Effect of heat treatment on inhibition of Monilinia fructicola and induction of disease resistance in peach fruit

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1. Introduction

Peach (Prunus persica (L.) Batsch) fruit has a short postharvest life at room temperature due to its high susceptibility to decay pathogens and rapid deterioration in quality (Sasaki et al., 2010). Brown rot caused by Monilinia fructicola (G. Wint.) Honey is the major postharvest disease of peach fruit in the United States (Janisiewicz et al., 2010; Karabulut et al., 2010). Although fungicides such as flucoxonil and fenhexamid are registered in USA for postharvest application to control decay of peach fruit, fungicide resistance and public concern over the potential impact of fungicides on the environment and human health have created interest in new strategies for the disease management (Ma et al., 2003; Karabulut and Baykal, 2004; Droby et al., 2009).

Among various non-chemical approaches, exposure to high temperature, either dry (Conway et al., 2005) or with hot water (Fallik, 2004), appears to be one of the most effective. Margosan et al. (1997) reported that immersion of peach fruit in water at 46°C for 2.5 min reduced the incidence of decay caused by M. fructicola and Rhizopus stolonifer (Ehrenb. Fr.) Vuill. Mari et al. (2007) reported that hot water dipping (HWD) of nectarines at 40°C for 2 min significantly reduced decay on fruit that had one hour previously been inoculated with Monilinia laxa (Aderh. et Ruhi) Honey, while Jemric et al. (2011) reported that brown rot decay of peach fruit that been inoculated for 24 h with M. laxa could be effectively controlled by HWD (48°C for 12 min) without affecting fruit quality.

The control of postharvest decay with a heat treatment (HT) involves effects on both plant pathogen and plant host (Schirra et al., 2000; Pavoncello et al., 2001). While it is known that HT can have a direct inhibitory effect on postharvest pathogens (Jemric et al., 2011; Zhang et al., 2008), the mode of action is not well understood. Fungi exposed to abiotic stress, including high temperature, will accumulate reactive oxygen species (ROS) intracellularly in a species-specific and dose-specific manner (Abrashkev et al., 2008; Liu et al., 2011). Mitochondria are responsible for the major portion of intracellular ROS, thus the undesirable ROS level is often accompanied by mitochondrial dysfunction, characterized by a collapse of mitochondrial membrane potential ΔΨm and a decrease of cellular ATP (Helmerhorst et al., 1999; Prabhakaran et al., 2005).

Changes in the proteome and transcriptome of peach fruit following HT have been studied in relation to their effect on chilling resistance and mealliness, when fruit are stored at low temperature (Lara et al., 2009; Zhang et al., 2011), but little to no information is available in relation to postharvest disease resistance. Disease...
resistance responses elicited by heat treatment included the production of lignin-like materials in lemon fruit (Nafussi et al., 2001) and the induction of pathogenesis-related genes in tomato and orange fruits (Schirra et al., 2000). However, specific responses in peach fruit in relation to disease resistance have not been studied. An additional advantage of HT is its positive effect on the quality of stored fruit (Malakou and Nanos, 2005; Lara et al., 2009). However, improper treatment temperature and duration can have a negative impact on fruit quality. Therefore, it is necessary to take fruit quality into account when HT is applied to control postharvest diseases.

The overall objective of the present study was to determine the effect of heat treatment on control of brown rot caused by M. fructicola in peach fruit and its mode of action. More specifically, we investigated the effect of HT on (i) in vitro growth of M. fructicola, (ii) changes in ROS, ∆Ψm, and ATP content of M. fructicola, (iii) control of brown rot caused by M. fructicola in peach fruit, (iv) the expression of the defense-related genes: chitinase (CHI), β-1,3-glucanase (GNS) and phenylalanine ammonia lyase (PAL) and their enzyme activity in peach fruit, and (v) fruit quality.

