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Biological and integrated control of postharvest blue mold (*Penicillium expansum*) of apples by *Pseudomonas syringae* and cyprodinil ☆

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Abstract

In an attempt to develop fungicide (thiabendazole)-resistance management strategies, experiments were carried out to determine if the control of blue mold (*Penicillium expansum*) in 'Empire' and 'McIntosh' apples could be achieved by a biocontrol agent, *Pseudomonas syringae* and enhanced by integrating a biocontrol agent and a reduced-risk fungicide, cyprodinil, in a cold storage, and in a subsequent shelf-life study. The *Ps. syringae* at a concentration of 1.4×10^8 CFU ml^{-1}, or cyprodinil at a concentration of 20 μg ml^{-1} were effective against blue mold caused by thiabendazole (fungicide)-sensitive and -resistant *P. expansum* in cold storage for 30 days and in the subsequent shelf-life study at 20 °C for 6 days in Empire' and 'McIntosh' apples. Cyprodinil was effective in both the co-treatment, where fungicide or biocontrol agent and pathogen inoculum were applied together, and the post-inoculation treatment, where fungicide or biocontrol agent applied 20 h after inoculation. *Ps. syringae* was more effective as a co-treatment than as a post-inoculation treatment. All of the eight combinations, two concentrations of the biocontrol agent (3.0×10^7 and 6.0×10^7 CFU ml^{-1}) and four concentrations of the fungicide (5, 10, 20, and 40 μg ml^{-1}) were more effective than either the *Ps. syringae* or cyprodinil alone on blue mold caused by the isolates of *P. expansum* collected from Ontario. Integrated control was efficient in controlling blue mold of apple and could be considered for disease control strategies to manage thiabendazole-resistant *P. expansum* and also for reducing fungicide residues on fruit.

Keywords: Apple; Bacterial antagonist; Biocontrol; Integrated control; Postharvest disease control; *Penicillium expansum*

1. Introduction

Blue mold in apple (*Malus domestica*, Borkh) is caused by wound-invading necrotrophic *Penicillium* spp. including *P. expansum* Link., (Sanderson and Spotts, 1995). Currently, thiabendazole, which belongs to the chemical class benzimidazoles, is the main fungicide used for the control of postharvest fungal fruit decay of apple and pear in Canada. The fungicide is applied as the drench treatment before the cold storage and/or spray treatment in packing lines (Koffmann and Penrose, 1987). The intensive use of the thiabendazole, along with antiscalding agent, diphenylamine (DPA), has resulted in the development of benzimidazole resistant pathogens in packing houses in the United States (Rosenberger and Meyer, 1985); and British Columbia

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(Sholberg and Haag, 1996); and Ontario (Errampalli, 2004) in Canada.

Fungicide resistance development has led to the search for alternative control measures including biocontrol for the management of storage diseases of pome fruit. Two biofungicides, BioSave 110 and Aspire have been registered for postharvest use on pome fruit in the USA (Fravel and Larkin, 1996; Janisiewicz and Jeffers, 1997), but not in Canada. Although the biocontrol agents were found effective, the control level could not reach the 95–98% that was often approached by the chemical fungicides. Also lack of consistency was reported as one of the drawbacks for the biocontrol (Chalutz and Droby, 1997). Cyprodinil, an anilinopyramidine fungicide was assessed as reduced risk fungicide by the United States Environmental Protection Agency (USEPA, 1998). It was shown that cyprodinil was effective against TBZ-resistant *P. expansum* on 'Empire' apples (Errampalli and Crnko, 2004). In a preliminary study it was shown that 5×10^{10} CFU ml^{-1} of *Pseudomonas syringae* (*Ps. syringae*; BioSave) reduced 89% of blue mold in 'Empire' apples in cold storage (Errampalli, 2003).

Integrating biocontrol agents with fungicides or exogenous chemicals have been proposed to enhance the efficacy of biocontrol agents (Chand-Goyal and Spotts, 1996, 1997; Sugar and Spotts, 1999; Zhou et al., 2002, 2005). Reduction in fungicide concentrations results in reduced residue levels on the fruit, as the fungicide residue levels retained by the fruit is directly proportional to the concentrations of the fungicide applied (Papadopoulou-Mourkidou, 1991).

