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Effect of three species of bacteria on damping-off, root rot development, and ectomycorrhizal colonization of lodgepole pine and white spruce seedlings

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Summary

Interactions between three species of bacteria (*Burkholderia cepacia*, *Pseudomonas chlororaphis* and *Pseudomonas fluorescens*), an ectomycorrhizal fungus (*Paxillus involutus*), and three root pathogenic fungi (*Fusarium moniliforme*, *Fusarium oxysporum* and *Rhizoctonia solani*) were studied. *Burkholderia cepacia* significantly reduced the *in vitro* mycelial growth of *P. involutus*, whereas, *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* reduced the mycelial growth of *F. moniliforme*, *F. oxysporum* and *R. solani*. Culture filtrates of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* reduced conidial germination and increased the formation of chlamydospores of *F. moniliforme* and *F. oxysporum*. *Burkholderia cepacia* also reduced the formation of ectomycorrhizal short roots by *P. involutus* on lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) seedlings 2 months after inoculation. However, no significance difference in mycorrhizal short roots was observed after 4 months. The fungicide Anchor (a mixture of carboxine and thiram) significantly reduced root rot severity and increased the survival of lodgepole pine seedlings grown in a growth mix infested with *F. moniliforme*, *F. oxysporum* and *R. solani*. Control of the diseases of white spruce caused by these pathogens was not as successful. Treatment of seeds with either *B. cepacia* or *P. involutus* alone significantly increased the survival of seedlings grown in a mix that was inoculated with *F. moniliforme* and reduced the root rot severity caused by *F. moniliforme* and *F. oxysporum*, but not *R. solani*. Higher seedling survival and lower root rot severity were observed when conifer seeds were concomitantly inoculated with one of the bacterial species, *P. involutus* and Anchor.

1 Introduction

In North American conifer nurseries, root diseases (damping-off, wilt and root rot) are responsible for seedling mortality and can cause further losses by disrupting reforestation plans (Bloomberg 1971, 1973; Filer and Peterson 1975; Sutherland et al. 1989; Hiratsuka et al. 1995). Various fungicides are used to control root diseases in conifer nurseries. However, in recent years, fungicides have become less effective due to the development of pathogen resistance (Ogawa et al. 1981). Biological control, using fungi and/or bacteria as either an alternative or a supplement to fungicides may be a viable alternative for root disease (Marx 1972, 1973; Baker 1987; Chakravarty and Unestam 1987a,b; Weller 1988; Chakravarty and Hwang 1991; Hwang and Chakravarty 1992; Hwang et al. 1995).

Paxillus involutus (Batsch.) Fr., is an ectomycorrhizal fungus known to partially protect seedlings of red pine (*Pinus resinosa* Ait.) under laboratory and greenhouse conditions (Duchesne et al. 1987, 1988a,b, 1989; Chakravarty et al. 1990, 1991). Although species of *Pseudomonas* are known to be antagonists against certain root pathogens (Kawamoto and Lorbeer 1976; Myers and Strobel 1983; Strobel and Myers 1981; Scheffer 1983; Elad

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and CHET 1987; WELLER 1988; PARKE 1990, 1991; PARKE et al. 1991; BULL et al. 1991; PENG et al. 1993; VIDHYASEKARAN and MUTHAMILAN 1995), their effects on controlling root diseases of lodgepole pine and white spruce seedlings are not known. HUBBARD et al. (1983)and BIN et al. (1991) stated that biocontrol bacteria may inhibit plant pathogens and plant-beneficial (i.e. biocontrol) fungi at the same time. FRAVEL (1988) discussed the possibility of toxic effects of antibiotic and other compounds, produced by biocontrol agents, on beneficial microorganisms. In an integrated system of biocontrol, greater success will be achieved if both bacterial and fungal biocontrol agents are compatible. Bacteria could then provide protection against specific pathogens at times when environmental conditions are not favourable for the activity of fungal biocontrol agents. Thus, the combination of biocontrol agents should give a higher level or a broader range of control than a single organism.

The first objective of this study was to investigate the effects of three species of bacteria, *Burkholderia cepacia* Burkholder, *Pseudomonas chlororaphis* (Guignard and Sauvageau) Bergey *et al.* and *Pseudomonas fluorescens* Migula on the *in vitro* growth of an ectomycorrhizal fungus *Paxillus involutus* (Batsch.) Fr. and on ectomycorrhizal colonization of lodgepole pine and white spruce seedlings. The second objective was to study the protective effect of these three species of bacteria, *P. involutus*, and a fungicide (Anchor: 6.7% each of carbathiin and thiram, Uniroyal Chemical Co. Inc., Middlebury, CT, USA) alone and in combination against three fungal root pathogens, *Fusarium moniliforme* Scheldon, *Fusarium oxysporum* Schlecht and *Rhizoctonia solani* Kühn on lodgepole pine and white spruce seedlings.

