

Efficacy of Various Biological Control Agents and Biorationals against *Pythium* Root Rot in Poinsettia

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SUMMARY. *Pythium* root rot (*Pythium* spp.) is a common disease of greenhouse-grown poinsettias (*Euphorbia pulcherrima*) that can cause serious plant loss or reduction in plant quality. Application of effective chemical fungicides to poinsettia plants has reduced losses due to *Pythium*; however, development of resistance to these fungicides is a legitimate concern, as well as the environmental implications of using chemical pesticides. In this study, a group of products of biological origin and known biocontrol agents were evaluated for their efficacy to control *pythium* root rot of poinsettia. These products and organisms were compared to metalaxyl (Ridomil), a fungicide commonly used to reduce losses to *Pythium*. The results showed

that two products based on two different species of *Streptomyces*, Mycostop and Actino-Iron, were as effective as metalaxyl at reducing the symptoms associated with *pythium* root rot when artificially inoculated with *Pythium ultimum* var. *ultimum* compared to the control plants. Many roots remained functional throughout the duration of the experiments and the overall appearance and number of bracts of commercial quality of the plants were similar for the three treatments mentioned above. In an additional experiment, Mycostop was tested in combination with a single application of metalaxyl either at 3, 7, or 11 weeks after transplanting. Plants inoculated with *P. ultimum* var. *ultimum* and treated with metalaxyl either on week 3 or 7 after transplanting in combination with two applications of Mycostop, had greater fresh root weight than those only treated with metalaxyl at week 11 or the chemical control (three applications of metalaxyl). However, there was no significant difference in the number of bracts or the bract diameter between plants treated with metalaxyl at weeks 3 or 7 followed by Mycostop and those plants treated with the fungicide alone. A reduction in the amount of fungicide used to control *pythium* root rot can be achieved when used in combination with a biocontrol agent without compromising the health of poinsettias.

Root rots caused by *Pythium* species are of great concern to growers in the floricultural industry particularly since the adoption of new irrigation technologies that prevent the run off of chemicals into the environment. At present, 25% to 30% of commercial greenhouses in the Niagara region of southern Ontario, Canada, are using recirculating irrigation systems. In addition, growers of potted floricultural crops are replacing the traditional overhead (drip) irrigation with subirrigation. These changes in irrigation practices have increased the concerns about pathogen contamination of fertilizer solutions and the reservoirs where these solutions are maintained. Poinsettia plants are often infected by the pathogen *Pythium ultimum* var. *ultimum*. Symptoms of plants infected with this pathogen are stunting, chlorosis, and a general wilting. Infected roots are brown with rotted tips and the cortex usually sloughs off easily, leaving the

vascular system exposed. Although *P. ultimum* var. *ultimum* seldom produces zoospores, the motile propagules produced by oomycetes such as *Pythium*, the fungus has been isolated from reservoirs where the fertilizer solutions are stored (J.A. Gracia-Garza, unpublished) and can easily be disseminated throughout a greenhouse operation that uses a recirculating subirrigation system.

Several chemical products are recommended for use against this pathogen. In Ontario, metalaxyl (Subdue, Syngenta Crop Protection Canada Inc., Guelph, Ont., Canada) and fosetyl-aluminum (Aliette, Rhone-Poulenc Canada, Mississauga, Ont., Canada) are two chemicals registered for the control of root rot caused by *Pythium* in ornamental crops. However, recent studies have indicated that some isolates of *Pythium* are becoming less sensitive to metalaxyl (Daughtrey, 1998). Thus, alternative strategies must be found before resistance to these pesticides is fully developed. Many different organisms, including actinomycetes, have been reported as potential biocontrol agents (BCA) against *Pythium* (Liang et al., 1996; Mahadevan and Crawford, 1997; McCullagh et al., 1996; Rankin and Paulitz, 1994). *Streptomyces* are aerobic, gram-positive actinomycetes with reported activity against several plant pathogens, including *Pythium* (Mahadevan and Crawford, 1997). The mechanisms by which *Streptomyces* are able to reduce losses due to soilborne pathogens include competition for nutrients, and production of antimicrobial products and lytic enzymes (Kortemaa et al., 1994; Mahadevan and Crawford, 1997). The objectives of the study presented here were 1) to evaluate several commercially available products of biological origin for their ability to reduce or eliminate root rot in poinsettia and 2) to determine if a single application of the fungicide metalaxyl in combination with a strain of *Streptomyces griseoviridis*, will provide satisfactory control of *P. ultimum*.

