Host–pathogen interactions modulated by heat treatment

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Abstract

Prestorage heat treatment appears to be a promising method of postharvest control of decay. Heat treatments against pathogens may be applied to fresh harvested commodities by hot water dips, by vapour heat, by hot dry air or by a very short hot water rinse and brushing. Heat treatments have a direct effect slowing germ tube elongation or of inactivating or outright killing germinating spores, thus reducing the effective inoculum size and minimising rots. Heat treatment can also indirectly affect decay development via physiological responses of the fruit tissue. These responses include inducing antifungal-like substances that inhibit fungal development in the fruit tissue, or enhancing wound healing. Heat treatment can induce PR proteins such as chitinase and β-1,3 glucanase, stabilise membranes, elicit antifungal compounds, or inhibit the synthesis of cell wall hydrolytic enzymes (polygalacturonases), and delay the degradation rate of pre-formed antifungal compounds that are present in unripe fruit. Additionally, curing, as a heat treatment can cause the disappearance of wax platelets normally present in untreated fruit and make the fruit surface relatively homogeneous. Thus, cuticular fractures, microwounds and most stomata are partially or completely filled, and early-germinated spores are encapsulated and inactivated by molten wax. The occlusion of possible gaps for wound pathogens as well as the encapsulation and inactivation of early-germinated spores have been considered as additional factors in fruit protection against decay. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Postharvest decay is the major factor limiting the extension of storage life of many fresh harvested commodities. All fresh fruits and vegetables for domestic or export markets should be free of dirt, dust, pathogens and chemicals before they are packed. The susceptibility of freshly harvested produce to postharvest diseases increases during prolonged storage as a result of physiological changes that enable pathogens to develop in the fruits (Eckert and Ogawa, 1988). The use of synthetic chemicals on harvested fresh produce is becoming more difficult to justify (How, 1991). Therefore, the interest in 'non-conventional' methods for postharvest decay control of horticul-
tural crops has been increasing. To minimise pre-
or postharvest treatments with agrochemicals (such as e.g. fungicides, insecticides, growth regulators, antioxidants), research efforts are currently focused on enhancement of host resistance to pathogens through physical, chemical or biological inducers (Ben-Yehoshua et al., 1988; Wilson et al., 1994; Ben-Yehoshua et al., 1997b). However, prestorage heat treatment appears to be one of the most promising in postharvest control of decay (Couey, 1989; Klein and Lurie, 1991; Schirra and Ben-Yehoshua, 1999). Besides physical methods, postharvest heat treatments (hot water treatments and curing) are currently practised and recognised as methods of controlling postharvest diseases by direct inhibition of the pathogen and by stimulating certain host-defense responses (Schirra and Ben-Yehoshua, 1999). Additionally, these treatments enhance fruit resistance to chilling injury in sensitive cultivars (Wild and Hood, 1989; McDonald et al., 1991; Rodov et al., 1994a; Schirra and D’hallewin, 1997; Schirra et al., 1997b).

In the first decades of the 20th century, postharvest heat treatment was used on a commercial scale to control fungal diseases and insect infestation of horticultural crops. However, with the development of selective synthetic fungicides, the use of heat treatment was abandoned because of the greater advantages of fungicide treatments in terms of effectiveness, lower cost and ease of application. Many factors, however, have recently contributed to the implementation of strategies for reducing dependence on agrochemicals. These include: the enhanced proliferation of resistant strains of fungus due to the improper and prolonged use of agrochemicals, thereby diminishing their efficacy (Holmes and Eckert, 1992; Eckert, 1995); the prohibitive costs of selecting, synthesising and testing new active ingredients; and difficulties in registering them, as well as the possibility of deregistration of approved fungicides.

The effect of heat therapy on horticultural crops has been reviewed during the last decade (Couey, 1989; Paull, 1990; Barkai-Golan and Phillips, 1991; Klein and Lurie, 1991; Artés, 1995; Lurie, 1998; Schirra and Ben-Yehoshua, 1999; Ben-Yehoshua et al., 2000). This paper deals with the relevant literature on postharvest heat therapy, using citrus fruit as the main example, with emphasis on host-defense reactions in fruits affected by heat treatment.

2. Postharvest heat treatment

Postharvest heat treatment is also known as curing or conditioning. Curing is the objective of most heat treatments, e.g. to cure wounds and injuries caused during postharvest handling. Conditioning is a specific application of heat so as to enable produce to withstand stronger specific stresses such as sub-optimal temperatures; sterilisation as a quarantine treatment against fruit flies is one example. These treatments have been classified as short-term (up to 60 min in water at 45–60°C) or long-term (12 h to 4 days in air at 38–46°C). The long-term treatments will be denoted here as curing treatments (Paull and McDonald, 1994).

