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Alternative Biological and Biorational Control of Botrytis Gray Mold in Containerized Confer Stock

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NODA/NORTHERN FORESTRY PROGRAM

FINAL REPORT

Alternative Biological and Biorational Control of Botrytis Gray Mold in Containerized Conifer Stock

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Abstract

Effective methods of biological and biorational control of gray mold, caused by *Botrytis cinerea*, were developed as alternatives to fungicide treatments in container-grown seedlings of black spruce and red pine. *Gliocladium roseum* was highly effective for biocontrol, and potassium carbonate, sodium carbonate, and sodium bicarbonate were the most effective biorational compounds. Freezing temperatures and low light intensity predisposed black spruce seedlings to infection by *B. cinerea*. Reduced seedling density suppressed disease progress. Quantitative models developed of environmental predisposition to infection and of biological control of *B. cinerea* have applications in optimizing disease management in seedling production systems.

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Introduction

Gray mold, caused by *Botrytis cinerea* Pers.: Fr., is a destructive disease of container-grown seedlings of black spruce (*Picea mariana* (Mill.) B.S.P.), red pine (*Pinus resinosa* Ait.) and other conifers in greenhouses in northern Ontario and elsewhere (Greifenhagen et al. 1990, Mittal et al. 1987). In conifer production systems in northern Ontario, gray mold frequently develops on the lower needles and stem portions of seedlings after the canopies become dense or close, and after the containers are transferred outdoors from greenhouses in the autumn. The disease can also become severe in subsequent cold storage and after the seedlings are outplanted. Affected tissues typically are tan or brown, and are often covered with grayish mycelium and conidiophores of *B. cinerea*. Needles, branches, and stems of affected seedlings are often killed. Because of costs of growing and outplanting seedlings, weakening and killing of seedlings by *B. cinerea* in the nursery and after outplanting can result in substantial economic losses. Control practices such as measures to lower humidity and increase light intensity in the greenhouses, careful fertilizer use, and good sanitation help to suppress the disease, but fungicide programs continue to be the mainstay of control (Glover et al. 1987; Greifenhagen 1989).

The heavy dependency on chemical fungicides to control gray mold has several serious disadvantages. A disadvantage from the disease management standpoint is that *B. cinerea* often develops resistance to the fungicides such that the disease is no longer controlled effectively (Bollen and Scholten 1971; Cooley 1981; James 1983; James et al. 1983; Barak and Edgington 1984; Northover and Matteoni 1986; Chiba and Northover 1988; Staub 1991). Resistance of the pathogen to benomyl and iprodione is suspected in several container nurseries in northern Ontario in which fungicide programs have failed to provide adequate control of gray mold (J. Juzwik, personal communication). Another disadvantage is that heavy use of fungicides in enclosed environments of

production greenhouses can result in high occupational exposure of greenhouse workers to fungicides, and residues of fungicides on the seedling foliage and in the greenhouse environment (Archibald 1993; Jarvis 1992). Tree planters are increasingly reluctant to handle fungicide-treated stock.

Reduced dependency on fungicides might be possible through use of biological control agents, applications of biorational compounds, and application of epidemiological information on gray mold to avoid environmental predisposition to disease and to optimize the timing of control treatments. From the effective biocontrol of *B. cinerea* developed in recent studies in strawberry, raspberry, and grape, biocontrol of the pathogen may be feasible also in conifer seedlings (Dubos 1987; Peng and Sutton 1991; Sutton and Peng 1993a,b; Sutton 1995). Control of *B. cinerea* using chemicals that sometimes are loosely grouped in the category of biorational substances has received only minor attention (Punja and Grogan 1982). However, several workers have reported that sodium bicarbonate, soluble silicon, and other biorational compounds suppress powdery mildews and other foliage diseases in several kinds of hosts (Volk et al. 1958; Homma et al. 1981; Leusch and Buchenauer 1989; Menzies et al. 1991; Horst et al. 1992; Elad and Volpin 1988,1993; Northover and Schneider 1993). Recent observations that high temperature and drought predispose black spruce seedlings to infection by *B. cinerea* has raised the possibility that other environmental stresses may predispose seedlings and promote gray mold in nurseries in northern Ontario (Zhang and Sutton 1994a).

In the present study, biological control of *B. cinerea* was investigated in container-grown seedlings of black spruce and red pine, and biorational control of *B. cinerea* was investigated on an agar medium and in black spruce seedlings. Low light intensity and freezing temperatures were investigated as potential factors that predispose black spruce seedlings to attack by *B. cinerea*, and seedling density was explored in relation to rates of progress of gray mold epidemics. A majority of the studies were quantitative, and in many

instances relationships of independent and dependent variables were described using mathematical models. Application of the findings in integrated management of gray mold in container-grown conifer seedlings is discussed.

Materials and Methods

M1. General procedures

M1-1. Production of seedlings of black spruce and red pine

Seeds were soaked in 5% hydrogen peroxide for 15 min immediately prior to sowing. To produce seedlings for biocontrol tests in the growth room, treated seeds were sown in a peat-vermiculite mix (3:1, v:v) in conifer seedling flats (Super Stubby Leach Tubes, Stuewe and Sons Inc., Corvallis OR) with one seed per tube in each flat. Seedlings for tests in the greenhouse were grown in a 2 : 1 (v:v) mix of peat and vermiculite in size 308 (for black spruce) and size 408 (for red pine) paper seedling flats (Lännen Tehtaat Oy, Finland) with one seedling per cell of each flat. All seedlings were grown on a bench in a greenhouse maintained at 20 - 35 °C and with the photoperiod extended to 16 h by means of high pressure sodium lamps that were positioned about 1.5 m above the bench and provided a minimum light intensity of 300 µmol $m^{-2}s^{-1}$. The seedlings were supplied once each week with soluble 20-20-20 (N-P-K) fertilizer containing microelements (Plant Products Ltd., Brampton ON) at 150 ppm in water.

M1-2. Production of inoculum of Botrytis cinerea

An isolate of *B. cinerea* (ZP-90-54) obtained from a black spruce seedling in Sault Ste. Marie in 1990, and a second isolate (SG224) from the Thessalon Tree Nursery, Ontario in 1993 were used in the tests. Isolate ZP-90-54 was moderately aggressive in conifer seedlings, and isolate SG224 was highly aggressive. The isolates were maintained and inocula were produced on potato dextrose agar (PDA) medium and on a strawberry agar medium (Zhang and Sutton 1994a). Conidia were recovered by flooding the cultures with sterile-distilled water plus surfactant (0.1 ml Triton X-100/100 mL water), rubbing the sporulating colony with a glass rod, and filtering the spore suspensions through four layers of cheese cloth. Spore concentration was estimated with

the aid of a haemacytometer and diluted to desired levels. Percent germination of spores used as an inoculum was estimated on PDA and consistently exceeded 98%.

M1-3. Collection and inoculum production of biocontrol organisms

Mycelial fungi, yeasts, and bacteria were isolated from the foliage of black spruce and other conifers at eight sites in Wellington County in southern Ontario in April 1990, and at eight sites near the Trans-Canada Highway between Espanola and Dryden in northern Ontario in June, July, August, and October 1990. One hundred and twelve of the isolates from spruce, plus twenty four fungal isolates from strawberry and wheat, were evaluated in preliminary studies in the growth room for effectiveness in suppressing *B. cinerea* in black spruce seedlings (Zhang et al. 1994d). The following biocontrol organisms were selected in the preliminary screening for further study: *Gliocladium roseum* Link: Bainier (AL710, from strawberry); *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Stendel. (ZP2005, from a black spruce tree), *Fusarium* sp. (ZP2009, from a black spruce seedling), and *Penicillium* sp. (ZP2047, from a black spruce seedling). These fungi were maintained on PDA at 2-3 C.

Conidia of the biocontrol agents for use as inoculum in small-scale studies in the growth chamber and growth room were produced by culturing the fungi on PDA in plastic petri dishes beneath cool-white fluorescent lamps (14-h photoperiod) at 20-23 C for 20-25 days. Conidia were recovered from colonies on agar by the same methods used for *B. cinerea* described in Section M1-2. Percent germination of spores of the biocontrol agents used as inocula was estimated on PDA and consistently exceeded 96%.

Conidia for use in larger scale studies in the greenhouse were produced on wheat seeds (Sutton and Yu 1994). Wheat grain was immersed in boiling water

for 20 min, strained, dispensed in 500 mL Mason Jars (100-150 g grain /jar), autoclaved at 121 C and 104 kPa for 60 min, then cooled. The sterilized grain was inoculated with conidial suspensions of the biocontrol agents and kept in the laboratory. Caps of the jars were loosened for a few seconds every day to allow air exchange. The jars were shaken manually every 1-2 days to prevent "matting" of the mycelium. After 14-18 days the metal lid inserts of the Mason jars were replaced with sterilized disks of photocopy paper, which allowed the cultures to dry slowly, and favored heavy spore production. At about 30 days the infested grain was immersed in water plus surfactant (about 30-50 g grain/L), vigorously shaken for 10 min., and the suspension was filtered though 3 layers of cheese cloth. Conidial concentrations were estimated withe the aid of hemacytometers and diluted as required.

M1-4. Inoculations

In studies in growth rooms and growth chambers inocula of *B. cinerea* and of the biocontrol agents were applied to incipient run-off on the seedling foliage using air-pressurized sprayers of 200 mL capacity (Home Hardware Stores, St. Jacobs, ON). Inoculated seedlings were immediately placed in a plastic humidity chamber (2.0 m long, 0.5 m wide, 0.5 m high) positioned on a bench in a growth room for 36 h. Relative humidity (RH) in the chamber was maintained near 100% by intermittent operation of an ultrasonic humidifier. The growth room was operated on a 12-h photoperiod (light intensity at plant height near 200 $\mu E\ m^{-2}s^{-1}$), at RH of 60 - 70%, and at 20 ± 1 °C. A sequence of 12 h light, 12 h dark, and 12 h light was followed during the humid period. After the humid period, seedlings were kept on the growth room bench. Temperature and RH in the humidity chamber and growth room were monitored by means of calibrated hygrothermographs (Lambrecht, model 252, Göttingen, Germany).

In greenhouse studies, inocula were applied to incipient runoff on seedling

foliage using a compressed-air sprayer of 4 L capacity and equipped with a single nozzle. Seedlings were inoculated at particular times of day as indicated in methods of specific experiments outlined below. Unless otherwise indicated, the seedlings received no special treatment to provide postinoculation humid periods.

M1-5. Estimation of infection

Infection and colonization of seedling needles by *B. cinerea* were quantified indirectly by estimating sporulation incidence and spore production of the pathogen. In studies conducted in growth rooms and growth chambers, seedling shoots were cut off at the root collar, surface sterilized by immersion in 70% ethanol for 10 s and in 0.3% sodium hypochlorite (5% Javex) for 60 s, and rinsed three times in sterile distilled water. Fifteen needles were removed at equidistant intervals between the base and apex of each seedling. A total of 30 needles from two seedlings constituted a sampling unit, and a 6-mm segment was cut from near the centre of each needle. Segments of each sampling unit were placed on water agar in one 9-cm diameter petri dish. The dishes were incubated beneath cool-white fluorescent tubes (12-h photoperiod) at 20 °C for 8 days after which the segments were examined on a dissecting microscope and incidence of sporulation of *B. cinerea* was recorded. In some studies, conidia were recovered in water containing 0.1% Triton X-100 (8 mL per dish), counted on a haemacytometer, and the number produced per segment was estimated. Sporulation incidence and numbers of spores were estimated for three replicate sampling units per treatment.

In greenhouse studies, twenty seedlings were taken arbitrarily from each replicate flat or half-flat of seedlings (or as otherwise indicated in description of specific experiments if the treatment unit was different) of each treatment and examined with a large (20 cm diameter) hand lens. Sporulation incidence of *B. cinerea* on the seedlings was recorded, and

severity of gray mold was assessed based on the estimated percent length of the shoot of each seedling on which sporulation of the pathogen was evident.

M1-6. Estimation of electrolyte leakage

Electrolyte leakage from foliage of seedlings was estimated as an indication of stress induced by environmental condition such as high temperature, freezing, drought, and low light intensity. The method used was modified from that of Colombo (1990). Electrolyte leakage was estimated for one seedling from each treatment replicate. The apical 2-3 cm of the shoot of each seedling was removed, weighed, placed in a 45 mL screw-capped bottle containing 25 mL deionized water and kept at 22 C in darkness. After 24 hr, the bottles were shaken vigorously by hand and the electrical conductivity of the water in each bottle containing a live shoot (EC_{live}) was measured using a conductivity meter (model CDM3, Back-Simpson Limited, London, Ont. N6A 4L6).

The shoots were then killed by immersing the bottles in boiling water for 10 min. Electrical conductivity of the water in each bottle (EC_{killed}) was measured again after the bottles were kept for a further 16-24 hr and manually shaken. Percent leakage of electrolytes (EL) was calculated using the following equation (Colombo 1990): $EL=(EC_{live}/EC_{killed}) \times 100\%$.

M1-7. Chlorophyll estimation

Chlorophyll content of seedlings of each treatment replicate was estimated using 20 6-mm needle segments taken randomly from 30-needle sampling units. The segments were immersed in 6 mL dimethyl sulfoxide (anhydrous mol wt 78.13) at 65 ± 1 C in darkness for 6 hours to extract chlorophyll. Absorbance (A) of the solution was measured at 663 and 645 mμ wavelengths on spectrophotometer (DU series 65, Beckman Instruments Inc., Fullerton CA) (Arnon 1949). Total chlorophyll in μg/mL (C) of the needle extracts was determined from the

equation $C = 8.02\ A_{663} + 20.2\ A_{645}$ and chlorophyll content was expressed in µg/needle segment (Lichtenthaler and Wellburn 1983; Rudiger and Schoch 1988). In some cases, the apical 2 cm of the shoot of each seedling was used to estimate chlorophyll content. The apical end was cut, and immersed in 35 mL dimethyl sulfoxide in a 45 mL screw-capped bottle at the same temperature for same periods. After measurement, the apet was placed in a paper bag at 70 C for at least three days, and then weighed on a balance with accuracy to 10^{-4} g. The unit of chlorophyll content in these cases was µg/g dry weight.

M1-8. Measurement of photosynthesis

Net photosynthetic rate (µmol CO_2 fixed$\cdot m^{-2}\cdot s^{-1}$) was also estimated as an indication of stress induced by gray mold. At each time of sampling, the net photosynthetic rate was measured for the entire shoot of one arbitrarily chosen seedling of each treatment replicate. A closed-loop photosynthesis system (model LI-6200, Li-Cor Inc, Lincoln, NE) was used, as described in Tan et al. (1992), and Tan and Hogan (1994). For making measurements, the apparatus was set up inside a growth room operated at 22 ± 1 C, 60 ± 1% RH, and with 1500 µmol$\cdot m^{-2}\cdot s^{-1}$ photosynthetically active radiation (PAR) at instrument level, which was provided by high pressure sodium lamps supplemented by a 50 W coolspot tungsten-halogen lamp (Osram Canada Ltd, Mississauga, ON). After the measurement of net photosynthetic rate the needle projected area of each seedling was measured using a Delta-T Area Measurement System (Delta-T Devices Ltd, Cambridge, UK), and net photosynthetic rate was calculated.

M1-9. Monitoring of greenhouse microclimates

Microclimates were monitored in the black spruce seedlings through the growth season and recorded using a data logger (model 21X, Campbell Scientific Inc., Logan UT 84321). Air temperature, RH and needle wetness were measured using

shielded thermistors, RH probes and , respectively (Models 107, 207, and 237, Campbell Scientific Inc.), positioned within the seedling canopies and 1 cm above the soil (Sutton et al. 1984).

M1-10. Data analyses

Statistical computations were performed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Data of disease (sporulation incidence and number of spores of *B. cinerea*) and plant physiology (chlorophyll content, leakage of electrolytes, and photosynthesis) were examined using analysis of variance (ANOVA). Observations of repeated experiments were subjected to analysis of homogeneity of variance and pooled accordingly. Treatment means were compared using the protected least significant difference test (protected LSD) (Snedecor and Cochran 1989). The level of significance (α) used in all analyses was 0.05. Regression analysis was used to characterize the effect of independent variables on disease and plant physiology.

M2. Biological control of *B. cinerea* in black spruce

M2-1. Evaluation of mycelial fungi as biocontrol agents

Biocontrol studies were conducted in a research greenhouse at the Canadian Forestry Service Laboratory, Sault Ste Marie, Ontario 1993 - 1994. Black spruce seeds were planted on 6 March in the first year and 25 March in the second year. Inoculum of *B. cinerea* (isolate ZP-90-54) and of biocontrol agents, water plus surfactant, and fungicides were applied to all above-ground portions of about 200 seedlings in each of six half flats for each replicate of each treatment and check. Seedlings were inoculated with *B. cinerea* at 92 and 106 days after planting in the first year and at 106 and 120 days after planting in the second year. Biocontrol agents, water plus surfactant, and recommended fungicides were applied at 107, 127, and 143 days after planting,

or at these times plus days 157 and 191 in the first year, and on days 120, 134, and 148 or at these times plus days 162, 176, and 190 in the second year. In both years treatments were initiated after the seedling canopies had become dense and a few of the lower needles had turned yellow or brown and showed signs of gray mold. Biocontrol agents used in the study were one isolate each of *Gliocladium roseum* (AL710), *Myrothecium verrucaria* (ZP2005), *Fusarium* sp. (ZP2009), and *Penicillium* sp. (ZP2047) that were previously found to suppress *B. cinerea* in black spruce seedlings in the growth room study. In a second water-plus-surfactant check, seedlings were sprayed at all times when *B. cinerea* and treatments were applied. In the fungicide program in the first-year test, chlorothalonil (Daconil 2787, 2 mL product /L) was applied twice and iprodione (Rovral 50 WP 0.01 *g* product/L) was applied three times. In the second-year test the same fungicide treatments were used plus one application of benomyl (Benlate 50 WP at 0.5 *g* product/L). Seedlings were covered with clear vinyl sheeting to maintain high humidity for 14 h after the first application of *B. cinerea* and also after the treatments were applied. Thirty seedlings were sampled arbitrarily from each replicate of each treatment on day 197 after planting in the first year and on day 235 in the second year. The seedlings were surface sterilized, kept in high humidity at 20 °C for 7 - 8 days, after which sporulation incidence of *B. cinerea* on the seedlings was estimated. The experiments were conducted as a randomized complete block design with six replications.

