Mode of Action of Hot-Water Dip in Reducing Decay of Lemon Fruit

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A hot-water dip for 2 min at 52−53 °C prevented decay for at least one week in lemon fruit inoculated with *Penicillium digitatum*. The mode of action of hot water in reducing decay was investigated by studying the effects of this treatment on the pathogen and on the resistance mechanisms of the fruit. The hot-water dip had a transient inhibitory effect on the pathogen, arresting its growth for 24−48 h. During this lag period, the combined effects of the pathogen and the hot-water dip induced the build up of resistance in the peel. Lignin production in the inoculated sites began within 24 h after inoculation or wounding. When inoculation was followed by the hot-water dip, lignin accumulation continued for a week. Inoculated lemons that were not dipped in hot water rotted completely within 3 days after inoculation and their lignin content did not rise or even decreased. The scoparone concentration in the inoculated sites of hot dipped fruit started to rise 24 h after treatment and reached a level sufficient to inhibit the pathogen within 2 days after treatment. Parallel to scoparone accumulation, scopoletin was detected in inoculated and heat-treated lemons. Without the pathogen challenge or wounding, heat treatment by itself was not able to induce any of the above-mentioned defensive effects. Our data do not support the involvement of ethanol-extractable aldehydes, associated in the literature with wound gum, or of citral in decay inhibition in hot-water dipped lemons.

Keywords: *Defense mechanisms, hot-water dip, lignin, scoparone, scopoletin, wound gum, ethanol-extractable aldehydes*

INTRODUCTION

Reduction of postharvest decay, with or without minimal use of synthetic fungicides, is one of the long-standing goals of the citrus industry in Israel. In citrus fruit, the green and blue molds, caused by *Penicillium digitatum* and *P. italicum*, respectively, are the main causal agents of postharvest decay. Both pathogens require wounds to enable them to penetrate the fruit through the exocarp (flavedo) (*1*). Heat treatments, such as curing (34−36 °C for 48−72 h in a saturated atmosphere) or hot-water dips, are promising nonchemical means for controlling postharvest decay of citrus fruit (*2*, *3*).

The presence of lignin in plant tissue is recognized as a factor that increases resistance to infections, as lignins serve as a strong mechanical barrier against pathogen invasion (*4*). Brown et al. (*5*, *6*) found that wound sites in fruit of sweet orange, *Citrus sinensis* (L.) Osbeck cured at 30 °C and 90−96% RH for 24−48 h developed resistance to *Penicillium digitatum*. They proposed that the accumulation of lignin-like materials in cell walls of injured citrus flavedo was the major factor involved in the resistance of healed tissue to penetration by *P. digitatum*. Ben-Yehoshua et al. (*7*) also suggested that the mode of action of heat treatment (curing) in citrus fruit was partly attributable to the rapid lignification of wound areas. Stange et al. (*8*) suggested that wound gum, rather than lignin, is deposited in infection-resistant injuries of citrus peel. Gums are polysaccharides and usually contain some aromatic aldehydes that give a red color with phloroglucinol/HCl (pg/HCl) at room temperature (*9*). Stange et al. (*8*) found antifungal activity in the healed injury sites of orange peels after curing, similar to the findings of Kim et al. (*10*) and Ben-Yehoshua et al. (*11*). However, he associated this antifungal activity with the accumulation of ethanol-extractable aromatic aldehydes. The predominant ethanol-extractable pg/HCl-positive antifungal compound has been identified as 3-[4-hydroxy-3-(3-methyl-2-butenyl)phenyl]-2-(*E*)-propenal (*8*).

The involvement of scoparone as an authentic phytoalexin of citrus has been previously demonstrated by Afek et al. (*12*) and by Ali et al. (*13*) in their study of *Phytophthora* infection of various genotypes of citrus. Other postharvest treatments, such as UV radiation (*11*, *14*, *15*) and yeast biocontrol agents (*16*, *17*), have been shown to elicit scoparone production in various citrus cultivars, in parallel with decay control. Heat treatment by itself did not induce the production of the phytoalexin scoparone in lemon fruit, but markedly enhanced its elicitation by *P. digitatum* (*10*). Scopoletin is another phytoalexin that has been found in citrus fruit (*16*).

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10.1021/jf000700n CCC: $20.00
Published on Web 12/09/2000

In addition to direct thermal inhibition of the pathogen and to induced defense mechanisms (*7*), the effect of curing was related to inhibition of the natural decline of the level of citral, which was shown to be the major constitutive antifungal compound in lemon fruit (*18*).