In this report, we have evaluated the effectiveness of a biocontrol agent, *Ps. syringae* alone or in combination with a reduced risk fungicide, cyprodinil, for the control of blue mold in 'Empire' and 'McIntosh' apples in cold storage and in the subsequent shelf-life study. The specific objectives of this study were, (1) to test the efficacy of a biocontrol agent, *Ps. syringae,* and a reduced risk fungicide, cyprodinil, against blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum* that were collected in Ontario; (2) to evaluate *Ps. syringae* or cyprodinil in a co-treatment together with pathogen inoculum and a post-inoculation treatment of *Ps. syringae* or cyprodinil on apples that had been wounded and inoculated for 20 h; and (3) to integrate *Ps. syringae* and cyprodinil for control of blue mold in 'Empire' and 'McIntosh' apples in cold storage and in the subsequent shelf-life study.

2. Materials and methods

2.1. Fungal isolates and fungicides

Three thiabendazole-resistant isolates (PS-1R, PS-2R, and P12-4AR) and three thiabendazole-sensitive isolates (P24-7AS, P28-8AS, and P9-3AS) of *P. expansum* used in this study were collected from 1998 to 2001 in Ontario (Errampalli, 2004). The isolates of *P. expansum* were maintained as single spore cultures on potato dextrose agar (PDA; Difco, Detroit, MI) in petri dishes at 4 °C and with periodic transfers through apples. The pathogen inoculum was prepared as previously described (Errampalli, 2004). For fruit inoculation, conidia, from the isolates grown on PDA at 20 °C for 10–14 days, were harvested by adding a small amount of sterile distilled water with 0.01% Tween 20 to each plate and gently rubbing the sporulating mycelial mat with a bent glass rod. Conidial suspension was filtered through a milk filter (Ederol filter No. 261, J.C. Binzer, Hatzfeld, Germany). The conidial concentration was adjusted with the aid of a haemocytometer. Fungal isolates were used either individually or as a mixture of either sensitive or resistant isolates. When isolates of *P. expansum* were used as a mixture of either sensitive or resistant isolates, conidia from each of the three thiabendazole-sensitive or -resistant isolates were combined in equal proportions.

The fungicides tested were cyprodinil (Vangard 75%, WG), and thiabendazole (Mertect, 50%, SC) from Syngenta Crop Protection Canada, Guelph, Ont., Biofungicide, *P. syringae* strain ESC-10 (BioSave 10 LP) was from JetHarvest Solutions, Fl. Fungicides or a biocontrol agent were suspended in reverse osmosis (RO) water and diluted to desired concentrations. The concentrations of fungicides were expressed as active ingredients per ml (μg ml^{-1}) and the biocontrol agent was expressed as colony forming units per ml (CFU ml^{-1}).

2.2. Fruit

In 2002 and 2004 growing seasons, 'Empire' and 'McIntosh' apples were harvested from a research orchard at Vineland, Ontario and stored at 2–4 °C for 2 weeks prior to use in experimental treatments. Both cultivars were harvested at commercial maturity. Apples were not treated with any postharvest fungicide before the experiments. Natural decay at the time of the treatments was 0%.

2.3. Inoculation and treatment procedures

Apples were disinfested in 10% household bleach (5% sodium hypochlorite) and 0.01% Tween 20 (Fisher Scientific, Nepean, Ont.) for 4 min and rinsed in tap water for 4 min. The fruits were drained and placed in plastic fruit inserts. Each apple fruit was punctured once with a standard nail-like tapered probe 5 mm deep and 4 mm wide at its base. Wounded apples were treated within 45 min of wounding. Twelve fruits were used for each post-inoculation treatment or co-treatment and each treatment had

four replicates. Following the treatment, fruits were placed on fruit inserts in plastic boxes and covered with plastic lids to maintain high relative humidity (95%) and held either in a growth chamber (20 °C, 100–120 Ein m^{-2} s^{-1}, 16 h of light per day) for 6 days or in a cold storage at 2 ± 2 °C for 4 weeks and in a growth chamber (20 (±1) °C, 100–120 Ein m^{-2} s^{-1}, 16 h of light per day) for 6 days for a subsequent shelf-life study. The temperature and light conditions in growth chambers were set to simulate shelf-life study. The treatments were completely randomized. Experiments were conducted with 'Empire' and 'McIntosh' apples in 2002–2003 and 2004–2005. Apples were evaluated for disease incidence after each specified incubation period. Fruits were considered diseased when a lesion developed on the fruit and percent disease incidence was calculated. The experiments were repeated at least once.

