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Infection risk of *Monilinia fructicola* on stone fruit during cold storage and immersion in the dump tank

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Abstract

Monilinia spp. is the main pathogen responsible for postharvest losses of stone fruit. Several studies have examined the conditions for *Monilinia* spp. infection in the field, but very limited information is available about postharvest. Storing fruit for 24 hours in cold room and water dump fruit in a water tank are the most common handling operations during the postharvest of fruit. Then, the aim of this study was to investigate the risk of *Monilinia fructicola* infection for peaches and nectarines during cold storage and water dump operations. The storage of fruit with the presence of *M. fructicola* conidia on their surface for up to 30 days at 0 or 4 ºC and 98% Relative Humidity (RH), did not suppose an important risk of infection since only 3.3% of fruit were already infected. *M. fructicola* was not able to infect fruit at 20 ºC when the RH was around 60%, however, it was possible to develop disease if fruit was already infected before the treatment applications. Conidia of *M. fructicola* present on the surfaces of nectarines was not able to infect fruit stored at 0 ºC and 100% RH for 24 hours and then immersed in the water dump tank, nevertheless it was able to infect 26.3% of peaches in the same conditions. When fruit was immersed in the dump tank with water containing the presence of viable conidia of *M. fructicola*, and then fruit was incubated at 20 ºC and 60 or 100% for 7 days, the infection recorded was between 66.7 and 90%, respectively. In addition, water dump operation free from *M. fructicola* conidia favours optimal conditions to develop infections produced on fruit before the treatment applications. Therefore, postharvest water dump would provide optimal conditions to infect inoculated and non-inoculated fruit, increasing the need for water disinfection.

Keywords; Brown rot, *Monilinia* spp., postharvest, cold storing, water dumping, peaches, nectarines

1. Introduction

The main pathogen responsible for stone fruit losses are *Monilinia fructicola* and *Monilinia laxa* both present in Europe and worldwide. However, in Spain, *M. fructicola* was not detected until 2009 (De Cal *et al.*, 2009) and it was included in the list of EU quarantine agencies until the end of 2014. Since its detection, *M. fructicola* has replaced *M. fructigena* and now *M. laxa* and *M. fructicola* coexist at the same frequency of occurrence (Villarino *et al.*, 2013).

In the field, brown rot incidence increases as harvest time approaches and similarly fruit is more susceptible to infections (Gell *et al.*, 2008; Villarino *et al.*, 2011). When climatic conditions are favorable for disease development, brown rot losses in postharvest may be more severe than preharvest, which can be as high as 80% (Usall *et al.*, 2015). During the postharvest period, brown rot routinely occurs during handling, storage and transport (Tian and Bertolini, 1999).

Favorable conditions for disease development refers to temperature and humidity factors that are considered to be the most important abiotic factors affecting germination (Casals *et al.*, 2010), infection (Biggs and Northover, 1988; Xu and Robinson, 2000) and the period of incubation and latency of the pathogen (Luo *et al.*, 2001). On the other hand, there are other factors to be considered on the development of brown rot disease such as maturity degree (Emery *et al.*, 2000; Lee and Bostock, 2006) or susceptibility of fruit to be infected by *Monilinia* spp. (Xu *et al.*, 2007).

Usually, fruit reaching packing houses is apparently healthy but they could actually be contaminated by *Monilinia* spp. conidia on their surface or conidia that have already infected fruit at the orchard but without visible symptoms. Therefore, fruit that arrives at packing houses can fit in three different scenarios: (i) really healthy fruit (without

