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UNIVERSITÀ DEGLI STUDI DI TORINO

Efficacy of yeast antagonists used individually or in combination with hot water dipping for control of postharvest brown rot of peaches

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Abstract

The efficacy of the antagonistic yeasts *Pseudozyma fusiformata* AP6, *Metschnikowia fructicola* AP47 and *Aureobasidium pullulans* PL5 were investigated, in combination with hot water dipping (HWD), to control brown rot of peaches caused by *Monilinia laxa*. Their effects on postharvest fruit quality were also evaluated under semi-commercial conditions. In potato dextrose broth, HWD at 50°C for more than 40 s or at 55-60°C for more than 10 s completely suppressed *M. laxa* spore germination and germ tube elongation. In fruits inoculated by wounding, HWD at 55°C for 50 s significantly reduced brown rot decay and maintained fruit quality. This treatment would be optimal for commercial application. The harvested fruits were dipped in HW at 55°C for 50 s, cooled to 1°C for 10 min, dipped in the selected yeast cell suspension (10^8 cells ml^{-1}) for 60 s, and then stored at 1°C and 95% relative humidity (RH). After 21 days, the disease incidence was measured and the postharvest quality parameters of the fruits, including firmness, total soluble solids, ascorbic acid and titratable acidity, were measured. When used alone, the antagonists AP6, AP47 and PL5 reduced brown rot incidence on the peaches to 28.3%, 30.0% and 25.8%, respectively,

compared with 74.2% for the untreated control. However, when applied together with HWD, AP6, AP47 and PL5 reduced brown rot incidences to 16.7%, 15.8% and 17.5%, respectively, while when HWD was used alone, disease incidence was 30.0%, suggesting that HWD greatly increased the effectiveness of the antagonists. In combination with HWD, the efficacies of AP6, AP47 and PL5 significantly increased from 61.8%, 59.6% and 65.2% to 77.5%, 78.7% and 76.4%, respectively, and they were similar to the efficacy of tebuconazole (77.5 %). Analysis of postharvest quality indicated that none of the treatments damaged fruit quality. Our results showed that the three antagonists combined with HWD at 55°C for 50 s exhibited the potential for commercial use, as an effective alternative to fungicides in controlling postharvest brown rot of peaches. This appears to be the first report on combined application of yeast strains *M. fructicola* or *P. fusiformata* with HWD to control *M. laxa* on peaches.

Key words: *Aureobasidium pullulans*, Biological control, *Metschnikowia fructicola*, *Monilinia laxa*, *Prunus persica*, *Pseudozyma fusiformata*.

1 Introduction

Brown rot caused by *Monilinia* spp. is among the most important postharvest diseases of commercially grown stone fruits. In the European Mediterranean areas brown rot of peaches is caused by three fungi, *Monilinia laxa, Monilinia fructigena* and *Monilinia fructicola* (PELLEGRINO *et al.*, 2009). The most common species isolated from brown rot-infected peaches and nectarines in Spain and Italy is *M. laxa* (LARENA *et al.*, 2005), which causes severe losses during the postharvest storage. *Monilinia* spp. infection can occur before or after harvest, but it is considered as a post-harvest disease because the symptoms develop in ripe fruit. From latent infections, fruit brown rot develops prior to harvest, during storage and transit (Hong *et al.*, 1998).

When permitted, use of synthetic fungicides is the best method to control the post-harvest diseases on fruits (ECKERT and OGAWA, 1988). However, consumers are increasingly concerned about safety, and prefer foods free from pesticide residues, toxins and

