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Biological Control of *Phytophthora infestans* of Potatoes using *Trichoderma atroviride*

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ABSTRACT

The efficacy of *Trichoderma atroviride* against late blight (*Phytophthora infestans*) was studied in trials conducted in growth chamber and *in vitro*. The growth chamber trials were randomized as complete blocks with four treatments replicated five times. The treatments included: 1) untreated control inoculated with *P. infestans*; 2) tubers inoculated with *P. infestans* and treated with *T. atroviride*; 3) tubers inoculated with *P. infestans* and treated with Bravo 500F; and 4) tubers inoculated with *P. infestans* and treated with both *T. atroviride* and Bravo 500F. Among the different treatments, Bravo 500F was significantly superior to *T. atroviride* alone or in combination with Bravo 500F. Late blight disease severity was reduced by 27 and 36%, respectively, as a result of treatments with *T. atroviride* alone or in combination with Bravo 500F. The efficacy of *T. atroviride* applied at various concentrations was tested *in vitro* for their efficacy in reducing disease severity in leaf disc assays. *T. atroviride* applied at high concentration provided complete control of late blight incidence on leaf discs. The results of the present studies suggest that higher disease control efficiency can be achieved if *T. atroviride* is used in an integrated late blight management approach.

Keywords: Chlorothalonil, efficacy, late blight, *Phytophthora infestans*, *Solanum tuberosum*, *Trichoderma atroviride*

INTRODUCTION

Late blight of potato caused by *Phytophthora infestans* (Mont.) deBary is an important disease in potato growing areas around the world. Crop losses due to this disease can reach 50% (Goodwin *et al*. 1994; Secor and Gudmestad 1999). Reports published earlier have predicted that potato late blight will continue to cause food shortages and hunger in several parts of the world (Schiermeier 2001; Garelik 2002). In the last decade, the occurrence of new genotypes of *P. infestans* has lead to an increase in incidence and severity of late blight and ultimately hampering the disease control process (Goodwin *et al*. 1995; Chycoski and Punja 1996; Goodwin *et al*. 1998; Cooke *et al*. 2003, 2006). Most of the *P. infestans* isolates belong to the US-8 (A2) mating type and are insensitive to the fungicide metalaxyl (Deahl *et al*. 1995). Studies conducted earlier have shown that US-8 isolates are more aggressive on potato foliage and tubers than the US-1 isolates (Inglis *et al*. 1996; Fry and Goodwin 1997; Kato *et al*. 1997; Lambert and Currier 1997; Miller *et al*. 1997). The US-1 genotype has been displaced by US-8 genotype in major parts of Canada, excluding British Columbia (Peters *et al*. 1998). Only a few potato varieties are considered to be moderately resistant to late blight (Secor and Gudmestad 1999). Fungicide resistance observed in *P. infestans* over the years prevented appropriate disease control (Smart and Fry 2001; Shattock 2002; Cooke *et al*. 2003). The production of sexual oospores by the mating types of *P. infestans* allows for survival of the pathogens in the soil between potato crops. This eventually acts as a source of primary inoculum and results in earlier epidemics (Drenth *et al*. 1995; Chycoski and Punja 1996; Andersson *et al*. 1998; Smirnov and Elansky 1999; Flier and Turkensteen 2000; Turkensteen *et al*. 2000; Zwankhuizen *et al*. 2000). The use of infected seed potato tubers should be avoided as it is also one of the important means of *P. infestans* transmission between potato crops (Boyd 1974). Using disease-free seed is necessary since the pathogen has the capability to spread throughout the storage area or the field from the infected seed pieces (Johnson *et al*. 1997; Secor and Gudmestad 1999; Johnson *et al*. 2000). Due to these reasons there is a need to look for alternative measures for late blight control.