Hot-water dips have been utilized in the past (*19*), and their use has recently been renewed as a postharvest decay-control treatment (*3*). Dipping citrus fruit in water at 52−53 °C for 2 to 3 min significantly reduced the decay and chilling injury of various citrus cultivars (*20*, *21*). A 2-min water dipping of cv. Oroblanco grapefruit at 52 °C (*22*) and a 2-min water dip of cv. Fortune mandarins at 50−54 °C (*21*), caused a clear redistribution of the epicuticular wax layer and a significant reduction of cuticular cracks, thus improving physical barriers to pathogen penetration. A similar response involving structural changes of epicuticular wax was found with apples subjected to heat treatment at 38 °C for 4 days (*23*). Scanning electron microscope observations of grapefruit fruit inoculated with *P. italicum* showed that exposure to 50 and 52 °C delayed spore germination for 48 h, but did not affect spore viability (*24*).

The purpose of the present study was to investigate the mode of action of a hot-water dip in reducing decay, by observing the effects of this treatment on the pathogen and on the resistance mechanisms of the fruit. The resistance mechanisms studied were lignin production, accumulation of phytoalexins such as scoparone and scopoletin, production of ethanol-extractable aldehyde, and changes in citral concentration.

MATERIALS AND METHODS

Plant Materials and Experimental Design. Mature green lemons *(Citrus limon (L.) Burm.*, cv. Eureka) were obtained from an orchard in Israel. Within 1 day of harvest, the fruit were surface sterilized in 70% ethanol and air-dried. The experiment involved 330 fruit, of which 220 were wounded by piercing the flavedo with a tool which inserted three 0.5-mm diameter needles to a depth of 1.5 mm. Each fruit was wounded in 8 sites, randomly distributed on the fruit surface. After 1 h, the wounds of 110 fruit were inoculated with 10 μL of *Penicillium digitatum* spore suspension (10^4 or 10^5 spores mL^{-1}) (referred to below as inoculated fruit) and 10 μL of sterile water was placed on the wounds of the other 110 fruit (referred to below as wounded fruit). The remaining 110 fruit were kept as unwounded and noninoculated control fruit. Fruit were stored at 21 °C for 20 h and then each of the three groups was then divided into two subgroups of 55 fruit: one subgroup was dipped in water at 52−53 °C for 2 min and the other subgroup was dipped in water at 25 °C. Thus, there were six treatments. The fruit were placed in covered plastic boxes and kept at 21 °C and 100% RH, and decay development was evaluated 2, 3, 4, and 6 days after the dip. In certain cases the inoculated fruit were left in storage as long as 3 weeks. In other experiments the time passed between inoculation and hot-water dip was increased up to 2 days to allow better elicitation.

Sampling. Peel disks excised with an 8-mm-diameter cork borer were used for the measurements of lignin, phytoalexin, and ethanol-extractable pg/HCl positive aldehydes. Samples were taken on the day of harvest (the day before dipping), just before dipping, 4 h after dipping, and 1, 3, 5, and 7 days after dipping. On each sampling day, eight peel disks were excised from each of nine fruit removed from each treatment. Twenty four disks from three fruit were pooled and considered a replication. Thus, the measurements on each sampling day involved three replications (nine fruit), each consisting of 24 disks. The disks from wounded or inoculated fruit were excised from the wounding or inoculation sites; those from the control fruit were excised from sites randomly distributed on the fruit surface.

Lignin Measurements. Lignin measurements were performed according to Morrison (*25*) by means of a modified acetyl bromide procedure. This method has the advantage that other components of the plant cell wall do not interfere with the estimation of lignin. Additionally, the modified method allows the use of very small (milligram) samples and the operating time is shorter.

Peel disks were frozen in liquid nitrogen and lyophilized for 3 days. The disks were then ground to a fine powder, 350 mg of which was washed thoroughly on Whatman No.1 filter paper with the solvents water, ethanol, acetone, and diethyl ether, in sequence, until the filtrate was colorless. The washed powder was transferred from the filter paper to small aluminum cups and dried at 70 °C for 1 h to remove final traces of the solvents. Samples of 20 mg of dried powder were placed in glass-stoppered bottles with a solution of 25% (w/w) acetyl bromide in acetic acid (2.5 mL) and $HClO_4$ (70%, 0.1 mL) and heated in a bath at 70 °C for 30 min with shaking at 10-min intervals to promote digestion. Then the reaction bottles were cooled with ice, and the contents were transferred to a 50-mL volumetric flask containing 2M sodium hydroxide (10 mL) and acetic acid (12 mL). The bottle was rinsed, and the solution was made up to 50 mL with acetic acid. The absorbance of the solution (diluted 1:10) was read at 280 nm.