harmful microorganisms (SPADARO and GULLINO, 2004). Moreover, many fungal strains are developing resistance to widely-used synthetic fungicides and more effective fungicides are no longer re-registered (RAGSDALE, 2000). Therefore, new alternative strategies need to be developed. Microbial biocontrol agents have shown great potential as alternatives to synthetic fungicides for control of postharvest decay of fruit and vegetables (DROBY *et al*., 2009). A variety of microbial antagonists have been reported to control different pathogens on various fruits and vegetables. *Aureobasidium pullulans*, a yeast-like fungus, has been successfully applied to control postharvest pathogens on a number of fruits and vegetables, for example *Botrytis cinerea* and *Rhizopus stolonifer* on strawberries, and *B. cinerea* on sweet cherries (IPPOLITO *et al.*, 2000). Different species of *Metschnikowia* are active against *Botrytis cinerea* and *Penicillium expansum* on some fruits (KURTZMAN and DROBY 2001; SPADARO *et al*., 2002). Several strains of *Pseudozyma fusiformata* have also shown antifungal activity (KULAKOVSKAYA *et al.*, 2003). In our previous studies, *A. pullulans* PL5, *M. fructicola* AP47 and *P. fusiformata* AP6 showed strong activity against *Monilinia* spp. on plums and peaches (ZHANG *et al*., 2010). However, as with other non-fungicide approaches, at present none of the biocontrol yeasts are as effective as synthetic fungicides (JANISIEWICZ and KORSTEN, 2002; DROBY *et al*., 2009). Therefore, it is necessary to combine antagonistic yeasts with other non-chemical or low-risk chemical methods to control postharvest diseases of fruits (LARRIGAUDIÈRE *et al*., 2002).

Hot water dipping can control postharvest diseases and is already widely used in the fruit industry (MALAKOU and NANOS, 2005). But when used alone, HWD does not give a consistent economic level of control that would warrant acceptance as a viable alternative to synthetic fungicides. Moreover, there are few studies of combining HWD and antagonists to control brown rot on peaches, compared with studies on apples and pears, under semi-commercial conditions, (SPADARO *et al*., 2004).

The objective of this research was to investigate, under semi-commercial conditions, the effect of the three antagonistic yeasts *Pseudozyma fusiformata* AP6, *Metschnikowia fructicola* AP47 and *Aureobasidium pullulans* PL5, used alone or in combination with hot water, on postharvest brown rot of peaches and to evaluate the impacts of these treatments on fruit quality.

2 Materials and methods

2.1 Microorganisms and culture conditions

Five strains of *Monilinia laxa* (Aderhold & Ruhland) Honey were isolated from rotted peaches and selected for their virulence by inoculation in artificially wounded peaches. They were used as a mixture (each strain accounted for 1/5 of the total final concentration) throughout this work, to ensure a high level of disease. Each strain was stored in slants on Potato Dextrose Agar (39 g L^{-1}; PDA; Merck, Darmstadt, Germany) with 50 mg L^{-1} of streptomycin (Merck) at 4°C. Spore suspensions were prepared by growing the isolates on Petri dishes at 25°C for 7 days on Peach Agar [PA; 500 ml L^{-1} peach juice + 20 g L^{-1} agar (Merck); pH 7.0] medium. *M. laxa* spores were collected and suspended in sterile Ringer solution (pH 6.9±0.1; Merck). After filtering through 8 layers of sterile cheese-cloth, spores were quantified with a Bürker chamber and brought to a final concentration of 10^5 spores/mL.

Three yeast strains (ZHANG *et al*., 2010), *A. pullulans* PL5 (isolated from the carposphere of an "Angeleno" plum), *P. fusiformata* AP6 and *M. fructicola* AP47 (both isolated from the carposphere of "Golden delicious" apples), were grown in 300 ml of YPD (20g l^{-1} dextro-glucose, 20g l^{-1} peptone casein, 10g l^{-1} yeast extract; Merck) at 25°C for 48 h on a rotary shaker (250 rpm). Yeast cells were harvested by centrifugation at 5000×g for 10 min and then re-suspended in sterile ringer solution (Merck). The final concentration of yeast cells was determined using a Bürker chamber as required.

Peaches [*Prunus persica* (L.) Batsch] cv. Springcrest harvested at commercial maturity were used throughout. Before treatment hey were disinfected in 1% commercial sodium hypochlorite.

2.2 Effect of HWD on spore germination of M. laxa in vitro

The effect of HWD on spore germination and germ tube elongation of *M. laxa* was studied in PDB according to KARABULUT *et al*. (2002) with small modifications. Sterile

rubber-sealed 35 ml glass tubes containing 4.9 ml PDB were placed in water baths (D-3508 Melsungen, Germany) at 45, 50, 55 and 60 °C for 30 min. Spore suspensions of *M. laxa* (100 µl aliquots of 5×10^5 conidia ml^{-1}) were then added to the tubes to reach a final concentration of 10^4 conidia ml^{-1}. After 10, 20, 30, 40, 50 and 60 s of incubation, the tubes were placed at 0°C. Tubes held at 20°C for 30 min served as controls. All tubes were then incubated at 25°C on a rotary shaker at 120 rpm for 20 h. One hundred spores were randomly selected from each tube and the percentage spore germination and germ tube length were measured microscopically. Each treatment had three replicates and the experiment was repeated twice.