The fungal species belonging to the genus *Trichoderma* occur throughout the world and can be easily isolated from soil, decaying wood, and organic matter. The potential of this genus in the biological control of pathogens was first noticed in the early 1930s (Weindling 1932). Over the years it has proven to be very effective in combating various plant diseases (Lifshitz *et al*. 1986; Chet 1987; Zhang *et al*. 1996; Elad and Kapat 1999; Yedidia *et al*. 1999; Harman 2000; Sharon *et al*. 2001; Tsror *et al*. 2001; Howell 2002; Sid Ahmed *et al*. 2003; Ezziyyani *et al*. 2007). The success of *Trichoderma* in plant disease control has lead to the commercial production of several *Trichoderma* species for crop growth and disease control (Lumsden *et al*. 1992; Harman *et al*. 1996; Samuels 1996; McSpadden Gardner and Fravel 2002). *Trichoderma atroviride* (Plant Helper®, AmPac Bio Tech Inc., Fresno, CA) is a fast growing fungus which produces profuse spores and is resistant to metalaxyl and captan while having high tolerance to mancozeb and other chemical fungicides. Plant Helper® containing living microorganisms and other naturally derived components has multiple ingenious functions to stimulate plant growth and enhances resistance in plants against various plant diseases (McBeath *et al*. 2000). *T. atroviride* forges a symbiotic relationship with plants and has been associated with plant growth promotion in addition to disease suppression (Wong and McBeath 1999). In the present study an attempt was made to test the efficacy of *T. atroviride* in controlling *P. infestans* under *in vitro* and growth chamber conditions.

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Original Research Paper

MATERIALS AND METHODS

Source of microorganisms used in the study

Phytophthora infestans (A2 mating type) was isolated from infected potato leaves collected from New Brunswick. The cultures were purified, properly identified, and deposited in the fungal culture bank at the Potato Development Centre, New Brunswick Department of Agriculture and Aquaculture, Wicklow, New Brunswick, Canada.

Experiment I. Efficacy of *Trichoderma atroviride* and Bravo 500F against late blight (*Phytophthora infestans*) in potato plants

The experiment was set as a randomized complete block design with four treatments which were replicated five times. The treatments were: 1) untreated control inoculated with *Phytophthora infestans*; 2) tubers inoculated with *P. infestans* and treated with *Trichoderma atroviride*; 3) tubers inoculated with *P. infestans* and treated with Bravo 500F; and 4) tubers inoculated with *P. infestans* and treated with both *T. atroviride* and Bravo 500F.

Twenty healthy seed potato tubers (cv. 'Shepody', Elite 2, Bon Accord Seed Potato Centre, New Brunswick, Canada) were cut in half and planted in square pots (Jumbo 55 - 5" size, Kord Co., Brampton, Ontario) containing Shultz® professional potting soil (N-P-K 0.08-0.12-0.08). The plants were allowed to grow for 4 weeks in the growth chamber. A 400 watt metal halide MHSS 408 light (Cooper Lighting Division, Peachtree city, Georgia, USA) was used to simulate natural sunlight throughout the growing period.

The plants were administered with various materials according to the treatment details 28 days after planting. Plant Helper [flowable concentrate, 10% *Trichoderma atroviride* (3×10^8 CFU/g), AmPac BioTech Inc., Fresno, CA] (0.454 g) was mixed with 250 mL of sterile distilled water (SDW). In case of Bravo 500F [500 g Chlorothalonil (Tetrachloroisophthalonitrile) per litre, Syngenta Crop Protection Canada, Inc., Guelph, Ontario], 2 mL were mixed with 250 mL of SDW. For plants treated with both *T. atroviride* and Bravo 500F, Bravo 500F was applied first and *T. atroviride* was applied in the subsequent two weeks. All treatments were applied until runoff using a hand held spray bottle.

Fresh potato leaves infected with *P. infestans* (A2 mating type) were collected from the field. Spores from the most symptomatic area of infected leaves were separated and suspended in approximately 50 ml sterile distilled water (SDW) chilled to 4°C by dipping the leaves several times in SDW. The spore suspension was filtered through miracloth® (Calbiochem®, VWR, Ontario) into a sterile tube. Spore counts were made using a hemacytometer (~11,000 spores mL^{-1}). The plants were inoculated with *P. infestans* spore suspension using a hand held spray bottle three days after application of treatments. Two weeks after inoculation, the plants were visually assessed for disease severity on a scale of 0-100% (0 = no disease observed; 1 = up to 10 spots per plant; 5 = about 50 spots per plant; 25 = nearly every leaflet infected; 50 = every plant affected and about 50% of leaf area destroyed; 75 = about 75% of leaf area destroyed; 95 = only a few leaves on plants are alive; 100 = all leaves are dead) (Cruickshank *et al*. 1982; Dorrance and Inglis 1997; James 1971). Disease severity values relative to the inoculated untreated control were calculated and the data were analyzed using CoStat (CoHort Software, Monterey, CA, USA). Means were separated using LSD test at P=0.1. The experiment was repeated one more time and data were averaged and presented in the tables.