2. Materials and methods

2.1. Fruit

Peach (P. persica (L.) Batsch cv. ‘June Prince’) fruit were harvested at commercial maturity. The average quality values of firmness, soluble solids content (SSC) and titratable acidity (TA) were 77.33 N, 12.17% and 0.55%, respectively. Fruit without wounds or rot were selected based on uniformity of size and absence of physical injury or disease infection. Before treatment, fruit were surface-disinfected with 2.5% (v/v) sodium hypochlorite for 2 min, rinsed with tap water and air-dried.

2.2. Pathogen inoculum

M. fructicola (CW-1) was isolated from infected peach fruit and maintained on potato dextrose agar (PDA) (Difco, Sparks, MD, USA) at 4 °C. In order to reactivate the culture and verify its ability to cause decay, the pathogen was inoculated into wounds of peach fruit and re-isolated onto PDA once an infection was established. Spore suspension was obtained from ten-day-old cultures at 25 °C. The number of spores was calculated using a C ellometer Vision (Nexcelom Bioscience, Lawrence, MA, USA), and the spore concentration desired was adjusted with sterile distilled water prior to use.

2.3. Measurement of spore germinability of M. fructicola

The effects of heat treatment (HT) on spore germination and germ tube elongation of M. fructicola was determined according to Jemric et al. (2011), with slight modification. Aliquots of 1 mL spore suspension (5 × 10^5 spores mL^-1) were distributed in 1.7-mL Eppendorf tubes. The spores were then incubated for 0, 5 or 10 min in separate water baths set at 40 °C. After the treatments, 20 µL of spore suspension from each tube was spread on PDA plates. The plates were incubated at 25 °C for 5 h. Approximately 200 spores of M. fructicola were measured for germination rate and germ tube length per treatment within each replicate under microscope. Each treatment was replicated three times and the experiment was repeated three times.

2.4. Assay of ROS accumulation

The oxidant-sensitive probe, 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA; Invitrogen, Eugene, OR, USA), was used to assess the intracellular ROS production in M. fructicola according to Qin et al. (2007), with a slight modification. M. fructicola spores were collected from samples exposed to 40 °C for 0, 5 or 10 min. Spores were washed with phosphate buffered saline (PBS) buffer (pH 7.0) and re-suspended in the same buffer containing 25 µM H2DCFDA (dissolved in dimethyl sulfoxide). The suspension was incubated in the dark at 30 °C for 1 h. After washing twice with PBS buffer, spores were examined under a Zeiss Axioskop microscope (Carl Zeiss, Oberkochen, Germany) equipped with a UV-light source using a 485-nm excitation and 530-nm emission filter combination. Five fields of view from each slide (at least 200 spores) were randomly chosen, the number of spores producing visible levels of ROS in response to heat stress was counted. The ROS level was calculated as a percentage (number of fluorescing spores divided by number of spores present in bright field image 100×). There were three replicates in each treatment, and the experiment was repeated three times.

2.5. Determination of mitochondrial membrane potential and ATP contents

Loss of the membrane potential (ΔΨm) is a hallmark for cellular impairment and was measured with a mitochondrial membrane potential detection kit (JC-1; Cell Technology, Minneapolis, MN, USA) which contains a cationic dye (5,5',6,6'-tetrachloro-1 to 1,3,3'-tetraethylbenzimidazolylcarbocyanine iodide, known as JC-1) that fluoresces red in the mitochondria of healthy cells. When the mitochondrial membrane potential collapses, the cationic dye remains in the cytoplasm as green fluorescence. The ratio of red fluorescence to green fluorescence is higher in healthy cells and decreases in impaired cells (Shah and Sylvester, 2005; Chwa et al., 2006). M. fructicola spores were resuspended in JC-1 reagent at a concentration of 1 × 10^6 spores mL^-1, and then incubated for 15 min at 37 °C. Afterwards, spores were centrifuged, and the supernatant was removed. Cell pellets were resuspended in 1 mL assay buffer provided by the kit, and the ratios of red (550-nm excitation and 600-nm emission) to green (485-nm excitation and 535-nm emission) fluorescence were detected immediately on a multi-mode microplate reader (Synergy™ HT, BioTek Instruments, Winooski, VT, USA).

