

New Strategies to Control Brown Rot Caused by *Monilinia* spp. of Stone Fruit

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Summary

The importance of brown rot caused by *Monilinia* spp. in all stone fruit growing areas is well recognized. The infection occurs in the field but the most dangerous fruit losses happen during storage and commercialization. The disease control depends mainly on integrated strategy based on cultural practices and fungicide spray programmes in the field. Among cultural practices, the sanitization of orchard by minimizing inoculum potential, reducing the risk of blossom and fruit infections is essential for brown rot management. However, the requirements in sustainable agriculture, integrated crop management and organic production are increased in the last few decades, resulting in the need to develop other methods than fungicide applications to disease control. Several studies focused on alternative strategies, defining three different approaches: i) biological control with microbial antagonists, ii) use of natural products and iii) use of physico-chemical methods. Significant progress has been achieved in the reduction of pesticide use with these methods; although a multidisciplinary methodology that integrates sanitary and alternative strategies has to be investigated more fully. The new strategy, starting from the field with agronomic interventions and the selection of cultivars tolerant to *Monilinia* spp. could be usefully integrated by postharvest treatments based on low risk chemical fungicides, natural antimicrobial substances and other physical means determining a sustainable approach to brown rot control.

Key words

biological control agents, natural compounds, physico-chemical methods, DA-meter

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Received: June 15, 2016 | Accepted: March 8, 2017

ACKNOWLEDGEMENTS

This study was funded by the LIFE financial instrument of the European Union (contract no. LIFE13 ENV/HR/000580). The authors are thankful for this financial support.

Introduction

Brown rot caused by *Monilinia* spp. represents an important fungal disease of stone fruit (Byrde and Willetts, 1977). The main species of *Monilinia* present in Europe are *M. laxa* (Aderhold and Ruhland), *M. fructigena* (Aderhold and Ruhland) and *M. fruticola* (Winter and Honey), however recently another specie: *M. polystroma* able to infect stone and pome fruits, has been detected in Hungary (Petroczy and Palkovics, 2009) and in Croatia on apple (Di Francesco et al., 2015), and in Italy on pear (Martini et al., 2015). These fungal pathogens attack aerial parts of host plant with a variety of symptoms such as blighting of blossoms, twig cankers and fruit rotting, however the prevalent fruit losses happen after harvest during storage and shelf life (Martini and Mari, 2014). Losses depend on weather conditions and are especially severe if high humidity, warm temperatures and abundant rainfall prevail prior to harvest (Bonaterra et al., 2003). At the consumer level, they can negatively affect consumer satisfaction and propensity to further consumption. Moreover, stone fruits are important in the agricultural economy of many countries; for example, the peach and nectarine world production reached 21 MT in 2013 (FAOSTAT, 2014), and by calculating an underestimated average percentage of losses (10%) due to brown rot, *Monilinia* losses can account for 2,1 M€ /year, for peach and nectarine alone. In organic stone fruit orchards, *Monilinia* rot is a major yield-limiting factor that is difficult to control (Larena et al., 2005; Xu et al., 2007), and in a wet season, for commercial organic orchards, it is not unusual to suffer 75% or more crop losses due to brown rot, despite the fact that copper-based fungicides are used. In some regions, organic cherry production is almost impossible because all attempts to control brown rot have failed.

The control of *Monilinia* rot depends on integrated strategy based on cultural practices and fungicide spray programmes in the field, however in the last two years, a postharvest treatment with fludioxonil, a phenylpyrrole fungicide, is allowed for European stone fruit productions. Among cultural practices, the sanitization of orchard by minimizing potential inoculum, reducing the risk of blossom and fruit infections is essential for brown rot management. The removal of all remaining fruit from the tree, after the final picking, limits the infection of fruit peduncles and twigs, reducing the appearance of brown rot cankers. In addition, this practice prevents the formation of mummies within the tree immediately adjacent to susceptible blossoms in the spring. Excessive fertilization can influence the overcrowding of branches, and limiting the air circulation prevents a rapid drying, favouring the proliferation of pathogen, while pre-harvest calcium applications significantly reduced the incidence of rotted fruit per tree at harvest and in postharvest phase (Elmer et al., 2007). Disproportionate irrigation, particularly during the last days before harvest, can produce microlesions on fruit and allow *Monilinia* spores to penetrate into the fruit. However, all these practices are partially successful but are not reliable for consistent, effective control, therefore fungicide applications are usually used to control brown rot in commercial orchards. In the past, the introduction of benzimidazole (MBC) fungicides dramatically improved the control of *Monilinia* spp. in the field, although their wide and almost exclusive use produced quickly the appearance of benomyl-resistant populations of *M. fruticola*