Experiment II. Effect of *Trichoderma atroviride* applied at various concentrations on late blight (*Phytophthora infestans*) severity using potato leaf discs

The trial was designed as randomized complete block with six treatments replicated three times each. The treatments were 1) untreated control; 2) *T. atroviride* (3 × 10 CFU/mL); 3) *T. atroviride* (3×10^2 CFU/mL); 4) *T. atroviride* (3×10^3 CFU/mL); 5) *T. atroviride* (3×10^4 CFU/mL); and 6) *T. atroviride* (3×10^5 CFU/mL). Healthy seed potato pieces were planted and were kept in a growth chamber at 21°C and 70% relative humidity. Mature leaves (29 days after emergence) were later removed from the plants and leaf discs were made using a #10 sterile cork borer (14 mm diameter, VWR, Ontario).

Spore suspension of *P. infestans* required for inoculating leaf discs was prepared as described in Experiment I. Thirty milligram (30 mg) of *T. atroviride* (Plant Helper® containing 3×10^8 CFU/g) was dissolved in 30 mL of SDW and a series of dilutions were then prepared.

Leaf discs were dipped in a 100 mL solution containing the appropriate concentration of *T. atroviride* and were placed in sterile plastic Petri plates (100 × 15 mm, Fisher Scientific Co., Ontario) containing moistened filter paper. After 3 hrs, 10 µL of *P. infestans* (11,000 spores mL^{-1}) was added to the centre of each leaf disc. The plates were then incubated at 18 °C for 2 weeks and disease severity was recorded once every two days. Data analysis was done using CoStat (CoHort Software, Monterey, CA, USA) and the means were separated using LSD test at P=0.1. The experiment was repeated and data were averaged and presented in the tables.

Experiment III. Effect of *Trichoderma atroviride* applied at various concentrations on the growth of *Phytophthora infestans in vitro*

The efficacy of various concentrations of *Trichoderma atroviride* in inhibiting the growth of *Phytophthora infestans* was assessed under *in vitro* conditions. The experiment contained six treatments which were replicated three times. The treatments were 1) untreated control; 2) *T. atroviride* (3 × 10 CFU/mL); 3) *T. atroviride* (3×10^2 CFU/mL); 4) *T. atroviride* (3×10^3 CFU/mL); 5) *T. atroviride* (3×10^4 CFU/mL); and 6) *T. atroviride* (3×10^5 CFU/mL).

The same procedure used in the previous experiment was followed in preparation of different concentrations of *T. atroviride*. One mL of solution of the appropriate concentration of *T. atroviride* was added to plates containing sterilized rye extract agar (REA). Plates were incubated overnight to allow the products to be absorbed in the media. Solutions of different concentrations of *T. atroviride* were added to corresponding plates. Plates amended with 1 mL of SDW served as controls. Agar plugs (5 mm in diameter) were cut from plates containing actively growing culture of *P. infestans* (A1 or A2 mating types) and placed in the centre of rye agar plates amended with the appropriate concentrations of *T. atroviride*. The plates were covered with parafilm and incubated at 18°C for one week Radial growth of the fungus was recorded using two perpendicular measurements and mean values were calculated. Percentage of fungal growth inhibition (PFGI) was calculated using the following formula: PFGI = [(growth in sterile distilled water treated plates - growth in fungicide treated plates)/ growth in sterile distilled water treated plates)] × 100. Data obtained were analyzed using CoStat (CoHort Software, Monterey, CA, USA) and the means were separated using LSD test at P=0.1. The experiment was repeated twice and three replicates were used for each particular treatment and data were averaged and presented in the tables.

RESULTS

Experiment I. Efficacy of *Trichoderma atroviride* and Bravo 500F against late blight (*Phytophthora infestans*) in potato plants

Late blight disease severity was reduced by 96% when Bravo 500F was used, which was significantly superior to the *Trichoderma atroviride* treatment alone or the combination of both *T. atroviride* and Bravo 500F (**Table 1**). Treatment with *T. atroviride* alone and in combination with Bravo 500F reduced late blight disease severity by 27 and 36%, respectively; relative to the *P. infestans* inoculated controls. The combined treatment of *T. atroviride* and Bravo 500F was more effective against late blight than the treatment with *T. atroviride* alone. Among all treatments tested, Bravo 500F was the most effective against late blight.