2 Material and methods

2.1 Organisms

Seeds of lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) and white spruce (*Picea glauca* [Moench] Voss), were used in this study. Potential biocontrol agents tested were the ectomycorrhizal fungus, *P. involutus* (0262), and three bacterial species, *B. cepacia* (Ral 3), *Ps. chlororaphis* (63–28), and *Ps. fluorescens* (63–49). Three fungal root pathogens were used, *F. moniliforme* (SW 06), *F. oxysporum* (UG 001) and *R. solani* (SW 09). All axenic cultures of root pathogens were maintained on potato dextrose agar (PDA), and *P. involutus* was maintained on modified Melin Norkrans' (MMN) agar medium (MARX 1969). Bacterial species were suspended in a proprietary liquid formulation (Agrium, Inc., Saskatoon, Saskatchewan, Canada) containing approximately 1×10^8 colony-forming units (cfu)/ml.

2.2 Interactions of *B. cepacia*, *Ps. chlororaphis* and *Ps. fluorescens* with *P. involutus in vitro*

Interactions of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens*, and *P. involutus* were studied on PDA medium in 90 mm Petri plates. *Paxillus involutus* was inoculated onto the PDA medium by placing 5 mm agar plugs (taken from the periphery of 2-week-old mycelial mats grown in the dark) at the centre of the plate and incubating at 20°C in the dark. Seven days later, *B. cepacia*, *Ps. chlororaphis* and *Ps. fluorescens* were streaked separately on both sides of the actively growing fungal colony. The control plates were streaked with sterile distilled water. The plates were incubated as described above. There were 20 replicates for each bacterial species. The colony diameter of *P. involutus* was measured after 7 days and the reduction in diameter was calculated by comparison with diameters from the control plates.

2.3 Interactions of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* against root pathogens

The level of antagonism of *B. cepacia*, *Ps. chlororaphis*, and *Ps. fluorescens*, to *F. moniliforme*, *F. oxysporum*, and *R. solani* was studied on PDA medium in 90 mm Petri plates. The plates

were inoculated with each pathogen by placing 5 mm mycelial plugs (taken from the periphery of 5-day-old mycelial mats grown in the dark) at the centre of the plate and incubating at 20°C in the dark. Four days later, *B. cepacia, Ps. chlororaphis,* and *Ps. fluorescens* were streaked separately on both sides of the growing colony and incubated as described above. Control plates were streaked with sterile distilled water. Antagonism between *P. involutus* and root pathogenic fungi was studied on MMN agar medium. Agar plugs (5 mm diameter) with *P. involutus* were inoculated at the margin of the plate and allowed to grow at 20°C in the dark. After 7 days, 5 mm mycelial plugs of *F. moniliforme, F. oxysporum,* and *R. solani* were placed on the plate opposite the growing colonies of *P. involutus*. There were 20 plates for each species of root pathogen. The colony diameters of the pathogens were measured after 5 days and reduction of the colony diameter was calculated by comparison with those of control plates.

2.4 Effect of *B. cepacia, Ps. chlororaphis, Ps. fluorescens* and *P. involutus* on mycelial dry weight of root pathogenic fungi

Paxillus involutus was grown in liquid MMN medium at 20°C in the dark on a shaker. After 7 days, the mycelium was harvested by filtering the culture through a Whatman No. 1 filter paper (Whatman Int. Ltd., Maidstone, UK); the culture filtrate was collected and stored at 5°C in the dark. *Burkholderia cepacia, Ps. chlororaphis* and *Ps. fluorescens* were grown in 250 ml Erlenmeyer flasks containing 100 ml of sterile nutrient broth (NB). The flasks were placed on a rotary shaker for 12 h at room temperature and culture filtrate was collected and stored at 5°C in the dark. Erlenmeyer flasks (250 ml) containing 100 ml of potato dextrose broth (PDB) were autoclaved for 20 min at 121°C. Single mycelial plugs (5 mm diameter) from colonies of *F. moniliforme, F. oxysporum,* and *R. solani* were added separately into the flasks containing PDB. The flasks were placed on a shaker and incubated at 20°C in the dark. Three days later, 5 ml of filter-sterilized culture filtrate of *P. involutus, B. cepacia, Ps. chlororaphis* and *Ps. fluorescens* were added into separate flasks containing cultures of each pathogen. Five millilitres of sterile distilled water was added to each control flask. There were 20 replicates for each combination of biocontrol agent and fungal pathogen. The flasks were placed back on the shaker for an additional 7 days. The mycelium of each pathogen was then harvested on a preweighed Whatman No. 1 filter paper, oven dried at 70°C for 48 h, and the dry weight was determined.

2.5 Effect of culture filtrates of *B. cepacia, Ps. chlororaphis, Ps. fluorescens* and *P. involutus* on conidial germination and chlamydospore formation of *Fusarium* spp.

