Synergistic effects of a cellulase-producing *Micromonospora carbonacea* and an antibiotic-producing *Streptomyces violascens* on the suppression of *Phytophthora cinnamomi* root rot of *Banksia grandis*

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Abstract: Three polyvalent *Streptomyces* phages were used to isolate four *Micromonospora* species (*M. carbonacea*, *M. chalcea*, *M. purpureochromogenes*, and *M. inositola*) from mine-site rhizosphere soils in Western Australia. *Streptomyces violascens* was isolated using selective isolation techniques from the same soils. The *Micromonspora* spp. were examined for their ability to produce cellulases. *Micromonospora carbonacea*, *M. chalcea*, and *M. purpureochromogenes*, which were found to produce the enzyme, caused lysis of *Phytophthora cinnamomi* hyphae. Glasshouse trials showed that the use of the cellulase-producing *M. carbonacea* isolate, in conjunction with the antibiotic-producing *S. violascens* isolate, had a synergistic effect on the suppression of the *Phytophthora* root rot and in promoting growth of *Banksia grandis*. The importance of using a number of antagonists with different antagonistic abilities to control plant pathogenic fungi is discussed.

Key words: biological control, *Micromonospora carbonacea*, *Streptomyces violascens*, cellulases, *Phytophthora cinnamomi*.

Résumé : Les auteurs ont utilisé trois phages polyvalents de *Streptomyces* pour isoler quatre espèces de *Micromonospora* (*M. carbonacea, M. chalcea, M. purpureochromogenes* et *M. inositola*) à partir de la rhizosphère de sites miniers, en Australie occidentale. En utilisant des techniques d'isolement sélectives, ils ont pu obtenir le *Streptomyces violascens* des mêmes sols. Ils ont examiné la capacité des *Micromonospora* spp. à produire des cellulases. Les espèces *M. carbonacea, M. chalcea* et *M. pupureochromogenes*, qui produisent l'enzyme, provoquent la lyse des hyphes du *Phytophthora cinnamomi*. Des essais en serre montrent que l'utilisation de l'isolat du *M. carbonacea* produisant l'enzyme, en conjonction avec l'isolat du *S. violascens* produisant un antibiotique, agit en synergie pour supprimer la pourriture racinaire causée par le *Phytophthora* et promouvoir la croissance du *Banksia grandis*. Les auteurs discutent l'importance d'utiliser plusieurs antagonistes aux propriétés différentes dans la lutte biologique contre les champignons phytopathogènes.

Mots clés : lutte biologique, *Micromonospora carbonacea, Streptomyces violascens*, cellulases, *Phytophthora cinnamomi*.
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Introduction

The soil-borne plant pathogen *Phytophthora cinnamomi* Rands is responsible for the widespread destruction of native plant communities in Australia (Shearer and Tippett 1989; Wills 1993). In Western Australia alone, 14% of the 2 million hectares of jarrah (*Eucalyptus marginata* Donn ex Smith) forest are infected by this pathogen (Davison and Shearer 1989). This introduced plant pathogen not only destroys the main commercial timber eucalyptus species but also many understorey plants, subsequently reducing the biodiversity of these sites. In the southwest of Western Australia, between 1500 to 2000 plant species of an estimated 9000 species of vascular plants may be susceptible to infection by *P. cinnamomi* (Wills 1993). *Phytophthora cinnamomi* also limits the re-establishment of native bushland following mining (Gardner and Rokich 1987).

The effective and long-term rehabilitation of areas infected with *P. cinnamomi* is greatly hindered. The use of chemicals to control the pathogen in most incidences is impractical and environmentally unfriendly. An alternative and promising method is the integration of management systems with biological control through the manipulation of the environment and the introduction of specific microorganisms. In-depth and systematic studies of the soil environments in which the pathogen exists but causes little or no disease are the basis for developing functionally stable biocontrol systems (Cook and Baker 1983).