M2-2. Effects of G. roseum on infection and on physiological changes associated with infection

Studies were conducted in controlled environment using black spruce seedlings, 4-months old. These seedlings had been grown in a greenhouse in which the temperature was often sufficiently high to predispose the seedlings to infection by *B. cinerea* (Zhang and Sutton 1994a). The seedlings were inoculated with *G. roseum* (5×10^7 conidia/mL water plus surfactant) or with

water plus surfactant only, and kept in the humidity chamber at 22 C for 24 h. Seedlings previously treated with *G. roseum* or with water only were then challenge inoculated with *B. cinerea* (isolate SG224, 5×10^5 conidia/mL water plus surfactant), or treated with water plus surfactant only, and returned to the humidity chamber for a further 48 h. The seedlings were subsequently kept in a growth chamber operated at 22-23 C on a 16-h photoperiod (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at seedling height) with RH 78 - 82% in the light period and 96-100% in the dark period. After 0, 4, 7, 10, and 13 days in the growth chamber, one seedling of each treatment replicate was taken arbitrarily and used to estimate sporulation incidence of *B. cinerea* on the needles and number of spores produced by the pathogen on the needle segments. The second, third and fourth seedlings were respectively used to estimate electrolyte leakage, chlorophyll content, and rate of photosynthesis. The experimental design was a split plot, with inoculation treatments as the main plots, and incubation period as subplots. Treatments were replicated four times and the study was repeated once.

M2-3. Concentration and time of application of G. roseum inoculum in relation to biocontrol

To investigate effects of inoculum concentration of *G. roseum* on incidence of *B. cinerea* and severity of gray mold in black spruce seedlings, the antagonist was applied at concentrations of 10^2, 10^4, 10^6, and 10^8 conidia/mL to all above-ground portions of seedlings on 20 June, 18 July, and 1 and 15 August. At the same times, chlorothalonil (Daconil 2787, 2 mL product/L) was applied as a standard fungicide treatment, and water plus surfactant was used as a check. All treatments were applied about 1-2 h before sunset. For each treatment replicate there was one flat which contained about 500 seedlings. All seedlings were sprayed with inoculum of *B. cinerea* (isolate SG224, 5×10^5 conidia/mL) at about 2100 hours on 7 July. Proportion of seedlings with sporulation of *B. cinerea* and proportion of foliage with sporulation of the

pathogen were estimated on 27 June, 11 and 26 July, and 8 and 22 August. The experimental design was a split plot, with inoculation treatments as the main plots, and time as subplots. Treatments were replicated three times. The experiment was conducted in Sault Ste. Marie once in the research greenhouse and once in a fibreglass-covered greenhouse. The fibreglass-covered greenhouse lacked sophisticated microclimate control, and was similar to greenhouses in which black spruce seedlings are produced in northern Ontario.

Inoculum of *G. roseum* (10^6 conidia/mL water plus surfactant) was applied to black spruce seedlings on the first one, two, three, four, five, and six of the following treatment dates: 20 and 27 June; 4, 18, and 31 July; and 15 August. Chlorothalonil (Daconil 2787, 2 mL product/L) and water plus surfactant were each applied to seedlings on all of the treatment dates. All treatments were applied to all above-ground portion of seedlings at about 1-2 h before sunset. For each treatment replicate there was one flat which contained about 500 seedlings. All seedlings were inoculated with *B. cinerea* (5×10^5conidia/mL) at about 2030 hours on 7 July. Proportion of seedlings with sporulation of *B. cinerea* and proportion of foliage with sporulation of the pathogen were estimated on 27 June, 12 and 26 July, and 8 and 23 August. The experimental design was a split plot, with treatment programs as the main plots, and time as subplots. The experiment had three replications, and was conducted once in the research greenhouse and once in the fibreglass-covered greenhouse.

M2-4. Population dynamics of *G. roseum* in relation to biocontrol

Temperature effects on G. roseum in dry needles

Seedlings that were predisposed or not predisposed to infection by *B. cinerea* were inoculated with *G. roseum*, allowed to dry in a growth room in darkness at 20 C for 1 h and subsequently kept in growth chambers in darkness at 12, 20,

and 28 C and 70 ± 4% RH. Temperature and RH in each chamber were recorded continuously with a hygrothermograph. Four arbitrarily chosen seedlings at each temperature were removed from the chambers after 0, 6, 12, 24, 48, 72, 96, 120, and 168 h, and used for estimating numbers of conidia of *G. roseum* recovered per seedling, percent germination of the recovered conidia, and sporulation incidence of the antagonist on needle segments. The experiment was a split-plot design in which temperature and treatment duration were main plot and sub-plot variables, respectively. The experiment was repeated twice and the three experiments were considered as replications in data analysis.

Greenhouse study of G. roseum on dry needles

Seedlings that were predisposed or not predisposed to infection by *B. cinerea* were inoculated with *G. roseum* at 0800 hours on 6 July (repetition 1) and 26 July (repetition 2) of 1993, allowed to dry in a growth room in darkness at 20 C for 1 h, and then placed in a research greenhouse. Temperature and RH were measured continuously with electronic sensors that were positioned in the seedling canopy and connected to a datalogger (model 21 X, Campbell Scientific Inc., Logan, UT) (Sutton et al. 1984). The greenhouse glass were sprayed with Liquid Shading (Plant Products Ltd., Brampton ON) prior to the studies. Four predisposed seedlings and four nonpredisposed seedlings, chosen arbitrarily, were recovered after 0, 6, 12, 24, 48, 72, 96, 120, and 168 h and used for estimating numbers of conidia per seedling, percent germination of recovered conidia, and sporulation incidence of the biocontrol agent on needle segments.

Time of application of *G. roseum* and of *B. cinerea* (isolate ZP-90-54) in relation to biocontrol of the pathogen was examined in black spruce seedlings in the growth room. The seedlings were predisposed to infection by the pathogen, inoculated with *G. roseum* (10^8 conidia/mL) or with water plus surfactant and allowed to dry. The seedlings were challenge-inoculated with *B. cinerea* (10^6 conidia/mL) at 0, 24, 48, 96, and 144 h after *G. roseum* was applied, and immediately kept in a clear plastic humidity chamber (2.0 m long

x 0.8 m wide x 0.8 m high) in a growth room at 20 - 22 C in darkness for 32 h. Relative humidity in the chamber was maintained at or near saturation by continuous operation of an ultrasonic humidifier. A 5-mm space among the sides of the chamber allowed air exchange and loss of water vapour. The seedlings were subsequently kept in a growth room (20 - 22 C, 50 - 70% RH, 16-h photoperiod) for 2 days, after which sporulation incidence of *B. cinerea* on the needles was estimated. The experiment was a completely randomized design in which each treatment (time between inoculation with *G. roseum* and with *B. cinerea*) was replicated three times. The experiment was repeated once.

M3. Biological control of *B. cinerea* in red pine

M3-1. Concentration and time of application of *G. roseum* in relation to biocontrol in the growth room

Inoculum concentration

Inoculum concentration of *G. roseum* was investigated in relation to suppression of gray mold in coinoculation tests of *B. cinerea* (isolate ZP-90-54) and the antagonist on red pine seedlings in the growth room. Freshly-prepared inoculum of the pathogen and of the antagonist were mixed (1:1, v:v) to provide final concentrations of 10^6 conidia of *B. cinerea*/mL, and 0, 10^2, 10^4, 10^6, 10^7, and 10^8 conidia of *G. roseum*/mL. Immediately before inoculation, the seedlings were kept in a growth room in darkness at 40 C for 3 days to predispose them to infection by *B. cinerea* (Zhang and Sutton, unpublished observations). After the inoculation treatments, the seedlings were kept in high humidity for 36 h and in the growth room for 3 days. Sporulation incidence of *B. cinerea* on needle segments was estimated using four replicate sampling units per treatment. The experiment was a completely randomized design with three replicates and repeated twice.

Inoculation timing

Effects of application time of *G. roseum* in relation to that of *B. cinerea* (isolate ZP-90-54) on effectiveness of the antagonist in suppressing gray mold was examined in red pine seedlings in the growth room. Immediately before treatments were initiated, the seedlings were kept at 40 C in darkness for 3 days to predispose them to infection by the pathogen (Zhang and Sutton, unpublished). The seedlings were inoculated with *G. roseum* (10^8 conidia/mL) at 6, 5, 4, 3, 2, and 1 days before, on the same day as, and at 1, 2, 3, 4, 5, and 6 days after they were inoculated with *B. cinerea* (10^6 conidia/mL). After the inoculations, the seedlings were kept in high humidity for 36 h and in the growth room for 3 days. The growth room was operated at 22 - 23 C on a 16-h photoperiod (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at seedling height) with RH 78 - 82% in the light period and 96-100% in the dark period. Sporulation incidence of *B. cinerea* on needle segments was estimated using four replicate sampling units per treatment. The experiment was a completely randomized design with four replicates and repeated once.

M3-2. Inoculum concentration of G. roseum in relation to biocontrol in the greenhouse and outdoors

Red pine seedlings from the Ont. Min. Nat. Resources Tree Nursery at Thessalon, ON, that were in the second year of growth, were inoculated with *G. roseum* at concentrations of 0, 10^2, 10^4, 10^6, and 10^8 conidia/mL water plus surfactant on 3 August 1993. The experimental design was a completely randomized design (CRD) with four replications each with 42 seedlings grouped in a portion of a seedling flat. Sporulation incidence of *B. cinerea* was estimated on 28 September 1993 using all of the seedlings. Inoculum of *B. cinerea* from natural sources was present in the nursery prior to the experiment, and in the greenhouse. The experiment was repeated once in a similar greenhouse.

Red pine seedlings from the nursery that were in the third year of growth were inoculated with *G. roseum* (10^5 and 10^7 conidia/mL water plus surfactant), chlorothalonil (Daconil 2787, 2 mL product/L), and water plus surfactant on 14 and 28 June, 26 July, and 9 and 23 August. One flat with about 300 seedlings was used for each of six replicates per treatment. Inoculum of *B. cinerea* was from natural sources as before. Incidence of gray mold was estimated on 24 August using 50 seedlings chosen arbitrarily in each flat. The experiment was conducted as a completely randomized design.

M4. Biorational compounds in relation to control of *B. cinerea* in black spruce

M4-1. Effects of inorganic salts, carbohydrates and nitrogenous compounds on growth of *B. cinerea* and of *G. roseum* on water agar

Effects of inorganic salts, carbohydrates, and nitrogenous compounds on growth of *B. cinerea* (isolate SG224) and of *G. roseum* were examined in water agar assays in 88-mm diameter petri dishes. The water agar was prepared using 15 g agar (purified grade)/L distilled water. The inorganic salts, carbohydrates, and nitrogenous compounds were added to the agar, after it was autoclaved and had cooled, at final concentrations of 50 mM, 100 mg/L, and 2 mM, respectively. Unamended water agar was used as a check substrate in all tests. To examine effects of the substances on conidial germination and germ tube growth, 0.15 mL inoculum (10^6 conidia/mL) of *B. cinerea* or *G. roseum* was spread across the surface of the medium in each petri dishes and the dishes were incubated in darkness at 22 C for 20 h. After incubation, conidia in each dish were examined microscopically for germination and germ tube growth. Conidia were considered germinated when the length of the germ tube exceeded the diameter of the conidium. Estimates of percent germination and germ tube length were based on observations of 100 conidia and 10 germ tubes in each of three replicate dishes per treatment.

To examine effects of the substances on colony growth, mycelial plugs, each 3 mm in diameter and about 1 mm thick, were cut near the margin of growing colonies of *B. cinerea* on PDA and positioned mycelium side down at the centre of the agar medium in each of 3 replicate dishes per treatment. A drop of 0.02 mL inoculum (10^6 conidia/mL) of *G. roseum* was placed on the centre of the agar medium in each of 3 replicate dishes per treatment. The petri dishes were incubated in darkness at 22 C for various periods. For *B. cinerea*, colony diameter, minus 3 mm for the plug and the drop of inoculum suspension, was measured. For *G. roseum*, mycelial density was estimated with aid of a hand-lens based on a 1 - 10 scales. The mycelial density rate was calculated from mycelial density in various treatment/mycelial density in water check. The experiment was repeated once.

M4-2. Effects of inorganic salts on infection

Effects of 16 inorganic salts on infection of black spruce seedlings by *B. cinerea* (isolate SG224) were examined in a growth room operated at 20 C and with a 12-h photoperiod (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Each salt was dissolved in sterile-distilled water and mixed with freshly prepared inoculum of *B. cinerea* to provide final concentrations of 50 mM for the salt and 10^6 conidia/mL for the pathogen. Black spruce seedlings 4 months old were kept in a growth cabinet at 40 ± 1 C in darkness for 2 days immediately before inoculation to predispose them to infection by *B. cinerea* (Zhang and Sutton 1994a). For inoculations, the salt-inoculum mixtures and inoculum diluted to 10^6 conidia /mL with sterile-distilled water plus surfactant (the check) were applied to incipient run-off on the foliage of the seedlings. Two seedlings were treated in each of 3 replicates per treatment. Inoculated seedlings were kept in a plastic humidity chamber in the growth room for 24 or 48 h and subsequently on the growth room bench. Sporulation incidence of *B. cinerea* on the needles, and numbers of conidia produced by the pathogen on the needles were estimated at 5 days after inoculation. The experiment was a completely randomized design and

was repeated once.

M4-3. Effects of inorganic salts on biocontrol

Effects of twenty two inorganic salts on biocontrol effectiveness of *G. roseum* against *B. cinerea* in black spruce seedlings were investigated in the growth room. Each salt was dissolved in sterile distilled water and mixed with inoculum of *G. roseum* to provide final concentrations of 50 mM for the salt and 10^6 conidia/mL for the antagonist. Foliage of 4-month-old seedlings was inoculated to incipient run-off with the salt-*G. roseum* mixtures, with inoculum of *G. roseum* only (10^6 conidia/mL water plus surfactant), or with water plus surfactant only. Inoculated seedlings were immediately kept in a humidity chamber in the growth room at 20 C for 66 h, then challenge-inoculated with *B. cinerea* (10^6 conidia of isolate SG224/mL water plus surfactant) and returned to the humidity chamber for a further 48 h. The seedlings were then moved to a bench in a growth room maintained at 20 C, with RH 78 - 82% in the light period and 96-100% in the dark period, and with a 12-h photoperiod (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at seedling height). Incidence of sporulation of *B. cinerea* on 10 shoots and on 30 needles, and numbers of spores of *B. cinerea* and *G. roseum* produced per seedling, were estimated at eight days after the seedlings were inoculated with *B. cinerea*. The experiment with a completely randomized design was repeated with the following minor changes: the interval between inoculations of *G. roseum* and *B. cinerea* was 46 hr; seedlings were kept in the growth room for 4 days before determination of sporulation incidence.

M4-4. Effects of sodium bicarbonate on infection

Effects of concentration and application time of sodium bicarbonate on the pathogen were conducted in a research greenhouse and a growth room at the Canadian Forestry Service Laboratory, Sault Ste Marie, Ontario in 1994. Three

and six-month-old seedlings were sprayed with sodium bicarbonate at concentrations 15, 30, 60, and 120 mM at 1, 7, and/or 14 days. Treatment periods were scheduled such that all ended simultaneously. After the treatments, seedlings were kept at 45 C in darkness for 3 hours to predisposed the seedlings to infection of *B. cinerea* (Zhang and Sutton 1994a), then inoculated and kept in the humidity chamber for 36 h and on the growth room bench for three days. Sporulation incidence and spore production by *B. cinerea*, and chlorophyll content, were estimated in needle segments as described in sections M1-5 and M1-7. The experimental design was a split-plot with treatment timing as main plots, and concentration of sodium bicarbonate as sub-plots. Treatments were replicated three times, and the study was repeated once with four replications.

M5. Epidemiology of gray mold in black spruce

M5-1. Low light intensity in relation to infection

In growth room studies, seedlings about 14-15 cm tall were placed at least 4 cm apart in a growth room at 30 °C and relative humidity (RH) of 40-60%. Light was provided on a 16-h photoperiod by cool-white fluorescent lamps. Intensity of PAR (400-700 nm wavelength band) was measured using a quantum sensor (model LI-188, Li-Cor Inc., Lincoln, NE). Intensity of PAR at seedling height was regulated at 7, 15, and 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by adjusting the height of the lamps above the seedlings, and by positioning neutral filters (plywood with 1-4 cm holes in a grid pattern) ≥0.8 m below the lamps. For treatment at 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ seedlings were kept in darkness in the same growth room. PAR was measured at seedling height every three days, and minor adjustments to the specified PAR levels were made when necessary. Seedlings were kept in the various light intensities for periods that increased by 3-day increments from 0 to 42 days. All treatment durations were scheduled to end simultaneously. The experimental design was a split-plot in which main-plot and sub-plot treatments were light

intensity and duration of treatment, respectively. There were three replicate seedlings per treatment, and the experiment was repeated twice. A set of seedlings was used for estimating chlorophyll content. Another set of treated seedlings was inoculated with *B. cinerea*, and immediately kept in the clear-plastic humidity chamber. After the humid period, the seedlings were kept in the growth room for 2 days, and then evaluated for infection by *B. cinerea*.