Filter-sterilized culture filtrates of *B. cepacia, Ps. chlororaphis, Ps. fluorescens,* and *P. involutus* were collected as described above. Conidial suspensions (1×10^5 conidia/ml) of *F. moniliforme* and *F. oxysporum* were prepared in saline solution (0.08% NaCl). A drop of each conidial suspension was placed on a separate cavity slide and 20 µl of a culture filtrate was added, so that each slide had a single combination of pathogen conidia and biocontrol agent filtrate. A 20 µl drop of sterile distilled water was added to the control slides. All the slides were incubated in a moist chamber at 20°C for 48 h. Germination of conidia and formation of chlamydospores of *F. moniliforme* and *F. oxysporum* were monitored by examination under a microscope. A total of 500 conidia were counted for each treatment.

2.6 Effect of *B. cepacia, Ps. chlororaphis, Ps. fluorescens* and *P. involutus* on ectomycorrhizal colonization of lodgepole pine and white spruce seedlings

Conifer seeds were surface sterilized by soaking in 30% H_2O_2 for 30 min, and washing several times with sterile distilled water. Seeds were then coated with either *B. cepacia, Ps.*

chlororaphis or *Ps. fluorescens* in the proprietary liquid formulation at a rate of 0.3 ml (1×10^8 cfu/ml) per gram, and air dried until slightly moist. The control seeds did not receive any treatment.

Erlenmeyer flasks (250 ml) were prepared by adding 100 ml of vermiculite moistened with liquid MMN medium. The pH of the medium was then adjusted to 5.0 by addition of 1N HCl and excess liquid was allowed to drain off. The tops of the flasks were stoppered with a foam plug, and wrapped with aluminium foil, and the flasks were then autoclaved for 30 min at 121°C. When cooled, two seeds were coated with *B. cepacia*, *Ps. chlororaphis* or *Ps. fluorescens* and sown, under aseptic conditions, into separate flasks. One week after germination, the seedlings were inoculated with *P. involutus* by adding 5 ml mycelial suspension to the flask. There were 20 replicates for each treatment. The flasks were kept in a growth chamber under a photoperiod of 16 h (100 μmol/m^2 per s PAR, provided by fluorescent lamps) at 20°C and were randomized every 15 days. No fertilizer or water was added to the flasks. The seedlings were harvested and evaluated at 2 and 4 months after inoculation. Ectomycorrhizal colonization of the roots of lodgepole pine and white spruce seedlings was evaluated by counting the number of ectomycorrhizal short roots per seedling.

2.7 Effect of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens*, *P. involutus* and Anchor on damping-off and root rot disease of lodgepole pine and white spruce seedlings

Seeds of lodgepole pine and white spruce were surface sterilized, washed and coated separately with *B. cepacia*, *Ps. chlororaphis* and *Ps. fluorescens* as described above. The seeds were then coated with fungicide (Anchor) by mixing 100 g of seeds and 0.5 ml of Anchor. The control seeds did not receive any treatment. For each treatment, 30 seeds were sown in each of three replicate plastic trays (18 cm × 45 cm × 15 cm) containing a sterile mixture of peat and vermiculite (3 : 1 w/w). After sowing, the trays were inoculated with a 10 ml conidial suspension (10^5 conidia per ml) of either *F. moniliforme* or *F. oxysporum*. Treatments receiving *R. solani* were inoculated with 5 g ground grain inoculum (130 cfu/g) per tray and those receiving *P. involutus* were inoculated with 10 ml mycelial suspension per tray. The trays were then placed randomly in a growth chamber with the same temperature and photoperiod as described previously. The treatments for each conifer species–pathogen combination were as follows: non-inoculated control; inoculated control (pathogen alone); *B. cepacia* + pathogen; *Ps. chlororaphis* + pathogen; *Ps. fluorescens* + pathogen; *P. involutus* + pathogen; Anchor + pathogen; *B. cepacia* + Anchor + pathogen; *Ps. chlororaphis* + Anchor + pathogen; *Ps. fluorescens* + Anchor + pathogen; *B. cepacia* + Anchor + *P. involutus* + pathogen; *Ps. chlororaphis* + Anchor + *P. involutus* + pathogen; *Ps. fluorescens* + Anchor + *P. involutus* + pathogen. Treatments with each biological control agent and Anchor alone were also included to ensure that there was no detrimental effect on conifer seedlings, however, these data were excluded from the analyses. Four months after sowing, seedlings

Table 1. **Interactions of *B. cepacia*, *Ps. chlororaphis* and *Ps. fluorescens* against *P. involutus in vitro***

Bacterial spp.	Reduction (%) of colony diameter of *P. involutus* from control
B. cepacia	40.0 a
Ps. chlororaphis	0.0 b
Ps. fluorescens	0.0 b

Values are the means of 20 replicates. Means followed by the same letter do not differ significantly ($p = 0.05$) from each other.

survival was assessed and the severity of root rot on each surviving seedling was rated using a 0–4 scale, where 0 = no root rot and 4 = severely necrotic root system.