73 conidia either on surfaces or infected), (ii) fruit with the presence of conidia on their
74 surface (an interaction between fruit-conidia has not been established) and (iii) fruit
75 already infected with *Monilinia* spp. conidia.
76 Once fruit has reached packing house, they will start an episode of several operations
77 where the objective is to maintain fruit quality and extend its shelf life. Field heat can
78 cause rapid deterioration and it is desirable to remove this heat as quickly as possible
79 after harvest (Dennis, 1984). The most common methods used to cool stone fruit in the
80 Ebro Valley area is storage in a pre-cooling room at 4 or 0 ºC because it is a simple
81 technique since it does not need large or special facilities. However, this method needs
82 around 24 hours to cool a whole load of fruit. After cooling, fruit is sorted starting with
83 the water dump operation, where water is used to avoid blows caused during fruit box
84 overturning. Then, fruit is transported from the tank to the lines with a conveyor belt
85 and rotten fruit is discarded manually (Bernat *et al.*, 2017a).
86 In addition, immersed fruit in the water dump tank with chlorine has also been used to
87 sanitize fresh products and could reduce decay by reducing the effective conidia
88 concentration (Bertrand and Saulie-Carter, 1979). During these operations, infected fruit
89 without visual symptoms can develop decay inside boxes during postharvest and
90 conidia or infected tissues could remain adhered to boxes. Therefore, healthy fruit in
91 contact with contaminated boxes could be infected by *Monilinia* spp. conidia or other
92 pathogens during postharvest handling (Tian and Bertolini, 1999) and secondary
93 inoculum could be epidemiologically important.
94 The main objective of this study was to investigate the infection risk of *Monilinia* spp.
95 on stone fruit during several postharvest operations in packing houses. Specific
96 objectives were to determinate whether *M. fructicola* is able to infect: (i) stone fruit with
97 conidia of *M. fructicola* on their surface during storage periods in cold rooms at 0 or 4

ºC, (ii) stone fruit with conidia of *M. fructicola* on its surface stored for 24 hours at 0 ºC and then immersed in the dump tank with water, and (iii) stone fruit without conidia of *M. fructicola* on its surface and immersed in water with or without conidia of *M. fructicola* during the water dump operation.

2. Material and methods

2. 1 Fruit

Fruit from peaches cultivars 'Baby Gold 6' and 'Baby Gold 9' and nectarine cultivar 'Fantasia' was harvested from organic orchards in Lleida (Catalonia). Harvest time for peaches cultivar 'Baby Gold 6' was approximately mid-August, for 'Baby Gold 9' mid-September and for nectarine cultivar 'Fantasia' early August. Healthy fruit was picked at an optimum stage of commercial maturation, and with approximately the same size. Fruit was immersed in 10% commercial chlorine for 1 min, rinsed with tap water for 3 min and, finally, air-dried for 24 hours at room temperature before the experiment. Fruit not used at the time of harvest was stored at 0 ºC for up to 5 days until use.

2. 2 Fungal isolate and inoculum preparation

The isolate of *M. fructicola* (CPMC1) used in this study come from the collection of the Postharvest Pathology Group, IRTA Centre of Lleida (Catalonia, Spain) and this strain was isolated and classified at the Department of Plant protection, INIA (Madrid, Spain). The strain was maintained in our laboratory on potato dextrose agar (PDA) medium (Biokar Diagnostic, 39 gL^{-1}) at 4 ºC in darkness for 5-7 days.

The strain CPMC1 was sub-cultured onto PDA Petri dishes and incubated in the dark at 25 ºC for approximately during 1 week. To ensure conidial production, peach and

nectarine fruit was inoculated with the isolate separately. Fruit was first wounded by a sterilized steel rod (1 mm wide and 2 mm long); then conidia and mycelia were transferred from the PDA culture onto each wound site previously carried out by a sterilized pipette tip. Fruit inoculated with *M. fructicola* was incubated at 25 ºC and 85% RH in the dark.

Conidia from infected fruit was scraped with a sterile loop and transferred to a test tube with 10 ml of sterile distilled water and one added droplet of 80% Tween per litre to break up conidia. The conidial concentration was adjusted to a desirable concentration using a haemocytometer.

2. 3 Fruit inoculation

The different scenarios of the fruit that reached packing houses was performed with two different inoculums; dry inoculum to simulate fruit with *Monilinia* spp. conidia on fruit surfaces and wet inoculum to simulate water tank contaminated with *Monilinia* spp. conidia.

2.3.1 Dry inoculum

Dry inoculum was prepared using sand from a quarry characterized as having a fine and homogeneous granulometry, sterilized in the autoclave for 20 min and dried in a stove at 100 ºC for 24 hours. Then, 10 grams of dried sand was mixed with 500 µl of a *M. fructicola* suspension concentrated to 10^7 conidia ml^{-1}. The mixture of sand and inoculum was placed in an open plastic Petri dish and was left to dry for 1 hour in a laminar hold. To check that the conidia mixture with sand was viable, a sample of sand was scattered onto Petri dishes with potato dextrose agar (PDA) medium and incubated for 48 hours at 25 ºC. Then, the number of viable conidia were recovered.

