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Postharvest Biology and Technology 36 (2005) 235–244

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit

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Received 4 October 2004; accepted 26 January 2005

Abstract

'Golden Delicious' apples were wound-inoculated with either *Colletotrichum acutatum* or *Penicillium expansum* and then treated with various combinations of heat (38 °C) for 4 days, 2% sodium bicarbonate, and two biocontrol agents alone or combined. The fruit were stored for 4 months at 1 °C and then at 20 °C for 2 weeks. Either heat or the antagonists reduced decay caused by *C. acutatum*, but a combination of the two was required to completely eliminate decay caused by this pathogen in most cases. Sodium bicarbonate alone or in combination with the antagonists had little effect on *C. acutatum*. The antagonists alone reduced decay caused by *P. expansum* but tended to be more effective when combined. Sodium bicarbonate increased the effectiveness of decay control by each antagonist alone or in combination. All of the treatments that included heat virtually eliminated decay caused by this pathogen. The proper combination of alternative control measures can provide an effective strategy to reduce postharvest decay of apple fruit.
Published by Elsevier B.V.

Keywords: Biological control; *Cryptococcus laurentii*; *Metschnikowia pulcherrima*

1. Introduction

The use of fungicides has been becoming increasingly more restricted because of health concerns due to contamination with chemical residues (Ragsdale and Sisler, 1994). A recent (October 21, 2003) report by the Environmental Working Group (www.ewg.org) indicated that apples are among the top four fruits and vegetables that are the most consistently contaminated with pesticides. Many of the fungicides such as benzimidazole and dicarboximide fungicides that are still available for use are losing their effectiveness because

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0925-5214/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.postharvbio.2005.01.006

of the development of resistance in many postharvest pathogens (Lennox and Spotts, 2003). Also, there are no postharvest fungicides registered for some fruits but postharvest pathogens continue to cause significant losses. It is therefore necessary to find alternatives to control postharvest pathogens. Various methods have been investigated, and although they show promise, none alone has been found to be as effective as fungicides. It is therefore necessary to develop a strategy which combines several of these alternatives that may equal the effectiveness of fungicides.

Prestorage heat treatment has been investigated to modify the ripening of commodities as well as control of fungal decay and insects (Lurie, 1998). Hot air treatment (38 °C for 4 d) of apples after inoculation with *Penicillium expansum* either reduced or completely eradicated decay caused by this pathogen (Leverentz et al., 2000). Decay of apple fruit by *Colletotrichum acutatum* was also reduced by this same heat treatment but was not eradicated as in the case of *P. expansum* (Janisiewicz et al., 2003). Heat treatment, while a good eradicant, has no residual activity (Fallik et al., 1996).

Biological control is another alternative to chemical control that shows effectiveness in controlling postharvest diseases (Janisiewicz et al., 2001; Janisiewicz and Jeffers, 1997; Korsten et al., 1994; Usall et al., 2001; Wilson and Wisniewski, 1989; Zhou et al., 2001). Gray mold caused by *Botrytis cinerea* and blue mold caused by *P. expansum* in decay of apples and pears, both in laboratory and large scale studies, have been controlled by bacterial and yeast antagonists (Chand-Goyal and Spotts, 1996; Janisiewicz, 1994; Janisiewicz and Marchi, 1992; Roberts, 1990). Postharvest pathogens of stone, citrus, and subtropical fruit have also been effectively reduced by biocontrol (Janisiewicz and Korsten, 2002). Biocontrol agents are being used currently to control postharvest decay of various fruits. Biosave (Ecoscience Corp., Orlando, FL) has been used on pome fruits, citrus fruits, cherries and potatoes.

Sodium bicarbonate (SBC, $NaHCO_3$) has also been used to reduce postharvest decay, mainly on citrus fruits (Barger, 1928). It is readily available, inexpensive, and poses little risk of phytotoxicity at the concentrations (1–4%) used (Palou et al., 2001).

Although many alternatives to chemical control have been investigated, none, when used alone, is as effective as fungicides. The reduction of decay by biological control is generally more variable than for fungicides since biocontrol is affected more by environmental factors. There is also a narrower spectrum of activity than is found with chemical control. While heat treatment is very effective in eradicating decay if infection occurs prior to heating, it has little protective effect if infection occurs after heating (Klein et al., 1997). Also, while heat treatment is effective in eradicating infection by *P. expansum*, it is much less effective against *C. acutatum* (Janisiewicz et al., 2003). Similarly, SBC is not effective in providing protection if fruit are infected after treatment (Smilanick et al., 1999).