Table 1 Effect of *Trichoderma atroviride* and Bravo 500F on the severity of late blight (*Phytophthora infestans*) on potato plants.

Treatments[1]	Disease severity (%)
Trichoderma atroviride	73 a[2]
Trichoderma atroviride + Bravo 500F	64 a
Bravo 500F	4 b

Data presented are an average of 2 experiments.
[1] Each treatment was replicated five times. Percent disease severity was calculated relative to the untreated, *Phytophthora infestans* inoculated controls.
[2] Within each column, means followed by same letter are not significantly different from each other at P=0.1.

Table 2 Effect of *Trichoderma atroviride*, applied at various concentrations, on the severity of late blight (*Phytophthora infestans*) using potato leaf discs.

Treatments[1]	Disease severity (%)
Untreated control	100 a[2]
Trichoderma atroviride (3 × 10)	40 ab
Trichoderma atroviride (3×10^2)	34 b
Trichoderma atroviride (3×10^3)	21 b
Trichoderma atroviride (3×10^4)	21 b
Trichoderma atroviride (3×10^5)	0 b

Data presented are an average of 2 experiments.
[1]Each treatment was replicated three times.
[2]Within each column, means followed by same letter are not significantly different from each other at P=0.1.

Table 3 Effect of *Trichoderma atroviride* applied at various concentrations on the growth of *Phytophthora infestans in vitro*.

Treatments[1]	Growth inhibition of *Phytophthora infestans* (%)	
	A1 mating type	A2 mating type
Untreated control	0.00 c[2]	0.00 d
Trichoderma atroviride (3 × 10)	89.90 b	84.13 c
Trichoderma atroviride (3×10^2)	90.24 ab	88.75 b
Trichoderma atroviride (3×10^3)	90.94 ab	90.20 ab
Trichoderma atroviride (3×10^4)	91.69 ab	90.95 ab
Trichoderma atroviride (3×10^5)	91.80 a	92.18 a

Data presented are an average of 2 experiments.
[1]Each treatment was replicated three times.
[2]Within each column, means followed by same letter are not significantly different from each other at P=0.1.

Experiment II. Effect of *Trichoderma atroviride* applied at various concentrations on late blight (*Phytophthora infestans*) severity using potato leaf discs

All concentrations of *T. atroviride* were able to suppress late blight at varying degrees (**Table 2**). Among the various concentrations tested, the 3×10^5 CFU/mL of *T. atroviride* gave complete control of late blight (**Table 2**). Treatments with *T. atroviride* tested (3 × 10, 3×10^2, 3×10^3, 3×10^4 and 3×10^5 CFU/mL) resulted in significantly lower disease severity values compared to the untreated inoculated control. The lowest late blight disease severity were obtained for the 3×10^5 CFU/mL of *T. atroviride* (0%) followed by 3×10^4 (21%), 3×10^3 (21%), 3×10^2 (34%) and 3 × 10 CFU/mL (40%) (**Table 2**).

Experiment III. Effect of *Trichoderma atroviride* applied at various concentrations on the growth of *Phytophthora infestans in vitro*

The ability of *T. atroviride* to inhibit the growth of *P. infestans* was tested *in vitro* using 2 strains of the fungus (both A1 and A2 mating type). All concentrations were effective in suppressing the growth of *P. infestans* (**Table 3**). The highest inhibition of the A1 mating type was obtained when the media was amended with 3×10^5 CFU/mL of *T. atroviride* (91.8%) followed by 3×10^4 CFU/mL (91.69%), 3×10^3 CFU/mL (90.94%), 3×10^2 CFU/mL (90.24%) and 3 × 10 CFU/mL (89.9%) all of which were significantly better than the control (**Table 3**). Similarly, the inhibition of the A2 mating type was higher at 3×10^5 CFU/mL of *T. atroviride* (92.18%), followed by 3×10^4 CFU/mL (90.95%), 3×10^3 CFU/mL (90.2%), 3×10^2 CFU/mL (88.75%) and 3 × 10 CFU/mL (84.13%) (**Table 3**).