For ATP assay, M. fructicola spores (about 1 × 10^7 spores) were extracted with 50 µL of 2.5% trichloroacetic acid (TCA) for 3 h at 4 °C (Li et al., 2010). After centrifugation at 10,000 × g for 15 min, 10 µL of supernatant was diluted with 115 µL of ATP-free H2O and 125 µL of ATP-free Tris–acetate buffer (40 mM, pH 8.0). ATP contents were determined with a luciferin/luciferase kit (ENLITEN® ATP Assay System, Promega, Madison, WI, USA) according to the protocol of manufacturer. The luminescence emission by the reaction was determined with the multi-mode microplate reader. There were three replicates in each treatment, and the experiment was repeated three times.

2.6. Effect of heat treatment on control of brown rot in peach fruit

In order to investigate the action of HT on control of brown rot caused by M. fructicola in peach fruit, we applied three treatment methods.

Method I: HT of M. fructicola followed by fruit inoculation. Three wounds (4 mm deep × 3 mm wide) were made on the equator of each fruit with a sterile nail. One mL of a spore suspension (5 × 10^6 spores mL^-1) in a 1.7 mL Eppendorf tube was incubated for either 5 or 10 min in a water bath at 40 °C, and 10 µL of HT spore suspension were administered into each wound. A non-HT M. fructicola spore suspension was used as a control.

Method II: HT was applied to fruit followed by inoculation of wounds with non-HT spore suspension of M. fructicola. Fruit were randomly grouped into three lots. Two lots of fruit were subjected
to a hot water immersion treatment of 40 °C for either 5 or 10 min. After HT treatment, fruit were immediately cooled to 25 °C using forced-air and then air-dried for 2 h. A third lot of fruit immersed in water (25 °C for 10 min) served as a control. Three wounds (4 mm deep × 3 mm wide) were made on the equator of each fruit with a sterile nail and each wound was inoculated with 10 μL of a non-HT M. fructicola suspension (5 × 10^4 spores mL⁻¹).

Method III: Inoculation of fruit with a non-HT spore suspension of M. fructicola followed by the application of a HT to the inoculated fruit. Three wounds (4 mm deep × 3 mm wide) were made on the equator of each fruit with a sterile nail and each wound was inoculated with 10 μL of a M. fructicola suspension (5 × 10^4 spores mL⁻¹). The fruit were air-dried for 2 h. Subsequently, fruit were randomly grouped into three lots. Two lots of fruit were subjected to hot water immersion at 40 °C for either 5 or 10 min. After HT treatment, fruit were immediately cooled to 25 °C using forced-air and then air-dried. The third lot of fruit was immersed in water at 25 °C for 10 min and served as a control.

After each treatment, fruit were placed in a covered plastic food tray, and each tray was enclosed with a polyethylene bag and stored at 25 °C. Disease incidence and lesion diameter of peach fruit caused by M. fructicola were determined after 3 d. Each treatment contained three replicates with ten fruit per replicate, and the experiment was repeated three times. Incidence represented the percentage of fruit displaying rot, while lesion diameter was measured only on those wounds that were infected.

2.7. Determination of fruit quality

In order to determine the effect of HT on peach fruit quality, firmness, soluble solids content (SSC), and titratable acidity (TA) of peach samples (control without HT, HT-5 min, and HT-10 min) were measured daily from 1 to 3 d according to Jemric et al. (2011). Fruit firmness was determined with a Digital Refractometer (Atago, PR-100, Tokyo, Japan). TA was determined by titrating 10 mL of juice with 0.1 M NaOH up to pH 8.2, and expressed as a percentage of malic acid. Each treatment contained three replicates with ten fruit per replicate, and the experiment was repeated three times.

