JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 4303–4309 4303
DOI:10.1021/jf904273m

Effects of Heat Treatment on Wound Healing in Gala and Red Fuji Apple Fruits

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This study investigated the effects of heat treatment (hot air at 38 °C for 4 days) on wound healing in Gala and Red Fuji apple fruits (*Malus domestica* Borkh.) and the possible mechanism. Wounded apples were healed at either 20 or 38 °C for 4 days. During the treatment, ethylene, phenylalanine ammonia-lyase (PAL), peroxidase (POD), polyphenol oxidase (PPO), hydrogen peroxide (H_2O_2), and phenolic and lignin contents were measured. Following the treatment, healed wounds were inoculated with *Penicillium expansum*, *Botrytis cinerea*, and *Colletotrichum acutatum*, and then the decay development was observed. Results revealed that the influence of heating on wound healing in apple fruit was cultivar dependent. Compared with fruits healed at 20 °C, heating at 38 °C had a pejorative effect on wound healing in Gala apples. However, identical treatment enhanced wound healing in Red Fuji apples. Heating sharply reduced ethylene evolution, PAL and POD activity, and the accumulation of phenolic compounds and lignin around wounds in Gala apples. Alternatively, in Red Fuji apples, treatment at 38 °C significantly improved ethylene evolution and peroxide (H_2O_2) content at the first two days of treatment. In addition, both PAL and POD activities, and contents of phenolic compounds and lignin around wounds increased. Our findings suggest that this discrepancy in the effect of heat treatment on wound healing is due to different effects on ethylene evolution in cultivars of apple fruit.

KEYWORDS: Disease resistance; hot air treatment; apple; wounding

INTRODUCTION

Decay caused by fungi results in the most common and devastating postharvest diseases in fruits. Blue mold, gray mold, and anthracnose rot are severe diseases caused by *Penicillium expansum*, *Botrytis cinerea*, and *Colletotrichum acutatum*, respectively. These diseases are present, in apple fruit, even in production areas where the most advanced storage technologies are used (*1*, *2*). As a safe physical treatment, heat treatment can reduce or control the decay development in many horticultural crops (*3*). Heat treatment at 38 °C for 4 days has been recognized as the optimal combination of temperature and time for apple fruit treatment (*4*), it can delay the ripeness, slow softening, improve consumer acceptability, and especially effectively control disease caused by *P. expansum*, *B. cinerea*, and *C. acutatum* on apple fruits (*1*, *4*−*6*). Heat treatment has been acknowledged having a direct inhibitory effect on fungi spores and indirectly affects the physiological responses of fruit tissue in preventing diseases (*7*).

In most cases, fungi require a wound to enter susceptible fruit tissue and initiate infection (*8*). Compared with freshly infected wounds, wounds healed at high or low temperature before fungi inoculation exhibited greater resistance to fungi, resulting in reduced decay development (*8*−*10*). It has been suggested that holding fruit at high temperature and humidity inhibits pathogen growth and is conducive to wound healing in holding fruits (*11*). Although heat treatment promotes wound healing response and reduces decay development in pears (*8*), it is not clear whether the same effect can be produced in apple fruit as a result of heat treatment. On Gala apples, no difference was found in the healing process between 38 and 1 °C (*10*). Lurie et al. reported that no decay was found on wounded Golden Delicious apples following heat-treatment at 38 °C for 4 days before inoculation with *P. expansum* (*12*). However, there were no other temperature treatments (e.g., 20 °C) as controls, and therefore, their reports on the specific effects of heat treatment on wound healing are not conclusive.

In recent years, Gala and Red Fuji apple fruits have become increasingly popular apple cultivars in the world market. Hot air treatment at 38 °C for 4 days is a suitable prestorage treatment for Gala and Red Fuji apples to control disease and/or delay ripeness according to our previous report (*6*). Fresh apple fruits are always transported, stored, or sold at around 20 °C in the Chinese market. The objectives of this study were to investigate the effect of heating (38 °C) on the wound healing process of these two cultivar apples compared to that at 20 °C and explore the possible underlying mechanisms involved.

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Published on Web 03/17/2010

MATERIALS AND METHODS

Fruits. Apple fruits (*Malus domestica* Borkh. cv. Gala; Red Fuji) were harvested at commercial maturity stage according to fruit size and skin background color in 2006 and 2007 seasons from Xuzhou, China. The climacteric rise in carbon dioxide and ethylene production in these apples had not yet occurred. For each cultivar, nearly 560 fruits per year were harvested from the same orchard. Fruit were placed in corrugated cardboard boxes and transported by temperature-controlled trucks (20 °C) to the laboratory within 5 h of harvest. All fruit were uniform in size and color with no physical injury or infection. Apples were surface disinfected with 2% sodium hypochlorite for 2 min, then rinsed with tap water, and air-dried at room temperature.