DISCUSSION

The occurrence of new genotypes of *Phytophthora infestans* and their resistance to metalaxyl have led scientists all over the world to look for alternative strategies and products to control this destructive pathogen of potato (Deahl *et al.* 1995; Goodwin *et al.* 1995; Chycoski and Punja 1996; Goodwin *et al.* 1998; Cooke *et al.* 2003, 2006). The genus *Trichoderma* has been successfully used to control a variety of plant pathogens in different crops (Harman 2000; Sharon *et al.* 2001; Tsror *et al.* 2001; Howell 2002; Sid Ahmed *et al.* 2003; Ezziyyani *et al.* 2007). In growth chamber studies, the use of *T. atroviride* alone reduced the severity of *P. infestans* by 27% but was inferior to Bravo 500F treatment which reduced the severity by 96%. However the combination of *T. atroviride* and Bravo 500F fared better than the individual treatment with *T. atroviride*. Late blight severity in leaf discs and the growth of the causal fungus on rye extract agar (REA) plates were reduced significantly by all tested concentrations of *T. atroviride*. Harman *et al.* (1996) cited that the use of *T. harzianum* alone or in combination with iprodione resulted in highly effective control of bunch rot in grapes. In another study *T. harzianum* strain 1295-22 used as a conidial suspension spray significantly reduced *Pythium* root rot, brown patch and dollar spot of creeping bentgrass in both greenhouse and field experiments (Lo *et al.* 1997). *T. hamatum* strain TRI-4 reduced Fusarium wilt incidence in tomato plants by 64% when compared to pathogen inoculated control (Larkin and Fravel 1998). In another study *T. harzianum* T39 applied at sites spatially separated from the *B. cinerea* inoculation resulted in a 25-100% reduction of grey mold symptoms in tomato, lettuce, pepper, bean, and tobacco (de Meyer *et al.* 1998). *T. harzianum* used alone or in combination with *Glomus intraradices* significantly reduced the incidence and severity of Fusarium crown and root rot of tomato (Datnoff *et al.* 1995).

Treatment with *T. harzianum* T4 or *T. harzianum* N47 reduced plant damage in pea plants caused by *Pythium ultimum* and improved the growth characteristics of the plants (Naseby *et al.* 2000). In studies conducted under *in vitro* conditions, the mycelial growth of *P. erythroseptica* was reduced by 49-71 and 49-54% as a result of treatment with *T. virens* DAR 74290 and *T. harzianum* T39, respectively (Etebarian *et al.* 2000). Trichodex (formulation containing *T. harzianum* T39, Makhteshim Chemical Works, Be'er Sheva, Israel) and *T. virens* DAR 74290 applied alone or in combination reduced the severity of pink rot in shoots and roots of potatoes 10 weeks after inoculation with the pathogen in glasshouse experiments (Etebarian *et al.* 2000). Similarly, *T. virens* G6-4 and *T. koningii* TK-7 gave effective biological control of pre-emergence damping-off of cotton plants in pathogen-infested soil (Howell 2002).

The mechanisms by which the isolates of *Trichoderma* control plant pathogens have been extensively studied and reports indicate the involvement of several mechanisms in pathogen suppression (Howell 2003). The ability of *T. virens* to inhibit pink rot in potato plants was attributed to the production of gliotoxin (Na Lampang 1994; Etebarian *et al.* 2000). The inhibitory effect of *T. virens* against *P. ultimum* and *Rhizoctonia solani* has been related to the production of gliotoxin by the biocontrol agent (Lumsden *et al.* 1992). Disease suppression of *P. ultimum* by *T. virens* has been associated with the production of antibiotic gliovirin (Howell 1991). The inhibition of *P. ultimum* in pea plants by strains of *Trichoderma* was related to mycoparasitism (Naseby *et al.* 2000). Similarly Ezziyyani *et al.* (2007) reported that the inhibition of *Phytophthora capsici* by *T. harzianum* follows a gradual process wherein *T. harzianum* grows rapidly at the outset and then invades the colony of *P. capsici* by a marked process of hyperparasitism. In most cases, the production of antibiotics by the *Trichoderma* strains was positively correlated to the inhibition of the pathogen except for a few cases wherein mutants of *T. virens* that were deficient in gliotoxin biosynthesis were just as effective as the wild type strains (Howell and Stipanovic 1995). In studies conducted earlier a mutant of *T. virens* deficient in both mycoparasitism and gliotoxin biosynthesis retained its biocontrol capacity to suppress *P. ultimum* and *R. solani* even after mutation (Howell *et al*. 2000; Howell 2002). The mutant strain of *T. virens* showed biocontrol efficacy similar to the parent strain.