2.1. Curing treatment

The first curing experiments on citrus fruit were performed by Fawcett, 1922 to reduce Phytophthora citrophthora (R.E. Sm. and E.H. Sm.) infections. As early as 1936, Brooks and McColloch (1936) reported that heat treatment alleviated chilling injury in grapefruit (Citrus paradisi M.) stored at 2 or 4.5°C. Ben-Yehoshua et al. (1987a,b) demonstrated that curing of seal-packaged fruit at 36°C and saturated humidity for 3 days effectively reduced decay without any damage to various citrus fruit species during storage at 17°C for 35 days. This treatment did not kill the already existing and growing mycelia of Penicillium digitatum Sacc., P. italicum Wehmer and Geotrichum candidum Lk.ex Pers., but suppressed their growth from conidia.

Curing at 32°C for 3 days was found to reduce P. digitatum decay in wounded and inoculated lemons [Citrus limon (L.) Burm. cv. Femminello Siracusano] and oranges [C. sinensis (L.) Osbeck cv. Valencia late], but higher temperatures (36°C) and longer exposure times were required to control this decay in ‘Tarocco’ oranges (Lanza and
Di Martino Aleppo, 1996). These curing conditions caused a delay in conidia germination of *P. digitatum* but did not prevent its growth. However, exposure times longer than 48 h at 36–37°C adversely affected taste and flavour in pigmented orange cultivars (Schirra and D'hallewin, unpublished results).

Investigations with ‘Star Ruby’ grapefruit (D'hallewin et al., 1997) have shown that when curing (37°C for 72 h) was carried out within 36 h post-inoculation, it effectively controlled decay during 30 days of storage at 8°C (optimal storage conditions for this cultivar in Italy, Schirra, 1992) and a subsequent 1 week of simulated shelf-life conditions at 20°C.

2.2. Hot water treatment

In spite of its beneficial effects in reducing decay and chilling injury in various fruit species, curing is not widely utilised on a commercial scale. This is probably because of the high cost of heating large volumes of fruit for up to 3 days. By contrast, water-dip treatments at 50–53°C for 2–3 min have proven to be as effective as curing and are much less expensive (Rodov et al., 1993, 1994a). More recently, interest has also been focused on a unique short hot water rinsing and brushing of agricultural fresh produce (Fallik et al., 1996a).

The beneficial effect of prestorage hot water dipping to prevent rot development has been shown in various fruit species world-wide (Madhukar and Reddy, 1990; Barkai-Golan and Phillips, 1991; Cheah et al., 1992; Jacobi and Wong, 1992; Garcia et al., 1995; Schirra et al., 1996a; Schirra and Ben-Yehoshua, 1999). Such an effect was thought to be partly dependent on the elimination of incipient infections by removing spores from wounds and acting directly on their viability (Couey, 1989). However, investigations at our laboratories (authors’ unpublished data) have demonstrated that spores of *Penicillium spp.* cannot be easily eliminated by water dips as the number of conidia removed from wound-inoculated fruit following 2 min washing with water at 52°C was found to be negligible.

Experimental evidence on grapefruit that was wounded and inoculated with *P. italicum* and incubated for 4 days at 27°C has shown that dipping the infected fruit hemisphere resulted in a complete inhibition of the pathogen development in the submerged hemisphere. However, 6 days after dip treatment, mycelia outbreak and sporulation occurred in the area around the equatorial side of the non-submerged hemisphere (Fig. 1). These results supported the evidence that heat treatment may induce fruit defense mechanisms in the outer layers of epicarp which inhibit pathogen spread (Ben-Yehoshua et al., 1988; Dettori et al., 1996; Fallik et al., 1996b; Ben-Yehoshua et al., 1997b; D'hallewin et al., 1997; Porat et al., 2000b). Although treatment conditions (temperatures and duration of hot water dipping) are very different from those of curing, the direct effect of heat on pathogens appears to be similar.

