Postharvest Biology and Technology 70 (2012) 25–31

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Control of a wide range of storage rots in naturally infected apples by hot-water dipping and rinsing

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ARTICLE INFO

Article history:
Received 6 January 2012
Accepted 2 April 2012

Keywords:
Malus domestica
Defence response
Heat damage
Latent infection
Postharvest disease

ABSTRACT

Hot-water rinsing (3 or 4 min) and dipping (15, 20 or 25 s) at a range of incubation temperatures was applied to apples (cv. 'Ingrid Marie' and 'Pinova') naturally infected with a range of North West European storage-rot fungi. Significant reductions in the incidence of fruit rot were achieved by incubation periods of 3 min at 50–54 °C (dipping) and 20 or 25 s at 55 °C (rinsing), followed by up to 100 d cold-storage at 2 °C and 14 d at 18 °C. Pathogens controlled in this way were *Neofabraea alba*, *N. perennans*, *Monilinia fructigena*, *Colletotrichum acutatum*, *Phacidiopycnis washingtonensis* and *Cladosporium* spp. *Neonectria galligena* was reliably controlled by dipping but not rinsing. No effects of either heat treatment on *Gibberella avenacea* and *Botrytis cinerea* were apparent. Following rinsing at 65 °C for 20 s, the incidence of *P. washingtonensis*, *Penicillium expansum*, *Mucor* spp. and *Phoma exigua* was higher than in untreated control fruit or in apples rinsed at lower temperatures, and was associated with heat damage. The relative contributions of heat effects on inoculum viability and activation of defence responses of apple fruit are discussed. Hot-water rinsing has several advantages over hot-water dipping related to the efficient processing of fruit either directly after harvest or after long-term storage.

1. Introduction

Mild and humid climatic conditions such as those prevalent in North Western Europe favour postharvest fruit rots caused by fungi. The most important pathogens which infect apples prior to harvest are *Neofabraea* spp. (*N. alba* and *N. perennans*), *Neonectria galligena* and *Monilinia fructigena* in descending order of importance (Palm and Kruse, 2005). *Penicillium* spp. and *Botrytis cinerea* may infect fruit before and also during storage (Jijakli and Lepoivre, 2004). Although modern storage technologies aimed at retarding fruit ripening have an effect on many fruit rots (Spotts et al., 2007; Lafer, 2010), repeated sprays with fungicides (e.g., captan and strobilurin-type compounds) during the 2 months preceding harvest remain an essential component of the current strategy to control storage-rot fungi (Palm and Kruse, 2005; Minar, 2006).

The use of fungicides shortly before harvest is under scrutiny because of retailers' demands to reduce pesticide residues well below the legally permissible thresholds, or to restrict the number of detectable residues (Poulsen et al., 2009). Furthermore, resistance development may impair the efficacy of fungicides against key pathogens (Weber and Palm, 2010). Alternative strategies to control fungal postharvest diseases are therefore required, and this is especially important for organic orchardists who may experience elevated storage losses because of the non-availability of chemical fungicides (Holb and Scherm, 2007; Granado et al., 2008).

Heat treatments of apples have shown promise in reducing the subsequent development of storage rots (Fallik et al., 2001). High efficacies against *Neofabraea* spp. and *Penicillium expansum* have been obtained after incubation in hot air (e.g., 72 h at 40 °C; Tahir et al., 2009; Fallik et al., 2001) or by hot-water dipping (HWD) for up to 3 min (Maxin et al., 2005; Amiri and Bompeix, 2011). Hot-water rinsing (HWR) for <30 s at temperatures above 50 °C has been developed in Israel to control postharvest pests and diseases of a range of horticultural products (Fallik, 2004). In Northern Germany, HWD has been introduced into organic apple production (Maxin et al., 2006), although acceptance of this technology by orchardists has been hampered by high energy costs and the need for added labour during the peak work time at harvest (Maxin and Klopp, 2004). Furthermore, there is only limited information on the range of fungi that can be controlled by HWD and especially HWR.

In preliminary studies, Maxin and Weber (2011) and Maxin et al. (2012) have shown that HWD could successfully control various storage rots on artificially inoculated apples. The aim of the present study was to characterise the full range of fungal pathogens susceptible to HWD and HWR as natural infections, and to evaluate the potential of HWR as an alternative to HWD in commercial organic fruit production.

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http://dx.doi.org/10.1016/j.postharvbio.2012.04.001

2. Materials and methods

2.1. Apples

On 22 September 2009 and 29 September 2010, apples (cv. 'Ingrid Marie') were harvested from an experimental orchard at Aarslev (Aarhus University, Denmark; 55°18′N, 10°26′E, altitude 47 m) because previous surveys at this site had shown a high incidence of storage rots (Maxin and Weber, unpublished data). Apples were harvested at a starch index of 3.5–4.0 according to Streif (1983) and a fruit flesh firmness of 6.5–7.5 kg cm^{-2} (measured with a GS20 fruit texture analyser, Güss Ltd., Strand, South Africa). In order to maximise natural infections by storage-rot fungi, this orchard was not exposed to any fungicide treatment after petal fall.

'Pinova' apples harvested on 4 October 2010 from the Esteburg experimental farm in Northern Germany (53°30′N, 9°45′E, altitude −2 m) were also included in the evaluations. The starch index at harvest was 4.0–5.0, and the fruit flesh firmness was 8.0–9.0 kg cm^{-2}. This orchard had been under organic management since 1995, and stored fruit from previous harvests had shown a reliable incidence of bull's-eye rot caused by *Neofabraea* spp. (Maxin, unpublished data).

