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

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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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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

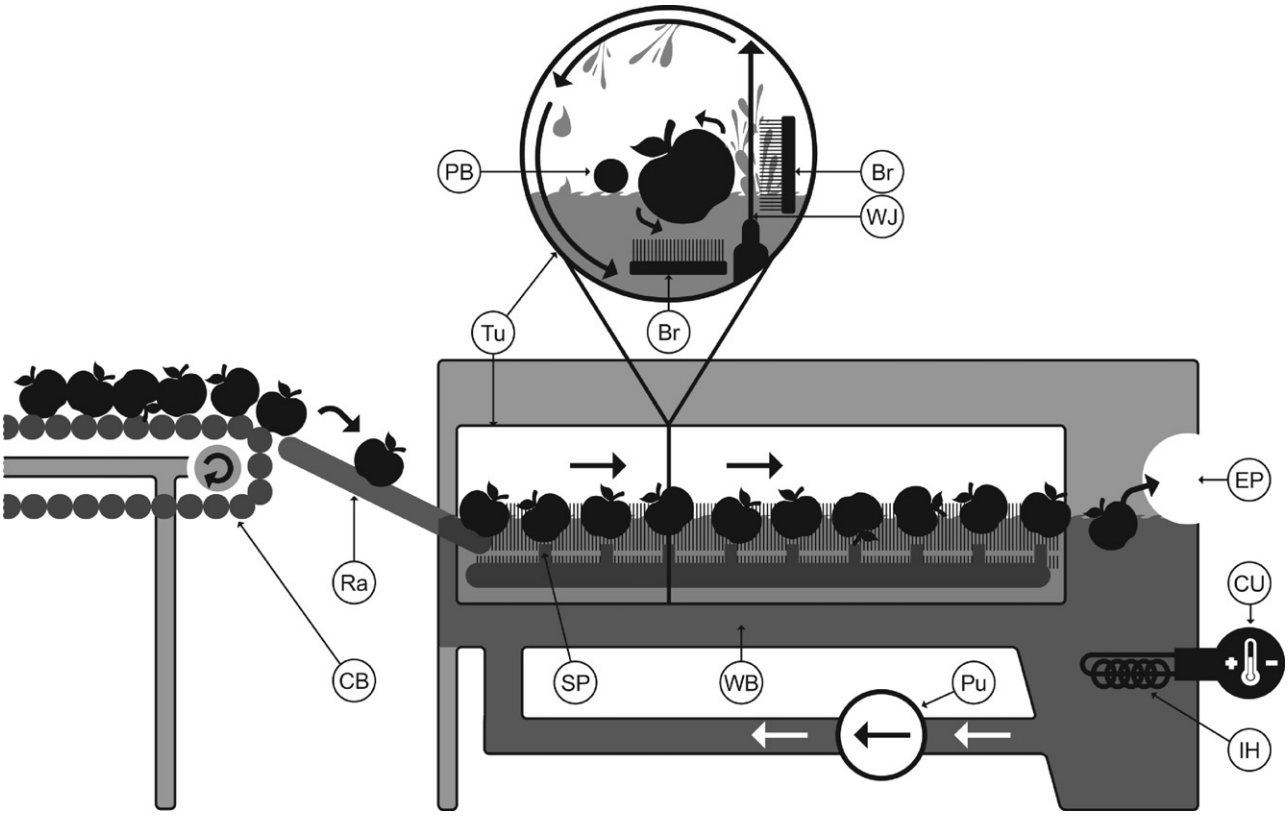

Fig. 1. Schematic drawing of the hot-water rinsing (HWR) equipment as used in the 2010 trials. Apples were transported by a conveyor belt (CB) towards a ramp (Ra) leading to the entry point of a hollow tube (Tu) immersed in a water bath (WB). Apples were moved forward in a single file by jets of water (not shown) until the first spinning position (SP). The insert shows a transverse section of the tube at a spinning position where a water jet (WJ) forced the apple to spin around its own axis, being maintained in single file by a passing barrier (PB) and brushes (Br). Apples were moved forward by new fruit entering the tube, and were removed manually at the exit point (EP). The water bath was heated by immersion heaters (IH) connected to a digital control unit (CU) and kept under circulation by a pump (Pu).

fungus on the same apple were counted as one. An analysis of variance (ANOVA) test of arcsin square root-transformed percentages was performed, and significant differences ($P < 0.05$) were calculated using the Tukey test. The computing environment 'R-project' (http://www.r-project.org) was used for all statistical analyses.