Materials and methods

PLANT MATERIAL, PATHOGENS AND SCREENED BCAs, AND BIORATIONALS. Cuttings from stock 'Freedom' poinsettia plants, were collected and rooted in Oasis (Smithers-Oasis, Kent, Ohio) for 4 weeks. Rooted cuttings were

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then transplanted into 10-cm-diameter (4-inch) plastic pots, using Sunshine Mix # 4, (SunGro Horticulture Inc., Westerville, Ohio), a peat-based soilless medium.

Plants were treated as follows (Table 1): 1) control; 2) Actino-Iron (Natural Industries, Inc., Houston, Texas) containing: *Streptomyces lydicus* WYEC 108, 18% to 20% iron, 30% to 32% fulvic acid blended into the potting medium at 3 g·L⁻¹ (label rate) before planting; 3) BTM (Earth Corp Environmental Ltd., Calgary, Alta., Canada) containing several genera of bacteria (*Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas*, and *Rhizobium*) in a humic acid solution applied as a monthly drench at 5.5 mL·L⁻¹ (label rate); 4) Modicell (DeruNed BV, Bergschenhoek, The Netherlands) containing enzymes extracted from several genera of fungi applied as a monthly drench at 1.5 mL·L⁻¹ (label rate); 5) Mycostop (Kemira Agro Oy, Helsinki, Finland) containing *S. griseoviridis* strain K61 applied as a monthly drench at 0.06 g·L⁻¹ (label rate); 6) *Pseudomonas fluorescens* strain 15 (T.C. Paulitz, collection) applied by dipping the Oasis cube in a solution containing 10⁶ cfu/mL at transplanting; 7) metalaxyl (Ridomil 240. E.C.; Syngenta Crop Protection Canada Inc., Guelph, Ont., Canada) applied as a monthly drench at 0.04 mL·L⁻¹ (label rate); 8) RootShield (Bioworks, Inc. Geneva, N.Y.) containing *Trichoderma harzianum* Rifai strain KRL-AG2 applied once as a drench shortly after transplanting at 0.6 g·L⁻¹ (label rate); 9) SoilGard 12G (Termo-Trilogy Corp. Columbia, Md.) containing *Gliocladium virens* GL-21 applied to the potting soil (1 d) before transplanting at 0.9 g·L⁻¹ of substrate (label rate); 10) *T. hamatum* strain TMCS 3 (TMCS 3) (J.A. Gracia-Garza, personal collection) applied as a drench once shortly after transplanting at 10⁶ cfu/mL. All drenches were applied at 100 mL (3.4 fl oz) per pot. Incorporation of Actino-Iron and SoilGard into the growing substrate took place 1 d before transplanting to allow incubation.

Cultures of *P. fluorescens* were grown on nutrient broth for 2 d on an orbital mechanical shaker at 125 rpm (Lab-Line Instruments, Inc., Melrose Park, Ill.) and 22 ± 2 °C (71.6 ± 3.6 °F). The concentration of bacteria was estimated using a spectrophotometer

(Beckman Du 640, Beckman Instruments, Inc. Fullerton, Calif.), to obtain a final absorptivity reading of 0.14 (wavelength of 650 μm). At this absorptivity, the solution was estimated to contain 6 × 10⁷ cfu/mL (1.8 × 10⁹ cfu/fl oz). The bacterial solution was diluted to 10⁶ cfu/mL (3.0 × 10⁷ cfu/fl oz) before inoculating the plants. The strain TMCS 3 was grown on Petri plates containing potato dextrose agar (PDA) (Difco Laboratories, Detroit, Mich.) for 5 d before harvesting conidia. Conidia were scraped from the surface of the plate with a metal rod after adding 15 mL (0.5 fl oz) of sterile water. The spore solution was diluted until a 10⁶ cfu/mL concentration was obtained.