2.3.1. Co-treatment experiments

In the co-treatment experiments, conidial suspension was mixed with different concentrations of fungicides or biocontrol agent solution immediately prior to the application on apples. Either a single or mixtures of thiabendazole-sensitive or -resistant isolates of *P. expansum* were used at a final concentration of 1×10^5 conidia ml^{-1}. In the control treatment, conidial suspension was mixed with an equal amount of water. Each of the wounded apples in each replicate were drop inoculated with fungicide or biocontrol agent and pathogen inoculum suspension. The drop application consisted of mixing the pathogen inoculum and the appropriate amount of fungicides or biocontrol agent in water and placing a 20 μl^{-1} drop in the wound.

2.3.2. Post-inoculation treatments

Wounded apples were inoculated with 20 μl^{-1} suspension of *P. expansum* at a concentration of 1×10^5 conidia ml^{-1}, incubated in closed plastic boxes to maintain high humidity, and held at 13(±1) °C for 20 h. After incubation, the apples were drop treated with different concentrations of fungicides by suspending appropriate amount of fungicides or biocontrol agent in water and placing a 20-μl^{-1} drop in the wound. Treated apples were incubated either in cold storage or growth chambers.

2.4. Effect of cyprodinil and biocontrol agent on blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum*

Two fungicides and a biocontrol agent were tested for their efficacy against blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum* in 'Empire' and 'McIntosh' apples. Three thiabendazole-resistant isolates (PS-1R, PS-2R, and P12-4AR) or three thiabendazole-sensitive isolates (P24-7AS, P28-8AS, and P9-3AS) of *P. expansum* at a concentration of 1×10^5 conidia ml^{-1} were used in a co-treatment along with different concentrations of fungicides. Cyprodinil at 5, 10, 20, and 40 μg ml^{-1}; and thiabendazole at 5, 50, 100, and 500 μg ml^{-1} were tested on 'Empire' and 'McIntosh' apples. *Ps. syringae* at 1.5×10^7; 3×10^7; 6×10^7; 1×10^8; 1.4×10^8; and 2.8×10^8 CFU ml^{-1} were tested on 'Empire' and 'McIntosh' apples. The rate recommended for use on pome fruit in the US is 1.4×10^8. In the control treatment, conidial suspension was mixed with an equal amount of water. Apples were evaluated for disease incidence after incubation in a growth chamber at 20 °C for 6 days or in cold storage and the subsequent shelf-life study.

2.5. Co-treatment and post-inoculation treatments

In a co-treatment, the protective effect of the *Ps. syringae*, a biocontrol agent or cyprodinil, a reduced risk fungicide, or combination of the both, together with the inoculum of *P. expansum* was tested on wounded 'Empire' and 'McIntosh' apples. The pathogen inoculum consisted of conidia of either thiabendazole-resistant or -sensitive isolates of *P. expansum* and the final concentration of pathogen inoculum suspension was adjusted to 1×10^5 conidia ml^{-1}. The treatments include: the inoculum only; four concentrations of cyprodinil (5, 10, 20, and 40 μg ml^{-1}); two concentrations, 3×10^7 and 6×10^7 CFU ml^{-1} of *Ps. syringae*; the combinations of two concentrations of the biocontrol agent (3×10^7 and 6×10^7 CFU ml^{-1}) with four concentrations of cyprodinil (5, 10, 20, and 40 μg ml^{-1}). These combinations were tested on blue mold in 'Empire' and 'McIntosh' apples. In a post-inoculation treatment, the apples were wounded, inoculated (with 1×10^5 conidia ml^{-1} of either thiabendazole-resistant or -sensitive isolates *P. expansum*) and incubated for 20 h at 13(±1) °C . The apples were then treated with either biological control agent, cyprodinil or a combination of the both. Cyprodinil at 20 μg ml^{-1} or *Ps. syringae* at 1×10^8 were tested in a post-inoculation treatment. All the treated apples were incubated in a cold storage at 4(±1) °C for 30 days and in a growth chamber at 20 °C for 6 days in a subsequent shelf-life. Apples were evaluated for disease incidence after cold storage and the subsequent shelf-life study. The percent disease reduction in relation to the inoculum only control was calculated.

2.6. Statistical analyses

All data was analyzed using SigmaStat (Sigma Stat 2.0 for Windows, SPSS Science, Chicago, IL). The figures were prepared using sigmaPlot (Sigma Plot 2.0 for Windows, SPSS Science, Chicago, IL). Percentage

data were subjected to arcsine-square-root transformation before the ANOVA. No disease symptoms were found in the sterile water control treatments, indicating little background infection, and these data were excluded from the analyses.