2.3 Effect of HWD on brown rot decay development in wound-inoculated fruits

Effects of HWD on brown rot decay development were investigated according to Zhang *et al*. (2007) with some modifications. Peaches were surface-sterilized with 1% commercial sodium hypochlorite for 1 min, and then rinsed with tap water. Three artificial wounds (about 3mm wide× 3mm deep) were made along the equatorial zone of the peach. Aliquots (30 µl) of *M. laxa* suspension at 1×10^5 conidia ml^{-1} were pipetted into each wound. After 2 h of air drying at 25°C, they were divided into three groups. Some fruits were immersed in a water bath at 50, 55 or 60°C for 10, 20, 30, 40, 50 or 60 s, respectively. After HWD treatment, the fruits were placed in a chamber at 0°C and dried for 10 min with forced air. A second group of fruits, inoculated with the pathogen, was immersed in a water bath at 20°C for 60 s and served as HWD control. A third group of peaches, inoculated with the pathogen, did not receive any water treatments and served as water control. All fruits were incubated at 20°C and 95% RH. After 6 days, the percentage of infected wounds was recorded and the diameter of lesions was measured. There were three replicates of fifteen fruits for each treatment, and the experiment was repeated twice.

2.4 Control of brown rot of peaches by three antagonists and HWD under semi-commercial conditions

The cells of each antagonist were diluted with 30 L tap water in a 50 L tank to a final

concentration of 10^8 cells ml^{-1} by adding 612.3 ml of suspension of antagonist (5×10^9 cells ml^{-1}) to 30 L tap water. Thirty litres of tebuconazole suspension containing 250 mL/100 L of Folicur (Bayer Crop Science; 25.0 % a. i.) was prepared according to the manufacturer's instruction. For the treatments of yeasts in combination with HWD, the peaches were first dipped in hot water at 55°C for 50 seconds and then cooled immediately to 0°C for 10 min. They were then dipped in individual antagonist suspensions for 1 min. The fruits were air-dried at 25°C for 2 h and then stored at 1°C and 95% RH. Fruits treated with tebuconazole served as chemical control while untreated peaches served as untreated control. There were three replicates of 25 fruits for each treatment. After 21 days of storage, the number of rotten peaches was recorded and the diameters of the lesions measured. The experiment was repeated twice.

2.5 Effects of the yeasts and HWD treatment on fruit quality

After 21 days of storage at 1°C and 95% RH, the quality of the healthy peaches in every treatment was assessed as described below. Ten peaches from each replication of each treatment were randomly selected for assessment. To assess the firmness parameter, the fruits were measured individually, while to assess the other quality parameters three fruits were pooled together for each measure.

Firmness was measured on two opposite sides along the equatorial region with a FT327 - Fruit Pressure Tester having an 11 mm probe (EFFEGI, Alfonsine, Italy). The probe descended toward the sample at 1.0 mm s^{-1} and the maximum force (*N*) was defined as firmness.

Total soluble solids (TSS) were determined by measuring the refractive index of the pressed juice with a digital refractometer (DBR95, Singapore) and the results expressed as percentages (g per 100g fruit weight) (LARRIGAUDIÈRE *et al*., 2002).

The 2,6-dichloroindophenol titrimetric method (AOAC, 1995) was employed to determine the ascorbic acid content of pressed peach juice. Results were reported as milligrams of ascorbic acid per 100 g sample (ÖZDEN and BAYINDIRLI, 2002).

Acidity was measured by titration to pH 8.0 with 0.1 N NaOH, using 5 ml of pressed juice

diluted with 5 ml of distilled water. Titratable acidity was calculated as percent malic acid (WRIGHT and KADER, 1997).

2.6 Statistic analysis

Replications of all the experiments, when the means were similar, were pooled and analyzed together. Means and standard errors for every experiment are reported. Data was subjected to analysis of variance using SPSS (SPSS Inc., version 17.0, Chicago, IL, USA) and statistical significance was assessed at the level of $P<0.05$. When the analysis of variance was statistically significant, Duncan's multiple range test was used to compare the means.

