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Postharvest Biology and Technology 27 (2003) 255–264

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Effect of combinations of hot water dips, biological control and controlled atmospheres for control of gray mold on harvested strawberries

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Received 21 August 2001; accepted 7 May 2002

Abstract

The efficacy of hot water, biological control and controlled atmospheres (CA), alone and in combinations, in controlling gray mold on harvested strawberry fruit was tested. All fruit were wound inoculated with *Botrytis cinerea* Pers.:Fr. Inoculated fruit were subsequently dipped in hot water at 63 °C for 12 s, inoculated with a biological control yeast, *Pichia guilliermondii* Wickerham, and/or immediately stored at 5 °C under air or 15 kPa CO_2 for 5 and 14 days followed by 2 days at 20 °C to simulate market conditions. Fruit treated with the combination of heat, biocontrol, and CA had significantly less decay than those in all of the other treatments after 5 days at 5 °C plus 2 days at 20 °C. After 14 days at 5 °C and 14 days at 5 °C plus 2 days at 20 °C, the heat + biocontrol + CA treatment continued to control decay though not significantly more than CA alone, biocontrol + CA, or heat + CA treatments. Some damage occurred following heat treatment; however, quality parameters did not differ between treatments. Overall, the combination treatments did not provide better control than the current commercially used treatment of 15 kPa CO_2.
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Keywords: *Fragaria* x *ananassa* Duch.; Carbon dioxide; *Botrytis cinerea*; Heat; Decay; *Pichia guilliermondii*

1. Introduction

Strawberry fruit have a very short postharvest life, due in part to gray mold caused by *Botrytis cinerea* Pers.:Fr. The efficacy of hot water treatments, biological control and controlled atmospheres (CA) has been evaluated for control of gray mold on strawberry. Hot water treatments have been tested on strawberry with varying results (Couey and Follstad, 1966; Yoshikawa et al., 1992; Garcia et al., 1995, 1996). Garcia et al. (1995) found that a hot water treatment of 45 °C for 15 min significantly reduced postharvest losses of strawberry fruit by delaying the onset of decay. Our preliminary results indicated that 45 °C for 15 min did indeed reduce decay; however, the fruit displayed shrivel and loss of shine. Unfortunately, heat treatments that are effective against pathogens are often close to the level of tolerance of the commodity. Recently, a short duration heat treat-

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PII: S0925-5214(02)00095-9

ment (10–30 s) at very high temperatures (56–62 °C) has shown promising results for citrus decay control without damage to the fruit (Porat et al., 2000a,b). This treatment is believed to sanitize the fruit as well as induce resistance to pathogens in some cases (Pavoncello et al., 2001).

CA including 12–15 kPa CO_2 are used commercially for both transport and storage of strawberries (Mitchell, 1992; Chambroy et al., 1993; Moyls et al., 1996). Elevated CO_2 effectively suppresses mycelial growth, spore germination, and germ tube elongation of *B. cinerea* and is fungistatic to most fungi. However, once the fruit is removed from the atmosphere, there is no residual protection from decay and fungal growth quickly resumes.

Several biological control agents are effective in reducing decay caused by gray mold on strawberry (Peng and Sutton, 1991; Swadling and Jeffries, 1996; Lima et al., 1997; Guinebretiere et al., 2000). However, few of these have been tested under postharvest storage conditions, and those that have would not be acceptable as a sole means for decay control. While these agents reduce decay, the level of control would neither be accepted by consumers nor be comparable to that achieved with the commercially used CA treatment. McLaughlin and Wilson (1992) isolated a yeast, *Candida* (*Pichia*) *guilliermondii*, which greatly reduced gray mold on apple. This yeast is a good candidate for postharvest biological control, as it continues to proliferate under storage and CA conditions, while growth of *B. cinerea* is hindered (author's unpublished data). Furthermore, *P. guilliermondii* has several modes of action; it acts by directly attaching itself to *B. cinerea* and by secreting cell wall degrading enzymes (Wisniewski et al., 1991). Multiple modes of action make this yeast a stronger candidate for biological control than antagonists with only a single mode of action.

Heat treatments, biological control and CA acting alone cannot completely control postharvest decay of strawberry. However, combinations of these treatments may achieve a satisfactory level of disease control. Combinations may have an additive or even synergistic effect on the berry for several reasons: (1) heat treatments may partially disinfect the fruit surface, allowing the biological control agent to become established more quickly and completely; (2) biological control agents may be favored under CA conditions allowing them to better compete with the pathogen; (3) combinations of other treatments with heat allow a less severe heat treatment to be used, thereby reducing the potential for fruit injury; and (4) biological controls may provide the residual protection that CA and heat treatments are lacking.

