*Tree and Forestry Science and Biotechnology* ©2009 Global Science Books

# Control of Citrus Postharvest Diseases by Physical Means

**Lluís Palou***

Centre de Tecnologia Postcollita, Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain

*Correspondence*: * **palou_llu@gva.es**

## ABSTRACT

Economic losses due to fungal postharvest diseases are among the most important concerns of the citrus industry worldwide. Typically, these diseases have been successfully controlled by the application of synthetic chemical fungicides. However, human health risks and environmental contamination associated with chemical residues and the proliferation of resistant strains of the pathogens are major problems associated with the continuous and widespread use of conventional postharvest fungicides. There is, therefore, an increasing need to find and implement alternatives such as physical, chemical, or biological postharvest treatments as part of integrated management programs for disease control. In this article, extensive research work based on the evaluation of physical means used alone or in combination with other control methods for citrus decay control is reviewed. Efficacy, general performance, direct and indirect modes of action, potential benefits, advantages and disadvantages, and commercial feasibility of direct antifungal physical treatments, such as heat (curing, hot water dips, and hot water rinsing and brushing) and irradiation (UV-C illumination and ionizing radiation), are discussed. The role of complementary physical means such as storage at low temperatures or in controlled atmospheres to minimize decay losses is also described.

**Keywords:** controlled atmospheres, curing, heat, hot water, integrated disease management, ionizing radiation, irradiation, ozone, UV-C illumination

**Abbreviations: CA**, controlled atmosphere; **GRAS**, generally regarded as safe; **HSP**, heat shock proteins; **HWRB**, hot water rinsing and brushing; **IDM**, integrated disease management; **PAL**, phenylalanine ammonia lyase; **PRP**, pathogenesis-related proteins; **RH**, relative humidity; **SOPP**, sodium ortho-phenil phenate; **US EPA**, United States Environmental Protection Agency; **US FDA**, United States Food and Drug Administration; **UV-C**, far ultraviolet; **λ**, wavelength

## CONTENTS



## INTRODUCTION

Postharvest diseases of citrus fruit are typically caused by filamentous fungi. Some of the most economically important pathogens infect the fruit in the field during the growing season and remain latent or quiescent until they resume growth after harvest because of significant changes in fruit characteristics and environmental conditions. The principal species in this group include *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. [syns.: *Diplodia natalensis* Pole-Evans, *Botryodiplodia theobromae* Pat.; teleomorph: *Botryosphaeria rhodina* (Cooke) Arx] and *Phomopsis citri* H. Fawc. non Sacc. Traverso & Spessa (teleomorph: *Diaporthe citri* F.A. Wolf), which cause the diseases commonly known as stem-end rots; *Alternaria citri* Ellis & N. Pierce in N. Pierce, the cause of alternaria rot or black rot; *Botrytis cine-*

Received: 9 June, 2008. Accepted: 11 October, 2008.

Invited Review

*rea* Pers.:Fr. [teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel], the cause of gray mold; *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. [teleomorph: *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk], the cause of anthracnose; or *Phytophthora* spp., which cause brown rot. Other economically important pathogens infect the fruit through rind wounds or injuries inflicted during harvest, transportation, and postharvest handling. These so-called wound pathogens include *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer, the cause of green and blue molds, respectively; *Geotrichum citri-aurantii* Ferraris E.E. Butler (teleomorph: *Galactomyces citri-aurantii* E.E. Butler), the cause of sour rot; *Trichoderma viride* Pers.:Fr. (syn.: *T. lignorum* Tode), which causes trichoderma rot; or *Aspergillus niger* van Tiegh, the cause of aspergillus rot. Another infrequent, but especially devastating disease, is rhizopus rot, usually caused by the fungus *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. (syn.: *R. nigricans* Ehrenb.). Nests of fungal mycelia are common in *Rhizopus*-infected fruit stored at room temperatures and leakage from decayed tissue, apart from being phytotoxic, carries inoculum that may easily infect adjacent healthy fruit by the action of pectolytic enzymes (Eckert and Eaks 1989; Snowdon 1990; Brown and Eckert 2000).