Pythium ultimum var. *ultimum* isolate Po20 (Po20) was originally isolated from a diseased poinsettia plant in the Niagara region. The pathogen was grown in a V8 liquid medium amended with calcium carbonate (CaCO₃) [10 g·L⁻¹ (1.34 oz/gal) of V8 juice]. The growing medium was similar to that described by Miller (1955) without adding agar. The cultures were incubated on an orbital mechanical shaker (150 rpm) for 10 d under constant incandescent light at 26 °C (79 °F). Cultures were then blended for 30 s to homogenize the sporangia and oospores in the culture. Sufficient inoculum was added directly to 25 L (6.6 gal) tanks used for recirculating fertilizer solution at a concentration of 10² sporangia/mL (3.8 × 10⁵ sporangia/gal). The concentration of the pathogen was an approximate to levels found in tanks for recirculating subirrigation systems in commercial greenhouses for this region. Tanks were infested twice with the sporangial solution namely, at 1 and 6 weeks after transplanting.

To determine the dynamics of the pathogen population in the recirculating tanks, samples [500 μL (17 × 10⁻⁶ fl oz)] of the fertilizer solution were taken on a weekly basis during the experiments. Samples (500 μL) were plated on 90 mm (3.5 in) Petri plates of a selective medium for *Pythium* spp. (Tsao and Ocaña 1969). Plates were incubated at 22 ± 2 °C for 3 to 5 d before counting colonies.

SCREENING OF BCAs AND BIORATIONALS. Each treatment was applied to two sets of 15 plants. The plants were 4-week-old rooted cuttings of 'Freedom' poinsettia. One set

was inoculated (2 weeks after transplanting) with Po20 through subirrigation with an infested fertilizer solution, and the other set served as a noninoculated control (i.e., *Pythium*-free). For those plants receiving a drench, each plant received 100 mL (3.4 fl oz) of the appropriate solution. Each treatment was placed in a separate trough and each trough was connected to a separate tank. Recirculating tanks were filled with a fertilizer solution containing, in mmol·L⁻¹ (ppm): 12.25 (760) nitrate (NO₃⁻), 2.25 (40.5) ammonium (NH₄⁺), 1.0 (97) dihydrogen phosphate (H₂PO₄⁻), 3.5 (136.5) potassium (K⁺), 3.75 (150) calcium (Ca²⁺), 1.0 (24) magnesium (Mg²⁺), 1.0 (96) sulphate (SO₄²⁻), 0.025 (1.4) iron (Fe²⁺), 0.005 (0.28) manganese (Mn²⁺), 0.0035 (0.23) zinc (Zn²⁺), 0.020 (1.2) borate (BO₃³⁻), 0.0008 (0.05) copper (Cu²⁺), and 0.0005 (0.08) molybdate (MoO₄²⁻). Irrigation of all plants was changed to clear water when cyathia began to produce pollen according to commercial practices. Experimental plants were grown in a greenhouse for 15 weeks with the following equal day/night temperature regimes: 21 °C (70 °F) during vegetative growth and flower initiation, 19 °C (66 °F) during flower development and 17 °C (63 °F) for final 2 weeks before harvest of the experiment. From transplanting to blackout, plants were maintained under long-day photoperiod using incandescent light from 2200 to 0200 HR daily. Plants were pinched (top of plant removed) 10 d before the beginning of blackout. Blackout was provided daily with black curtains for photoperiodic control for 13 h (1800 to 0700 HR) to induce flower initiation, starting 4 weeks after transplanting until the end of experiment.

At the end of the experiment (15-week duration), the following measurements or ratings were taken on 10 randomly selected plants per experimental unit: number of bracts of commercially acceptable quality, diameter of bracts (bract diameter was determined by measuring across the uppermost inflorescence from tip to tip of two of the primary bracts) and overall appearance of root system. Root rating was done as follows: 1 = healthy root system with white roots; 2 = few roots showing discoloration and/or tissue maceration; 3 = root system discolored with some dead roots; 4 =

root system totally disintegrated/dead. In addition, on five plants (first five plants in the trough closer to the tanks) per experimental unit: fresh and dry weights of the plants (only dry weights reported in the results) were measured.