Actinomycetes isolated from naturally suppressive soils were reported to be antagonistic to *Phytophthora* species

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(Weste and Vithanage 1977; Hardy and Sivasithamparam 1991*b*). Reduced incidence of root infection was correlated with an increase in numbers of actinomycetes in the rhizosphere (Malajczuk et al. 1977). In soils with low populations of actinomycetes, root rots can develop very rapidly (Weste and Vithanage 1977). Certain strains belonging to *Actinoplanes* Couch, *Amorphosporangium* Couch, *Ampullariella* Couch, *Micromonospora* Ørskov, and *Spirillospora* Couch were shown to parasitize the oospores of *Phytophthora megasperma* Drechs. f.sp. *glycinea* Kuan and Erwin (Sutherland and Lockwood 1984). In the U.S.A., *Actinoplanes missouriensis* Couch, *Actinoplanes utahensis* Couch, and *Micromonospora* species were reported to reduce *P. megasperma* f.sp. *glycinea* root rot of soybean (Filonow and Lockwood 1985). Although these studies indicated a correlation between the presence of actinomycetes and reduced disease incidence, little work has been done on the ecology, identity, and mechanisms of action of these actinomycetes in Australia. Most studies utilized streptomycetes as potential biological control agents of *Phytophthora* species (Broadbent et al. 1971; Malajczuk 1983; Hardy and Sivasithamparam 1995), and information on other genera of the order *Actinomycetales* is lacking in Australia.

Hydrolytic enzymes including cellulases were suggested to play a role as a mechanism of biological control (Schrempf 1995), and micromonosporae were reported to produce cellulases (Sandrak 1977; Van Zyl 1985; Kawamoto 1989). Since the cell walls of *Phytophthora* species consist largely of cellulose (Bartnicki-Garcia and Wang 1983), it is postulated that cellulase-producing micromonosporae could be involved in the suppression of *Phytophthora*. In the present study, therefore, the cellulolytic micromonosporae were targeted for selective isolation from rehabilitated mine-site soils in south Western Australia where *Phytophthora* root rot is a significant problem. The micromonosporae were screened for their activity to degrade, in vitro, the cell walls of *P. cinnamomi* and to suppress the pathogen in glasshouse trials with and without an antibiotic-producing streptomycete.

Materials and methods

Pathogen

The *P. cinnamomi* isolate MU 94048 (Murdoch University Culture Collection), which had been isolated in 1994 from the collar of a naturally infected jarrah tree at a rehabilitated mine site in south Western Australia, was used throughout this study. It was maintained on V8 agar slants at 4°C (Ayers and Lumsden 1975).

Isolation and identification of micromonosporae

Rhizosphere soil samples were collected from the root zone 25 cm below the surface under 2-year-old jarrah trees growing on rehabilitated bauxite mine sites (near Dwellingup, south Western Australia). The soil was air dried at 25°C for 4 days, mixed to ensure uniformity, and passed through a 3-mm sieve to remove stones and root fragments. The pH of the soil was 6.5 (in 0.01 M $CaCl_2$).

The selective isolation technique described by Kurtböke et al. (1992) was used to isolate micromonosporae. Polyvalent *Streptomyces* phages were used to reduce the numbers of *Streptomyces* species growing on isolation plates to facilitate the recoveries of micromonosporae. The characteristics of the three phages used in this study were previously described by El-Tarabily et al. (1995). The stock suspension prepared by mixing high-titre phage suspensions ($\times 10^{12}$ pfu/mL) was then used to treat rhizosphere soil suspensions in dilution tubes as described by Kurtböke et al. (1992). Plates without phages were used as control treatments.

Starch casein agar was used for isolation of micromonosporae (Küster and Williams 1964) and contained cycloheximide (50 μg/mL) (Sigma Chemical Co., St. Louis, Mo.) (Williams and Davies 1965). The plates were then inoculated with 0.3 mL of the selected dilutions of the soil samples treated with and without phages and dried in a laminar flow cabinet for 30 min (Vickers and Williams 1987). After incubation at 28°C in the dark for 3 weeks, all colonies that resembled micromonosporae on the phage-treated soil-dilution plates were transferred onto oatmeal agar plates supplemented with 0.1% yeast extract (BBL, Becton Dickinson, Cockeysville, Md.) (Williams and Wellington 1982). Isolates were tentatively identified on the basis of their morphological descriptions and physiological characteristics (Kawamoto 1989). They were then stored in 10% glycerol at −20°C (Wellington and Williams 1978).