Investigations on ‘Fortune’ mandarin (*Citrus reticulata* Blanco) a hybrid of the ‘Clementine’ (mandarin group) and ‘Dancy’ tangerine (Young, 1986) have shown that the core temperature of

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Fig. 1. ‘Oroblanco’ grapefruit hybrid with: (a) visible infection of *P. italicum* in the surface of hemisphere of fruit wounded-inoculated, incubated for 4 days at 27°C, submerged in water at 52°C for 2 min and subsequently held at 27°C for 6 days; (b) mycelia outbreak in the area around the equatorial side of non-submerged hemisphere. The arrow indicates the wound-inoculations point.
fruit subjected to curing at 37°C reaches room set-point temperature approximately 3 h after starting treatment (author's unpublished data). By contrast, only the outer cell layer of the flavedo reaches equilibrium with water temperature during 3 min dipping in water at 50–58°C. In fact, 2 min after treatment the albedo temperature in fruits dipped at 50°C was 29.3°C and 33.0°C in those treated at 58°C (Schirra and D'hallewin, 1997). Thereafter temperatures continued to drop, rapidly reaching ambient values (about 18°C) after 90 min. In the same period, the fruit core temperature rose gradually over about 20 min to a maximum of 27.5 and 29.8°C in fruit submerged at 50–58°C respectively, and then decreased, reaching ambient temperature approximately 2 h later.

Like curing, hot water dips at 50–53°C have proven to be ineffective in killing dormant spores but do cause a delay in conidia germination, growth and sporulation (Dettori et al., 1996; D’hallewin et al., 1997). These latter effects may allow fruit to build up or improve defense systems against pathogens. Studies on wounded grapefruit inoculated with *P. digitatum* (D’hallewin et al., 1997) have shown that a 2 min water dip at 50°C is effective against the pathogen when treatment is carried out within 36 h after inoculation. The mycelium was visibly damaged and infection was arrested. Similar results were found on ‘Star Ruby’ grapefruit inoculated with *P. italicum* (Dettori et al., 1996). These findings appear to be important from a practical viewpoint as postharvest treatments in the packing house are usually carried out the day after harvesting. Treatment 48–72 h after incubation did not effectively control decay development. However, inoculum potential was greatly affected by treatment, the incidence of fruit soiled by mold spores being reduced (Dettori et al., 1996). Water dipping at 50°C for 2 min was found to be effective only against superficial infections (3–4 mm deep) and ineffective on deeper infections (> 5 mm). The mycelium morphogenesis pattern of *Penicillium spp.* was markedly different for in vivo than for in vitro growth (Dettori et al., 1996; D’hallewin et al., 1997). In fact, in vivo morphological studies with *P. italicum* growing on ‘Star Ruby’ grapefruit have shown that following water dipping at 50°C 1 h after wounding and inoculation, mycelium development was seriously affected, becoming thinner, with reduced branching, and unable to spread into the albedo. Pathogen growth was also hindered when dip treatments were carried out from 5 to 72 h after wounding and inoculation. By contrast, treatments in vitro with the pathogen growing in Petri dishes did not affect mycelium thickness and branching. Similar results were obtained when the hot water dip was applied to green lemons inoculated with *P. digitatum* (Ben-Yehoshua et al., 1998). Mycelium of *P. digitatum* from peel discs excised from inoculated fruit (in vivo) dipped at 25°C, began to grow 24 h after placing the discs on PDA, while those from hot water (52°C for 2 min) dipped fruit began to grow after 48–72 h. After this initial lag, the hot-water dipped fungus started to grow, and after 6 days reached approximately the same growth radius as that of the fungus that was not exposed to heat. This growth inhibition was most pronounced when the discs were excised from the inoculated fruit on the day of heat treatment and gradually declined when the discs were excised from the fruit taken 1 and 3 days after the heat treatment. When the heat treatment was applied directly to a spore suspension of *P. digitatum* (in vitro), a lag of 48 h occurred as compared to the spores exposed to 25°C. After this initial lag, the mycelia grew at a similar or even higher rate than that of mycelia not exposed to heat. When lemons were inoculated with these heat-treated conidia, decay occurred 24–48 h later as compared to lemons inoculated with conidia, which were not heat-treated. However, after 6 days of storage, approximately 90% of the fruit inoculated with heat-treated conidia, developed decay (Ben-Yehoshua et al., 1998). This means that the pathogen retained its infectivity and, as reported above in the in vivo experiments, spores were capable of recovery when transferred to nutrient medium. Yet no decay was observed in the inoculated fruit that were hot-water dipped. This prevention of decay by the potentially infective conidia might be interpreted to mean that the fruit dipped in hot water exerted some additional inhibitory mechanisms against the pathogen extending beyond the
direct heat effect on the fungus. These results suggest that host–pathogen interactions in vivo may be affected by heat-induced changes in the wounded tissue of the host.

