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Brown rot on stone fruit: from epidemiology studies to the development of effective control strategies

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Highlights

- Brown rot is highly affected by abiotic factors, mainly related to the climatology of each production area, and consequently, the disease behaviour can slightly change.
- The risk of postharvest contamination of healthy fruit by *Monilinia* spp. conidia is generally low.
- The combined treatment based on dipping fruit at 60 ºC for 40 s plus the application of the antagonist CPA-8 (Bacillus amyloliquefaciones) at 107 cfu/mL

showed a synergistic effect to control brown rot in comparison with each treatment alone.

- Curing treatment at 50 ºC for 2 h and 95–99% RH effectively controlled brown rot caused by *M. laxa* and *M. fructicola* on peaches and nectarines.
- It is essential to integrate all the generated knowledge to design sustainable control strategies to control brown rot and obtain competitive, sustainable, healthy, and high-quality fruit.

Abstract

In the last years, our research has focused on the study of brown rot on stone fruit caused by *Monilinia* spp. in the 'Valle del Ebro' (Spain). The epidemiology of this disease was thoroughly investigated in the field and one of the main outcomes was the development of a prediction model that indicates the risk of infection. Furthermore, the epidemiology was also studied in the postharvest phase, providing a lot of information regarding the relevance of the main postharvest handling operations, the identification of fungal population in packing houses, the fruit infection risks and the influence of temperature and humidity on conidia survival.

Additionally, many efforts have been oriented to the development of control strategies for both pre and postharvest periods. Traditionally, chemical fungicides have been used to preserve fruit quality over extended periods of storage or transportation. However, the growing public concern over health and environmental hazards associated with high levels of pesticides has resulted in restrictions imposed by legislation and also by distribution companies. In this context, our main goal has been the development of environmentally friendly alternative strategies to synthetic fungicides in order to control brown rot on stone fruit. Although many studies have demonstrated the efficacy of these alternative treatments, only a few of them are currently applied under commercial conditions. Biocontrol agents, natural compounds from different origins and physical means are the main approaches which have been studied, with different success levels. There are several reasons for the limited success of these treatments, such as the inconsistency of results, variability of the efficacy under commercial conditions, low persistence, a narrow spectrum range of activity, the difficulties in developing a shelf-stable formulated product that retains efficacy (in the case of biocontrol agents), and economical and regulatory limitations. Generally, it is accepted that the combination of different strategies is necessary to improve the control of postharvest diseases and that the real solution needs to integrate different tools to achieve satisfactory disease control. The aim of this review is to describe the main efforts conducted by our research group regarding the control of brown rot on stone fruit, from epidemiology studies to the development of effective control strategies.

Keywords

Monilinia spp., epidemiology, preharvest, postharvest, alternative treatments, stone fruit

1. Introduction

Peaches and nectarines are included on the list of the most produced fruits in Europe. France, Spain, Italy, and Greece are the main producers with 2.03, 1.54, 1.2 and 0.92 million tons, respectively (FAOSTAT, 2021). These fruits are well accepted by consumers because of their high nutritional value and wonderful taste. However, they are perishable products susceptible to pathogen attack in the field, but mostly during postharvest, storage, transportation, and the market. In Spain, the main causal agents of fungal disease on stone fruit are *Monilinia*, firstly the species *M. laxa* (Aderh. & Ruhland) Honey and *M. fructigena* (Aderh. &Ruhland) Honey (De Cal and Melgarejo, 1992), and later the species *M. fructicola* (G. Wint.) Honey (De Cal et al., 2009), all of them causing agents of the disease called brown rot. Moreover, other fungal pathogens, such as *Rhizopus stolonifer* (Ehrenb.:Fr.), *Geotrichum candidum* Link and *Penicillium expansum* Link, have also been reported as causal agents of minor postharvest diseases on stone fruit (Mari et al., 2004; Yaghmour et al., 2012).