3. Results

3.1. Effect of cyprodinil and biocontrol agent on blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum*

Cyprodinil, a reduced risk fungicide, and *Ps. syringae*, a biocontrol agent, were effective against thiabendazole-sensitive and -resistant isolates of *P. expansum* in 'Empire' apples in the cold storage for 33 days (Table 1). Disease reduction of blue mold was positively correlated with concentrations of cyprodinil, and *Ps. syringae*. The concentration of 40 μg ml^{-1} of cyprodinil or 3.0×10^8 CFU ml^{-1} of *Ps. syringae* gave complete control of blue mold in the cold storage and in the subsequent shelf-life. The lowest concentration of cyprodinil at 5 μg ml^{-1} and a concentration of 6.0×10^7 CFU ml^{-1} of *Ps. syringae* gave 50–55% of reduction in blue mold caused by both thiabendazole-sensitive and -resistant isolates of *P. expansum* in cold storage. An increase of disease was observed in the subsequent shelf-life study. As expected, thiabendazole gave 100% reduction of blue caused by the thiabendazole-sensitive isolates of *P. expansum* and was totally ineffective against thiabendazole -resistant isolates of *P. expansum*.

After incubation at 20 °C for 6 days, cyprodinil and *Ps. syringae* were effective against blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum* in 'McIntosh' apples (Table 1). The concentration of 40 μg ml^{-1} of cyprodinil and 2.8×10^8 CFU ml^{-1} of *Ps. syringae* gave 98–100% control of blue mold. The lowest concentrations tested, 1.5×10^7 CFU ml^{-1} of *Ps. syringae* gave 8–11% of reduction in blue mold caused by both thiabendazole-sensitive and -resistant isolates of *P. expansum*. As expected, thiabendazole gave 95–100% reduction of blue mold caused by the thiabendazole-sensitive isolates of *P. expansum* and it was totally ineffective against thiabendazole-resistant isolates. In this study, cross-resistance between thiabendazole-resistant isolates of *P. expansum* and *Ps. syringae* was not observed.

Table 1
Effect of cyprodinil, thiabendazole, and *Ps. syringae* on blue mold caused by thiabendazole-sensitive and -resistant *P. expansum* in 'Empire' and 'McIntosh' apples

Treatment	Blue mold reduction (%)[a]					
	Empire				McIntosh	
	Cold storage at 4 °C for 30 days		Cold storage at 4 °C for 30 days + at 20 °C for 6 days		Cold storage at 20 °C for 6 days	
	TBZ-sensitive[b]	TBZ-resistant[c]	TBZ-sensitive[b]	TBZ-resistant[c]	TBZ-sensitive[b]	TBZ-resistant[c]
Pathogen inoculum only	0 (0)[d]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cyprodinil (μg ml^{-1})						
5	70 (2.2)	63 (5.1)	0 (0)	4 (3.2)	54 (2.6)	58 (2.9)
10	85 (4.0)	89 (2.2)	30 (3.1)	35 (11.1)	28 (7.2)	35 (3.4)
20	100 (0)	100 (0)	90 (3.3)	95 (2.1)	96 (3.9)	96 (1.5)
40	100 (0)	100 (0)	100 (0)	98 (3.5)	98 (2.1)	98 (1.6)
Thiabendazole (μg ml^{-1}l)						
5	100 (0)	6 (4.8)	100 (0)	6 (3.1)	95 (3.6)	0 (0)
50	100 (0)	0 (0)	100 (0)	0 (0)	100 (0)	0 (0)
100	100 (0)	0 (0)	100 (0)	0 (0)	100 (0)	0 (0)
500	100 (0)	0 (0)	100 (0)	0 (0)	100 (0)	0 (0)
Pseudomonas syringae (CFU ml^{-1})						
1.5×10^7	17 (1.2)	19 (2.4)	0 (0)	0 (0)	8 (2.1)	11(2.9)
3.0×10^7	35 (2.2)	37 (2.6)	0 (0)	0 (0)	18 (3.2)	20 (3.2)
6.0×10^7	55 (2.4)	50 (2.8)	0 (0)	0 (0)	44 (2.1)	42 (2.3)
1.0×10^8	89 (2.8)	90 (3.2)	NT	NT	63 (2.9)	68 (4.4)
1.4×10^8[e]	92 (4.7)	93 (3.6)	10 (6.2)	15 (8.1)	100 (0)	100 (0)
2.8×10^8	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)

[a] The biocontrol agent or each of the fungicides were applied together with the pathogen inoculum in the co-treatment. Disease incidence was assessed after incubation in a cold storage and subsequent shelf-life study.
[b] A mixture of three TBZ-sensitive isolates, P24-7AS, P28-8AS, and P9-3AS in equal proportions was used.
[c] A mixture of three TBZ-resistant isolates, PS-1R, PS-2R, and P12-4AR in equal proportions was used.
[d] Values are means of six replicates from two experiments with standard errors in parenthesis.
[e] Recommended concentration, where registered.