Actual losses due to all these diseases are quite variable and depend upon the area of production, citrus variety, tree age and condition, weather conditions during the growing and harvest season, the extent of physical injury to the fruit during harvest and the subsequent handling, the effectiveness of antifungal treatments, and the postharvest environment. In general, the incidence of postharvest decay is higher in production areas with abundant summer rainfall, such as Florida, Brazil, or southeastern Asia. The principal diseases in these regions are stem-end rots caused by *L. theobromae* and *P. citri*, which require rain and humid weather for inoculum production and dispersal and subsequent fruit colonization and infection. In production areas with a Mediterranean-type climate, such as Spain and other Mediterranean countries, California, or South Africa, where summer rainfall is scant, the total postharvest decay incidence is considerably lower and the most prevalent causal agents are *Penicillium* spp. and other wound pathogens (Tuset 1987; Smilanick *et al.* 2006a). Penicillium molds, however, are also very important in humid areas (the second cause of decay losses after stem-end rots) because both *P. digitatum* and *P. italicum* reproduce very rapidly and their spores are ubiquitous in the atmosphere and on fruit surfaces and are readily disseminated by air currents. Therefore, the source of fungal inoculum in citrus groves and packinghouses is practically continuous during the season and the fruit can become contaminated and infected in the grove, the packinghouse, and during distribution and marketing. Furthermore, healthy citrus fruit can become unmarketable when "soiled" with conidia of these two fungi that are loosened during handling of diseased fruit. For these reasons, postharvest disease management programs for fresh citrus fruit are primarily based on the control of green and blue molds. Typically, these and other citrus postharvest diseases have been controlled worldwide for many years by the application of conventional fungicides such as imazalil, sodium orthophenyl phenate (SOPP), thiabendazole, or mixtures of these compounds. Currently, new active ingredients such as fludioxonil, pyrimethanil, azoxystrobin, or trifloxystrobin, most of them classified by the United States Environmental Protection Agency (US EPA) as 'reduced-risk' fungicides, have been extensively assayed (Schirra *et al.* 2005, 2006; Smilanick *et al.* 2006b; Kanetis *et al.* 2007; Zhang 2007) and some are already available to citrus growers and packers. For example, Philabuster® 400 SC (Janssen PMP, Beerse, Belgium), a new commercial mixture containing 20% imazalil and 20% pyrimethanil, has been recently registered in Spain for postharvest application against penicillium molds, anthracnose, and gray mold. Postharvest treatments with these synthetic chemicals are typically relatively inexpensive, easy to apply, have curative action against pre-existing or established infections and persistent preventive action against potential new infections that can occur after their application in the packinghouse, and many also inhibit the sporulation from lesions on decaying fruit. However, concerns about environmental contamination and human health risks associated with fungicide residues periodically lead to regulatory reviews and potential restrictions or cancellations. Likewise, traditional citrus export markets are increasingly demanding products with lower levels of pesticides in order to satisfy the safety demands from the general public. In addition, new higher-value markets based on organically-grown, sustainable, environmentally-friendly, ecological, or green agricultural produce are currently arising and becoming more popular. Furthermore, the widespread and continuous use of these synthetic compounds has led to a build-up of resistant biotypes of the pathogens in commercial packinghouses that seriously compromise the effectiveness of these treatments (Holmes and Eckert 1999; Kinay *et al.* 2007). There is, therefore, a clear and increasing need to find and implement methods alternative to conventional fungicides as part of integrated disease management (IDM) programs for the control of postharvest diseases of citrus fruit (Narayanasamy 2006).

According to their nature, these alternative decay control methods can be physical, chemical, or biological (Palou *et al.* 2008b). In this article, physical means evaluated alone or in combination with other treatments for the control of citrus postharvest diseases are reviewed and their potential benefits, disadvantages, and commercial feasibility are discussed. The most important benefits from the use of such treatments as direct antifungal treatments to replace the use of conventional fungicides are undoubtedly the total absence of residues of any kind on/in treated produce and their minimal environmental impact. Some of these treatments, moreover, have shown the ability to initiate, under certain conditions, defense mechanisms in citrus fruit tissues that may contribute to the maintenance of natural fruit resistance to fungal infection. On the other hand, the adequate application during the postharvest phase of physical technological tools such as storage at low temperatures or in controlled atmosphere (CA) conditions may also have great impact in the reduction of decay losses, primarily by inhibiting or reducing the development of the pathogens and also by maintaining the resistance of the fruit to infection.

