Efficacy of Bio-Save 10LP and Bio-Save 11LP (*Pseudomonas syringae*) for management of potato diseases in storage

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Abstract

Silver scurf and dry rot are two diseases of economic importance in potato- growing areas of the world. In recent years, the pathogens that cause these diseases (namely *Helminthosporium solani* and *Fusarium* spp., respectively) have developed resistance to thiabendazole, a post-harvest product commonly used for their control. Therefore, alternative disease control strategies are needed. The present study was undertaken to assess the efficacy of the biopesticides Bio-Save 10LP (*Pseudomonas syringae* strain ESC-10) and Bio-Save 11LP (*Pseudomonas syringae* strain ESC-11) against silver scurf and dry rot of potatoes. Potato samples showing symptoms of silver scurf and Fusarium dry rot were collected from New Brunswick (NB), Prince Edward Island (PEI) and Alberta (AB) and used to obtain a total of 30 isolates belonging to the *Fusarium* or *Helminthosporium* genera. The species represented were *Fusarium sambucinum*, *F. tumidum*, *F. coeruleum*, *F. culmorum*, *F. avenaceum* and *Helminthosporium solani.* The efficacy of Bio-Save 10LP and Bio-Save 11LP against *F. sambucinum* and *H. solani* isolates representing NB, PEI and AB were tested *in vitro*. Bio-Save 11LP inhibited the growth of *F. sambucinum* and *H. solani* while Bio-Save 10LP was effective against *H. solani* only. Application of Mertect (thiabendazole) alone or in combination with either Bio-Save 10LP or Bio-Save 11LP significantly reduced the growth of *F. sambucinum* and *H. solani* in culture compared to the control. *F. sambucinum* from NB was more sensitive to thiabendazole than *F. sambucinum* from AB and PEI. Isolates of *H. solani* from AB were more sensitive to thiabendazole than those from NB and PEI. Storage trials were conducted in NB and PEI to assess the efficacy of Bio-Save applied after harvest against dry rot and silver scurf. The application of Bio-Save or Mertect SC alone or in combination was effective in significantly reducing the incidence and severity of dry rot and silver scurf in both NB and PEI. The results of the present trial indicate that the use of Bio-Save (10LP or 11LP) may play a role in resistance management and in the management of both silver scurf and Fusarium dry rot.

Key words: Biopesticide, Bio-Save, thiabendazole, postharvest diseases, silver scurf, Fusarium dry rot

Introduction

Silver scurf and Fusarium dry rot are two important postharvest diseases of potatoes throughout the world. These two diseases are capable of causing losses in stored tubers ranging from 25 to 60%. Silver scurf, caused by *Helminthosporium solani* Durieu & Mont. is characterized by a dark, metallic discoloration of the periderm in irregular patterns. The lesions increase in size, coalesce, and may cover a major portion of the tuber surface appearing as a 'silvery sheen' when the tubers are wet.This disease often adversely affects the appearance and skin color of potato tubers, ultimately resulting in reduced consumer acceptance (Secor and Gudmestad, 1999). Infected tubers are also more difficult to peel, thus creating problems for processors. Primary infection occurs in the field and secondary lesions develop from conidia dispersed during storage. It is considered a problem of stored potatoes, even though infection often takes place before harvest (Jellis and Taylor, 1977; Lennard, 1980; Carnegie et al. 2003). The primary sources of inoculum are diseased seed-pieces, infested crop residues and contaminated field soil. Soil-borne inoculum infects tubers through the lenticels or directly through the skin. The severity of the disease can increase in storage if relative humidity levels are above 90% and temperatures are greater than 3°C. The disease will continue to develop and spread in storage as aerial spores are produced at the margins of tuber lesions. Over a period of time, the diseased tubers may lose moisture and shrivel resulting in weight loss (Tsror and Peretz-Alon 2002). Very few fungicides are effective against the silver scurf pathogen (Errampalli et al. 2001) and pathogen resistance has developed for some fungicides such as thiabendazole (Mertect) (Merida and Loria, 1994).

Similarly, Fusarium dry rot caused by *Fusarium* spp. is characterized by an internal light to dark brown or black rot of the potato tuber. Tuber infection usually occurs due to tuber injury caused by bruises or cuts on the tuber surface. In this case, the pathogen penetrates the tuber often causing rotting in the pith of the tuber. Extensive rotting causes tissues to shrink and collapse, while leaving a dark sunken area on the outside of the tuber showing internal cavities. For many years, management of Fusarium dry rot has included the use of thiabendazole applied as a postharvest treatment. However, in recent years,, resistance to thiabendazole in isolates of *F. sambucinum* has been recorded in Europe, the United States and Canada (Hide et al. 1992; Hanson et al. 1996; Platt 1997; Peters et al. 2001). No other options for post-harvest disease management have proven to be sufficiently efficacious against Fusarium dry rot.

The limited availability of post-harvest fungicides has prompted the search for new and efficient methods to control silver scurf and Fusarium dry rot of potato. Biopesticides have shown increasing promise as tools for safe and sustainable disease management. The identification and registration of effective biopesticides is urgently needed to control post-harvest potato diseases and to curb growers' reliance on synthetic chemicals. In the U.S.A., Bio-Save 10LP and 11LP (*Pseudomonas syringae*) are registered for control of silver scurf and Fusarium dry rot. The registrant, JET Harvest Solutions (Longwood, Florida) is also pursuing registration of these products in Canada. The objective of this study was to assess the efficacy of the biopesticides Bio-Save 10LP and Bio-Save 11LP, alone and in combination with Mertect, against silver scurf and Fusarium dry rot of

potatoes caused by pathogen strains occurring in important potato production regions in Canada. Results from this study have provided data to support the registration of these biopesticides in Canada.