One carton washer (25.4 cm^2 of hole) was stuck on the surfaces of each piece of fruit selected for the experiment and then the fruit was inoculated with 0.10 g of the dry inoculum of *M. fructicola* and was deposited in the hole of each washer stuck. Fruit weas placed in plastic trays to run the experimental treatments described later.

2.3.2 Wet inoculum

Wet inoculum was prepared in a tank with 15 litters of water solution and a final concentration of 10^4 conidia ml^{-1} of *M. fructicola*. Then, a set of fruit previously superficially disinfected (proceedings described in 2.1) and apparently healthy without damage was immerse for 30 seconds in the water tank with *M. fructicola* conidia.

2. 4 Experimental treatments

All treatments, including the fruit controls described below were performed with four replicates (each replicate included five fruits) and the tial was performed three times; with two peach cultivars ‘Baby Gold 9’ and ‘Baby Gold 6’ and one nectarine cultivar ‘Fantasia’.

2.4.1 Effect of cold room operation at 0 or 4 ºC on the infection of inoculated fruit

To determine if *M. fructicola* conidia can infect fruit during the cold chamber storage, fruit was inoculated with dry inoculum as was described previously. Then, inoculated fruit was stored for 3, 9, 15 or 30 days at 0 or 4 ºC and high RH (98%). After each storage period, fruit was incubated for up to 14 days at 20 ºC and 60% RH (conditions where no new infections might be made) and the incidence of infected fruit on the inoculated area was recovered after 7 and 14 days of incubation.

Three sets of fruit were used as a control of the cold room treatment and that were directly incubated after dry inoculation at; (i) 20 ºC and 100% RH for up to 14 days, or (ii) 20 ºC and 60% RH for up to 14 days, or (iii) 20 ºC and 98% RH for 72 hours and then at 20 ºC and 60% RH for up to 14 days to ensure that (i) dry inoculum prepared is viable and it is able to infect healthy fruit when it is incubated at optimal conditions, (ii) dry viable inoculum prepared is not able to infect fruit when it is incubated at non-optimal humidity conditions and (iii) fruit infections produced during incubation at optimal conditions are able to develop when it is incubated at non-optimal humidity conditions

2.4.2 Effect of water dump operation on the infection of inoculated fruit

Fruit was dry inoculated with $2x10^5$ conidia $fruit^{-1}$ as was described previously and stored at 0 ºC and 98% RH for 24 hours. After storage, fruit was immersed in a tank of 15 litres of tap water at 15 ºC for 30 seconds with a slight manual shake. Then, fruit was left to dry and placed again on plastic trays. A set of fruit was incubated for 14 days at 20 ºC and 60% RH (conditions where no new infections of *M. fructicola* might be made) and another set was incubated at 20 ºC and 100% RH (optimal conditions for conidial infection) for 14 days. Finally, the incidence of superficially infected fruit on the inoculated area was recovered after 7 and 14 days.

2.4.3 Effect of water dumping operation on the infection of non-inoculated fruit

Fruit was immersed in 15 litres of tap water at 15 ºC containing *M. fructicola* at 10^4 conidia ml^{-1} for 30 seconds with a slight manual shake. Then, fruit was left to dry and placed on plastic trays. The experiment was repeated exactly as describe above but this

188 time the water used in the tank was free from *M. fructicola* conidia. Fruit was left to dry
189 and placed on plastic trays.
190 In both experiments a set of immersed fruit was incubated at 20 ºC and 60% RH
191 (conditions where no new infections might be made) and another set of immersed fruit
192 was incubated at 20 ºC and 100% RH (optimal conditions for conidia infection) for 14
193 days. The incidence of infected fruit was recovered after 7 and 14 days of incubation.

