs + St + Fm	25.0 b	3.2 c	2.0 c	57.0 b	10.0 c

† Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Scheffé's test.

Table 3. Population density of *Fusarium moniliforme* (Fm) in the rhizosphere of jack pine seedlings inoculated with *Paxillus involutus* (Pi), *Suillus tomentosus* (St), and *Bacillus subtilis* (Bs)

Treatment	Population density of <i>Fusarium moniliforme</i> (cfu†)	
	Erlenmeyer flask	Tray system
Control	0 c§	0 c
Fm	4.0 a	4.0 a
Bs	0 c	0 c
Pi	0 c	0 c
St	0 c	0 c
Bs + Fm	2.0 b	2.1 b
Pi + Fm	2.2 b	2.4 b
St + Fm	3.9 a	3.7 a
Bs + Pi + Fm	2.2 b	2.0 b
Bs + St + Fm	2.0 b	2.2 b

† Colony forming units.

§ Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to the Scheffé's test.

numbers of cfu of *F. moniliforme* was observed when seedlings were inoculated with *P. involutus* + *B. subtilis* + *F. moniliforme* and *S. tomentosus* + *B. subtilis* + *F. moniliforme* (Table 3).

DISCUSSION

In our study, the mycelium of *P. involutus* or cells of *B. subtilis* did not penetrate the mycelium or spores of *F. moniliforme*. The formation of dormant spores was observed when *F. moniliforme* was treated with culture filtrate of *P. involutus*. When jack pine seedlings were inoculated with *P. involutus*, it was observed that not only the mantle-covered short-root tips of jack pine were protected, but also the long tap roots without a mantle were protected against *F. moniliforme*. Many short roots without a mantle were also protected, while the roots not inoculated with *P. involutus* were susceptible. Thus, the mantle barrier hypotheses cannot explain the mechanism of protection. Our *in vitro* studies suggest that *P. involutus* produced inhibitory substances in the liquid medium which was toxic to *F. moniliforme*. *Suillus tomentosus*, on the other hand, did not produce substances that are inhibitory to *F. moniliforme*.

Several authors have reported that disease suppression by ectomycorrhizal fungi is associated with plant-produced antimicrobial substances (Chakravarty and Hwang 1991; Chakravarty and Uneshtam 1986, 1987a,b; Duchesne *et al.* 1987a,b, 1988, 1989a,b; Sampangi and Perrin 1985; Sylvia and Sinclair 1983a,b). Suppression of *F. moniliforme* damping-off by *P. involutus* likely results from the production of antifungal compounds by *P. involutus* or production of antimicrobial compounds by the plant.

The number of cfu of *F. moniliforme* was significantly reduced in the rhizosphere of jack pine seedlings inoculated with *P. involutus*. This indicates that *P. involutus* produced antifungal substances in the rhizosphere of jack pine seedlings that limited the numbers of cfu of *F. moniliforme*. It has been suggested by several authors that ectomycorrhizae produce solvent-extractable volatile compounds which can inhibit several root

pathogens (Graham and Linderman 1980; Krupa and Fries 1971; Krupa and Nylund 1972; Krupa *et al.* 1973; Schisler and Linderman 1989a,b). The production of volatiles by ectomycorrhizal fungi may be deleterious to several species of *Fusarium* (Chakravarty *et al.* 1991; Schisler and Linderman 1989b; Stack and Sinclair 1975). The formation of ectomycorrhizae on jack pine seedlings was also reduced when challenged with *F. moniliforme*. This suggests that *F. moniliforme* also produced antifungal compounds in the rhizosphere of jack pine seedlings that inhibited ectomycorrhiza formation by *P. involutus*. Chakravarty *et al.* (1991) made similar observations with several root pathogenic fungi including *Fusarium* spp.

As with *P. involutus*, *B. subtilis* also significantly inhibited *in vitro* growth of *F. moniliforme* and reduced mortality of jack pine seedlings. *B. subtilis* is known to be an active antagonistic bacterium against several root pathogens (Broadbent *et al.* 1971; Gordon *et al.* 1973; Hall and Davis 1990; Pusey *et al.* 1988; Singh and Deverall 1984; Utkhede and Sholberg 1986). The bacterium protects against or prevents root pathogens by secreting antimicrobial substances including several antibiotics such as bacilysin, fengycin, bacitracin, bacillin, bulboformin, mycosubtilin, and subtilin (Loeffler *et al.* 1986; Vanittanakom *et al.* 1986). In this study, *B. subtilis* significantly inhibited the *in vitro* growth of *F. moniliforme*. The culture filtrate of *B. subtilis* strongly inhibited spore germination but stimulated chlamydospore formation of *F. moniliforme*. Similar observations were also reported by Singh and Singh (1983). Our *in vitro* study indicates that inhibition of growth of *F. moniliforme* and formation of chlamydospores was related to the production of antifungal compounds by *B. subtilis*. The bacterium also reduced seedling mortality and decreased the population density of *F. moniliforme* in the rhizosphere of jack pine seedlings.

The biological protection provided by *B. subtilis* in this study was similar to that of *P. involutus*. When *P. involutus* and *B. subtilis* were co-inoculated, the effectiveness of both these organisms, however, remained the same. *P. involutus* and *B. subtilis*, on the other hand, did not

show any inhibitory effect against each other. This suggests that these two organisms are compatible and can function in the presence of each other. In this study, *P. involutus* did not reduce the effectiveness of *B. subtilis* and vice versa. Thus *P. involutus* and *B. subtilis* are potentially compatible for biological protection against *F. moniliforme* damping-off of jack pine. In this integrated system, both *P. involutus* and *B. subtilis* could provide protection at times when environmental factors are not favourable for the activity of either *P. involutus* or *B. subtilis*. *S. tomentosus* is not considered a potential candidate for biological control of *F. moniliforme*. Further field studies are in progress to determine the effectiveness of *P. involutus* and *B. subtilis* and their interactions with other soil microorganisms, pathogens, and naturally occurring mycorrhizal fungi.

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