2.3. Application of heat with other postharvest treatments

The combination of curing over 48–72 h at 34–35°C within 48 h of harvest and individual seal-packaging of fruit in plastic film has greatly enhanced the beneficial effects of heat treatment by providing a water-saturated atmosphere and protecting the fruit from damage caused by high temperature (Ben-Yehoshua et al., 1987a,b, 1988, 1989a; Miller et al., 1990). Indeed, sealed and cured pomelo (Citrus grandis (L.) Osbeck) fruit that was not treated with any fungicides was kept for 8 weeks without any damage and decay and its appearance was judged as fresh. Fruit that was sealed but not cured had about 20% decay; fruit that was not sealed and not cured (control fruit) had about 45% decay and the non-decayed control fruit was shrivelled and unmarketable (Ben-Yehoshua et al., 1987a,b). Similar results have been reported with other citrus fruit species (Ben-Yehoshua et al., 1989a,b; Kim et al., 1991; Ben-Yehoshua et al., 1992).

Mayberry and Hartz (1992) reported that the combination of polyethylene bags and water dips at 60°C for 3 min retained quality of muskmelon [Cucumis melo (L.)] for at least 28 days of storage at 3°C.

Investigations on ‘Fortune’ mandarin have demonstrated that a 1.5 min dip treatment with 2,6-dichloro-4-nitroaniline (DCNA) at 51.5°C was much more effective in postharvest decay control than treatments with hot water or DCNA applied separately (Wells and Harvey, 1970). Indeed, after treatment with 225 mg l⁻¹ DCNA at 51.5°C, the residue levels on fruit were found to be similar to those of 900 mg l⁻¹ DCNA dips at room temperature.

Schirra et al. (1996b) have shown that dipping lemons for 3 min in imazalil (IMZ) fungicide mixtures at concentrations ranging from 250 to 1500 mg l⁻¹ at 50°C produced four to fivefold higher levels of fungicide residue on fruit than that dips at 20°C. Treatment with 250 mg l⁻¹ at

3. Heat and fungicide treatments

Extensive work has been conducted in recent years to control postharvest decay of horticultural crops by using heat treatment in combination with agrochemicals. The focus of these efforts is to enhance the effectiveness of the active ingredient (a.i.) in decay control and to minimise the doses of the chemicals being used with respect to conventional treatments. Positive synergistic effects of combined chemical and hot water treatments have been corroborated on various fruit species (Wells and Harvey, 1970; Gutter, 1978; Sharma and Kaul, 1990; Barkai-Golan and Apelbaum, 1991; McDonald et al., 1991; Cohen et al., 1992; Coates et al., 1993; McGuire and Campbell, 1993; Schirra and Mulas, 1993; Conway et al., 1994; Rodov et al., 1994b; Schirra and Mulas, 1995b; Smilanick et al., 1995).

The mechanism of fungicide diffusion through the plant cuticle plays a major role in the uptake of a.i. (Riederer and Schreiber, 1995). During this process, the cuticle acts as a diffusion barrier: as the temperature increases, so does the diffusion and fungicide uptake (Cabras et al., 1999). It is thus possible to reduce the levels of chemicals usually employed in conventional postharvest treatments at ambient temperature without compromising fruit quality and treatment efficacy in reducing decay.

Investigations on peach [Prunus persica (L.) Batsch], plum [P. salicina Lindl.] and nectarine [P. persica (L.) Batsch var. nectarina (Alt.) Maxim.] have demonstrated that a 1.5 min dip treatment with 2,6-dichloro-4-nitroaniline (DCNA) at 51.5°C was much more effective in postharvest decay control than treatments with hot water or DCNA applied separately (Wells and Harvey, 1970). Indeed, after treatment with 225 mg l⁻¹ DCNA at 51.5°C, the residue levels on fruit were found to be similar to those of 900 mg l⁻¹ DCNA dips at room temperature.

Schirra et al. (1996b) have shown that dipping lemons for 3 min in imazalil (IMZ) fungicide mixtures at concentrations ranging from 250 to 1500 mg l⁻¹ at 50°C produced four to fivefold higher levels of fungicide residue on fruit than that dips at 20°C. Treatment with 250 mg l⁻¹ at
50°C was as effective as 1500 mg/l at 20°C in suppressing *Penicillium* rots during 13 weeks of cold storage at 9°C plus 1 week of simulated shelf-life at 20°C. Given the linear relationship between the level of fungicide employed and the residue deposition on fruit, it was postulated in this study that 50 mg/l IMZ should be the minimum concentration at 50°C to achieve a reasonable control of *Penicillium* decay in lemons. This hypothesis was corroborated by later studies (Schirra et al., 1997a).