Relationships of light intensity in seedling canopies and infection of needles by *B. cinerea* were investigated in research greenhouses at Guelph in 1992 and at Sault Ste. Marie, Ontario, in 1993. Seeds of black spruce were sown on 15 March (1992) and 17 May (1993), and seedlings were used for light intensity studies when the canopies closed. Light intensity at 400-700 nm was measured between 11:30 and 13:30 hours of various days when there was no immediate cloud cover. Measurements were made at 2-8 cm above the growing medium at 28 and 40 sites in the seedling canopies of 1992 and 1993, respectively, using the quantum sensor. When PAR at a given site had decreased to low levels (20 and 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 1992 and 1993 respectively), 50 needles on seedlings near the sensor position were marked with small ribbons that were tied on the stems below and above the needles. Further measurements of PAR were made at the same sites at 9-11 day intervals for 40 days. At the end of the 40-day period, the marked needles were evaluated for infection by *B. cinerea*. In 1992, seedlings with marked needles were inoculated with *B. cinerea* using a hand sprayer, and kept under postinoculation conditions described for the growth room study. In 1993, seedlings received only natural inoculum under conditions for infection that occurred in the greenhouse. In both years, incidence of sporulation of *B. cinerea* on 6-mm segments of marked needles was estimated by the methods used in the growth room study.

M5-2. Freezing conditions in relation to infection

Relationships of preinoculation freezing treatments to injury and to infection

of black spruce by *B. cinerea* was examined using 2-, 3-, and 4-month-old seedlings that had been grown in the research greenhouse described before. For freezing tests the seedlings were preconditioned in a growth room at 5 C in darkness for 12 h, then placed in the dark in growth rooms in which air temperature was decreased from 5 C to desired test temperatures at a rate of 5 C/h. Check seedlings were preconditioned at 5 C only, and were not cooled to freezing temperatures. Seedlings were kept at test temperatures of -2, -6, and -10 C in darkness for 1, 2, 4, 6, 10, and 14 h. Immediately after the freezing treatments, the seedlings were placed in styrofoam chests and moved to the growth room operated at 5 C. The chests were left unopened for 12 h to allow a slow change in air temperature within the chests, which increased to 5 C during 6 to 8 h. One seedling of each replicate of each treatment was used to estimate electrolyte leakage and another seedling was used for infection tests. Electrolyte leakage was used as an indicator of freezing injury. To estimate infection, the seedlings were inoculated with *B. cinerea* (isolate ZP-90-54, 10^6 conidia/mL) and kept in the humidity chamber for 36 h. Sporulation incidence of *B. cinerea* on the needles, and number of conidia produced by the pathogen were estimated after 8 days. The experimental design was a split plot, with temperatures as the main plots, and treatment period and seedling age as subplots. Treatments were replicated three times and the study was repeated once.

M5-3. Planting density on black spruce seedlings in relation to progress of gray mold

Seeds of black spruce were soaked in 5% hydrogen peroxide for 15 min and immediately sown, on 9-11 March 1994, in size 308 paper seedling flats (Lännen Tehtaat Oy, Finland) containing a 2:1 (v:v) mix of peat and vermiculite. Three seeds were sown per cell of each flat and seedlings were thinned at 2 weeks after emergence to produce densities of 0.25, 0.5, 1 and 2 seedlings/cell (119, 238, 476, and 952 seedlings per flat), respectively. The seedlings were

grown in a research greenhouse (repetition 1) and in a fibre-glass covered greenhouse similar to those used commercially in northern Ontario (repetition 2). The seedlings were inoculated with *B. cinerea* (5×10^5 conidia/mL) at dusk (2030 - 2100 hours) on 7 July when the seedlings were 12 - 15 cm tall and the seedling canopies were closing. Twenty seedlings were selected arbitrarily from each replicate of each treatment on 27 June, 12 and 26 July, and 8 and 23 August and used to estimate incidence and severity of gray mold on the needles. The experiment was a completely randomized design with one flat of seedlings for each of three replicates per treatment.

Results

R1. General overview

Gliocladium roseum and *M. verrucaria* effectively suppressed *B. cinerea* in container-grown seedlings of black spruce in greenhouses, and were at least as effective as recommended fungicide treatments. Applied at concentrations of 10^6 -10^8 conidia/mL, *G. roseum* consistently suppressed *B. cinerea* in black spruce seedlings under a wide range of microclimatic conditions in the growth room, greenhouse, and outdoors. As few as one application of *G. roseum* was sufficient to control *B. cinerea* in the seedlings in the greenhouse over the growth season. *Gliocladium roseum* did not adversely affect treated seedlings, but did prevent, or almost prevent, pathogen-induced loss of electrolytes and chlorophyll, and reduced photosynthesis rates in the foliage of seedlings inoculated with *B. cinerea*. However *G. roseum* only survived for several days if no wetness appeared on seedlings after *G. roseum* was applied. Similar biocontrol performances of *G. roseum* were observed on red pine seedlings.

The biorational compounds potassium carbonate, sodium carbonate, and sodium bicarbonate effectively suppressed *B. cinerea* in the tests on an agar medium and on black spruce seedlings in the greenhouse. Calcium carbonate and potassium carbonate effectively reduced spore production of the pathogen and promoted spore production of *G. roseum*. However, inorganic salts, Sugars, amino acids, and several other carbohydrates and nitrogenous compounds in most instances did not enhance biocontrol of *B. cinerea* by *G. roseum*.

Light intensity (400 - 700 nm wavelength) of ≤10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and low temperatures of -6 and -10 C predisposed black spruce seedlings to infection by *B. cinerea*. Conventional planting density of black spruce seedlings was strongly conducive to rapid progress of gray mold epidemics in the seedlings, and epidemic rates could be markedly reduced by using lower planting

densities.

R2. Biocontrol of B. cinerea in black spruce

R2-1. Evaluation of mycelial fungi as biocontrol agents

Incidence of *B. cinerea* in seedlings of the water plus surfactant checks that received only natural inoculum of the pathogen, and in check seedlings that were artificially inoculated with the pathogen only, did not differ significantly in the first year or the second year. Incidence of the pathogen in the inoculated and noninoculated checks was high (83 - 97%) in the first year, and moderate (34 - 36%) in the second year.

In the first year, three applications of *G. roseum*, M. *verrucaria*, and *Fusarium* sp. significantly suppressed incidence of *B. cinerea* in the black spruce seedlings by 69, 60, and 64%, respectively (Fig. 1). Five applications of *G. roseum* and of M. *verrucaria* significantly suppressed the pathogen by 72 and 55%, respectively, and did not differ in effectiveness from three applications of the respective fungi ($P > 0.05$). The suppressive biocontrol treatments were each as effective as the recommended fungicides. Five applications of *Fusarium* sp. and three and five applications of *Penicillium* sp. were each ineffective.

In the second year, three and six applications of *G. roseum* significantly suppressed incidence of *B. cinerea* by 50 - 55% relative to that in the water plus surfactant check and in the check seedlings treated only with *B. cinerea* (Fig. 1). Three applications of M. *verrucaria* significantly suppressed incidence of the pathogen relative to the *B. cinerea*-treated check but not in comparison to the water plus surfactant check. Six applications of M. *verrucaria* were ineffective. Three and six applications of *Fusarium* sp. numerically suppressed the pathogen by 28 - 31% and 25 - 27%, respectively,

compared to the checks, but the differences were not significant ($P > 0.05$). *Gliocladium roseum* suppressed incidence of *B. cinerea* in seedlings 12 - 18% more than the fungicides did, however this difference also was not significant ($P > 0.05$).

R2-2. Effects of G. roseum on infection and on physiological changes associated with infection

Observations and statistics of the two repetitions of the study did not differ significantly ($P > 0.05$). Therefore the data were combined for presentation.

Sporulation incidence of *B. cinerea* in needle segments of seedlings that were sprayed with water and challenge-inoculated with *B. cinerea* was low (7.4%) for seedlings sampled at 2 days after the pathogen was applied, but high (56-64%) for seedlings sampled after 6-15 days (Fig. 2). In contrast, sporulation incidence was at or near zero in seedlings of the water checks, in those treated only with *G. roseum* and, except for seedlings sampled at 15 days, in those treated with *G. roseum* and challenge-inoculated with *B. cinerea*. Sporulation incidence in seedlings inoculated with *G. roseum* plus *B. cinerea* and sampled at 2, 6, and 15 days after the pathogen was applied was 1, 2, and 8%, respectively.

Patterns of conidial production of *B. cinerea* on the seedling shoots were similar to those of sporulation incidence (Fig. 2). Number of conidia produced on seedlings treated with water and challenged with *B. cinerea* was low (6.6×10^5 spores/seedling) at 2 days after the pathogen was applied but high (4.1 to 5.7×10^6 spores/seedling) in seedlings of subsequent sampling times. Conidial production was zero in water checks and in seedlings treated with *G. roseum* only. In those treated with *G. roseum* and inoculated with *B. cinerea*, 0.6×10^5, 1×10^5, and 9.3×10^5 conidia were produced per seedling in samples taken at 2, 6, and 15 days after the pathogen was applied.

Electrolyte leakage from foliage increased markedly in seedlings treated with water and challenged with *B. cinerea* but was low (5.6 to 13.3%), and did not differ significantly, among seedlings treated with water only, with *G. roseum* plus water, and with *G. roseum* plus *B. cinerea* (Fig. 2). Leakage from foliage in the water plus *B. cinerea* treatment was low (12.4 - 12.7%) when the seedlings were sampled at 2 and 6 days after the pathogen was applied, but high (47.3 - 51.3%) in those sampled at 9, 12, and 15 days.

Chlorophyll content of seedling shoots and rate of photosynthesis of the shoots were generally stable with time after the challenge inoculations, and did not differ significantly among treatments, in seedlings treated with water only, with *G. roseum* plus water, and with *G. roseum* plus *B. cinerea* (Fig. 2). However, in seedlings of the water plus *B. cinerea* treatment, chlorophyll content progressively declined from 9.6 to 5.1 mg/g dried shoot, and rate of photosynthesis declined from 9.0 to 0.4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in the period of 2 to 15 days after the pathogen was applied. At 2 days after the challenge inoculations, chlorophyll content of the shoot in the water plus *B. cinerea* treatment was similar to those of the other treatments but rate of photosynthesis in seedlings treated with water plus the pathogen was already about 33 - 43% lower than in those of the other treatments.

R2-3. Concentration and time of application of G. roseum inoculum in relation to biocontrol

Data of the two repetitions of the study were statistically similar and were combined for analysis and presentation. Proportion of black spruce seedlings with sporulation of *B. cinerea* and proportion of foliage with sporulation of the pathogen increased chiefly in the period from week 15 (22 June) to week 23 (17 August) after planting in patterns resembling sigmoid curves (Fig. 3). The first phase of the epidemics at weeks 15 - 17 coincided with initial closure of the seedling canopies. In the period from week 15 to

week 23, the canopies increased in height from ?? cm to ?? cm, and markedly increased in density. From the ANOVA of general effects of *G. roseum*, inoculum concentration of the antagonist significantly affected sporulation incidence of *B. cinerea* ($P = 0.0001$) and foliage with sporulation ($P = 0.0001$) in the seedlings.

Logistic regression equations were selected that adequately described effects of inoculum concentration (C) and time after planting of *G. roseum* (T), and of chlorothalonil treatment programs on proportion of seedlings with sporulation of *B. cinerea* (YI) and proportion of foliage with sporulation of the pathogen (YS) (Table 1). In the model for inoculum concentrations and time of application of *G. roseum* in relation to YI, terms of b_0 and b_2 were significant ($P < 0.001$) based on stepwise regression but terms of b_1 and b_3 were not significant ($P > 0.05$), which indicated that T affected YI but that C did not. The relationship between logits of YI and T was significantly linear. There was no significant difference among the treatments of chlorothalonil and inoculum concentrations ($P > 0.05$). In the model for inoculum concentration in relation to YS, terms of b_0 - b_2 were significant ($P < 0.001$) based on stepwise regression which indicated that both T and C affected YS. The relationships between logits of YS and T and between logits of YS and C were significantly linear. There was a significant difference among various concentrations of *G. roseum* and chlorothalonil. ($P < 0.01$). *Gliocladium roseum* and chlorothalonil significantly suppressed *B. cinerea* compared with water checks ($P < 0.01$). From the interactions between T and C, change in logits of YI and YS with change in C was not consistent for all T values. Although some parameters (b values) were negative, this did not indicate a decline in logits of YI and YS with increase in T, which was clarified by Zhang and Sutton (1994b).

Treatment programs of *G. roseum* suppressed incidence of seedlings but not foliage with sporulation of *B. cinerea* on the spruce seedlings (Fig. 4).

Logistic and Gompertz regression equations were selected that adequately described the effects of the time after planting (T) at various treatment programs in relation to incidence of seedlings (YI) and foliage (YS) with sporulation, respectively (Table 2). In the logistic models for YI, terms of b_0 and b_1 were significant ($P < 0.001$) based on stepwise regression. The relationship between logits of YI and T was significantly linear. There was no significant difference among the treatments of chlorothalonil and biocontrol programs ($P > 0.05$). In the Gompertz model for YS, terms of $b_0 - b_1$ were significant ($P < 0.001$) based on stepwise regression which indicated T affected YS. The relationship between transformed YS and T was significantly linear. There was a significant difference among the treatments of chlorothalonil and inoculum concentrations ($P < 0.01$). *Gliocladium roseum* and chlorothalonil significantly suppressed *B. cinerea* compared with water checks ($P < 0.01$).

R2-4. Population dynamics of *G. roseum* in relation to biocontrol

Observations of the three repetitions of the study in the growth room did not differ significantly and were combined for analysis. Number of conidia of *G. roseum* recovered per seedling and sporulation incidence of *G. roseum* on the seedling needles were not affected significantly by temperature or by time after the conidia were applied to the seedling foliage (Table 3). Mean numbers of conidia recovered from foliage of seedlings that were predisposed and not predisposed to *B. cinerea* ranged from 4.8 to 6.6 $\times 10^7$ spores/seedling and from 4.2 to 6.7 $\times 10^7$ spores/seedling, respectively. The respective values for mean sporulation incidence of *G. roseum* were 0 to 6.7% and 0 to 7.8%.

Percent germination of *G. roseum* conidia recovered from inoculated foliage decreased sharply with time after inoculation at all tested temperatures in seedlings that did or did not receive the high temperature predisposition treatments to *B. cinerea* (Fig. 5, Table 3). In general, germination decreased

more rapidly at 20 and 28 C than at 12 C, and in predisposed seedling than in those that were not predisposed. At 48 h after inoculation, for example, percent germination of conidia from seedlings kept at 12, 20, and 28 C was 50, 15, and 10%, respectively for predisposed seedlings, and 89, 57, and 50% for nonpredisposed seedlings. At 168 h after inoculation, the respective values were 5, 2, and 1% for predisposed seedlings, and 40, 11, and 0.3% for nonpredisposed seedlings.

Regression analysis was used to ascertain relationships between temperature (T), time after inoculation (D), and percent recovered conidia that did not germinate (100 - % germinated conidia) (Y) in seedlings that had or had not received predisposition treatments (Table 4). The Weibull regression model fit the data better than did other models tested and can be written as follows:

$$\ln[\ln(100/(100-Y))] = b_o + b_1T + b_2T\ln(D) + b_3T^2\ln(D) \quad (1)$$

The b_s values were estimates of the unknown parameters (Table 4). Based on stepwise regression the terms of the model were significant in the predisposed and nonpredisposed seedlings. An F-test indicated results for regressions among the three experimental repetitions did not differ significantly for predisposed seedlings or for nonpredisposed seedlings, so the data in each instance were combined. The ANOVAs (Table 3) and the Weibull regression analysis (Table 4) indicated that T, D, and their interaction significantly affected Y. The relationship between D and Y had only a linear component, but that between T and Y had linear and squared components. The negative values of b_s parameters (Table 4) did not indicate that Weibull transformed values of percent ungerminated conidia ($\ln[\ln(100/(100-Y))]$) decreased with increase in D. To demonstrate these points, equation 2 can be rewritten as:

$$\ln[\ln(100/(100-Y)] = (b_o + b_1T) + (b_2T + b_3T^2)\ln(D) \quad (2)$$

In this equation, the new term $b_2T + b_3T^2$ was the slope for the change in the Weibull transformation with ln(D). The net slope was positive for temperatures analyzed, therefore, the equation demonstrated that there was a positive relationship between the Weibull transformed value and ln(D) at the temperatures considered. From the lower standard deviations and higher coefficients of determination, data from the test on predisposed seedlings had less variability than did those of nonpredisposed seedlings (Table 4). Values of coefficient of determination (R^2) and coefficient of determination adjusted for degrees of freedom (R^2_a) in the linearized Weibull regression equation were 0.92 and 0.91 for predisposed seedlings and 0.77 and 0.76 for nonpredisposed seedlings, respectively. Coefficients of determination between back-transformed predicted and observed values were 0.92 and 0.88 for stressed and green seedlings, respectively (Table 4).

Parameters (b_s) that were estimated from observations (Table 4) were used to calculate predicted values of ln[ln(100/(100-Y))], and subsequently Y, for constant temperatures of 12, 20, and 28 C during exposure duration of 0 - 168 hr. The predicted values of Y for predisposed and nonpredisposed seedlings are presented in Fig. 6.

In the greenhouse studies, number of conidia of *G. roseum* recovered per seedling did not change significantly, but percent germination of the recovered conidia decreased sharply, with time after predisposed and nonpredisposed seedlings were inoculated with the antagonist (Fig. 7).

