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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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18	Yeasts and hot water to control brown rot of peaches		
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20	Efficacy of yeast antagonists used individually or in combination with hot		
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23	D. Zhang ¹ , J. G. Lopez-Reyes ¹ , D. Spadaro ^{1,2} * A. Garibaldi ¹ & M. L. Gullino ¹		
24			
25	¹ Centre of Competence for the Innovation in the Agro-environmental Sector, Università degli		
26	Studi di Torino, Grugliasco (TO), Italy;		
27	² DiVaPRA-Plant Pathology, Università degli Studi di Torino, Grugliasco (TO), Italy.		
28			
29	*Corresponding author, Tel.: +39-011-6708942; fax: +39-011-6709307; E-mail:		
30	davide.spadaro@unito.it		

- 31
- 32 Abstract
- 33

The efficacy of the antagonistic yeasts Pseudozyma fusiformata AP6, Metschnikowia 34 fructicola AP47 and Aureobasidium pullulans PL5 were investigated, in combination with hot 35 water dipping (HWD), to control brown rot of peaches caused by Monilinia laxa. Their 36 effects on postharvest fruit quality were also evaluated under semi-commercial conditions. In 37 38 potato dextrose broth, HWD at 50°C for more than 40 s or at 55-60°C for more than 10 s 39 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 40 maintained fruit quality. This treatment would be optimal for commercial application. The 41 harvested fruits were dipped in HW at 55°C for 50 s, cooled to 1°C for 10 min, dipped in the 42 selected yeast cell suspension (10⁸ cells ml⁻¹) for 60 s, and then stored at 1°C and 95% 43 relative humidity (RH). After 21 days, the disease incidence was measured and the 44 postharvest quality parameters of the fruits, including firmness, total soluble solids, ascorbic 45 acid and titratable acidity, were measured. When used alone, the antagonists AP6, AP47 and 46 47 PL5 reduced brown rot incidence on the peaches to 28.3%, 30.0% and 25.8%, respectively,

48 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, 49 while when HWD was used alone, disease incidence was 30.0%, suggesting that HWD 50 greatly increased the effectiveness of the antagonists. In combination with HWD, the 51 52 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 53 54 (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 55 exhibited the potential for commercial use, as an effective alternative to fungicides in 56 controlling postharvest brown rot of peaches. This appears to be the first report on combined 57 58 application of yeast strains M. fructicola or P. fusiformata with HWD to control M. laxa on peaches. 59

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

63

64 **1 Introduction**

65

Brown rot caused by Monilinia spp. is among the most important postharvest diseases of 66 commercially grown stone fruits. In the European Mediterranean areas brown rot of peaches 67 is caused by three fungi, Monilinia laxa, Monilinia fructigena and Monilinia fructicola 68 69 (PELLEGRINO et al., 2009). The most common species isolated from brown rot-infected 70 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 71 after harvest, but it is considered as a post-harvest disease because the symptoms develop in 72 73 ripe fruit. From latent infections, fruit brown rot develops prior to harvest, during storage and 74 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 78 harmful microorganisms (SPADARO and GULLINO, 2004). Moreover, many fungal strains are 79 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 80 81 developed. Microbial biocontrol agents have shown great potential as alternatives to synthetic 82 fungicides for control of postharvest decay of fruit and vegetables (DROBY et al., 2009). A 83 variety of microbial antagonists have been reported to control different pathogens on various 84 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 85 Botrytis cinerea and Rhizopus stolonifer on strawberries, and B. cinerea on sweet cherries 86 (IPPOLITO et al., 2000). Different species of Metschnikowia are active against Botrytis cinerea 87 and Penicillium expansum on some fruits (KURTZMAN and DROBY 2001; SPADARO et al., 88 2002). Several strains of *Pseudozyma fusiformata* have also shown antifungal activity 89 90 (KULAKOVSKAYA et al., 2003). In our previous studies, A. pullulans PL5, M. fructicola AP47 91 and P. fusiformata AP6 showed strong activity against Monilinia spp. on plums and peaches 92 (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, 93 94 2002; DROBY et al., 2009). Therefore, it is necessary to combine antagonistic yeasts with 95 other non-chemical or low-risk chemical methods to control postharvest diseases of fruits (LARRIGAUDIÈRE et al., 2002). 96

97 Hot water dipping can control postharvest diseases and is already widely used in the fruit 98 industry (MALAKOU and NANOS, 2005). But when used alone, HWD does not give a 99 consistent economic level of control that would warrant acceptance as a viable alternative to 100 synthetic fungicides. Moreover, there are few studies of combining HWD and antagonists to 101 control brown rot on peaches, compared with studies on apples and pears, under 102 semi-commercial conditions, (SPADARO *et al.*, 2004).