The growth chamber experiments were repeated once. Thus, the experiments were arranged in a two-replicate completely randomized design. Data from each conifer species–pathogen combination were analysed separately by one-way ANOVA using SAS software (SAS INSTITUTE 1990). The individual means were compared using Scheffe's test for multiple comparison. All percentage data were analysed after arcsine transformation (ZAR 1984).

3 Results

3.1 Interactions of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus in vitro*

In dual culture, *B. cepacia* significantly reduced the colony diameter of *P. involutus* by 40% compared with the control (Table 1). *Pseudomonas chlororaphis* and *Ps. fluorescens*, on the other hand, had no effect on the colony diameter of *P. involutus*.

3.2 Interactions of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens*, *P. involutus* and root pathogens

Burkholderia cepacia, *Ps. chlororaphis*, *Ps. fluorescens*, and *P. involutus* reduced the colony diameter of *F. moniliforme*, *F. oxysporum*, and *R. solani* (Table 2). The highest reduction in

Table 2. **Interactions of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* against three species of root pathogenic fungi**

Antagonists	Reduction (%) of colony diameters of root pathogens from control		
	F. moniliforme	*F. oxysporum*	*R. solani*
B. cepacia	68.0 a	75.0 a	50.0 a
Ps. chlororaphis	35.0 c	42.0 c	11.0 c
Ps. fluorescens	43.5 b	55.0 b	20.0 b
P. involutus	25.0 d	18.0 d	10.0 c

Values are the means of 20 replicates. Means in each column followed by the same letter do not differ significantly (p = 0.05) from each other.

Table 3. **Effects of culture filtrates of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* on mycelial dry weight of *F. moniliforme*, *F. oxysporum* and *R. solani***

Antagonists	Mycelial dry wt. (mg)		
	F. moniliforme	*F. oxysporum*	*R. solani*
Control	200 a	182 a	262 a
B. cepacia	122 b	120 c	120 c
Ps. chlororaphis	151 b	161 ab	260 a
Ps. fluorescens	150 b	150 abc	180 b
P. involutus	120 b	122 bc	178 bc

Values are the means of 20 replicates. Means in each column followed by the same letter do not differ significantly (p = 0.05) from each other.

growth of these pathogens (50–75%) was observed when they were challenged with *B. cepacia*. *Paxillus involutus* had the least effect on colony diameter.

3.3 Effects of culture filtrates of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens* and *P. involutus* on mycelial dry weight of three species of root pathogens

Only the culture filtrates of *B. cepacia* and *P. involutus* significantly reduced the mycelial dry weights of all three pathogens (Table 3). *Pseudomonas fluorescens* reduced the mycelial dry weight of *F. moniliforme* and *R. solani*, whereas *Ps. chlororaphis* reduced the mycelial dry weight of *F. moniliforme* alone.

Table 4. **Effects of culture filtrates of *B. cepacia*, *Ps. chlororaphis*, *Ps. fluorescens*, and *P. involutus* on conidial germination and chlamydospore formation of *F. moniliforme* and *F. oxysporum***

	F. moniliforme		*F. oxysporum*	
Antagonists	Spore[1] germination	Chlamydospore formation	Spore[1] germination	Chlamydospore formation
Control	95.0 a	0.0 c	91.0 a	0.0 c
B. cepacia	35.0 c	23.0 b	29.5 d	30.0 a
Ps. chlororaphis	50.0 b	23.5 b	45.0 c	16.5 b
Ps. fluorescens	48.0 b	33.0 a	52.0 b	15.0 b
P. involutus	35.0 c	25.0 b	30.0 d	30.0 a

[1] Values are the means of 500 conidia. Values in each column followed by the same letter do not differ significantly ($p = 0.05$) from each other.

Table 5. **Effect of *B. cepacia*, *Ps. chlororaphis*, and *Ps. fluorescens* on ectomycorrhizal colonization of lodgepole pine and white spruce seedlings**

	Ectomycorrhizal short roots by *P. involutus* (%)			
	Experiment no. 1		Experiment no. 2	
Bacterial species	Lodgepole pine	White spruce	Lodgepole pine	White spruce
Two months after inoculation				
Control	76.0 a	70.0 a	77.5 a	68.0 ab
B. cepacia	37.0 b	45.0 b	35.0 b	40.0 b
Ps. chlororaphis	75.5 a	70.5 a	79.0 a	70.0 a
Ps. fluorescens	76.0 a	68.5 a	78.0 a	68.0 a
Four months after inoculation				
Control	87.5 a	75.0 a	85.5 a	76.0 a
B. cepacia	88.0 a	77.0 a	87.0 a	77.5 a
Ps. chlororaphis	88.5 a	75.5 a	86.0 a	78.0 a
Ps. fluorescens	88.0 a	77.0 a	87.0 a	77.0 a

Values are the means of 20 seedlings. Means in each column followed by the same letter do not differ significantly ($p = 0.05$) from each other.