Observations of the greenhouse studies were used to test the validity of the Weibull models derived from the data obtained in the growth room. Values for % germination of conidia recovered in the greenhouse study were predicted using the Weibull equation and estimated parameter values obtained for predisposed and nonpredisposed seedlings in the growth room studies, and temperature values from the greenhouse. Hourly temperature values, measured in the

greenhouse, were used to compute mean temperature values of the various postinoculation treatment periods used in the study. These values varied from 20.0 C to 27.1 C with an exception of 30.6 C. Hourly RH values in the greenhouse were also averaged fro the postinoculation treatment periods and ranged from 65.0% to 82.8% with an exception of 55.9%. Because the average RH values were similar to those in the controlled experiments, RH was not considered in the equation.

The predicted values for % germination in the greenhouse (Y_p) were then regressed on observed values for % germination in the greenhouse (Y_o), for both predisposed and non-predisposed seedlings, using the linear model $Y_p = b_0 + b_1Y_o$ (Fig. 7). The linear regression equations obtained were significant ($P < 0.001$) and the R^2 values were high. Theoretically, an unbiased result would produce an intercept of 0 and a slope of 1. The estimated intercepts (b_0) were 4.40 and 13.54 for predisposed and nonpredisposed seedlings respectively, and significant higher than 0 ($P < 0.05$), indicating that % initial germination was generally overpredicted. The estimated slope (b_1) was 0.89 and 0.79, respectively, for predisposed and nonpredisposed seedlings, and lower than 1.00, indicating that germination was increasingly underpredicted when the observed value increased.

An ANOVA indicated that the interval between application of *G. roseum* and *B. cinerea* to black spruce seedlings in the growth room significantly affected sporulation incidence of the pathogen on the needles ($P = 0.0001$), and that observations of the two repetitions of the experiment did not differ significantly ($P = 0.0897$). The data of the two repetitions were therefore pooled. *Gliocladium roseum* reduced sporulation incidence of *B. cinerea* markedly when the antagonist was applied at 0 to 24 h before the pathogen, slightly when applied 48 or 96 h before the pathogen, but not at all when applied 144 h before the pathogen (Fig. 8).

Relationship between the germination rate of *G. roseum* on black spruce seedlings at 20 C in darkness and sporulation incidence of *B. cinerea* was examined by linear correlation procedure. Values obtained for Pearson's coefficient (r) of the correlation between the conidial germination rate and the sporulation incidence in repetition 1 was -0.68 (P = 0.0052), between the germination rate and the incidence in repetition 2 was -0.67 (P = 0.0060).

R3. Biological control of *B. cinerea* in red pine

R3-1. Concentration and time of application of *G. roseum* in relation to biocontrol in the growth room

In the coinoculation studies, *G. roseum* suppressed incidence of sporulation of *B. cinerea* in needle segments of the red pine seedlings when inoculum concentration of the antagonist was 10^7 and 10^8 conidia/mL, or 10 and 100 times greater, respectively, than that of *B. cinerea* (Fig. 9). *Gliocladium roseum* did not suppress sporulation incidence of the pathogen when applied at the same concentration (10^6 conidia/mL) or lower concentrations than that of the pathogen. Sporulation incidence in seedlings treated with 10^2 and 10^4 conidia of *G. roseum*/mL was higher than in the water check.

In the study of inoculation timing in the growth room, incidence of sporulation of *B. cinerea* on the needles of the red pine seedlings was suppressed to zero when *G. roseum* was applied to the foliage at 6, 5, 4, or 3 days before *B. cinerea* was applied, and to 1, 5, and 17% when applied 2, 1, 0 days before the pathogen (Fig. 10). Sporulation incidence of *B. cinerea* increased sharply, however, as the time of application of *G. roseum* was increased after that of *B. cinerea*, and was at or near 100% when the antagonist was applied 2 - 6 days after the pathogen.

R3-2. Inoculum concentration of *G. roseum* in relation to biocontrol in the

greenhouse and outdoors

Gliocladium roseum applied once at 10^6 and 10^8 conidia/mL to second-year seedlings of red pine, that were grown in the presence of *B. cinerea* from natural sources, suppressed incidence of the pathogen in the seedlings by 21% and 43%, respectively (Fig. 11). Lower concentrations of antagonist (10^2 and 10^4 conidia/mL) were ineffective.

Programs of five applications of *G. roseum* (10^7 conidia/mL) and of chlorothalonil in third-year seedlings that were grown in the presence of *B. cinerea* from natural sources respectively suppressed sporulation incidence of the pathogen in the seedlings by 39 and 49% in the greenhouse test, and 40 and 57% in the outside compound (Fig. 12). Effectiveness of *G. roseum* and of chlorothalonil did not differ significantly in each instance ($P > 0.05$). Five applications of *G. roseum* at 10^5 conidia/mL, however, did not significantly suppress sporulation incidence of the pathogen in the greenhouse or in the outside compound.

R4. Biorational compounds in relation to control of *B. cinerea* in black spruce

R4-1. Effects of inorganic salts, carbohydrates and nitrogenous compounds on growth of *B. cinerea* and of *G. roseum* on water agar

Inorganic salts that suppressed conidial germination and germ tube growth of *B. cinerea* on water agar were all the ammonium salts tested, all calcium salts (except calcium sulfate in relation to germination), potassium carbonate, dibasic potassium phosphate, sodium carbonate, sodium bicarbonate, and dibasic sodium phosphate (Table 5). In addition, potassium carbonate, potassium nitrate, sodium nitrate, and sodium sulfate suppressed germ tube growth but not germination. Salts that suppressed colony growth of the pathogen included all carbonates, sodium bicarbonate, ammonium chloride, dibasic potassium

phosphate, sodium nitrate, dibasic sodium phosphate. Salts that strongly suppressed both germination and growth of *B. cinerea* on water agar were ammonium carbonate, ammonium chloride, potassium carbonate, sodium carbonate, and sodium bicarbonate.

Inorganic salts that suppressed conidial germination, germ tube growth, and colony growth of *G. roseum* on water agar were all of the ammonium salts tested (except ammonium chloride in relation to colony growth), potassium carbonate, sodium carbonate, and sodium bicarbonate (Table 6). In addition, potassium chloride and dibasic sodium phosphate suppressed germination, monobasic potassium phosphate, sodium sulfate, and monobasic sodium phosphate suppressed germ tube growth, and monobasic sodium phosphate suppressed colony diameter. In general, ammonium carbonate, potassium carbonate, sodium carbonate and sodium bicarbonate suppressed *G. roseum* very strongly.

Among the carbohydrates evaluated, the monosaccharides D-fructose, D-galactose, D-glucose, and D-mannose, and the disaccharide D-cellobiose strongly promoted colony growth of *B. cinerea* from PDA disks, and D-arabinose was inhibitory (Table 7). Increase in mycelium density rate (mycelium density in treatment/mycelium density in water check) of *G. roseum* was high in response to the monosaccharides D-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, and D-xylose, the disaccharide D-cellobiose, and the trisaccharide D-trehalose. None of the tested carbohydrates significantly suppressed the mycelium density rate of *G. roseum*. Among all the carbohydrates, only D-arabinose strongly stimulated the biocontrol agent and suppressed the pathogen.

In the study of amino acids and other nitrogenous compounds on *B. cinerea*, L-glutamic acid and L-aspartic acid strongly stimulated growth of the pathogen. Nitrogen compounds which were suppressive in comparison to growth of the pathogen from disks of PDA placed on water agar were ammonium nitrate, L-

alanine, L-arginine, L-asparagine, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophane, and L-valine (Table 8). Other amino acids and nitrate salts had only minor or no effects. Growth rate of *G. roseum* was promoted strongly by L-isoleucine, L-phenylalanine, L-threonine, L-tryptophane, and L-tyrosine, and moderately by L-aspartic acid, L-glutamic acid, L-leucine, L-proline, L-serine, and L-valine. Other amino acids and the nitrate salts had little or no effect on the antagonist.

R4-2. Effects of inorganic salts on infection

Potassium carbonate, sodium carbonate, and sodium bicarbonate suppressed infection incidence of black spruce needles by 57, 43, and 76%, respectively, when the wet period after inoculation with *B. cinerea* was 24 h, and by 40, 14, 32% when the postinoculation wet period was 48 h (Table 9). Dibasic sodium phosphate also decreased infection incidence (by 43%) when wetness lasted 48 h. In contrast, the ammonium salts (except dibasic ammonium phosphate), calcium chloride, and monobasic potassium and sodium phosphates increased infection incidence 150 - 200% when postinoculation wetness lasted 24 h, but did not do so significantly after 48 h wetness when infection incidence in the checks was also very high (82%).

Addition of potassium carbonate, sodium carbonate, and sodium bicarbonate to the inoculum of *B. cinerea* numerically reduced conidial production of the pathogen on the needles by 90, 87, and 97%, respectively, when the postinoculation wet period was 24 h, but the reductions were not statistically significant (Table 9). Ammonium chloride, ammonium sulfate, and monobasic sodium phosphate significantly increased conidial production when the wet period was 24 h, and ammonium nitrate, ammonium sulfate, dibasic ammonium phosphate, calcium nitrate, and monobasic sodium phosphate did so after 48 h wetness.

Pearson's correlation coefficients between agar (R4-1) and seedling (R4-2) tests were 0.17 - 0.52 (Table 10), which indicated observations on agar and on the host correlated weakly.

R4-3. Effects of inorganic salts on biocontrol

The inorganic salts variously affected infection of the seedlings by *B. cinerea* and biocontrol of the pathogen by *G. roseum* (Table 11). Though none of the salts significantly suppressed sporulation incidence of *B. cinerea* on seedling shoots and needles, calcium carbonate and potassium carbonate effectively reduced spore production of the pathogen and promoted spore production of *G. roseum*.

R4-4. Effects of sodium bicarbonate on infection

Sodium bicarbonate, foliar sprayed to black spruce seedlings 1, 7, and 14 days before the seedlings were subjected to high temperature plus darkness and inoculated with *B. cinerea*, variously reduced sporulation incidence and number of spores produced by the pathogen in the needles. From the ANOVA concentration of sodium bicarbonate affected sporulation incidence ($P = 0.0507 - 0.0001$) and number of spores produced ($P = 0.0339 - 0.0045$) in 3- and 6-month-old seedlings; time of sodium bicarbonate application variously affected sporulation incidence ($P = 0.0087 - 0.3014$), and number of spores ($P = 0.0090 - 0.8911$). However, sporulation incidence and number of spores produced by the pathogen on the seedlings of given treatments was highly variable and not uniform.

In the ANOVA, general effects of concentration of sodium bicarbonate on chlorophyll content of needle segments from seedlings subjected to high temperature predisposition treatments were significant ($P \leq 0.0148$). Effects of application time of sodium bicarbonate on the chlorophyll content were

nonsignificant ($P > 0.1597$). However, chlorophyll content of seedlings of given treatments was highly variable and lacked uniformity.

R5. Epidemiology of gray mold in black spruce

R5-1. Low light intensity in relation to infection

Observations of three experimental repetitions did not differ significantly ($P > 0.01$) and were pooled. Sporulation incidence in seedlings kept in light (PAR) intensity of 0 and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was zero when treatment duration was 3 and 9 days respectively, but 0.7% and 0.4% after 6 and 12 days, respectively. Sporulation incidence progressively increased with longer treatment periods to maximum, or near maximum, levels of 45.6% (dark treatment) and 32.5 (seedlings at 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) after 21 and 27 days of preinoculation treatment (Fig. 13). Mean values for seedlings kept at 0 and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ declined, however, when treatment durations were ≥27 and ≥39 days, respectively. Sporulation incidence was zero in seedlings kept at 15 and 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for up to 24 and 39 days, respectively, but the pathogen sporulated on a few needles (≤6.3% and ≤0.4%, respectively) when the preinoculation treatment lasted 30 and 42 days.

Chlorophyll content of needles progressively decreased to about 50% of original levels during the initial 15 days of treatment at all of the light intensities evaluated (Fig. 13). In seedlings kept for 15-45 days at 30 and 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, chlorophyll content was stable in the range of 1.4-2.0 µg/needle segment. However, in seedlings kept at 7 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, chlorophyll content continued to decrease with treatment period to about 39 and 27 days, respectively, and each reached levels near 0.7-0.8 µg/needle segment.

The regression model selected to describe sporulation and chlorophyll content as a function of light intensity and duration of light treatment was

$$Ln[Y/(100-Y)] = b_o + b_1L + b_2D + b_3LD \quad (3)$$

in which b_0 - b_3 were unknown parameters. Data used in the model were for seedlings treated at light intensity of 7 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 0-27 days (sporulation incidence) and 0-45 days (chlorophyll content). The regression equation obtained for sporulation was

$$ln(Y_s/(100-Y_s)) = -9.499 - 0.202L + 0.391D - 0.016LD \quad (4)$$

for which the coefficient of determination (R^2) was 0.59, and R^2 adjusted for degree of freedom (R_a^2) was 0.58. From the model, logits of sporulation incidence increased with decrease in light intensity (slope of -0.202-0.016D), and increased with duration of light treatment (slope of 0.391-0.016L). The regression equation for chlorophyll was

$$ln(Y_{ch}/(100-Y_{ch})) = 0.805 + 0.065L - 0.068D - 0.001LD \quad (5)$$

for which $R^2 = 0.72$, and $R_a^2 = 0.71$. From this model, logits of chlorophyll content of needle segments decreased with duration of light treatment at slope of -0.068-0.001L. Sporulation incidence correlated moderately and negatively with chlorophyll content of needle segments in seedlings kept at 7 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for between 0 and 27 days in all three experimental repetitions (r = -0.58 to -0.61).

In 1992, the seedling canopies began to close in mid July and light (PAR) intensity at the measurement sites in the canopies was <20 and <8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on 26 August and 15 October, respectively. In 1993, canopy closure began on 15 September and light intensity at measurement sites was <15 and <6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on 15 October and 10 December. Canopy height in the period when light was monitored was 18-25 cm in 1992 and 20-30 cm in 1993.

Sporulation incidence ranged from 0-100% in segments of needles from sites in the canopies at which the average light intensity during the 40-day monitoring periods were in the range of 0-12 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Sporulation incidence (Y_s)

decreased as light intensity at the monitoring sites in the canopy increased in both the 1992 and 1993 studies (Fig. 14). In simple regression analysis using the equation $Y_s = b_o + b_1L$, the coefficient of determination (R^2) was significant in both years, but was higher in 1992 than in 1993 (Fig. 14). In the analysis of the 1992 data, the decrease in sporulation with increased light intensity had a slope of -5.11 and sporulation incidence reached zero when the average light intensity was 9.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In 1993, the slope was -5.76 and sporulation incidence reached zero at 10.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

R5-2. Freezing conditions in relation to infection

Botrytis cinerea sporulated on needles of 2-, 3-, and 4-month-old seedlings that were kept at -2, -6, and -10 C) in darkness before inoculation (Fig 15), but did not sporulate on needles of check seedlings that received no freezing treatment. Sporulation incidence in needles increased with duration of preinoculation freezing, but the pattern of increase over time differed markedly with treatment temperature and seedling age. Sporulation incidence in seedlings kept at -2 C before inoculation was low (<5%) except in seedlings 2 months old that were kept at -2 C for 14 h (32%). In 2- and 3-month-old seedlings treated at -6 C, sporulation incidence increased from 1 to 80% and from 23 to 86%, respectively when the treatment period was increased from 1 to 10 h, but only slightly more when the treatment period was increased from 10 to 14 h. Sporulation incidence in 4-month-old seedlings treated at -6 C, however, was <3.3% when the treatment period was 1 - 10 h, and only 8.3% after 14 h treatment. In 2- and 3-month-old seedlings treated at -10 C, sporulation incidence increased abruptly to 87% and 69% respectively, when the treatment period was only 1 h, and remained high after longer treatment. The increase in sporulation incidence in 4-month-old seedlings kept at -10 C was more gradual than in the younger seedlings and reached high levels (86 - 90%) only when the seedlings were treated for 10 - 14 h.

Conidia of *B. cinerea* were produced chiefly on 2- and 3-month-old seedlings that were kept at -6 or -10 C before inoculation, and on 4-month-old seedlings of the -10 C treatment (Fig. 15). Few or no conidia were produced on seedlings treated for various periods at -2 C, or in 4-month-old seedlings treated at -6 C. Patterns of conidial production in relation to temperature and duration of the preinoculation temperature treatment generally resembled those of sporulation incidence, except for extraordinarily high production in 2-month-old seedlings treated at -6 or -10 C for 14 h.

Patterns of electrolyte leakage from the seedlings strongly resembled those of sporulation incidence of *B. cinerea*, and were similar to those of spore production by the pathogen. In general, electrolyte leakage increased as temperature of the preinoculation treatment was reduced, and as the period of the treatment was increased.

R5-3. Planting density on black spruce seedlings in relation to progress of gray mold

Observations and statistics in the two repetitions of the experiment were similar ($P > 0.05$), and were combined for analysis and presentation (Fig. 16). From the ANOVA, estimated incidence of seedlings and area of foliage with sporulation of *B. cinerea* differed significantly in response to seedling density. Incidence and area foliage of sporulation in seedlings of the various plant densities increased sigmoidally with time. Rates of disease progress increased with seedling density (Fig. 17, Table 12).

Logistic regression equations were selected that adequately described incidence of seedlings (YI) and area of foliage (YS) with sporulation in relation to time after planting (T) and planting density (D) (Table 12). In the models for YI and YS, terms of b_0 and b_3 were significant ($P < 0.002$) based on stepwise regression. The relationship between logits of the dependent

variables (YI and YS) and T was significantly linear. There was a linear and cubic relationship between YI and D and a linear and quadratic relationship between YS and D.