103 The objective of this research was to investigate, under semi-commercial conditions, the 104 effect of the three antagonistic yeasts *Pseudozyma fusiformata* AP6, *Metschnikowia* 105 *fructicola* AP47 and *Aureobasidium pullulans* PL5, used alone or in combination with hot 106 water, on postharvest brown rot of peaches and to evaluate the impacts of these treatments on 107 fruit quality. 108

109 2 Materials and methods

110

- 111 2.1 Microorganisms and culture conditions
- 112

Five strains of Monilinia laxa (Aderhold & Ruhland) Honey were isolated from rotted 113 peaches and selected for their virulence by inoculation in artificially wounded peaches. They 114 were used as a mixture (each strain accounted for 1/5 of the total final concentration) 115 throughout this work, to ensure a high level of disease. Each strain was stored in slants on 116 Potato Dextrose Agar (39 g L⁻¹; PDA; Merck, Darmstadt, Germany) with 50 mg L⁻¹ of 117 streptomycin (Merck) at 4°C. Spore suspensions were prepared by growing the isolates on 118 Petri dishes at 25°C for 7 days on Peach Agar [PA; 500 ml L^{-1} peach juice + 20 g L^{-1} agar 119 (Merck); pH 7.0] medium. M. laxa spores were collected and suspended in sterile Ringer 120 solution (pH 6.9±0.1; Merck). After filtering through 8 layers of sterile cheese-cloth, spores 121 were quantified with a Bürker chamber and brought to a final concentration of 10^5 spores/mL. 122 Three yeast strains (ZHANG et al., 2010), A. pullulans PL5 (isolated from the carposphere 123 of an "Angeleno" plum), P. fusiformata AP6 and M. fructicola AP47 (both isolated from the 124 carposphere of "Golden delicious" apples), were grown in 300 ml of YPD (20g l⁻¹ 125 dextro-glucose, 20g l⁻¹ peptone casein, 10g l⁻¹ yeast extract; Merck) at 25°C for 48 h on a 126 rotary shaker (250 rpm). Yeast cells were harvested by centrifugation at 5000×g for 10 min 127 and then re-suspended in sterile ringer solution (Merck). The final concentration of yeast cells 128 was determined using a Bürker chamber as required. 129

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

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134 2.2 Effect of HWD on spore germination of M. laxa in vitro

135

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 138 Melsungen, Germany) at 45, 50, 55 and 60 °C for 30 min. Spore suspensions of M. laxa (100 139 ul aliquots of 5×10^5 conidia ml⁻¹) were then added to the tubes to reach a final concentration 140 of 10⁴ conidia ml⁻¹. After 10, 20, 30, 40, 50 and 60 s of incubation, the tubes were placed at 141 0°C. Tubes held at 20°C for 30 min served as controls. All tubes were then incubated at 25°C 142 on a rotary shaker at 120 rpm for 20 h. One hundred spores were randomly selected from 143 each tube and the percentage spore germination and germ tube length were measured 144 microscopically. Each treatment had three replicates and the experiment was repeated twice. 145

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147 2.3 Effect of HWD on brown rot decay development in wound-inoculated fruits