3.4 Effect of culture filtrate of *B. cepacia, Ps. chlororaphis, Ps. fluorescens* and *P. involutus* on conidial germination and chlamydospore formation of *F. moniliforme* and *F. oxysporum*

Germination of conidia of *F. moniliforme* and *F. oxysporum* was significantly reduced when treated with culture filtrates of *B. cepacia, Ps. chlororaphis, Pseudomonas fluorescens*, or *P. involutus* (Table 4). The highest reduction in spore germination was observed following treatment with culture filtrates of either *B. cepacia* or *P. involutus*. An increase in chlamydospore formation by both fungal pathogens was also observed.

3.5 Effect of *B. cepacia, Ps. chlororaphis* and *Ps. fluorescens* on ectomycorrhizal colonization of lodgepole pine and white spruce seedlings by *P. involutus*

Two months after inoculation, *B. cepacia* significantly reduced the formation of ectomycorrhizal short roots on lodgepole pine and white spruce seedlings. *Pseudomonas chlororaphis* and *Ps. fluorescens* had no effect on ectomycorrhizal colonization of either conifer species (Table 5). Four months after inoculation, none of the three bacterial species had an inhibitory effect on the formation of ectomycorrhizal short roots.

3.6 Effect of *B. cepacia, Ps. chlororaphis, Ps. fluorescens, P. involutus* and Anchor on damping-off and root rot disease of lodgepole pine and white spruce seedlings

Fusarium moniliforme, F. oxysporum, and *R. solani* significantly reduced seedling survival and resulted in severe root rotting of lodgepole pine and white spruce seedlings 4 months after inoculation. None of the three bacterial species, *P. involutus*, or Anchor had any adverse effects on either the lodgepople pine or white spruce seedlings (data not presented). Anchor alone significantly increased the survival of lodgepole pine seedlings grown in a

Table 6. **Effects of *B. cepacia, Ps. chlororaphis, Ps. fluorescens, P. involutus*, and Anchor on seedling survival and root rot severity on lodgepole pine and white spruce caused by *Fusarium moniliforme* 4 months after inoculation**

Treatment	Lodgepole pine		White spruce	
	Seedling survival (%)	Root rot severity[1]	Seedling survival (%)	Root rot severity[1]
Non-inoculated control	97 a	0.0 e	100 a	0.0 e
Inoculated control (Fm)	13 d	3.6 a	24 e	3.4 a
Bc + Fm	58 c	1.5 cd	49 cd	1.5 c
Pc + Fm	12 d	3.6 a	35 de	3.5 a
Pf + Fm	12 d	3.6 a	34 de	3.5 a
Pi + Fm	86 b	0.3 e	83 b	0.6 d
Anchor + Fm	67 c	1.7 c	61 c	1.7 c
Bc + Anchor + Fm	66 c	0.9 d	56 cd	0.5 d
Pc + Anchor + Fm	49 c	2.4 d	44 cde	2.6 b
Pf + Anchor + Fm	56 c	1.5 cd	50 cd	1.6 c
Bc + Anchor + Pi + Fm	95 ab	0.0 e	83 b	0.6 d
Pc + Anchor + Pi + Fm	92 ab	0.2 e	84 b	0.2 de
Pf + Anchor + Pi + Fm	91 ab	0.2 e	84 b	0.2 de

[1] 0 = no root rot symptoms; 4 = severely necrotic root. Values are the means of survived seedlings. Means followed by the same letters in columns do not differ significantly (p = 0.05) from each other. Bc, *B. cepacia*; Pc, *Ps. chlororaphis*; Pf, *Ps. fluorescens*; Pi, *P. involutus*; Fm, *F. moniliforme*.

Table 7. **Effects of *B. cepacia, Ps. chlororaphis, Ps. fluorescens, P. involutus*, and Anchor on seedling survival and root rot severity on lodgepole pine and white spruce caused by *Fusarium oxysporum* 4 months after inoculation**

Treatment	Lodgepole pine		White spruce	
	Seedling survival (%)	Root rot severity[1]	Seedling survival (%)	Root rot severity[1]
Non-inoculated control	97 a	0.0 e	100 a	0.0 e
Inoculated (Fo)	14 d	3.7 a	24 e	3.5 a
Bc + Fo	50 b	1.2 c	49 e	1.6 c
Pc + Fo	16 c	3.4 a	33 e	3.5 a
Pf + Fo	12 d	3.6 a	34 e	3.4 a
Pi + Fo	91 a	0.4 de	84 bc	0.5 d
Anchor + Fo	49 bc	1.5 c	51 de	1.7 c
Bc + Anchor + Fo	56 b	1.0 cd	57 cde	0.6 d
Pc + Anchor + Fo	43 bc	2.5 b	50 e	2.6 b
Pf + Anchor + Fo	56 b	1.6 c	50 e	1.5 c
Bc + Anchor + Pi + Fo	94 a	0.0 e	85 bc	0.2 de
Pc + Anchor + Pi + Fo	91 a	0.2 de	90 ab	0.2 de
Pf + Anchor + Pi + Fo	92 a	0.2 de	84 bcd	0.2 de

