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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**

19

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

22

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31

32 **Abstract**

33

34 The efficacy of the antagonistic yeasts *Pseudozyma fusiformata* AP6, *Metschnikowia*
35 *fruticola* AP47 and *Aureobasidium pullulans* PL5 were investigated, in combination with hot
36 water dipping (HWD), to control brown rot of peaches caused by *Monilinia laxa*. Their
37 effects on postharvest fruit quality were also evaluated under semi-commercial conditions. In
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
40 inoculated by wounding, HWD at 55°C for 50 s significantly reduced brown rot decay and
41 maintained fruit quality. This treatment would be optimal for commercial application. The
42 harvested fruits were dipped in HW at 55°C for 50 s, cooled to 1°C for 10 min, dipped in the
43 selected yeast cell suspension (10^8 cells ml⁻¹) for 60 s, and then stored at 1°C and 95%
44 relative humidity (RH). After 21 days, the disease incidence was measured and the
45 postharvest quality parameters of the fruits, including firmness, total soluble solids, ascorbic
46 acid and titratable acidity, were measured. When used alone, the antagonists AP6, AP47 and
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,
49 AP6, AP47 and PL5 reduced brown rot incidences to 16.7%, 15.8% and 17.5%, respectively,
50 while when HWD was used alone, disease incidence was 30.0%, suggesting that HWD
51 greatly increased the effectiveness of the antagonists. In combination with HWD, the
52 efficacies of AP6, AP47 and PL5 significantly increased from 61.8%, 59.6% and 65.2% to
53 77.5%, 78.7% and 76.4%, respectively, and they were similar to the efficacy of tebuconazole
54 (77.5 %). Analysis of postharvest quality indicated that none of the treatments damaged fruit
55 quality. Our results showed that the three antagonists combined with HWD at 55°C for 50 s
56 exhibited the potential for commercial use, as an effective alternative to fungicides in
57 controlling postharvest brown rot of peaches. This appears to be the first report on combined
58 application of yeast strains *M. fructicola* or *P. fusiformata* with HWD to control *M. laxa* on
59 peaches.

60

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

63

64 **1 Introduction**

65

66 Brown rot caused by *Monilinia* spp. is among the most important postharvest diseases of
67 commercially grown stone fruits. In the European Mediterranean areas brown rot of peaches
68 is caused by three fungi, *Monilinia laxa*, *Monilinia fructigena* and *Monilinia fructicola*
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
71 severe losses during the postharvest storage. *Monilinia* spp. infection can occur before or
72 after harvest, but it is considered as a post-harvest disease because the symptoms develop in
73 ripe fruit. From latent infections, fruit brown rot develops prior to harvest, during storage and
74 transit (Hong *et al.*, 1998).

75 When permitted, use of synthetic fungicides is the best method to control the
76 post-harvest diseases on fruits (ECKERT and OGAWA, 1988). However, consumers are
77 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
80 no longer re-registered (RAGSDALE, 2000). Therefore, new alternative strategies need to be
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
85 applied to control postharvest pathogens on a number of fruits and vegetables, for example
86 *Botrytis cinerea* and *Rhizopus stolonifer* on strawberries, and *B. cinerea* on sweet cherries
87 (IPPOLITO *et al.*, 2000). Different species of *Metschnikowia* are active against *Botrytis cinerea*
88 and *Penicillium expansum* on some fruits (KURTZMAN and DROBY 2001; SPADARO *et al.*,
89 2002). Several strains of *Pseudozyma fusiformata* have also shown antifungal activity
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
93 the biocontrol yeasts are as effective as synthetic fungicides (JANISIEWICZ and KORSTEN,
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
96 (LARRIGAUDIÈRE *et al.*, 2002).

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

113 Five strains of *Monilinia laxa* (Aderhold & Ruhland) Honey were isolated from rotted
114 peaches and selected for their virulence by inoculation in artificially wounded peaches. They
115 were used as a mixture (each strain accounted for 1/5 of the total final concentration)
116 throughout this work, to ensure a high level of disease. Each strain was stored in slants on
117 Potato Dextrose Agar (39 g L⁻¹; PDA; Merck, Darmstadt, Germany) with 50 mg L⁻¹ of
118 streptomycin (Merck) at 4°C. Spore suspensions were prepared by growing the isolates on
119 Petri dishes at 25°C for 7 days on Peach Agar [PA; 500 ml L⁻¹ peach juice + 20 g L⁻¹ agar
120 (Merck); pH 7.0] medium. *M. laxa* spores were collected and suspended in sterile Ringer
121 solution (pH 6.9±0.1; Merck). After filtering through 8 layers of sterile cheese-cloth, spores
122 were quantified with a Bürker chamber and brought to a final concentration of 10⁵ spores/mL.