Similar results on increased activity and a.i. uptake were observed after hot thiabendazole (TBZ) treatments (Schirra et al., 1998a,b). It is worth noting that, in addition to its antifungal activity, TBZ displays physiological properties in reducing chilling injury (Schiffman-Nadel et al., 1972, 1975), and these effects were enhanced when TBZ was used in combination with hot water (see reviews: Paull and McDonald, 1994; Schirra and Ben-Yehoshua, 1999). The increased efficacy of heated compared to unheated fungicide, at the same chemical doses, was related to the increased fruit a.i. uptake (Schirra et al., 1998a), although factors other than TBZ concentration are also involved in the CI-alleviating effects. For example, it has been demonstrated that postharvest treatments with 1200 mg/l TBZ at room temperature (19°C) or 200 mg/l TBZ at 50°C produced similar TBZ uptake in ‘Tarocco’ (blood) oranges, but treatment at 50°C was the most effective in reducing CI (Schirra et al., 1998b).

It is interesting to note that fruit harvested in April (late season) and treated with 1200 mg/l TBZ at room temperature contained significantly more TBZ than fruit picked earlier, probably because of the presence of gaps in the epicuticular wax of the more mature fruit (Freeman et al., 1979; El-Otmani et al., 1989; D’hallewin and Schirra, 2000). In contrast, TBZ deposition following 200 mg/l dipping at 50°C was not significantly dependent on maturity stage due to the enhanced mobility and penetration of the a.i. through the epicuticular wax during treatment. The great persistence of TBZ in fruit treated at 50°C with respect to fruit treated at room temperature was related to the better encapsulation and coverage of the a.i. by epicuticular wax, thus providing better protection to the chemical.

4. Heat treatment and structural changes of epicuticular wax

Investigations on apples have shown that important structural changes of epicuticular wax occurred as a result of curing (Roy et al., 1994). The epicuticular wax of non-heated fruit displayed a number of deep surface cracks that formed an interconnected network on peel surface. In fruit harvested before the climacteric, these cracks became wider and deeper during long-term storage (Roy et al., 1999). A positive relationship occurred between increase in cracking and calcium uptake by fruit subjected to calcium chloride pressure infiltration (Roy et al., 1999). Following postharvest heat treatment at 38°C for 4 days, the cuticular cracks disappeared, probably as a result of the ‘melting’ of the wax platelets that had occurred in the cracks (Roy et al., 1994).

Similar changes in epicuticular wax structure have been observed in various fruit species subjected to heating, such as 2 min water dipping at 52°C of ‘Oroblanco’ (*C. grandis* Osb. x *C. paradisi*, cv. Oroblanco, Syn. Sweety) grapefruit hybrid (Rodov et al., 1996b), 2 min water dip at 50–54°C of ‘Fortune’ mandarins (Schirra and D’hallewin, 1996, 1997), and 3 min water dips at 52°C or curing at 37°C for 24–72 h cactus (prickly) pears (*Opuntia ficus-indica* Miller (L.)) (D’hallewin et al., 1999), as well as after hot water rinsing and brushing (HWRB) sweet pepper (*Capsicum anuum*) (Fallik et al., 1999), melons (*Cucumis melo*) (Fallik et al., 2000) and organically grown grapefruits (Porat et al., 2000a). Thus, fruit response to various types of heat treatments, in terms of changes of ultrastructure of epicuticular wax, appears to be quite similar.

Investigations on ‘Marsh seedless’ grapefruit (D’hallewin and Schirra, 2000) revealed that cuticular cracks became wider and deeper during long-term storage, in accordance with previous results on apples (Roy et al., 1999). Additionally, the outer stomata chamber displayed severe alterations, becoming important invasion sites for wound pathogens such as *Penicillium spp.* (Eckert and Eaks, 1988). By contrast, in fruit subjected to water dips at 50°C, platelets flattened while cracks and most stomata appeared partially (Fig. 2a) or...
prior to penetration, presumably because of topographical stimuli (Mendegen et al., 1988; Isaac, 1992).

Similar results were observed on cactus pears (Schirra et al., 1999): platelets, cracks and stomata which disappeared as a result of heat treatment at 37°C for 30 h appear again after 45 days of storage and subsequent 3 days simulated shelf-life.