Combinations of various postharvest treatments have been studied with promising results in several laboratories, although little of this work has been with strawberry (Wells, 1970; Burmeister et al., 1997; Dock et al., 1998; Spotts et al., 1998; Conway et al., 1999). Wells (1970) combined modified atmosphere (20 kPa CO_2 or 1 kPa O_2), chemical (sodium salt solution of dehydroacetic acid or captan) and hot air (41 °C for 30 min) treatments to control postharvest decay of strawberry. However, only the high CO_2 treatment greatly reduced total losses after a 36-h treatment period, as the heat treatment discolored some berries. Our objectives were to examine the growth and survival of biological control populations when combined with hot water treatment and CA storage and to investigate the efficacy of these combination treatments for decay control on strawberry fruit to effectively control *B. cinerea*.

2. Materials and methods

2.1. *B. cinerea* inoculation

'Aromas' strawberries were sorted to remove injured berries, washed in 75 µl l^{-1} sodium hypochlorite and dried with a fan. Berries were punctured with a sterilized nail mounted in a board creating a 2-mm wide by 2-mm deep cylindrical wound. Once wounded, three replications of ten berries each per evaluation time were inoculated with 10 µl of a *B. cinerea* conidial suspension (4×10^4 conidia ml^{-1}). The conidial suspension was made from a culture of *B. cinerea* that was isolated from strawberry and was plated on potato dextrose agar (Difco Laboratories, Detroit, MI) amended with 100 mg l^{-1} streptomycin (Sigma, St Louis, MO) (SPDA). A 2-week-

old, sporulating plate (1 week in black light/1 week in 12 h fluorescent light and 12 h dark cycle) was flooded with sterilized distilled water containing one drop of Tween 20 (Fisher Scientific, Pittsburgh, PA). After inoculation, the fruit were dried for 1 h in a laminar flow hood and then either submerged in a hot water dip, treated with a biological control yeast, placed directly into air or CA storage, or treated with various combinations thereof.

2.2. Treatments

There were eight treatments, (1) air (control); (2) heat; (3) CA; (4) biocontrol; (5) heat+CA; (6) biocontrol+CA; (7) heat+biocontrol; and (8) heat+biocontrol+CA. Fruit were stored at 5 °C in sterilized 19-l glass jars for 5 or 14 days, and an additional 2 days at 20 °C in sterilized 6.5-l covered plastic containers, to simulate handling and market conditions.

2.3. Heat treatment

The heat treatment consisted of a 12-s dip in 63 °C water in the HWH-2 Laboratory Scale Hot Water Fruit Heating System (Gaffney Engineering, Gainesville, FL). Fruit were then forced-air cooled to 5 °C and inoculated with the antagonist or directly stored under air or CA.

2.4. Biological control

The yeast isolate, *P. guilliermondii*, was obtained from Charles Wilson (USDA, Agricultural Research Service, Kearneysville, WV) and maintained on silica gel crystals for long-term storage. The crystals were plated on SPDA for 72 h under a 12 h light and 12 h dark cycle. The yeast suspension was prepared with sterilized distilled water and one drop of Tween 20. Concentrations of the antagonist were adjusted to 3.1×10^7 colony-forming units (CFU) ml^{-1}. Each wound for the biological control treatments, either alone or in combination, was inoculated with 10 μl of the yeast cell suspension. Fruit were then dried for 1 h in a laminar flow hood and stored under air or CA.

2.5. Controlled atmosphere

A CA of 15 kPa CO_2+18 kPa O_2+67 kPa N_2 was established using micro-metering valves. The CA and air supplied to each 19-l treatment jar was at a constant flow of 300-ml min^{-1}. The atmospheres were humidified to ~90% RH by bubbling through water. Gas mixtures were monitored daily using a rapid gas analyzer (model VIA-510 Infrared CO_2 Analyzer; Horiba Instruments, Irvine, CA) and concentrations were maintained at ±1 kPa for the duration of the experiment.

2.6. Fruit decay and quality

Whole berry decay, including lesions originating from quiescent infections or other wounds, was evaluated subjectively and scored as none (0, no decay), slight (1, one to three small spots of decay), moderate (2, one-quarter to one-half of berry decayed) or severe (3, one-half to full berry rot). The lesion diameter of each inoculated berry wound was measured in millimeters.