Pathogens. *P. expansum*, *B. cinerea*, and *C. acutatum* were isolated from rotten apples during storage and maintained separately on potato dextrose agar (PDA, extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g; and deionized water, 800 mL). Spores from a 7-day-old culture were suspended in 0.05% Tween-20 and adjusted to 10^5 conidia/mL using a hemacytometer. In the 2006 season, the concentrations of *P. expansum* spores were adjusted to 10^4 and 10^3 conidia/mL with sterile water.

Effect of Heat Treatment on the Wound Healing of Apple Fruits. All fruits were wounded twice, on opposite sides to a depth of 2 mm by pressing them down with the head of a nail (4 mm in diameter), then wounded apples (Gala, Red Fuji) were randomly divided into two groups each containing 280 fruits. The control group (W-CK), wounded apples were healed at 20 °C for 4 days with relative humidity (RH) of 90%. The experimental group (W-HT): wounded apples were healed at 38 °C for 4 days with 90% RH. Ethylene, phenylalanine ammonia-lyase (PAL), peroxidase (POD), polyphenol oxidase (PPO), hydrogen peroxide (H_2O_2), and phenolic and lignin content were measured during 4 days treatment with 20 apples for each test. All measurements were preformed in triplicate with the experiments repeated in both the 2006 and 2007 seasons.

According to the literature (*8*−*10*, *12*), the effects of wound healing could be revealed by the decay development on the wounded apples that healed at different conditions before fungal inoculation. *P. expansum* was chosen as an indicant pathogen to investigate the resistance ability of healed wounds among two cultivar apples in the 2006 season. After healing at 20 or 38 °C for 4 days, apples of each group (W-CK or W-HT) were subdivided into three smaller samples (20 each) and then inoculated with 15 μL of *P. expansum* spores at different concentrations (10^3, 10^4, or 10^5 conidia/mL, respectively). After inoculation, apples were incubated at 20 °C for 10 days, and the incidence of decay and lesion diameters were measured to indicate the effects of wound healing. This part of the trials was repeated three times in 2006.

On the basis of the obtained results in 2006, the main pathogens of postharvest apples, *P. expansum*, *B. cinerea*, and *C. acutatum* with same concentration, were chosen to investigate the resistance ability of healed wounds to different fungi. After the different healing treatments (20 or 38 °C for 4 days) in the 2007 season, apples of each group (W-CK or W-HT) were also subdivided into three smaller samples (20 each), and then inoculated with 15 μL of different fungi (*P. expansum*, *B. cinerea*, or *C. acutatum*, respectively) at a concentration of 10^5 conidia/mL. The inoculated apples were incubated at 20 °C for 8 days to observe the decay development. This part of the trials was also repeated three times in 2007.

Measurement of Decay Development on Healed Wounds Inoculated with Fungi. Following the healing treatment, healed wounds were inoculated with different fungal suspensions and then incubated at 20 °C to observe the decay incidence and lesion diameters. The decay incidence was expressed as the percentage of infected wounds over the total number of wounds, while the lesion diameter was expressed as the mean of the width and length of decay area as described by Leverentz et al. (*10*).

Measurement of Wound-Induced Ethylene of Wounded Apples during Treatment. Ethylene evolution was measured in W-CK and W-HT apples during the healing treatment at 20 or 38 °C in a 12 h interval up to 96 h. Every 12 h, five pairs of fruits from each group were weighed and sealed in jars at 20 °C for 1 h. Gas samples were collected with a syringe from these sealed jars and injected into a glass Poropak N column of a gas chromatograph (GC-14C, Shimadzu, Tokyo, Japan). Ethylene was determined by using an FID detector as reported previously (*6*).

Measurement of PAL, POD, and PPO Activity of Wounded Apples during Treatment. In order to evaluate the changes of these enzymes (PAL, POD, and PPO) under the different healing treatments (38 or 20 °C, 4 days), cylindrical fresh tissues (5.0 mm distance from the edge of the wound, 10 mm height from the peel) were obtained from 20 W-CK or W-HT apples (Gala or Red Fuji) at the following time points: 0, 1, 2, 3, and 4 days during treatment and then used for determining enzyme activity.

Extraction of Enzymes. All procedures of enzyme extraction were performed at 4 °C. For phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), fresh tissue (2 g) was ground with 5 mL of 100 mM sodium borate buffer (PBS) at pH 8.7 containing 0.037% ethylene diamine tetraacetic acid (EDTA) (w/v), 0.137% β-mercaptoethanol (v/v), and 3% polyvinyl polypyrrolidone (PVPP) (w/v). For peroxidase (POD, EC 1.11.1.7) and polyphenoloxidase (PPO, EC 1.10.3.1), 2 g of fruit tissue was ground with 5 mL of PBS (100 mM, pH 6.4) containing 3% PVPP (w/v). The extracts were homogenized and centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected for enzyme assays.