Detection of cellulolytic activity of *Micromonospora* isolates

The preliminary detection of cellulose decomposition was investigated in liquid Kadota medium by using the technique developed by Sandrak (1977). Tubes (15 mL) containing mineral salts with starch-free Whatman No. 1 filter paper (Whatman, Maidstone, England) were inoculated with the *Micromonospora* isolates. After 3 weeks of incubation in the dark at 28°C, the contents of the tubes were centrifuged (3500 × *g* per 10 min) and filtered through sterile 0.45-μm Millipore filters (Millipore, Australia Ltd.) and stored at 4°C.

Potential cellulase-producing *Micromonospora* isolates were then screened for the production of cellulolytic enzymes according to the method of Mandels et al. (1976). To induce cellulase production, the submerged cultures were grown in 1% (w/v) microcrystalline cellulose (Sigmacell S-5504, Sigma) as the sole carbon source. After 3 weeks of growth in the dark at 28°C, the contents of each flask were centrifuged (3500 × *g* per 10 min at 4°C) to separate the cell mass and residual microcrystalline cellulose from the aqueous phase. The supernatants were then filtered through glass wool, and the preparation used as the extracellular (crude) enzyme solution. The enzyme solution was stored at 4°C in the presence of toluene.

Cellulase activity

Cellulase activity was assayed using both filter paper (FP-cellulase) and carboxymethyl-cellulose (CM-cellulase) as substrates and measuring the release of reducing sugars by the dinitrosalicylic acid method according to Bailey et al. (1992), using glucose as the standard. The unit of FP- and CM-cellulase activity is defined as the amount of enzyme required to liberate 1 μmol reducing sugars (as glucose) per minute under the assay conditions.

β-Glucosidase activity

β-glucosidase activity was assayed by measuring the amount of *p*-nitrophenol liberated from *p*-nitrophenyl-β-glucoside (PNPG) (Sigma) as substrate (Dekker 1981). The amount of *p*-nitrophenol was determined spectrophotometrically (Hitachi U-1100 spectrophotometer, Tokyo, Japan) at 400 nm from a standard curve using *p*-nitrophenol. The unit of β-glucosidase activity is the amount of enzyme required to produce 1 μmol *p*-nitrophenol per minute under the conditions of assay.

Effects of the filtrates of *Micromonospora* isolates on *P. cinnamomi* hyphae

Sterilized glass slides were coated with V8 agar and placed into sterile plastic Petri dishes containing moist sterile filter paper. *Phytophthora cinnamomi* agar plugs (6 mm diameter) were then placed on the coated slides and incubated in the dark for 4 days

at 25°C (Shankar et al. 1994). The cellulolytic action of the *Micromonospora* isolates on the cell wall of *P. cinnamomi* was then studied by dropping (0.2 mL) of the filtrate onto actively growing hyphae of the pathogen. After 7 h of incubation in the dark at 25°C the hyphae of *P. cinnamomi* were stained with lactophenol-cotton blue and any subsequent changes in the hyphal morphology were observed using a light microscope (×100 magnification). Controls consisted of (*i*) liquid Kadota's medium without the presence of the *Micromonospora* isolates and (*ii*) a commercial cellulase preparation derived from *Trichoderma* (1.0 cellulase unit/mL; Celluclast 1.5L, Novo/Nordisk A/S, Denmark).

Detection of the antibiotic activity of *Micromonospora* isolates

The *Micromonospora* isolates were also examined for the production of inhibitory compounds active against *P. cinnamomi*. The isolates were streak-inoculated to one side of fish-meal agar plates (El-Tarabily 1992). The plates were then incubated for 8 days to allow the production and diffusion of metabolites into the agar. An agar disk (6 mm diameter) containing *P. cinnamomi* mycelium was then placed onto the opposite side of the *Micromonospora* inoculated plates and incubated in the dark at 25°C. Inhibition was indicated when *P. cinnamomi* mycelial growth in the direction of the actinomycete colony was retarded (Crawford et al. 1993).