External berry color was measured with a Minolta Chromameter (model CR-300; Ramsey, NJ) in CIE $L^*a^*b^*$ mode under CIE Standard Illuminant C. Changes in hue angle ($h°$) were calculated as $h° = \arctan b^*/a^*$ (°) (McGuire, 1992). Two readings per fruit were taken on opposite cheeks of the berry for both color and firmness. Firmness was measured with a penetrometer (Ametek, Largo, FL), using a 3-mm tip. Juice was extracted from a composite of three berries per replicate to determine soluble solids and titratable acidity. Soluble solids were assessed using a temperature-compensating digital refractometer (Abbe model 10450; American Optical, Buffalo, NY). Titratable acidity was determined by automatic titration and calculated using citric acid to determine acid equivalents (PHM85 precision, ABU80; Radiometer, Copenhagen, Denmark). Berry deterioration (damage), including shrivel, loss of shine, darkening between achenes, and calyx browning, was evaluated subjectively and scored as none (0, no deterioration), slight (1), moderate (2) or severe (3).

2.7. Population dynamics

The population dynamics of *P. guilliermondii* in storage were evaluated after 1, 5 and 14 days at 5 °C, and 5 or 14 days at 5 °C plus 2 days at 20 °C. The wounded area of six fruit per treatment, which had been inoculated with *P. guilliermondii* (biocontrol; biocontrol+CA; heat+biocontrol; and heat+biocontrol+CA) per storage duration was removed with a sterile 4-mm diameter cork borer. Each wounded area was placed individually in a 473-ml container with 25 ml of sterilized distilled water and one drop of Tween 20. The containers were placed on a rotary shaker for 20 min at 180 rpm. Ten microliter of the suspension were plated on SPDA and incubated for 3 days at 23 °C, then the colonies were counted. Three plates were incubated per berry.

2.8. Statistical analysis

Statistical analysis of fruit quality attributes, population dynamics, total berry decay and lesion diameter differences by treatment were analyzed using analysis of variance, and means were separated using LSD (SAS Institute, Cary, NC).

3. Results

3.1. Fruit decay and quality

Whole berry decay was minimal for all treatments after 5 days at 5 °C (Fig. 1A). After 5+2 days, fruit in the heat+CA treatment had the least decay, although not significantly different from decay in the heat+biocontrol+CA and heat alone treatments. After 14 days at 5 °C, fruit from the heat+biocontrol+CA treatment had significantly less decay than fruit from the control, heat, biocontrol, and heat+biocontrol treatments (Fig. 1B). After 14+2 days, fruit from both the heat+CA and heat+biocontrol+CA treatments had less decay than fruit from all other treatments.

Wound decay was minimal after 5 days at 5 °C and was not different between the treatments (Fig. 2A). After 5 days at 5 °C plus 2 days at 20 °C (5+2 days), fruit from the heat+biocontrol+CA treatment combination had the smallest lesion diameter. The control treatment had the largest lesion diameter, significantly larger than all of the other treatments, except for the biocontrol and CA treatments alone. After 14 days at 5 °C, all of the combination treatments as well as CA alone had significantly smaller wound lesions than the control, heat alone and biocontrol alone treatments (Fig. 2B). After 14 days at 5 °C plus 2 days at 20 °C (14+2 days), all four combinations and the