Ethanol-Extractable pg/HCl Positive Aldehydes Measurements. Peel disks were frozen in liquid nitrogen and lyophilized for 3 days. The measurements were conducted according to Stange et al. (*8*). The disks were ground to a fine powder, 100 mg of which was suspended in 0.8 mL of 75% ethanol and vortexed three times at high speed for 30 s. Solids were precipitated by centrifugation (2 min at 10 000 rpm). The supernatant, 200 μL, was added to 1 mL of 0.2% (w/v) phloroglucinol in 70% ethanol. Five drops of concentrated HCl were added and mixed quickly by swirling. The absorbance of the solution at 545 nm was measured 15 min after mixing.

Phytoalexin Measurements. Scoparone and scopoletin measurements were performed according to Kim et al. (*10*). Peel disks were extracted with dichloromethane at a ratio of 1:10 (w/v) for 1 day and homogenized in an Ultra Turrax TPI8−10 (Janke & Kunkel KG, Germany) for 1 min at full speed. The homogenate was filtered in vacuo through Whatman No.1 filter paper. The residue was washed with solvent twice, and the filtrate was concentrated up to a volume of 2 mL. Fifty μL was pipetted onto a TLC aluminum plate covered with silica gel (Merck) with toluene/ethyl acetate (4:1) as the carrier. The developed sheets were air-dried and exposed to UV light (366 nm). Individual spots were collected (after comparison with authentic scoparone and scopoletin standards on the plate). The scoparone spot was eluted with methanol and the scopoletin spot was eluted with methanol/water (1:1). The analysis for quantitative determination of the phytoalexins was performed on an FL 600 Microplate fluorescence reader by measuring the relative fluorescence intensities for excitation at 355 nm and emission at 460 nm. Standardization of the analysis was performed with authenticated scoparone and scopoletin samples.

Spore Suspension Treatment (in vitro). To simulate the conditions in inoculated fruit, a spore suspension of *Penicillium digitatum* (10^5 spores mL^{-1}) in Czapek Dox medium (containing 33 g sucrose/ L) with 10% orange juice was incubated for 24 h at 21 °C. Eppendorf tubes (1.5 mL), containing 900 μL of sterile water, were placed in a floating rack on a water bath (52 °C). When water in the tubes reached 52 °C (measured by thermometer) 100 μL of the 10^5 spores mL^{-1} suspension of *P. digitatum* was added, and the tubes were kept at 52 °C for 2 min. The measured temperature fluctuation during this period did not exceed 1 °C. The unheated control spore suspension was kept at 25 °C in a water bath for 2 min.

Fungal Growth Measurements. *After in vivo Fruit Treatment.* After the fruit had been dipped, disks from the inoculated sites were excised with a cork borer and scalpel and placed on potato dextrose agar (PDA) in Petri dishes. Three disks were placed in each of three Petri dishes, which were kept at 21 °C. Mycelial growth was measured daily for 1 week.

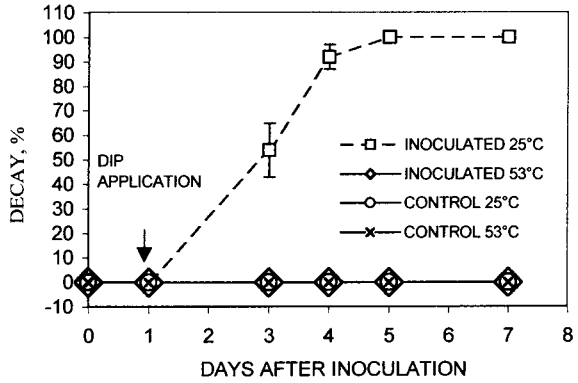

Figure 1. The effect of hot-water dip on decay of inoculated lemons. On the day of harvest, lemons were either inoculated with spore suspension of *P. digitatum* (10^5 spores mL^{-1}) or not treated (control). Fruit were stored at 21 °C for 20 h and then fruit from each group were dipped in water at 52−53 or 25 °C for 2 min. Decay development was evaluated 2, 3, 4, and 6 days after the dip. Bars indicate the confidence interval ($p = 0.05$).

After in vitro Spore Suspension Treatment. After the spore suspension had cooled, 50 μL of the suspension (heated or control) was placed aseptically on PDA plates and the radius of the growing mycelium was measured daily for 1 week. In a parallel experiment, 10 μL of the spore suspension (heated or unheated control) was placed in the wound sites of lemons 1 h after wounding, and decay percentages were measured daily for 1 week.

Citral Quantification. Lemons were dipped in hot water or were dipped in water at 25 °C for 2 min. Fruit were kept at 20 °C for six weeks. Citral concentration was measured on the day of the dip and after 2 and 6 weeks.