The treatments described above have been shown to be complementary to one another when applied in combination, and therefore the combinations are more effective than any individual treatment. When an antagonist specific for *P. expansum* was combined with one specific for *B. cinerea*, the resulting mixture inhibited the development of both types of lesions (Janisiewicz, 1988). In another study, a combination of yeasts resulted in better control of decay caused by *B. cinerea* than either antagonist alone and also reduced the variability in control levels (Guetsky et al., 2001). In a recent study, 'Golden Delicious' apples were inoculated with either *C. acutatum* or *P. expansum* and then treated with heat (38 °C) for 4 days, SBC (3 or 1%), and/or heat tolerant antagonistic yeasts. Both antagonists reduced decay caused by *P. expansum*, whereas heat or heat in combination with either antagonist eliminated decay (Conway et al., 2004). Either heat or the antagonists alone reduced decay caused by *C. acutatum*, but a combination of the two was required to completely eliminate decay caused by this pathogen. Adding SBC to the heated or antagonist treated fruit had little effect on decay caused by either pathogen, but on non-heated fruit, it slightly reduced decay caused by *P. expansum* (Conway et al., 2004). An increase in control of decay on oranges caused by *Penicillium digitatum* and *Penicillium italicum* occurred when *Bacillus subtilis* antagonists were combined with SBC (Obagwu and Korsten, 2002). Combining SBC with another antagonist also improved decay control of *P. digitatum* on oranges and grapefruit (Porat et al., 2003).

Our research goal is to combine several alternatives to develop a control strategy that will be highly effective and reliable in reducing postharvest decay

of apples. The objective of this investigation was to improve upon our previous decay control strategies by studying the effect of a mixture of antagonists, heat, and SBC treatments, alone and combined, on reducing postharvest decay caused by *C. acutatum* and *P. expansum*.

2. Materials and methods

2.1. Fruit

Apples ('Golden Delicious') were harvested from a commercial orchard in Pennsylvania in the preclimacteric stage (the climacteric rise in CO_2 and ethylene production had not yet occurred) and randomized 1 day after harvest. The respiration and ethylene production rates were measured over a 7-day period at 20 °C at harvest and after storage for 2 and 4 months at 1 °C followed by an overnight equilibration period at 20 °C. The respiration rate was measured using a gas chromatograph (GC, 5890a Series II; Hewlett Packard, Rockville, MD) with a thermalconductivity detector (TCD) for CO_2 detection and a flame ionization detector (FID) for ethylene detection (Izumi et al., 1996). Fruit firmness was measured using a manually controlled digital penetrometer (EPT-1 with an 11.1-mm-tip; Lake City Technical Products, Kelowna, BC, Canada) set in the Magness–Taylor mode (Saftner et al., 1998). The starch content of the non-treated fruit at harvest was measured using the Cornell generic starch scale of 1–8 (Blanpied and Silsby, 1992).

2.2. Pathogens

The *P. expansum* isolate (MD-8) is an aggressive pathogen from our collection that has been used in previous studies (Conway et al., 2004; Janisiewicz et al., 2003; Janisiewicz and Jeffers, 1997; Janisiewicz and Marchi, 1992). The *C. acutatum* isolate was obtained from Kenneth D. Hickey, Penn State Fruit Research Laboratory and Extension Service, Biglerville, Pennsylvania and has also been used in previous tests (Conway et al., 2004; Janisiewicz et al., 2003). Both pathogens were grown on potato-dextrose-agar (PDA) with virulence being maintained by periodic transfers through apple fruit. The 1×10^4 conidia/ml suspensions used to inoculate the fruit were prepared from 10-day-old PDA cultures as previously described (Janisiewicz and Marchi, 1992).

2.3. Antagonists

The two antagonists used in this study, *Cryptococcus laurentii* (ST4-E14) and *Metschnikowia pulcherrima* (FMB-24H-2), were originally isolated from the surfaces of apple fruit. FMB-24H-2 was shown to be heat resistant (Conway et al., 2004) while ST4-E14 is not. The yeasts were grown in 50 ml of nutrient yeast-dextrose broth medium in 250 ml-Erlenmeyer flasks at 26 °C on a gyratory shaker at 150 rpm for 24 h. Then the cells were harvested by centrifuging at $7000 \times g$ for 10 min, resuspended in water, and the concentration was adjusted to 3×10^7 CFU ml^{-1} with a spectrophotometer at 420 nm.