3. Results

3.1. Identification of storage-rot fungi

During the 2 years of these experiments, approx. 2000 rotten apples were examined visually and by microscopy for the occurrence and identity of pathogenic fungi. *Botrytis cinerea*, *Monilinia fructigena* and *Neonectria galligena* were unequivocally identifiable by these means (Jones and Aldwinckle, 1990). For other common or unusual species, microscopic identification was confirmed by ITS sequence analysis of representative isolates (Table 1). In the case of minor rots occurring as species complexes (*Mucor*, *Cladosporium*), identification to species level was not attempted.

In both years of the trials, *Neofabraea alba* was the dominant storage-rot fungus on 'Ingrid Marie' fruit from the Aarslev site. An exceptionally wide range of additional pathogens was identified in these apples (Table 2). Because only traces of *N. perennans* were discovered in 'Ingrid Marie' fruit harvested from Aarslev, we obtained 'Pinova' apples from another orchard (Esteburg site) which in previous years had shown infections by *N. perennans*. The 2010 harvest from this orchard was heavily colonised by both *N. alba* and *N. perennans* (Table 2).

Table 1
Important or unusual storage-rot fungi on 'Ingrid Marie' apples from Aarslev (Denmark) identified by ITS sequence analysis.

Species	Esteburg accession number	Reference for microscopic identification	Representative GenBank sequences (% identity)
Colletotrichum acutatum	OVB11-001	Johnston and Jones (1997)	AJ301906, AJ301914, AJ301917, AJ301956, AJ301963, AJ301971, AJ301987 (all 100%)
Neofabraea perennans	OVB11-006	Verkley (1999)	AF281389, AF281390, AF281391, AF281392, AF281393, AF281395, AF281396, AF281397 (all 100%)
Neofabraea alba	OVB11-007	Verkley (1999)	AF141190, AF281366, AF281367, AF281368, AY359235, AY359236, EU098116, EU098124, HQ166293, HQ166318, HQ166319, HQ166337, HQ166339, HQ166387, HQ166390, HQ166503 (all 100%)
Penicillium expansum	OVB11-008	Pitt (1979)	DQ339547, DQ339548, DQ339552, DQ339556, DQ339558, DQ339562 (all 100%)
Phacidiopycnis washingtonensis	OVB10-012	Weber (2011)	See Maxin and Weber (2011)
Gibberella avenacea	OVB11-004 and 11-005	Booth (1971)	AY147282, AY147283, AY147284 (all 100%)
Phoma exigua	OVB11-003	–	EU343139, EU167567, AJ608976, EF136400 (all 100%)

Table 2
Incidence of different storage-rot fungi (percent of total fruit examined) in untreated control fruit of cv. 'Ingrid Marie' (Aarslev, Denmark) and cv. 'Pinova' (Esteburg, Germany) after storage for 100 d at 2 °C and 14 d at 18 °C (n.d., not determined).

Pathogen	'Ingrid Marie' 2009 (%)	'Ingrid Marie' 2010 (%)	'Pinova' 2010 (%)
Neofabraea alba	28.6	37.0	62.0
Neofabraea perennans	0	0.8	24.5
Neonectria galligena	5.3	4.5	0
Monilinia fructigena	4.1	0.3	0
Cladosporium spp.	3.3	1.4	0
Penicillium expansum	2.3	1.9	0
Phacidiopycnis washingtonensis	n.d.	2.4	0
Colletotrichum acutatum	0.3	1.8	0
Gibberella avenacea	0.6	0.3	0
Botrytis cinerea	0	0.5	0

3.2. Heat damage associated with HWD and HWR

No heat damage was observed on 'Ingrid Marie' fruit subjected to HWD at temperatures up to 50 °C, or to HWR up to 58 °C. HWD caused significant ($P < 0.05$) skin damage (categories 3 and 4) at 52 and 54 °C (Fig. 2A). Significant skin damage was also caused by HWR at 60 °C or above (Fig. 3A).

3.3. Effect of HWD and HWR on storage rots

HWD in the range of 48–54 °C controlled *N. alba* infections at efficacies above 80% (Fig. 2B). The commercially applied conditions of HWD for 3 min at 50 °C reduced *N. alba* by 86% and 84% in 2009 and 2010, respectively. HWR also significantly reduced fruit rot due to *N. alba* (Fig. 3B). The highest efficacy was 82% in 'Ingrid Marie' fruit in 2010 (Table 3) and 77% in 'Pinova' fruit (Table 4) following HWR for 25 s at 55 °C. When 'Pinova' apples from Northern Germany were treated by HWD for 3 min at 54 °C, *N. perennans* fruit rot was reduced by 96% (Table 4). Reduced control of *N. perennans*, at efficacies of 59–73%, was obtained by HWR (Table 4). Therefore both *Neofabraea* spp. responded similarly to hot-water treatments.