Limpel's formula, as described by RICHER (1987), was used to determine synergistic interactions between antagonist and HWD treatments. The formula is $Ee = X + Y-(XY/100)$, where *E*e is the expected effect from additive responses of two treatments and *X* and *Y* are the percentages of decay reduction obtained when each agent was used alone. Thus, synergism exists if the combination of the two agents produces a value of decay reduction greater than *E*e.

3 Results

3.1 Effects of HWD on M. laxa *spore germination* in vitro

Under *in vitro* conditions, spore germination was measured and germ tube elongation were measured after 20 h growth in PDB at 25°C on a rotary shaker. During the experiment setting up, longer incubation times, up to 48 h, were assessed, but the spore germination rate was not changing significantly, while the germ tubes became too much long to be measured under microscope conditions. Hot water treatments at 50°C for at least 20 s and at 55 °C for at least 10 s could significantly inhibit spore germination and germ tube elongation. With treatments at 50°C for more than 40 s or above 50°C for more than 10 s, spore germination was completely inhibited. With HWD at 45°C for 10-60 s, spore germination rates (98.3%) were similar to those of controls, while germ tube elongation was still significantly inhibited (Table

1). With HWD at 50°C for more than 20 s, both spore germination and germ tube elongation were significantly lower than in controls. With HWD at 55°C for more than 40 s or at 60°C for 10 s, spore germination and germ tube elongation were completely suppressed. Thus spore germination and germ tube elongation consistently became lower with increasing HWD temperature and incubation time.

3.2 Effects of HWD and antagonists on brown rot development of wound-inoculated fruits

When wound-inoculated fruits were subjected to HWD at 50, 55 or 60°C for 10-60s, decay was inhibited to various extents. However, at 60°C for more than 50 s, the fruit pericarp became bronzed (Table 2). *In vivo* test indicated that brown rot induced by wounding and inoculation with *M. laxa* was significantly inhibited or prevented by HWD at 55°C for more than 30 s or at 60 °C for more than 10 s. Moreover, as for incidence of rotten lesions on fruits, significant difference was observed only between the treatments at 55°C for more than 40 s or at 60°C for more than 20 s and the control treatments (Table 2). Therefore, in our investigation the optimal hot water dipping treatment was determined to be 55°C for 50 s.

3.3 Control of brown rot by three antagonists and HWD under semi-commercial conditions

Applications of the three antagonistic yeasts (AP6, AP47 and PL5) and HWD to control brown rot were evaluated after 21 days of storage at 1°C and 95% RH. When each antagonist was used alone, disease incidences in the treatments and the untreated control were 28.3%, 30.0%, 25.8% and 74.2%, respectively. However, when combined with HWD, the three yeasts performed better and the disease incidences were greatly decreased as seen in Table 3. The disease incidence after HWD alone was lower than that of the untreated control but higher than the disease incidence following tebuconazole treatment. When combined with HWD, the biocontrol efficacy of AP6, AP47 and PL5 was correspondingly increased by 25.4%, 32.0%, and 17.2%, respectively, reaching the level of tebuconazole.

The use of Limpel's formular did not indicate synergism between the antagonists and HWD treatments in reducing brown rot. The mean lesion diameters on fruits treated with the

combination were lower than on fruits treated with the antagonist or HWD alone.Only the diameters of lesions in the treatments with *A. pullulans* PL5 or *M. fructicola* AP47 plus HWD and those of the tebuconazole treatments were significantly lower than in the untreated control (untreated peaches). No significant difference of lesion diameter was observed between the other treatments and the control treatments.

3.4 Effects of antagonists in combination with HWD on fruit quality

No significant effect of the three antagonists and HWD was observed on fruit firmness, total soluble solids, ascorbic acid or titratable acidity (Table 4), after 21 days of storage at 1°C and 95% RH, compared with the fruit quality of control peaches.

4 Discussion

When used alone, microbial antagonists usually do not perform as well as fungicides in controlling postharvest fruit pathogens. However, use of antagonists integrated with hot water treatment has emerged as a viable alternative to the application of synthetic fungicides (KARABULUT *et al*., 2002; LARRIGAUDIÈRE *et al*., 2002; SPADARO *et al*., 2004; ZHANG *et al*., 2007). Our research shows that the antagonistic yeasts *A. pullulans* PL5, *M. fructicola* AP47 and *P. fusiformata* AP6 combined with HWD can control brown rot on peaches in semi-commercial conditions.