MATERIALS AND METHODS

Collection of samples and isolation of pure cultures

Potato tubers showing symptoms of silver scurf or Fusarium dry rot were collected from the provinces of New Brunswick (NB), Prince Edward Island (PEI), Nova Scotia (NS), and Alberta (AB), Canada, and were used to obtain pathogen cultures. Tubers were collected, washed thoroughly and examined for specific disease symptoms. The procedure reported by Holley and Kawchuck (1996) was followed for isolation of both silver scurf and dry rot pathogens. Briefly, conidia of *H. solani* were removed from skin surfaces of infected tubers using a dissecting needle and then transferred to Petri dishes containing acidified potato dextrose agar (PDA) amended with penicillin and streptomycin. Petri dishes were then incubated for 5-8 weeks at 20º C. Colonies of *H. solani* were then subcultured on malt agar and incubated for 5-8 weeks at 20º C.

For dry rot, tubers with cuts or pressure bruises were sliced open and wefts of mycelia inside dry rot cavities were removed and placed on PDA plates amended with penicillin and streptomycin. In cases where mycelia were not present, the infected tuber tissue was removed from the edges of lesions and surface- sterilized with 70% ethanol for 2 minutes. These were then placed on acidified PDA plates or PDA plates amended with penicillin and streptomycin and incubated for 7-14 days at 20º C. Macroconidia were then transferred to fresh PDA plates and allowed to germinate and grow for 7-14 days at 20º C.

Identification of *Helminthosporium* isolates

Pure cultures were grown in 9 cm polystyrene Petri dishes onto potato dextrose agar (PDA, Difco) for 5 days at 22 C in dark. Fungal mycelia were scraped from the surface of agar using a sterilized scalpel and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Inc.,CA) according to manufacturer's instructions and one µL of aliquot was then analyzed on 1.5% agarose gel in 0.5X Tris-borate-EDTA buffers to estimate the concentration and the quality of the DNA. 10 ng of the total DNA were used to get amplicons of β-tubulin and Glyceraldhyde-3-phosphate dehydrogenase (*gpd1*), and the ITS region (including ITS1& 2, and 5.8S rRNA. Primers pair SS-for 5' AGCATAGGCTGATGCTCGT-3' and SS-rev 5'- ACCTTACCACGGAAGATACCAC-3' previously reported by McKay & Cooke (1997) was used for partial amplification of β-tubulin gene. For partial of *gpd 1* we designed a primer pair (forward: Up-gpd 5' CAACGGCTTCGGTCGCATTG-3' and reverse: Low-gpd 5'-GCCAAGCAGTTGGTTGTGC -3') from a gene recovered from *Cochliobus heterostrobus* and deposited in GenBank under accession X63516. We used a primer set Un_Up18S42 5'- CGTAACAAGGTTTCCGTAGGTGAAC -3' and Un-Lo28S22 5'-GTTTCTTTTCCTCCGCTTATTGATATG -3') previously reported by Allain-Boulé et

al. (2004) to amplify a portion of internal translation spacer (ITS) gene. The PCR amplification was carried out in an Eppendorf cycler, Brinkmann Instruments, Inc. using the no clean method described by Alain-Boulé *et al* (2004). Briefly amplification was performed using the following programs: (a) initial denaturation step of 95°C for 2min was followed by 40 amplification cycles of 95°C for 30 s, 58°C for 45 s, and 72°C for 90 s. After the 40 cycles were completed, the amplicons were incubated for an additional eight min at 72°C for a final extension and a 10°C soak for β-tubulin, (b) (a) initial denaturation step of 95°C for 3 min was followed by 40 amplification cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 60 s. After the 40 cycles were completed, the amplicons were incubated for an additional eight min at 72°C for a final extension and a 10°C soak for *gpd1* and (c) initial denaturation step of 95°C for 3 min was followed by 40 amplification cycles of 95°C for 45 s, 68°C for 45 s, and 72°C for 90 s. After the 40 cycles were completed, the amplicons were incubated for an additional eight min at 72°C for a final extension and a 10°C soak for *ITS*. To estimate the concentration and quality of PCR, 1 μL of amplicon from the PCR reaction was run on a 1.5 % agarose gel with 0.5 μg of ethidium bromide per ml and 0.5 x Tris borate EDTA buffer for 45 min. DNA bands were visualized on a UV trans-illuminator.

PCR amplicons were sequenced using the using the BigDye Terminator V2.0 ready reaction Kit from Applied Biosystems (Forster City, CA) and 0.5x reactions. An estimated 10-20 ng of each fragment was sequenced in both directions using the same primers described for PCR above. To obtain sequences of β-tubulin and gpd1, cycle sequencing was executed with the following program: one cycle 95°C for 3 min followed by 40 cycles of 95°C for 30s, 50°C for 30s, and 60°C for 120s. For ITS amplicon, we used the same cycling program with slight modifications. The temperature of 50°C was changed to 60°C. Sequencing products were precipitated before they were analyzed with the ABI 3100-Avant automated sequencer (Applied Biosystems⁄Hitachi). Contigs were assembled and sequences were edited using SeqMan version 6.1 (DNASTAR, Madison, WI). Sequences were used to establish identity of the isolates based on sequences similarity in GenBank using the BLASTn program for nucleotide-nucleotide analysis (version 2.0, National Center for Biotechnology Information (NCBI), United States National Institutes of Health, Bethesda, MD).

In vitro efficacy of Bio-Save 10LP and 11LP against *Fusarium sambucinum* and *Helminthosporium solani*

The efficacies of Bio-Save 10LP [Biological fungicide, 29.8% *Pseudomonas syringae* strain ESC-10 (9×10^{10} CFU/g of formulated product), JET Harvest Solutions, Longwood, Florida] and Bio-Save 11LP [Biological fungicide, 29.8% *Pseudomonas syringae* strain ESC-11 (9×10^{10} CFU/g of formulated product), JET Harvest Solutions, Longwood, Florida] against different isolates of *F. sambucinum* and *H. solani* were tested *in vitro*. Mertect® SC [thiabendazole (500 gL^{-1}), Syngenta Crop Protection Canada, Inc., Guelph, Ontario] alone or in combination with either Bio-Save 10LP or Bio-Save 11LP was also tested. One isolate of *F. sambucinum* from each province was selected for the *in vitro*

studies. For silver scurf, one isolate of *H. solani* was selected from NB and two isolates were selected from both AB and PEI.