EVALUATION OF APPLICATION OF DIFFERENT MYCOSTOP/METALAXYL COMBINATIONS. Rooted cuttings of 'Freedom' poinsettias were grown as described above and all treatments were either inoculated with Po20 (2 weeks after transplanting) or remained *Pythium*-free. The treatments consisted of the following application combinations, metalaxyl (R) on week 3 followed by two applications of Mycostop (M) at weeks 7 and 11 (R/M/M). The other treatments were M/R/M and M/M/R. Control treatments consisted of plants treated only with metalaxyl or Mycostop at the three application times (R/R/R, M/M/M) and nontreated plants. At each application date, a drench of only one product was applied (100 mL of solution overhead). Concentrations for metalaxyl and Mycostop were 0.04 mL·L⁻¹ (0.005 fl oz/gal) and 0.06 g·L⁻¹ (0.008 oz/gal), respectively.

The same parameters as in the previous experiment were measured.

EXPERIMENTAL DESIGN. All experiments were arranged in a split-plot design with three replications per treatment. There were 15 plants per replication (irrigation trough with separate tank). The main plot factor was the presence or absence of *Pythium* and the subplots were the products tested (treatments). All experiments were repeated once; the first experiments (i.e., screening of BCAs and biorationals) took place during Fall 1999 and Win-

ter 2000; the second (i.e., evaluation of applications of different Mycostop/metalaxyl combinations) during the fall of 2000 and Winter 2001.

STATISTICAL ANALYSIS. Statistical analyses were carried out using the PROC GLM procedure (SAS Institute, Cary, N.C.) to establish significance of the effects of all factors ($P \leq 0.05$). The analyses were done separately for inoculated and noninoculated treatments. Rating values for root appearance were first converted into ranks (PROC RANKS) and then analyzed using PROC GLM. Differences among treatments were identified using protected LSD.

Results

SCREENING OF BCAs AND BIORATIONALS. Less than 1% of the plants died throughout these experiments as a result of *P. ultimum* infection; however, symptoms of root rot were evident in many plants inoculated with Po20. *Pythium* infection reduced plant dry weight and the number of bracts commercial quality in nontreated control plants ($P \leq 0.05$). This indicates that the Po20 isolate used in this experiment was pathogenic to poinsettias.

Compared to control plants, plants treated with Mycostop or Actino-Iron showed an improvement in the overall appearance (number of bracts and plant dry weight) when the fertilizer solution was infested with Po20 (Table 2). Plants treated with Actino-Iron had plant dry weights similar to plants treated with metalaxyl. Plants treated with Actino-Iron or metalaxyl had similar shoot numbers (data not shown) and plant growth.

Plant dry weight and number of bracts of Mycostop-treated plants were not significantly different than those plants treated with either metalaxyl or Actino-Iron (Table 2). Based on root evaluations, plants treated with metalaxyl had significantly healthier root systems than those treated with Modicell, Actino-Iron, or Mycostop (Table 2).

On *Pythium*-free plants (noninoculated), a growth enhancing effect was observed on plants treated with the mycoparasite *Trichoderma* (i.e., TMCS 3 and RootShield), as well as with *Streptomyces* (Mycostop) compared to the other treatments. Plants were more vigorous and bracts of commercial quality and bract diameters were significantly better than the rest of the treatments, including metalaxyl (Table 2).

Populations of *P. ultimum* in the irrigation tanks generally decreased over time after each infestation. However, tanks connected to troughs with diseased plants maintained a consistent population [27 cfu/mL (8.0×10^2 cfu/fl oz) of fertilizer solution] during the course of the experiments, indicating that *Pythium* propagules were carried with the recirculating fertilizer solution from diseased plants into the holding tanks. However, recent experiments by the authors suggest that movement of *P. ultimum* appears to be limited (unpublished data). *Pythium* population density in *Pythium*-free tanks was zero throughout the experiments.

EVALUATION OF APPLICATIONS OF DIFFERENT MYCOSTOP/METALAXYL COMBINATIONS. Plant mortality of *Pythium*-inoculated plants was highest in the control treatment (25%),

Table 1. Listing of treatments, method and rate of application and frequency of application for the control of pythium root rot during the cropping cycle of poinsettias.