5. Heat treatment and host pathogen interactions

5.1. Pathogen response to heat treatment

The efficacy of heat treatment on the pathogen completely plugged by melted wax (Fig. 2b), thereby providing a mechanical barrier against wound pathogens. Moreover, in cactus pears subjected to curing at 37°C for 30 h, germinated spores, conidia and hyphae appeared covered and mummified by molten wax (Schirra et al., 1999). Such beneficial effects, however, may be thwarted during long-term storage or shelf-life conditions as cracks tend to reappear (Fig. 3a) and a number of stomata are seriously damaged (D’hallewin and Schirra, 2000), becoming vulnerable to invasion by fungal hyphae (Fig. 3b). While in freshly harvested fruit hyphae may often grow near or even over stomatal pores, without any attraction or penetration, after prolonged fruit storage, hyphae seem to be attracted toward damaged stomata.
is usually measured by reduced viability of the heated propagules. However, heat effects may be lethal or sublethal (Castejon-Munoz and Bollen, 1993). The response of decay-causing agent to heat can be influenced by several factors such as the moisture content of spores, age of the inoculum and inoculum concentration (Barkai-Golan and Phillips, 1991), as well as the host (Klein and Lurie, 1991).

Although reports have indicated a linear relationship between the logarithm of the reduction time and temperature of heat treatment (Pullman et al., 1981; Roebroeck et al., 1991), pathogen kill is not always proportional to the temperature-time product of the treatment. The sensitivity of fungal spores to heat treatments does not necessarily depend upon the spore size, shape or inoculum age. Fungi express considerable species variation in sensitivity to high temperatures (Sommer et al., 1967; Castejon-Munoz and Bollen, 1993). In vitro experiments showed that Botrytis cinerea was found to be more sensitive to heat than Alternaria alternata (Fallik et al., 1996b), while A. alternata was more sensitive than Fusarium solani (Fallik et al., 2000). Colletotrichum gloeosporioides was found to be more heat-sensitive than Dothiorella dominicana (Rap pel et al., 1991). The effective time–temperature regime that killed 50% of the spores (ET50) for spore germination and germ tube elongation for B. cinerea was significantly shorter than that for A. alternata (Fallik et al., 1996b). Heating germinated spores for a very short time can also affect spore viability. Fallik et al. (2000) reported that the ET50 for Allernaria was 25 and 16 s at 55 and 65°C, respectively, whereas for Fusarium the ET50 was 18 s at 60°C.

Germinating spores were found to be more sensitive to heat treatment than was mycelial growth (Fallik et al., 1993). Penicillium expansum, the main decay-causing agent on stored apples in Israel, was found to be relatively resistant to high temperature. The time to reduce spore germination of P. expansum by 50% (ET50) was found to be 12, 23 and 45% shorter than mycelial growth at 38, 42 and 46°C, respectively (Fallik et al., 1995). Barkai-Golan (1973) reported that germinated spores of Alternaria al-

ternata (formely A. tenuis) were more sensitive to heat than non-germinated spores. Hot water treatments were found to be ineffective in killing dormant spores (Barkai-Golan, 1973; D’hallewin et al., 1997).

The above results obtained by in vitro experiment could explain the direct effect of heat treatment in vivo. A certain threshold of inoculum level is needed to initiate decay development (Yao and Tuite, 1989; Trapero-Casas and Kaiser, 1992). As a result of heat treatments, which reduce fungal viability, the effective inoculum concentration which causes decay development is reduced, thus reducing rot development. Hot water rinsing and brushing (HWRB) was reported to reduce significantly decay development on several fresh harvested commodities (Ben-Yehoshua et al., 1997a; Fallik et al., 1999; Prusky et al., 1999; Fallik et al., 2000; Porat et al., 2000a). Employing HWRB resulted in a 3–4 log reduction of the total microbial counts (CFU) of the epiphytic microorganism population, compared to untreated fruit as was revealed by scanning electron microscopy analysis (Fallik et al., 2000; Porat et al., 2000a). Another explanation for the reduction in rot development could be a reduction in spore survival of various decay causing agents such as with Penicillia in ‘Valencia’ oranges (Williams et al., 1994). Heat may also cause changes in nuclei and cell walls, denature proteins, destroy mitochondria and outer membranes, disrupt vacuolar membranes and formation of gaps in the spore cytoplasm (Barkai-Golan and Phillips, 1991).