3.2. Comparison of co-treatment and post-inoculation treatment

After an incubation of 33 days at 4 °C, a statistically significant difference was observed between co-treatment of *Ps. syringae* applied together with pathogen inoculum, and the post-inoculation treatment, of *Ps. syringae* on the wounded and inoculated apples ($P \leqslant 0.001$; Fig. 1). No reduction in blue mold was observed in the shelf-life study that followed incubation in the cold storage.

Both the co-treatment of fungicide, at concentration of 20 μg ml^{-1}, together with pathogen inoculum and the post-inoculation treatment of the wounded inoculated apples were effective against thiabendazole-sensitive and -resistant isolates of *P. expansum* in the cold storage ($P \leqslant 0.001$; Fig. 2). There was a significant difference between co-treatment and post-inoculation treatment in cold storage and in the subsequent shelf-life study. An increase of disease was observed in the shelf-life study. Because the co-treatment was found more effective than the post-inoculation treatment, in the subsequent experiments, only the co-treatment was tested on blue mold caused by thiabendazole resistant isolates of *P. expansum*.

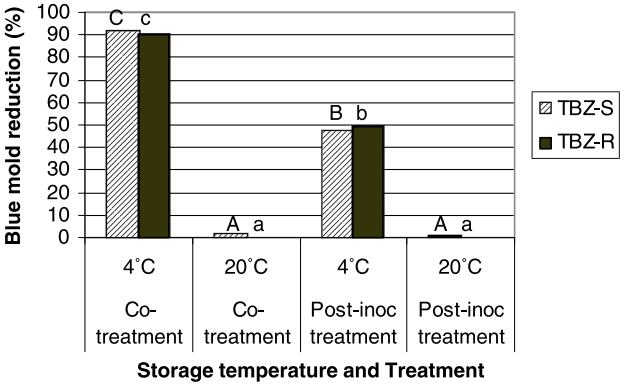

Fig. 1. Comparison of a co-treatment and a post-inoculation treatment with 1×10^8 CFU ml^{-1} of *Ps. syringae* on the reduction (%) of blue mold caused by thiabendazole-sensitive (diagonal bar) and resistant (solid bar) isolates of *P. expansum* in 'Empire' apple at 4 °C for 33 days and in the subsequent shelf-life study for 6 days at 20 °C. The values are means of four replicates. Means are not significantly different from each other according to the Tukey's test ($P = 0.05$) when followed by the same small or capital letter.

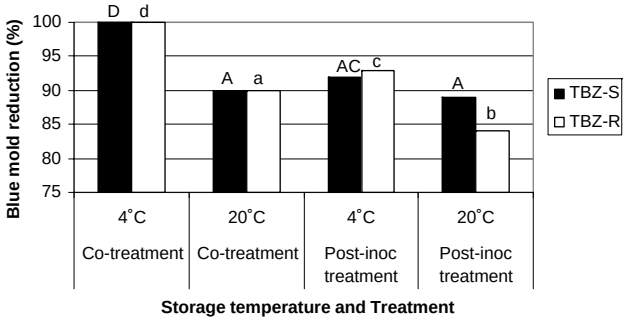

Fig. 2. Comparison of a co-treatment and a post-inoculation treatment with 20 μg ml^{-1} concentration of reduced risk cyprodinil on the reduction (%) of blue mold caused by thiabendazole-sensitive (sphere bar) and resistant (solid bar) isolates of *P. expansum* in 'Empire' apple at 4 °C for 33 days and in the subsequent shelf-life study for 6 days at 20 °C. The values are means of four replicates. Means are not significantly different from each other according to the Tukey's test ($P = 0.05$) when followed by the same small or capital letter.