Several studies have shown that hot water treatments have the potential to control postharvest diseases of peaches (MARGOSAN *et al*., 1997; FALLIK *et al*., 2000) with increased efficacy against postharvest fruit pathogens when hot water and yeast antagonist treatments are combined (KARABULUT *et al*., 2002). The results of the present study show that combining hot water dipping and yeast antagonists (*A. pullulans* PL5, *M. fructicola* AP47 or *P. fusiformata* AP6) gives improved control of postharvest decay in peaches.

FALLIK *et al*. (2000) and SMILANICK *et al*. (2003) reported that hot water treatment could significantly reduce the decay development in fresh harvested produce due to reduction of the total microbial colony forming units of the epiphytic microorganism population. In our

investigation, all the hot water treatments at 50°C for 20-60 s and at 55-60°C for 10-60 s significantly inhibited *M. laxa* spore germination and germ tube elongation. HWD at 50°C for longer than 40 s or above 55°C for more than 10 s completely suppressed *M. laxa* spore germination, *in vitro*, suggesting that increasing both the temperature and incubation time of HWD enhanced the efficacy. However, when the temperature was above 55°C for more than 50 s, the fruit pericarp was damaged. This shows that high temperatures not only inhibit the spores germination on the fruit surface but also damage the epidermis tissues of the fruits. *In vivo* tests indicated that brown rot of the peaches was significantly inhibited or prevented by HWD at 55-60°C for more than 10 s. Therefore, in our research the optimal hot water treatment was a dip at 55°C for 50 s.

SCHIRRA and D' HALLEWIN (1997) showed that HWD caused redistribution of the epicuticular wax layer and significant reduction in cuticular cracks which improved physical barriers to pathogen penetration in citrus fruit. FALLIK *et al*. (1999) showed that hot water treatment could lower respiration rate and ethylene production, inhibiting ripening in sweet pepper. However, how to best use HWD to reduce decay development of fruit is not yet fully clear, and needs further investigation.

An ideal antagonist should not impair fruit quality (ZHANG *et al*., 2008). Our results show that indeed none of the three antagonists in combination with HWD impaired fruit quality. Thus the three yeasts in combination with HWD could be a good alternative to fungicide in controlling postharvest brown rot of peaches. Our trials were carried out under semi-commercial conditions, thus reinforcing this conclusion.

Antagonistic activity can be expressed in a number of ways, the most common being production of metabolites, competition for nutrients and direct parasitism, but other mechanisms can also be involved, for example induced resistance, sometimes associated with reduction of pathogen enzyme activity (SPADARO *et al*., 2004; DROBY *et al*., 2009). Our previous research has shown that the efficacy of *A. pullulans* PL5 and *P. fusiformata* AP6 in controlling *M. laxa* depends on the concentrations applied, implying that nutrition competition may play an important role in control. However, the efficacy of *M. fructicola* AP47 against the pathogen was not proportional to the concentrations applied, and in addition, neither inactivated cells nor antagonist culture filtrate affected pathogen spore

germination or germ tube elongation, suggesting that production of antifungal metabolites was not involved in the modes of action of the three antagonists against *M. laxa* (ZHANG *et al.*, 2010). Better understanding of the modes of action is essential for developing appropriate commercial formulation and application methods to maximize the potential of biocontrol agents (SPADARO and GULLINO, 2010). In future research, more importance will be attached to elucidating mechanisms by which the three antagonists suppress brown rot on peaches.

The severity of brown rot on postharvest peaches is dependent on a series of factors including peach cultivars, degree of ripeness of postharvest fruits, and preharvest environmental conditions (KARABULUT *et al.*, 2002). Some peach cultivars, such as cv. "Elberta" are resistant to brown rot, while others such as cv. "Southhaven" and cv. "Summercrest" are highly susceptible. In this research, control of brown rot by HWD in combination with the three antagonists was tested only on peaches cv "Springcrest" which is an important early cultivar susceptible to brown rot. In the future, the varieties monitored should be chosen with different ripening periods, to cover all the harvesting season. Particular attention should be posed in choosing early and late varieties. Experiments should also be repeated on fruits harvested from different orchards and at different harvesting times. Peaches and nectarines are generally harvested twice in Italy: the time between first and second harvest was 2-6 days for peaches and 4–7 days for nectarines. Despite the few days between the two harvests, significant differences can be noticed in the postharvest rots because the fruit of the second harvest could be more susceptible to diseases.