Lemon flavedo was removed with a scalpel, and extracted with dichloromethane at ratio 1:10 (w/v). The tissue was homogenized in an Ultra Turrax TP 18-10 and the homogenate was filtered in vacuo, rehomogenized with the same volume of dichloromethane, and filtered in vacuo again. The filtrate was dried over anhydrous magnesium sulfate and concentrated in vacuo on a Buchi Rotavapor RE 120. The analysis was performed with a Varian 3300 gas chromatograph equipped with a flame ionization detector and fitted with an OV-17, Chrom W-HP steel column (6 feet and 1/4 in. O.D). Nitrogen was used as the carrier gas at flow rate of 50 mL min^{-1}. The initial temperature of 120 °C was held for 5 min, the temperature was then increased to 250 °C at 2.5 °C min^{-1}, and then held at 250 °C for an additional 20 min. The temperatures of the injector and detector were 250 and 270 °C, respectively. Quantification of the analysis was done with authentic samples of citral as an external standard. The relative areas of peaks were in positive linear correlation with the concentrations of the citral standard.

Statistical Analyses. Each experiment was repeated three times and analyzed separately by regression analysis. The results of the three experiments were similar, and the data presented are those from one representative experiment.

RESULTS

Effect of Hot-Water Dip on Decay Development. The hot-water dip for 2 min at 52−53 °C inhibited the development of decay in lemons inoculated with *P. digitatum*. When the fruit were heat-treated 1 day after the inoculation, no decay occurred within 6 days after the treatment, whereas fruit dipped in water at 25 °C reached 100% decay after 4 days (Figure 1). When the hot-water dip was applied 2 days after the inoculation, 90% of the treated fruit remained healthy.

In other experiments, decay incidence on hot dipped lemons ranged between 0 and 8% after 7 days, and did not exceed 12% after 3 weeks. Fruit that had not been inoculated did not develop decay during this period whether dipped at 53 °C or at 25 °C.

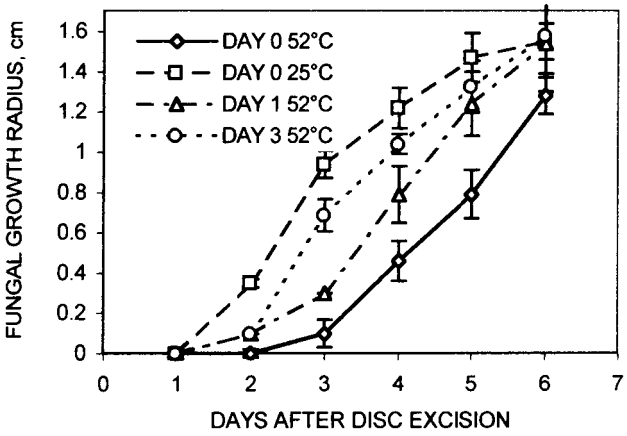

Figure 2. The effect of in vivo hot-water dip on *Penicillium digitatum* growth on disks cut from inoculated fruit. Lemons were inoculated with spore suspension of *P. digitatum* (10^5 spores mL^{-1}). Fruit were stored at 21 °C for 20 h and then dipped in water at 52−53 or 25 °C for 2 min. After dipping (day 0) *P. digitatum* was grown in vitro from inoculated excised lemon peel disks. This was repeated 1 day and 3 days after the hot-water dip (days 1 and 3). The mycelial growth was measured daily for 6 days. Bars indicate the confidence interval ($p = 0.05$).

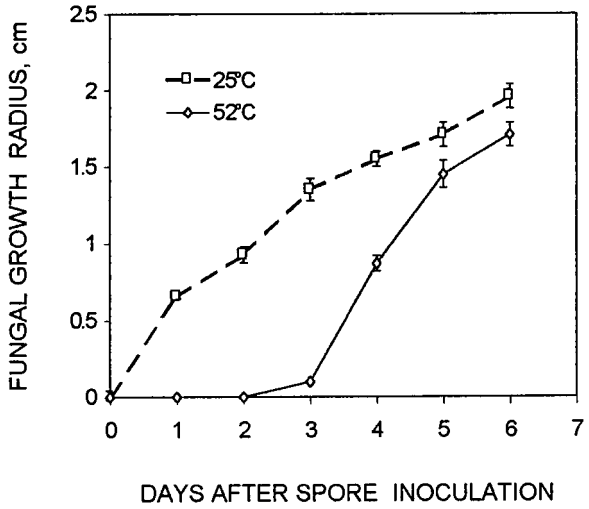

Figure 3. The effect of in vitro heat treatment of *Penicillium digitatum* spores on fungal growth. A spore suspension of *P. digitatum* (10^5 spores mL^{-1}) incubated for 20 h at 21 °C, was exposed in vitro to 52 or 25 °C (control) for 2 min. The spores (heated or control) were placed on Petri dishes containing PDA and the radius of the growing mycelium was measured daily for 6 days. Bars indicate the confidence interval ($p = 0.05$).