3.3. Integration of Ps. syringae and cyprodinil for control of blue mold

The growth rate of *Ps. syringae* was not affected by cyprodinil in the cyprodinil amended growth medium (data not shown). The results from the experiments on the effect of integrated control, with two concentrations of *Ps. syringae* combined with four concentrations of cyprodinil, on blue mold are presented (Fig. 3). The data from experiments in 2002–2003 and 2004–2005 were homogeneous for variance and, therefore, were pooled prior to statistical analyses. The overall observations show that the eight combinations with, two concentrations of the biocontrol agent and four concentrations of the reduced risk fungicide, cyprodinil, were more effective than the cyprodinil or *Ps. syringae* alone. Blue mold disease reduction was positively correlated with concentrations of cyprodinil, and *Ps. syringae*. Low incidence of blue mold was observed in the cold storage and an increase in the disease was observed in the subsequent shelf-life study.

After the cold storage, some reduction of blue mold was observed in the treatment with 10 μg ml^{-1} of cyprodinil while the two higher concentrations, 20 and 40 μg ml^{-1}, reduced the disease by 98–100% in 'Empire' apples and by 96% in 'McIntosh' apples (Figs. 3A1–A2). In the shelf-life study, 20 μg ml^{-1} of cyprodinil gave higher control of blue mold in 'Empire' apples than in 'McIntosh' apples (Figs. 3A1–A2). In the *Ps. syringae* only treatment, 23 and 50% of reduction in blue mold was observed at concentrations of 3.0×10^7 and 6.0×10^7 CFU ml^{-1}, respectively. In the control treatments, inoculum only control showed 0% reduction in the cold storage and in the shelf-life study (data not shown).

The ANOVA on the reduction of blue mold from studying the effects of eight combinations, two concentrations of *Ps. syringae* and four concentrations of fungicide shows that there was a statistically significant difference among the combinations of the treatments ($P \leqslant 0.001$). The combinations of 6.0×10^7 CFU ml^{-1} of *Ps. syringae* with four concentrations of cyprodinil were more effective than the combinations that involved the lower concentration, 3.0×10^7 CFU ml^{-1}, of *Ps. syringae* in 'Empire apples' (Figs. 3A1–C1) and in 'McIntosh' apples (Figs. 3A2–C2). Of the four cyprodinil concentrations tested, 5 and 10 μg ml^{-1} of cyprodinil