Enzyme Assays. PAL activity was analyzed by using the method of Assis et al. (*13*). Briefly, 0.5 mL of enzyme extract was incubated with assay medium containing 3.5 mL of 100 mM sodium borate buffer (pH 8.7) and 1 mL of 10 mM L-phenylalanine as substrate at 37 °C for 1 h. The reaction was terminated by adding 0.2 mL of 6 mol L^{-1} HCl. PAL activity was measured by the change in absorbance at 290 nm. Results were expressed as units per gram fresh weight (FW). One unit is defined as the change of 0.01 absorbance at 290 nm per h.

POD activity was assayed by using a modified method of Valentines et al. (*14*). The assay mixture contained 1 mL of enzyme extract, 1 mL of PBS (100 mM, pH 6.4), 1 mL of 0.25% guaiacol (w/v), and 0.01 mL of 0.75% H_2O_2 (v/v). POD activity was measured by an increase in absorbance at 460 nm. Results were expressed as units of g^{-1} FW. One unit of POD activity is defined as a 0.001 increase in absorbance at 460 nm per min.

PPO activity was examined by using a modified method of Valentines et al. (*14*). The assay mixture contained 0.1 mL of enzyme extract, 2 mL of PBS (100 mM, pH 6.4), and 1 mL of catechol (65 mM). PPO activity was determined by an increase in absorbance at 400 nm. Results were expressed as units of g^{-1} FW. One unit of PPO activity is defined as a 0.01 increase in absorbance at 400 nm per min.

Measurement of H_2O_2, Phenolic Compounds, and Lignin Content of Wounded Apples during/after Treatment. Fruit samples were collected as described above. For the determination of H_2O_2 content, 2 g of fresh tissue was homogenized with 5 mL of chilled 100% acetone and centrifuged at 10,000*g* at 4 °C for 20 min. The supernatant was immediately collected for analysis of H_2O_2 content by a method of Patterson et al. (*15*). H_2O_2 content was expressed as μmol g^{-1} FW.

At the end of 4 days of treatment, phenolic compounds and lignin content were measured. For the determination of phenolic compound content, 2 g of fresh tissue was homogenized with 5 mL of 80% ethanol and centrifuged at 10,000*g* at 4 °C for 20 min. The supernatant was immediately collected for analysis of phenolic content by a method of Pan et al. (*16*). The content was calculated by using gallic acid as the standard, and results were expressed as mg kg^{-1} FW.

Lignin content was determined gravimetrically by using a modified method of Femenia et al. (*17*). Fresh sample (2 g) was dispersed in 10 mL of 98% H_2SO_4 at room temperature for 12 h, diluted to 200 mL with deionized water, and heated in a boiling water bath for 6 h. Insoluble material was recovered by vacuum filtration and washed thoroughly with hot water (90 °C) until acid free before drying at 105 °C overnight. The residual weight was recorded as a percentage of lignin content.

Statistical Analysis. Random samples were used in all experiments. Data were expressed as the mean ± SD. SAS Software (version 8.2; SAS Institute, Cary, NC, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) was used to test the statistical difference between groups. Mean separations were performed by Duncan's multiple range tests. A P value < 0.05 was considered as significant.

RESULTS

Effect of Heat Treatment on Wound Healing of Apple Fruits. The effect of heat treatment on wound healing in Gala and Red Fuji harvested in the 2006 and 2007 seasons is shown in **Figure 1** and **Table 1**, respectively. A positive correlation was observed between decay incidence and concentrations of *P. expansum* spore suspension in W-CK and W-HT Gala apples ($p < 0.05$),