(Jones and Ehret, 1976; Whan, 1976). Other new fungicides were registered such as demethylation inhibitors (DMI, e.g. myclobutanil and fenexamide), succinate dehydrogenase inhibitors (SdhI, e.g. boscalid), dicarboximides (e.g. iprodione) and quinine outer binding site inhibitors (QoI, e.g. pyraclostrobin and azoxystrobin). Although *Monilinia* species are classified by the FRAC (Fungicide Resistance Action Committee) as pathogens with a moderate risk of developing resistance to fungicides, the appearance of resistant isolates to the above mentioned fungicides in some regions, after several years of exposure, was reported (Elmer and Gaunt, 1994; Luo et al., 2008; Zehr et al., 1999). Across the world, recommendations for brown rot control with field fungicide treatments vary depending on climatic conditions, the presence of *Monilinia* species and the legislative measures allowed in each country.

Since the main fruit losses caused by *Monilinia* spp. are produced after harvest, during storage and commercialization, the management of fruit at postharvest phase acquires a considerable importance. Some practices help to prevent infections at harvest and during storage and transit, as picking and handling fruit with the greatest care to avoid punctures and skin abrasions, which enable the fungal pathogen to gain entrance more easily. Fruit should be protected from heating after harvest and cooled as soon as possible to avoid excessive flesh softening while fruit with brown rot spots have to be discarded (Crisosto et al., 1995).

The requirements in sustainable agriculture, integrated crop management and organic production are increased in the last few decades, resulting in the need to develop other methods to control brown rot (Nunes, 2012). Several studies focused on alternative strategies defining three different approaches: i) biological control with microbial antagonists, ii) use of natural compounds and iii) use of physico-chemical methods. All three approaches have been widely reviewed in the past (Droby et al., 2009; Nunes, 2012; Mari et al., 2015).

Biological control

Nevertheless, the postharvest phase is suited to the application of biological control agents (BCAs), because of restricted environments such as storage rooms, where parameters like temperature, relative humidity RH and gas composition can be altered to favour antagonist growth, their use is not already routinely entered in postharvest handling. Probably the cause of this delay lies with the long and expensive process of registration and with some inconsistent results (Janisiewicz, 2010). Among biofungicides, those active against *Monilinia* spp. are even fewer: 'Biosave' 10LP and 110 based on *Pseudomonas syringae* (strain 10LP and 110); 'Serenade' based on *Bacillus subtilis*, are commercialized for brown rot control on stone fruits but not yet registered for postharvest treatments in Europe. However, some BCAs appear interesting such as *Bacillus* spp. that, among bacteria, represents a promising candidate for biological control of *Monilinia* spp. due to its ability to produce a wide variety of antimicrobial compounds, including iturin (Gueldner et al., 1988) and lipopeptide antibiotics (Yanez-Mendizibal et al., 2011). While yeast and yeast-like microorganisms that show an antifungal activity not generally depend on the production of toxic metabolites are more appreciated as BCAs. Two *Aureobasidium pullulans* strains (L1 and L8) were active on *Monilinia* spp. at

low temperature (0°C) on peach (Mari et al., 2012). Several trials performed to understand their mechanisms of action revealed that the production of volatile compounds such as phenethyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-methyl-1-propanol could provide a contribution although limited, to the biocontrol of pathogens as *Monilinia* spp., however more frequently with fungistatic than fungicidal activity (Di Francesco et al., 2011). The main postharvest fungal pathogens as *Monilinia* spp. are wound parasites, therefore the ability of antagonists to colonize these niches and rapidly increase their population appears strategic for the success of control. A study on the population dynamics of two antagonistic strains showed different rates of development with respect to fruit species; in apple wounds, the population size increased the initial concentration 7-fold, while in peach wounds only a weak growth was observed, although it was enough to control *Monilinia* rot (Mari et al., 2012). Significant progress has been made in understanding the various aspects of biocontrol agents that allow them to inhibit or prevent pathogen development. However, the main conclusion indicates that there is a lack of a single universal action mechanism and that the interactions between antagonist, pathogen and environment, in particular postharvest conditions, could play a strategic role in the efficacy of BCAs (Di Francesco et al., 2016).