2.8. RNA isolation and semi-quantitative RT-PCR analysis of defense gene expression

Total RNA from peach samples at each time point (0, 1, 2 and 3 d after inoculation) was isolated using Concert™ Plant RNA Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions (Wisniewski et al., 2011). Extracted RNA was treated with TURBO™ DNase (Ambion, Austin, TX, USA) and purified again with RNeasy Mini Kit (Qiagen Science, Germantown, MD, USA). Aliquots of 1 μg total RNA were used for first strand cDNA synthesis in 20 μL reaction volume with 100 units of M-MLV reverse transcriptase (Ambion, Austin, TX, USA). Transcript levels of tubulin-α served as an internal control gene (Li et al., 2009). Cycling parameters for each gene amplification were 95 °C for 5 min; 25 cycles of 95 °C for 30 s, specific annealing temperature for 30 s, and 72 °C for 30 s; and finally 72 °C for 10 min. The primers of the defense genes of CHI, GNS and PAL, and annealing temperatures were shown in Table 1. PCR products were cloned and sequenced to verify the identity. Quantification of transcript expression level was based on the band intensity on a ethidium-bromide-stained gel using Scion Image Software (Scion Corp., Frederick, MD, USA) according to Vinagre et al. (2006). There were three replicates in each treatment, and the experiment was repeated three times.

2.9. Assay of enzyme activities in peach fruit

Peach samples were obtained from 20 fruit stored at 25 °C containing the pericarp and flesh at 0, 1, 2 and 3 d after treatment (DAT). Each treatment consisted of three replicates and the experiment was repeated three times.

For chitinase (CHI) and β-1,3-glucanase (GNS), enzymes were extracted according to Cao and Jiang (2006). Fruit tissue samples (5 g) were homogenized in 20 mL of ice-cold sodium acetate buffer (100 mM, pH 5.0) containing 5 mM β-mercaptoethanol and 1 mM ethylenediaminetetraacetic acid (EDTA) at 4 °C. The homogenate was centrifuged at 13,000 × g for 20 min at 4 °C, and the resulting supernatant was collected for the enzyme assay. CHI activity was determined with chitin azide (Sigma–Aldrich, St. Louis, MO, USA) as the substrate, according to the method of Xu and Du (2011). β-1,3-Glucanase activity was assayed with laminarin as the substrate, following the method described by Ippolito et al. (2000). Reaction production were measured spectrophotometrically at 550 nm (for CHI) or 500 nm (for GNS) using the multi-mode microplate reader. The specific activity of CHI was expressed as U kg⁻¹ of protein, where one unit was defined as the amount of the enzyme producing 1 μmol of azide per second. The specific activity of GNS was expressed as U kg⁻¹ of protein, where one unit was defined as the production of 1 μmol of glucose equivalent per second. For phenylalanine ammonia lyase (PAL) was extracted by the method of Jin et al. (2009). Tissue sample (5 g) was homogenized with 20 mL of ice-cold sodium borate buffer (100 mM, pH 8.7) containing 5 mM β-mercaptoethanol and 4% (w/v) polyvinylpyrrolidone (PVP) at 4 °C. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C, and the resulting supernatant was collected for the enzyme assay. Enzyme extract (0.1 mL) was incubated with 0.2 mL of sodium borate buffer and 0.1 mL of l-phenylalanine (20 mM) for 60 min at 37 °C. The reaction was stopped with 0.1 mL of 1 mol L⁻¹ HCl. PAL activity was determined by the production of cinnamate, with the absorbance change at 290 nm (Assis et al., 2001). The blank was the crude enzyme preparation mixed with l-phenylalanine with zero time incubation. The specific enzyme activity was defined
U kg⁻¹ of protein, where one unit was defined as the production of 1 nmol of cinnamic acid per second.

Protein content was measured using the Bradford assay (Bradford, 1976). Bovine serum albumin (Sigma–Aldrich, St Louis, MO, USA) was used as a standard.

2.10. Data analysis

All statistical analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way ANOVA. Mean separations were performed by Duncan’s multiple range tests. Differences at P<0.05 were considered to be significant. Data presented in this paper were pooled across three independent repeated experiments, as the interaction between treatment and experiment variables was not significant.