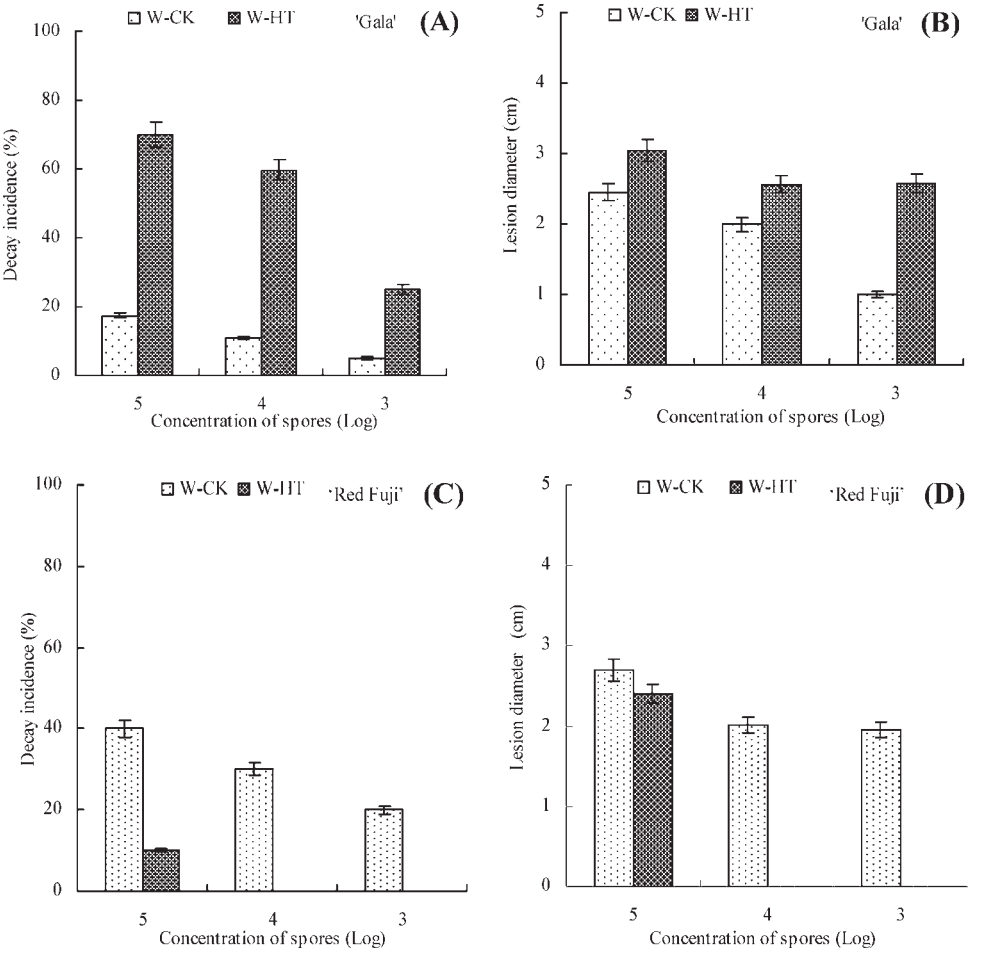

Figure 1. Effects of heat treatment on wound healing in Gala or Red Fuji apples (in 2006). W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples healed at 38 °C for 4 days. Following treatment, healed wounds were inoculated with different concentrations of *P. expansum* and then incubated at 20 °C for 10 days; the incidence of decay (**A**,**C**) and lesion diameters (**B**,**D**) were measured. Data are expressed as means ± SD (n = 6). Vertical bars represent the standard errors of the means.

Table 1. Effects of Heat Treatment on Wound Healing of Gala or Red Fuji Apples (in 2007)[a]

		P. expansum (10^5 conidia/mL)		*B. cinerea* (10^5 conidia/mL)		*C. acutatum* (10^5 conidia/mL)	
cultivars	treatment	decay incidence (%)	lesion diameter (cm)	decay incidence (%)	lesion diameter (cm)	decay incidence (%)	lesion diameter (cm)
Gala	W-CK	5.0 ± 0.4 b	1.0 ± 0.07 b	67.5 ± 4.7 b	3.8 ± 0.26 a	12.5 ± 2.7 b	1.0 ± 0.10 a
	W-HT	42.5 ± 3.0 a	2.3 ± 0.16 a	97.5 ± 1.8 a	3.9 ± 0.25 a	22.5 ± 1.8 a	1.1 ± 0.15 a
Red Fuji	W-CK	50.0 ± 3.5 a	2.1 ± 0.14 a	36.8 ± 2.6 b	3.4 ± 0.24 a	15.0 ± 1.7 a	0.7 ± 0.34 a
	W-HT	35.0 ± 2.8 b	1.9 ± 0.18 a	22.5 ± 1.6 a	3.2 ± 0.23 a	10.0 ± 1.2 b	0.8 ± 0.38 a

[a] W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples heated at 38 °C for 4 days. Following treatment, healed wounds were inoculated with different fungi and then incubated at 20 °C for 8 days; the incidence of decay and lesion diameters were measured. Values followed by a different letter for the same cultivar indicate significant difference using Duncan's multiple range test (p = 0.05).

between decay size and concentrations of *P. expansum* spore suspension in W-CK Gala apples ($p < 0.05$), and between decay incidence and concentrations of *P. expansum* spore suspension in W-CK Red Fuji apples ($p < 0.05$). Therefore, the highest concentration (10^5 conidia/mL) of fungus spore suspension was used in fruits harvested in the 2007 season. Anthracnose rot, caused by *C. acutatum*, in W-HT Red Fuji apples was less obvious than other fungi infections (**Table 1**). Gray mold, caused by *B. cinerea*, in W-HT Gala apples showed the most severe decay development (**Table 1**).

For Gala apples, the decay in the W-HT group was more severe than that in the W-CK group ($p < 0.05$) (**Figure 1** and **Table 1**). In contrast, for Red Fuji apples, both the decay incidence and lesion diameter of the W-HT group was significantly lower than that in the W-CK group ($p < 0.05$) (**Figure 1** and **Table 1**). The data show that the effectiveness of heat treatment varies between apple cultivars. Heat treatment (38 °C) is conducive to the wound healing process in Red Fuji apples but not in Gala apples.