2.4. Sodium bicarbonate

Solutions of SBC (Sigma-Aldrich, St. Louis, MO) at concentrations of 0% (control, water only) or 2% (w/v) at pH 8.3–8.6 were used. Preliminary experiments were conducted to determine if the SBC concentrations selected were compatible with the antagonists.

2.5. Fruit inoculation, heat treatment and lesion measurement

The fruit were wounded with a six-penny nail (4 mm in diameter tapering to a point) to a depth of 4 mm at the equator, and 25 μl of either *C. acutatum* or *P. expansum* conidia alone, or the individual pathogens in combination with one or both of the antagonists and/or either 0 or 2% of the SBC solutions was placed in each wound. Following inoculation, one lot of the fruit was stored in 1 °C and a second similarly inoculated lot was heat treated as follows. Inoculated apples were tray-packed and placed in boxes with perforated polyethylene bag liners and then heated in a thermostatically controlled (±1 °C) walk-in chamber. The fruit were heated for 4 d at 38 °C and the relative humidity in the chamber was maintained at >85%. The storage conditions were monitored with a hygrothermograph (Belfort Instrument Co., Baltimore). Following heat treatment, the fruit were stored at 1 °C until removal for evaluation. Both lots of fruit were evaluated for decay incidence

and severity after 2 and 4 months at 1 °C and again after an additional 14 days at 20 °C. The non-heated *P. expansum* inoculated control fruit totally decayed after 2 months in storage and were discarded following evaluation. There were 45 fruit per treatment in a completely randomized design. Severity of decay was determined by measuring the lesion diameters at each evaluation period.

2.6. Antagonist recovery

The populations of ST4-E14 and FMB-24H-2 in the wounds were determined immediately after inoculation and after the 4-day heat treatment (38 °C) or cold storage (1 °C). Populations were determined again after 2 and 4 months at 1 °C, and after 4 months at 1 °C followed by 2 weeks at 20 °C. The antagonists were recovered from four wounds per treatment by a procedure previously described (Conway et al., 2000).

2.7. Statistical analysis

2.7.1. Decay severity and incidence

The severity of decay caused by either pathogen was determined by measuring lesion diameter at each time of evaluation. Since there was no significant decay on the fruit inoculated with *C. acutatum* after 4 months at 1 °C, the fruit were stored for an additional 2 weeks at 20 °C before determining decay incidence and severity. The resulting data were analyzed as a one-factor linear model using PROC MIXED (SAS Inst.) with treatment as the effect. The assumptions of the linear model were checked and the variance grouping technique was used to correct for variance heterogeneity. The mean comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error rate was 0.05. For the lesion incidence, a χ^2-analysis of the treatments was done using STATXACT 6 (Cytel Software Corp., 2000).

2.7.2. Antagonist recovery

The populations of the yeasts resulting from the various treatments were analyzed as a one-factor linear model using PROC MIXED (SAS Inst.). The assumptions of the linear model were checked and the variance grouping was used to correct variance heterogeneity.

3. Results

3.1. Fruit

The respiration and ethylene production rates of the preclimacteric fruit at harvest were 35.5 ± 5.6 nmol $kg^{-1}s^{-1}$ and 0.4 ± 0.0 pmol $kg^{-1}s^{-1}$, respectively. Between 8 and 12 days after harvest, the fruit entered the climacteric stage of development, as indicated by rapid increases in the respiration and ethylene production rates (data not shown). Heat treatment reduced climacteric respiration and ethylene production rates following 2 months at 1 °C plus 7 days at 20 °C and 4 months at 1 °C plus 1 or 14 days 20 °C (Table 1). While heat treatment initially had little or no effect on fruit firmness, heat-treated fruit were firmer following 4 months at 1 °C plus 1 or 14 days at 20 °C. Heat treatment increased the starch score, i.e., decreased the starch content of the fruit at harvest (Table 1).

3.2. Effect of treatments on decay

3.2.1. Decay severity

The mean lesion diameters on the non-heated and heat-treated control fruit were 29.1 and 4.6 mm, respectively (Fig. 1). The lesion diameters of the FMB-24H-2 and ST4-E14 treated fruit were 8.8 and 6.1 mm, respectively, and there was no significant difference in the effectiveness of either antagonist alone in reducing decay, although the trend seemed to favor ST4-E14, the heat sensitive antagonist. Heat in combination with FMB-24H-2, the heat tolerant antagonist, provided better control than either heat or the antagonist alone, since no lesions developed. Heat in combination with ST4-E14 provided better control than the antagonist alone with a lesion diameter of 1.2 mm but was not significantly better than heat alone with a lesion diameter of 4.6 mm. SBC did not improve decay control when applied alone or in combination with heat or the antagonists alone or in combination.