Phytophthora cinnamomi control in vivo

Banksia grandis Willd., which is highly susceptible to *P. cinnamomi* (Shearer and Tippett 1989), was used in this study. *Micromonospora carbonacea* Luedemann and Brodsky (M1) and *Micromonospora inositola* Kawamoto, Okachi, Kato, Yamamoto, Takahashi, Takasawa, and Nara (M4), a cellulase producer and a noncellulase producer, respectively, were selected to determine whether they were able to suppress *P. cinnamomi* root rot of *B. grandis* under glasshouse conditions. Another noncellulolytic actinomycete, *Streptomyces violascens* Pridham, Hesseltine, and Benedict (S97), which had been isolated from the soils described above by the use of the dry heat technique (Nonomura and Ohara 1969), was included in the study since it produced antibiotics that were more inhibitory to *P. cinnamomi* than any of the *Micromonospora* isolates. The antibiotics were identified as actinomycin D and actinomycin X_2 (V) (K.A. El-Tarabily, C. Loane, M. Favas, I.D. Kurtböke, G.E.St.J. Hardy, G. Roos, and R. Giles, unpublished data). *Streptomyces violascens* was also included to determine whether biological control could be enhanced by the dual inoculation of a cellulase-producing actinomycete and an antibiotic-producing actinomycete. *Micromonospora chalcea* Ørskov (M2) and *Micromonospora purpureochromogenes* Luedemann (M3) were not used in the glasshouse trials because their cellulase activity was less than that of M1.

Inoculum preparation

The fungal inoculum was prepared by placing 50 g of moist wheat bran into 500-mL flasks; these were autoclaved at 121°C for 20 min on three successive occasions as described by Rouiger and Jeffers (1991). This mixture was then inoculated with 8 agar plugs (6 mm diameter) from the freshly growing margins of the *P. cinnamomi* hyphae under aseptic conditions. The flasks were incubated at 25°C in the dark for 10 days and were occasionally shaken by hand to ensure uniformity of fungal growth.

The actinomycetes inocula were prepared on wheat bran as described above; the flasks were inoculated with spore suspensions (10 mL) of each actinomycete in 10% glycerol and incubated at 28°C in the dark for 3 weeks.

Container medium, plants, and inoculation

A pine bark based container medium (pine bark to *Eucalyptus marginata* sawdust to coarse sand (3:1:1)) was steamed at 60°C for 30 min prior to inoculation. This pine bark based medium has been shown to be conducive to *Phytophthora* spp. (Hardy and Sivasithamparam 1991*a*). The contents of each flask were then mixed with the required amount of container medium, which was placed in 15 cm diameter plastic pots (Smith and Nephew, Australia). Wheat bran infested with the test organisms (0.5% w/w) was mixed with the container medium in the following combinations: A, *P. cinnamomi* alone; B, *P. cinnamomi* + M1; C, *P. cinnamomi* + M4; D, *P. cinnamomi* + M1 + M4; E, *P. cinnamomi* + S97; F, *P. cinnamomi* + M1 + S97; G, *P. cinnamomi* + M4 + S97; H, *P. cinnamomi* + M1 + M4 + S97; I, M1; J, M4; K, S97; L, M1 + M4; M, M1 + S97; N, M4 + S97; O, M1 + M4 + S97; and P, control (sterilized wheat bran).

After inoculating the soil with the appropriate treatments, 18-month-old *B. grandis* plants were planted into the containers. Each treatment was replicated five times with two plants per replicate, and the experiment was repeated once. The seedlings were maintained in a 25 ± 2°C glasshouse and watered twice daily and fertilized twice weekly with a modified Hoagland's nutrient solution (Arnon and Hoagland 1940). Plants were observed daily for symptom development and rated on a scale of 1–5 (1, healthy; 2, few and small lesions on the main tap root; 3, shoot yellowing and lesions covering up to half of the main tap root; 4, shoot wilting and lesions covering more than half of the main tap root; and 5, dead shoot and extensive lesions covering entire tap root) to establish a disease index and progress curve. The disease progress curve was plotted for each treatment as mean percent mortality corrected for multiple infections; log [1/(1-*y*)] (in which y = mean portion mortality) versus time (Spencer and Benson 1982). Dead plants were removed and plated onto P_{10}ARPH (Jeffers and Martin 1986, except that ampicillin was reduced from 250 to 25 mg), a *Phytophthora*-selective agar medium. After 11 weeks the trial was terminated, the remaining symptomless plants were removed and the roots were washed under running tap water; 100 randomly selected roots (2–3 cm long) and a 2- to 3-cm section of the collar of each plant were also plated onto P_{10}ARPH. Colonies of *P. cinnamomi* were examined microscopically to confirm Koch's postulates.