Effect of Hot-Water Dip on Growth of *P. digitatum*. The effect of the hot-water dip on *P. digitatum* growth was examined both in vivo and in vitro.

In vivo Heat Application. Mycelium of *P. digitatum* from the peel disks excised from inoculated fruit dipped at 25 °C began to grow 24 h after the disks had been placed on PDA, whereas that from disks excised from hot-dipped fruit began to grow only after 48−72 h. After this initial lag, the fungus from the hot-dipped fruit started to grow and after 6 days reached approximately the same growth radius as the fungus that had not been exposed to heat (Figure 2). This growth inhibition was most pronounced when the disks were excised on the day of heat treatment and it gradually declined when

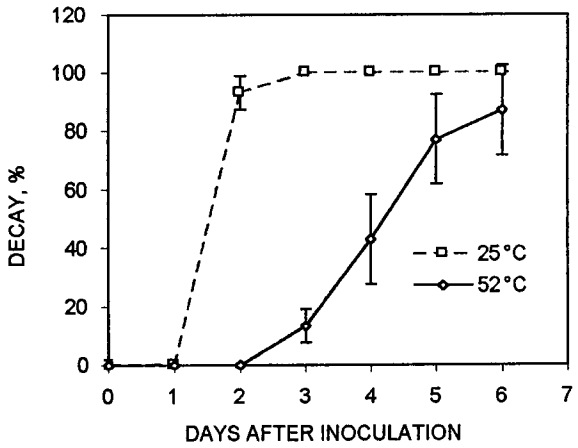

Figure 4. Decay development of lemons inoculated with previously heat-treated *Penicillium digitatum* spores. A spore suspension of *P. digitatum* (10^5 spores mL^{-1}) incubated for 24 h at 21 °C, was exposed in vitro to 52 or 25 °C for 2 min. Lemons were inoculated with the heat-treated or the control spore suspension. Decay development was evaluated daily for 6 days after the inoculation. Bars indicate the confidence interval ($p = 0.05$).

the disks were taken one or 3 days after the heat treatment (Figure 2).

In vitro Heat Application. When the heat treatment (52 °C) was applied directly to spore suspensions of *P. digitatum*, there was a 48 h lag in development when compared with the spores exposed to 25 °C. After this initial lag, the growth rate of the heat-exposed spores was higher than that of the nonexposed spores (Figure 3). When lemon fruit were inoculated with these heat-treated conidia, decay symptoms were observed on approximately 10% of the fruit 72 h after inoculation. In comparison, 90% of the lemons inoculated with nonheat treated conidia developed decay 48 h after inoculation. However, after 6 days of storage, approximately 90% of the fruit inoculated with heat-treated conidia developed decay (Figure 4).

Effect of Hot-Water Dip on Lignin Production. Dipping inoculated lemon fruit in water at 53 °C for 2 min significantly ($p = 0.01$) increased the amount of lignin deposited in the inoculation sites (Figure 5). Within the first 24 h, inoculation induced an increase of 59% in lignin level as compared with that of noninoculated fruit. When inoculation was followed by a hot-water dip, this lignin accumulation continued for an entire week, after which the lignin amount had increased by 200% compared with the initial level. Inoculated lemons that were not dipped in hot water rotted completely 4 days after inoculation and their lignin amount did not rise or even decreased. Wounding the fruit peel in the absence of the pathogen also induced a small rise in lignin level during the first 24 h after wounding. However, this increase was less pronounced than that in the case of inoculated fruit. The hot-water dip induced an additional increase in the lignin level of wounded fruit (Figure 5).

A slow increase of lignin level occurred in unwounded, noninoculated fruit (both heated and not heated) during their 7 days of storage, during which time the lignin level increased by 67%. This increase was not affected by the hot-water dip.

Effect of Hot-Water Dip on Ethanol-Extractable pg/HCl Positive Aldehydes. The accumulation of ethanol-extractable aldehydes in inoculated and subsequently heat-treated fruit did not exceed that of fruit which had only been wounded, whether heat-treated or not (Figure 6). The level of these aldehydes in inoculated and hot-dipped fruit rose 1.7-fold during 7 days. In wounded fruit, the ethanol-extractable aldehyde levels rose 1.9-fold and 2.1-fold in hot-dipped and nonhot-dipped fruit, respectively. In inoculated, nonhot-dipped fruit, ethanol-extractable aldehyde levels doubled 3 days after the inoculation, but all the lemons rotted afterward.

No increases of the aldehyde levels were observed in unwounded and noninoculated fruit, irrespective of hot-water treatment.