Storage rots caused by *N. galligena* were present in 'Ingrid Marie' fruit in both years. In 2009, all HWD and HWR treatments except 15 s at 55 °C significantly ($P < 0.05$) reduced fruit rot caused by this fungus (not shown). In 2010, *N. galligena* rot was significantly reduced by moderate HWD treatments such as 3 min at 50 °C, but not by HWR. The highest incidence of *N. galligena* was associated with the most severe HWR treatment of 20 s at 65 °C which caused major heat damage.

Blue mould caused by *P. expansum* was not controlled by any hot-water treatment, and its incidence significantly ($P < 0.05$) increased following exposure of the fruit to high temperatures, such as HWD at 54 °C for 4 min or HWR at 62 °C for 20 s in 2009 (not shown), or HWR for 20 s at 65 °C in 2010 (Table 3). In line with this finding, *P. expansum* infections were positively correlated with increasing severity of physiological heat damage in 'Ingrid Marie' fruit (Fig. 4).

Due to low infection rates in 2010, no clear-cut effects of hot-water treatments were obtained for *M. fructigena*. However, in 2009 a significant suppression of Monilinia fruit rot was obtained by HWD for 3 min at 54 °C, or HWR for 20 s at 58 °C and 62 °C (Table 3).

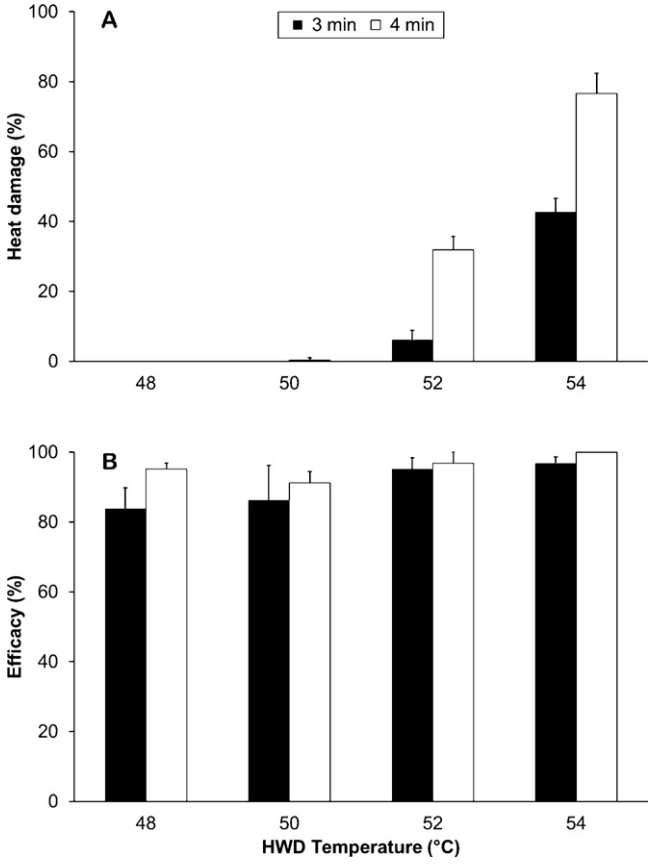

Fig. 2. Effects of hot-water dipping (HWD) at various temperatures for 3 min (black bars) and 4 min (white bars) in apples cv. 'Ingrid Marie'. (A) Heat damage presented as percentage of fruit with lesions >5 mm × 5 mm, and (B) control efficacy (%) of *Neofabraea alba* storage rot on naturally infected fruit. Means of experimental replicates for 2009 are shown ($n = 4$). Error bars indicate standard deviation.