At all rating periods, *P. expansum* caused significant decay (Fig. 2A–C). The mean lesion diameter of the control was 23.1 mm. Heat treatment was the most effective treatment and it alone eliminated decay caused by *P. expansum* after the 2-month storage period at 1 °C (Fig. 2A). SBC alone was better than the control but only at the 2-month rating period with a lesion diameter of 11.4 mm. FMB-24H-2 and ST4-E14

Table 1
Maturity-related indices of non-heated and heat-treated 'Golden Delicious' apples stored in air at 1 °C for various periods of time

Treatment	CO_2 production (nmol kg^{-1} s^{-1})	Ethylene production (pmol kg^{-1} s^{-1})	Firmness (*N*)	Starch
At harvest				
Non-heated	35.5 ± 5.6a[a]	0.4 ± 0.0a	89.9 ± 5.9a	5.2 ± 1.0a
Heated	38.2 ± 7.5a	0.7 ± 0.1a	85.9 ± 5.0a	7.4 ± 0.5c
6 d cold storage plus 7 d at 20 °C				
Non-heated	80.6 ± 8.7b	6.3 ± 3.3a	86.7 ± 4.9a	5.8 ± 0.9b
2 Months cold storage plus 7 d at 20 °C				
Non-heated	114.2 ± 6.1c	806.7 ± 20.8e	73.9 ± 7.0b	
Heated	85.4 ± 4.0b	744.4 ± 29.2d	67.1 ± 6.2c	
4 Months cold storage plus 1 d at 20 °C				
Non-heated	122.0 ± 6.0d	625.7 ± 26.3c	58.3 ± 5.8d	
Heated	77.4 ± 7.6b	105.9 ± 22.6b	68.0 ± 6.5c	
4 Months cold storage plus 14 d at 20 °C				
Non-heated	105.9 ± 3.6c	917.0 ± 35.2f	51.4 ± 3.7e	
Heated	80.8 ± 7.7b	714.2 ± 65.4d	57.4 ± 3.9d	

[a] Within columns, symbols labeled with the same letter are not significantly different at $\alpha = 0.05$ using Holm–Sidak-adjusted multiple comparisons.

treated fruit had lesion diameters of 0.3 and 0.2 mm, respectively. There was no significant difference in the effect of either antagonist alone and the addition of SBC to either antagonist did not improve decay control. After 4 months in cold storage, all of the non-heated control fruit were considered totally decayed (Fig. 2B). There was a significant reduction in decay when FMB-24H-2 was combined with SBC on non-heated fruit, but there was no significant effect when SBC was combined with ST4-E14 although the combination tended to result in smaller lesions. The combination of the two antagonists with SBC was the most effective treatment on non-heated fruit. After 4 months at 1 °C plus 2 weeks at 20 °C, FMB-24H-2 or ST4-E14 in combination with SBC was significantly better than the antagonist alone on non-heated fruit (Fig. 2C). A combination of the antagonists was significantly more effective than FMB-24H-2 alone, but not better than ST4-E14 alone, although again the combination tended to result in smaller lesions. The most effective treatment on non-heated fruit, as it was on fruit stored for 4 months, tended to be a combination of the antagonists with SBC.

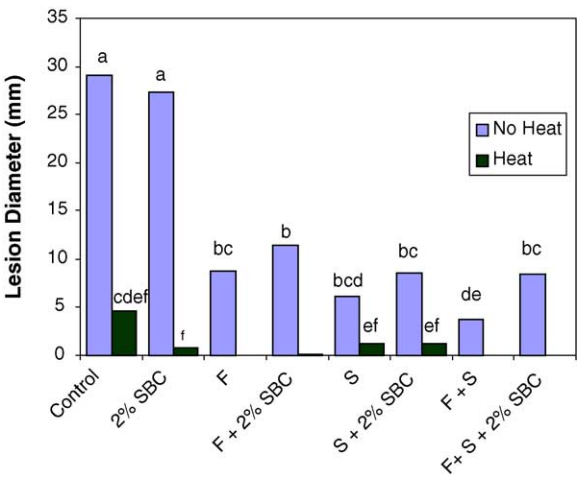

Fig. 1. Decay severity on 'Golden Delicious' apples inoculated with *C. acutatum* and then subjected to treatments or treatment combinations of heat (38 °C, 4 days), two antagonists, *M. pulcherrima* strain FMB-24H-2 (F) and/or *C. laurentii* strain ST4-E14 (S), or 2% sodium bicarbonate (SBC) after 4 months at 1 °C plus 2 weeks at 20 °C. Means with different letters are different at the 0.05 significance level. Bars without letters were not included in the analysis as there was zero variance.