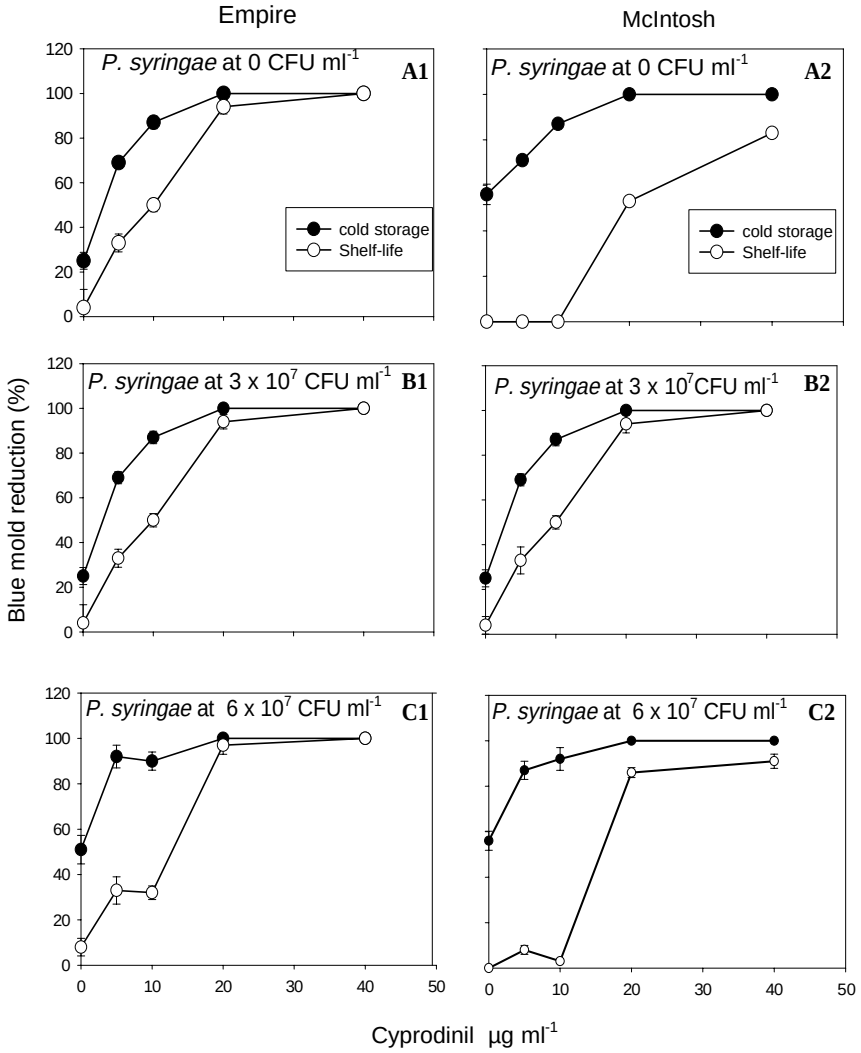

Fig. 3. Percent reduction of blue mold incidence from the interaction between two concentrations (3×10^7 or 6×10^7 CFU ml^{-1}) of *Ps. syringae* and four concentrations (5, 10, 20, and 40 µg ml^{-1}) of cyprodinil on the postharvest control of blue mold caused by thiabendazole-resistant *P. expansum* in 'Empire' (A1–C1) and 'McIntosh' (A2–C2) apples. The biocontrol agent or the fungicides or the combinations of biocontrol agent or the fungicides were applied together with the pathogen inoculum in a co-treatment. A mixture of three TBZ^R isolates, PS-1R, PS-2R, and P12-4AR in equal proportions were used in 2002–2003 and TBZ^R isolate, PS-1R was used in 2004–2005. Disease incidence was assessed after cold storage and in a subsequent shelf-life. The data from experiments in 2002–2003 and 2004–2005 were homogeneous for variance and therefore were pooled prior to statistical analyses. Means of six replicates from two experiments were pooled. The bars are standard errors.

and 3.0×10^7 CFU ml^{-1} of *Ps. syringae* had higher disease than the apples treated with 5 and 10 µg ml^{-1} of cyprodinil and 6.0×10^7 CFU ml^{-1} of *Ps. syringae*. A higher disease incidence was observed with 5 and 10 µg ml^{-1} than with 20 and 40 µg ml^{-1} of cyprodinil in the combinations. The combination involving 3.0×10^7 CFU ml^{-1} and 10 µg ml^{-1} gave 89% control in the cold storage and 53–59% reduction in the subsequent shelf-life studies. There was no significant difference between 'Empire' and 'McIntosh' in the combinations with higher concentrations, 20 and 40 µg ml^{-1} of cyprodinil and 3.0×10^7 or 6.0×10^7 CFU ml^{-1} of *Ps. syringae* ($P = 0.397$). Although the combinations of 6.0×10^7 CFU ml^{-1} and 20 and 40 µg ml^{-1} were most effective against blue mold in cold storage, combinations involving 3.0×10^7 CFU ml^{-1} of *Ps. syringae* and 20 µg ml^{-1} of cyprodinil gave complete control in the cold storage, and 97 and 86% reduction was observed in 'Empire' and 'McIntosh,' respectively, in the subsequent shelf-life studies. Phytotoxicity was not observed in the eight combinations of cyprodinil, and *Ps. syringae* tested.

4. Discussion

The data reported here shows that *Ps. syringae* gave significant reduction of blue mold caused by thiabendazole-sensitive and -resistant isolates of *P. expansum* collected from Ontario. Similarly, control of blue mold of

apple with the commercial biocontrol agent, *Ps. syringae,* was reported in the US (Janisiewicz and Jeffers, 1997). The efficacy of cyprodinil as postharvest treatment confirms the findings from a previous study (Errampalli and Crnko, 2004). Resistance of pathogens to the benzimidazole class of fungicides, which includes thiabendazole, is well documented (Rosenberger and Meyer, 1985; Smith, 1988). Some of the management strategies for benzimidazole (thiabendazole)-resistant populations of *P. expansum* include rotation with non-benzimidazoles fungicides alone or application of a combination of products that have different modes of action (Delp, 1988; Eckert, 1988). Thiabendazole binds to microtubules assembly in cell wall and a mutation in the β-tublulin in fungal cells results in resistance to the fungicide (Davidse and Ishii, 1995). Cyprodinil belongs to a chemical class different from benzimidazoles, which include thiabendazole (Forster and Staub, 1996). The mode of action for cyprodinil, an anilinopyramidine fungicide, is that it blocks the excretion of hydrolytic enzymes and inhibits methionine biosynthesis in fungal cells (Leroux, 1996). It was reported that the *Pseudomonas cepacia* produced pyrrolnitrin, a phenylpyrroles, (Janisiewicz et al., 1991) and the mode of action of phenylpyrroles is that they affect cell wall synthesis and induce glycerol in mycelial cells (Leroux, 1996). Although, the mode of action for *Ps. syringae* ESC-10 and ESC-11 (BioSave) has not been conclusively determined, a study on the mechanism of this biocontrol agent had shown that syringomycin E produced by *Ps. syringae* was found to be antagonistic against *P. digitatum*, the causal agent of green mold in lemons (Bull et al., 1998). In this context, the role of phenlypyrroles have been excluded. Competition for nutrients and space may have played a major role in the control of *P. digitatum* with the biocontrol agents, *Ps. syringae* ESC-10 and ESC-11 (Bull et al., 1997). Nutrient competition was cited between *Sporobolomyces roseus* and *P. expansum* and *B. cinerea* on apple (Janisiewicz, 1994).