Petri dishes containing PDA amended with Bio-Save 10 LP or 11 LP at concentrations of 0, 0.1, 1.0, 4.412 (label rate), 10, 100 and 1000 g/L were prepared. In addition, Petri dishes containing PDA amended with Mertect alone (label rate of 44 mL product/L of water) or in combination with Bio-Save 10 LP or 11 LP (at label rates) were prepared. Dishes were incubated overnight to allow the biopesticides to be absorbed into the media. Plates receiving sterile distilled water only served as controls. Agar plugs (5 mm in diameter) were taken from the margins of actively growing cultures of *F. sambucinum* or *H. solani* and placed in the centre of PDA plates amended with the various products at different concentrations. The plates were then incubated at room temperature (approximately 7 weeks of incubation for *H. solani* and 1 week for *F. sambucinum*). Two perpendicular measurements of radial mycelial growth were made using a digital caliper (VWR Scientific Products, Mississauga, Ontario, Canada) once every two days. Growth was expressed as colony diameter in mm and mean values were used in calculating percent inhibition. Radial growth of the fungus was recorded until the untreated control reached the margin of the plate. Percent inhibition was calculated using the following formula: Inhibition (%) = [(growth of the pathogen in untreated control plates – growth of the pathogen in treated plates)/ growth of the pathogen in untreated control plates] x 100 (Daouk et al. 1995; Al-Mughrabi 2003). Each treatment for each fungus at each concentration was replicated three times. Data analysis was done using CoStat (CoHort Software, Monterey, CA, USA) and the means were separated using LSD test at *P*=0.1.

Post Harvest Storage Trials

Replicated storage trials using a randomized complete block design with 4 replications of treatments were established in NB and PEI. Separate experiments were conducted to assess the impact of postharvest treatments on the incidence and severity of silver scurf (SS) and Fusarium dry rot (FDR). Six treatments were assessed: 1) Inoculated, untreated control 2) Bio-Save 10 LP (label rate) 3) Bio-Save 11 LP (label rate) 4) Mertect SC (label rate) 5) Bio-Save 10 LP (label rate) + Mertect (label rate) and 6) Bio-Save 11 LP (label rate) + Mertect (label rate).

Dry rot trial

In NB, tubers of cultivar Yukon Gold were dry-brushed to remove any adhering soil, and then were wounded in a bruise box. In PEI, tubers of cultivar Shepody were wounded at one discrete site using a custom-made wounding tool which created a surface wound of 2 mm in width (5 mm depth). All tubers were sprayed with 2 mL of a suspension of *F. sambucinum* (10^5 conidia/mL) prepared by scraping the surface of 14 day old cultures growing on PDA. After inoculation, tubers were placed in plastic crates and allowed to incubate in the storage chamber until treatments were applied the next day. Each set of 25 tubers (per replication) were then placed on a conveyor belt and sprayed with the

respective treatments at label rates using a CO_2 sprayer mounted at the end of the conveyor to simulate application of postharvest spray to tubers entering storage. Equipment was thoroughly cleaned prior to the next treatment application with distilled water. For each treatment, tubers were placed in individual plastic crates and stored in a defined storage facility set at 10ºC and 95% RH for a period of 16 weeks. Following storage, dry rot incidence (number of diseased tubers) and severity (depth of internal necrosis) were assessed.

Silver scurf trial

Tubers of cultivar Yukon Gold (NB) or Shepody (PE) harvested from local sources and naturally infected with the silver scurf pathogen were used. Tubers were dry-brushed to remove any adhering soil. Each set of 25 tubers were then placed on a conveyor belt and sprayed with the respective treatments at label rates using a CO_2 sprayer mounted at the end of the conveyor to simulate application of postharvest spray to tubers entering storage. Equipment was thoroughly cleaned prior to the next treatment application with distilled water. For each treatment, tubers were placed in individual plastic crates and stored in a defined storage facility set at 10ºC and 95% RH for a period of 6 months. After storage, silver scurf disease incidence (number of diseased tubers) and severity (% tuber surface diseased) were assessed.

The data collected for both silver scurf and dry rot trials were analyzed using CoStat (CoHort Software, Monterey, CA, USA) and the means were separated using a protected LSD test (P=0.05).

Analysis of antagonist populations

During the trial conducted in NB, some tuber samples were marked and treated with either Bio-Save 10LP or Bio-Save 11LP. After incubating the tubers for two days in storage, they were sent to JET Harvest Solutions in Florida for analysis of *Pseudomonas syringae* population levels in the treated samples. Bio-Save solution (500 g of Bio-Save in 30 gallons of water) was applied to naturally infected tubers showing symptoms of silver scurf. For dry rot, tubers were artificially wounded (1 X 3 mm wound), inoculated with a spore suspension of *F. sambucinum* and then treated with Bio-Save solution. The wounds were excised 48 hours after treatment application and then tested for density of *P. syringae* populations on KBBC agar plates (Schaad et al., 2001). The population density of *P. syringae* in both Bio-Save 10LP or Bio-Save 11LP was approximately $1x10^5$ cfu mL^{-1}.

Results

Identification of pathogen isolates

Isolates obtained from the infected tuber samples were identified and culture collections were deposited at the National Fungal Identification Service (NFIS) in Ottawa and at the

Potato Pathology unit, Potato Development Centre, Department of Agriculture and Aquaculture, NB. Of the 30 isolates collected, 13 were from NB, 12 from PEI, 3 from AB, and 2 from NS. Identification was carried out using morphological and molecular techniques as described above. A total of 30 isolates belonging to *Fusarium* or *Helminthosporium* genera were identified. The *Fusarium* isolates were identified as *F. sambucinum*, *F. tumidum*, *F. coeruleum*, *F. culmorum*, or *F. avenaceum*. Based on micro-morphological observations, the *Helminthosporium* isolates were identified as *H. solani* (Table 1). By analysis of sequence data from *ITS, β-tubulin and gpd1* genes, we also found that isolates of *Helminthosporium solani* from PEI and AB belonged to the same group.