Natural compounds

Generally, any biological molecule could be considered a natural product, but normally, the term is reserved for secondary metabolites produced by an organism-like plant, bacterium, yeast, or fungus (Mari et al., 2015). These compounds have received attention as one of several non-chemical options in the control of postharvest diseases; in fact, many data have reported the antimicrobial properties of natural compounds derived from plants. Plants produce a myriad of secondary metabolites important for their interaction with the environment and many of them are associated with the defence system and can function as fungal inhibitors. One of the first works on the use of plant extracts to control postharvest fruit pathogens appeared in 1959 by Ark and Thompson. The authors reported the activity of the garlic (*Allium sativum* L.) extract on the inhibition of *M. fructicola* in peach fruits; moreover they suggested the possibility that garlic extract could produce off-flavours in peach fruit. After this first attempt, a wide literature reported the activity of natural compounds on fruit postharvest pathogens. These compounds are generally grouped as plant extracts but they are also classified in subgroups depending on their origin and chemical composition as essential oils (EOs), isothiocyanates (ITCs), jasmonates, aroma compounds, etc. Among aroma compounds *trans*-2-hexenal showed fungicidal activity against many postharvest pathogens including *M. laxa* of stone fruits; fumigation with *trans*-2-hexenal (20 µl l⁻¹) applied immediately after inoculation (2h) stopped decay and did not cause any visible injury on plum (Neri et al., 2007). In addition, postharvest application of EOs as carvacrol and thymol was effective in controlling *B. cinerea* and *M. fructicola* in cherries (Tsao and Zhou, 2000) and *M. fructicola* on apricots and plums (Liu et al., 2002), although these treatments caused phytotoxic symptoms and off-flavours in cherries and apricots. ITCs, a large group of bioactive compounds derived from the enzymatic hydrolysis of

glucosinolates, showed a high activity against a wide range of pathogens affecting food (Delaquis and Mazza, 1995). Numerous studies have evaluated the vapour-phase activity of allyl isothiocyanate (AITC) *in vitro* and *in vivo* tests. In particular, the exposure for 6 h to AITC (0.04 mg l⁻¹) controlled *Monilinia* rot on peach and nectarine without revealing any apparent detrimental effect (Mari et al., 2008). Nevertheless, there are several evidences that make possible the use of natural compounds in fruit postharvest disease control, more researches are needed before a routinely application of them. The main issues remain their strong aromas even at low concentrations that can affect the taste of fruit and the setting of best conditions of treatment (dose and duration of treatment) that is an inexorable necessity to avoid phytotoxic effects on treated fruit. On the other hand, the added value of these biobased products is unquestionable, in fact if compared to conventional fungicides, natural compounds can give clear environmental benefits due to their renewability, biodegradability, hypotoxicity and, in addition, to the reduction of greenhouse gas emission during their production and use (Mari et al., 2015).

Physico-chemical methods

In recent years, physical treatments have gained great interest to control many postharvest diseases because of the total absence of residues in the treated product and minimal environmental impact (Usall et al., 2015). Among them heat represents the main known treatment producing the inactivation of fungal propagules; generally its effectiveness is related to temperature and duration of treatment, although excessive temperatures should be avoided due to detrimental effects on produce quality (i.e., phytotoxic effects, discoloration, water loss). Heat treatments can be applied to fruit in the form of hot water, vapour heat, hot air and hot water rinse-brushing. Curing fruit with hot wet air (50°C for 2 h at 95-99 % RH) completely inhibited *M. laxa* and *M. fructicola* in four peach and nectarine varieties, without affecting internal and external fruit appearance (Casals et al., 2010). However, hot water (HW) appears to be one of the most effective and promising methods; in fact recent applications of HW showed a good control of brown rot of peach and nectarine naturally infected; the fruit dipping in water at 60°C for 1 min reduced the disease incidence of more than 78% in four trails out of six (Spadoni et al., 2013). More studies should be carried out to determine the effect of heat treatments (HW and curing) on *Monilinia* spp. latent infections. The conidia on the fruit surface can be easily inactivated by a dip treatment, while the suppression of viable conidia or hyphae localized below the epidermal cell appears more difficult with certain methods that act on the surface of fruit, such as BCAs and volatile compounds. However, the heat can stimulate certain host defence mechanisms active also against latent infections (Martini and Mari, 2014).

The use of radio frequency (RF) energy not only to heat food but also to disinfect commodities and to control postharvest diseases deals with the need to achieve fast and effective thermal treatment. The first study on RF heating to control brown rot showed the efficacy of irradiation for 18 min and an increase of effectiveness was obtained by dipping the fruit in water during RF treatment (Sisquella et al., 2013).