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

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

133

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

135

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

138 rubber-sealed 35 ml glass tubes containing 4.9 ml PDB were placed in water baths (D-3508
139 Melsungen, Germany) at 45, 50, 55 and 60 °C for 30 min. Spore suspensions of *M. laxa* (100
140 µl aliquots of 5×10^5 conidia ml⁻¹) were then added to the tubes to reach a final concentration
141 of 10^4 conidia ml⁻¹. After 10, 20, 30, 40, 50 and 60 s of incubation, the tubes were placed at
142 0°C. Tubes held at 20°C for 30 min served as controls. All tubes were then incubated at 25°C
143 on a rotary shaker at 120 rpm for 20 h. One hundred spores were randomly selected from
144 each tube and the percentage spore germination and germ tube length were measured
145 microscopically. Each treatment had three replicates and the experiment was repeated twice.

146

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

148

149 Effects of HWD on brown rot decay development were investigated according to Zhang *et*
150 *al.* (2007) with some modifications. Peaches were surface-sterilized with 1% commercial
151 sodium hypochlorite for 1 min, and then rinsed with tap water. Three artificial wounds (about
152 3mm wide× 3mm deep) were made along the equatorial zone of the peach. Aliquots (30 µl)
153 of *M. laxa* suspension at 1×10^5 conidia ml⁻¹ were pipetted into each wound. After 2 h of air
154 drying at 25°C, they were divided into three groups. Some fruits were immersed in a water
155 bath at 50, 55 or 60°C for 10, 20, 30, 40, 50 or 60 s, respectively. After HWD treatment, the
156 fruits were placed in a chamber at 0°C and dried for 10 min with forced air. A second group
157 of fruits, inoculated with the pathogen, was immersed in a water bath at 20°C for 60 s and
158 served as HWD control. A third group of peaches, inoculated with the pathogen, did not
159 receive any water treatments and served as water control. All fruits were incubated at 20°C
160 and 95% RH. After 6 days, the percentage of infected wounds was recorded and the diameter
161 of lesions was measured. There were three replicates of fifteen fruits for each treatment, and
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

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

179

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

181

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

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

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

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

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

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

200

201 2.6 Statistic analysis

202

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

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

214

215 3 Results

216

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

218

219 Under *in vitro* conditions, spore germination was measured and germ tube elongation were
220 measured after 20 h growth in PDB at 25°C on a rotary shaker. During the experiment setting
221 up, longer incubation times, up to 48 h, were assessed, but the spore germination rate was not
222 changing significantly, while the germ tubes became too much long to be measured under
223 microscope conditions. Hot water treatments at 50°C for at least 20 s and at 55 °C for at least
224 10 s could significantly inhibit spore germination and germ tube elongation. With treatments
225 at 50°C for more than 40 s or above 50°C for more than 10 s, spore germination was
226 completely inhibited. With HWD at 45°C for 10-60 s, spore germination rates (98.3%) were
227 similar to those of controls, while germ tube elongation was still significantly inhibited (Table

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

233

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

235

236 When wound-inoculated fruits were subjected to HWD at 50, 55 or 60°C for 10-60s,
237 decay was inhibited to various extents. However, at 60°C for more than 50 s, the fruit
238 pericarp became bronzed (Table 2). *In vivo* test indicated that brown rot induced by wounding
239 and inoculation with *M. laxa* was significantly inhibited or prevented by HWD at 55°C for
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
242 40 s or at 60°C for more than 20 s and the control treatments (Table 2). Therefore, in our
243 investigation the optimal hot water dipping treatment was determined to be 55°C for 50 s.

244

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

246

247 Applications of the three antagonistic yeasts (AP6, AP47 and PL5) and HWD to control
248 brown rot were evaluated after 21 days of storage at 1°C and 95% RH. When each antagonist
249 was used alone, disease incidences in the treatments and the untreated control were 28.3%,
250 30.0%, 25.8% and 74.2%, respectively. However, when combined with HWD, the three
251 yeasts performed better and the disease incidences were greatly decreased as seen in Table 3.
252 The disease incidence after HWD alone was lower than that of the untreated control but
253 higher than the disease incidence following tebuconazole treatment. When combined with
254 HWD, the biocontrol efficacy of AP6, AP47 and PL5 was correspondingly increased by
255 25.4%, 32.0%, and 17.2%, respectively, reaching the level of tebuconazole.

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

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

263

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

265

266 No significant effect of the three antagonists and HWD was observed on fruit firmness,
267 total soluble solids, ascorbic acid or titratable acidity (Table 4), after 21 days of storage at
268 1°C and 95% RH, compared with the fruit quality of control peaches.

269

270 4 Discussion

271

272 When used alone, microbial antagonists usually do not perform as well as fungicides in
273 controlling postharvest fruit pathogens. However, use of antagonists integrated with hot water
274 treatment has emerged as a viable alternative to the application of synthetic fungicides
275 (KARABULUT *et al.*, 2002; LARRIGAUDIÈRE *et al.*, 2002; SPADARO *et al.*, 2004; ZHANG *et al.*,
276 2007). Our research shows that the antagonistic yeasts *A. pullulans* PL5, *M. fructicola* AP47
277 and *P. fusiformata* AP6 combined with HWD can control brown rot on peaches in
278 semi-commercial conditions.