To determine whether the pathogen survived in the treatments that contained *P. cinnamomi* but in which no disease symptoms were observed, the soil was baited for the presence or absence of the pathogen. Approximately 100 g of container medium from each treatment was placed into transparent containers (11.5 wide × 17.5 long × 4.5 cm deep). The medium was then covered with deionized water and young leaves of *Pimelia ferrugina* Labill. were floated on the surface. After 4 days at 22 ± 2°C the leaves were blotted dry on paper towels and plated onto P_{10}ARPH. Plates were then incubated in the dark for 4 days at 25°C and presence or absence of *Phytophthora* was recorded. On completion of the trials, the plants were assessed for fresh root and shoot weights.

Statistical analysis

A randomized complete block design was used and analysis of variance was carried out using SUPERANOVA (Abacus Concepts, Inc., Berkeley, Calif.) to evaluate the effect of the different treatments on total plant weight and disease index. Significant differences between means were determined by Duncan's new multiple range test at $P = 0.05$. Linear regression equations for the incidence of mortality were carried out using STATVIEW 4.0 (Abacus Concepts, Inc., Berkeley, Calif.).

Results

Isolation and identification of Micromonosporae

The use of polyvalent phages significantly ($P < 0.001$) reduced the numbers of streptomycetes on the isolation plates (Table 1). Subsequently the numbers of micromonosporae were significantly ($P < 0.01$) higher on the plates treated

Table 1. Comparison of number of colony forming units (cfu) of streptomycetes and micromonosporae from starch casein agar plates treated with and without phage.

	Without phage		With phage	
	cfu/plate*	cfu/g dry wt. soil	cfu/plate*	cfu/g dry wt. soil
Streptomycetes	26.8±1.97	13.4×10^5	5.83±1.24	3.0×10^5
Micromonosporae	2.16±0.47	1.08×10^5	11.0±1.18	5.5×10^5

Note: t test is significant at $P < 0.001$ for reduction of streptomycetes and significant at $P < 0.01$ for the increase for the micromonosporae numbers on phage treated plates.
*Values are means ± SE.

Table 2. The effect of selected actinomycetes on *Phytophthora* root rot caused by *P. cinnamomi* on *Banksia grandis* plants.

Treatment[a]	Disease index[b]	Linear regression equation[c]	R^d	$T_{50}{}^e$
A. *P. c.*	4.9*e*	$y = 0.035x-0.38$	0.95	19
B. *P. c.* + M1	2.0*c*	$y = 0.007x-0.061$	0.96	51
C. *P. c.* + M4	4.8*e*	$y = 0.023x-0.18$	0.97	21
D. *P. c.* + M1 + M4	2.0*c*	$y = 0.006x-0.053$	0.96	58
E. *P. c.* + S97	2.9*d*	$y = 0.009x-0.077$	0.95	41
F. *P. c.* + M1 + S97	1.1*ab*	—	—	—
G. *P. c.* + M4 + S97	2.8*d*	$y = 0.009x-0.063$	0.97	40
H. *P. c.* + M1 + M4 + S97	1.3*b*	—	—	—

[a]*P. c.*, *Phytophthora cinnamomi*, isolate number 94048; M1, *Micromonospora carbonacea*; M4, *Micromonospora inositola*; and S97, *Streptomyces violascens*.
[b]Disease index based on a scale of 1−5, where 1 is healthy, 2 is few and small lesions on the main tap root, 3 is shoot yellowing and lesions covering up to half of the main tap root, 4 is shoot wilting and lesions covering more than half of the main tap root, and 5 is dead shoot and extensive lesions covering the entire tap root. Values with the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[c]Linear regression equations of log [1/(1-y)], in which y = proportion of diseased plants versus time (days after transplanting from day 2 through 80). —, no mortality on any day.
[d]The linear correlation coefficient (R) was significant at $P = 0.001$.
[e]T_{50} is the number of days required for 50% of plants to develop aboveground symptoms.

with phage (Table 1), and the isolates were identified as *M. carbonacea* (M1), *M. chalcea* (M2), *M. purpureochromogenes* (M3), and *M. inositola* (M4).

Enzyme production

Three of the *Micromonospora* isolates (M1, M2, and M3) grew vigorously in Kadota's medium and caused maceration of the Whatman filter paper strips after 3 weeks of incubation. The isolates also grew well on the basal medium containing microcrystalline cellulose. The cellulase activities for the three isolates M1, M2, and M3 were 0.09, 0.04, and 0.05 IU/mL for FP-cellulase, respectively, and 0.05, 0.02, and 0.03 IU/mL for CM-cellulase, respectively. The respective β-glucosidase activities for the three isolates were 0.03, 0.007, and 0.002 IU/mL. Isolate M4 did not produce significant amounts of any of the cellulolytic enzymes assayed. These results revealed that M1 produced the highest cellulolytic activity of the four isolates examined.