In vitro efficacy of Bio-Save 10LP and 11LP against *Fusarium sambucinum* and *Helminthosporium solani*

The growth of all isolates of *H. solani* was significantly inhibited by both Bio-Save 10LP and Bio-Save 11LP (Table 2). The use of Mertect alone or in combination with either Bio-Save 10LP or Bio-Save 11LP significantly reduced the growth of all isolates of *H. solani* compared to the untreated, inoculated control. The *H. solani* isolates from AB were more sensitive to Mertect than those from PEI and NB. Bio-Save 11LP performed significantly better than Bio-Save 10LP in inhibiting the activity of *H. solani*. The highest inhibition of all isolates of *H. solani* was obtained by use of Mertect alone or when it was combined with either Bio-Save 11LP or Bio-Save 10LP.

Bio-Save 11LP significantly inhibited the growth of *F. sambucinum* under *in vitro* conditions. However, Bio-Save 10LP was unable to inhibit the growth of *F. sambucinum* (Table 3). Similarly, the use of Mertect alone or in combination with either Bio-Save 10LP or Bio-Save 11LP significantly reduced the growth of all isolates of *F. sambucinum* compared to the untreated, inoculated control. The *F. sambucinum* isolate from NB was more sensitive to Mertect than those from AB and PEI. The highest inhibition of all isolates of *F. sambucinum* was obtained by use of Mertect alone or when it was combined with either Bio-Save 11LP or Bio-Save 10LP.

Post Harvest Storage Trials

Dry Rot trial

In PEI, the lowest disease incidence was recorded from the treatment Bio-Save 10LP followed by Mertect SC, Bio-Save 11LP + Mertect SC, Bio-Save 11LP and Bio-Save 10LP + Mertect SC (Table 4). Disease incidence values for these treatments differed significantly from the inoculated, untreated control (Table 4).

In NB, the lowest disease incidence of dry rot was recorded for the Bio-Save 11LP + Mertect SC treatment (24%). All treatments tested significantly reduced the incidence of dry rot compared to the inoculated, untreated control (93%) (Table 5). Disease incidence

values recorded for Bio-Save 11LP + Mertect SC (24%) differed significantly from Bio-Save 11LP (60%) and Mertect SC (42%) (Table 5).

The lowest external severity of dry rot in PEI was recorded for Bio-Save 10LP (0.42%) followed by Bio-Save 11LP + Mertect SC (0.48%), Bio-Save 11LP (0.92%), Mertect SC (0.99%) and Bio-Save 10LP + Mertect SC (1.21%) treatments (Table 6). All treatments differed significantly from the untreated control inoculated with *Fusarium sambucinum*. As well, all treatments significantly reduced internal disease severity compared to the inoculated, untreated control (Table 6). The external disease severity of dry rot in the NB trial was lowest in tubers receiving Bio-Save 11LP + Mertect SC (1.4%) followed by Bio-Save 10LP (1.8%), Bio-Save 10LP + Mertect SC (1.9%), Mertect SC (2.6%) and Bio-Save 11LP (3.1%) (Table 7). All treatments differed significantly from the inoculated, untreated control treatment (8.2%). The external disease severity values recorded for Bio-Save 11LP + Mertect SC (1.4%) differed significantly from the Bio-Save 11LP (3.1%) and Mertect SC treatment (2.6%) (Table 7). Similarly, the lowest internal disease severity was recorded for Bio-Save 11LP (1.3 mm) and Bio-Save 11LP + Mertect SC (1.3 mm) followed by Bio-Save 10LP (1.5 mm), Bio-Save 10LP + Mertect SC (1.8 mm) and Mertect SC (1.9 mm) treatments (Table 7). All five treatments differed significantly from the inoculated, untreated control treatment (6.4 mm) with regard to internal disease severity.

Silver Scurf trial

The incidence of silver scurf was 100% for all treatments tested in PEI and therefore, no significant difference among treatments was observed. However, among all treatments tested, the severity of silver scurf was lowest in tubers treated with Bio-Save 11LP (7.02%) which differed significantly from the inoculated, untreated control (19%) and Bio-Save 10LP + Mertect SC (9.09%)(Table 8). The mean disease severity recorded for Bio-Save 10LP, Bio-Save 10LP + Mertect SC and Mertect SC treatments were significantly less than that observed for the inoculated, untreated control (Table 8).

The incidence of silver scurf in the NB trial was lowest for the Bio-Save 10LP + Mertect SC treatment (10%) followed by Bio-Save 10LP (11%), Bio-Save 11LP + Mertect SC (19%), Bio-Save 11LP (21%) and Mertect SC (22%) treatments (Table 9). The treatments Bio-Save 10LP + Mertect SC, Bio-Save 10LP, Bio-Save 11LP + Mertect SC, Bio-Save 11LP and Mertect SC differed significantly from the inoculated, untreated control treatment (79%) (Table 9). Disease incidence values recorded for Bio-Save 10LP + Mertect SC treatment (10%) were significantly different from the Mertect SC (22%) and Bio-Save 11LP (21%) treatments. Similarly, the lowest severity of silver scurf was recorded for the Bio-Save 10 LP + Mertect SC treatment (0.7%), followed by Bio-Save 10 LP (0.9%), Bio-Save 11LP (1.4%), Bio-Save 10LP + Mertect SC (1.5%) and Mertect SC (1.8%) (Table 9). All treatments significantly reduced the severity of silver scurf compared to the inoculated, untreated control.