194 *2. 5 Statistical analysis*

195 The incidences of infected fruit were recovered at each assessment time described
196 before and the percentages of infected fruit were calculated. Data from the three
197 repeated experiments was used for statistical analysis in all the experiments except for
198 the water dump operation with fruit previously dry inoculated and stored for 24 hours in
199 a cold room at 0 ºC. In this case, data was separated between peaches and nectarines
200 because significant differences between cultivars were observed. All analysese were
201 done using the JMP®9 statistical software (SAS Institute, Cay, NC, USA). Non-
202 parametric test was selected because incidences of fruit infection data were discrete due
203 to the experimental design and the Kurskal-Wallis test was used to identify the
204 significance of treatments. When the analysis was statistically significant, the Tukey
205 (HSD) test was performed for separation of the means. Statistical significance was
206 judged at the level $P<0.05$.

207 **3. Results**

3.1 Effect of cold storage on the infection of inoculated fruit

In any cold storage period, fruit with infection was no higher than 3.3% after 30 days of storage at 0 (Fig. 1A) or at 4 ºC (Fig. 1B) and then incubated for 14 days at 20 ºC and 60% RH (conditions where no new infections might be made). In addition, no significant differences were found between the percentages of infections in fruit stored for 3, 9, 15 or 30 days both at 0 and 4 ºC and then for 7 and 14 days incubated at 20 ºC and 60% RH. There were also no significant differences between incidences in fruit stored at 0 or 4 ºC.

Disease incidences of the three sets of fruit used as controls are shown in Figure 2. Inoculated fruit with dry inoculum of *M. fructicola* and incubated at 20 ºC and 100% RH showed 10.8 and 71.4% of incidence after 7 and 14 days of incubation respectively, whereas fruit dry inoculated and incubated at 20 ºC and 60% RH for up to 14 days was not able to develop brown rot disease . In addition, with fruit superficially inoculated and stored for 72 hours at 20 ºC and 98% RH and then incubated for 14 days at 20 ºC and 60% RH, the incidence of infected fruit recovered was 10 and 31.7% after 7 and 14 days of incubation respectively.

3.2 Effect of water dump operation on the infection of inoculated fruit

The incidence of infected fruit was statistically higher in peaches than in nectarines superficially inoculated with dry inoculum of *M. fructicola* conidia after 24 hours at 0 ºC and 98% RH and then immersed in clean water at 15 ºC for 30 seconds (Figure 3). Nectarines were not infected by *M. fructicola* after 14 days of incubation at 20 ºC and 60% RH (restricted conditions to infect) (Fig. 3A). However, when nectarines were incubated at 20 ºC and 100% RH (optimal conditions for infection), 31.3% of the fruit was infected after 14 days of inoculation. On peaches, the incidence of infected fruit

was 26.3% after 14 days at 20 ºC and 60% RH (Fig. 3B). When peaches were incubated at 20 ºC and 100% RH, the incidence of infected fruit recovered after 7 days of incubation was 26.9% and 81.9% after 14 days.

3.3 Effect of water dump operation on the infection of non-inoculated fruit

Overall, the incidence of infected fruit was less on fruit immersed in water free from inoculum than on fruit immersed in water with *M. fructicola* conidia (Figure 4). After 7 days of incubation, the infected fruit recorded from fruit immersed in water free of inoculum and incubated at 20 ºC and 100 and 60% RH was 36.7% and 11.7%, respectively. However, when fruit was immersed in water with the presence of *M. fructicola* and then incubated at 20 ºC and 100 or 60% RH, the incidences of infected fruit were statistically higher and increased to 90% and 66.7% respectively.

After 14 days of incubation at 20 ºC, the differences between treatments were lower and only the incidence of infected fruit immersed with water free of inoculum and stored at 20 ºC and 60% RH was statistically lower (51.7% incidence of infected fruit) than the others.

4. Discussion

This is the first time to our knowledge, that the behaviour of *Monilinia* spp. in relation to its risk to infected fruit in postharvest has been studied. In this sense, this paper provides valuable information about the effect of postharvest operations such as cold storage and water dump on the risk of *M. fructicola* infecting peaches and nectarines. Our results have shown that during the storage period in cold rooms, the probability of *M. fructicola* present on fruit surfaces of infecting peaches and nectarines was