5.2. Fruit responses affecting pathogen defense

The mechanisms of fruit defense against pathogens involve complex interactions with many lines of response such as mechanical barriers to microorganisms and antimicrobial chemical compounds and pathogenesis related (PR) proteins (Bell, 1981; Couey, 1989). Part of these are constitutive while others are induced by many factors; heat treatment was shown to affect both mechanisms (Ben-Yehoshua et al., 1995, 1997b, 1998). Loss of fruit resistance to decay has been found to
parallel the breakdown of antifungal compounds that are constitutively present in the exocarp. Heat treatment is known to inhibit ripening in climacteric fruit such as tomato (Lurie, 1998), but to accelerate the ripening in mango fruit (Prusky, 1996). Plumbley et al. (1993) reported that levels of an antifungal diene, constitutively present in avocado epicarp, decreased and disappeared as the fruit ripened; at the same time quiescent infections of Colletotrichum gloeosporioides Penz. occurred. Dry heat treatment delayed decay development in the avocado (Lurie et al. 1997b) whereas water dipping at 55°C for 10 min accelerated both the appearance of disease symptoms and antifungal diene breakdown (Plumbley et al., 1993). Mature green tomatoes are strongly resistant to infections. The increased susceptibility of tomatoes to decay during ripening has been associated with the decrease and disappearance of mRNA encoding an anionic peroxidase (Sherf and Kolattukudy, 1993). Heat treatment of mature green tomatoes was proven to delay the degradation rate of such mRNA and to maintain antifungal resistance in the fruit tissue to decay (Lurie et al. 1997a).

Oil cavities of citrus flavedo (exocarp) constitutecantly contain certain compounds (such as citral in lemons), having antifungal activity (Kim et al., 1991; Ben-Yehoshua et al., 1992; Rodov et al., 1995). Mechanical wounding releases the content of the cavities, which may come into contact with such penetrating wound pathogens as P. digitatum. In young mature-green citrus fruit, constitutive antifungal compounds act as a first line of defense against pathogens. Ben-Yehoshua et al. (1995) reported that curing lemon fruit (3 days at 36°C, 97% R.H.) inhibited the decline of antifungal activity in the flavedo tissue, reduced the loss of citral and suppressed decay development. Such effect of heat has been described as modulation of the ageing-associated decline of naturally occurring antifungal compounds (Ben-Yehoshua et al., 1995).

The presence of lignin in plant tissue is recognised as increasing its resistance to infections as most plant pathogens cannot degrade lignin and it serves as a strong mechanical barrier against pathogen invasion (Friend 1976). Accumulation of lignin-like material and/or antifungal compounds may be induced in response to injuries or infections (Friend, 1976; Stange et al., 1993). Heating wounded pear fruits at 37°C reduced Mucor rot and Phialophora side rot even when fruits were inoculated after heating, demonstrating a wound healing response (Spotts and Chen, 1987). Curing at 32°C and 90% RH for 2 days enhanced fruit resistance to infection by induction of the healing process involving the biosynthesis of lignin-like compounds, which are catalysed by phenyl ammonialyase (PAL), a key enzyme in the phenylpropanoid pathway (Ismail and Brown, 1975; Brown et al., 1978; Ismail and Brown, 1979; Ben-Yehoshua et al., 1987a,b). This lignin-like production creates a physical barrier in the wound, which hinders pathogen penetration. Mulas et al. (1996) reported that various factors such as wounding, chilling injury and senescence increased the accumulation of ethanol-extractable phloroglucinol/HCl positive compounds in flavedo tissue of ‘Oroval’ clementines, suggesting that wound gum deposition rather than lignin was more likely related to the healing process. Investigations with green lemons inoculated with P. digitatum have shown that production of lignin-like material in the inoculation sites started rising within 24 h (Ben-Yehoshua et al., 1998). Wounding induced a smaller rise in lignin level during the first 24 h in comparison to inoculation. Hot water dip, applied 1 day after the inoculation, promoted an additional increase in lignin production, which continued for an entire week after the hot-water dip. By the end of this period, the lignin amount increased by 200% as compared to the initial level. Inoculated lemons that were not hot dipped rotted completely after 3 days. It is interesting that beyond the effect of inoculation in inducing the lignin build up during the first day, the lignin content did not rise and even decreased in the inoculated fruit that did not receive any heat treatment (Ben-Yehoshua et al., 1998; Nafussi et al., 2000). Penicillum expansum inoculated into apple tissue before implementing a dry heat treatment failed to develop rot after the treatment. Spores that were re-isolated from the wound and plated out on potato dextrose agar (PDA) were viable but growth rate was slow with distorted
hyphae (Fallik et al., 1995). The partial recovery that was obtained, the lag in initial germination, and the decreased rate of growth all indicate that there is a residual ‘shock effect’ of heating well after application of the treatment. The physiological phenomenon is strengthened by the finding that adding crude extract from the peel of heated fruit to PDA inhibits fungal growth but also results in distorted mycelia (Fallik et al., 1995). The effect of crude extract on fungal growth supports the hypothesis that antifungal-like substances are also involved in the resistance of heated apple fruit to *P. expansum.*