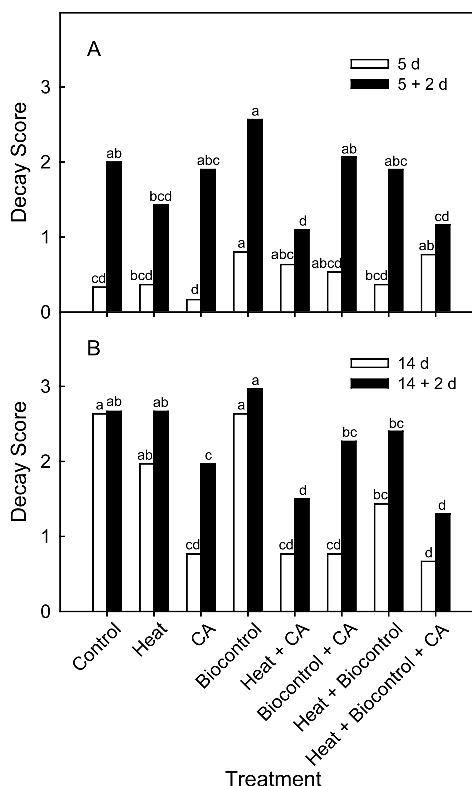

Fig. 1. Whole berry decay after (A) 5 days at 5 °C (5 days) plus an additional 2 days at 20 °C (5+2 days) and (B) 14 days at 5 °C (14 days) plus an additional 2 days at 20 °C (14+2 days). All fruit were wound inoculated with *B. cinerea* and then treated with various combinations of heat, biological control and CA (15 kPa CO_2). Decay score: 0, none; 1, slight; 2, moderate; 3, severe. Mean separation within storage period by LSD test. Means within storage period followed by the same letter are not significantly different at $P \leq 0.05$.

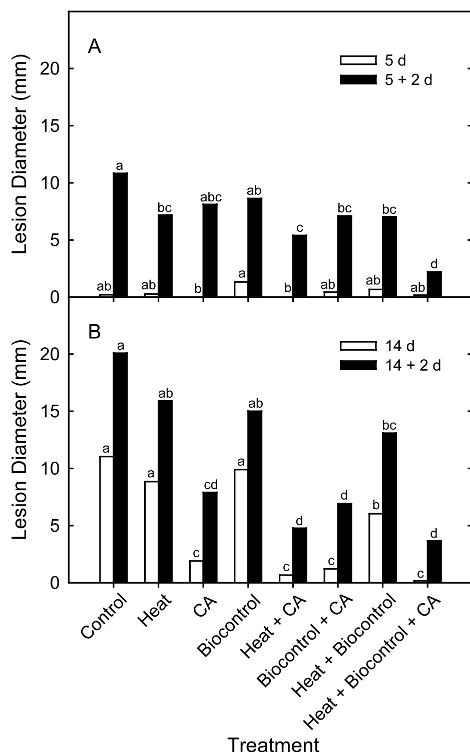

Fig. 2. Wound decay as measured by changes in lesion diameter (mm) over time during storage at 5 °C (A) for 5 days (5 days) plus an additional 2 days at 20 °C (5+2 days) and (B) for 14 days (14 days) plus an additional 2 days at 20 °C (14+2 days). All fruit were wound inoculated with *B. cinerea* and then treated with various combinations of heat, biological control and CA (15 kPa CO_2). Mean separation within storage period by LSD test. Means within storage period followed by the same letter are not significantly different at $P \leq 0.0004$.

CA treatment still had significantly smaller wound lesions than the control.

Fruit deterioration, including shrivel, loss of shine, darkening between achenes, and calyx browning, was not significantly different among treatments after 5 days at 5 °C (Fig. 3A). After 5+2 days, deterioration was greater in the heat+biocontrol +CA treatment than all of the other treatments, except for the heat+CA treatment. After 14 days, deterioration was greater in the heat+CA treatment than all of the other treatments, except for the heat+biocontrol+CA treatment (Fig. 3B). After 14+2 days, deterioration was not significantly different among treatments. The biocontrol and control fruits tended to have the least deterioration across all evaluation times.

After 5 days at 5 °C, the control treatment had significantly ($P \leq 0.01$) higher soluble solids (7.9%) than all of the other treatments (6.9–7.5%) except for the biocontrol+CA treatment (7.8%), although this difference did not persist after 2 days at 20 °C (data not shown). After 14 days, fruit from the heat alone treatment had significantly higher soluble solids (7.6%) than fruit from all of the other treatments (6.6–7.1%) except for CA alone (7.5%) and heat+CA (7.4%). After 14+2 days, most of the fruit had decayed due to