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Effects of HWD on brown rot decay development were investigated according to Zhang et 149 al. (2007) with some modifications. Peaches were surface-sterilized with 1% commercial 150 sodium hypochlorite for 1 min, and then rinsed with tap water. Three artificial wounds (about 151 3mm wide× 3mm deep) were made along the equatorial zone of the peach. Aliquots (30 µl) 152 of *M*. laxa suspension at 1×10^5 conidia ml⁻¹ were pipetted into each wound. After 2 h of air 153 drying at 25°C, they were divided into three groups. Some fruits were immersed in a water 154 bath at 50, 55 or 60°C for 10, 20, 30, 40, 50 or 60 s, respectively. After HWD treatment, the 155 fruits were placed in a chamber at 0°C and dried for 10 min with forced air. A second group 156 of fruits, inoculated with the pathogen, was immersed in a water bath at 20°C for 60 s and 157 158 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 159 and 95% RH. After 6 days, the percentage of infected wounds was recorded and the diameter 160 of lesions was measured. There were three replicates of fifteen fruits for each treatment, and 161 162 the experiment was repeated twice.

163

164 2.4 Control of brown rot of peaches by three antagonists and HWD under semi-commercial
 165 conditions

166

167 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 (5×10⁹ cells 168 ml⁻¹) to 30 L tap water. Thirty litres of tebuconazole suspension containing 250 mL/100 L of 169 Folicur (Bayer Crop Science; 25.0 % a. i.) was prepared according to the manufacturer's 170 instruction. For the treatments of yeasts in combination with HWD, the peaches were first 171 dipped in hot water at 55°C for 50 seconds and then cooled immediately to 0°C for 10 min. 172 They were then dipped in individual antagonist suspensions for 1 min. The fruits were 173 air-dried at 25°C for 2 h and then stored at 1°C and 95% RH. Fruits treated with tebuconazole 174 served as chemical control while untreated peaches served as untreated control. There were 175 three replicates of 25 fruits for each treatment. After 21 days of storage, the number of rotten 176 peaches was recorded and the diameters of the lesions measured. The experiment was 177 repeated twice. 178

179

180 2.5 Effects of the yeasts and HWD treatment on fruit quality

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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).

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201 2.6 Statistic analysis

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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.

214

215 **3 Results**

216

217 3.1 Effects of HWD on M. laxa spore germination in vitro

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219 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 220 up, longer incubation times, up to 48 h, were assessed, but the spore germination rate was not 221 changing significantly, while the germ tubes became too much long to be measured under 222 microscope conditions. Hot water treatments at 50°C for at least 20 s and at 55 °C for at least 223 10 s could significantly inhibit spore germination and germ tube elongation. With treatments 224 at 50°C for more than 40 s or above 50°C for more than 10 s, spore germination was 225 completely inhibited. With HWD at 45°C for 10-60 s, spore germination rates (98.3%) were 226 227 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.

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234 3.2 Effects of HWD and antagonists on brown rot development of wound-inoculated fruits

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236 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 237 pericarp became bronzed (Table 2). In vivo test indicated that brown rot induced by wounding 238 and inoculation with M. laxa was significantly inhibited or prevented by HWD at 55°C for 239 240 more than 30 s or at 60 °C for more than 10 s. Moreover, as for incidence of rotten lesions on 241 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 242 investigation the optimal hot water dipping treatment was determined to be 55°C for 50 s. 243

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245 3.3 Control of brown rot by three antagonists and HWD under semi-commercial conditions

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Applications of the three antagonistic yeasts (AP6, AP47 and PL5) and HWD to control 247 248 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%, 249 30.0%, 25.8% and 74.2%, respectively. However, when combined with HWD, the three 250 yeasts performed better and the disease incidences were greatly decreased as seen in Table 3. 251 The disease incidence after HWD alone was lower than that of the untreated control but 252 higher than the disease incidence following tebuconazole treatment. When combined with 253 HWD, the biocontrol efficacy of AP6, AP47 and PL5 was correspondingly increased by 254 25.4%, 32.0%, and 17.2%, respectively, reaching the level of tebuconazole. 255

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.

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264 3.4 Effects of antagonists in combination with HWD on fruit quality

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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.

269

270 4 Discussion

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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

288 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 289 for longer than 40 s or above 55°C for more than 10 s completely suppressed M. laxa spore 290 291 germination, in vitro, suggesting that increasing both the temperature and incubation time of 292 HWD enhanced the efficacy. However, when the temperature was above 55°C for more than 293 50 s, the fruit pericarp was damaged. This shows that high temperatures not only inhibit the 294 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 295 HWD at 55-60°C for more than 10 s. Therefore, in our research the optimal hot water 296 treatment was a dip at 55°C for 50 s. 297

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.