Discussion

Biological control using postharvest biocontrol products has great potential because postharvest environmental parameters, namely temperature and humidity, can be rigidly controlled to suit the needs of the biocontrol agent, as long as crop quality parameters are not compromised. In addition, harvested commodities offer a concentrated target for the application of biocontrol agents. The use of biocontrol agents in managing postharvest diseases of fruits and vegetables has been reported to be effective and feasible (Janisiewez, 1988; Wisniewski and Wilson, 1992). The use of Bio-Save 110 (*P. syringae*) on pears reduced the diameter of lesions caused by *Penicillium expansum* by 32 to 72% and disease incidence was redcued by 21 to 40% over the 2 years of the study (Sugar and Spotts, 1999). Similarly, in a packinghouse trial, the control of *P. expansum* in pears with Bio-Save 110 + 100 ppm of thiabendazole was equivalent to the control obtained with 569 ppm of thiabendazole. In several trials conducted using Bio-Save 11, Jeffers and Hankinson (1995) reported that Bio-Save 11 was as good as or better than thiabendazole for controlling postharvest decay in pears. In another study, the use of Bio-Save 10 significantly reduced the incidence of blue mold in apples by 50 to 73% (Janisiewicz and Jeffers, 1997; Zhou et al., 2001). Earlier studies with *P. syringae* (strain MA-4) showed efficacy in controlling two postharvest diseases of peach, namely brown rot and rhizopus rot (Zhou et al. 1999; Northover and Zhou 2000). As well, postharvest treatment of pears with *P. syringae* ESC10 reduced rhizopus rot by 97% in Redhaven pears and 90% in Harrow Beauty pears relative to the control (Northover and Zhou, 2002). Bio-Save 10LP and Bio-Save 11LP have been shown to significantly reduce the incidence of decayed sweet potato roots (Holmes and Adams, 2003; Edmunds and Holmes, 2004) and Rhizopus soft rot (*Rhizopus stolonifer*) relative to inoculated control treatments (Clarke et al., 2006).

The isolates of *Fusarium* spp. obtained from infected tuber samples were identified as *F. sambucinum*, *F. coeruleum*, *F. culmorum*, *F. avenaceum* and *F. tumidum*. It should be noted that *F. tumidum* has not been reported as a causal agent of dry rot and yet, it was isolated from 2 samples collected from NB. Further work to complete Koch's postulates must be carried out to determine the pathogenicity of these isolates. Of all isolates collected, *F. sambucinum* (31.25%) was the most frequently isolated species followed by *F. culmorum* (25%) and *F. coeruleum* (25%). Other researchers have recovered *F. sambucinum*, *F. culmorum*, *F. avenaceum*, *F. solani*, *F. oxysporum*, *F. acuminatum*, *F. equiseti* and *F. crookwellense* from infected potato tubers (Hanson et al., 1996). The most commonly isolated fungus in the latter study was also *F. sambucinum,* comprising 35.5% of isolates. The majority of the *H. solani* isolates obtained were from PEI (64%) while very few were from NB (21%) and AB (15%).

Results from the *in vitro* assays indicated that the isolates of *H. solani* from all provinces were generally suppressed by Bio-Save 10LP and 11LP.. By contrast, isolates of *F. sambucinum* were suppressed by Bio-Save 11LP but not by Bio-Save 10LP. Thus, variation in the response of this pathogen to the different bacterial strains was observed, which underscores the necessity of broad-based efficacy screening. Isolates of *H. solani* and *F. sambucinum* varied in their sensitivity to thiabendazole *in vitro,* with some isolates

displaying insensitivity to this chemical. The insensitivity to thiabendazole of isolates of *H. solani* and *F. sambucinum* recovered from diverse potato production regions in Canada has been well documented (Kawchuk et al. 1994; Peters et al. 2001; Platt 1997).

The results of storage trials conducted in NB and PEI suggest that Bio-Save 10LP, Bio-Save 11 LP and Mertect SC used alone or in combination are effective for the control of Fusarium dry rot and silver scurf in storage. Thiabendazole has been widely used in the past for control of *H.solani* and *F. sambucinum* (French, 1973; Copeland and Logan, 1975; Cayley et al., 1979; Hide, 1986; Hide et al., 1988; Maughan et al., 1991; Secor and Gudmestad, 1999). Thiabendazole is one of the few chemicals registered for post-harvest application to potatoes entering storage. This compound initially provided good control of several post-harvest diseases including Fusarium dry rot caused by *Fusarium* spp. and silver scurf. However, resistance to thiabendazole in isolates of *F. sambucinum* was recorded in Europe (Hide et al. 1992) and became widespread in the United States (Desjardins et al. 1993; Hanson et al. 1996) and Canada (Kawchuk et al. 1994; Peters et al. 2001; Platt 1997) in the 1990s. In addition, thiabendazole-resistant strains of *H. solani* have also become commonplace (Platt 1997). Conversely, populations of other *Fusarium* spp. causing potato tuber dry rot, including *F. coeruleum*, *F. avenaceum*, and *F. graminearum*, have generally been found to be sensitive to thiabendazole (Ali et al. 2005; Hide et al. 1992; Kawchuk et al. 1994; Platt 1997). As well, recent surveys have found fluctuation for thiabendazole resistance in pathogen populations in Canada, that are largely driven by the frequency of product use in a growing region (R.D. Peters, unpublished data). Therefore, thiabendazole may still play a role in postharvest disease management, particularly if it is rotated with other products and combined with biocontrol products as part of a resistance management strategy.

The effectiveness of biological control agents against Fusarium dry rot and silver scurf pathogens has been previously documented. The treatment of potato tubers with *Pseudomonas fluorescens* S22:T:04 and *Gibberella pulicaris* prior to storage reduced the severity of Fusarium dry rot by 19% when compared to the control and the thiabendazole treatments (Schisler et al., 2000). In a storage trial conducted using 12 different isolates of *P. fluorescens* and *Enterobacter*, nine bacterial isolates were able to reduce the severity of Fusarium dry rot more than thiabendazole treatments. Among the different isolates tested, *P. fluorescens* bv. V P22:Y:05 gave the best control (Slininger et al., 2003). In another study, treatment of potato tubers with isolates I32 of *Bacillus licheniformis* and X16 of *B. cereus* also resulted in reduced Fusarium dry rot severity and improved yield parameters under greenhouse conditions (Sadfi et al., 2002). The use of *P. fluorescens* and *Enterobacter cloacae* applied to potato tubers enetring storage has been shown to significantly reduce the severity of Fusarium dry rot and silver scurf compared to control treatments (Al-Mughrabi, unpublished data).