3.2.2. Decay incidence

The incidence of decay was rated by counting the number of fruit inoculated with either pathogen that had no lesions. The number of apples without lesions inoculated with *C. acutatum* by treatment and storage time is shown in Table 2. A χ^2-analysis of the 16 treatments using STATXACT 6 (Cytel Software Corp.) showed that there was no difference in the frequency distributions after 2 months in storage. After 4 months, the frequency distributions were not all

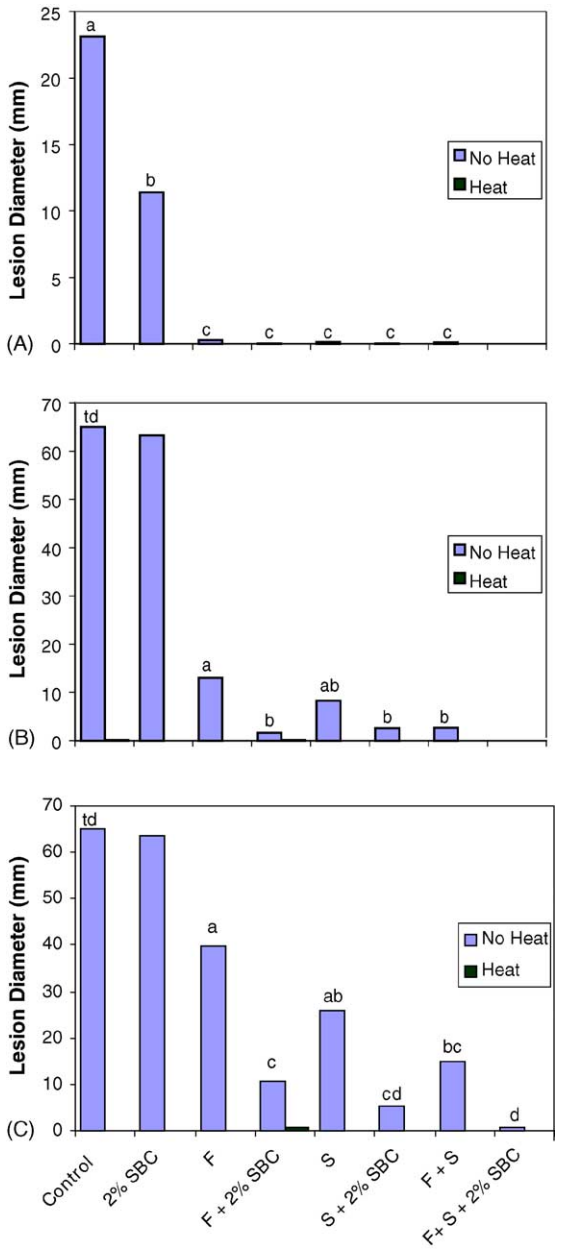

Fig. 2. Decay severity on 'Golden Delicious' apples inoculated with *Penicillium expansum* and then subjected to treatments or treatment combinations of heat (38 °C, 4 days), two antagonists, *M. pulcherrima* strain FMB-24H-2 (F) and/or *C. laurentii* strain ST4-E14 (S), or 2% sodium bicarbonate (SBC) after 2 months (A) and 4 months (B) at 1 °C and after 4 months at 1 °C plus 2 weeks at 20 °C (C). Bars with different letters are different at the 0.05 significance level. Bars with no letters were not included in the analysis as there was zero variance.