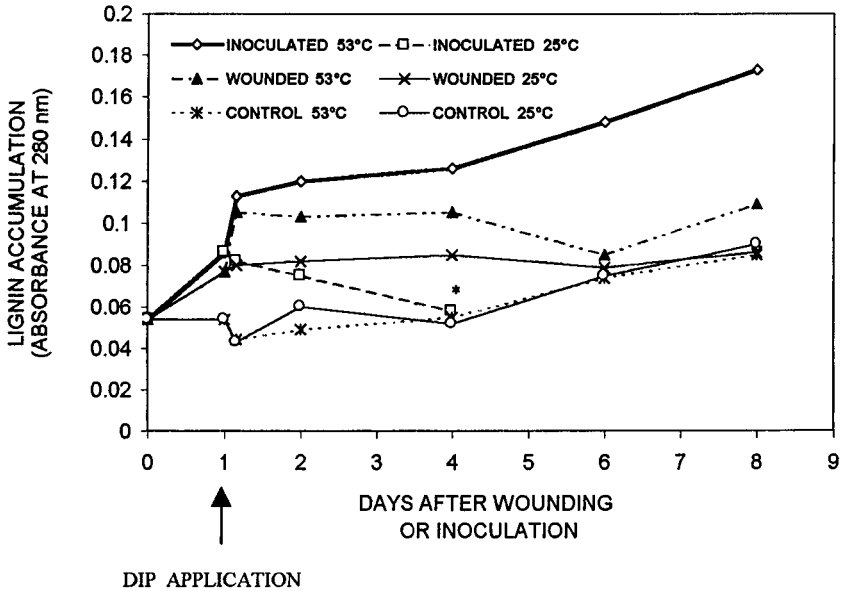

Figure 5. The effect of hot-water dip on lignin accumulation in inoculated or wounded lemons. On the day of harvest lemons were either wounded or inoculated with spore suspension of *P. digitatum* (10^5 spores mL^{-1}) or not treated (unwounded and noninoculated) (control). Fruit were stored at 21 °C for 20h and then dipped in water at 52−53 or 25 °C for 2 min. Peel disks for lignin measurements were taken on the day of harvest (the day before dipping), just before dipping, 4 h after dipping and 1, 3, 5, and 7 days after dipping. *, fruit rotten. Linear regression was performed. Residual standard deviation was 0.029 of lignin accumulation (absorbance at 280 nm) at $p = 0.01$.

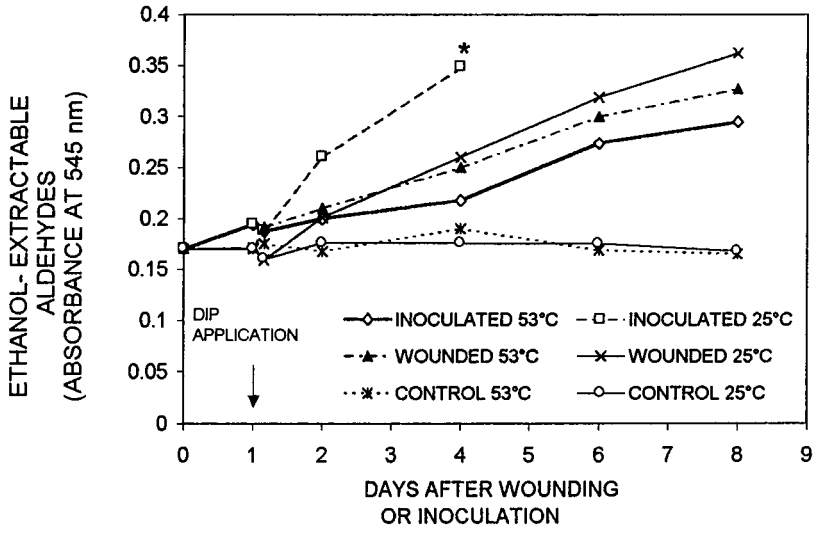

Figure 6. The effect of hot-water dip on accumulation of ethanol-extractable aldehydes in inoculated or wounded lemons. On the day of harvest lemons were either wounded or inoculated with spore suspension of *P. digitatum* (10^5 spores mL^{-1}) or not treated (unwounded and noninoculated) (control). Fruit were stored at 21 °C for 20 h and then dipped in water at 52−53 or 25 °C for 2 min. Peel disks for ethanol-extractable measurements were taken on the day of harvest (the day before dipping), just before dipping, 4 h after dipping, and 1, 3, 5, and 7 days after dipping. *, fruit rotten.