Effects of Heat Treatment on Wound-Induced Ethylene of Wounded Apples during Treatment. Figure 2 presents the effect of heating on wound-induced ethylene evolution during the 4 days of treatment. There was a positive correlation between ethylene evolution and the time of treatment in W-CK groups of both Gala and Red Fuji apples. During the 4 days at 20 °C, ethylene evolution increased sharply from 15 to 147 nL kg^{-1} h^{-1} and from 15 to 85 nL kg^{-1} h^{-1} in W-CK Gala and Red Fuji fruits, respectively. This increase was not found in W-HT Gala apples but was observed in W-HT Red Fuji apples when wounded apples healed at 38 °C. Heating decreased the levels of ethylene in Gala apples from 15 to 5 nL kg^{-1} h^{-1} after the first 12 h. This decreased

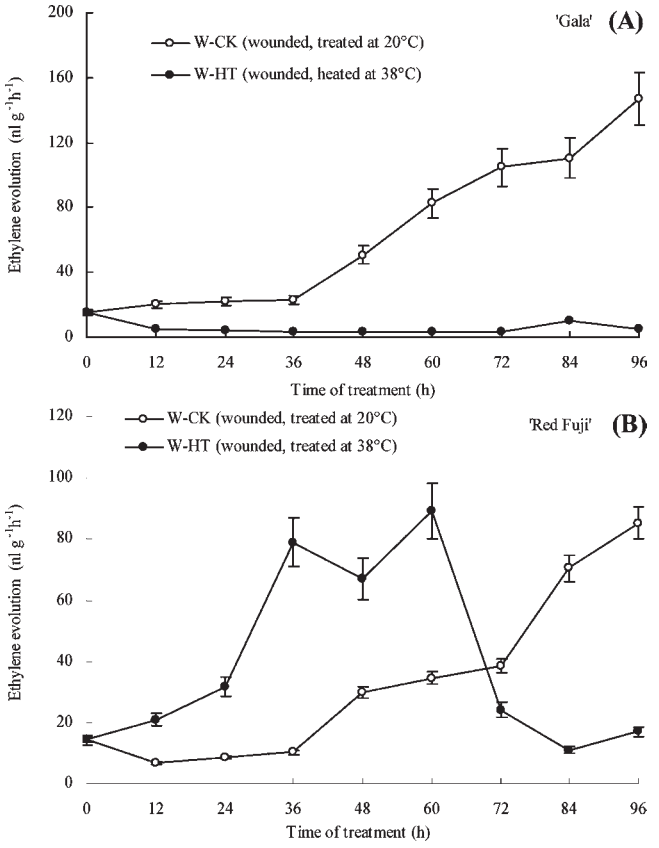

Figure 2. Effects of heat treatment on ethylene evolution of wounded Gala (**A**) or Red Fuji (**B**) apples during the 4 days of treatment. W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples healed at 38 °C for 4 days. Data are expressed as means ± SD (n = 6). Vertical bars represent the standard errors of the means.

level of ethylene was sustained throughout the duration of treatment (**Figure 2A**). Interestingly, ethylene evolution progressively increased in W-HT Red Fuji apples up to 60 h of treatment and reached a peak value of 89 nL kg^{-1} h^{-1} before declining to the baseline (**Figure 2B**). Thus, in terms of ethylene content, the influence of heat treatment on wounded apples is cultivar dependent.

Effect of Heat Treatment on the Activity of PAL, POD, and PPO during Treatment. The effect of heating on PAL, POD, or PPO activity during the 4 days of treatment is summarized in **Figure 3**. For Gala apples, PAL activity increased in the W-CK group until reaching the maximum on day 2 (**Figure 3A**). Alternatively, Gala apples treated at 38 °C (W-HT), showed significantly reduced PAL activity throughout treatment ($p < 0.05$). Red Fuji apples showed a negative correlation between PAL activity and the time of treatment in both W-CK ($r = -0.84$, $p < 0.05$) and W-HT ($r = -0.89$, $p < 0.05$) groups (**Figure 3B**). PAL activity of W-HT was significantly ($P < 0.05$) higher than that of W-CK at the first 2 days and then was lower the rest of the time.

The values of POD activity of Gala apples increased in the first 2 days before declining to the baseline. Peak values were seen on day 2 in both W-CK and W-HT groups (**Figure 3C**). Compared to treatment at 20 °C, heat treatment at 38 °C reduced POD activity in Gala apples. The difference was significant ($p < 0.05$) on day 1, and POD activity values for W-CK and W-HT groups were 15 and 11 Ug^{1-} FW, respectively. Red Fuji apples showed a positive correlation between POD activity and the time of treatment in both W-CK ($r = 0.82$, $p < 0.05$) and W-HT ($r = 0.92$, $p < 0.01$) groups (**Figure 3D**). Higher values of POD activity were observed in the W-HT group than in the W-CK group consistently throughout treatment ($p < 0.05$).