Table 2
Number of apples without decay (out of 45 per treatment) at various sampling times on fruit inoculated with *C. acutatum* and subjected to various treatments

Treatment			Sampling time		
Antagonist[a]	SBC[b]	Heat[c]	2 Months	4 Months	4 Months + 2 weeks
S+F	+	+	45	45	45
S+F	–	+	45	45	45
F	–	+	45	45	45
F	+	+	45	45	44
S	+	+	45	45	41
S	–	+	45	45	40
C	+	+	45	45	39
C	–	+	45	45	29
S+F	–	–	45	45	24
F	–	–	45	45	15
S+F	+	–	45	43	13
S	–	–	45	45	12
S	+	–	45	44	12
F	+	–	45	45	4
C	+	–	45	27	0
C	–	–	45	21	0

[a] S, antagonist ST4-E14; F, antagonist FMB-24H-2; C, control.
[b] Concentration (2%) of sodium bicarbonate (SBC); +, treated, –, not treated.
[c] Heat treatment (38 °C, 4 days); +, treated, –, not treated.

the same ($\chi^2 = 302.81$, P-value < 0.0000). The top 14 treatments were not statistically different ($\chi^2 = 12.02$, P-value = 0.2069). The top 15 treatments were statistically different ($\chi^2 = 220.9$, P-value < 0.0000). After 4 months in cold storage plus 2 weeks at 20 °C, the frequency distributions were also not all the same ($\chi^2 = 411.5$, P-value < 0.0000). The top four treatments were not statistically different ($\chi^2 = 3.02$, $P = 0.3890$). However, the top five treatments were statistically different ($\chi^2 = 12.27$, P-value = 0.0335). In general, on heated fruit, if no antagonist was present, then SBC was helpful. However, if either or both antagonists were present, SBC did not improve control of *C. acutatum*.

In the case of *P. expansum* (Table 3), a χ^2-analysis of the 16 treatments showed that the frequency distributions were not all the same after 2 months in storage ($\chi^2 = 631.5$, P-value < 0.0000). Further analysis showed that the top 13 treatments were not statistically different ($\chi^2 = 18.76$, P-value = 0.2149). The top 14 treatments were statistically different ($\chi^2 = 45.07$, P-value = 0.0002). After 4 months, the frequency distributions were not all the same ($\chi^2 = 485.8$,

Table 3
Number of apples without decay (out of 45 per treatment) at various sampling times on fruit inoculated with *P. expansum* and subjected to various treatments.

Treatment			Sampling time		
Antagonist[a]	SBC[b]	Heat[c]	2 Months	4 Months	4 Months + 2 weeks
C	–	+	45	45	45
C	+	+	45	45	45
F	–	+	45	45	45
S	–	+	45	45	45
S	+	+	45	45	45
S+F	–	+	45	45	45
S+F	+	+	45	45	45
F	+	+	45	44	44
S+F	+	–	45	45	43
S	+	–	45	42	41
F	+	–	45	40	36
S+F	–	–	44	37	32
S	–	–	43	30	25
F	–	–	40	25	13
C	+	–	3	1	1
C	–	–	0	0	0

[a] S, antagonist ST4-E14; F, antagonist FMB-24H-2; C, control.
[b] Concentration (2%) of sodium bicarbonate (SBC); +, treated, −, not treated.
[c] Heat treatment (38 °C, 4 days); +, treated, −, not treated.

P-value < 0.0000). Further analysis showed that the top nine treatments were not significantly different ($\chi^2 = 8.02$, *P*-value = 1.000). The top 10 treatments were statistically different ($\chi^2 = 23.36$, *P*-value = 0.0054). Similarly, after 4 months plus 2 weeks, the frequency distributions were not all the same ($\chi^2 = 474.3$, *P*-value < 0.0000). Further analysis showed that the top 9 treatments were not statistically different ($\chi^2 = 12.09$, *P*-value = 0.1473). However, the top 10 treatments were statistically different ($\chi^2 = 23.36$, *P*-value = 0.0054). Interestingly, after 4 months and after 4 months plus 2 weeks, the combination of the two antagonists plus SBC was significantly more effective than either antagonist alone, a combination of the two antagonists, or either antagonist alone combined with SBC on non-heated fruit.

3.3. Antagonist recovery

The populations of the antagonists in the wounds determined immediately after inoculation ranged from 4.65 to 5.01 log CFU/wound and there was no significant treatment effect at this time. The antagonist ST4-E14 was not tolerant to heat and was therefore eliminated on fruit subjected to heat treatment (Table 4). The

Table 4
Recovery (log CFU/wound) of *M. pulcherrima* strain FMB-24H-2 (heat resistant) or *C. laurentii* strain ST4-E14 (heat sensitive) from 'Golden Delicious' apples which were heated (38 C, 4 days) (+) or non-heated (−) and/or treated with 0 (−) or 2% (+) solutions of sodium bicarbonate (SBC) and stored for various times