Chemical compounds with low toxicity are considered as GRAS (generally recognized as safe) compounds and receive an increasing interest for the control of postharvest pathogens. GRAS are allowed with very few restrictions for many industrial and agricultural applications by regulations worldwide and offer a considerable promise in postharvest technology. Among them, peracetic acid, K-sorbate, sodium bicarbonate, and calcium salts are some example of GRAS used to control brown rot. A K-sorbate treatment reduced over 80% the *Monilinia* rots on peaches and nectarines naturally infected (Gregori et al., 2008). Adversely, K-sorbate and sodium benzoate lacked effectiveness and persistence when tested against *M. fructicola* in small-scale trials, showing a reduction of brown rot incidence of less than 40% (Palou et al., 2012). In 1997, ozone was declared to be GRAS for food contact application (EPRI Expert Panel, 1997) and used for fruit postharvest treatments with satisfactory results. In a preliminary report, *M. fructicola* conidia were inhibited by ozone exposure at a concentration of 200 $\mu\text{l l}^{-1}$ and 4000 $\mu\text{l l}^{-1}$ under humid and dry conditions respectively (Margosan and Smilanick, 1998). Unfortunately, the concentrations of ozone that inactivated conidia were relatively high and could not be used without complete containment of the gas and protection of workers.

Electrolyzed water (EW) generated by the electrolysis of salt solution through an electrolytic cell showed a strong antifungal activity against postharvest disease such as brown rot of peaches (Guentzel et al., 2010). In this investigation, electrolysis was conducted with the addition of sodium chloride as electrolyte with a consequent formation of free-chlorine and chlorinated organic compounds like chloramines, dichloramines and trichloromethanes, creating drawbacks for handlers and consumers. Free chlorine is also quickly inactivated by the heavy inorganic load presents in the wash water of commercial packinghouses; therefore, the use in the electrolysis reaction of salts not containing chlorine might be particular interesting (Fallanaj et al., 2013).

Future perspectives

The alternative methods (biological, physical and chemical) cited above showed a control of brown rot not always at acceptable levels when used individually, in addition they have a poor effect against established infection before treatment (Droby et al., 2009). Therefore, to overcome these drawbacks, the adoption of integrated strategies have been widely investigated. The combination of HW treatment and ethanol improved the control of *M. fructicola* in peaches and nectarines compared the single treatment (Margosan et al., 1997). The combination of peracetic acid and HW showed a greater reduction of brown rot in peaches inoculated with *M. fructicola* than that observed in fruit treated only with peracetic acid or HW (Sisquella et al., 2013). A possible additive effect between *Debaromyces hansenii*, an antagonist yeast, and UV-C irradiation in controlling brown rot incidence on both artificially inoculated and naturally infected peaches was observed by Stevens et al. (1997). The superiority of a combined treatment was probably due to the ability of UV-C to control deep-seated infections such as a latent infections, whereas the yeast controlled only superficial infections originating in recent wounds.

Recently, the possibility of sorting asymptomatic peaches, harvested at a commercial maturity, by DA-meter, into two

ripening classes that will show different brown rot incidence during storage was investigated. The DA-meter is a hand-held instrument developed from vis/NIR spectroscopy that measures the Index of Absorbance Difference (I_{AD}) correlated with the flesh chlorophyll- α content of fruit. After 3 days of shelf-life, fruit natural infected belonging to a class with high I_{AD} values (less ripening) showed an incidence of *Monilinia* rots significantly lower than fruit belonging to a class with low I_{AD} values (more ripening). Inserting DA-meter in sorting lines, each fruits can be evaluated for the I_{AD} and sorted for specific I_{AD} classes, revealing immediately after harvest, which fruit could be destined to a short shelf-life, not too long of 1-2 days and commercialized in close markets (low I_{AD}). While the fruit sort for high I_{AD} could be stored in shelf-life for more time (6-8 days) and sold in distant markets (Spadoni et al., 2016).

Conclusions

The importance of brown rot is evident worldwide, such as the difficulties for controlling the disease development (www.monilinia.org). Traditionally, the fungicide treatments in the pre and postharvest phases are considered the main mean for disease control although they have a considerable impact on the environment and on human health. As reviewed, even though significant progress has been achieved in the reduction of pesticide use with alternative methods, it seems to be unrealistic to assume that these methods have the same fungicidal activity as fungicides. In this scenario, a multidisciplinary approach that integrates sanitary and alternative strategies has to be investigated more fully. This new strategy, starting from the field with agronomic interventions such as orchard sanitization to reduce inoculum sources and the selection of cultivars tolerant to *Monilinia* spp. if not resistant, could be usefully integrated by postharvest treatments based on BCAs, natural antimicrobial substances and other physic-chemical means. The tailoring of a complete specific strategy for each situation (species: cherry, peach, apricot, nectarine, plum; climatic and seasonal conditions; conventional or organic production; destination market, etc.) should be implemented. In particular, since stone fruits are characterized by a relatively short postharvest life, the destination of fruits after harvest (time between harvest and sale, distance between the site of production to the point of sale, etc.) must drive the implementation of programmes for brown rot management (Martini and Mari, 2014).

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