254 extremely low. In addition, the immersion of fruit in the dump tank, increased the risk
255 of *M. fructicola* infection.
256 In the present study we simulated fruit with the presence of *M. fructicola* conidia on its
257 surface. The source of this conidia could come from both the field and the packing
258 house but in a study carried out by Bernat *et al.* (2016) it is shown that the presence of
259 *Monilinia* spp. in the environment of packing houses or on their surface facilities is
260 really low. In addition, Villarino *et al.* (2012) reported that the maximum number of
261 *Monilinia* spp. airborn conidia registered in the field occurs near harvest or immediately
262 after harvest. In order to simulate fruit with non-germinated conidia on its surface, in
263 this study paper we have developed a new methodology to apply dry conidia and avoid
264 the interference of the water when conidia is applied as a wet inoculum.
265 The storage of stone fruit with the presence of *M. fructicola* on its surface coming from
266 field in cold rooms at 0 or 4 ºC and high humidity for up to 30 days would not suppose a
267 high risk of infection since only less than 4% of fruit artificially inoculated with dry
268 conidia was infected during this period in our experiment. Humidity provided during
269 cold storage is optimal for conidia germination and infection. The maximum
270 germination which correspond to 90% of *M. fructicola* conidia in PDA at 0 and 5 ºC
271 occurred at 99% of a_w (water activity) after 4 and 2 days, respectively and, at 87% of a_w,
272 no germination was registered at these temperature conditions (Casals et al., 2010).
273 Nevertheless, Garcia-Benitez *et al.* (2017) reported that less than 30% of conidia
274 germinated on culture medium containing a skin extract of mature fruit at 4 ºC and
275 100% RH. This difference in the percentage of conidia germination in both studies
276 should be due to the different substrates of germination indicating that other factors than
277 temperature and humidity are also involved in conidia germination. In addition, conidia
278 germination is only the first step to infect fruit and the infection process is more

279 complex. Maybe the interaction between temperature and humidity and other factors
280 such as fruit variety or *Monilinia* specie, are not entirely known. Infection processes at
281 low temperatures should be rather slow because Bernat *et al.* (2017b) reported more
282 than 40 and 20 days at 0 and 4 ºC, respectively to observe the first symptoms of decay
283 on stone fruit artificially infected by *M. fructicola*.
284 In our study, fruit incubated at optimal environmental conditions (20 ºC and 100% RH)
285 and at optimal fruit development resulted in all fruit being infected after few days of
286 incubation. Our results agree with Biggs and Northover (1988) who reported optimal
287 temperature for peach infection by *M. fructicola* conidia between 22.5-27.5 ºC in a
288 wetness chamber. However, fruit incubated at 20 ºC and 60% with *M. fructicola* conidia
289 on their surface was not able to be infect but if infection was already produced, brown
290 rot disease could develop in those conditions.
291 As far as we know, there are no other *Monilinia* spp. infection studies with such
292 extreme humidity tested as most studies of infections are done in field conditions. It
293 could be that environmental conditions reach such low humidity but it usually happens
294 for only a short period of time since temperature and humidity fluctuate in orchards. On
295 the other hand, studies are normally focused on knowing the shortest time with wetness
296 duration required for infection (Kreidl *et al.*, 2015; Luo et al., 2001; Luo and
297 Michailides, 2001; Xu et al., 2007). Kreidl et al. (2015) and Xu et al. (2007) concluded
298 that 3 hours of wet period may be long enough for *M. fructicola* and *M. laxa*
299 respectively to germinate and infect fruit at field temperatures. During postharvest fruit
300 storage, humidity must be well controlled and kept constant at 60% in order to avoid
301 new infections. Unfortunately, the conditions of relative humidity at which our results
302 indicated that no infections occur (60%) are not a recommended practice because fruit