3. Results

3.1. Effect of heat treatment on spore germination of M. fructicola

Fig. 1 illustrates the inhibitory effect of HT on spore germination and germ tube elongation of M. fructicola. After 5 h of incubation on PDA at 25 °C, the heat-treated (40 °C for 5 or 10 min) M. fructicola spores exhibited a significantly (P<0.05) lower level of germination, as compared to the non-heat-treated control (Fig. 1A). Likewise, a similar pattern was observed for germ tube elongation (Fig. 1B). The inhibitory effect of HT (40 °C) was enhanced when the treatment time increased from 5 to 10 min. Importantly, after 18 h of incubation of PDA at 25 °C, there was no significant difference in the level of spore germination between control and HT (40 °C for 5 or 10 min) spores. The germination rates in all the treatments reached 95% (data not shown), indicating that the HT (40 °C for 5 or 10 min) only retarded the growth of M. fructicola spores and was not lethal.

3.2. Effect of heat treatment on ROS accumulation, ΔΨm and ATP content of M. fructicola

At time 0, prior to the heat treatment, the percentage of M. fructicola spores exhibiting a visible ROS level, as determined by use of the fluorescent dye H₂DCFDA, was less than 10% (Fig. 2). However, the percentage significantly (P<0.05) increased with time of exposure to 40 °C. After 10 min, the percentage of spores stained with H₂DCFDA reached 45%. To investigate whether or not the increased levels of ROS in M. fructicola spores under heat stress were associated with mitochondrial dysfunction, ΔΨm and ATP content were measured (Fig. 3). Data indicated that the level of ΔΨm and ATP in M. fructicola spores were similar. Both of them markedly (P<0.05) decreased with treatment time, and showed the lowest levels after 10 min at 40 °C.

3.3. Efficacy of heat treatment on control of brown rot and fruit quality

Three different methods of heat treatment were employed: (I) spores were heated and inoculated in wounds of unheated fruit; (II) wounded fruit were heated and unheated spores were applied to wounds; and (III) unheated spores were inoculated in fruit wounds and then the fruit were heated. In all three different experimental treatments, HT (40 °C for 10 min) showed better control effect than 40 °C for 5 min. This was true for both diseases incidence and lesion diameter (Fig. 4). Among the HT treatments, Method III, where fruit were inoculated and then heated, gave the best level of control effect with the greatest impact being on lesion diameter. Disease incidence reached 100% after 3 days in the control fruit. At that time, incidence for the three treatments (I, II, and III) was 85, 92, and 75%, respectively, when heat had been applied for 10 min (Fig. 4B). While lesion diameter was significantly reduced by HT for 5 and 10 min in all HT methods, the largest reduction was obtained by HT (40 °C for 10 min) in Method III (Fig. 4C). Regarding fruit quality, no visual symptoms of heat damage were observed during the duration of the experiment. Moreover, heat-treated fruit had no significant differences (P>0.05) in fruit firmness, SSC and TA, compared to untreated control fruit during the 3-d period in which the heat treatment experiment was conducted (Table 2).

3.4. Effect of heat treatment on induction of defensive gene expression in peach fruit

The transcript level of the three defensive genes chitinase (CHI), β-1,3-glucanase (GNS) and phenylalanine ammonia lyase (PAL) was increased by HT (5 and 10 min) (Fig. 5). CHI expression in control fruit increased slightly during the experimental period (three days).
Table 2
The quality parameters of peach fruit stored at 25 °C for 3 d.

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Heat treatment (min)</th>
<th>Quality indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Firmness (N)</td>
</tr>
<tr>
<td>Day 1</td>
<td>0</td>
<td>31.33 ± 0.25a</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>34.36 ± 0.26a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30.76 ± 0.18a</td>
</tr>
<tr>
<td>Day 2</td>
<td>0</td>
<td>22.67 ± 0.20a</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>25.12 ± 0.35a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24.85 ± 0.27a</td>
</tr>
<tr>
<td>Day 3</td>
<td>0</td>
<td>17.67 ± 0.25a</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15.89 ± 1.89a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.06 ± 3.14a</td>
</tr>
</tbody>
</table>

Values are the means of pooled data ± standard deviations (n = 9). The values followed by the same letter at each day are not significantly different according to Duncan’s multiple range test (P > 0.05).