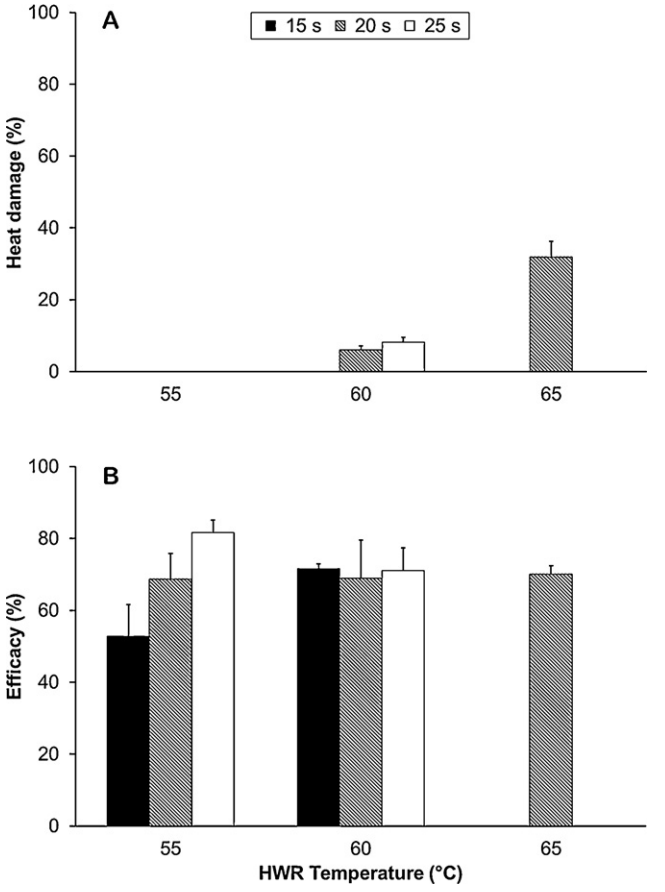

Fig. 3. Effects of hot-water rinsing (HWR) at various temperatures for 15 s (black bars), 20 s (striped bars) and 25 s (empty bars) in apples cv. 'Ingrid Marie'. (A) Heat damage as percentage of fruit with lesions >5 mm × 5 mm, and (B) control efficacy (%) of *Neofabraea alba* storage rot on naturally infected fruit. Means of experimental replicates for 2010 are shown ($n = 4$). Error bars indicate standard deviation.

Table 3
Incidence of storage-rot fungi (percent infected fruit) on 'Ingrid Marie' apples treated by hot-water dipping (HWD) and hot-water rinsing (HWR) relative to the untreated control after 100 d storage at 2 °C, followed by 14 d at 18 °C (n.d., not determined).

Storage-rot fungus	Year of treatment	Control	Hot-water dipping (HWD)			Hot-water rinsing (HWR)				
			3 min at 50 °C	3 min at 52 °C	3 min at 54 °C	20 s at 55 °C	25 s at 55 °C	20 s at 58 °C	20 s at 62 °C	20 s at 65 °C
Neofabraea alba	2010	37.0a	5.8bc	5.5c	3.8c	11.6b	6.8bc	n.d.	n.d.	11.1b
Neonectria galligena	2010	4.5a	2.5ab	1.0b	2.7ab	3.5ab	2.0ab	n.d.	n.d.	6.7a
Monilinia fructigena	2009	4.1a	1.4ab	0.6c	0.9b	2.9ab	n.d.	1.1b	1.1b	n.d.
Phacidiopycnis washingtonensis	2010	2.4b	0.5c	0.3c	0.0c	0.0c	0.0c	n.d.	n.d.	9.3a
Penicillium expansum	2010	1.9b	4.8ab	1.3b	6.3ab	2.3b	2.5b	n.d.	n.d.	12.2a
Colletotrichum acutatum	2010	1.8a	0.0b	0.5b	0.8ab	0.3b	0.3b	n.d.	n.d.	0.7ab
Cladosporium spp.	2010	1.4a	0.0b	0.0b	0.0b	0.0b	0.2b	n.d.	n.d.	0.0b
Gibberella avenacea	2009	0.6	1.1	0.6	0.9	0.6	n.d.	0.6	1.7	n.d.
Botrytis cinerea	2009	0.0	0.3	0.0	0.6	0.0	n.d.	0.3	0.3	n.d.
Mucor spp.	2010	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	n.d.	n.d.	9.1a
Phoma exigua	2010	0.0	0.0	0.0	0.0	0.5	0.3	n.d.	n.d.	1.3

For each fungus, data are presented as means ($n = 4$), and significant differences of treatments relative to the control ($P < 0.05$; Tukey test) are indicated by different letters.

Table 4
Incidence of *Neofabraea alba* and *N. perennans* (percent infected fruit) on apples (cv. 'Pinova') treated by hot-water dipping (HWD) and hot-water rinsing (HWR) relative to the untreated control after 100 d storage at 2 °C and a further 14 d at 18 °C.