[1] 0 = no root rot symptoms; 4 = severely necrotic root. Values are the means of survived seedlings. Means followed by the same letters in columns do not differ significantly (p = 0.05) from each other. Bc, *B. cepacia*; Pc, *Ps. chlororaphis*; Pf, *Ps. fluorescens*; Pi, *P. involutus*; Fo, *F. oxysporum*.

Table 8. **Effects of *B. cepacia, Ps. chlororaphis, Ps. fluorescens, P. involutus*, and Anchor on seedling survival and root rot severity on lodgepole pine and white spruce caused by *Rhizoctonia solani* 4 months after inoculation**

Treatment	Lodgepole pine		White spruce	
	Seedling survival (%)	Root rot severity[1]	Seedling survival (%)	Root rot severity[1]
Non-inoculated control	97 a	0.0 c	100 a	0.0 b
Inoculated control (Rs)	22 c	3.5 a	34 b	3.4 a
Bc + Rs	26 c	3.4 a	34 b	3.4 a
Pc + Rs	25 c	3.5 a	34 b	3.4 a
Pf + Rs	21 c	3.5 a	33 b	3.5 a
Pi + Rs	23 c	3.5 a	33 b	3.4 a
Anchor + Rs	59 b	1.5 b	51 b	1.5 ab
Bc + Anchor + Rs	59 b	1.5 b	55 b	1.0 b
Pc + Anchor + Rs	59 b	1.6 b	54 b	1.0 b
Pf + Anchor + Rs	59 b	1.5 b	58 b	0.5 b
Bc + Anchor + Pi + Rs	59 b	1.5 b	54 b	1.0 b
Pc + Anchor + Pi + Rs	60 b	1.6 b	55 b	1.0 b
Pf + Anchor + Pi + Rs	60 b	1.5 b	55 b	1.0 b

[1] 0 = no root rot symptoms; 4 = severely necrotic root. Values are the means of survived seedlings. Means followed by the same letters in columns do not differ significantly (p = 0.05) from each other. Bc, *B. cepacia*; Pc, *Ps. chlororaphis*; Pf, *Ps. fluorescens*; Pi, *P. involutus*; Rs, *R. solani*.

mix that was infested with *F. moniliforme, F. oxysporum*, or *R. solani*, and white spruce seedlings in a mix that was infested with *F. moniliforme* (Tables 6, 7, 8). Anchor also reduced root rot severity on both conifer species infected with either Fusarium pathogen. The ectomycorrhizal fungus, *P. involutus*, gave the highest degree of protection among all the biocontrol agents tested. *Paxillus involutus* significantly increased seedling survival and reduced root rot severity caused by the two Fusarium pathogens, but not *R. solani*. Seed treatment with *B. cepacia* had a similar effect although the reduction in root rot severity on white spruce that was infected with *F. oxysporum* was not significant. *Pseudomonas chlororaphis* and *Ps. fluorescens* alone had no effect on seedling survival or the root rot severity caused by the three pathogens. In some cases, particularly for *B. cepacia*, the effectiveness of the bacterial control agents was increased when used in combination with the fungicide Anchor. The highest seedling survival and lowest root rot severity were observed when seed was concomitantly inoculated with a bacterial agent, *P. involutus* and Anchor.

4 Discussion

Biological control of soil-borne plant pathogens by fungi and bacteria has been suggested by several authors (BAKER 1987; WELLER 1988). Since these beneficial microorganisms grow in the rhizosphere, they can provide a front-line defence for roots against attack by root pathogens. Antagonistic microorganisms are often applied to seeds before planting and colonize the rhizosphere as seedling roots develop. Root pathogens must encounter these antagonistic microorganisms in the rhizosphere before and during primary infection and subsequent disease development.