303 would lose its firmness and quality reducing the shelf life, however, it could be used as
304 non-infected conditions for several experiments.
305 Under commercial postharvest conditions, fruit is usually stored in cold rooms for 24
306 hours and then immersed in a dump tank with water to avoid blows caused during fruit
307 box overturning and to clean the fruit surface, which is a common practice in packing
308 houses of many production areas. Our study has indicated that if the fruit has the
309 presence of *M. fructicola* conidia on its surface, during these operations, 26.3% of peach
310 fruit was infected, while on nectarine fruit no infection was produced. The observed
311 differences could be due to fruit skin; nectarines are smoother however, peaches are
312 fuzzier and therefore it is easier to clean nectarine surfaces than peaches. This
313 explanation agrees with (Scheper *et al.*, 2007) who reported that washing apples with
314 clean water significantly reduces the number of fungi on apple surfaces. In addition, the
315 drying period for peach surfaces is longer than for nectarines because peaches are able
316 to keep higher humidity and consequently it increased the risk of conidia infection. Dry
317 operation after water dumping would play an important step to remove humidity on fruit
318 surfaces and decrease the infection probability at packing houses due to reduce surface
319 fruit humidity.
320 Our study has also indicated that immersing fruit with non-presence of *Monilinia* spp.
321 on their surface in the water tank with clean water should not produce new infection.
322 Conditions of humidity and temperature are supposed to be optimal for infection and for
323 developing established brown rot infections. From our results we could conclude that
324 brown rot disease developed on fruit superficially disinfected and immersed in clean
325 water is due to infections produced before superficial disinfections, maybe in the field
326 during the fruit growing season or just before harvest, since *Monilinia* spp. conidia

327 produces infections on fruit but disease is not expressed until conditions become
328 favourable (Bryde and Willetts, 1977; Gell et al., 2008).
329 During water dump operations, it is likely that circulating water will become
330 contaminated due to conidia from infected fruit which are detached in water or when
331 dirty fruit bins contaminated with conidia from the field are immersed in water. Conidia
332 detached in water could adhere to healthy fruit immersed and increase decay incidence.
333 This dynamic of water contamination in packing houses has been reported previously
334 by different authors (Michailides and Spotts, 1986; Spotts and Cervantes, 1986; Sugar
335 and Spotts, 1993). This study shows that immersing healthy fruit in a water tank with
336 viable *Monilinia* spp. conidia supposes a high risk of infection for fruit after few days
337 regardless of the subsequent incubation conditions (even 60 % of humidity). This may
338 be because during water dump operations, conidia adheres to the fruit surface and
339 infection is produced during immersion water dump or during the subsequent fruit
340 drying period, in which humidity and temperature are still optimal for infection. Sugar
341 and Spotts (1993) also reported an increase of *Phialophora malorum* conidia on pear
342 surfaces after immersion in an infested water tank. In addition, recirculated used water
343 during postharvest operations need to be disinfected to prevent new infections. Water
344 disinfection with 50 mg L^{-1} of sodium hypochlorite for 3 min was effective to kill 100%
345 of *M. fructicola* conidia for the tested temperatures between 4 and 25 ºC (Bernat et al.,
346 unpublished data).
347 In conclusion, our results showed that stored fruit with *Monilinia* spp. conidia on its
348 surface in cold rooms do not suppose a risk of infection and therefore of developing
349 brown rot symptoms. However, whether fruit is stored in cold room and then immersed
350 in clean water, infection could develop either because water dump conditions are
351 optimal for fruit infection or because infections previously produced develop during

these postharvest operations since water dump operations provide optimal conditions. Therefore, our results increase the knowledge of the epidemiology of *Monilinia* spp. in postharvest helping the packing houses to improve effective methods of water dump management to avoid infection risks and minimize brown rot development in postharvest.

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References

Bernat, M., Segarra, J., Casals, C., Teixido, N., Torres, R., Usall, J., 2017a. Relevance of the main postharvest handling operations on the development of brown rot disease on stone fruits. Journal of the science of food and agriculture 97, 5319-5326.

Bernat, M., Segarra, J., Casals, C., Torres, R., Teixido, N., Usall, J., 2016. Identification of fungal population in the environment and on surfaces of stone fruit packinghouses. European Journal of Plant Pathology 148, 723-731.

Bernat, M., Segarra, J., Xu, X.M., Casals, C., Usall, J., 2017b. Influence of temperature on decay, mycelium development and sporodochia production caused by *Monilinia fructicola* and *M. laxa* on stone fruits. Food Microbiology 64, 112-118.

Bertrand, P., Saulie-Carter, J., 1979. Postharvest decay control of apples and pears after immersion dumping. Agricultural Experiment Station. Oregon State University, Corvallis.

Biggs, A.R., Northover, J., 1988. Influence of temperature and wetness duration on infection on peach and sweet cherry fruits by *Monilinia fructicola*. Phytopathology 78, 1352-1356.

Bryde, R.J.W., Willetts, H.J., 1977. The brown rot fungi of fruit: Their biology and control. Pergamon Press, Oxford.