Storage-rot fungus	Control	Hot-water dipping (HWD)			Hot-water rinsing (HWR)	
		3 min at 50 °C	3 min at 52 °C	3 min at 54 °C	20 s at 55 °C	25 s at 55 °C
Neofabraea alba	62.0a	13.0 cd	7.5d	4.7d	20.5c	14.0cd
Neofabraea perennans	24.5a	8.5bc	4.0bc	1.0c	10.0b	6.5bc

Data are presented as means ($n = 4$), significant differences of treatments relative to the control ($P < 0.05$; Tukey test) are indicated by different letters.

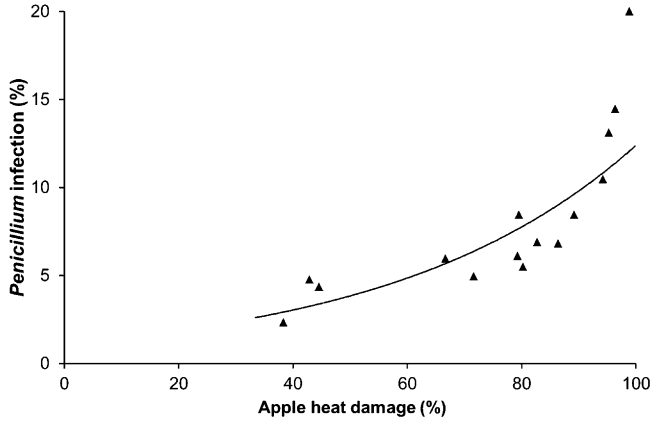

Fig. 4. Correlation of *Penicillium expansum* fruit rot (percent infected fruit) with heat damage (percent of apples with lesions >5 mm × 5 mm) on 'Ingrid Marie' apples subjected to hot-water dipping (HWD) at temperatures above 51 °C (2009 data). The regression equation was $y = 1.19\,e^{0.0234x}$ ($R^2 = 0.76$).

In 2009, *Colletotrichum acutatum* occurred as a minor storage rot in 'Ingrid Marie' apples, resulting in low degrees of infection that failed to produce any significant result. However, in 2010 HWD for 3 min at 50 °C and HWR for 20 s or 25 s at 55 °C gave significant control (Table 3). In 2010, fruit rot associated with *Cladosporium* spp. was observed almost exclusively on untreated fruit, and this was significantly controlled by all hot-water treatments (Table 3).

In marked contrast, an increase in the incidence of fruit rots was observed for several fungal pathogens in heat-damaged apples (Table 3). The incidence of *Mucor* spp. was significantly ($P < 0.05$) confined to fruit with severe heat damage treated by HWR for 20 s at 65 °C, but was not associated with heat damage caused by HWD. A similar observation was made for *Phoma exigua* which caused a sporadic fruit rot with black lesions physically closely associated with heat-damaged areas (not shown). Significant ($P < 0.05$) control of rubbery rot caused by *Phacidiopycnis washingtonensis* was achieved by all HWD treatments and by all HWR treatments except at 65 °C where the incidence of rubbery rot was significantly higher than in the untreated control.

4. Discussion

The present study has shown that HWD of apples is capable of controlling natural infections by *Colletotrichum acutatum*, *Neonectria galligena* and *Cladosporium* spp. for which no critical data have been reported previously, in addition to confirming HWD susceptibility of *Neofabraea* spp. (Burchill, 1964), *Monilinia fructigena* (Maxin et al., 2005) and *Phacidiopycnis washingtonensis* (Maxin and Weber, 2011). Whereas excessive HWD conditions (3 min at 54 or 56 °C) had significant deleterious effects on the fruit surface in terms of heat damage (this study) and pigment bleaching (Maxin, unpublished), no effects on internal quality parameters such as flesh firmness or sugar and starch contents were observed (Maxin, unpublished data). Lower temperatures (50 or 52 °C) did not produce such surface defects whilst still affording high efficacies of storage-rot control. Further, our study is the first report of the successful use of HWR to control apple storage-rot fungi other than *P. expansum* (Fallik et al., 2001) and *P. washingtonensis* (Maxin and Weber, 2011). Although different kinds of equipment were used in 2009 and 2010, both approaches gave rise to comparable results.