During the healing treatment, Gala fruits demonstrated a positive correlation between PPO activity and the time of treatment in the W-HT group ($r = 0.86$, $p < 0.05$; **Figure 3E**). A higher PPO activity was noted in the W-CK group on day 1 but lower from day 2 to day 4 ($p < 0.05$). For Red Fuji fruit, only day 4 showed significant differences between W-HT and W-CK groups in PPO activity (**Figure 3F**).

Effect of Heat Treatment on the Content of H_2O_2, Phenolic Compounds, and Lignin during/after Treatment. After wounding, the level of H_2O_2 progressively increased in Gala apples, and heat treatment had no influence on the H_2O_2 level (**Figure 4A**). However, levels of H_2O_2 in heat-treated Red Fuji apples (W-HT) were higher than those in unheated control fruits (W-CK). The difference was significant during the first two days of healing treatment ($p < 0.05$; **Figure 4B**).

Table 2 exhibits the effect of heat treatment on phenolic compounds or lignin. At the end of healing treatment, heat-treated (W-HT) Gala fruit showed lower levels of phenolic compounds and lignin than nontreated (W-CK) fruit ($p < 0.05$). In contrast, Red Fuji apples showed significant increase in phenolic compounds and lignin content after treatment (W-HT) ($p < 0.05$). These results suggest that the effect of heat treatment, regarding the accumulation of these materials, in Gala and Red Fuji apples is cultivar dependent. Specifically, heat treatment was conducive to wound healing in Red Fuji but not in Gala apples.

DISCUSSION

This study aimed to clarify specific effects of heating at 38 °C on the wound healing process in different apple cultivars (Gala and Red Fuji). Our results correlate with previous findings that heat treatment has a beneficial effect on the wound healing processes in pears (*8*, *9*). However, the effect of heat treatment on wound healing is not limited to enhancing this process but can also impair it. It was found that the effect of heat treatment on wound healing is cultivar dependent. For Gala apples, decay development was in fact more severe following healing at 38 °C (W-HT) than healing at 20 °C (W-CK) when inoculated with different concentrations of *P. expansum* or three different pathogens (**Figure 1**; **Table 1**). In contrast, decay development of heat-treated (W-HT) Red Fuji apples was significantly lower than that of unheated (W-CK) fruit. Thus, heat treatment at 38 °C impaired the wound healing process on Gala apples but enhanced this process in Red Fuji apples.

Ethylene is a modulator of disease resistance in plants. It plays an important role in mediating different types of induced resistance (*18*) and is required in the transduction path leading from injury (*19*). Production of ethylene has been shown to be an indicator of lignification in wound periderm cell layers in the roots of several sweet potato cultivars (*20*). Thus, ethylene production is an indicator of wound healing. Previous study had shown that wounding induced a marked and rapid increase in the rate of ethylene production in citrus fruits (*21*). In this study, we found that the ethylene evolution of wounded Gala or Red Fuji apples in the control group (W-CK) sharply increased during the treatment (**Figure 2A** and **B**). However, ethylene evolution significantly decreased in Gala apples (**Figure 2A**) within the first 12 h of heat treatment. In wounded Red Fuji apples, heating had the opposite effect and significantly increased ethylene evolution up to 60 h into treatment, after which it decreased (**Figure 2B**). Research suggested that ethylene production was induced within 8 h of wounding accompanied by increased enzyme activity and increased ethylene biosynthesis-related gene expression (*22*), and