It is important to note that *Monilinia* spp. infect fruit in the field and losses can occur there, but disease symptoms mainly appear in postharvest in the packing house where losses can be as high as 80 % (Larena et al., 2005). In this context, the control of *Monilinia* spp. must be undertaken, firstly, in the orchard. Currently, the control of *Monilinia* spp. on stone fruit is based on a program of chemical fungicide applications in the field, complemented, in some countries, with fungicide applications at postharvest. This conventional fruit production is unsustainable and could be greatly improved using tools such as: 1) a warning system to detect the most efficient moment to apply fungicides (Holb, 2013), 2) cultural practices applied in the field to reduce the inoculum pressure and 3), the use of alternative strategies to chemical fungicides both in the field and at postharvest (Casals et al., 2021a; Usall et al., 2015). It will be essential in the near future to go deep into these issues to observe legislation and enforce the requirements for fruit production for consumer and distribution chains. The present review describes the most recent knowledge for improving the control of brown rot in the field and at postharvest, in an attempt to achieve more sustainable fruit production based on observing the current legislation requirements, but also those that will be imposed in the coming years.

2. Understanding the disease for sustainable control

In the framework of sustainable control strategies for diseases in general, and specifically for brown rot, estimating the possible risk of infections is essential. Insights regarding the epidemiology of brown rot in the field and in postharvest are essential to understanding the disease in each climatic area of stone fruit production. This knowledge is fundamental to designing the most sustainable control strategies for managing the disease.

Epidemiology in the field

In 1977, Byrde and Willetts already reported that brown rot in peaches can be caused by any of the three previously mentioned *Monilinia* spp. (Willetts et al., 1977). They described two infection phases: the blossom blight phase in spring and the fruit rot phase in summer (Figure 1)(Obi et al., 2018). However, the behaviour of the diseases is highly affected by abiotic factors, mainly related to the climatology of each production area, and consequently, the disease behaviour can slightly change.

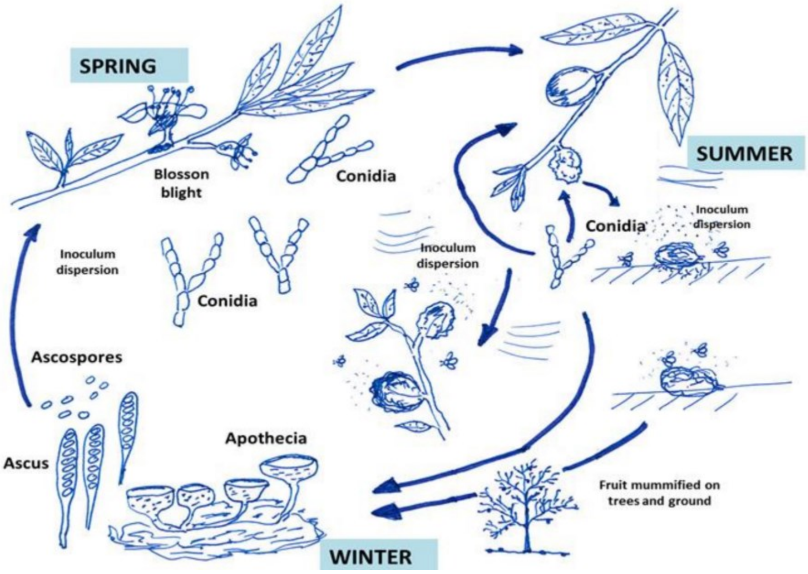

Figure 1. Brown rot cycle (Obi et al., 2018).

Monilinia spp. have been throroughly studied in the climatological conditions of the Ebro Valley over the last 15 years. Our studies have demonstrated that overwintered *Monilinia* spp. on mummified fruit together with inoculum from necrotic twigs, act as the main source of primary inoculum. For mummified fruit, Casals et al. (2015) elucidated that conidia on ground mummies had less viability through time in the following growing season in comparison with tree mummies, although both can act as a source of inoculum for primary infection on flowers, or later on fruit (Casals et al., 2015). Besides this, studies from Villarino et al. (2010) demonstrated the existence of a positive relationship between mummified fruit and the incidence of brown rot in postharvest (Villarino et al., 2010).