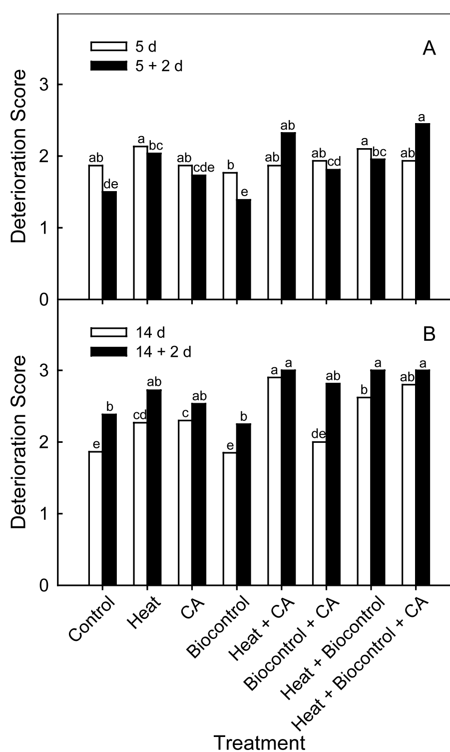

Fig. 3. Berry deterioration after (A) 5 days at 5 °C (5 days) plus an additional 2 days at 20 °C (5+2 days) and (B) 14 day at 5 °C (14 days) plus an additional 2 days at 20 °C (14+2 days). All fruit were wound inoculated with *B. cinerea* and then treated with various combinations of heat, biological control and CA (15 kPa CO_2). Deterioration score: 0, none; 1, slight; 2, moderate; 3, severe. Deterioration symptoms include shrivel, loss of shine, darkening between achenes and browning of the calyx. Mean separation within storage period by LSD test. Means within storage period followed by the same letter are not significantly different at $P \leq 0.0002$.

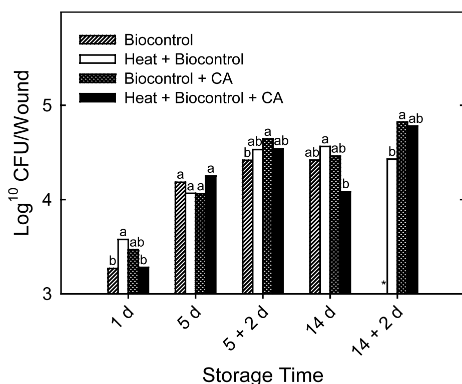

Fig. 4. Population dynamics measured as the $\log^{10}$ of CFU per wound after 1, 5 and 14 days storage at 5 °C plus an additional 2 days at 20 °C (5+2 days and 14+2 days). All fruit were wound inoculated with *B. cinerea* and then treated with various combinations of heat, biological control and CA (15 kPa CO_2). Mean separation within storage period by LSD test. Means within storage period followed by the same letter are not significantly different at $P \leq 0.05$.

quiescent infection, therefore, soluble solids, titratable acidity, color and firmness were not measured. Titratable acidity tended to be highest in control fruit across all of the storage periods (data not shown). Fruit color and firmness did not vary significantly between treatments, regardless of storage time.

3.2. Population dynamics

The biological control agent populations significantly increased over time in cold storage ($P \leq 0.04$) (Fig. 4). The population of the antagonist in the biocontrol alone treatment increased by 8-fold between 1 and 5 days at 5 °C and almost doubled again by 14 days of storage. The biocontrol+CA treatment and heat+biocontrol treatment resulted in antagonist populations which more than doubled and tripled in number between 5 and 14 days of cold storage, respectively. The population of the antagonist in the heat+biocontrol+CA treatment increased 9-fold between 1 and 5 days at 5 °C, but did not increase between 5 and 14 days in cold storage.

The 2 days at 20 °C following either cold storage period significantly enhanced the yeast populations in the fruit wounds (Fig. 4). The antagonist population in the biocontrol alone treatment increased almost 60% between 5 and 5+2 days. All of the biocontrol alone treated fruit had completely decayed from quiescent infection after 14+2 days; therefore, no population data was recorded for this storage period. Between 5 and 5+2 days, antagonist populations in the heat+biocontrol treated fruit nearly tripled, although they remained nearly constant between 14 and 14+2 days. The biocontrol+CA treatment antagonist populations increased nearly 4-fold between 5 days at 5 °C and 2 days at 20 °C, and more than doubled between 14 and 14+2 days. The heat+biocontrol+CA antagonist populations nearly doubled between 5 and 5+2 days and increased 5-fold between 14 and 14+2 days.

4. Discussion

4.1. Fruit decay and quality

The heat treatments, especially heat+CA and heat+biocontrol+CA, were most effective in reducing total berry decay and restricting lesion diameter after short storage periods (5+2 days). After a longer storage period (14-days), the heat plus CA combinations were still effective; however, all of the CA treatments, both alone and in combination with the biocontrol, were comparable in reducing total berry decay and restricting lesion diameter. It is possible that, initially, the heat treatment reduced decay and restricted lesion diameter by partially disinfecting the fruit, as suggested by Fallik et al. (1999), while exposure to CA reduced decay and restricted lesion diameter for the duration of storage.