The mode of action of Bio-Save 10LP and Bio-Save 11LP for control of postharvest diseases of citrus and pome fruits is believed to be mainly through competition for nutrients and space (Zhou et al., 2001), although syringomycin E production may also play a role (Bull et al., 1998). The mode of action involved in the suppression of silver scurf and dry rot by Bio-Save 10LP and Bio-Save 11LP noted in this study has not been

thoroughly investigated. However, it is probable that after application to the tubers, *P. syringae* enters wounds and outcompetes germinating fungal spores for the nutrients found there (Zhou et al., 2001). A form of competitive inhibition by which the bacterial cells interrupt the normal metabolism of the disease-causing organism to prevent its growth and multiplication is likely also involved. Further research is needed to understand the exact mechanism by which control of silver scurf and dry rot is conferred with postharvest applications of Bio-Save 10LP and Bio-Save 11LP.

In conclusion, Bio-Save (10LP or 11LP) alone or in combination with thiabendazole (Mertect) may provide growers with new tools for the successful management of both silver scurf and Fusarium dry rot in storage. As well, the compatibility of Bio-Save and Mertect for postharvest application provides a resistance management strategy for Mertect, and may prolong its usefulness even though resistance to thiabendazole in H. solani and F. sambucinum is widespread in Canada. The application of biocontrol products such as Bio-Save also fits well into a pest management program that reduces reliance on synthetic chemicals with concomitant benefits for human and environmental health.

Acknowledgements

References

Allain-Boulé, N., C.A. Lévesque, C. Martinez, R.R. Bélanger and R.J. Tweddell. 2004. Identification of *Pythium* species associated with cavity-spot lesions on carrots in eastern Quebec. Can. J. Plant Pathol. 26:365-370.

Ali S, V.V. Rivera and G.A. Secor. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis 89:105.

Al-Mughrabi, K.I. 2003. Antimicrobial activity of extracts from leaves, stems and flowers of *Euphorbia macroclada* against plant pathogenic fungi. *Phytopathologia Mediterranea* 42: 245-250.

Bull, C.T., Wadsworth, M.L., Sorensen, K.N., Takemoto, J.Y., Austin, R.K., and Smilanick, J.L. 1998. Syringomycin E produced by biological control agents controls green mold on lemons. Biol. Control, 12: 89–95.

Carnegie, S.F., J.W. Choiseul, and A.M.I. Roberts. 2003. Detection of *Colletotrichum coccodes* and *Helminthosporium sol*ani in soils by bioassay. Plant Pathology 52: 13–21.

Cayley GR, Hide GA, Lord KA, Austin DJ, Davies AR, 1979. Control of potato storage diseases with formulations of thiabendazole. Potato Research 22, 177-90.

Clark, C. A., Gray, E. D. and Hoy, M. W. 2006. Evaluation of fungicide dips for post-harvest control of Rhizopus soft rot of sweetpotato. Plant Disease Management Reports 1:18

Copeland RB, Logan C, 1975. Control of tuber diseases, especially gangrene, with benomyl, thiabendazole and other fungicides. Potato Research 18, 179-88.

Daouk, R.K., Dagher, S.M. and E.J. Sattout, 1995. Antifungal activity of the essential oil of *Origanum syriacum* L. *Journal of Food Protection* 58, 1147-1149.

Desjardins AE, EA Christ-Harned, SP McCormick and GA Secor. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83:164-170.

Edmunds, B.A. and Holmes, G.J. 2004. Effect of postharvest dip treatments on Rhizopus soft rot of sweetpotato. Fungicide and Nematicide Tests, 60:6

Errampalli, D., J.D. Saunders, and J.D. Holley. 2001. Emergence of silver scurf (*Helminthosporium solani*) as an economically important disease of potato. Plant Pathology 50:141-153.

French WM, 1973. Some effects of benomyl and thiabendazole on a number of potato cultivars. Journal of National Institute of Agricultural Botany 13, 21-30.

Hanson, L.E., S.J. Schwager, and R. Loria. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86: 378-384.

Hide GA, 1986. Treatment of potatoes used for seed. In: Jeffs KA, ed. Seed Treatment. Croydon, UK: British Crop Protection Council, 263-89.

Hide GA, Hall SM, Boorer KJ, 1988. Resistance to thiabendazole in isolates of Helminthosporium solani, the cause of silver scurf disease of potatoes. Plant Pathology 37, 377-80.

Hide, G.A., P.J. Read, and S.M. Hall. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected with dry rot. Plant Pathology 41: 745-748.

Holley, J.D. and L.M. Kawchuk, 1996. Distribution of thiabendazole and thiophanate–methyl resistant strains of Helminthosporium solani and Fusarium sambucinum in Alberta potato storages. Canadian Plant Disease Survey, 76: 21–27.

Holmes, G. J. and Adams, M. L. 2003. Effect of postharvest dip treatments on Rhizopus soft rot of sweetpotato. Fungicide and Nematicide Tests, 59:16

Janisiewez WJ. (1988) Biocontrol of postharvest diseases of apples with antagonist mixtures. *Phytopathology* 78: 194–198.

Janisiewicz, W.J., and Jeffers, S.N. 1997. Efficacy of commercial formulation of two biofungicides for control of blue and gray mold of apples in cold storage. Crop Prot. 16: 629–633.

Jeffers, S. N., and Hankinson, T. R. 1995. Biological control of postharvest diseases of apples and pears: Preliminary results from the 1994 Bio-Save field trial program. Proc. Annu. Wash. Tree Fruit Postharvest Conf., 11th, Wenatchee.

Jellis, G.J. and G.S. Taylor. 1977. The development of silver scurf (*Helminthosporium solani*) disease of potato. Annals of Applied Biology 86: 19–28.

Kawchuk LM, JD Holley, DR Lynch and RM Clear. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Amer Potato J 71:185-192.

Lennard, J.H., 1980. Factors affecting the development of silver scurf (*Helminthosporium solani*) on potato tubers. Plant Pathology 29: 87-92.

Maughan, J.P., Shanmuganathan, N. and G. Hepworth, 1991. Fungicide treatments for the control of storage roots of seed potatoes. Australasian Plant Pathology, 20: 142-145.

Merida, C. L. and R. Loria. 1994. Comparison of Thiabendazole-sensitive and –resistant *Helminthosporium solani* isolates from New York. Plant Disease 78:187-192.

McKay, G.L. and L.R. Cook. 1997. A PCR-based methiod to characterize and identify benzimidazole resistance in *Helminthosporium solani*. FEMS Microbiol. 152: 371-378.