Discussion

D1. General discussion

Principal contributions of the studies were: 1, development of effective biological control of *B. cinerea* in container-grown seedlings of black spruce and red pine; 2, quantitative observations of the chief biocontrol agent, *G. roseum* in relation to biological control of *B. cinerea* and in suppressing stress in seedlings associated with infection by the pathogen; 3, discoveries that several inorganic salts can markedly suppress *B. cinerea* in black spruce seedlings; 4, quantitative observations of low light intensity in relation to predisposition of black spruce seedlings to infection by *B. cinerea* (a 'first' for any disease); 5, the discovery that temperatures below freezing can strongly predispose black spruce seedlings to infection by *B. cinerea*; and 6, establishment of quantitative relationships between seedling density of black spruce and rate of increase of gray mold caused by *B. cinerea*. The observed effects of independent variables such as preinoculation low light or freezing conditions, biocontrol agents, and biorational compounds on sporulation incidence and spore production by *B. cinerea* in the seedlings were indirect, and presumably mediated by direct influence of these variables on infection and colonization of the foliage by the pathogen.

D2. Biocontrol of *B. cinerea* in black spruce

D2-1. Evaluation of mycelial fungi as biocontrol agents

The selected isolates of *G. roseum* and M. *verrucaria* markedly suppressed *B. cinerea* in seedlings of black spruce in the greenhouse in both years of evaluation. The isolates suppressed the pathogen as or more effectively than did the programs of recommended fungicides, which were similar to those used for managing gray mold in container production of various conifers. The

isolates were effective under conditions that favored high infection incidence by the pathogen in the first year, and moderate infection incidence in the second year. Levels of naturally-occurring inoculum of *B. cinerea* were probably high in the greenhouses in both years in view of the high sporulation incidence of the pathogen in check seedlings that were not artificially inoculated with the pathogen and the failure of artificially-applied inoculum to significantly increase sporulation incidence. The higher incidence of *B. cinerea* in seedlings in the first year probably resulted in part from greater predisposition of seedlings by the greenhouse environment in that year than in the second year (Zhang and Sutton 1994a). The isolate of *G. roseum* (AL710) used in the greenhouse tests originated from strawberry and was previously found to be highly effective against *B. cinerea* in strawberry and raspberry in the greenhouse and in the field (Peng and Sutton 1991; H. Yu and J.C. Sutton 1991, unpublished observations). An isolate of M. *verrucaria* from strawberry suppressed *B. cinerea* in strawberry in the greenhouse but not in the field (Peng and Sutton 1991). Recovery of *G. roseum* and M. *verrucaria* from spruce in the present study and from strawberry in a previous investigation (Peng and Sutton 1991) indicated that both fungi probably are adapted ecologically to these plants which may be important for effective biocontrol of *B. cinerea* (Sutton and Peng 1993a). Biocontrol of other pathogens by *G. roseum* and M. *verrucaria* was reported (Walker and Maude 1975; Moody and Gindrat 1977; Munnecke 1984; Teyes and Dirks 1985; Inglis and Boland 1992).

Observations in the greenhouse tests that three applications of biocontrol agents were as or more effective than when two or three additional applications were made, indicating that it could be advantageous to suppress *B. cinerea* at early stages of the epidemic. Later treatments may not be needed for effective biocontrol, at least by *G. roseum* and M. *verrucaria*, possibly because these antagonists become established and maintain biocontrol activity in the seedlings after initial introductions.

From the observations in the research greenhouses, *G. roseum* and *M. verrucaria* have potential for managing gray mold in container-grown seedlings of black spruce in production greenhouses. Further investigations are needed, however, to improve strategies for timing applications of the isolates and to optimize biocontrol.

D2-2. Effects of *G. roseum* on infection and on physiological changes associated with infection

From the estimates of sporulation incidence and conidial production by *B. cinerea*, *G. roseum* almost completely suppressed infection of the black spruce seedlings by the pathogen. These observations supported those in section R2-1 and those reported previously (Zhang et al. 1994d). The moderately high incidence of infection (56 - 63%) of seedlings that were treated only with *B. cinerea* and water plus surfactant, and sampled at 6 to 16 days after inoculation, implied that a majority of the seedlings were predisposed to infection by the pathogen (Zhang and Sutton 1994a). Because the seedlings were not predisposed artificially, it is likely the predisposition was effected by microclimate factors such as high temperature which the seedlings were growing in the greenhouse (Zhang and Sutton 1994a). It is not known why sporulation incidence and conidial production by *B. cinerea* were low in seedlings sampled at 2 days after inoculation; possibly the infection process was insufficiently advanced at that time to allow the pathogen to progressively colonize the tissues and sporulate during the sporulation tests.

Besides the strong suppression of *B. cinerea*, *G. roseum* largely prevented the marked increase in electrolyte leakage, the loss of chlorophyll, and the reduction in photosynthesis that were associated with infection by the pathogen. To our knowledge, this is the first demonstration of suppression by a biocontrol agent of pathogen-induced physiological changes in a host. The mechanism by which *G. roseum* suppressed the physiological changes is not

known, but presumably was indirect and associated with the suppression by the antagonist of infection and colonization by *B. cinerea*. In the absence of the pathogen, *G. roseum* did not significantly affect electrolyte loss, chlorophyll content, or rate of photosynthesis in the seedling foliage.

From the increased electrolyte leakage and progressive decline in chlorophyll content and in photosynthesis in seedlings infected by *B. cinerea*, pathogenesis probably was progressive even though no obvious symptoms were present, at least during the first 2 days after the seedlings were inoculated with the pathogen. The marked physiological changes should be interpreted within the context that the isolate of *B. cinerea* (SG224) was highly aggressive in black spruce; it is possible that less aggressive isolates would cause less marked changes in the host. Each of the physiological variables evaluated has potential value as a yardstick of infection, whether or not symptoms are evident. However, measurement of electrolyte loss has the advantages of ease, rapidity, and low cost compared to available methods for estimating chlorophyll and photosynthesis rates.

D2-3. Concentration and time of application of *G. roseum* inoculum in relation to biocontrol

Success of *G. roseum* as a biocontrol agent of gray mold depends on the concentration applied. High concentrations of the agents were effective. Although it is important to examine the concentrations for biocontrol, it is unacceptable to rely on excessive concentration for biocontrol because of costs. Good timing and reasonable concentrations are needed to be figured out.

Application timing is important, and one application of *G. roseum* before infection of pathogen can protect the seedlings against gray mold over the same growing season in this study. Subsequent application of biocontrol agent was not useful, which is similar to the result in the earlier studies (Zhang

et al. 1994d).

D2-4 showed that the biocontrol effectiveness only remained couple of days if *G. roseum* does not establish itself on the seedlings (for example, no or no enough wetness after *G. roseum* was applied). D3 showed that the biocontrol is not effective if *G. roseum* at concentration of 10^6 spores/mL was applied at the same time and after infection of *B. cinerea*. In this study, one application of *G. roseum* at concentration of 10^6 spores/mL protected seedlings over the growing season, which indicated that *G. roseum* had established itself on the seedlings before the pathogen arrived.

Logistic and Gompertz models adequately described the disease progress, and the increase rates of the disease were adequate to present the treatments.

The smooth curves (or the uniform observations) indicated no many other variances occurred in the experiments in the greenhouses.

D2-4. Population dynamics of *G. roseum* in relation to biocontrol

The stability in number of *G. roseum* conidia recovered from the foliage of the black spruce seedlings at various time after the seedlings were inoculated with the antagonist in the growth room and in the greenhouse indicated that the conidia did not germinate on the needles during the week-long studies. The *G. roseum* conidia apparently did not germinate regardless of whether the inoculated seedlings had received the high temperature treatment that is used to predispose seedlings to infection by *B. cinerea*. The RH tested in this experiment on the foliage surface of seedlings in the growth room (70 ± 4%) and greenhouse probably prevented germination.

While the number of recovered conidia was stable, the germinability of the conidia declined rapidly in patterns typical of biological extinction curves

(Zadoks and Schein 1979; Campbell and Madden 1990), both on seedlings in the growth room and on those in the greenhouse. Within 4 days in the growth room, 77 - 96% of conidia had lost germinability, except for those on nonpredisposed seedlings kept at 12 C (28% loss), while in the greenhouse the loss was 79 to 100%. For comparison, germinability of *G. roseum* conidia declined only slowly in storage in glass jars at 3 - 4 C (J.C. Sutton, unpublished observations). Thus, germinability of the conidia is retained longer in cool or cold temperatures than at moderate or warm temperatures. Water potential, irradiance, availability of nutrients, other microbes, and probably other variables also likely influence the rate at which *G. roseum* conidia lose the ability to germinate on leaves or other microhabitats, as occurs with many other fungi (Papavizas 1982,1985; Hildebrand and Sutton 1984; Dickinson 1986; Dubos 1987; Peng et al. 1992). Differences among certain of these variables probably accounted for the lower rates of germination loss of spores on the nonpredisposed seedlings compared to those on the predisposed seedlings.

The loss in germinability of the conidia has major implications in the use strategies of *G. roseum* for biological control of *B. cinerea* in seedlings of black spruce and probably other conifers. Present evidence indicates that *G. roseum* affects biological control of *B. cinerea* after the antagonist has germinated and penetrated the foliage of black spruce (P.G. Zhang and J.C. Sutton 1994, unpublished observations), as it does in strawberry and raspberry (Sutton and Peng 1993; H. Yu and J.C. Sutton, 1993 unpublished observations). Thus, for effective biocontrol, it is important that microclimatic conditions favor germination and penetration before the conidia lose the ability to germinate. At least one period of leaf wetness is probably needed to allow *G. roseum* to germinate, penetrate, and establish in the needles, but relationships of wetness period and temperature to these processes have not been established. Greenhouse microclimate may have to be managed to assure that an adequate wet period occurs after *G. roseum* has been applied to the foliage.

The Weibull regression models of temperature and time after inoculation in relation to germination have potential application for predicting whether *G. roseum* conidia applied to black spruce foliage is sufficient at the time of a postinoculation wet period for the antagonist to effectively suppress *B. cinerea*. From the coefficients of determination, standard error and plotting of residuals, the regressions fit the data of the growth room study very well, and accurately predicted germination of conidia on black spruce foliage in the greenhouse. Even though the percent germination of the conidia may decline rapidly, the data indicated that germination could be sufficient for effective biological control even after three to four days, depending on the concentration of inoculum applied and the postinoculation microclimate. A loss of 99% germination would not be serious, for example, when 10^8 conidia/mL are applied to the foliage and 10^6 conidia/mL would be sufficient for adequate biological control.

D3. Biological control of *B. cinerea* in red pine

D3-1. Concentration and time of application of G. roseum in relation to biocontrol in the growth room

Gliocladium roseum effectively suppressed infection of red pine seedlings by *B. cinerea* in the growth room. The antagonist applied at 10^8 conidia/mL suppressed incidence of the pathogen in the seedling needles by about 87% even though the isolate of the pathogen (SG224) used in the study was highly aggressive to red pine, the inoculum concentration of *B. cinerea* was high (10^6 conidia/mL), and postinoculation wetness and temperature were highly favorable for infection of conifer seedlings by the pathogen (Zhang and Sutton 1994b). The observations that *G. roseum* suppressed *B. cinerea* only when inoculum concentration of the antagonist was ten or one hundred times higher than that of the pathogen was consistent with findings in similar studies of *G. roseum* against *B. cinerea* in strawberry (Peng and Sutton 1991, Sutton and Peng 1993b)

and raspberry (H. Yu 1994 unpublished observations). The basis of the apparent stimulation of infection of red pine seedlings by *B. cinerea* when inoculum concentration of *G. roseum* was low (10^2 or 10^4 conidia/mL) is not known.

Time of application of *G. roseum* in relation to that of *B. cinerea* was critical for effective suppression of the pathogen. The antagonist (10^8 conidia/mL) effectively suppressed infection only when applied 0 - 6 days before the seedlings were inoculated with *B. cinerea* (10^6 conidia/mL). Infection was suppressed slightly or not at all when *G. roseum* was applied 2 - 6 days after the pathogen. The complete or near-complete suppression when *G. roseum* was applied before, or at the same time as, the pathogen was consistent with observations of the preceding experiment and with findings in biocontrol studies of *B. cinerea* in black spruce (P.G. Zhang unpublished observations). The wet period after each application of *G. roseum* was favorable for conidia germination and penetration of the needles by the antagonist (R2-4, Zhang et al. 1994c), a factor that was probably important of suppression of *B. cinerea*. It can be concluded from the observations that for biocontrol of *B. cinerea* on red pine seedlings, inoculum of *G. roseum* should be applied before conidia of the pathogen are dispersed to the foliage and the microclimate becomes favorable for the pathogen to infect the needles.

D3-2. Inoculum concentration of *G. roseum* in relation to biocontrol in the greenhouse and outdoors

The substantial suppression of *B. cinerea* by *G. roseum* in second- and third-year seedlings produced in the presence of natural sources of pathogen inoculum underscored the potential value of the antagonist in managing *B. cinerea* in red pine production systems. The high incidences of infection of untreated check plants indicated that inoculum of *B. cinerea* was present in the nursery and in the greenhouse used for the studies. The inoculum concentrations of *G. roseum* needed to effectively suppress *B. cinerea* in the

red pine seedlings (10^7 - 10^8 conidia/mL) were similar to those required to control the pathogen in black spruce (R2-3). One application of *G. roseum* (10^8 conidia/mL) was highly effective in the initial study of inoculum concentration conducted in the greenhouse, and the program of 5 applications (10^7 conidia/mL) of the antagonist were as effective as the standard fungicide program both in the greenhouse and in the outdoor section of the nursery. Collectively, the observations point to value of *G. roseum* for suppressing *B. cinerea* in naturally-infected seedlings, including those that were infected prior to treatment with the antagonist.

D4. Biorational compounds in relation to control of B. cinerea in black spruce

D4-1. Effects of inorganic salts, carbohydrates and nitrogenous compounds on growth of *B. cinerea* and of *G. roseum* on water agar

The studies of biorational compounds in relation to germination, germ tube growth, and colony growth of *B. cinerea* and *G. roseum* on water agar provided a perspective of substances that may have potential for direct suppression of the pathogen on conifer seedlings and other hosts, or for interacting with *G. roseum* to enhance biological control (Chou 1972; Clark and Lorbeer 1977). Many of the monosaccharides and other tested carbohydrates stimulated germination and growth of *B. cinerea*, as anticipated from numerous previous reports (Blakeman 1975,1980), and also of *G. roseum* (Jackson et al. 1991). D-arabinose, however, suppressed *B. cinerea* but stimulated *G. roseum*, and thus may have value for direct control of the pathogen, or as an adjuvant with *G. roseum* in biological control (??search literature on D-arabinose vs. fungal growth/development). Among the amino acids and other nitrogenous compounds evaluated L-lysine was inhibitory to *B. cinerea* but moderately stimulated *G. roseum*, and thus could have value as a biorational compound or biocontrol adjuvant with *G. roseum*.

The most promising biorational compounds were certain of the inorganic salts, especially the ammonium salts, potassium carbonate, sodium carbonate, sodium bicarbonate, and dibasic sodium phosphate which suppressed *B. cinerea* completely or almost completely. These compounds also suppressed germination and /or growth of *G. roseum*, so are not expected to be of value as adjuvants with the antagonist in biological control. The high effectiveness of sodium carbonate and sodium bicarbonate is consistent with earlier observations of these substances in relation to sclerotial germination of *Sclerotium rolfsii* (Punja and Grogan 1982), cucumber powdery mildews caused by *Sphaerotheca fuliginea* (Homma et al. 1981) and rose powdery mildew and black spot caused by *Sphaerotheca pannosa* (Wallr.:Fr.) Lev. var. rosae Woronichin and *Diplocarpon rosae* F.A. Wolf, respectively (Horst et al. 1992).

D4-2. Effects of inorganic salts on infection

From the strong suppression of *B. cinerea* in black spruce seedlings by potassium carbonate, sodium carbonate, and sodium bicarbonate, these salts appear to have promise as biorational compounds against the pathogen. These salts were highly effective even though a highly aggressive isolate of the pathogen was used, and the inoculum concentration of the isolate was high (10^6 conidia/mL). The strong performance of the carbonates and bicarbonate against *B. cinerea* was similar to the effects of these salts on powdery mildew of cucumber and roses (Homma et al. 1981; Horst et al. 1992).

The strong suppression of *B. cinerea* in seedlings by potassium carbonate, sodium carbonate, and sodium bicarbonate agreed with the observed effects of these salts against the pathogen on water agar. However, several other salts that performed well in the agar tests were ineffective or only marginally effective in the seedling tests. Inconsistencies in effectiveness between the tests were reflected by the moderate to low values of Pearson's coefficients in the correlation analyses of data obtained on agar and on the seedlings.

Effectiveness of agar tests for predicting performance of biorational compounds against *B. cinerea* in conifer seedlings is thus highly questionable, as is often the case also with fungicides and candidate organisms for biological control.

There are a few reports describing the effects of carbonate (CO_3^{-2}) and bicarbonate (CO_3^{-1}) on fungi. Leach and Davey (1942) reported that ammonium was toxic to mycelium of *Sclerotium rolfsii*. In the studies on effect of bicarbonate on morphogenesis in the aquatic mold *Blastocladiella emersonii*, low levels of bicarbonate in the medium triggered the formation of brown, thick-walled, resistant sporangia from thin-walled, colorless thalli that in the absence of bicarbonate would have developed into thin-walled, papillate sporangia (Cantino 1956,1966). CO_2 or dissolved CO_3^{-1} induced formation of sclerotia of *Phymatotrichum omnivorum* (Lyda and Burnett 1971,1975). Macauley and Griffin (1969) observed a reduction in mycelial dry weight of *Fusarium acuminatum*, *Cochliobolus spicifer*, and *Gibberella zeae* in the presence of 10% CO_2, but the decrease was most pronounced only when the pH of the medium was increased from 4 to about 7. Horst et al. (1992) postulated effective control of Sphaerotheca *pannosa* var. *rosae* and *Diplocarpon rosae* in roses by sodium bicarbonate was attributable to fungicidal characteristics of bicarbonate ions.