In view of the highly localised occurrence of storage-rot inoculum on individual trees (Spolti et al., 2012; Maxin and Weber, unpublished data), apples from different trees were mixed after harvest. Aliquots of 90–110 fruit (cv. 'Ingrid Marie') or 40–46 fruit (cv. 'Pinova') were packed in perforated plastic boxes (40 L volume; 60 cm × 40 cm × 17 cm; 35% perforated area in side walls and bottom), stored for 5 d at 2 °C at ambient atmosphere, and then subjected to HWD or HWR. All treatments were replicated four times, each replicate comprising apples from one box.

2.2. Hot-water dipping (HWD)

Plastic boxes containing apples were dipped in 350 L heated water. The top of each box was covered with another box containing a 5 kg weight, thereby ensuring that all fruit remained entirely submerged throughout the HWD period. Heat loss and cooling effects were buffered by adding 95 °C water from a commercial steam-jet blower that introduced water currents into the dipping unit to ensure that a uniform temperature was maintained around the apples within 30 s of submersion. During dipping, temperatures were monitored between apples in the centre of the dipped box using an electric thermometer (Voltcraft K 204; Conrad Electronic SE, Hirschau, Germany). Prior to each HWD step, the temperature of the water bath was equilibrated and checked with a certified analogue mercury thermometer scaled to 0.1 °C (Carl Roth, Karlsruhe, Germany). In 2009, HWD was carried out at four temperatures (48, 50, 52, 54 °C) in combination with two dipping times (3 and 4 min) which were chosen on the basis of previous results with apples showing reduced efficacies after 1 or 2 min HWD (Trierweiler et al., 2003; Maxin et al., 2005). In 2010, HWD was carried out for 3 min at 50, 52 and 54 °C.

2.3. Hot-water rinsing (HWR)

For the 2009 trial, single apples were removed from the plastic boxes, placed on a conveyor belt with rotating elements, and sprayed with hot water from 12 flat fan nozzles (Type DG 005 VS TeeJet; Spraying Systems Co., Wheaton, USA). Hot-water consumption was 2 L nozzle^{-1} min^{-1}, and each apple was treated with 2 L hot-water during a 20 s exposure. High losses of energy were observed, a 10 cm spraying distance between the nozzles and the apple surface reducing the temperature by approx. 10 K. The actual treatment temperature (T_1) was measured with an analogue mercury thermometer in water samples collected from the processing line. The adjusted water temperature ($T_2 = T_1 + 10$ K) was controlled with a second thermometer incorporated in the water supply unit upstream of the nozzles. In the 2009 season, apples were rinsed for a standard period (20 s) at different temperatures (55, 58 or 62 °C).

For the 2010 trial, equipment modifications and parameter changes were introduced (Fig. 1). To ensure that temperatures were within ±1 K of the required values, a volume of 400 L water was heated in a closed system to the specified treatment temperature by electronic heaters connected to an automatically regulated digital control unit (ELK 38, EL.CO. S.r.l., Pievebelvicino, Italy). During HWR processing, apples were rotated and floated in a row formed by water currents at 16 positions on one side and a border of fixed plastic brushes on the other side. The addition of a new apple at the beginning of the row resulted in a forward movement of the row of apples by one position. The last fruit leaving the row at the end of the line was removed manually. Experimental repeats were separated by inserting green dummy apples. The duration of HWR treatments was determined by the speed of adding apples into the process line which was controlled by using the regulated conveyor belt from the 2009 trial. In the 2010 trial, HWR temperatures were combined with different exposure times, i.e. 55 °C for 15, 20 and 25 s, 60 °C for 7, 15, 20 and 25 s, and 65 °C for 20 s.

2.4. Storage after hot-water treatments

Following HWD or HWR, apples were stored for 100 d at 2 °C and 14 d at 18 °C in ambient atmosphere, and examined at 14-d intervals. Apples showing incipient fruit rot were isolated from healthy fruit, labelled, and kept at 2 °C until the onset of sporulation.

2.5. Identification of fruit rots

Fungi associated with fruit rots were identified for each infected apple by the appearance of macroscopic symptoms, sporulating structures and microscopy of spores produced. Pure-culture isolates were obtained from representative infections by streaking out spores onto potato dextrose agar augmented with 200 mg penicillin G and streptomycin sulphate L^{-1} agar (supplied by Carl Roth). These isolates were incorporated into the culture collection, Esteburg Fruit Research and Advisory Centre, Germany. DNA extraction from mycelium, PCR amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA were carried out as described in detail by Weber (2011). Sequence searches were performed in GenBank using the BLASTN function (Zhang et al., 2000).

2.6. Assessment of heat damage

Physiological damage due to heat was examined after 70 d at 2 °C. Heat damage was identified as slightly sunken regions of brownish discolouration which did not spread during further incubation at 2 °C. Four categories were distinguished, i.e. 1 (no damage), 2 (small occasional spots <5 mm × 5 mm), 3 (spots >5 mm × 5 mm covering <50% of the fruit surface), and 4 (severe damage covering >50% of the fruit surface).

2.7. Statistical analyses

Data were expressed as percentages of heat-damaged fruit (categories 3–4) or apples infected by a given fungal pathogen. Efficacies of HWD or HWR treatments against fruit-rot development were calculated according to Abbott (1925). In case of fruit showing multiple infections, each identifiable fungus was recorded as a separate infection event, whereas multiple infections by the same