Treatment			Sampling time			
Antagonist[c]	SBC	Heat	4 Days	2 Months	4 Months	4 Months + 2 weeks
F	–	–	5.59 cd[a]	5.59 de	6.33 c	6.43 ab
F	–	+	5.32 de	6.48 a	6.96 a	6.90 a
F	+	–	5.73 c	5.92 bcd	6.57 bc	6.73 ab
F	+	+	5.57 cde	5.92 bcd	6.87 ab	6.78 ab
S	–	–	6.22 ab	5.57 de	6.42 bc	6.24 ab
S	–	+	–[b]	–	–	–
S	+	–	6.42 a	6.05 bc	6.41 bc	6.48 ab
S	+	+	–	–	–	–
S+F	–	–	6.31 ab	5.43 e	6.11 abc	6.29 b
S+F	–	+	5.18 e	6.24 ab	6.85 abc	6.79 a
S+F	+	–	6.27 bcd	5.88 ab	6.47 bc	6.44 b
S+F	+	+	5.45 bcde	5.83 cd	6.44 bc	6.65 ab

For each time period shown in the table, the treatments were analyzed as a one-factor linear model using PROC MIXED (SAS Inst.). The results were statistically significant and were: 4 days, $F = 46.38$, $P < 0.0001$; 2 months, $F = 16.37$, $P < 0.0001$; 4 months, $F = 6.27$, $P = 0.0003$; 4 months + 2 weeks, $F = 7.75$, $P = 0.0001$. Mean comparisons were done with Sidak adjusted *P*-values so the experiment-wise error was 0.05.
[a] Means with different letters are different at the 0.05 significance level.
[b] Treatment not in analysis as all values were zero so variance was zero. Antagonist ST4-E14 is heat sensitive.
[c] F, antagonist FMB-24H-2; S, antagonist ST4-E14.

populations of this antagonist remained stable throughout the study on non-heated fruit. FMB-24H-2 was heat tolerant and the populations of this antagonist remained stable or slightly increased on both heated and non-heated fruit by the end of the experiment (Table 4). SBC had no clear effect on antagonist survival.

4. Discussion

Since no alternative to chemical control alone is as consistently effective as fungicides in reducing postharvest decay, promising alternatives of biological control, heat treatment, and SBC were tested alone and in combination to develop a strategy to provide satisfactory control of *C. acutatum* and *P. expansum* on apple fruit in storage. It was previously shown that apple fruit inoculated with *C. acutatum* did not develop decay during cold storage (Conway et al., 2004; Janisiewicz et al., 2003), therefore fruit were stored for an additional 2 weeks at 20 °C for decay to develop to determine the relative effectiveness of the various treatments and treatment combinations. In this study, low temperature was also sufficient to stop decay of apple fruit by *C. acutatum*, but only slowed decay by *P. expansum*.

Heat treatment was the most effective treatment in controlling both *C. acutatum* and *P. expansum* when used alone. It completely eradicated *P. expansum* and reduced decay caused by *C. acutatum*. However, it was necessary to add either or both of the antagonists to eradicate decay caused by *C. acutatum*. *M. pulcherrima* strain FMB-24H-2 was one of a number of *M. pulcherrima* strains found to be effective in reducing decay caused by *P. expansum* (Janisiewicz et al., 2001; Janisiewicz et al., 2003). More recently, it was also shown to be effective against *C. acutatum* and to be heat tolerant as well. In this as well as an earlier study (Conway et al., 2004) in combination with heat, FMB-24H-2 eliminated decay caused by this *C. acutatum*, which heat or the antagonist treatments alone were unable to accomplish. *C. laurentii* strain ST4-E14 had not been previously reported to be tested against *C. acutatum*. In this study, it was effective in reducing decay caused by either fungus on non-heated fruit. Unlike FMB-24H-2, it was not heat tolerant. On non-heated fruit, the combination of the two antagonists tended to be among the most effective treatments against either pathogen.

The addition of 2% SBC had no significant effect in reducing decay caused by *C. acutatum*. On non-heated fruit inoculated with *P. expansum*, fruit treated with SBC alone had significantly less decay after 2 months in storage than the control fruit. SBC was also helpful when non-heated fruit were evaluated for decay severity after 4 months at 1 °C plus 2 weeks at 20 °C. Either antagonist, when combined with SBC was significantly more effective than the antagonists alone. The smallest lesions on non-heated fruit tended to occur on fruit treated with the antagonist mixture combined with SBC. The 2% SBC concentration was more effective in reducing decay severity by *P. expansum* than the 0.3 or 1% SBC used in an earlier study (Conway et al., 2004). The 2% SBC seemingly had no clear negative effect on antagonist survival in this present study and was therefore compatible with both antagonists.