In conclusion, an integrated approach that includes biological control and hot water treatment has proved to be a good strategy for controlling postharvest diseases of peaches in storage. Hot water treatment is simple and can be easily used in packing houses, so in controlling postharvest brown rot of peaches, the use of yeast antagonists and hot water treatment have the great potential to be adopted on a commercial scale.

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Table 1: Effects of HWD at different temperatures and for different incubation times on spore germination and germ tube elongation of *M. laxa* (10^5 conidia mL^{-1}) in PDB.*

HWD treatment	Spore germination (%)	Germ tube length (µm)
45°C for 10 s	98.0 ± 1.00 d	261.8 ± 9.00 d
45°C for 20 s	96.3 ± 1.53 d	261.3 ± 13.61 d
45°C for 30 s	96.3 ± 2.08 d	249.0 ± 21.6 d
45°C for 40 s	95.3 ± 0.58 d	246.4 ± 6.70 d
45°C for 50 s	95.3 ± 1.15 d	246.0 ± 20.17 d
45°C for 60 s	95.3 ± 2.52 d	245.3 ± 14.62 d
50°C for 10 s	93.7 ± 1.53 d	121.7 ± 11.55 c
50°C for 20 s	74.3 ± 6.03 c	101.1 ± 12.69 c
50°C for 30 s	35.7 ± 4.04 b	72.0 ± 13.9 b
50°C for 40-60 s	0.0 ± 0.00 a	0.0 ± 0.00 a
55 or 60°C for 10-60 s	0.0 ± 0.00 a	0.0 ± 0.00 a
M. laxa control	98.3 ± 1.53 d	291.4 ± 13.67 e

* The results are the mean of two independent experiments. "±" stands for standard error of the mean. Values of each column followed by different letters show significant difference ($P<0.05$) according to analysis by Duncan's Multiple Test.

Table 2: Effect of HWD at different temperatures for different incubation times on the control of brown decay of on peaches cv. 'Springcrest' artificially wound-inoculated (3 mm×3 mm wounds; *M. laxa* at10^5 conidia mL^{-1}) and stored at 20°C and 95 % RH for 6 days.*

HWD treatment	Diameter of brown decay (mm)	Decay incidence (%)	Damage to pericarp[a] (+/-)
50°C for 10 s	41.5±1.1 l	100.0±0.0 e	-
50°C for 20 s	40.1±1.0 i	100.0±0.0 e	-
50°C for 30 s	39.3±1.3 k	99.5±0.89 de	-
50°C for 40 s	38.9±0.9 k	99.5±0.89 de	-
50°C for 50 s	38.4±1.4 jk	99.0±0.89 de	-
50°C for 60 s	37.9±1.3 ij	98.5±1.54 cde	-
55°C for10 s	38.5±0.6 jk	100.0±0.0 e	-
55°C for 20 s	37.8±1.5 hij	99.0±0.89 cde	-
55°C for 30 s	37.1±1.4 ghi	98.5±1.54 cde	-
55°C for 40 s	36.5±1.5 fg	97.9±0.89 bcd	-
55°C for 50 s	35.5±1.1 e	97.9±0.89 bcd	-
55°C for 60 s	35.3±1.2 de	97.3±1.66 bc	+
60°C for 10 s	37.0±1.0 gh	98.5±0.89 cde	+
60°C for 20 s	35.9±0.9 ef	97.9±1.54 bcd	+
60°C for 30 s	34.6±0.8 cd	97.4±0.89 bcd	+
60°C for 40 s	33.8±1.0 c	96.9±1.54 bc	+
60°C for 50 s	32.9±1.0 b	95.9±0.89 ab	+
60°C for 60 s	31.3±1.4 a	94.4±1.78 a	+
HWD control[b]	42.3±0.6 n	100.0±0.0 e	--
Water control[b]	42.5±1.0 n	100.0±0.0 e	--

[a] "+" pericarp damaged and "-" pericarp undamaged.