Casals, C., Vinas, I., Torres, R., Griera, C., Usall, J., 2010. Effect of temperature and water activity on in vitro germination of *Monilinia* spp. Journal of Applied Microbiology 108, 47-54.

De Cal, A., Gell, I., Usall, J., Viñas, I., Melgarejo, P., 2009. First report of brown rot caused by *Monilinia fructicola* in peach orchards in Ebro Valley, Spain. Plant Disease 93, 763-763.

Dennis, C., 1984. Effect of storage and distribution conditions on the quality of vegetables. Acta Hort 163, 85-104.

Emery, K.M., Michailides, T.J., Scherm, H., 2000. Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. The American Phytopathological Society 84, 853-857.

Garcia-Benitez, C., Melgarejo, P., De Cal, A., 2017. Fruit maturity and post-harvest environmental conditions influence the pre-penetration stages of *Monilinia* infections in peaches. International Journal of Food Microbiology 241, 117-122.
Gell, I., De Cal, A., Torres, R., Usall, J., Melgarejo, P., 2008. Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. European Journal of Plant Pathology 121, 487-498.
Kreidl, S., Edwards, J., Villalta, O., 2015. Assessment of pathogenicity and infection requirements of *Monilinia* species causing brown rot of stone fruit in Australian orchards. Australasian Plant Pathology 44, 419-430.
Lee, M.-H., Bostock, R.M., 2006. Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. Phytopathology 96, 1072-1080.
Luo, Y., Ma, Z., Michailides, T.J., 2001. Analysis of factors affecting latent infection and sporulation of *Monilinia fructicola* on prune fruit. Plant disease 85, 999 - 1003.
Luo, Y., Michailides, T.J., 2001. Factors affecting latent infection of prune fruit by *Monilinia fructicola*. Phytopathology 91, 864-872.
Michailides, T.J., Spotts, R.A., 1986. Factors affecting dispersal of *Mucor piriformis* in pear orchards and into the packinghouse. Plant Disease 70, 1060-1063.
Scheper, R.W.A., Rogers, D.J., Walker, J.T.S., Manning, M.A., Wood, P.N., 2007. The incidence of storage rots after postharvest apple washing. New Zealand Plant Protection 60, 7-14.
Spotts, R.A., Cervantes, L.A., 1986. Population, pathogenicity and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in packinghouses. Plant Disease 70, 106-108.
Sugar, D., Spotts, R.A., 1993. Dispersal of inoculum of *Phialophora malorum* in pear orchards and inoculum redistribution in pear immersion tanks. Plant Disease 77, 47-49.
Tian, S.P., Bertolini, P., 1999. Effect of temperature during conidial formation of *Monilia laxa* on conidial size, germination and infection of stored nectarines. Phytopathology 147, 635-641.
Usall, J., Casals, C., Sisquella, M., Palou, L., De Cal, A., 2015. Alternative technologies to control postharvest diseases of stone fruits. Stewart Postharvest Review 11, 1-6.
Villarino, M., Eguen, B., Lamarca, N., Segarra, J., Usall, J., Melgarejo, P., De Cal, A., 2013. Occurrence of *Monilinia laxa* and *M. fructigena* after introduction of *M. fructicola* in peach orchards in Spain. European Journal of Plant Pathology 137, 835-845.
Villarino, M., Larena, I., Martinez, F., Melgarejo, P., De Cal, A., 2012. Analysis of genetic diversity in *Monilinia fructicola* from the Ebro Valley in Spain using ISSR and RAPD markers. European Journal of Plant Pathology 132, 511-524.
Villarino, M., Sandín-España, P., Melgarejo, P., De Cal, A., 2011. High chlorogenic and neochlorogenic acid levels in immature peaches reduce *Monilinia laxa* infection by interfering with fungal melanin biosynthesis. Journal of Agricultural and Food Chemistry 59, 3205-3213.
Xu, X., Robinson, J.D., 2000. Epidemiology of brown rot (*Monilinia fructigena*) on apples: infecion of fruits by conidia. Plant Pathology 49, 201-206.
Xu, X.M., Bertone, C., Berrie, A., 2007. Effects of wounding, fruit age and wetness duration on the development of cherry brown rot in the UK. Plant Pathology 56, 114 - 119.