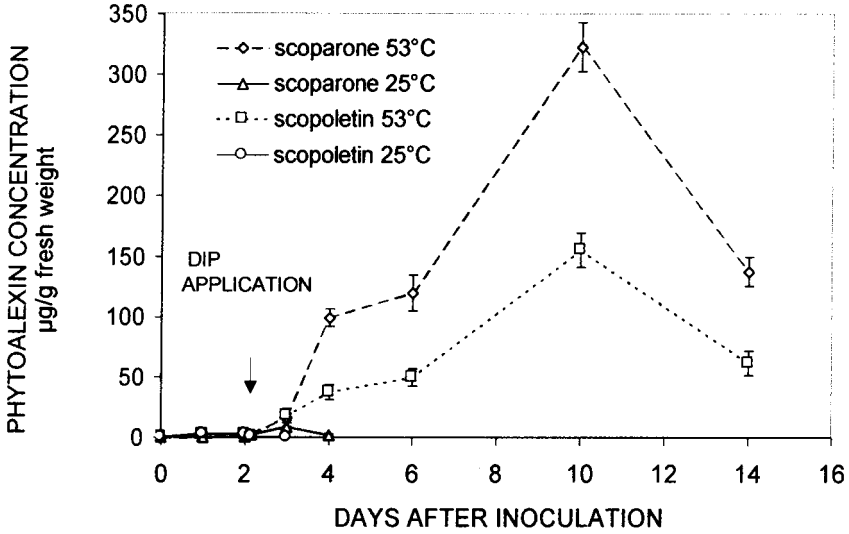

Figure 7. The effect of hot-water dip on scoparone and scopoletin concentrations in inoculated lemons. On the day of harvest lemons were inoculated with a spore suspension of *P. digitatum* (10^4 spores mL^{-1}). Fruit were stored at 21 °C for 48 h and then dipped in water at 52−53 or 25 °C for 2 min. Peel disks for phytoalexins measurements were taken on the day of harvest (two days before dipping), just before dipping, and 1, 2, 4, 8, and 12 days after dipping. Bars indicate the confidence interval ($p = 0.05$).

Effect of Hot-Water Dip on Phytoalexin Accumulation. The scoparone level in lemon peel on the day of harvest was negligible (about 0.5 μg/g fresh weight). In inoculated and nonhot-dipped fruit, scoparone production reached about 9 μg/g fresh weight (FW) 3 days after the inoculation, but already on the third day about 90% of the fruit were rotten and on the fourth day all the fruit had rotted. When the hot-water dip was applied 1day after inoculation, scoparone and scopoletin concentrations did not rise above 10 μg/g FW 7 days after the treatment (data not shown).

However, if the hot-water dip was applied 2 days after inoculation, the scoparone concentration started to rise as early as 24 h after the treatment, and reached a level of about 100 μg/g FW after 2 days (Figure 7). The scoparone concentration continued to increase and reached a peak of 323 μg/g FW after 8 days and then declined to 137 μg/g FW 12 days after treatment. Parallel to scoparone accumulation, another citrus phytoalexin, scopoletin, was detected in inoculated, heat-treated lemons, and it attained concentrations of up to 125 μg/g FW (Figure 7).

Wounding, whether followed by a hot-water dip or not, induced much less scoparone production than the combination of inoculation and heat treatment (less than 20 μg/g FW). In fruit that were not inoculated or wounded, phytoalexins were not observed whether the lemons were dipped in hot water or not.

Effect of Hot-Water Dip on Citral Concentration. No significant effect of hot-water treatment on citral concentration was observed in the present study. In lemons dipped in water at 25 °C for 2 min (control), citral concentration declined from 1150 μg/g FW on the day of harvest to 500 μg/g FW after storage for 6 weeks. A similar decline to 400 μg/g FW was observed in lemons that were dipped in hot water at 52−53 °C for 2 min (data not shown).

DISCUSSION

A hot-water dip at 52−53 °C for 2 min markedly inhibited development of *P. digitatum* in lemon fruit (Figure 1). This dip had a transient inhibitory effect on the pathogen (Figures 2 and 3), temporarily arresting its growth, but not causing its total death. A similar effect of delayed spore germination of *P. italicum* by hot-water dip was found by Dettori et al. (*24*). At least some of the spores treated for 2 min at 52 °C survived the treatment: they were capable of recovery when transferred to the nutrient medium at 21 °C, and were also capable of infecting fruit (Figure 4). This means that the surviving pathogen retained its infective ability. Nevertheless, no decay was observed in the inoculated fruit that had been hot-water dipped (Figure 1). This prevention of decay might be interpreted to mean that in addition to the direct effect of heat on the pathogen, the hot-water dip induced a defense response in the wounded or inoculated fruit. Several compounds, recognized as involved in the lemon fruit defense response, were investigated concerning their role, if any, in the decay-inhibiting effect of a hot-water dip. The earliest effect found in the inoculated fruit in the present study was lignin production, which was elicited by pathogen challenge (Figure 5). This rapid response has been reported previously for many crops (*26*). This initial rapid increase in lignin level was insufficient to prevent decay by itself, as can be seen in the results obtained with inoculated but not-heat-treated fruit, and needed reinforcement. This reinforcement was provided by the hot-water dip, which allowed the fruit to mobilize additional defense mechanisms during the period of temporary arrest of the pathogen growth. The enhanced lignin production and the accumulation of scoparone and scopoletin may be considered as the later-stage components of wound-site reinforcement. The lignin production was already markedly accelerated in the first 4 h after dipping in hot water. Later, the lignin level continued to increase, but at a much slower pace. Lignin production was induced only if the fruit was inoculated or wounded; heat treatment, per se, did not induce lignin production as shown in Figure 5.