In general terms, HWR was not quite as effective as HWD in our trials, although we acknowledge that this technique has not yet been fully optimised. From Fig. 3 it is apparent that further experiments should test prolonged exposure times up to 30 s at 55 °C, or explore temperatures between 55 and 60 °C at a rinsing time of 20 s. Such an approach may be worthwhile because many apple varieties are much less prone to heat damage than the highly sensitive 'Ingrid Marie' fruit (Schirmer et al., 2004).

HWD and HWR were able to control a similar range of fungal pathogens. A few species such as *G. avenacea*, *B. cinerea* and

P. expansum were relatively indifferent to both kinds of heat treatment (Table 3). In the case of the latter two species, this result was unexpected because our previous HWD experiments with artificially infected fruit had given good control (Maxin et al., 2012). Poor or variable efficacies of hot-water treatments in naturally infected fruit may be due to deep-seated centres of natural infection located in the apple core in the case of *G. avenacea* (Weber, unpublished data) or in the blossom end of the fruit (*B. cinerea*, *N. galligena*; Jijakli and Lepoivre, 2004). Although *Penicillium expansum* is widely regarded as being tolerant to hot-water treatments (Vorstermans et al., 2008), critical experiments have demonstrated that inoculum present at the time of HWD can be effectively controlled (Amiri and Bompeix, 2011; Maxin et al., 2012). Therefore, problems in *P. expansum* control by hot-water treatments are perhaps best explained by late infections during prolonged storage (Amiri and Bompeix, 2005).

There are at least two components which may contribute to the mode of action of hot-water treatments: (1) a direct and lethal effect of heat on fungal inoculum within or outside the apple, and (2) an indirect effect mediated by a stress-induced physiological response of the fruit. Support for the latter aspect has been provided by studies in which the production of heat-shock or pathogenesis-related proteins was induced by heat treatments in different kinds of fruit (Schirra et al., 2000; Pavoncello et al., 2001; Widiastuti et al., 2011). Further, in inoculation experiments hot-water treatments had a retarding effect on fungal pathogens even when fruit were inoculated shortly after the heat shock (Pavoncello et al., 2001; Widiastuti et al., 2011; Maxin et al., 2012).

It is plausible that both direct and indirect heat effects on fungal inoculum may influence the efficacy of HWD and HWR. In particular, prolonged exposure times above 1 min are required to permit subcuticular regions of the fruit to be exposed to significant increases in temperature (Trierweiler et al., 2003). The heat destruction of superficial apple tissues, without deeper heat penetration, may explain why certain pathogens such as *P. washingtonensis*, *P. exigua* and *Mucor* spp. caused an elevated incidence of storage rot in association with heat damage in HWR- but not HWD-treated fruit (Table 3). At least in the slowly growing *P. exigua*, infections were clearly co-located with heat-damaged areas on individual apples. Therefore, the association of pathogens with heat damage caused by HWR can be likened to the effect of the herbicide paraquat, which releases fungal endophytes from dormancy by killing plant tissues while sparing fungal inoculum (Biggs, 1995).

Pathogens with a high heat tolerance might be expected to be able to grow on apple tissue killed by HWD, and this was indeed observed for *P. expansum* in artificially inoculated fruit which showed a significantly elevated incidence of infection after HWD at destructive temperatures of 56–60 °C as compared to 50–54 °C (Maxin et al., 2012). For a complete inhibition of *P. expansum* rot, temperatures of 70 °C were required (Maxin et al., 2012). Taken together, therefore, several lines of evidence support induced resistance as the primary factor determining the efficacy of HWD and HWR in apple.

HWR is particularly attractive to fruit producers because the method could be incorporated into fruit grading lines directly after harvest without extensive technical modifications. This would result in substantial economic savings as compared to HWD which requires specialised equipment and expertise (Maxin and Klopp, 2004). There is also the possibility to introduce a HWR step at pack-out because storage-rot fungi retarded by controlled-atmosphere conditions (Lafer, 2010) can break out in cold-storage during the remainder of the marketing chain. Further, HWR of cold fruit may provide a substantial saving of heat energy as compared to HWD. In view of the short duration of the heat-shock response at room temperature (Pavoncello et al., 2001), it is uncertain if HWR is able to prolong shelf-life during the retail phase.

5. Conclusions

A wide range of fungal pathogens of stored apples can be effectively controlled by HWD and HWR. High efficacies of HWR have been demonstrated for the first time on naturally infected apples. HWR has potential to become a sustainable alternative for fruit orchardists and packers because it is less costly than HWD and because its short treatment times enable it to be integrated into existing fruit grading lines. There is scope for further optimisation of HWR parameters.

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