Northover, J., and T. Zhou, 2000. Biological and fungicidal control of rhizopus rot of peaches. Abstracts. 4th International Conference on Postharvest Science, 26–31 March 2000, Jerusalem, Israel. The International Society for Horticultural Science (ISHS), Leuven, Belgium. p. 63.

Northover, J. and T. Zhou, 2002. Control of rhizopus rot of peaches with postharvest treatments of tebuconazole,fludioxonil, and *Pseudomonas syringae*. Canadian Journal of Plant Pathology, 24: 144–153

Peters, R.D., I.K. Macdonald, K.A. MacIsaac, and S. Woodworth. 2001. First report of thiabendazole-resistant isolates of *Fusarium sambucinum* infecting stored potatoes in Nova Scotia, Canada. Plant Disease 85: 1030.

Platt, H.W. 1997. Resistance to thiabendazole in *Fusarium* species and *Helminthosporium solani* in potato tubers treated commercially in eastern Canada. Phytoprotection 78: 1-10.

Sadfi N, Cherif M, Hajlaoui MR, Boudabbous A. (2002) Biological control of the Potato tubers dry rot caused by *Fusarium roseum* var. *sambucinum* under greenhouse, field and storage conditions using *Bacillus* spp. isolates. *J Phytopathol* 150: 640–648.

Schaad, N.W., Jones, J.B. and W. Chun, 2001. Laboratory guide for identification of plant pathogenic bacteria. 3rd edition, American Phytopathogical Society, St. Paul, MN.

Schisler DA, Slininger PJ, Kleinkopf G, Bothast RJ, Ostrowski RC. (2000) Biological control of Fusarium dry rot of potato tubers under commercial storage conditions. *Am J Pot Res* 77: 29 - 40.

Secor, G.A. and N.C. Gudmestad. 1999. Managing fungal diseases of potato. Canadian Journal of Plant Pathology 21: 213-221.

Slininger PJ, Schisler DA, Burkhead D, Bothast RJ. (2003) Postharvest biological control of potato sprouting by Fusarium dry rot suppressive bacteria. *Biocontrol Sci Techn* 13: 477-494.

Sugar, D., and Spotts, R.A. 1999. Control of postharvest decay in pear by four laboratory-grown yeasts and two registered biocontrol products. Plant Dis. 83: 155–158

Tsror, L. and I. Peretz-Alon. 2002. Reduction of silver scurf on potatoes by pre- and post-storage treatment of seed tubers with imazalil. American Journal of Potato Research 79: 33-37.

Wisniewski ME, Wilson CL. (1992) Biological control of postharvest diseases of fruits and vegetables: recent advances. *Hort-Science* 27: 94–98.

Zhou, T., Northover, J., and Schneider, K.E. 1999. Biological control of postharvest diseases of peach with phyllosphere isolates of *Pseudomonas syringae*. Can. J. Plant Pathol. 21: 375–381.

Zhou, T., Chu, C.L., Liu, W.T., and Schneider, K.E. 2001. Postharvest control of blue mold and gray mold on apples using isolates of *Pseudomonas syringae*. Can. J. Plant Pathol. 23: 246–252.

Table 1: List of silver scurf and dry rot pathogens isolated from tubers collected from NB, AB, PEI and NS.

Strain Number	Genus	Species	Province
2007-029 (2007M-73)	*Helminthosporium*	*solani*	NB
2007-037 (2007M-74)	*Helminthosporium*	*solani*	NB
2007-067 (2007M-75)	*Helminthosporium*	*solani*	NB
2007-042 (2007M-85)	*Fusarium*	*tumidum*	NB
2007-054 (2007M-86)	*Fusarium*	*sambucinum*	NB
2007-060 (2007M-87)	*Fusarium*	*coeruleum*	NB
2007-063 (2007M-88)	*Fusarium*	*tumidum*	NB
2007-112 (2007M-89)	*Fusarium*	*culmorum*	NB
2007-124 (2007M-90)	*Fusarium*	*culmorum*	NB
2007-131 (2007M-101)	*Fusarium*	*culmorum*	NB
2007-132 (2007M-102)	*Fusarium*	*sambucinum*	NB
2007-133 (2007M-103)	*Fusarium*	*culmorum*	NB
2007-134 (2007M-104)	*Fusarium*	*coeruleum*	NB
2007-103 (2007M-76)	*Helminthosporium*	*solani*	PEI
2007-104 (2007M-77)	*Helminthosporium*	*solani*	PEI
2007-105 (2007M-78)	*Helminthosporium*	*solani*	PEI
2007-106 (2007M-79)	*Helminthosporium*	*solani*	PEI
2007-107 (2007M-80)	*Helminthosporium*	*solani*	PEI
2007-108 (2007M-81)	*Helminthosporium*	*solani*	PEI
2007-109 (2007M-82)	*Helminthosporium*	*solani*	PEI
2007-110 (2007M-83)	*Helminthosporium*	*solani*	PEI
2007-111 (2007M-84)	*Helminthosporium*	*solani*	PEI
PL1Col1 (2007M-100)	*Helminthosporium*	*solani*	AB
PL2Col3 (2007M-99)	*Helminthosporium*	*solani*	AB
FS001-Alb (2007M-97)	*Fusarium*	*sambucinum*	AB
PEF378 (2007M-92)	*Fusarium*	*sambucinum*	PEI
PEF918 (2007M-96)	*Fusarium*	*sambucinum*	NS
PEF400 (2007M-93)	*Fusarium*	*coeruleum*	PEI
PEF888 (2007M-95)	*Fusarium*	*coeruleum*	NS
PEF423 (2007M-94)	*Fusarium*	*avenaceum*	PEI

Table 2: *In vitro* **efficacy of Bio-Save 10LP and Bio-Save 11LP (*Pseudomonas syringae*) against *Helminthosporium solani* and *Fusarium sambucinum*.**