D4-3. Effects of inorganic salts on biocontrol

Effects

D4-4. Effects of sodium bicarbonate on infection

Effects

D5. Epidemiology of gray mold in black spruce

D5-1. Low light intensity in relation to infection

Black spruce seedlings were predisposed to attack by *B. cinerea* chiefly when the intensity of PAR before inoculation was extremely low or zero ($\leq$10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for several days or weeks. From the observations of sporulation in the growth room study, the pathogen infected one third or more of the needles of seedlings that were kept at 0 and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for about 3-5 weeks, but few needles of the seedlings that were kept at 15 and 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and only after the light treatment was prolonged (39-42 days). The prolonged treatment periods increased the probability that seedlings were predisposed by confounding factors, such as natural senescence and the moderate treatment temperature (30 °C), as opposed to low light intensity (Jarvis 1977; Zhang and Sutton 1993; Zhang and Sutton 1995). From the observations in the greenhouse, the highest light intensity at which seedlings were predisposed to *B. cinerea* was near 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However this value was probably an overestimate because light measurements used to produce the response curves (Fig. 14) were taken in the canopies near midday under sunny conditions, and thus represented peak, or near peak, daily values. Continuous measurement of light in canopies would be needed to examine daily patterns of light intensity in relation to predisposition. Low light conditions were reported to predispose several other hosts to attack by *B. cinerea* (cited by Jarvis 1977, 1992), but to our knowledge, the present study is the first in which the relationship of light intensity and predisposition was quantified and a threshold value of light intensity for predisposition estimated (Jarvis 1992).

The patterns of increase and subsequent decline in sporulation incidence of *B. cinerea* with increasing duration of preinoculation treatment in PAR of 0 and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 13) indicated that needles that become predisposed to infection were later less susceptible to attack or invasion by the pathogen. The observation of sporulation incidence thus included needles that were never predisposed to infection, those that were predisposed, and those that had been but were no longer predisposed. The increases in sporulation incidence in

needles of seedlings kept at 0 and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ before inoculation in each instance coincided with extreme chlorophyll loss, while the subsequent declines in sporulation coincided with development of reddish brown discoloration, partial desiccation, and a high incidence of various other fungi in the needles. The patterns of sporulation incidence observed in response to the low light levels were similar to those reported in seedlings of black spruce that were subjected to preinoculation drought stress (Zhang and Sutton 1994a). Possible explanations for the declining incidence of predisposition after prolonged preinoculation treatment include physiological changes in affected needles and invasion of the severely stressed needles by various microorganisms. Perhaps, for example, cell membranes in affected tissues become increasing permeable and disrupted, resulting in loss of nutrients and water. *Botrytis cinerea* normally attacks tissues with high levels of sugars (Jarvis 1977,1992) so that loss of sugars, and perhaps other nutrients, from damaged tissues would be expected to reduce invasion by the pathogen. Similarly loss of water retention ability in affected tissues could result in water potentials, and the physical relationships of water with the tissues, becoming unfavorable for growth and development of the pathogen. From the observations of sporulation of various fungi, many affected needles were invaded by weak pathogens or saprotrophs (Schoeneweiss 1975; Bernstein and Carroll 1977; Cooke and Rayner 1984) which could have suppressed development of *B. cinerea* in the tissues. Several microfungi and bacteria were reported recently to suppress growth and sporulation of the pathogen in needles of black spruce seedlings (Zhang et al. 1994c).

Chlorophyll loss probably plays a key role in predisposition induced by low light intensity. Seedlings generally were predisposed to *B. cinerea* only when light intensity was sufficiently low that the chlorophyll content fell below 1.3-1.4 µg/6 mm segment (Fig. 13). In these treatments, light intensity values below this threshold coincided with the predisposition. The moderate correlations between chlorophyll content and sporulation incidence in response

to low light intensity are similar to those observed previously in response to high temperature plus darkness, and to drought (Zhang and Sutton 1994a).

The observations that needles were frequently predisposed to *B. cinerea* when light intensity was extremely low ($\leq$10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for several days or weeks, but infrequently when light intensity was higher for up to 42 days, are of epidemiological importance and provide a basis for improved control of the disease. Maintenance of daily light intensity within canopies at or above 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, such as by removing side and roof coverings of the greenhouse, by increasing spacing of seedlings, or through use of a subcanopy light source, probably has considerable potential for managing the disease (Tuller and Peterson 1988; Sutherland et al. 1989). Another potential application is to predict predisposition of needles from periodic measurements of light intensity in seedling canopies as a means to optimize timing of application of fungicides, biological control agents, or other treatments.

D5-2. Freezing conditions in relation to infection

The observations of sporulation of *B. cinerea* on the needles indicated that freezing conditions predisposed black spruce seedlings to attack by *B. cinerea*. The pathogen infected seedlings with completely green foliage only when they were subjected to freezing conditions before inoculation with the pathogen. No signs of *B. cinerea* or symptoms of gray mold were observed in similar seedlings that did not receive the freezing treatments.

The predisposition of the seedlings to *B. cinerea* was quantitatively a function of the level and duration of the freezing treatment and seedling age. In general, the period of preinoculation freezing required for a given level of infection and spore production by *B. cinerea* decreased as the temperature was decreased, but increased as seedling age increased. Treatment at -6 and -10 C strongly predisposed seedlings 2 and 3 months old, but 4-month-old

seedlings were strongly predisposed only at -10 C. Treatment at -2 C resulted in little predisposition. In view of the high incidence of predisposition of 2- and 3-month-old seedlings after only one hour at -10 C, it is likely that some seedlings would be predisposed after only a few minutes at this temperature. The striking similarities in patterns of electrolyte leakage with those of sporulation incidence and spore production underscored the value of electrolyte measurements as and indicator of infection of seedling foliage, especially before signs or symptoms develops.

The low-temperature predisposition of black spruce seedlings to infection by *B. cinerea* was probably mediated by physiological changes in the host associated with injurious strain caused by the low temperature (Levitt 1972; Schoeneweiss 1975; Creighton et al. 1986). With warm-temperature plants, chilling may induce greater leakiness of cells, lower oxidative activity (and hence energy supply), and greater accumulation of the products of fermentation, which might account for the greater susceptibility of the plants to pathogens such as *Fusarium*, *Pythium*, and *Rhizoctonia* at lower temperatures (Cook and Baker 1983). Generally cold-hardy plants such as conifers are naturally protected from freeze damage in at least two ways (Burke et al. 1976). In most hardy plants, water moves out of the cells and then freezes extracelluarly. Another mechanism of hardiness involves supercooling of the water within the protoplasm. Ice formation in the plant tissues may destroy cell structure and increase susceptibility of the plant when thawed.

The observations of low temperature predisposition to *B. cinerea* have implications in the production of black spruce seedlings in northern Ontario. Fluxes of cold air entering greenhouses through ventilation or doorways could predispose seedlings to infection by the pathogen, for example during the early portion of the production season in February, March, and April. Container-grown seedlings could also be predisposed by low temperature after they are transferred from greenhouses to outdoor sections of nurseries in the

autumn. This could lead to infection of the foliage by *B. cinerea* during the autumn and winter. Potential methods for protecting seedlings against infection include avoidance of low temperatures in seedling production systems; application of an effective biocontrol agent such as *G. roseum*; treatment of seedlings with a suitable fungicide; and use of chemicals, such as paclobutrazol, that reduce environmental stresses in black spruce seedlings (Markhart 1984; Zhang et al. 1994b). When feasible hardening-off of seedlings could help to reduced predisposition, however the relationship between hardening off and low temperature predisposition to *B. cinerea* remains unknown.

D5-3. Planting density on black spruce seedlings in relation to progress of gray mold

The observations of sporulation of *B. cinerea* on the black spruce seedlings indicated that the standard density of one seedling per cell of the seedling flats was highly conducive to gray mold epidemics. This density is equivalent to 1555 seedlings/m^2 seedling flat, and stems of seedlings planted at this density are about 2.5 cm apart in the two directions of a grid arrangement. Doubling of the density to two seedlings per cell increased sporulation incidence of *B. cinerea*, and reducing the density to one seedling in every two or four cells decrease the sporulation incidence. The rate of disease progress significantly increased with the increase in planting density (Table 12).

Wetness duration and light intensity were probably key variables that contributed to the increased rate of disease progress as planting density was increased. From the microclimatic measurements in the seedling canopies, wetness periods generally increased and light intensity decreased with increased in planting density. Long periods of high RH or leaf wetness, and low light intensity, favor gray mold in various kinds of container-grown conifer seedlings (Mittal et al. 1987; Peterson et al. 1988; Dugan and Blake

1989; Peterson and Sutherland 1990). In black spruce seedlings, single wetness periods of at least 8 - 12 h are needed at 12 - 28 C for *B. cinerea* to infect the foliage, and infection progressively increases when wetness duration is increased above the threshold values (Zhang and Sutton 1994b). Whether or not *B. cinerea* is able to infect seedlings during successive wetness periods that are shorter than the thresholds for single wetness periods is not known. Wetness is required also for sporulation of the pathogen on the foliage but quantitative relationships of wetness periods, temperature, and sporulation have not been determined. Low light intensity predisposes black spruce seedlings to infection (Section D5-1; Zhang et al. 1995), but does not influence infection directly (Zhang and Sutton 1994b). Seedlings can be predisposed when light intensity (400 - 700 nm) is $\leq$ 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for at least 6 - 12 days, but are not predisposed at higher intensities (Section D5-1; Zhang et al. 1995). Light intensity in the seedling canopies of the present study was below the threshold of 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for longer periods as the seedling density, and thus canopy density, increased.

The observations indicate that reduction in density of black spruce seedlings in production systems would reduce severity of gray mold, but this measure is unlikely to be cost effective and practical given the premium importance of space in greenhouses. Favorableness of the standard canopy density in prolonging wetness periods could be reduced by increasing the circulation of air through the canopies and possibly by using a layer of Perlite (Plant Products Ltd., Brampton ON) or other relative material on the surface of the growth medium. Peterson and Sutherland (1990) recently found that styroblocks (growing container) modified with vertical ventilation holes which allow increased air circulation in seedling canopies, reduced severity of gray mold epidemics in Douglas-fir seedlings. Such effects of the seedling trays would be expected also in black spruce and other conifers. Good air circulation is critical for managing gray mold in many kinds of crops grown in greenhouses (Jarvis 1992). Use of a strongly reflective material on the growth medium

could increase light intensity in seedling canopies sufficiently to avoid or reduce low light predisposition of seedlings to infection by *B. cinerea*, and thereby suppress disease, but requires investigation.

D6. Integrated considerations

The results of this study contribute to a framework of information that can allow major improvement in the management of gray mold in black spruce seedlings which reducing or avoiding fungicide use. Methods for improved management of gray mold fall into three principal categories: biological control, biorational control, and partial regulation of canopy microclimate of seedlings in greenhouses and outdoors.

D6-1. Biological control

The findings that *G. roseum* effectively suppressed *B. cinerea* in seedlings of black spruce and red pine indicated that biological control of gray mold is feasible, and has excellent potential as an alternative to chemical fungicides for managing gray mold in seedling production systems. Applied in suitable inoculum concentrations, the antagonist was consistently effective under a substantial range of conditions in the growth room, greenhouse, and outdoors. Without exception, *G. roseum* suppressed *B. cinerea* as or more effectively than did the standard fungicides benomyl, chlorothalonil, and iprodione. The observations that one application of *G. roseum* to black spruce seedlings at the canopy closure stage was as effective as programs of 2 to 6 *G. roseum* treatments or 6 fungicide treatments underscored the high level of biocontrol efficiency that can be expected with the antagonist. While *G. roseum* was generally more effective when applied prior to inoculation with *B. cinerea*, the antagonist nonetheless suppressed the pathogen as effectively as the fungicide chlorothalonil when applied to seedling populations that were infected from natural sources before and after the biocontrol treatment. To

our knowledge, our studies represent the first successful demonstration of biological control of gray mold in container-grown conifer seedlings. Application of *G. roseum* against *B. cinerea* in black spruce ranks as one of the most effective biocontrol systems against foliage pathogens ever reported (Sutton and Peng 1993a).

Gliocladium has major advantages as a biocontrol agent in conifer seedlings. Inoculum of the antagonist is easy to produce on cheap substrates such as wheat grain (Sutton and Yu 1994), and has good longevity when suitably stored (J.C. Sutton, unpublished observations). The fungus is consistently effective against *B. cinerea* and, given suitable humid periods, appears to have a long period of activity against the pathogen after application in container-grown black spruce seedlings. Because of its activity over a wide temperature range in the present studies and in earlier work (Sutton and Peng 1993b), *G. roseum* should prove effective at most times of the year in conifer seedling production systems. When handled by normal procedures, *G. roseum* should not represent any risk to human health or the environment. Spores of the pathogen are sticky and do not readily become airborne except by splashing water or in aerosols; thus risks of allergies in those who handle the fungus should be extremely small given that common-sense precautions are taken. From the observations in the present study (especially section R2-2), *G. roseum* did not in any way adversely affect the growth and vigor of black spruce and red pine seedlings.

D6-2. Biorational control

Among the compounds evaluated, potassium carbonate, sodium carbonate, and sodium bicarbonate have potential for use as biorational compounds against *B. cinerea* in container-grown seedlings of black spruce and probably of other conifers. Each of these salts strongly suppressed *B. cinerea* when tested on water agar and on black spruce seedlings in the growth room. Further studies

are needed to determine relationships of independent variables such as concentration and time of application of the salts and of *B. cinerea* inoculum, and microclimatic conditions, in relation to dependent variables such as infection of seedlings by the pathogen, progress of gray mold, and growth of the seedlings. Some of the evaluations should be done in greenhouses under conditions similar to those of reasonably well-managed seedling production systems. The growth-room studies did not reveal any obvious adverse effects of the carbonates on seedling growth, which may bode well for the value of these compounds in greenhouses and outdoors. Although the carbonates also suppressed the biocontrol agent *G. roseum*, it may nonetheless be possible to integrate use of these biorational compounds with biological control. For example, the carbonates may not reduce the effectiveness of *G. roseum* when applied after the antagonist has penetrated into the foliage.

The studies on water agar identified D-arabinose and L-lysine as possible candidates for biorational control of *B. cinerea* in conifer seedlings. These results should be regarded as preliminary, and the compounds require testing against the pathogen on seedlings to determine whether they have any true value for disease control.

Many of the inorganic salts, carbohydrates, and nitrogenous compounds that stimulated germination and growth of *B. cinerea*, infection of black spruce seedlings by the pathogen may threaten to seriously increase gray mold should they be introduced into seedling production systems. Strong promotion of infection, for example, by several of the ammonium salts raises the question as to whether fertilizers, especially the soluble formulations, may increase gray mold should they be sprayed or splashed onto the seedling foliage. Similarly, the stimulation of *B. cinerea* by many sugars and amino acids may indicate that infection or spore production by the pathogen would increase should these compounds increase on the surface of seedling foliage, as may happen through exudation from seedlings that are environmentally stressed or

attacked by aphids.

D6-4. Microclimate regulation

Our present and earlier studies have identified environmental predisposition as a major factor in epidemics of gray mold in container-grown seedlings of black spruce. Seedlings that are not environmentally predisposed to attack by the pathogen are unlikely, or much less likely, to be infected, even by highly aggressive strains of *B. cinerea* (Zhang and Sutton 1994a). In our earlier studies, high temperature plus darkness, and drought were identified as powerful predisposing factors (Zhang and Sutton 1994a). Additional predisposing factors identified and quantified in the present work were low light intensity and temperatures below freezing.

Avoidance of environmental stresses in seedling production system is an important goal in order to protect seedlings against *B. cinerea* and optimize production of good quality seedlings. Complete avoidance of environmental conditions that predispose seedlings may not be feasible in some production situations, however simple and practical measures may suffice and be advantageous in many instances (Jarvis 1989). For example, it would likely be feasible to avoid predisposition by freezing temperatures simply by protecting seedlings against cold draughts from ventilators when outdoor temperatures are low, or against low temperatures shortly after containers of seedlings are transferred outdoors from greenhouses in the Autumn. Commercially-available row covers, such as those used in strawberry fields for frost protection, could have value against freezing stress in black spruce in the fall. Effectiveness of such measures justify study.

Application of stress protectant chemicals is another potential approach to preventing environmental predisposition of seedlings to attack by *B. cinerea*, and thus controlling gray mold. Many stress protectants are triazoles, of

which paclobutrazol has already been shown to protect black spruce seedlings against predisposition to gray mold induced by high temperature and drought (Marshall et al. 1991; Zhang et al. 1994b). Paclobutrazol and other triazoles would also be likely to protect seedlings against stresses induced in black spruce seedlings by low light intensity and freezing temperatures. While stress protectants represent a form of chemical control, they are often effective for long periods when used in extremely small doses, for example on seeds (Fletcher and Hofstra 1985,1988). Quantities of stress protectant required to manage gray mold would probably be magnitudes smaller than those of conventional fungicide treatments.

D6-4. Integrated control

It is anticipated that highly flexible and effective control programs against gray mold could readily be developed by integration of biocontrol treatments, biorational treatments, and measures to minimize environmental predisposition, with other key practices such as sanitation and humidity control. Use of chemical fungicides can almost certainly be markedly reduced or eliminated altogether while maintaining adequate disease control and optimizing production of high quality seedlings.

Conclusions and Recommendations

C1. General conclusions

1. *Botrytis cinerea* was controlled effectively in container-grown seedlings of black spruce and red pine by means of the biological control agent *Gliocladium roseum*.

2. Applied at appropriate inoculum concentrations, *G. roseum* consistently suppressed *B. cinerea* in container-grown seedlings of black spruce under a wide range of microclimatic conditions in the growth room, greenhouse, and outdoors, and as or more effectively than conventional fungicide treatments.

3. As few as one application of *G. roseum* was sufficient to control *B. cinerea* in black spruce seedlings in the greenhouse over the growth season.