Effect of filtrates on the cell wall of *P. cinnamomi*

When aliquots of the filtrates of *Micromonospora* isolates M1, M2, and M3 grown in Kadota's medium were dropped onto growing *P. cinnamomi* hyphae, hyphal lysis was observed. When a commercial cellulase preparation expressing high cellulase activity was used, severe disruption of the hyphal cell occurred, with the hyphal walls rarely seen in full integrity. The isolate M4 and the control treatment (Kadota's medium without the presence of *Micromonospora* isolates) had no effect on cell walls of the pathogen *P. cinnamomi*.

Detection of the antibiotic activity of *Micromonospora* isolates

The *Micromonospora* isolates M1 and M4 did not produce inhibitory compounds active against *P. cinnamomi* in vitro. However, isolates M2 and M3 produced compounds that inhibited the growth of the pathogen.

Glasshouse trials and disease control

The treatments (treatments I−P) that did not contain *P. cinnamomi* did not show any disease symptoms (Table 2). Treatments F (*P. cinnamomi* + M1 + S97) and H (*P. cinnamomi* + M1 + M4 + S97) completely suppressed disease expression up to 80 days as observed by the T_{50} values (Table 2). It is apparent that the combination of the *S. violascens* (S97) with the cellulase-producing micromonospora isolate M1 greatly enhances the suppressive effect. However, the presence of M1 or S97 alone with the pathogen (treatments B and E, respectively) did not completely suppress disease (Table 2). The treatment that contained *P. cinnamomi* and the noncellulase-producing isolate (M4) failed to

Table 3. Effect of actinomycetes on fresh shoot and root weight (g) of *Banksia grandis* plants grown in soil infested with and without *P. cinnamomi* in greenhouse tests.

Treatment[a]	Fresh wt. (g)[b] Shoot	Root
A. *P. c.*	4.54*a*	1.13*a*
B. *P. c.* + M1	19.02*ef*	9.04*d*
C. *P. c.* + M4	4.75*a*	1.05*a*
D. *P. c.* + M1 + M4	18.01*de*	8.80*d*
E. *P. c.* + S97	17.51*d*	6.44*bc*
F. *P. c.* + M1 + S97	21.46*i*	12.12*e*
G. *P. c.* + M4 + S97	16.27*c*	6.17*bc*
H. *P. c.* + M1 + M4 + S97	21.03*hi*	11.76*e*
I. M1	20.77*ghi*	9.65*d*
J. M4	14.13*b*	5.50*b*
K. S97	19.62*fg*	7.23*c*
L. M1 + M4	20.01*fgh*	9.92*d*
M. M1 + S97	23.02*j*	13.90*f*
N. M4 + S97	18.81*ef*	7.04*c*
O. M1 + M4 + S97	22.71*j*	14.10*f*
P. Control (uninfested)	14.81*b*	5.32*b*

[a]*P. c.*, *Phytophthora cinnamomi*, isolate number 94048; M1, *Micromonospora carbonacea*; M4, *Micromonospora inositola*; and S97, *Streptomyces violascens*.

[b]Fresh weight 11 weeks after transplanting. Values with the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

control root rot (Table 2). All regression lines had significant linear correlation coefficients ($P = 0.001$). Slope values increased as T_{50} values decreased (Table 2).

A significant ($P = 0.05$) reduction in plant shoot and root weight was observed when plants were grown in soil infested with *P. cinnamomi* alone (treatment A) or in combination with the noncellulase-producing *Micromonospora* (treatment C), compared with the control (treatment P) (Table 3). All other treatments, except treatment J, increased shoot growth significantly ($P = 0.05$) compared with the control (Table 3). Treatments that included actinomycetes alone or in combination (treatments I–O, excluding J) gave significantly greater fresh shoot weights than the same treatments in combination with *P. cinnamomi* (treatments B–H).