Cyprodinil and *Ps. syringae* were tested for their effectiveness in a co-treatment and in a post-inoculation treatment. The fungicides and the biocontrol agent may control the infections that occurred at the time of drenching in a co-treatment, while the infection that may have occurred at the time of harvest in orchard or in transit prior to the treatment at apple storages may be controlled with post-inoculation treatment. Cyprodinil was effective in both the co-treatment and the post-inoculation treatment. Similarly, cyprodinil controlled blue mold in a co-treatment (Errampalli and Crnko, 2004). The biocontrol agent, *Ps. syringae*, was more effective in a co-treatment (90% reduction) than in a post-inoculation treatment (50% reduction).

Following the advent of the commercial preparations of biocontrol, researchers have experimented with products, biocontrol and chemicals, that control blue mold in apples and pears (Zhou et al., 2005). In this study, the integration of lower concentrations of *Ps. syringae* (registered in the US as BioSave, but not in Canada) and cyprodinil was effective against blue mold caused by *P. expansum*, collected from Ontario. Similarly, integration of biocontrol agents with lower concentrations of fungicides was reported (Chand-Goyal and Spotts, 1996, 1997; Sugar and Spotts, 1999; Zhou et al., 2002). Sugar and Spotts (1999) reported that two biocontrol products, registered in the US, BioSave and Aspire combined with 100 $\mu g\ ml^{-1}$ of thiabendazole were as effective as thiabendazole at 569 $\mu g\ ml^{-1}$ in controlling blue mold of pears. A combination of *Ps. syringae* MA-4 at $1–3 \times 10^7$ CFU ml^{-1} with cyprodinil at 5–10 $\mu g\ ml^{-1}$ controlled blue mold (tested on 1×10^4 conidia ml^{-1} of *P. expansum*) by >90% in 'Northern Spy' apples (Zhou et al., 2002). The higher concentration of incoulum 1×10^5 conidia ml^{-1} of the pathogen used, in this study, required higher concentrations of the 6×10^7 CFU ml^{-1} biocontrol agent and also 20 $\mu g\ ml^{-1}$ of cyprodinil.

A combination of a biocontrol agent, such as *Ps. syringae* and reduced concentrations of fungicide may greatly reduce their residues on fruit. Studies have shown that residue levels of chemicals and biocontrol agents were proportional to the concentrations applied in many cases (Papadopoulou-Mourkidou, 1991; Usall et al., 2001). Usually, high concentrations, 1×10^8 CFU ml^{-1} of yeast and 1×10^9 CFU ml^{-1} of bacteria, have been used to obtain adequate postharvest disease control (Chand-Goyal and Spotts, 1997). In the integrated control, lowering concentrations of the biocontrol agent may possibly reduce treatment cost and the use of reduced concentrations of fungicide would significantly reduce the fungicide residues on fruit and the cost of the treatment. Mixture partners may be combined to limit fungicide residues but caution should be taken not to reduce rates to the point where neither fungicide nor biocontrol agent is effective.

The integrated control experiments reported here have been conducted under laboratory conditions, using mixtures of either thiabendazole-sensitive or -resistant isolates of *P. expansum* in vivo on 'Empire' and 'McIntosh' apples. Since the controlled tests in the laboratory do not reflect commercial conditions these integrated control methods must be tested under commercial conditions. To be commercially acceptable, the application of the biocontrol agent and the fungicide also must be compatible with packing house operations. In some storages, 'Empire,' 'McIntosh,' 'Ida Red,' and 'McIntosh' apples, that are kept in controlled atmosphere storages (CA) for over six months, are drench treated with antiscalding agent, diphenylamine (DPA) and/or thiabendazole prior to storing under CA. Since the apples destined for long-term storage will be treated with DPA, integrated products must be tested with DPA in a drench in full-scale

commercial trials and the efficacy of the integrated control must be evaluated under CA storage conditions.

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