When climatic conditions are favourable, conidia sporulation occurs on the overwintered infected tissues by *Monilinia* spp., and then the dispersion of conidia can occur through microorganisms, wind, water, insects, birds, and man. Then, the relationship between the number of airborne conidia of *Monilinia* spp. and the presence of overwintered infected tissues (mummified fruit and pruned branches) on the floor orchards in Ebro Valley was reported (Villarino et al., 2010). According to Figure 1 (brown rot disease cycle), sexual spores (ascospores) of *Monilinia* spp. (mainly *M. fructicola*) produced on ground mummified fruit can also be an occasional source of inoculum (Holtz et al., 1998). However, in Spanish orchards, apothecial were not found, reducing the sources of primary inoculum (Villarino et al., 2010).

Thus, these new generated conidia on overwintered infected tissues, that are dispersed in the orchards by different means, will be the responsibility of the secondary inoculum, infecting flowers and fruit. The number of infection cycles depends on several factors, mainly related to the weather conditions. Accordingly, an *in vitro* study demonstrated that the germination of *Monilinia* spp. conidia was markedly influenced by water activity and temperature, requirements that will also take place under natural conditions (Casals et al., 2010e). Conidia on flowers or on fruit will infect if the required climatological conditions occur. In this sense, short wetness durations in the range 2-6 h at 15 or 20 ºC is sufficient for infection of immature or ripe peaches and nectarines (Kreidl et al., 2015). Once the infection has occurred, the disease development can be stopped if there is a lack of nutrients, unfavourable climatic conditions, or chemical compounds in fruit that inhibit the disease. The number of conidia of *Monilinia* spp. dispersed on the fruit surface also had a significant positive correlation with the incidence of latent infections (Gell et al., 2008). Furthermore, it has also been demonstrated that an average of 5-17 % of latent infections at preharvest implied more than 55 % of fruit with brown rot at postharvest (Villarino et al., 2012).

All the information related to the epidemiology of *Monilinia* spp. in Ebro Valley has been used by IRTA, INIA and UdL to develop a warning system (unpublished). Its implementation under commercial conditions has had several positive implications on stone fruit production, such as: i) a reduction of the number of chemical applications in the field; ii) the optimization of the treatments applied; and iii) a decrease in the residue level on the fruit surface, without affecting the control of *Monilinia* spp. Our warning system depends on 4 factors: 1) the presence of *Monilinia* spp. inoculum in the field, 2) rainfall, 3) period of leaf wetness, and finally 4) temperature.

Epidemiology in postharvest

When fruit is transported from the orchard to the packing house, the main objective is maintaining the fruit quality and extending its shelf life as much as possible. Fruit in packing houses could already be contaminated or even infected by conidia on their fruit surface, without visible symptoms. Then, different scenarios can occur: (i) healthy fruit (without conidia either on surfaces or infected), (ii) fruit with the presence of conidia on its surface (an interaction between fruit-conidia has not been established), so fruit is contaminated but not infected, and (iii) fruit already infected with *Monilinia* spp. conidia both with and without visible symptoms (Bernat et al., 2019a). In all of the conditions, fruit at postharvest will be submitted to different processes, including fruit reception, sorting, cooling tunnel, cold storage, and transport (Figure 2).