Studies on various citrus fruit cultivars (Kim et al., 1991) have shown that curing of *Penicillium spp.*-inoculated fruit prevented the development of the pathogen and promoted biosynthesis of the phytotoxins scoparone by cells adjacent to the wound. Hot water dipping induces the occurrence of scoparone and scopoletin in wounds of grapefruit approximately 12–14 h after treatment (D’hallewin et al., 1997). However, their appearance proved not to depend only on pathogenic infections. Ultra-violet illumination, gamma-irradiation, and biological antagonists also induce the production of phytotoxins (Ben-Yehoshua et al., 1992; Rodov et al., 1992; Wilson et al., 1994; D’hallewin et al., 1999). When heat treatment was carried out before wounding, neither scoparone nor scopoletin was detected in post-treated inoculated wounds. Hot water dip applied to green lemons 2 days after inoculation with *P. digitatum* induced scoparone production as soon as 24 h after treatment, and scoparone and scopoletin rose to an effective dose to inhibit the pathogen within 2 days of the treatment. Wounding, either followed by a hot water dip or not, induced scoparone production to a much smaller extent than the combined inoculation and heat treatment (Ben-Yehoshua et al., 1998).

Recently, Porat et al. (2000b) reported that hot water drench of 62°C for 20 s with additional brushing applied to ‘Star Ruby’ grapefruit before the inoculation with the pathogen, induced resistance against decay. This resistance was most effective when the inoculation was carried out 1 day after the brushing and was less effective when the fruit were inoculated at the same day or 7 days after the brushing. The heat treatment was essential to decay control as brushing with cold water did not enhance any resistance.

It has been demonstrated that pathogen-related proteins such as chitinases, which are known to play an important role in hyphae degradation (Bucheli et al., 1990), are constitutively present in orange peel and that their levels increased following pathogen inoculation and subsequent heat treatment (Rodov et al., 1996a). Porat et al. (personal communication) recently found that both β-1,3-glucanase and chitinase were induced by a hot water drench with additional brushing of ‘Star Ruby’ grapefruit applied before the inoculation with *P. digitatum.* They suggested also that various heat shock proteins may be involved in the hot water brushing-induced resistance responses.

6. Concluding remarks

Heat treatments have been developed as a non-chemical method of disinfection of fresh harvested fruits and vegetables. Postharvest heat treatments to control decay are often applied for a relatively short time (seconds to minutes) because the target pathogens are found on the surface or in the first few layers under the skin of the fruit or vegetable. However, in quiescent infection where fungi are located within the tissue fruit, decay development is not necessarily controlled by heat treatment (Johnson et al., 1992). Heat treatments against decay-causing agents may be applied to fruits and vegetables in several ways: by hot water dips, by vapour heat, by hot dry air (Lurie, 1998; Schirra and Ben-Yehoshua, 1999) or by a very short hot water rinse and brushing (Fallik et al., 1996a).

All the methods currently being employed to reduce decay by heat treatment are fungistatic but not fungicidal. The pathogen is markedly inhibited by both thermal inhibition, as well as by the enhanced resistance of the fruit against the pathogen. An important part of this enhanced resistance is related to the ‘welding’ of the epicuticular surface, filling the cracks of the cuticle and preventing the use of these occluded cracks as invasion sites of various pathogens.
Fruit dipping for 2 min in water at 53°C arrested the growth of \textit{P. digitatum} and \textit{P. italicum} for at least 24 h, allowing the infected fruit to build up resistance mechanisms against the pathogen. The following protective mechanisms were elicited in inoculated and subsequently hot-dipped fruit: production of lignin-like material within a few hours and on the inoculation site, followed by accumulation of the phytoalexins scoparone and scopoletin and the production of pathogen-related proteins such as chitinase and heat shock proteins. Hot water dip, by itself, did not elicit lignification or phytoalexin production in lemons and grapefruits unless the fruit was pathogen-challenged or wounded.

Hot drench with such fungicides as imazalil or thiabendazole appears to be an attractive practical solution and is already being implemented in many packing houses. The possibility of drastically reducing the use of fungicides in postharvest treatments of fruit is of considerable practical importance. Indeed, given the enormous amount of these chemicals being employed today throughout the world, fungicide residues may well represent a major threat to human health, with unpredictable consequences for the economy and the environment. The higher costs incurred by the heating of these mixtures with respect to ‘conventional’ treatments should be more than offset by the marked savings on chemicals and the lower costs involved in treatment facilities for packing house wastewater.

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