309 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 310 mechanisms can also be involved, for example induced resistance, sometimes associated with 311 reduction of pathogen enzyme activity (SPADARO et al., 2004; DROBY et al., 2009). Our 312 previous research has shown that the efficacy of A. pullulans PL5 and P. fusiformata AP6 in 313 controlling *M. laxa* depends on the concentrations applied, implying that nutrition 314 competition may play an important role in control. However, the efficacy of M. fructicola 315 AP47 against the pathogen was not proportional to the concentrations applied, and in 316 317 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.

324 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 325 conditions (KARABULUT et al., 2002). Some peach cultivars, such as cv. "Elberta" are 326 resistant to brown rot, while others such as cv. "Southhaven" and cv. "Summercrest" are 327 highly susceptible. In this research, control of brown rot by HWD in combination with the 328 three antagonists was tested only on peaches cv "Springcrest" which is an important early 329 330 cultivar susceptible to brown rot. In the future, the varieties monitored should be chosen with 331 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 332 harvested from different orchards and at different harvesting times. Peaches and nectarines 333 334 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, 335 significant differences can be noticed in the postharvest rots because the fruit of the second 336 harvest could be more susceptible to diseases. 337

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.

343

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- 427 combination with hot water dips on postharvest Rhizopus rot of strawberries. J. Food Eng.
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429 Table 1: Effects of HWD at different temperatures and for different incubation times on spore 430 germination and germ tube elongation of *M. laxa* (10^5 conidia mL⁻¹) in PDB.*

431

HWD treatment	Spore germination (%)	Germ tube length (μ m)
45°C for 10 s	$98.0 \pm 1.00 \text{ d}$	$261.8 \pm 9.00 \text{ d}$
45°C for 20 s	$96.3 \pm 1.53 \text{ d}$	$261.3 \pm 13.61 \text{ d}$
45°C for 30 s	$96.3 \pm 2.08 \text{ d}$	$249.0 \pm 21.6 \text{ d}$
45°C for 40 s	$95.3 \pm 0.58 \text{ d}$	$246.4 \pm 6.70 \text{ d}$
45°C for 50 s	95.3 ± 1.15 d	$246.0 \pm 20.17 \text{ d}$
45°C for 60 s	$95.3 \pm 2.52 \text{ d}$	$245.3 \pm 14.62 \text{ d}$
50°C for 10 s	93.7 ± 1.53 d	121.7 ± 11.55 c
50°C for 20 s	$74.3 \pm 6.03 \text{ c}$	$101.1 \pm 12.69 \text{ c}$
50°C for 30 s	$35.7\pm4.04~b$	$72.0 \pm 13.9 \text{ b}$
50°C for 40-60 s	$0.0 \pm 0.00 \ a$	0.0 ± 0.00 a
55 or 60°C for 10-60 s	$0.0 \pm 0.00 \text{ a}$	0.0 ± 0.00 a
M. laxa control	98.3 ± 1.53 d	291.4 ± 13.67 e

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* The results are the mean of two independent experiments. " \pm " 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* at10⁵ conidia mL⁻¹) and stored at 20°C and 95 % RH for 6 days.*

439

HWD treatment	Diameter of brown decay	Decay incidence	Damage to pericarp ^a
	(mm)	(%)	(+/-)
50°C for 10 s	41.5±1.11	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	

440 ^a "+" pericarp damaged and "-" pericarp undamaged.

* The results are the mean of two independent experiments. " \pm " 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.

^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.

447 Table 3: Biocontrol efficacy parameters of three antagonists applied at 10^8 cells mL⁻¹ and 448 HWD at 55°C for 50 s against postharvest brown rot of peaches cv. 'Springcrest'. The disease

- 449 was rated after storage for 21 days at 1°C and 95% RH.*
- 450

Treatment	Disease incidence	Limpel's value <i>Ee</i> **	Lesion diameter
	(%)	Ee = X + Y - (XY/100)	(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

451

^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. " \pm " 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	Titratable acidity
			(mg/100g)	(% 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

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

* The results are the means of two independent experiments. " \pm " 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.