In contrast, HT for both 5 and 10 min substantially increased CHI expression. Compared to HT for 5 min, HT for 10 min showed a greater increase compared to the HT 5 min treatment as evidenced by the band density values. Both 5 min and 10 min HT induced a higher GNS expression than that in control fruit, and GNS expression was induced to the highest level by 10 min HT on all three days. PAL gene expression was the least affected by the HT but did increase over the three-day period.

3.5. Effect of heat treatment on enzyme activity in peach fruit

Corresponding to the pattern of gene expression observed for the three defense related genes examined, HT (5 and 10 min) also increased the activity of chitinase (CHI), β-1,3-glucanase (GNS) and phenylalanine ammonia lyase (PAL) enzymes in peach fruit stored.

Fig. 2. Effect of heat treatment (40 °C) on intracellular ROS accumulation of M. fructicola. (A) Microscope images of M. fructicola spore under bright field; (B) microscope images of M. fructicola spore stained with the fluoroprobe H2DCFDA; (C) percentage of M. fructicola spores exhibiting visible ROS accumulation. Data presented are the means of three independent experiments where each experiment consisted of three biological replicates for a total of n = 9. Two hundred spores were counted on each replicate. Error bars indicate standard deviations of the mean. Columns with different letters indicate significant differences according to Duncan’s multiple range test (P < 0.05).

Fig. 3. Effect of heat treatment (40 °C) on mitochondrial membrane potential (ΔΨm) (A) and ATP content (B) of M. fructicola. Data presented are the means of three independent experiments where each experiment consisted of three biological replicates for a total of n = 9. Error bars indicate standard deviations of the mean. Columns with different letters indicate significant differences according to Duncan’s multiple range test (P < 0.05).
at 25 °C (Fig. 6). The highest level of enzyme activity was observed in fruit that received the HT (10 min). The activity of CHI in the control fruit kept relatively low. The pattern of enzyme activity both in 5 min- and 10 min-HT fruit were similar. CHI activity in both treated fruit reached its highest level at 3 DAT (Fig. 6A). During the storage period, 10 min-HT fruit showed significantly higher GNS activity than the control, while 5 min-HT fruit had significantly higher activity at 2 and 3 DAT (Fig. 6B). PAL activity was stimulated by HT treatment, increasing rapidly at 1 DAT and reaching a maximum at 3 DAT. PAL activity increased in both HT treated fruit as compared to the control during the entire storage period, but PAL activity in 10-min HT fruit were higher than that in 5-min HT fruit (Fig. 6C).

4. Discussion

Heat treatment (HT) of fruit has been reported to be an effective method of managing postharvest diseases by both its inhibitory or germicidal effect on decay pathogens and eliciting defense mechanisms in plant host (Schierra et al., 2000; Fallik, 2004). In the present study, it was found that HT directly inhibited both spore germination and germ tube elongation (Fig. 1). The results confirmed previous findings about the effect of HT on M. fructicola and M. laxa reported by Margosan et al. (1997) and Jemric et al. (2011). When fungi are exposed to severe abiotic stresses including heat stress, large amounts of intracellular ROS are generated (Singh et al., 2011). Abrashev et al. (2008) reported that heat stress of 40 °C significantly increased the levels of ROS in Aspergillus niger van Tieghem and caused cellular oxidative damage. We also observed intracellular ROS in M. fructicola when exposed to 40 °C (Fig. 2). The number of cells exhibiting intracellular ROS accumulation increased significantly with exposure time.

Levels of mitochondrial dysfunction closely followed intracellular ROS accumulation (Helmerhorst et al., 2001; Herrera et al., 2001). Impairment of mitochondria would result in decreased levels of ATP (Heazlewood et al., 2004). In the present study, oxidative stress in M. fructicola, as evidenced by an increase in intracellular ROS resulted in a collapse of mitochondria membrane potential (ΔΨm) and decreased levels of ATP (Fig. 3), resulting in delayed growth. This is in agreement with a recent study by Qin et al. (2011) on the effects of hydrogen peroxide on Penicillium expansum. The ATP decrease in M. fructicola spores may explain why heat-treated M. fructicola grew more slowly than unheated controls.