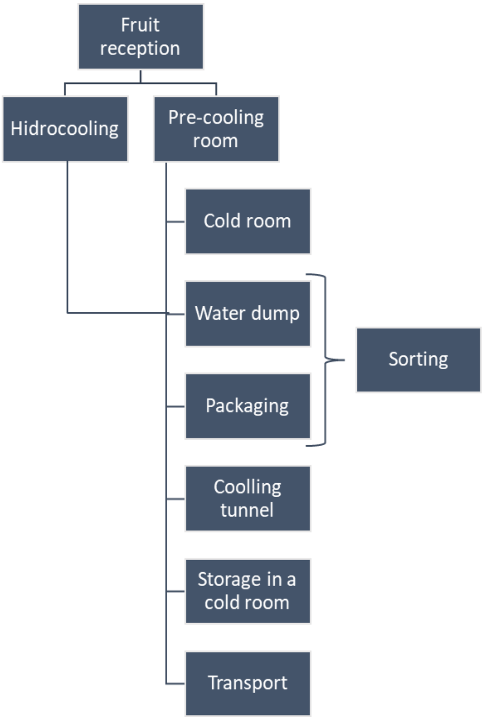

Figure 2. Diagram of a typical postharvest handling operation of peaches and nectarines in a packing house.

Basic knowledge on these processes in *Monilinia* spp. development on peaches and nectarines was provided (Bernat et al., 2017a). It was concluded that as a general trend, hydrocooling and water dump reduced the incidence of brown rot in fruit with recent infections (2 or 24 h before operation), however, when infections had been produced 48 h before operation, disease was not reduced.

For fruit that arrives at packing houses healthy, it will be fundamental to avoid contamination during the postharvest period. In a cold room, healthy fruit can be contaminated by direct contact with a decayed fruit or by airborne conidia deposited on fruit (Dutot et al., 2013). In this sense, the knowledge related to the fungal population in packing houses both in the environment and on inert surfaces is essential. Bernat et al. (2017) sampled environments, surfaces of floors, walls, containers, and lines, all in dirty and clean zones (Bernat et al., 2017b). The main genera identified were *Penicillium* spp., *Cladosporium* spp. and *Rhizopus* spp. However, *Monilinia* spp. was rarely detected, indicating a low risk of fruit infection in packing houses by this pathogen, and suggesting that most infected fruit in packing houses comes from the orchards. Regarding *Pencillium* spp., it was reported that it can cause significant losses on wounded prunes (Wells et al., 1994), and also on commercial nectarine and plum (Pitt and Hocking, 2009). For *Rhizopus* spp., which was highly detected, it has been described as an unpredictable variable accounting for heavy losses and sometimes destroying entire shipments in severe cases (Taheri et al., 2018).

In the case for *Monilinia* spp. conidia found in packing houses, its risk for fruit infection was determined by evaluating the conidia viability when located on different surfaces of the packing houses (floors, packages, equipment, conveyor belts…). Results indicated that the viability of these conidia is lower than 24 h at temperatures of 20 ºC or 30 ºC (Bernat et al., 2018). Based on all these results it can be concluded that the risk of contamination of healthy fruit by *Monilinia* spp. conidia may be low. Nevertheless, optimal and rigorous measures of prophylaxis in packing houses will still be essential to reduce the infection risk of other pathogens that also affect stone fruit.

Fruit with the presence of conidia on its surface, but without infection, has a real risk of finally being infected and consequently developing the disease. The most common handling operations in packing houses are storing fruit for 1 day in the cold room or dumping fruit in a water tank. It has demonstrated that conidia of *Monilinia* spp. on the surface of stored fruit in cold rooms do not suppose a risk of infection and therefore, brown rot symptoms are not developed (Bernat et al., 2019a). However, whether fruit is stored in a cold room and then immersed in a dump tank, or directly immersed in a dump tank, optimal conditions are provided for both infection and developing previous infections (Bernat et al., 2019b, 2017c; Xu and Robinson, 2000). They also indicated that water dump conditions favour the germination of *Monilinia* spp. and the following infection, because of the high relative humidity that remains on the fruit.