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

285 FALLIK *et al.* (2000) and SMILANICK *et al.* (2003) reported that hot water treatment could
286 significantly reduce the decay development in fresh harvested produce due to reduction of the
287 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
289 significantly inhibited *M. laxa* spore germination and germ tube elongation. HWD at 50°C
290 for longer than 40 s or above 55°C for more than 10 s completely suppressed *M. laxa* spore
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*
295 *vivo* tests indicated that brown rot of the peaches was significantly inhibited or prevented by
296 HWD at 55-60°C for more than 10 s. Therefore, in our research the optimal hot water
297 treatment was a dip at 55°C for 50 s.

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

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

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

318 germination or germ tube elongation, suggesting that production of antifungal metabolites
319 was not involved in the modes of action of the three antagonists against *M. laxa* (ZHANG *et*
320 *al.*, 2010). Better understanding of the modes of action is essential for developing appropriate
321 commercial formulation and application methods to maximize the potential of biocontrol
322 agents (SPADARO and GULLINO, 2010). In future research, more importance will be attached
323 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
325 peach cultivars, degree of ripeness of postharvest fruits, and preharvest environmental
326 conditions (KARABULUT *et al.*, 2002). Some peach cultivars, such as cv. “Elberta” are
327 resistant to brown rot, while others such as cv. “Southhaven” and cv. “Summercrest” are
328 highly susceptible. In this research, control of brown rot by HWD in combination with the
329 three antagonists was tested only on peaches cv “Springcrest” which is an important early
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
332 posed in choosing early and late varieties. Experiments should also be repeated on fruits
333 harvested from different orchards and at different harvesting times. Peaches and nectarines
334 are generally harvested twice in Italy: the time between first and second harvest was 2-6 days
335 for peaches and 4–7 days for nectarines. Despite the few days between the two harvests,
336 significant differences can be noticed in the postharvest rots because the fruit of the second
337 harvest could be more susceptible to diseases.

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

343

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349

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

432

433 * The results are the mean of two independent experiments. “ \pm ” stands for standard error of
 434 the mean. Values of each column followed by different letters show significant difference
 435 ($P < 0.05$) according to analysis by Duncan’s Multiple Test.

436 Table 2: Effect of HWD at different temperatures for different incubation times on the control
 437 of brown decay of on peaches cv. 'Springcrest' artificially wound-inoculated (3 mm×3 mm
 438 wounds; *M. laxa* at 10^5 conidia mL⁻¹) and stored at 20°C and 95 % RH for 6 days.*
 439

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 for 10 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.

441 ^b “HWD control” means the fruits wound-inoculated with the pathogen were just immersed in
 442 a water bath at 20°C for 60, while “water control” means the fruits wound-inoculated with the
 443 pathogen did receive any water treatments.

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

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 21days at 1°C and 95% RH.*

450

Treatment	Disease incidence (%)	Limpel's value <i>Ee</i> ** $Ee = X + Y - (XY/100)$	Lesion diameter (mm)
<i>P. fusiformata</i> AP6	28.3±3.8 b	-	42.9±9.4 bc
<i>P. fusiformata</i> AP6+HWD	16.7±1.4 a	84.6	36.8±5.9 abc
<i>M. fructicola</i> AP47	30.0±5.0 b	-	41.3±2.4 abc
<i>M. fructicola</i> AP47 +HWD	15.8±1.4 a	83.7	33.1±5.9 ab
<i>A. pullulans</i> PL5	25.8±2.9 b	-	40.1±5.7 abc
<i>A. pullulans</i> 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
 452 ^a Peaches were dipped in a suspension containing 250 mL/100 L of Folicur (Bayer Crop
 453 Science; tebuconazole: 25.0 %).

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

457 ** Limpel's formula was used to determine synergistic interactions between each antagonist
 458 and HWD treatments. Limpel's formula is $Ee = X + Y - (XY/100)$, where *Ee* is the expected
 459 effect from additive responses of two treatments, and *X* and *Y* represent the percentages of
 460 reduction of disease incidence obtained by each antagonist and HWD, respectively, when
 461 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)	Titrateable acidity (% malic acid)
<i>P. fusiformata</i> AP6	1.9±0.15a	10.6±1.22a	2.4±0.17ab	0.42±0.018ab
<i>P. fusiformata</i> AP6+HWD	2.3±0.22ab	10.9±0.16a	2.4±0.10ab	0.41±0.012ab
<i>M. fructicola</i> AP47	2.1±0.26ab	10.9±0.06a	2.6±0.22b	0.40±0.042a
<i>M. fructicola</i> AP47+HWD	2.5±0.62ab	10.6±0.97a	2.4±0.10ab	0.40±0.025a
<i>A. pullulans</i> PL5	2.4±0.17ab	10.4±0.33a	2.5±0.22ab	0.44±0.032b
<i>A. pullulans</i> 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.