4. *Gliocladium roseum* did not adversely affect treated seedlings, but did prevent, or almost prevent, pathogen-induced loss of electrolytes and chlorophyll, and reduced photosynthesis rates in the foliage of seedlings inoculated with *B. cinerea*.

5. Logistic and Gompertz regression models effectively predicted effects of *G. roseum*, applied at different inoculum concentrations at various times, on sporulation, and by inference infection, of *B. cinerea* in black spruce seedlings.

6. Weibull regression models effectively described relationships between temperature, time of inoculation, and germinability of conidia of *G. roseum* applied to foliage of black spruce seedlings.

7. The biorational compounds potassium carbonate, sodium carbonate, and sodium bicarbonate effectively suppressed *B. cinerea* in the tests on an agar medium and on black spruce seedlings in the greenhouse, and may have value for controlling gray mold in container-grown seedlings in greenhouses or outdoors.

8. Sugars, amino acids, and several other carbohydrates and nitrogenous compounds in most instances had little effect on or markedly increased, growth and sporulation of *B. cinerea* on water agar, and thus probably would have no value or prove disadvantageous against the pathogen on coniferous seedlings. Among the substances tested, only D-arabinose and L-lysine merit further study.

9. Light intensity (400 - 700 nm wavelength) of ≤10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ predisposed black spruce seedlings to infection by *B. cinerea*.

10. Regression models adequately described sporulation (and by inference, infection) of *B. cinerea* and chlorophyll content in black spruce needles as a function of light intensity and duration of light treatment.

11. Temperatures of -6 and -10 C strongly predisposed black spruce seedlings 2- and 3-months-old to infection by *B. cinerea*, and -10 C strongly predisposed 4-month old seedlings.

12. Conventional planting density of black spruce seedlings in size 308 paper seedling containers was strongly conducive to rapid progress of gray mold epidemics in the seedlings, and epidemic rates could be markedly reduced by using lower planting densities.

13. Measurement of electrolyte leakage was a sensitive and efficient method for indirect estimation of symptomless and signless infection of black spruce seedlings by *B. cinerea*.

14. Collectively, the observations of biocontrol, biorational compounds, and environmental predisposition provided a framework for efficient and flexible control programs against gray mold in container-grown seedlings of black spruce without the use of chemical fungicides.

C2. Recommendations

1. *Gliocladium roseum* should be developed and registered for use as a biological control agent against *Botrytis cinerea* in container-grown seedlings of black spruce and other conifers.

2. Additional isolates of *G. roseum* and isolates of other mycelial fungi should be evaluated for biocontrol of *B. cinerea* in an attempt to identify other effective agents that could add flexibility in biocontrol of the pathogen in seedling production systems.

3. Potassium carbonate, sodium carbonate, and sodium bicarbonate should be evaluated for effectiveness as biorational substances in controlling *B. cinerea* in container-grown seedlings of black spruce and other conifers in greenhouses under conditions similar to those of commercial production systems.

4. The feasibility for integrating biological control by means of *G. roseum* and biorational control by means of potassium carbonate, sodium carbonate, and sodium bicarbonate should be explored.

5. Methods to increase light intensity and to reduce humidity within canopies of container-grown conifer seedlings without sacrificing planting density, and effects of the methods on seedling predisposition to, and infection by, *B. cinerea* should be explored.

6. Stress-protectant substances should be explored further in relation to protecting seedlings of black spruce and other conifers against predisposition to *B. cinerea* induced by freezing temperatures, high temperatures, low light intensity, drought and other environmental factors.

7. The large amount of quantitative data on the epidemiology and control of gray mold, that has accumulated in the present and earlier studies, should be integrated into practical systems to optimize disease control within the context and realities of seedling production. We visualize development of a flexible system of wide applicability that is driven by information on the microclimate, pathogen, and host seedlings, and predicts when specific measures should be taken, which could include microclimate adjustment, introduction of a biocontrol agent, application of a biorational compound, use of a conventional fungicide, and other practices.

8. A practical manual should be produced for the industry to serve as a guide in managing gray mold in seedling production systems. The manual should include predictive systems developed under recommendation 7, and be structured such that it can easily be updated as new information becomes available.

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Table 1. Estimated parameter values and coefficients from logistic models for sporulation incidence of *Botrytis cinerea* (YI) and area of foliage with sporulation of the pathogen (YS) in black spruce seedlings as functions of inoculum concentration of *Gliocladium roseum* (C) and time (T).

	Estimated parameters						
	b_0	b_1	b_2	b_3	R^2	R^2_a	s
Sporulation incidence of *B. cinerea* (YI)							
G. roseum	-35.937 (2.1432)	0.0083 (0.1900)	1.9380 (0.1116)	-0.0048 (0.00989)	0.86	0.85	2.231
Chlorothalonil	-36.791 (2.4825)	1.9514 (0.1292)			0.89	0.89	2.002
Area of foliage with sporulation of B. cinerea (YS)							
G. roseum	-25.911 (1.4420)	0.2340 (0.1279)	1.2621 (0.0751)	-0.0168 (0.00665)	0.82	0.82	1.501
Chlorothalonil	-24.001 (2.0848)	1.1165 (0.1085)			0.79	0.78	1.681

Note: values are presented of estimated parameters (b_0 - b_3), coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R^2_a), and the standard deviation about the regression line (s). The logistic regression equation $\ln[Y/(1-Y)] = b_0 + b_1C + b_2T + b_3CT$ is used for Y (YI and YS) in relation to inoculum concentration (C) and time (T), and $\ln[Y/(1-Y)] = b_0 + b_1T$ for Y (YI and YS) in relation to T at treatment of chlorothalonil. Numbers in parentheses are standard errors of the corresponding parameter values. The intercept b_0 is the value of $\ln[\ln(1/(1-Y))]$ when T = 0 and ln(D) = 0. YI and YS are the combined data of repetitions 1 and 2.

Table 2 Estimated parameter values and coefficients from logistic models for increase in sporulation incidence of *Botrytis cinerea* (YI) and from Gompertz models for increase in area of foliage with sporulation of the pathogen (YS) in black spruce seedlings as functions of time (T) under various times of treatment of *Gliocladium roseum* and chlorothalonil.

Times of treatments						Estimated parameters				
June		July			Aug.	b_0	b_1	R^2	R^2_a	s
20	27	4	18	31	15					
Sporulation incidence of *B. cinerea* (YI)										
W	W	W	W	W	W	-36.016 (3.2312)	1.8945 (0.1682)	0.82	0.81	2.606
G	W	W	W	W	W	-38.176 (3.6517)	2.0390 (0.1901)	0.80	0.80	2.945
G	G	W	W	W	W	-35.366 (3.5186)	1.8770 (0.1832)	0.80	0.78	2.838
G	G	G	W	W	W	-37.402 (2.7211)	1.9866 (0.1417)	0.88	0.87	2.195
G	G	G	G	W	W	-33.072 (3.2050)	1.7199 (0.1668)	0.79	0.78	2.585
G	G	G	G	G	W	-36.566 (3.1964)	1.9198 (0.1664)	0.83	0.82	2.578
G	G	G	G	G	G	-35.831 (3.3298)	1.9040 (0.1733)	0.81	0.81	2.685
F	F	F	F	F	F	-37.035 (3.0772)	1.9815 (0.1610)	0.85	0.84	2.482
Area of foliage with sporulation of *B. cinerea* (YS)										
-	-	-	-	-	-	-9.459 (0.4868)	0.4874 (0.0253)	0.93	0.93	0.393
G	-	-	-	-	-	-7.669 (0.5395)	0.3676 (0.0281)	0.86	0.86	0.435
G	G	-	-	-	-	-7.276 (0.4906)	0.3460 (0.0255)	0.87	0.86	0.396
G	G	G	-	-	-	-7.373 (0.5715)	0.3510 (0.0297)	0.83	0.83	0.461
G	G	G	G	-	-	-7.532 (0.5392)	0.3602 (0.0281)	0.86	0.85	0.435
G	G	G	G	G	-	-7.983 (0.5380)	0.3835 (0.0280)	0.87	0.87	0.434
G	G	G	G	G	G	-7.232 (0.6128)	0.3430 (0.0319)	0.81	0.80	0.494
F	F	F	F	F	F	-8.250 (0.4331)	0.4064 (0.0226)	0.92	0.92	0.349

Note: values are presented of estimated parameters (b_0 - b_1), coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R^2_a), and the standard deviation about the regression line (s). The logistic equation $\ln[YI/(1-YI)] = b_0 + b_1T$ for sporulation incidence of *B. cinerea* in relation to time under various times of treatments, Gompertz equation $-\ln[-\ln(YS)] = b_0 + b_1T$ for area of foliage with sporulation of the pathogen in relation to time under various times of treatments. Numbers in parentheses are standard errors of the corresponding parameter values. The intercept b_0 is the value of $\ln[\ln(YI/(100-YI))]$ and $-\ln[-\ln(YS)]$ when $T = 0$. In the treatment programs, 'W'' presents one application of water plus surfactant, 'G' one application of G. roseum, and 'F' one application of chlorothalonil.

Table 3. F tests and significance levels (Prob > F) from analysis of variance of the effects of replication, temperature, and time after inoculation of black spruce seedlings with *Gliocladium roseum*, on number of conidia of *G. roseum* recovered per seedling, percent germination of the recovered conidia, and sporulation incidence of *G. roseum* on needles of the seedlings

Variable[a]	Predisposed		Non-predisposed	
	F	Prob>F	F	Prob>F
Number of recovered conidia				
Replication	1.54	0.2273	1.24	0.2986
Temperature	0.88	0.4815	1.06	0.4283
Time	0.42	0.9032	0.55	0.8099
Temperature x time	0.72	0.7353	0.40	0.9636
Percent germination of recovered conidia				
Replication	1.56	0.2209	1.42	0.2529
Temperature	38.56	0.0024	367.37	0.0001
Time	380.18	0.0001	376.83	0.0001
Temperature x time	16.60	0.0001	16.36	0.0001
Sporulation incidence of G. roseum on seedling needles				
Replication	0.15	0.8640	1.51	0.2328
Temperature	3.80	0.1191	2.30	0.2161
Time	1.09	0.3899	0.50	0.8500
Temperature x time	0.55	0.8764	0.23	0.9970

[a] Error terms were temperature x replication for temperature and temperature x duration x replication for other variables.

Table 4. Estimated parameters values and coefficients from Weibull regression model for incidence of ungerminated conidia of *Gliocladium roseum* recovered from seedlings of black spruce (Y), which received or did not receive high temperature treatment for predisposition to infection by *Botrytis cinerea*, as a function of temperature (T) and time after the seedlings were inoculated with the antagonist (D)

	Estimated parameters							
	b_0	b_1	b_2	b_3	R^2	R^2_a	R^{*2}	s
Predisposed seedlings	-2.2121 (0.2738)	0.0307 (0.0126)	0.0525 (0.0043)	-0.0012 (0.00017)	0.916	0.912	0.923	0.350
Nonpredisposed seedlings	-4.4101 (0.6122)	0.0602 (0.0282)	0.0555 (0.0096)	-0.0011 (0.00039)	0.773	0.763	0.875	0.783

Note: values are presented of estimated parameters (b_0 - b_3), coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R^2_a), coefficient of determination for the back transformed observation ($R^{*2} = 1 - \Sigma(\text{observed value - predicted value})^2/\Sigma(\text{observed value - mean observed value})^2$), and the standard deviation about the regression line (s). The Weibull regression equation is $\ln[\ln(100/(100-Y))] = b_0 + b_1T + b_2T\ln(D) + b_3T^2\ln(D)$, in which Y is the combined data of repetitions 1, 2, and 3. Numbers in parentheses are standard errors of the corresponding parameter values. The intercept b_0 is the value of $\ln[\ln(100/(100-Y))]$ when $T = 0$ and $\ln(D) = 0$.

Table 5. Effect of inorganic salts on conidial germination, germ tube length and colony growth of *Botrytis cinerea* on water agar medium inoculated with the pathogen

Salt[a]	Formula	Conidial germination (%)[b]	Germ tube length (μm)[b]	Colony diameter (mm)[c]
ammonium carbonate	$(NH_4)_2CO_3$	0.0 e[d]	0.0 i	0.0 i
ammonium chloride	NH_4Cl	0.0 e	0.0 i	0.0 i
ammonium nitrate	NH_4NO_3	0.0 e	0.0 i	44.2 d
ammonium sulfate	$(NH_4)_2SO_3$	14.5 d	10.7 h	41.4 e
ammonium phosphate dibasic	$(NH_4)_2HPO_4$	0.0 e	0.0 i	0.0 i
calcium carbonate	$CaCO_3$	67.5 b	30.6 g	16.0 h
calcium chloride	$CaCl_2$	74.5 b	27.1 g	49.0 c
calcium nitrate	$Ca(NO_3)_2$	55.2 c	30.7 g	48.0 c
calcium sulfate	$CaSO_3$	94.5 a	43.7 f	32.2 g
potassium carbonate	K_2CO_3	0.2 e	0.0 i	0.0 i
potassium chloride	KCl	93.8 a	59.4 e	45.5 d
potassium nitrate	KNO_3	97.5 a	80.4 d	40.2 e
potassium sulfate	K_2SO_3	98.5 a	92.3 c	40.8 e
potassium phosphate monobasic	KH_2PO_4	98.5 a	106.5 b	51.8 b
potassium phosphate dibasic	K_2HPO_4	47.0 c	33.7 g	14.8 h
sodium bicarbonate	$NaHCO_3$	0.2 e	1.1 i	0.0 i
sodium carbonate	Na_2CO_3	0.2 e	0.9 i	0.0 i
sodium chloride	NaCl	97.5 a	94.3 c	44.1 d
sodium nitrate	$NaNO_3$	97.8 a	65.2 e	0.0 i
sodium sulfate	Na_2SO_3	98.5 a	83.4 d	38.2 f
sodium phosphate monobasic	NaH_2PO_4	98.8 a	111.9 a	52.7 b
sodium phosphate dibasic	Na_2HPO_4	0.2 e	0.0 i	0.0 i
None (water)	H_2O	99.0 a	95.6 c	33.2 g

[a] Inorganic salts were tested at concentration of 50 mM in 1.5% water agar.

[b] Percent germination and germ tube length were determined after 18 h of incubation at 20 °C. Data are the means of three replications and the experiment was repeated once.

[c] Radial growth of mycelium-initiated colonies was determined after three days of incubation at 22 °C. Data are the means of three replications and the experiment was repeated once.

[d] Assignment of the same letter in a column for salt effects on germination, germ tube length and radial growth indicated that the observations did not differ significantly (protected LSD test, α = 0.05).

Table 6. Effect of inorganic salts on conidial germination, germ tube length and colony growth of *Gliocladium roseum* on water agar medium inoculated with the antagonist

Salt[a]	Formula	Conidial germination (%)[b]		Germ tube length (μm)[b]		Colony diameter (mm)[c]	
ammonium carbonate	$(NH_4)_2CO_3$	0.0	d[d]	0.0	d	2.1	j
ammonium chloride	NH_4Cl	16.2	d	4.9	d	42.1	bcde
ammonium nitrate	NH_4NO_3	26.1	cd	17.7	cd	40.1	de
ammonium sulfate	$(NH_4)_2SO_3$	21.2	d	23.5	cd	38.3	e
ammonium phosphate dibasic	$(NH_4)_2HPO_4$	0.6	d	0.7	d	33.3	f
calcium carbonate	$CaCO_3$	61.7	ab	122.2	abcd	40.8	cde
calcium chloride	$CaCl_2$	62.3	ab	111.3	abcd	44.3	bcde
calcium nitrate	$Ca(NO_3)_2$	73.6	ab	136.0	abc	42.1	bcde
calcium sulfate	$CaSO_3$	63.0	ab	165.5	ab	41.0	cde
potassium carbonate	K_2CO_3	5.1	d	3.0	d	17.8	h
potassium chloride	KCl	53.0	bc	73.0	abcd	46.6	b
potassium nitrate	KNO_3	90.2	ab	101.6	abcd	44.6	bcd
potassium sulfate	K_2SO_3	85.4	ab	108.8	abcd	45.1	bcd
potassium phosphate monobasic	KH_2PO_4	71.6	ab	52.2	bcd	42.0	bcde
potassium phosphate dibasic	K_2HPO_4	84.1	ab	86.1	abcd	44.9	bcd
sodium bicarbonate	$NaHCO_3$	13.0	d	12.0	cd	23.8	g
sodium carbonate	Na_2CO_3	0.0	d	0.0	d	14.1	i
sodium chloride	NaCl	91.4	ab	129.2	abcd	42.8	bcde
sodium nitrate	$NaNO_3$	83.3	ab	105.4	abcd	50.4	a
sodium sulfate	Na_2SO_3	59.0	ab	43.6	bcd	43.2	bcde
sodium phosphate monobasic	NaH_2PO_4	75.7	ab	49.4	bcd	39.6	de
sodium phosphate dibasic	Na_2HPO_4	52.0	bc	80.6	abcd	41.4	bcde
None (water)	H_2O	96.8	a	194.8	a	45.8	bc

[a] Inorganic salts were tested at concentration of 50 mM in 1.5% water agar.

[b] Percent germination and germ tube length were determined after 20 h of incubation at 20 °C. Data are the means of three replications and the experiment was repeated once.

[c] Colony diameter of mycelium-initiated colonies was determined after 9 days of incubation at 22 °C. Data are the means of three replications and the experiment was repeated once.

[d] Assignment of the same letter in a column for salt effects on germination, germ tube length and radial growth indicated that the observations did not differ significantly (protected LSD test, $\alpha = 0.05$).