Several genera of bacteria and ectomycorrhizal fungi have been shown to have potential for the biological control of plant pathogens. In most cases, the mechanisms of pathogen suppression by bacteria are related to the production of antibiotics or antifungal substances, induced resistance, and competition between the bacteria and plant pathogens for nutrients (WELLER 1988). It is also possible that more than one mechanism may operate to suppress a pathogen and a particular mechanism may vary with the physical, biological, and chemical conditions in the rhizosphere (WELLER 1988). The genus *Pseudomonas* has been shown to have potential for biological control of root pathogens (WELLER 1988). Primarily, the establishment of *Pseudomonas* in the rhizosphere of plants is due to an improved capacity to compete for root exudates. WELLER (1983, 1984) and GAMLIEL and KATAN (1992) stated that disease suppression by the introduced bacteria depends on their ability to colonize roots and rhizospheres. Root protection by ectomycorrhizal fungi is postulated to be the result of a barrier effect caused by the presence of a fungal mantle around roots, nutrient competition in the rhizosphere, and antimicrobial substances produced either by the ectomycorrhizal fungus or by the host plants (MARX 1972, 1973). According to SCHISLER and LINDERMAN (1987), all these factors may act simultaneously or synergistically to suppress disease.

For successful biological control of root pathogens, the system must be compatible with other beneficial rhizospheric microorganisms. In the present study, *Ps. chlororaphis* and *Ps. fluorescens* did not reduce *in vitro* mycelial growth or ectomycorrhizal colonization by *P. involutus* on lodgepole pine and white spruce seedlings 2 and 4 months after inoculation. On the other hand, *B. cepacia* inhibited *in vitro* mycelial growth of *P. involutus* and ectomycorrhizal colonization 2 months after inoculation. However, this adverse effect was short-lived as there was no significant difference in ectomycorrhizal short roots compared with the control seedlings 4 months after inoculation. This suggests that *B. cepacia* has no long-term inhibitory effect on *P. involutus*. GARBAYE et al. (1990) suggested that some pseudomonads might help the growth of young mycelia of *Laccaria laccata* by excreting organic acids and, thereby, providing a carbon source until the mycorrhizal association is established. DANELL et al. (1993), reported a reduction in the mycelial growth of *Cantharellus cibarius* Fr.: Fr. when cocultured with *Ps. fluorescens*, but the study was restricted to *in vitro* growth and did not examine the effect on ectomycorrhizal colonization of root systems.

Species of *Fusarium* form dormant chlamydospores when environmental conditions are not favourable or when exposed to toxic substances. In the present study, both *F. moniliforme* and *F. oxysporum* formed chlamydospores when treated with culture filtrates of *P. involutus, B. cepacia, Ps. chlororaphis* and *Ps. fluorescens*. These culture filtrates also significantly reduced the germination of conidia of *F. moniliforme* and *F. oxysporum*. The present *in vitro* studies suggest that *P. involutus* and the three bacterial species produced inhibitory substances in liquid medium which were toxic to *F. moniliforme* and *F. oxysporum*. *Paxillus involutus* is known to inhibit *Fusarium* spp. and give partial protection of pine seedlings against damping-off fungi by producing antifungal compounds (DUCHESNE et al. 1987, 1988a,b, 1989; CHAKRAVARTY et al. 1990, 1991; HWANG et al. 1995) and by inducing the seedling roots to produce their own antifungal compounds (DUCHESNE et al. 1987; CHAKRAVARTY et al. 1991). Interestingly, the bacterial control agents in this study also reduced the colony diameter of pathogens grown on a solid agar medium, whereas *P. involutus* did not. It appears that the inhibitory substances produced by the bacteria effectively diffuse through the agar medium and that those of *P. involutus* may not.

The use of fungicides to control root pathogens in forest nurseries is a common practice. In Canadian forest nurseries, conifer seedlings are treated with a variety of fungicides during the growing season (WALL and CARMIER 1976). In the present study, Anchor was effective in controlling *F. moniliforme*, and *F. oxysporum* by increasing seedling survival and decreasing root rot severity. *Paxillus involutus* and *B. cepacia* also increased survival and decreased root rot severity of lodgepole pine and white spruce seedlings grown in a growth mix that was infested with *F. moniliforme*, or *F. oxysporum*. Although *P. involutus* and *B. cepacia* reduced the *in vitro* growth of *R. solani*, seedling survival and root rot severity was not improved. However, when *P. involutus* was coinoculated with Anchor and with either *B. cepacia, Ps. chlororaphis*, or *Ps. fluorescens* the survival of seedlings was improved and root rot severity was decreased compared with treatment with Anchor alone. These results suggests that these three bacterial species can function simultaneously with a seed treatment fungicide and with *P. involutus* against these three root pathogens in the rhizosphere of lodgepole pine and white spruce seedlings.

ELAD et al. (1980), SCHROTH and HANCOCK (1981), and CHAKRAVARTY et al. (1990) stated that synergistic phenomena involved in integrated control using pesticides and biological control agents may result in a more efficient and longer lasting control than that achieved through pesticides or biological control agents alone. In the present study, *P. involutus* alone often gave better protection to seedlings than the bacterial species or Anchor alone. However, the highest level of disease control was obtained when either *B. cepacia, Ps. chlororaphis*, or *Ps. fluorescens* was combined with *P. involutus* and Anchor. Additional field studies are necessary to determine the interactions of these species of bacteria and *P. involutus* and other effective fungicides for the integrated control of *Fusarium* and *Rhizoctonia* root rot of lodgepole pine and white spruce seedlings and to determine the economic benefit of the integrated system.