Finally, for fruit that arrives at postharvest already infected by *Monilinia* spp., the rates of decay and mycelium development increase with temperature from 0 ºC to 25 ºC (Bernat et al., 2018). Considering that the temperature of harvested peaches and nectarines in Lleida in the field can reach 30 ºC, it will be essential to remove this heat as quickly as possible in order to slow down the metabolism and reduce fruit deterioration including physiological and pathological issues (Bernat et al., 2018). This process of cooling down is mainly conducted by using hydro-cooling equipment or a pre-cooling room. In this context, it is important to highlight that when storing fruit already infected in the field at 0-0.5 ºC, the conidia germination will be slowed, but not stopped (Casals et al., 2010e).

Conidia coming from fields, produced in chambers due to the development of latent infections or recent infections, can lead to secondary infections increasing the contamination inside of packing houses. Then, measures adopted to reduce the level of inoculum on fruit surfaces but also in different zones in packing houses, together with adopting the optimal measures for prophylaxis and fruit management, will contribute to reducing the disease.

3. Sustainable control of *Monilinia* spp.

At present, the most common strategy is based on the use of chemicals applied by calendar and, specifically in the Ebro Valley, the applications start from 45 days before harvest (Casals et al., 2021b). Currently, there is a wide range of chemical active ingredients available worldwide which are applied in the field to control brown rot. These include boscalida, cyprodinil, diphenoconazole, fenbuconazole, fenparazamine, fhenexamide, fludioxonil, fluopyram, pyraclostrobine, tebuconazole, etc. At this point, it is important to note that some of these active ingredients are recognized as a reduced risk fungicide, because they are characterised by a minimal impact on the environment, high specificity to target organisms, low potential for groundwater contamination, and minimal human health risk from their residues. In any case, the authorization of theses fungicide applications in the field, doses, security period and number of treatments for each active ingredient depend on each country. Mostly, they are used to control brown rot, and moreover, some of them have already shown to be effective for controlling *Rhizopus* spp. Additionally, during the postharvest period, the application of chemical treatments can also be used, since it is authorised in some European countries (Di Francesco et al., 2017). These postharvest treatments are advised as complementary to the field strategy for only mid-late varieties, or in the event of adverse meteorological conditions (Casals et al., 2021b).

However, this way of plant protection must start to change because of different issues: i) social pressure made by consumer demands for environmentally friendly fruit production that has dramatically increased in recent years, ii) stricter legislation on authorised chemical active ingredients, and the allowable level of chemical residues on fruit, and iii) the risk of these pesticides for developing resistant strains. In addition, the new European plant health regulation (EU 2016/2031), that aims to strengthen compliance with health and safety standards throughout the agri-food chain, has highlighted the use of safer products for consumers in the end results. And, recently, in the framework of the European Green Deal, the "farm to fork" strategy, which aims to accelerate the transition to a sustainable food system, considers the reduction of 50 % of pesticides (use and risk) and at least an increment of 25 % of the agricultural land under organic farming, in 2030. Obviously, the latter gives even more meaning to the different approaches for controlling brown rot described in the following parts of this review.

3.1. Control strategies addressed in the field

In the field, alternative strategies to the chemicals used for controlling brown rot, are mainly focused on the use of biological control agents (BCA). In this sense, it was demonstrated that a field calendar strategy based on the use of BCA formulated products (*Bacillus amyloliquefaciens*-CPA-8 or *Penicillium frequentans*-Pf909) effectively controlled brown rot in most cases (Casals et al., 2021a). However, their efficacy clearly depended on the disease pressure in the field. In this study, authors pointed out the need for tools such as warning systems for the prediction of infection risks to obtain basic information regarding the expected disease pressure in each orchard, and then the best control strategy could be decided. The decision support systems could also be implemented in the framework of fungicide rationalization, to minimize the number of applications in comparison with strategies based on a spray schedule. It is another way to meet the societal and legislative demands.