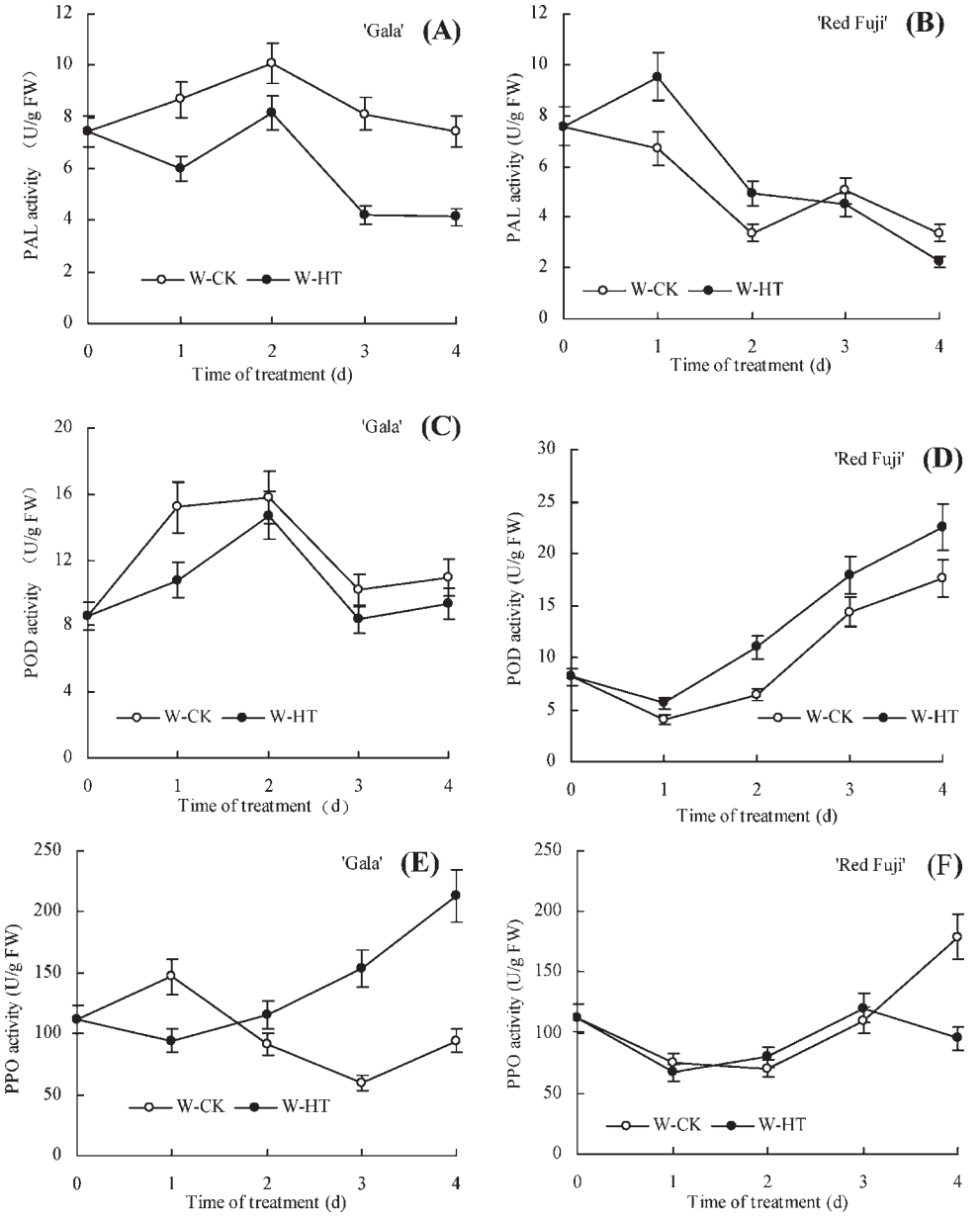

Figure 3. Effects of heat treatment on the PAL (**A**,**B**), POD (**C**,**D**), PPO (**E**,**F**) activity of wounded Gala or Red Fuji apples during the 4 days of treatment. W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples healed at 38 °C for 4 days. Data are expressed as means ± SD ($n = 6$). Vertical bars represent the standard errors of the means.

the wound signal is rapidly synthesized and propagated into adjacent tissue(*23*). On the basis of the literature, it was postulated that heating at 38 °C affects ethylene evolution and alters this resistance induction in apples, resulting in a different response to wound healing. Our results of PAL and POD activity support this point of view (in the following text).

PAL is a key enzyme in the first step of the phenylpropanoid pathway, which is related to the synthesis of phenolic and lignin. It was suggested that enhanced production of phenolic substances near wounded tissue is known to be a common response to wounding, and phenolic substances can be directly toxic to the pathogen or are incorporated into lignified cell walls (*7*, *10*). Besides PAL, POD is involved in the last step in the polymerization of cinnamyl alcohols to form lignin and directly involved in the induction of defense mechanisms (*14*). Lignin can create a physical barrier in the wound, which hinders pathogen penetration and increases the disease resistance ability in fruit (*7*). Researchers found that the increased PAL transcript level can promote the wound healing response (*24*), and anionic POD mRNA appeared at a higher level one day earlier after wound-healing (*25*). Staining of cell walls near healed wounds of apples showed the presence of phenolic substances, lignins, tannins, and callose, which is in agreement with a previous report on pears (*8*). Furthermore, heat treatment can enhance the accumulation of lignin in the wounded sites in lemon fruits (*26*). In our study, heating significantly reduced PAL and POD activity in Gala apples during the healing treatment (**Figure 3A** and **C**). As a result, there was a reduction in accumulated phenolic compounds and lignin around wounds (**Table 2**), providing a weak physical barrier against pathogens. In contrast, heating (W-HT) significantly improved PAL activity two days earlier and POD activity throughout treatment (**Figure 3B** and **D**). This corresponds with increased accumulation of phenolic compounds and lignin (**Table 2**), which provided a strong physical barrier against fungi on wounds of Red Fuji apple. PPO catalyzes the formation of lignin and phenols, providing defense for plant cells in order to

