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# Control of Monilinia rots on fruit naturally infected by hot water treatment in commercial trials

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ABSTRACT

In recent years, safer methods for the control of fruit postharvest pathogens have been investigated and heat treatment could represent an effective and safe approach for managing postharvest decay such as *Monilinia* rots. In the present study, the effect of hot water treatment (HWT) (60 °C for 30 and 60 s) on brown rot was investigated. More specifically, the influence of HWT was determined in *in vitro* trials on conidial germination of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* and in peach and nectarine fruit, naturally infected. The effect of hot water application on fruit quality was also assessed. *M. fructicola* showed a greater resistance to heat than *M. laxa* and *M. fructigena*, however conidia germination of all three species was completely inhibited by a dipping in hot water for 1 min at 55 °C. The results of a large scale experiment under commercial conditions and several pilot trials showed a good antifungal activity of HWT in naturally infected fruit. After 6 days at 0 °C and 3 days at 20 °C, in both semi-commercial and commercial trials, the inhibition of decay was higher than 78% in four trials out of six. In addition, the treated fruit showed an acceptable commercial quality and no visual damage was observed as a consequence of HWT. The results demonstrated that HWT is a promising method to control Monilinia rots of peach and nectarine, and is safe and readily available for conventional and organic production under commercial conditions.



## 1. Introduction

Brown rot is an economically important disease in warm, humid climates areas where stone fruit are cultivated. The disease is mainly caused by *Monilinia laxa* (Aderhold and Ruhland) Honey and *Monilinia fructicola* (Winter) Honey, while *Monilinia fructigena* (Aderhold and Ruhland) is prevalent on pome fruit (Ogawa et al., 1995). In European countries, in the last century, only *M. laxa* and *M. fructigena* were isolated from fruit affected by brown rot while *M. fructicola* was considered a quarantine pathogen; however, following some studies that appeared in the last decade (Lichou et al., 2002; De Cal et al., 2009; Pellegrino et al., 2009), it was moved from A1 to A2 in the list of quarantine organisms (EPPO, 2003). The pathogen is primarily a wound pathogen and in some cases, infections that occur in the field remain quiescent until the fruit reaches maturity, allowing *Monilinia* to overcome host defences. Eradication of these latent or incipient infections, which often develop within 24–48 h after harvest, is required. Consequently, marketing of decay-free fruit, especially to long-distance markets, can be problematic (Mari et al., 2009). The control of *Monilinia* rot depends on an integrated strategy based on cultural practices, orchard fungicide spray programme and, after harvest, on the maintenance of proper storage conditions in the packinghouse and during commercialization. Moreover, no chemical treatments are allowed on stone fruit after harvest in European countries (Jemric et al., 2011). Alternative strategies proposed for the control of Monilinia rots include treatments based on biocontrol agents (BCAs) such as yeasts (Mari et al., 2012), bacteria (Casals et al., 2010) and fungi (Mari et al., 2007), chemical products with low toxicity as food additives (Gregori et al., 2008), natural substances as biofumigants (Mari et al., 2008; Neri et al., 2007) or physical methods such as hot water treatments or modified atmosphere packaging (Karabulut and Baykal, 2004). A previous paper reported a significant reduction of *M. laxa* growth as conidia germination *in vitro* and as brown rot on peaches and nectarines artificially infected after a water dipping at 48 °C for 12 min (Jemric et al., 2011). Similar results were also obtained by Liu et al. (2012) on conidia germination and rot caused by *M. fructicola* using water at 40 °C for 10 min. However, only a few studies have evaluated this method under commercial conditions on naturally infected fruit. Laboratory and pilot-scale tests are essential to the development of treatment protocols, although optimal information can be found easily and quickly using small-scale experiments, it is important to transfer the pilot-scale or laboratory research results to large-scale industrial implementations.

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The overall aim of the present study was to investigate the influence of hot water (HW) treatment on brown rot in naturally infected peaches. More specifically, the effect of HW treatment was determined in *in vitro* trials on conidial germination of *M. laxa*, *M. fructicola* and *M. fructigena* and *in vivo* on different cultivars of peach and nectarine cultivated under conventional or organic management in laboratory and in semi-commercial and commercial-scale trials. Finally, the effect of hot water application on fruit quality was also assessed.

## 2. Materials and methods

### 2.1. Pathogen

*M. laxa, M. fructicola* and *M. fructigena* strains were obtained from our collection, previously identified by sequencing of ribosomal DNA ITS regions (Mari et al., 2012) and maintained on potato dextrose agar (PDA) at 4 °C until use. In order to obtain a good sporulation of pathogens they were inoculated on V-8 agar (V8A: 250 ml of pure V8 juice and 40 g of agar in 1 L of distilled water) and incubated at 25 °C with 12 h dark, 12 h light cycles for 10 days. Conidial suspensions were prepared by washing the colonies with sterile distilled water containing 0.05% (v/v) of Tween 80, quantified with a hemacytometer and diluted to the concentration of $10^6$ conidia per mL.