Table 7. Effects of carbohydrates on estimated colony diameter of *Botrytis cinerea* and estimated mycelial density of *Gliocladium roseum* on water agar medium inoculated with the fungi respectively

Carbohydrates[a]	Formula	Colony diameter of *B. cinerea* (mm)[b]		Mycelial density of *G. roseum*[c]	
D-Arabinose	$C_5H_{10}O_5$	38.9	j[d]	2.90	c
D-Cellobiose	$C_{12}H_{22}O_{11}$	75.7	bc	3.00	c
D-Fructose	$C_6H_{12}O_6$	78.9	ab	3.60	c
D-Galactose	$C_6H_{12}O_6$	73.2	bcd	2.70	cde
D-Glucose	$C_6H_{12}O_6$	77.1	bc	2.84	cd
Inositol	$C_6H_{12}O_6$	61.0	gh	2.24	fg
D-Lactose	$C_{12}H_{22}O_{11}$	61.3	gh	2.80	cd
D-Lyxose	$C_5H_{10}O_5$	53.1	i	1.98	g
D-Maltose	$C_{12}H_{22}O_{11}$	65.3	efg	2.34	efg
D-Mannitoal	$C_6H_{14}O_6$	57.8	hi	2.24	fg
D-Mannose	$C_6H_{12}O_6$	72.2	cd	2.50	def
Melezitose	$C_{18}H_{32}O_{16}$	60.1	gh	2.70	cde
A-Melibiose	$C_{12}H_{22}O_{11}$	68.8	def	2.30	efg
D-Raffinose	$C_{18}H_{32}O_{16}*5H_2O$	68.8	def	2.24	fg
L-Rhamnose	$C_6H_{12}O_5*H_2O$	57.2	hi	2.32	efg
Sucrose	$C_{12}H_{22}O_{11}$	70.5	cde	2.48	efg
D-Trehalose		61.9	gh	2.70	cde
D-Xylose	$C_5H_{10}O_5$	62.8	fgh	2.90	cd
None (water)	H_2O	56.6	hi	2.00	g

[a] Carbohydrates were tested at concentration of 100 mg/L in 15% water agar.

[b] Colony diameter of *B. cinerea* from agar plugs was determined after 9 days of incubation at 22 °C. Data are the means of three replications and the experiment was repeated once.

[c] Mycelial density rate of *G. roseum* from spore suspension (treatment/check) was determined after 16 days of incubation at 22 °C using a 1 - 10 scale. Data are the means of three replications and the experiment was repeated once.

[d] Values in a column assigned the same letter did not differ significantly (protected LSD test, α = 0.05).

Table 8. Effects of nitrogenous compounds on estimated colony diameter of *Botrytis cinerea* and estimated mycelial density of *Gliocladium roseum* on water agar medium inoculated with the fungi respectively

Nitrogenous compounds[a]	Colony diameter of *B. cinerea* (mm)[b]		Mycelial density of *G. roseum*[c]	
Calcium nitrate	54.2	def[d]	1.96	i
Potassium nitrate	54.4	de	2.00	i
Sodium nitrate	-	-	-	-
Ammonium nitrate	50.8	fg	1.94	i
L-Alanine	41.2	jk	3.10	h
L-Arginine	47.2	hi	1.96	i
L-Asparagine	48.2	gh	2.20	i
L-Aspartic Acid	68.3	b	4.80	f
L-Cysteine	38.8	l	2.20	i
L-Glutamic Acid	83.1	a	5.00	f
L-Glutamine	53.3	def	3.60	gh
L-Glycine	40.9	jk	3.60	gh
L-Histidine	44.2	ij	2.00	i
L-Isoleucine	50.9	gf	7.80	cd
L-Leucine	47.8	gh	5.20	ef
L-Lysine	17.6	m	3.00	h
L-Methionine	39.7	k	1.86	i
L-Phenylalanine	45.5	hi	7.90	cd
L-Pronine	53.6	def	5.90	e
L-Serine	48.4	gh	4.00	g
L-Threonine	39.8	k	7.20	d
L-Tryptophane	53.1	ef	8.00	bc
Tyrosine	58.3	c	8.70	b
L-Valine	40.9	jk	4.00	g
None (water)	56.6	dc	2.00	i

[a] Nitrogenous compounds were tested at concentration of 2 mM in 15% water agar.
[b] Colony diameter of *B. cinerea* from agar plugs was determined after 9 days of incubation at 22 °C. Data are the means of three replications and the experiment was repeated once.
[c] Mycelial density rate of *G. roseum* from spore suspension (treatment/check) was determined after 16 days of incubation at 22 °C using a 1 - 10 scale. Data are the means of three replications and the experiment was repeated once.
[d] Values in a column assigned the same letter did not differ significantly (protected LSD test, α = 0.05).

Table 9. Effects of inorganic salts coapplied with *Botrytis cinerea* to black spruce seedlings, on sporulation incidence and number of spores produced by the pathogen in needle segments of the seedlings

	Sporulation incidence (%)		Number of conidia/segment (x 10^4)	
Salt[a]	24 h[b]	48 h	24 h	48 h
ammonium carbonate	72.2 ab[c]	97.8 a	14.7 bc	17.6 def
ammonium chloride	79.4 a	95.6 a	16.9 ab	28.0 cd
ammonium nitrate	60.0 bc	96.7 a	10.9 bcd	36.9 bc
ammonium sulfate	80.6 a	98.9 a	16.7 ab	62.4 a
ammonium phosphate dibasic	46.7 cd	96.7 a	9.8 bcde	49.3 ab
calcium carbonate	46.1 cd	96.7 a	3.8 def	22.9 de
calcium chloride	67.8 ab	72.8 bc	13.8 bc	27.6 cd
calcium nitrate	45.0 de	96.7 a	12.7 bc	43.6 b
calcium sulfate	51.1 cd	93.3 a	12.2 bc	27.3 cd
potassium carbonate	17.8 gf	48.9 d	0.7 f	10.4 ef
potassium phosphate monobasic	68.9 ab	91.7 a	11.3 bcd	28.0 cd
potassium phosphate dibasic	47.2 cd	83.3 ab	3.3 def	16.0 def
sodium bicarbonate	10.0 g	70.6 bc	0.2 f	13.6 ef
sodium carbonate	23.3 gf	55.6 cd	0.9 f	11.6 ef
sodium phosphate monobasic	81.7 a	98.3 a	24.4 a	37.1 bc
sodium phosphate dibasic	30.6 ef	46.7 d	2.4 ef	5.8 f
None (water)	41.1 de	81.7 ab	7.1 cdef	16.0 def

[a] Inorganic salts were added to spore suspension of *B. cinerea* (10^6 conidia/mL) at a concentration of 50 mM. The mixtures were then applied to the foliage of the seedlings.

[b] Post-inoculation wetness duration.

[c] Values in a column assigned the same letter did not differ significantly (protected LSD test, α = 0.05).

Table 10. Correlation analysis of conidial germination, germ tube length and radial growth of *Botrytis cinerea* on water agar containing various inorganic salts, and sporulation incidence and number of spores produced by B. cinerea in 4-month-old seedlings of black spruce that were inoculated with conidia of the pathogen in 50 mM solutions of salts.

	1	2	3	4	5	6	7
1. Germination (%)	1[a] 0[b]						
2. Germ tube length (μm)	0.85 0.0001	1 0					
3. Radial growth (mm)	0.65 0.0001	0.73 0.0001	1 0				
4. Incidence (%) 24 h wetness	0.27 0.0057	0.28 0.0040	0.48 0.0001	1 0			
5. Incidence (%) 48 h wetness	0.25 0.0124	0.23 0.0182	0.36 0.0002	0.51 0.0001	1 0		
6. Spores/segment 24 h wetness (ln)	0.39 0.0001	0.34 0.0004	0.52 0.0001	0.66 0.0001	0.52 0.0001	1 0	
7. Spores/segment 48 h wetness (ln)	0.20 0.0397	0.17 0.0854	0.45 0.0001	0.49 0.0001	0.75 0.0001	0.48 0.0001	1 0

[a] Pearson's correlation coefficient.
[b] P value of correlation coefficient.

Table 11. Effect of coapplications of inorganic salts and *Gliocladium roseum* to black spruce seedlings, 46 and 66 h prior to inoculation of the seedlings with *Botrytis cinerea*, on sporulation incidence of the pathogen on shoots and needles of the seedlings, and on number of spores produced on the seedlings by the pathogen and by the antagonist

	Sporulation incidence of *B. cinerea* (%)		Number of spores/seedling (x 10^5)	
Salt[a]	Shoots	Needles	*B. cinerea*	*G. roseum*
ammonium carbonate	21.7 gh[b]	2.1 gh[c]	5.75 abcd	6.48 abcd
ammonium chloride	86.7 a	34.2 ab	6.50 a	7.27 a
ammonium nitrate	88.3 a	32.4 b	6.36 abc	7.19 ab
ammonium sulfate	88.3 a	43.9 a	6.41 ab	7.36 a
ammonium phosphate dibasic	73.3 ab	24.4 bc	6.44 ab	7.32 a
calcium carbonate	16.7 h	0.9 h	5.00 ef	6.06 cd
calcium chloride	33.3 defgh	4.5 efgh	6.20 abcd	6.41 abcd
calcium nitrate	47.2 cde	5.0 efgh	5.85 abcd	6.76 abc
calcium sulfate	17.0 h	3.4 fgh	5.76 abcd	6.23 bcd
potassium carbonate	35.0 defgh	2.4 gh	4.64 f	6.70 abcd
potassium chloride	40.0 cdefg	11.6 defgh	5.96 abcd	6.44 abcd
potassium nitrate	44.1 cdef	8.9 defgh	5.99 abcd	6.69 abcd
potassium sulfate	41.1 cdefg	18.4 cd	6.01 abcd	6.44 abcd
potassium phosphate monobasic	60.0 bc	13.9 cdef	6.09 abcd	6.67 abcd
potassium phosphate dibasic	28.3 efgh	3.8 efgh	5.91 abcd	6.24 bcd
sodium bicarbonate	30.6 efgh	6.4 efgh	5.53 de	6.26 bcd
sodium carbonate	41.7 cdefg	4.7 efgh	5.58 de	6.47 abcd
sodium chloride	24.1 fgh	5.0 efgh	5.74 bcd	6.08 cd
sodium nitrate	51.7 cd	14.2 cde	5.88 abcd	6.58 abcd
sodium sulfate	38.3 defg	11.8 defg	6.34 abc	6.65 abcd
sodium phosphate monobasic	42.9 cdef	10.1 ed	6.19 abcd	6.87 abc
sodium phosphate dibasic	47.4 cde	6.4 efgh	5.67 cde	4.23 e
None (water only)	27.2 efgh	5.4 efgh	5.92 abcd	5.76 d

[a] Inorganic salts were added to spore suspension of *Gliocladium roseum* (10^6 spores/mL) to concentration of 50 mM. The mixtures were then applied to the foliage of black spruce seedlings. *Botrytis cinerea* was challenge-inoculated to the seedlings 46 and 66 hours later.

[b] Values in a column assigned the same letter did not differ significantly (protected LSD test, α = 0.05).

Table 12. Estimated parameter values and coefficients from logistic models for sporulation incidence of *Botrytis cinerea* (YI) and area of foliage with sporulation of the pathogen (YS) in black spruce seedlings as functions of planting density (D) and time (T).

	Estimated parameters						
	b_0	b_1	b_2	b_3	R^2	R^2_a	s
Sporulation incidence of B. cinerea	-41.863 (2.0670)	-1.013 (0.2651)	2.0257 (0.1212)	0.3547 (0.06840)	0.829	0.825	3.188
Area of foliage with sporulation	-20.985 (0.8487)	-0.9513 (0.2904)	0.8389 (0.0516)	0.1650 (0.03568)	0.855	0.851	1.220

Note: values are presented of estimated parameters (b_0 - b_3), coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R^2_a), and the standard deviation about the regression line (s). The logistic regression equations are $\ln(YI/(100-YI)) = b_o + b_1D^3 + b_2T + b_3DT$ and $\ln(YS/(100-YS)) = b_o + b_1D^2 + b_2T + b_3DT$, in which YI and YS are the combined data of repetitions 1 and 2. Numbers in parentheses are standard errors of the corresponding parameter values. The intercept b_o is the value of $\ln(Y_s/(100-Y_s))]$ when T = 0 and D = 0.

Fig. 1. Effects of various fungal isolates and of fungicide programs on incidence of *Botrytis cinerea* in seedlings of black spruce in the greenhouse in 1991 and 1992. Identities of the isolates were as follows: *Gliocladium roseum* (AL710), *Myrothecium verrucaria* (ZP2005), *Fusarium* sp. (ZP2009), and *Penicillium* sp. (ZP2047). The water check included surfactant. Data bars of a given year assigned the same letter are not significantly different (protected LSD, $\alpha = 0.05$).

Fig. 2. Effect of *Botrytis cinerea* and *Gliocladium roseum*, applied alone and in combination to the foliage of black spruce seedlings, on sporulation incidence and number of spores produced by the pathogen, electrolyte leakage, chlorophyll content, and rate photosynthesis in the seedling foliage at various times after inoculation.

Fig. 3. Observed and predicted effects of treatment programs (4 applications) of *Gliocladium roseum* applied at various conidial concentrations, and of chlorothalonil applied at 1.4 g a.i./L, on proportion of seedlings with sporulation of *Botrytis cinerea* and proportion of foliage with sporulation of the pathogen in container-grown seedlings of black spruce at various times after planting in greenhouses. Predicted values were calculated from the relevant equations in Table 1.

Fig. 4. Observed and predicted effects of treatment programs of one to six applications of *Gliocladium roseum* (10^6 conidia/mL), and six applications of chlorothalonil (1.4 g a.i./L), on proportion of seedlings with sporulation of *Botrytis cinerea* and proportion of foliage with sporulation of the pathogen in container-grown seedlings of black spruce at various times after planting in greenhouses. Predicted values were calculated from the relevant equations in Table 2.

Fig. 5. Percent germination of *Gliocladium roseum* conidia at various times

after application to foliage of black spruce seedlings, which received or did not receive high temperature treatments for predisposition to *Botrytis cinerea*, and were kept at various temperatures after inoculation with *G. roseum*.

Fig. 6. Predicted values for percent germination of *Gliocladium roseum* conidia at various times after application to foliage of black spruce seedlings, which received or did not receive high temperature treatments for predisposition to *Botrytis cinerea*, and were kept at various temperatures after inoculation with *G. roseum*. Curves were produced using the Weibull equation with the estimated parameters showed in Table 4.

Fig. 7. Simple regressions of predicted germination incidence (Y_p) of conidia of *Gliocladium roseum* on foliage of black spruce seedlings, which received or did not receive high temperature predisposition to *Botrytis cinerea*. Observed germination values were obtained at various times after the seedlings were inoculated with *G. roseum* and maintained in the greenhouse. Predicted germination values were obtained using the Weibull equation and parameters that were derived from growth room studies (Table 4).

Fig. 8. Effects of the interval between inoculation of black spruce seedlings with *Gliocladium roseum* (10^8 conidia/mL) and challenge inoculation with *Botrytis cinerea* (10^6 conidia/mL) on sporulation incidence of the pathogen on needles of the seedlings in the growth room. Data bars assigned the same letters were not significantly different (Protected LSD test, $\alpha = 0.05$).

Fig. 9. Effects of inoculum concentration of *Gliocladium roseum* on sporulation incidence of *Botrytis cinerea* in needles of red pine seedlings that were coinoculated with the antagonist and the pathogen (10^6 conidia/mL) in the growth room. Standard errors are shown.

Fig. 10. Effects of time of inoculation of *Gliocladium roseum* (10^8 conidia/mL) in red pine seedlings in the growth room in relation to that of *Botrytis cinerea* (10^6 conidia/mL) on sporulation incidence of the pathogen on needles of the seedlings. Standard errors are shown.

Fig. 11. Effects of inoculum concentration of *Gliocladium roseum*, applied once to red pine seedlings in the greenhouse, on sporulation incidence of *Botrytis cinerea* in the seedlings. Standard errors are shown.

Fig. 12. Comparative effects of treatment programs of five applications of *Gliocladium roseum* (10^5 and 10^7 conidia/mL), of chlorothalonil (1.4 g a.i./L), and of water plus surfactant to red pine seedlings on sporulation incidence of *Botrytis cinerea* in the seedlings in a greenhouse and in an outside nursery compound. Data bars of a given experiment site assigned the same letter are not significantly different (protected LSD, $\alpha = 0.05$).

Fig. 13. Effect of low light intensity (400-700 nm wavelength band) for various preinoculation treatment periods on sporulation incidence of *Botrytis cinerea* and on chlorophyll content in 6-mm needle segments from black spruce seedlings that were inoculated with the pathogen (10^6 conidia/mL water plus surfactant) and kept in high humidity for 36 h.

Fig. 14. Effect of low light intensity (L) in canopies of container-grown seedlings of black spruce on sporulation incidence of *Botrytis cinerea* (Y_s) in needle segments from the seedlings, which were grown in greenhouses at Guelph in 1992 and at Sault Ste. Marie in 1993, and inoculated, respectively, with a spore suspension of the pathogen (10^6 conidia of *B. cinerea*/mL water) and from natural sources.

Fig. 15. Effects of period of preinoculation treatment at various freezing temperatures on incidence of sporulation and number of spores produced by

Botrytis cinerea, and on leakage of electrolytes, in black spruce seedlings 2-, 3-, and 4-months old that were inoculated with the pathogen (10^6 conidia/mL water plus surfactant) and kept in high humidity for 36 h.

Fig. 16. Effects of planting density of black spruce seedlings in cellular seedling flats on estimated incidence of sporulation and estimated area of foliage with sporulation of *Botrytis cinerea* in the seedlings at various times after planting in the greenhouse.

Fig. 17. Predicted effects of planting density of black spruce seedlings in cellular seedling flats on incidence of sporulation and area of foliage with sporulation of *Botrytis cinerea* in the seedlings at various times after planting in the greenhouse. Curves were produced using the logistic equation with the estimated parameters showed in Table 12.