Alternatively, Pavoncello et al. (2001) found that high heat treatments (62 °C for 20 s) induced resistance against green mold decay in grapefruit. They found that the induction of fruit resistance was temporary and inoculation 7 days after heating was much less effective in controlling decay than inoculation soon after heating. Perhaps similar resistance was induced in strawberry, which also declined during storage. The heat treatment may have initially produced a heat shock protein, giving the heat treatments an initial

advantage over CA storage alone, but the protein may have declined during 14 days in storage, thereby, CA alone provided similar levels of control.

When examining total berry decay, we cannot speculate on the effectiveness of the yeast, as it was only applied in the wound. Further tests, involving whole fruit sprays or dips, are necessary to determine the effects of the antagonist on total berry decay, including quiescent infection.

With regard to the decline in effectiveness of the biological control in storage, Janisiewicz et al. (1998) found that higher levels of biological control were needed to reduce decay on mature apple fruit than on less mature fruit. As fruit senesce in storage, they become more susceptible to pathogen attack, therefore, higher concentrations of the antagonist may be needed for longer storage. However, others have found a sustained synergism between biocontrol yeasts and CA on pear and cherry. Benbow and Sugar (1997) found that after 4 weeks, decay incidence was three times greater in CA (5 kPa O_2+12 kPa CO_2) treated pears as compared with CA plus *Cryptococcus laurentii*. Spotts et al. (1998) found that brown rot was reduced by 75% on sweet cherry by modified atmosphere packaging (5.1 kPa O_2+11.4 kPa CO_2), and further reduced by 23% as a result of a biological control (*Cryptococcus infirmo-miniatus*)-modified atmosphere package synergism. Powell (1969) reported that about 10% CO_2 enhanced sporulation in yeast. This could explain the synergism that results from the combination of CA and biological control, though we did not see this synergism in our study. As other researchers used slightly lower CO_2 concentrations (10–12 kPa), perhaps the level of CO_2 (15 kPa) that we used was above the yeast sporulation threshold, and, therefore, we did not observe the additive effect that they observed.

Fruit deterioration, including shrivel, loss of shine, darkening between achenes, and calyx drying, was similar for all treatments after 5 and 14+2 days. After 5 and 5+2 days, the deterioration was slight to moderate and fruit would have been acceptable for marketing. After 14 and 14+2 days, deterioration was moderate to severe and fruit was unmarketable, due to a combination of dehydration and decay. The berries that had been heated tended to show slightly drier calyces and, in a few cases, had mild water soaked areas compared with fruit that had not been heated.

Fruit from treatments combining CA with heat had the most deterioration after several evaluation times. The CA and heat combinations appeared to have had an additive damaging effect on fruit tissue. Exposure of plants to elevated temperatures produces a stress response, including increased respiration rates, decreased mRNA synthesis and increased damage to proteins and membranes (Paull and Jung Chen, 2000). These stress indicators generally resume control levels upon return to ambient temperatures, if the heat level was not above the fruit's temperature threshold. Perhaps the heat-stressed berries were more sensitive to CA storage than the unheated fruit and may have produced more fermentative metabolites due to the elevated respiration rate. Hence, these fruit could not recover from the initial stress and senesced more quickly under CA storage.

Our inability to effectively control *B. cinerea* growth in this study may be, in part, due to the use of late season fruit. As the season progresses, inoculum accumulates in the field, creating more infective opportunities for the fungus and higher rates of quiescent infection. In preliminary studies with early season strawberry fruit, we found that combinations of heat and CA sustained smaller lesion diameters and less total berry decay than either alternative alone over the course of the storage period.

Moreover, the ability of the antagonist to control decay may depend on fruit ripeness. After 14 days, strawberries are reaching the end of their postharvest life and senescence has begun. Chand-Goyal and Spotts (1997) found that *Rhodotorula glutinis* controlled decay on pears in storage at −1 °C in air or CA, but after ripening for 5 days at 22 °C in air, blue mold was no longer controlled.

In addition, Huang et al. (1995) found that effective biocontrol was dependent upon the successful establishment of a bacterial antagonist (*Pseudomonas glathei*) at the wound site prior to challenge by the pathogen. In our study, the fungus was applied before the antagonist to simulate field infection. Therefore, perhaps the

yeast did not successfully establish itself in the presence of the fungus. As strawberry fruit are prone to quiescent infection, field application of the antagonist could potentially permit the yeast to become established before fungal infection occurs and, hence, provide greater postharvest decay control.