Treatment	Application technique	Rate	Application frequency
Control (nontreated)			
Actino-Iron	Incorporate to soil mix	3 g·L ⁻¹ (5 lb/yard ³) of media	Once before transplanting
BTM ^a	Drench	5.5 mL·L ⁻¹ (0.7 fl oz/gal)	Monthly
Modicell ^b	Drench	1.5 mL·L ⁻¹ (0.2 fl oz/gal)	Monthly
Mycostop	Drench	0.06 g·L ⁻¹ (0.008 oz/gal)	Monthly
<i>Pseudomonas fluorescens</i>	Dip	10 ⁶ cfu/mL (3.0×10^7 cfu/fl oz)	Once at transplanting
Metalaxyl (Ridomil 240 EC)	Drench	0.04 mL·L ⁻¹ (0.5 fl oz/100 gal)	Monthly
RootShield	Drench	0.6 g·L ⁻¹ (8 oz/100 gal)	Once after transplanting
SoilGard 12 G	Soil mix	0.9 g·L ⁻¹ (1.5 lb/yard ³) of media	Once before transplanting
TMCS 3 ^c (<i>Trichoderma hamatum</i>)	Drench	10 ⁶ cfu/mL (3.0×10^7 cfu/fl oz)	Once after transplanting

^aBTM = a product containing several genera of bacteria (*Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas*, and *Rhizobium*) in a humic acid solution.

^bThe authors consider this product a biorational since no organism is alive in the product but only the product extracted from an organism is being used.

^cIsolate of the mycoparasite *Trichoderma hamatum* labelled TMCS 3 (J.A. Gracia-Garza personal collection).

Table 2. Average number of bracts (20 plants), plant dry weight (10 plants) and root rating (10 plants) of 'Freedom' poinsettia (*Euphorbia pulcherrima*) inoculated with *Pythium ultimum* var. *ultimum* isolate Po20 (Po20) or noninoculated and treated with one of several products.

Treatment	Po20 inoculated			Noninoculated	
	Bracts (no.) ^z	Plant dry wt (g) ^y	Avg root rating ^x	Bracts (no.)	Plant dry wt (g)
Control (nontreated)	1.3 c ^w	6.5 c	3.55 cd	2.4 ab	9.8 bcd
Actino-Iron	2.0 b	9.3 ab	3.33 c	1.9 c	9.0 bcd
BTM ^v	1.7 bc	6.6 c	3.40 cd	2.1 c	9.5 bcd
Modicell	1.6 bc	7.6 abc	2.96 b	2.3 b	10.7 bc
Mycostop	2.2 ab	8.1 ab	3.37 cd	2.4 abc	13.9 a
<i>Pseudomonas fluorescens</i>	1.2 c	5.9 c	3.67 d	2.1 c	9.0 bcd
Metalaxyl (Ridomil 240 E.C.)	2.7 a	9.6 a	1.97 a	1.9 c	7.8 d
RootShield	1.9 b	7.4 bc	3.57 cd	2.8 ab	11.1 b
SoilGard 12 G	1.3 c	6.0 c	3.56 cd	1.3 d	8.3 cd
TMCS 3 (<i>Trichoderma hamatum</i>) ^u	1.7 bc	6.8 c	3.40 cd	2.9 a	11.2 b
LSD ($P \leq 0.05$)	0.58	2.1		0.54	2.43

^xNumber of bracts of commercially acceptable quality.

^y28.4 g = 1.0 oz.

^zRoot rating was done as follows: 1 = healthy root system with white roots; 2 = few roots showing discoloration and/or tissue maceration; 3 = root system discolored with some dead roots; and 4 = root system totally disintegrated.

^wTreatments followed by different letters differed statistically at the 0.05 level of significance according to a protected LSD.

^vBTM = A product containing several genera of bacteria (*Bacillus*, *Clostridium*, *Enterobacter*, *Pseudomonas*, and *Rhizobium*) in a humic acid solution.