### 2.2. Culturable conidia test

Conidia viability was measured as colony forming units (CFU) on PDA (Casals et al., 2010). Aliquots of 0.5 mL spore suspension ($10^6$ spores $mL^{-1}$) were added to 4.5 mL of water pre-warmed at 45, 50, 55 and 60 °C. Immediately after 1, 5 and 10 min of exposure, 0.5 mL of the warmed conidia suspension was diluted 100-fold in cold water. Aliquots (0.1 mL) of treated *M. laxa, M. fructicola* and *M. fructigena* conidia suspensions were spread on petri dishes and incubated for 3 days at 25 °C. A suspension of untreated conidia ($10^3$ conidia per mL) was used as the control. Approximately 100 spores of each pathogen per treatment were evaluated by the culturable conidia test, expressing the results as number of CFU. The sample unit was represented by five plates (replicate) and the experiment was conducted twice.

### 2.3. Fruit

Peaches (*Prunus persica* (L.) Batsh) and nectarines [*P. persica* var. nectarina (Ait.) Maxim.] were obtained from a local packinghouse (Cesena, Italy) according to availability. Fruit free of visible wounds and rot and homogeneous in size were stored at 0 °C and used within a couple of days after harvest. For laboratory trials, 'Caldesi 2010' nectarines and 'Benedicte', 'Royal Summer' and 'Symphony' peaches, cultivated in orchards under conventional management, were used; for semi-commercial and commercial trials, 'Royal Glory', 'Royal Mayestic', 'Red Moon' peaches derived from two orchards under organic management were used.

### 2.4. Influence of hot water treatment on brown rot in fruit with natural inoculum

#### 2.4.1. Laboratory trials

Selected fruit were treated by dipping in a 10 L stainless steel tank. The water temperature was 60 °C, the duration of treatment was 20 s. Control fruit were dipped in water at room temperature for the same time. After treatment, fruit were stored for 10 days at 0 °C, followed by another 4–7 days at 20 °C (shelf-life). Disease incidence was recorded after refrigeration storage and after 4 days at 20 °C, when the incidence of brown rot was low (< 5% in the control), fruit were kept for another 3 days at 20 °C: the percentage of infected fruit was then evaluated for the second time. The sample unit was represented by 4 replicates of 25 fruit each per treatment and each trial was performed twice.

#### 2.4.2. Semi-commercial trials

Plastic perforated boxes (51 cm × 31 cm × 26 cm) containing 60 fruit each were used for the semi-commercial trials. The HW treatment was carried out with the same parameters (temperature and duration) described above; a 50 L stainless tank was used for the treatment, the water was heated by a digital thermostat (ScanVac SHC 2000, Linge, DK) with temperature stability ±0.01 °C, heater wattage 2 KW and pump having a flow rating of 15 L/min. Control fruit were dipped in the same tank containing water at room temperature. After treatments, fruit were stored at 0 °C for 6 days and subsequently another 3 days at 20 °C. The disease incidence was only recorded at the end of the experiment. The sample unit was represented by three replicates of one box each. The experiment was conducted twice.

#### 2.4.3. Commercial trials

The commercial trials were carried out on peaches harvested from three different orchards and placed in bins, each containing 250 kg of fruit (1100–1200 fruits). A dipping machine (Xeda International, S. Andiol, France) was used for the treatment. The machine consisted of a tank containing 450 L of water, heated by electric resistances dipped in the water and regulated by a thermostat with temperature stability ±1 °C. The machine has a work capacity of 40 bins per hour. The treatment parameters of were set at 60 °C for 60 s, since 1 min is the minimum time allowing complete dipping of the bin, the subsequent treatment and the removal of the bin. Moreover, the top of the bin was covered with another empty bin, thereby ensuring that all fruit remained entirely submerged throughout the treatment. Prior to each HW treatment, the temperature of the bath was checked with a digital thermometer (HD8605, Delta Ohm, Padova, Italy) scaled to 0.1 °C. Control fruit were treated in the same machine before water heating. After treatment, fruit were stored in the same conditions as the semi-commercial trials, as previously described. The disease incidence was only recorded at the end of the experiment. The sample unit was represented by three replicates of one bin each. The experiment was conducted twice.

### 2.5. Fruit quality

The effect of HWT on fruit quality was investigated on nectarine and peach fruit used for the laboratory trials. Visual fruit skin damage and physico-chemical parameters were evaluated at the end of each trial, after 10 days at 0 °C followed by 4 or 7 days at 20 °C, according to the experiment. Firmness (N, 8-mm fruit tester probe), soluble solids content (%) and titratable acidity (meq 100 $mL^{-1}$) were analyzed on 20 healthy fruits as reported by Neri et al. (2006).

The sensory profile was assessed by a trained panel after the period of shelf-life on fruit with the same firmness, taken from 20 fruit used for the instrument analysis as reported by Neri et al. (2007).

The sensory profile was determined using a 9 point category scale. The intensity of the sensory attributes increased from 1 (none) to 9 (extreme) for juiciness, mealiness, sweetness, sourness, fruitiness, off-odours and off-flavours. For hardness: 1: extremely hard and 9: extremely soft; for sweet-acid balance 1: mainly sour; 5: balanced and 9; mainly sweet; for colour: 1: green and 9: dark red.