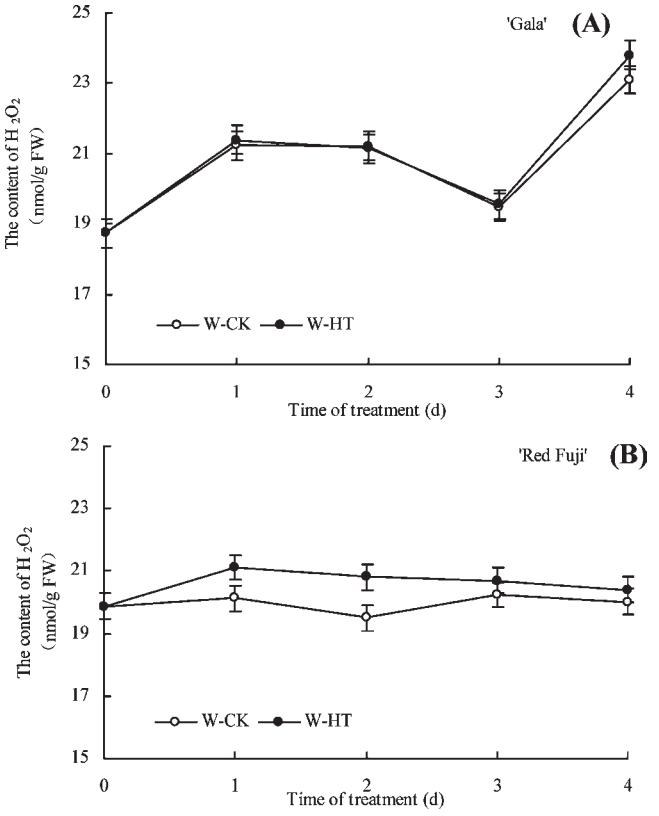

Figure 4. Effects of heat treatment on the content of H_2O_2 of wounded Gala (**A**) or Red Fuji (**B**) apples during the 4 days of treatment. W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples heated at 38 °C for 4 days. Data are expressed as means ± SD ($n = 6$). Vertical bars represent the standard errors of the means.

Table 2. Effects of Heat Treatment on Total Phenolic Compounds and Lignin around the Wounds of Gala or Red Fuji Apples at the End of Treatment[a]

cultivars	treatment	phenolic (mg kg^{-1} FW)	lignin (%)
Gala	W-CK	259.41 a	6.08 a
	W-HT	232.33 b	4.47 b
Red Fuji	W-CK	304.00 b	7.02 b
	W-HT	326.69 a	8.62 a

[a] W-CK, wounded apples healed at 20 °C for 4 days; W-HT, wounded apples heated at 38 °C for 4 days. Values followed by a different letter for the same cultivar indicate significant difference using Duncan's multiple range test ($p = 0.05$).

avoid pathogen infection. However, PPO may be not involved in the biological synthesis of lignin in apple and tomato fruits (*14*, *27*).The changes of PPO by treatments (**Figure 3E** and **F**) were not related to the changes of wound healing in Gala and Red Fuji apples.

One of the most prominent and earliest fruit defense responses is oxidative burst, which generates reactive oxygen intermediates (ROI), such as the superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). H_2O_2 is involved in plant defense responses following mechanical damage or wounding, and its accumulation is induced during wound healing processes, which also involves the lignification process (*28*, *29*). H_2O_2 either as a substrate of the lignification process or as a signal molecule plays a role in the defense mechanisms of apple fruit (*29*), and the inhibited wound-induced H_2O_2 production will strongly reduce lignin and suberin polyphenolic domain deposition along the wound (*28*). Furthermore, the combined heat and *Pichia guilliermondii* treatment stimulated a rapid increase of H_2O_2 and higher lignin deposition (*27*), and the changes of H_2O_2 were related to the changes of superoxide dismutase (SOD) and catalase (CAT) (*27*, *30*). In this study, heat treatment did not significantly alter the H_2O_2 level of Gala apples (**Figure 4A**). However, heating significantly improved the H_2O_2 level of Red Fuji apples after the first two days (**Figure 4B**), which may be contributed to the deposition of lignin. This discrepancy in H_2O_2 levels between Gala and Red Fuji fruit further illustrates evidence of cultivar related differences in effect of heat treatment on wound healing.

In conclusion, the present study found that the influences of heat treatment on wound healing in apple fruits was cultivar dependent. Heat treatment (hot air at 38 °C) impaired the wound healing process in Gala apples but enhanced this process in Red Fuji apples compared to healing at 20 °C. It is suggested that this difference in the wound healing process is due to different effects of heat treatment on ethylene evolution in cultivars of apple fruits.

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Received for review December 8, 2009. Revised manuscript received March 5, 2010. Accepted March 10, 2010. This study was supported by a project grant from the National Natural Science Foundation of China (No. 30771512), International Foundation for Science (No. E/4412-1), and Natural Science Foundation of Zhejiang Province (No. Y3090537).