[b] "HWD control" means the fruits wound-inoculated with the pathogen were just immersed in a water bath at 20°C for 60, while "water control" means the fruits wound-inoculated with the pathogen did receive any water treatments.

* The results are the mean of two independent experiments. "±" stands for standard error of the mean. Values of each column followed by different letters show significant difference (P<0.05) according to analysis by Duncan's Multiple Test.

Table 3: Biocontrol efficacy parameters of three antagonists applied at 10^8 cells mL^{-1} and HWD at 55°C for 50 s against postharvest brown rot of peaches cv. 'Springcrest'. The disease was rated after storage for 21days at 1°C and 95% RH.*

Treatment	Disease incidence (%)	Limpel's value *E*e** *E*e = *X* + *Y*−(*XY*/100)	Lesion diameter (mm)
P. fusiformata AP6	28.3±3.8 b	-	42.9±9.4 bc
P. fusiformata AP6+HWD	16.7±1.4 a	84.6	36.8±5.9 abc
M. fructicola AP47	30.0±5.0 b	-	41.3±2.4 abc
M. fructicola AP47 +HWD	15.8±1.4 a	83.7	33.1±5.9 ab
A. pullulans PL5	25.8±2.9 b	-	40.1±5.7 abc
A. pullulans PL5 +HWD	17.5±4.3 a	85.9	34.8±2.5 ab
HWD at 55°C for 50 s	30.0±5.0 b	-	40.7±5.1 abc
Tebuconazole [a]	16.7±2.9 a	-	31.0±3.4 a
Untreated control	74.2±3.8 c	-	46.7±7.4 c

[a] Peaches were dipped in a suspension containing 250 mL/100 L of Folicur (Bayer Crop Science; tebuconazole: 25.0 %).

* Results are the means of two independent experiments. "±" stands for standard error of the mean. Values of each column followed by different letters show significant difference (P<0.05) according to analysis by Duncan's Multiple Test.

** Limpel's formula was used to determine synergistic interactions between each antagonist and HWD treatments. Limpel's formula is $Ee = X + Y-(XY/100)$, where Ee is the expected effect from additive responses of two treatments, and X and Y represent the percentages of reduction of disease incidence obtained by each antagonist and HWD, respectively, when each agent was used alone.

462 Table 4: Effect of three antagonists applied at 10^8 cells mL^{-1} and HWD at 55°C for 50 s on
463 postharvest quality of peaches cv. 'Springcrest'. After the treatments the peaches were stored
464 at 1°C and 95% RH for 21 days. Ten fruits from each treatment were randomly selected for
465 quality assessment.*
466

Treatment	Firmness (Kg)	TSS (%)	Ascorbic acid (mg/100g)	Titratable acidity (% malic acid)
P. fusiformata AP6	1.9±0.15a	10.6±1.22a	2.4±0.17ab	0.42±0.018ab
P. fusiformata AP6+HWD	2.3±0.22ab	10.9±0.16a	2.4±0.10ab	0.41±0.012ab
M. fructicola AP47	2.1±0.26ab	10.9±0.06a	2.6±0.22b	0.40±0.042a
M. fructicola AP47+HWD	2.5±0.62ab	10.6±0.97a	2.4±0.10ab	0.40±0.025a
A. pullulans PL5	2.4±0.17ab	10.4±0.33a	2.5±0.22ab	0.44±0.032b
A. pullulans PL5+HWD	2.6±0.26b	10.5±0.58a	2.5±0.14ab	0.42±0.022ab
HWD	2.3±0.43ab	10.6±0.81a	2.2±0.14a	0.39±0.010a
Tebuconazole [a]	2.2±0.18ab	10.8±0.39a	2.4±0.10ab	0.40±0.023a
Untreated control	2.3±0.34ab	10.3±0.28a	2.5±0.14ab	0.43±0.037ab

467

468 [a] Peaches were dipped in a suspension containing 250 mL/100 L of Folicur (Bayer Crop
469 Science; tebuconazole: 25.0 %).
470 * The results are the means of two independent experiments. "±" stands for standard error of
471 the mean. Values of each column followed by different letters show significant difference
472 (P<0.05) according to analysis by Duncan's Multiple Test.