Phytophthora cinnamomi was isolated from the roots and collars of all treatments except P, F, and H (uninfested control, *P. cinnamomi* + M1 + S97, and *P. cinnamomi* + M1 + M4 + S97, respectively). *Phytophthora cinnamomi* was not isolated from any of the treatments that contained the antagonists alone (treatments I–O). In addition, treatments F and H reduced the recovery of the pathogen, since *P. cinnamomi* was rarely isolated from the soils containing these treatments by baiting. In all other treatments that contained the pathogen, *P. cinnamomi* was frequently isolated from the baits. At no time was it obtained from treatments that did not include the pathogen (treatments I–O).

Discussion

This study indicates that there is potential for the utilization of cellulase-producing *Micromonospora* species to degrade the cell walls and cause lysis of *P. cinnamomi* hyphae, similar to that observed by Malajczuk (1983). Once cell wall damage has occurred, the pathogen is more likely to be susceptible to attack by other biological, physical, and chemical agents. This was effectively shown by the addition of a known antibiotic-producing streptomycete alongside the cellulase-producing *Micromonospora* isolate. Actinomycete enzymes have been shown to play an important role in the hydrolysis of fungal cell walls and to act as natural biocontrol agents (Sneh 1981; Schrempf 1995).

The lytic antifungal mechanisms become effective only if the selected microorganisms are established as a stable component in the ecosystem (Sneh 1981). Pathogens such as *P. cinnamomi* are difficult to control once penetration of the host root has occurred. Consequently, promising biological control organisms may need to be rhizosphere associated to antagonize the pathogen prior to infection. The cellulolytic capabilities of such an antagonist may be a significant factor in allowing it to compete in the soil and to colonize the rhizosphere of the susceptible plant (Gees and Coffey 1989).

The cellulase activities of the *Micromonospora* isolates examined were rather low by comparison with highly efficient cellulase systems reported in the literature, e.g., *Trichoderma reesei* Simon and its mutant strains. It is well known that different cellulosic substrates affect the levels of cellulases produced when microorganisms are grown in culture (Chahal 1985; Dekker 1987). Furthermore, the low extracellular activities of cellulase and β-glucosidase can be attributable to their association with the cell wall (cell wall bound) (Yamane and Suzuki 1988; Van Zyl 1985) or, in the case of cellulases, their strong adsorption onto undigested cellulose (Kim et al. 1994). Moreover, the low β-glucosidase activity causes cellobiose to accumulate during cellulose degradation, which can lead to the inhibition of the cellulase complex (Dekker 1989). The low levels of cellulase produced by the micromonosporae isolates in this study may therefore be explained by the aforementioned factors.

Despite its low cellulolytic enzyme activities, the *M. carbonacea* isolate used in the glasshouse trials was, nevertheless, capable of causing partial damage and lysis of *P. cinnamomi* hyphae. As the cell walls of *Phytophthora* spp. contain cellulose that is poorly crystalline by nature (Bartnicki-Garcia and Wang 1983), it would be expected to be more easily degraded enzymatically than native cellulose. This assumption is based on the observation that the *Micromonospora* isolates were able to degrade filter paper, a representative cellulose that closely approximates native cellulose consisting of both amorphous and crystalline cellulose (Wood and Bhat 1988). We conclude that the cellulases (albeit cell-bound or extracellular) are capable of causing lysis of *P. cinnamomi* hyphae even at low levels and are thus involved in antagonism. However, factors other than cellulases may also be involved in the suppression of *P. cinnamomi*.

The greater disease suppression observed, when the cellulase-producing *M. carbonacea* and the antibiotic-producing *S. violascens* were used in conjunction to control *P. cinnamomi*, may suggest that this effect could be due to the co-antagonism as suggested by Papavizas (1985).

In addition to the observed disease suppression, the selected actinomycetes also significantly stimulated plant growth compared with the control. This was observed for all combinations of actinomycetes with or without *P. cinna-*

momi, except when the noncellulase-producing *M. inositola* was used alone or with *P. cinnamomi*. Therefore, it appears that the actinomycetes not only suppressed disease but also stimulated plant growth, even in the presence of *P. cinnamomi*. This study supports other observations in which actinomycetes stimulated plant growth in the presence of plant pathogens (Turhan 1981; Tahvonen and Avikainen 1990). This study highlights the importance of using polyvalent *Streptomyces* phages for the selective isolation of non-streptomycete actinomycetes as potential biological control agents.

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