^uIsolate of the mycoparasite *Trichoderma hamatum* labelled TMCS 3 (J.A. Gracia-Garza personal collection).

followed by the treatment consisting of a single metalaxyl application at week 11 (8.5%). There was no plant mortality when treated with metalaxyl either at week 3 or 7 (Table 3). None of the noninoculated plants died. Analysis of the overall appearance of the plant (plant and root rating) for the inoculated treatments indicated significant differences ($P \leq 0.05$) among the treatments. Plants treated with R/M/M, M/R/M or R/R/R appeared healthier (visually) than the rest of the treatments as well as quantitatively by the number and size of the bracts (Table 3). However, only the root fresh weights of the treatments R/M/M and M/R/M were significantly

larger ($P \leq 0.05$) than the other treatments (Fig. 1), including the chemical control (R/R/R). There were no differences in plant and root characteristics for the noninoculated treatments (root data shown in Fig. 1).

Similar trends in the population dynamics of *P. ultimum* in the tanks was observed in these experiments as in the previous test.

Discussion

Use of BCAs or products of biological origin is a feasible alternative to the use of chemicals for some pathosystems, as seen in these tests. However, often companies producing biologically based products recom-

mend to introduce the BCAs early in the cropping system to allow for adequate colonization of the root systems and/or the growing substrate. BCAs are better suited as a preventative rather than a curative treatment. Some BCAs such as those containing *Trichoderma*, appear to have a growth-enhancing effect when the population of the pathogen is either low or nonexistent as shown in Table 2. Some of these BCAs might not be strong competitors against aggressive pathogens but might be deleterious to weaker organisms, competing in the rhizosphere for nutrients. In addition, plants may react to the colonization of some BCAs by releasing some defense

Table 3. Mortality rate, number of bracts and bract diameter (cm) and root fresh weight (g) of 'Freedom' poinsettia (*Euphorbia pulcherrima*) inoculated with *Pythium ultimum* var. *ultimum* isolate Po20 (Po20), as well as mortality rate and root fresh weight (g) for noninoculated treatments. Plants were nontreated (control) or treated with three drench applications of metalaxyl (R/R/R), three applications of Mycostop (M/M/M) or a combined application of metalaxyl (R) and Mycostop (M) at 3, 7 or 11 weeks after transplanting.

Treatment	Po20 inoculated				Noninoculated	
	Mortality (%)	Bracts (no.) ^z	Bract diam (cm) ^y	Root fresh wt (g) ^x	Mortality (%)	Root fresh wt (g)
Control	25	2.3 bc ^w	24.0 b	7.7 b	0	10.9 b
R/R/R	0	2.8 a	31.4 a	7.6 b	0	13.1 b
M / M / M	4.2	2.1 c	22.9 b	11.2 ab	0	13.5 b
R/M/M	0	2.6 ab	31.2 a	13.4 a	0	12.7 a
M/R/M	0	2.7 ab	31.4 a	13.5 a	0	14.5 a
M/M/R	8.5	2.1 c	26.1 b	8.8 b	0	10.6 b

^xNumber of bracts of commercially acceptable quality.

^y2.5 cm = 1.0 inch.

^z28.4 g = 1.0 oz.

^wTreatments followed by different letters differ statistically at the 0.05 levels of significance according to a protected LSD.

mechanisms that indirectly suppress weak pathogens resulting in more vigorous plants.

In these tests, some products/organisms did not perform well; however, these should not be considered as an ineffective biological product for all pathosystems. Often, BCAs require specific conditions for optimal performance. What can be effective in one system may be ineffective against another system; for instance, a product may perform better at controlling damping-off in bedding plants than in a system such as the one studied in this project (Honeycutt and Benson, 2001). Moreover, BCAs are in many instances specific to a particular plant pathogen. In tests with other soil-borne pathogens, similar to those presented here we have obtained a large range of responses depending on the pathogen against which they are being used (Gracia-Garza, 2001). A thorough investigation of the pathogen and the natural environmental conditions where the BCAs are expected to perform should be conducted in order to fully explore the usefulness of the few biological alternatives presently available against plant pathogens. At present, in Canada, only two biocontrol agents have been registered against ornamental crops, Mycostop, and more recently, Rootshield. In the U.S., several of the biocontrol agents used in this study are already commercially available for growers of poinsettia and other ornamental crops.

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