The lack of the BCAs' efficacy under some circumstances can also be overcome by their integration with chemical treatments. Currently, little information is available regarding the integration of BCAs into conventional cropping systems. Data reported by Curtis (2019) showed that BCAs integrated with chemicals to control brown rot on stone fruit dramatically reduced, even to zero, the level of chemical residues in derived juices (De Curtis et al., 2019). This approach for controlling the disease must contemplate that the conventional crop production management could affect the BCAs' viability or efficacy. Therefore, it is essential that the BCAs must be compatible with the pesticides commonly used in field management. This issue was studied on the BCAs CPA-8 and pf99, which were found to be compatible with the majority of chemical products that are applied to stone fruit orchards under conventional production strategies (Guijarro et al., 2019). This information is important to widen their application spectrum. Currently, only three BCA products based on *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* are authorized in some countries for their field applications to control brown rot on stone fruit. Finally, it is important to highlight the registration of BCAs as another major obstacle. The registration process requires a large number of studies concerning human and environmental safety, basic toxicological tests, and an effective evaluation, including semi-commercial validations (Droby et al., 2009).

On the path to controlling brown rot in more sustainable stone fruit production, the integration of all available tools will be the key to a successful result. Therefore, cultural practices must be valued and integrated as long as they are economically viable:

- *Removing inoculum from the field.* The elimination of all diseased parts (mummies, shoots, branches, fruit) that are sources of primary and secondary inoculum will be essential to reducing inoculum density, especially mummies and brown rotted fruit found in trees (Villarino et al., 2010). It has to be noted that in production areas where the sexual form of *M. fructicola* occurs, mummies located on the ground will favour apothecia formation, and they should also be removed. These practices must be conducted at the beginning of the season for the primary inoculum removal, and 10-15 days before harvest for decayed fruit removal, when fruit is more susceptible to infection and disease development, thereby preventing the dissemination of conidia to healthy fruit.

- *Microclimate management*. Another cultural practice to apply in the field is maintaining an optimum microclimate by pruning trees and achieving higher insolation and ventilation, and so avoiding humid conditions that favour *Monilinia* spp. and other fungi development (Michailides and Morgan, 1997).

- *Nutritional elements*. Cultural practices in the field also include the provision of nutritional elements needed by the crop. In this sense, it has been demonstrated that applications of chemical elements, such as silica or calcium, have an effect in reducing brown rot. In fact, the incidence of fruit affected by *Monilinia* spp. is significantly reduced after the applications of 6 preharvest foliar treatments of Calcium, and this practice is adopted by stone fruit growers in New Zealand (Elmer et al., 2007).

- Fruit management. The use of clean boxes, avoiding the exposure of fruit to insolation, and caring for handled fruit to minimize injuries during harvest and transportation, are some practices that will certainly reduce the incidence of disease during postharvest.

3.2. Control strategies addressed in postharvest

In the European Union, chemical postharvest treatments have been forbidden for many years. In 2012, fludioxonil started to be commercialized in Spain to be applied in the postharvest of stone fruit to control *Monilinia* spp. as an exceptional authorization, and in 2015 this active ingredient was registered as it continues to be, together with pirymetanil. Before that, as no strategies were available to apply in postharvest, and control brown rot on stone fruit, postharvest pathologist researchers had focused deeply on developing efficient and alternative strategies. Then, the interest in this issue was slightly reduced, since there was an efficient solution to control brown rot at postharvest. However, in 2021, interest has drastically increased again, because of the reason already described in this review, mainly related to achieving sustainable stone fruit production in the framework of the European Green Deal and the need to mitigate climate change.

Alternative postharvest strategies to control *Monilinia* spp. have been numbered and thoroughly described by Usall et al., giving relevance to: 1) Susceptibility of varieties; 2) Food additives (organic and inorganic salts); 3) Natural compounds; 4) Biological control; 5) Physical treatments; 6) Inductions of resistance; and 7) Integrated approaches (Usall et al., 2015). In this review, we will focus on physical treatments, and specifically, on heat strategies, which are recognized as effective methods for controlling postharvest diseases by direct pathogen inhibition, and by stimulating the host defence mechanism.