Treatments	% Fungal Growth Inhibition*							
	H. solani					*F. sambucinum*		
	AB I	**AB II**	**PEI I**	**PEI II**	**NB**	**AB**	**PEI**	**NB**
Sterile Distilled Water (Control)	0[f]	0[f]	0[i]	0[h]	0[f]	0[h]	0[f]	0[g]
Bio-Save 10 LP (label rate = 4.4 g/L of water)	65[b]	60[b]	50[cd]	44[d]	68[a]	5[fgh]	2[ef]	7[de]
Bio-Save 10 LP (0.1 ppm)	0[f]	3[ef]	9[ghi]	4[h]	6[ef]	0[h]	0[f]	0[g]
Bio-Save 10 LP (1 ppm)	5[ef]	5[def]	17[gh]	5[gh]	16[cde]	2[gh]	0[f]	0[g]
Bio-Save 10 LP (10 ppm)	9[def]	8[def]	22[fgh]	20[f]	29[bc]	2[gh]	0[f]	3[fg]
Bio-Save 10 LP (100 ppm)	11[def]	19[de]	32[ef]	3[h]	39[b]	3[gh]	0[f]	5[ef]
Bio-Save 10 LP (1000 ppm)	40[c]	42[c]	43[de]	40[de]	40[b]	5[fgh]	1[ef]	5[ef]
Bio-Save 11 LP (label rate = 4.4 g/L of water)	66[b]	45[bc]	73[a]	67[bc]	72[a]	43[c]	28[d]	54[b]
Bio-Save 11 LP (0.1 ppm)	0[f]	0[f]	0[i]	2[h]	5[ef]	1[h]	0[f]	3[fg]
Bio-Save 11 LP (1 ppm)	1[ef]	8[def]	8[hi]	9[gh]	10[def]	8[fg]	0[f]	2[fg]
Bio-Save 11 LP (10 ppm)	18[def]	13[def]	8[hi]	8[gh]	6[ef]	10[ef]	1[ef]	5[ef]
Bio-Save 11 LP (100 ppm)	19[de]	15[def]	9[ghi]	14[fg]	14[def]	15[e]	1[ef]	10[d]
Bio-Save 11 LP (1000 ppm)	24[cd]	21[d]	23[fg]	31[e]	22[cd]	26[d]	3[e]	29[c]
Mertect (label rate = 44 ml/L of water)	100[a]	100[a]	53[bcd]	64[c]	70[a]	86[a]	88[b]	100[a]
Mertect (label rate) + Bio-Save 10 LP (label rate)	100[a]	100[a]	65[ab]	77[a]	79[a]	74[b]	77[c]	100[a]
Mertect (label rate) + Bio-Save 11 LP (label rate)	100[a]	100[a]	63[abc]	74[ab]	74[a]	83[a]	100[a]	100[a]

* Average values of 3 replicates. Mean followed by the same letter within each column are not significantly different from each other at *P=0.05*.
AB: Alberta; PEI: Prince Edward Island; NB: New Brunswick.

Table 3: The disease incidence and severity of dry rot on tubers treated with Bio-Save (*Pseudomonas syringae*), Mertect or the combination of both in a storage trial conducted in Prince Edward Island and New Brunswick.

Treatments	Prince Edward Island			New Brunswick		
	Disease Incidence (%)[1]	External Disease Severity (%)[2]	Internal Disease Severity (mm)[3]	Disease Incidence (%)[1]	External Disease Severity (%)[2]	Internal Disease Severity (mm)[3]
Inoculated, untreated control	64^{a*}	6.28^{a*}	7.05^{a*}	93^{a*}	8.2^{a*}	6.4^{a*}
Bio-Save 10 LP	8^{d}	0.42^{b}	0.52^{b}	34^{cd}	1.8^{cd}	1.5^{b}
Bio-Save 11 LP	21^{bc}	0.92^{b}	0.61^{b}	60^{b}	3.1^{b}	1.3^{b}
Mertect SC	10^{d}	0.99^{b}	1.00^{b}	42^{c}	2.6^{bc}	1.9^{b}
Bio-Save 10 LP + Mertect SC	24^{b}	1.21^{b}	0.81^{b}	34^{cd}	1.9^{cd}	1.3^{b}
Bio-Save 11 LP + Mertect SC	11^{cd}	0.48^{b}	0.41^{b}	24^{d}	1.4^{d}	1.8^{b}
LSD @ 0.05%	10	1.24	1.36	12	0.94	1.5

* Average values of 4 replicates. Each replicate contained a set of 25 tubers. Means followed by the same letter within each column are not significantly different from each other at *P=0.05*.

[1]Disease incidence means were based on the percentage of tubers evaluated per treatment that displayed symptoms of infection.

[2]External disease severity was measured on a scale of 0-100% (Cruickshank *et al*., 1982; Dorrance and Inglis, 1997).

[3]Internal disease severity (depth of rubber decay) was measured using a digital caliper (VWR Scientific Products, Mississauga, Ontario, Canada).

Table 4: The disease incidence and severity of silver scurf on tubers treated with Bio-Save (*Pseudomonas syringae*), Mertect or the combination of both in a storage trial conducted in Prince Edward Island and New Brunswick.

Treatments	Prince Edward Island	New Brunswick	
	Disease Severity (%)[1]	Disease Incidence (%)[2]	Disease Severity (%)[1]
Inoculated, untreated control	64[a*]	8.2[a*]	6.4[a*]
Bio-Save 10 LP	8[d]	1.8[cd]	1.5[b]
Bio-Save 11 LP	21[bc]	3.1[b]	1.3[b]
Mertect SC	10[d]	2.6[bc]	1.9[b]
Bio-Save 10 LP + Mertect SC	24[b]	1.9[cd]	1.3[b]
Bio-Save 11 LP + Mertect SC	11[cd]	1.4[d]	1.8[b]
LSD @ 0.05%	10	0.94	1.5

* Average values of 4 replicates. Each replicate contained a set of 25 tubers. Means followed by the same letter within each column are not significantly different from each other at *P=0.05*.
[1]Disease severity was measured on a scale of 0-100% (Cruickshank *et al*., 1982; Dorrance and Inglis, 1997).
[2]Disease incidence means were based on the percentage of tubers evaluated per treatment that displayed symptoms of infection.