In order to investigate whether the mode of action of HT on control of brown rot involved an effect on both the pathogen and the

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**Fig. 4.** Effect of heat treatment (40 °C) on control of brown rot caused by M. fructicola in peach fruit stored at 25 °C. Disease incidence and lesion diameter were measured 3 d after inoculation with M. fructicola. Inoculation I: heat treatment of M. fructicola followed by wound inoculation of fruit; Inoculation II: heat treatment of fruit followed by inoculation with non-HT suspension of M. fructicola; Inoculation III: inoculation of fruit with non-HT spore suspension of M. fructicola followed by heat treatment of the inoculated fruit. (A) A representative picture showing disease symptom of brown rot in peach fruit following Inoculation III; (B) disease incidence and (C) lesion diameter in peach fruit following the three treatment methods. Data presented are the means of nine pooled replicates. Error bars indicate standard deviations of the mean. Within methods columns with different letters indicate significant differences according to Duncan’s multiple range test (P<0.05).

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**Fig. 5.** Effect of heat treatment (40 °C) on gene expression of chitinase (CHI), β-1,3-glucanase (GNS) and phenylalanine ammonia lyase (PAL) in peach fruit. The tubulin gene was used as a control for normalizing mRNA quantity. The level of target gene expression relative to the sample at Day 0 is shown above each band. Data presented are the means of nine pooled replicates. Abbreviations: C, control; HS, heat treatment (40 °C for 5 min); H10, heat treatment (40 °C for 10 min).
of chitin which is an essential cell wall component of many fungal pathogens, and GNS is one of the most fully characterized pathogenesis-related (PR) proteins that act directly by degrading cell walls of pathogens or indirectly by releasing oligosaccharide and eliciting defense reactions (Lee et al., 2006). Additionally, PAL is the first enzyme in the phenylpropanoid pathway and is involved in the biosynthesis of phenolics, phytoalexins and lignins (Dixon et al., 2002). All these processes are potential defense mechanisms against fungal infection (Tian et al., 2007). The results of the present study indicated that HT markedly induced gene expression of CHI, GNS and PAL (Fig. 5), and their enzyme activities (Fig. 6). Moreover, the 10-min HT showed better inductive effect than the 5-min one, which corresponded well to the control effect of brown rot (Fig. 4). Similar inductive effects of HT have been reported on other fruits. Benitez et al. (2006) reported that hot water dipping treatment at 55 °C for 5 min induced resistance in mature green stage mango fruit against Colletotrichum gloeosporioides (Penz) Penz & Sac. by enhancing the activity of GNS and PAL. Pavoncello et al. (2001) observed that hot water brushing treatment at 62 °C for 20 s promoted the accumulation of CHI and GNS proteins in grapefruit and an increase in resistance to Penicillium digitatum Sac. The present report, however, is the first report demonstrating the inductive effect of HT on defensive enzymes at both the transcript and enzyme activity level in peach fruit.

In conclusion, we found that heat treatment could have a significant impact on brown rot control in peach fruit when both the fruit and pathogen were exposed to the treatment. The control effect was associated with the inhibition of M. fructicola germination and growth, intracellular ROS accumulation, mitochondrial impairment leading to a reduction in ATP, and the induction of defense-related enzymes in peach fruit.

Acknowledgements

This research was partially supported by a grant (IS-4268-09) from the U.S.-Israel Binational Agricultural Research and Development (BARD) Fund to S.D. and M.W., and a grant (31030051) from the National Natural Science Foundation of China (NNSFC) to S.T.

References


Fig. 6. Effect of heat treatment (40 °C) on enzyme activity of chitinase (CHI), β-1,3-glucanase (GNS) and phenylalanine ammonia lyase (PAL) in peach fruit. Data presented are the means of nine pooled replicates.