Hot water treatment

Hot water treatment (HWT) is the most studied heat treatment for the control of fruit diseases and is classified as a non-sophisticated strategy. It is based on fruit immersion in previously heated water for an exposure time. This technique is completely safe for humans and the environment (residue-free and environment-friendly) and has a feasible use without registration rules. Likewise, these treatments are easily adaptable to the packing houses that allow their application on just-harvested fruit, and they are currently commercially applied in some countries such as Italy. For these reasons, HWT appears to be especially recommended for organic crops or to comply with the strict regulations of markets and legislations that require minimal or no chemical postharvest treatment on commodities.

Many factors can influence the effect of HWT, such as the commodity (cultivars, size, shape, thermal conductivity of tissue, maturity at harvest), the aimed pathogen (species, location on or within the host) and the treatment conditions (temperature, fruit exposition and, quantity of product treated). Consequently, it will be fundamental to achieve the proper treatment conditions in each case. Taking into account all the commodities where the HWT has been studied, the temperature ranged between 46 and 64 ºC and exposure time between 15 seconds and 30 min (Usall et al., 2016). Table 1 lists the most relevant works published on the effective conditions to control postharvest disease in stone fruit. Most of them identify 60 ºC as the optimum temperature to control brown rot without affecting fruit quality. Some of these studies combined HWT with other alternative strategies such as low toxicity chemical products, natural extracts, or BCAs. In our work, the combined treatment based on dipping fruit at 60 ºC for 40 s plus the application of the antagonist CPA-8 (*Bacillus amyloliquefaciones*) at 10^7 cfu/mL showed a synergy effect to control the disease in comparison with when treatments were applied separately (Casals et al., 2010c). Thus, combining treatments with different modes of action becomes the most effective strategy.

Table 1. Hot water treatments for postharvest disease control in stone fruit.

Source	**Commodity**	**Pathogen target**	**HWT***	**Product added to water solution**
(Wells, 1970)	Plum Peach Nectarine	*Monilinia* spp. *Rhizopus* spp.	51.5 °C for 1.5 min	2,6-dicholro-4nitroaniline 225 ppm
(Karabulut et al., 2004)	Sweet Cherry	*Penicillium expansum* *Botrytis cincerea*	60 °C for 30 s	Ethanol (10 %)
(Mari et al., 2004)	Peach Nectarine	*Monilinia* spp.	60 °C for 20 s	Sodium bicarbonate (1 %)
(Casals et al., 2010c)	Peach Nectarine	*Monilinia* spp.	60 ºC for 40 s	Sodium bicarbonate (2 %) *Bacillus amyloliquefacien*s CPA-8 (10 cfu/mL)
(Karabulut et al., 2010)	Plum	*Monilinia* spp.	60 °C for 60 s	
(Sisquella et al., 2013b)	Peach Nectarine	*Monilinia* spp.	40 °C for 40 s	Peracetic acid (200 mg/L)
(Spadoni et al., 2013)	Peach	*Monilinia* spp.	55 °C for 1 min	

	Nectarine			
(Spadoni et al., 2014)	Peach Nectarine	*Monilinia* spp.	60 ºC for 20 s	
(Pazolini et al., 2016)	Peach	*Monilinia* spp.	50 ºC for 30 s	Canola extract Indian mustard extract

*Hot water treatment

Hot air treatments

Another technique to control postharvest disease in stone fruit by heat treatments is the use of hot air, otherwise called curing treatment. It is based on the exposure of fruit for several hours or days in an air atmosphere heated to temperatures higher than 30 ºC and at a high relative humidity (RH > 90 %). Curing treatment has been widely researched, providing numerous studies that have shown satisfactory disease reductions in a wide variety of citrus cultivars. However, only our 3 papers have reported controlling *Monilinia* spp in stone fruit (Casals et al., 2012, 2010a, 2010b). Our studies demonstrated that exposing peaches and nectarines to 50 ºC for 2 h and 95–99 % RH, effectively controlled brown rot caused by *M. laxa* and *M. fructicola* (Casals et al., 2010b). In a subsequent piece of research, the combination of this treatment with chitosan or *Bacillus amyloliquefaciens* CPA-8 was also studied to enhance fruit protection after heat treatment (Casals et al., 2012).