The possible role of enzymes in inhibition of the pathogen in biocontrol by *Trichoderma* has been suggested by some authors (Harman *et al*. 1993; Lorito *et al*. 1993; Haran *et al*. 1996; Elad and Kapat 1999; Lahsen *et al*. 2001). Elad and Kapat (1999) suggested the involvement of protease in the biocontrol of *B. cinerea* in bean by *T. harzianum* NCIM1185 and *T. harziaum* T39. The ability of *T. koningii* to control white rot caused by *Sclerotium cepivorum* in onion was ascribed to the production of endo- and exo-chitinases (Metcalf and Wilson 2001). The use of *T. virens* strains with disrupted or over expressed chitinase gene revealed that biocontrol activity was reduced in gene disrupted strain while in over expressed strain the activity was increased, indicating the involvement of chitinase in biocontrol of *R. solani* (Baek *et al*. 1999). The disruption of endochitinase gene in *T. harzianum* reduced the ability of the strain to control *B. cinerea* in bean leaves (Woo *et al*. 1999). However, the same mutant strain of *T. harzianum* was able to control *P. ultimum* and *R. solani* effectively and the authors concluded that interactions between *T. harzianum* strains and other fungal pathogens were based on different mechanisms. The transformants of *T. longibrachiatum* over expressing a gene encoding β-1,4-endoglucanase were more effective in controlling *P. ultimum* in cucumber compared to the wild type strain (Migheli *et al*. 1998).

Another mechanism thought to be involved in pathogen suppression by *Trichoderma* is the induction of resistance in the host plant upon treatment with a biocontrol strain. Application of *T. harzianum* reduced grey mold symptoms caused by *B. cinerea* in tomato, lettuce, pepper, bean and tobacco plants (de Meyer *et al*. 1998). They attributed the disease reduction to the induction of systemic resistance by *T. harzianum* T39 since there was spatial separation between *B. cinerea* and *T. harzianum* T39. Similarly the induction of defense response by terpenoid synthesis in cotton roots by *T. virens* is believed to be an important mechanism in the biocontrol of *R. solani* incited cotton seedling disease by *T. virens* (Howell *et al*. 2000). Inoculation of 7-day old cucumber seedlings with *T. harzianum* spores to a final concentration of 10^5 mL^{-1} in an aseptic hydroponic system induced plant defense responses in roots and leaves of treated plants (Yedidia *et al*. 1999).

The other proposed mechanism in *Trichoderma* biocontrol is the competition through rhizosphere competence (Howell 2003). The biocontrol strain should be able to compete with the pathogen for space and nutrients and survive in the rhizosphere. Additions of *Trichoderma* to the soil and seed have shown that it can grow readily with the developing root system of the treated plants (Harman 2000; Howell *et al*. 2000). *T. harzianum* reduced the severity of Fusarium crown and root rot in tomatoes and the reduction in disease severity were associated with possible competition for nutrients in the rhizosphere between *Trichoderma* and Fusarium (Sivan and Chet 1993). After following the different mechanisms involved in biocontrol with *Trichoderma* it can be noted that enzymes and antibiotics produced by *Trichoderma* species that is believed to be involved in biocontrol are affected by substrate on which the fungus is grown. In addition, the conditions in the laboratory probably occur very rarely in nature or not at all. The profound effect the temperature has on the production and activities of enzymes and antibiotics associated with *Trichoderma* biocontrol should also be taken into consideration (Howell 2003). Another reason could be the presence of other members of soil microflora which may affect biocontrol activity by inhibition of growth and development of antagonist or by metabolizing its enzymatic products. It can be concluded that the mechanisms involved in inhibition of pathogens by biocontrol agents are more complex and it varies with antagonist, pathogen and the host which are involved in the interaction. However, the mechanisms are affected by soil type, temperature, pH, plant moisture, soil environment and presence of other members of soil microflora. It should also be noted that the grower acceptance will be more for a biocontrol product which is effective when applied at the time of planting than with the ones which need additional cultivation procedures (Etebarian *et al*. 2000). Although the application of *T. atroviride* alone did not give better results as compared to application of Bravo 500F alone or combination of both, *T. atroviride* can be very useful if used in an integrated control approach of late blight. The success of *T. atroviride* alone or in combination with other treatments can be better judged by conducting field trials.

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