Résumé

Effet de trois espèces de bactéries sur la fonte, la pourriture des racines et la colonisation mycorhizienne de semis de Pinus contorta et de Picea glauca

On a étudié les interactions entre trois espèces de bactéries (*Burkholderia cepacia, Pseudomonas chlororaphis, Pseudomonas fluorescens*), un champignon mycorhizien (*Paxillus involutus*) et trois champignons parasites des racines (*Fusarium moniliforme, Fusarium oxysporum, Rhizoctonia solani*). *Burkholderia cepacia* réduisait significativement la croissance mycélienne *in vitro* de *P. involutus* alors que *B. cepacia, Ps. chlororaphis, Ps. fluorescens* et *P. involutus* réduisaient celle de *F. moniliforme, F. oxysporum* et *R. solani*. Des filtrats de culture de *B. cepacia, Ps. chlororaphis, Ps. fluorescens* et *P. involutus* réduisaient la germination des conidies et augmentaient la formation de chlamydospores de *F. moniliforme* et *F. oxysporum*. *Burkholderia cepacia* réduisait aussi la formation des racines courtes mycorhizées par *P. involutus* chez des semis de *Pinus contorta* et *Picea glauca*, 2 mois après l'inoculation. Cependant, aucune différence significative de racines courtes mycorhizées n'a été observée après 4 mois. Le fongicide Anchor (mélange de carboxine et de thirame) réduisait significativement la sévérité de la pourriture des racines et augmentait la survie des semis de *P. contorta* élevés sur un mélange infecté par *F. moniliforme, F. oxysporum* et *R. solani*. La lutte contre les maladies de *P. glauca* causée

par ces parasites n'a pas été un succès. Le traitement des semences avec *B. cepacia* ou *P. involutus* séparément augmentait significativement la survie des semis élevés sur substrat inoculé par *F. moniliforme* et réduisait la pourriture racinaire causée par *F. moniliforme* et *F. oxysporum*, mais pas par *R. solani*. Une survie plus élevée des semis et une pourriture racinaire plus faible ont été observées quand les graines de résineux étaient traitées au même moment avec une des espèces de bactéries, *P. involutus* et Anchor.

Zusammenfassung

Wirkung von drei Bakterienarten auf Umfallkrankheit, Wurzelfäule und Mykorrhizierung von Pinus contorta und Picea glauca

Es wurden Wechselwirkungen zwischen 3 Bakterienarten (*Burkholderia cepacia, Pseudomonas chlororaphis, Pseudomonas fluorescens*), einem Ektomykorrhiza-Pilz (*Paxillus involutus*) und 3 pathogenen Wurzelpilzen (*Fusarium moniliforme, Fusarium oxysporum* und *Rhizoctonia solani*) untersucht. *Burkholderia cepacia* reduzierte das Myzelwachstum von *P. involutus in vitro* signifikant. Das Myzelwachstum von *F. moniliforme, F. oxysporum* und *R. solani* wurde durch *B. cepacia, Ps. chlororaphis, Ps. fluorescens* und *P. involutus* gehemmt. Kulturfiltrate von *B. cepacia, Ps. chlororaphis, Ps. fluorescens* und *P. involutus* reduzierten die Konidienkeimung und förderten die Bildung von Chlamydosporen bei *F. moniliforme* und *F. oxysporum*. *Burkholderia cepacia* verminderte auch die Bildung von Mykorrhizen durch *P. involutus* an Sämlingen von *Pinus contorta* und *Picea glauca* 2 Monate nach der Inokulation. Nach 4 Monaten waren jedoch keine signifikanten Unterschiede in der Mykorrhizierung mehr feststellbar. Das Fungizid Anchor (ein Gemisch aus Carboxin und Thiram) reduzierte die Wurzelfäule signifikant und erhöhte die Überlebensrate von *P. contorta*-Sämlingen, die in einem mit *F. moniliforme, F. oxysporum* und *R. solani* infizierten Substrat kultiviert wurden. Bei *Picea glauca* war die Wirkung weniger ausgeprägt. Die Behandlung von Samen mit *B. cepacia* oder *P. involutus* alleine erhöhte die Überlebensrate von Sämlingen signifikant, die in einem Substrat kultiviert wurden, das mit *F. moniliforme* inokuliert worden war. Ausserden reduzierte diese Behandlung Wurzelfäulen, die von *F. moniliforme* und *F. oxysporum* verursacht wurden; auf *R. solani* hatte sie jedoch keinen Einfluss. Höhere Überlebensraten und eine geringere Wurzelfäule wurden nach Inokulation der Samen mit einem Gemisch aus einer der Bakterienarten, *P. involutus* und Anchor beobachtet.

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