Most of the studies using SBC to control postharvest decay were concerned with citrus fruit (Obagwu and Korsten, 2002; Palou et al., 2001; Porat et al., 2003; Smilanick et al., 1999). While significant control of *P. italicum* was achieved by 2, 3, and 4% SBC solutions, 1% was ineffective (Palou et al., 2001). It was concluded that the SBC treatment was primarily fungistatic since it delayed spore germination but did not kill the *P. italicum* spores. The SBC effect was therefore not very persistent. Another study showed that a 2% SBC solution killed germinating spores of *P. digitatum* in citrus fruit wounds (Porat et al., 2003), indicating that germinating spores are more susceptible to SBC action than non-germinating spores (Marloth, 1931).

Combining SBC with other non-fungicidal control measures improved the effectiveness of these measures. A significant increase in the biocontrol activity of *Bacillus subtilus* isolates against *P. digitatum* and *P. italicum* on citrus fruit was observed when the isolates were combined with SBC (Obagwu and Korsten, 2002). The antagonist may have been given a competitive advantage by the delay in pathogen development by SBC at the wound site. The control of *P. digitatum* on citrus fruits was significantly improved by combining *Pseudomonas syringae* strain ESC10 (the active ingredient in BioSave 10; Village Farms, BioSave Division, Orlando, FL) with a 3% SBC solution (Smilanick et al., 1999). In the present study, the 2% solution was not of sufficient strength to affect *C. acutatum*, but in combination with either or both antagonists, it made the antagonists more effective against *P. expansum* in

non-heated fruit. Increasing the concentration of SBC further may be an option, but doing so may also affect antagonist survival (Spadaro et al., 2004).

Heat treatment may reduce fungal decay by two modes of action in that it may affect survival of pathogen spores and modify the physiology of the host. The viability of *P. expansum* conidia in sporulating culture declined rapidly when exposed to 38 °C prior to inoculation of apple fruit and the resulting lesions were smaller than those on fruit inoculated with non-heated conidia (Conway et al., 1999). Similar results were obtained with *P. expansum* on apple where a reduction in decay development was seemingly a result of the heat treatment, which, by reducing germination, effectively reduced the inoculum concentration (Fallik et al., 1996). Heat treatment may also enhance antifungal defense reactions in fruit tissue since a compound with antifungal activity was induced at 38 °C in apple fruit (Fallik et al., 1996). This may explain the slower decay development on apples inoculated after heat treatment compared to non-heated fruit (Fallik et al., 1996; Sams et al., 1993).

Heat treatment also delays ripening characteristics of apple fruit by inhibiting volatile production (Fallik et al., 1997) and maintaining fruit firmness (Klein and Lurie, 1992). In the present study, the firmness of heated fruit was significantly higher than non-heated fruit after 4 months in storage. The fruit used in this study were preclimacteric when heated. Postclimacteric fruit were more sensitive than preclimacteric fruit to heat damage as evidenced by browning of the flesh at the distal end of the fruit (Lurie et al., 1998). It may therefore be advisable to heat treat fruit when they are preclimacteric.

Chemical control of pathogens usually affords complete control in that it also eradicates fungal spores already on the host and protects the host from infection in storage. Currently, to provide an equivalent level of control, it will take a combination of alternative methods if chemicals cannot be used. Heat treatment and SBC can help to eradicate fungal spores at the time of application, but they do little to protect against future infections. In contrast, the various antagonists can help protect against future infection, but do little to eradicate infections that are already present. However, recent initial reports on the eradicative effect of a biocontrol agent against postharvest decays of pome and citrus fruits appear very promising (Mercier and Jimenez, 2004). A combination of these alternatives, then, is complementary and provides a complete strategy to successfully protect fruit from decay in storage. This alternate strategy will require more steps to implement than the one step chemical treatment. However, if postharvest fungicides are no longer effective or are disallowed as in various European countries, a successful strategy combining various alternatives is available to protect fruit in storage.

Acknowledgements

We would like to acknowledge Amy Blodgett, Willard Douglas, Michelle Orton, and Patricia Parmley for valuable technical assistance. Mention of trade names or commercial products in the publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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