As in the case of lignin production, the hot-water dip by itself did not induce scoparone production. However, in inoculated and hot-water-treated fruit, the scoparone content rose, within 2 days after the heat treatment, to a level 3-fold higher than the median effective dose value for the inhibition of germ tube elongation of *P. digitatum* spores (*10*). The present results are in agreement with previous findings of Kim et al. (*10*) and Ben-Yehoshua et al. (*11*) regarding scoparone accumulation in citrus fruit subjected to curing at 36 °C for 72 h. The involvement of scoparone and scopoletin in defense mechanisms was more pronounced when the pathogen was allowed to grow for 2 days before hot water was applied. However, in the case of curing, the production of scoparone occurred when hot air was applied 1 day after inoculation. It is possible that the induction of scoparone and scopoletin production requires sufficient levels of fungal elicitors and these continue to accumulate during the early stages of curing. Probably the pathogen continued to grow for a longer time at the curing temperature of 36 °C before being inhibited by heat, whereas the 52−53 °C hot-water dip caused faster inhibition of the pathogen growth.

Thus, we suggest that the mode of action of the hot-water dip in reducing the decay of lemon fruit is partly related to the thermal inhibition of the pathogen growth, that was arrested for 24−48 h, allowing the infected fruit to build up resistance responses. The resistance mechanisms elicited in inoculated and subsequently hot-dipped fruit included fast lignin production on the inoculation site, later followed by accumulation of the phytoalexins scoparone and scopoletin. In addition to the stimulation of lignin and scoparone production, the effects of the hot-water dip may also involve other factors, such as chitinase and other pathogenesis-related proteins. Rodov et al. (*27*) showed that hot air treatment of *P. digitatum*-inoculated Valencia oranges for 72 h at 36 °C restored the accumulation of one of the chitinases and induced massive scoparone production in the inoculation sites, which acquired local pathogen resistance.

Ethanol-extractable aldehydes reacting positively with pg/HCl developed in the lemon peel as a consequence of wounding and of inoculation (Figure 6). No correlation was found between the inhibition of *P. digitatum* development and the level of ethanol-extractable aldehydes. Furthermore, the highest level of ethanol-extractable aldehydes was observed in fruit that developed decay (Figure 6). The present study suggests that the production of these aldehydes, associated in the literature (*8*) with the wound gum, may be merely a response of the citrus fruit to wounding and not involved in the decay-inhibiting effect of hot-water treatment.

Unlike hot air curing (*18*), the hot-water dip did not inhibit the natural decline of citral, the major constitutive antifungal compound in lemons.

It should be stressed that all the defense responses discussed above were confined to the wounded and the inoculated site. Wounding per se could also induce scoparone and lignin production, but to a smaller extent than wounding combined with inoculation (*10*). We also found that the hot-water dip enhanced the lignin production of wounded fruit, but that this enhancement, as in the case of scoparone, was smaller than that in inoculated and hot-water-dipped fruit. In other words, pathogen challenge or, to a lesser extent, wounding, elicited defense mechanisms in lemon fruit that were reinforced by the hot-water dip.

The hot-water dip, by itself, did not elicit lignin or phytoalexin production in lemons unless the fruit was pathogen-challenged or wounded. The defense mechanisms are activated only when fruit is inoculated. This saves much of the energy and other resources required for the production of all these defense-related compounds. Energy is also saved by concentrating the build-up of the protective mechanisms within the confined volume of the wounded tissue, at just the location where the pathogen is actually present. Furthermore, the restriction of the de novo synthesis of the defensive compounds to only the wounds, presents another advantage of the hot-water treatment for the consumer in that the composition of these defense compounds studied in the fruit changes only negligibly.

ACKNOWLEDGMENT

Special thanks to Ury Zehavi for his great help to the senior author and to Dov Prusky for reading the manuscript. The current paper is a contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel.

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Received for review June 6, 2000. Revised manuscript received October 13, 2000. Accepted October 17, 2000. This research was partially funded by the European Union FAIR CT-4096.

JF000700N