Radio frequency and microwave treatments

In recent years, the need to achieve fast and effective heat treatments has increased the study of radio frequencies and microwave heating strategies, also referred to as dielectric heating. Dielectric heating has been widely studied as a rapid pest control treatment. However, little information is available about the use of these new technologies to control postharvest diseases of fruits and vegetables (Guo et al., 2019). Casals (2010) demonstrated the efficacy of radio frequency heating for 18 min to control brown rot, caused by *Monilinia* spp., in peaches (Casals et al., 2010d). However, this treatment was not effective at controlling brown rot in nectarines and its effectiveness depended on fruit size. In the next study, the effectiveness of this radiofrequency treatment to control brown rot in both peaches and nectarines was improved by applying radio frequency heating with fruit immersed in water at 20 ºC (Sisquella et al., 2013a). The immersion of fruit in water solved the lack of efficacy in nectarines as the influence of fruit size on treatment efficacy. In the same study, the radio frequency treatment time required to effectively control brown rot was even reduced to 4.5 min by using water temperature at 40 ºC. Besides, radiofrequency treatment with fruit immersed in water at 40 ºC for 4.5 min did not affect fruit quality.

Microwave treatments using and industrial equipment also demonstrated the efficacy using a continuous treatment at 10 kW for 95 s to control *Monilinia* spp. in peaches and nectarines. Once again, fruit size influenced the final fruit temperature, which affected both treatment efficacy and internal fruit appearance (Sisquella et al., 2013c). The improvement of this microwave treatment was also conducted with the immersion of fruit in water (Sisquella et al., 2014). Then, it was studied at 20 kW for 60 s with fruit immersed in water at 40 ºC. These conditions controlled brown rot without affecting internal and external appearance and reduced brown rot incidence over 91 % in naturally infected peaches and nectarines, and moreover, its efficacy was not affected by fruit size, time between infection or inoculum concentration of *M. fructicola*. Finally, it was suggested that the lower influence of fruit size on the treatment effectiveness may be due to the similar temperatures achieved, since the temperature reached by smaller fruit in radio frequency and microwave treatments were, respectively, 3 and 4.5 ºC lower than those reached by larger fruit. Different dielectric properties between food and the surrounding air cause reflection and refraction phenomena at the interface, resulting in non-uniform electric field distribution (Guan et al., 2002). Therefore, the immersion of fruit in water can improve both radio frequency and microwave treatment (Sisquella et al., 2014, 2013a).

4. Conclusions

In the last 20 years, *Monilinia* spp., as main pathogens of stone fruit, have been thoroughly studied in our group. Its epidemiology has been studied in preharvest and postharvest, generating a wide range of information, fundamental to designing the best control strategies. Furthermore, it is important to highlight the warning system developed which, nowadays, is a tool already implemented by stone fruit producers in the Ebro Valley. In parallel, several control strategies have had their applications evaluated, again in both pre and postharvest. These strategies include chemicals, but also alternative strategies, and cultural practices to be applied during crop management. The outcomes of our research have been generated at the right moment, with legislation requirements for fruit production and social awareness about climate change being more established than ever. Therefore, it will be essential to integrate all this knowledge into designing sustainable control strategies to control brown rot, with minimum impact on the environment, farmers, and consumers, and following the Guidelines on Agriculture of the European Green Deal to obtain competitive, sustainable healthy and high-quality fruit.

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