4.2. Population dynamics

There has been little research done on antagonist recovery under postharvest storage conditions. Leverentz et al. (2000) followed populations of two antagonistic yeasts, before and after heat treatment of 38 °C for 4 days, on 'Gala' apples in storage at 1 °C for 3 months. They found that the populations increased during the heat treatment and continued to increase in cold storage. After 3 months at 1 °C, the population increases were almost two logs for both yeasts. Like Leverentz et al. (2000), we found that *P. guilliermondii* populations continued to increase during storage for all of the treatments. The population on fruit treated with the antagonist alone consistently increased in storage and increased equally well in the 2 days at 20 °C as it did during the 9 days at 5 °C (between 5 and 14 days). Conversely, the population on fruit from the heat+biocontrol treatment remained nearly the same between 14 days at 5 °C and the additional 2 days at 20 °C. After 14+2 days, the yeast may have not been able to compete with the heavy growth of *B. cinerea* and/or the fruit condition (damage from heat or senescence) was now favoring the growth of the fungus over that of the yeast.

While the CA treatment did not hinder the growth of the antagonist in cold storage, there was a marked spike in these populations on fruit from both the biocontrol+CA and heat+biocontrol+CA treatments upon removal to air at 20 °C. There were no significant differences in antagonist populations between fruit treated with the combination of the antagonist and CA and with the antagonist alone while under cold storage. When removed from CA and placed in air at 20 °C; however, there was a growth spurt in the population that allowed the biocontrol+CA antagonist population to surpass that of the biocontrol alone. This indicates that the increase in temperature was not solely responsible for the population increase.

The solubility of CO_2 in water produces carbonic acid and, in turn, lowers the solution pH (Pederson et al., 1961). A lower pH favors yeast growth over *B. cinerea* (Spotts et al., 1998). Perhaps a reduction in pH in the CA stored fruit worked in conjunction with the ambient temperature, causing a spike in the population in air. However, Holcroft and Kader (1999) found that pH in the external tissue of strawberry tended to increase slightly with 20 kPa CO_2 treatment (from $\sim$3.45 to 3.6 during 10 days of storage at 5 °C), and normalized upon return to air. Still, the naturally low pH of strawberry fruit would favor the yeast, as *B. cinerea* prefers a more neutral pH. Nevertheless, the increase in the antagonist population upon transfer to room temperature air could have contributed to the enhanced wound decay control of the heat, biocontrol+CA treatment after 5+2 days storage as compared with all of the other treatments. The heat+biocontrol+CA and biocontrol+CA treatments also showed reduced decay after 14+2 days, although there was no difference from the heat+CA or CA alone treatments. This phenomenon deserves further investigation.

5. Conclusion

While the combination of a hot water dip followed by the application of a biological control yeast, *P. guilliermondii*, and CA storage under 15 kPa CO_2 showed better wound decay control than single or double treatment combinations of the same after 5 days at 5 °C plus an additional 2 days at 20 °C, this difference was not maintained after 14 days at 5 °C or 14 days at 5 °C plus 2 days at 20 °C. The benefits of the three-combination treatment for *B. cinerea* control do not justify the added cost inputs necessary to implement the treatment. In addition, the combination treatments did not greatly surpass the commercially-used CA including 15 kPa CO_2. Still, the problem of the lack of residual protection upon removal from CA storage remains. Unpublished data from

our laboratory shows a large temperature range for the growth of *P. guilliermondii*. Zahavi et al. (2000) found that a strain of *C. (Pichia) guilliermondii* (A42) survived well under field conditions on table and wine grapes in Israel. This indicates that our strain may also be a good candidate for field application, and could potentially reduce quiescent infections at the flowering stage as well as provide enhanced postharvest decay control. The lack of decay control with the biological control agent in the late season fruit used in this study indicates the need to apply the antagonist as early as possible for the best control. Also, the sharp increase in the growth of the biological control agent upon transfer from CA to air is intriguing. Additional studies on combinations of CA, biological control and heat for postharvest decay control are needed.

Acknowledgements

Our sincere thanks to Charles Wilson for providing the *P. guilliermondii*, Bill Biasi and Kari Mansager for technical assistance, and Adel Kader, Joe Smilanick and Trevor Suslow for critical reading of the manuscript and helpful suggestions.

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