## HEAT TREATMENTS

Heat treatments are the most common and popular physical means used to control postharvest diseases of fresh fruits and vegetables because they are relatively effective, simple, inexpensive, and easy to apply. Despite their limitations, heat treatments increasingly play a key role in any integrated strategy for nonpolluting postharvest decay control and, as it will be discussed later in this review, their appropriate combination with other physical, chemical, or biological control methods has substantially increased the effectiveness of the resulting integrated treatments. As a postharvest treatment, heat can be applied to the commodities in different ways: hot water dips and sprays, moist (hot vapor) or dry hot air (curing), or infrared or microwave radiation (Lurie 1998; Barkai-Golan 2001). Originally, hot water and vapor heating were developed for postharvest fungal and insect control, respectively, but specific research later showed that in some cases both technologies might be extended for both types of application (Couey 1989; Coates and Johnson 1993). Heat treatments against fungal pathogens are often applied for relatively short periods of time (from several seconds to several minutes) because at the time of treatment most infection structures are present either on the fruit surface or in the outer cell layers of the fruit tissue and heat is need only to affect these tissues to achieve a significant degree of control (Barkai-Golan and Phillips 1991). In general, the primary obstacle to the widespread use of heat against decay-causing microorganisms is the sensitivity of treated produce to the temperatures required

for effective treatment (Couey 1989). The response of fresh produce to heat depends on the commodity treated, the condition of the produce prior to treatment, the temperature and duration of treatment, and the mode of heat application. The physiological responses of different fruits and vegetables to prestorage heat treatments can vary by season and growing location, and can be due to differences in climate, soil type, season, production practices, maturity at harvest, and produce size (Fallik 2004, 2007). In the particular case of fresh citrus fruit, the systems of postharvest heat application that have been more extensively evaluated for fungal decay control include curing, hot water dips, and hot water rinsing and brushing (HWRB).

## Curing

Typical procedures for a thermal curing treatment of citrus employ exposure of the fruit for 2-3 days to an air atmosphere heated to temperatures higher than 30°C at high relative humidity (RH >90%). It appears that the term curing was adopted for this treatment after evidence that a significant number of rind wounds healed (cured) and resisted infection following exposure to hot air (Ben-Yehoshua and Porat 2005). The first citrus curing experience was conducted by Fawcett (1922) against brown rot caused by *Phytophthora* spp. In 1948, Hopkins and Loucks reported significant reductions of the incidence of green mold, caused by *P. digitatum*, on oranges after exposure to 30°C and 90-100% RH for several days. Since then, numerous studies have demonstrated the intense curative activity of these treatments against postharvest penicillium molds in a variety of citrus species and cultivars (Ben-Yehoshua *et al.* 1987; Stange and Eckert 1994; Porat *et al.* 2000b; Lanza *et al.* 2004; Erkan *et al.* 2005; Kinay *et al.* 2005). For instance, research work in Catalonia (Spain) showed that after 1 week of incubation at 20°C, both green and blue molds were reduced by more than 95% in artificially inoculated oranges exposed to 33°C for 65 h. Furthermore, this treatment reduced the incidence of total decay by more than 90% on naturally infected oranges. However, on treated fruit stored for 2 months at 4°C and held at 20°C for 1 week as a period of shelf life simulation, control of blue mold was significantly lower than that of green mold and was not satisfactory (Plaza *et al.* 2003). Since *P. italicum* grows faster than *P. digitatum* at lower temperatures and the incidence of blue mold is higher than that of green mold on fruit stored at temperatures lower than 10°C (Brown and Eckert 2000), this result suggests that curing treatments would be less effective on citrus fruit cold-stored for long periods, which is an additional handicap for commercial adoption of this technology. In other trials in València (Spain), Tuset *et al.* (1996) found that curing naturally infected 'Washington navel' oranges at 35°C for 72 h reduced the total incidence of decay by 86% after storage at room temperature for 21 days. After this period of time, decayed fruit were infected in order of decreasing importance by *P. digitatum*, *P. italicum*, *A. citri*, and *B. cinerea*.

In contrast to penicillium molds, other important citrus postharvest diseases such as stem-end rots or sour rot are not effectively controlled by curing treatments. In humid production areas like Florida, usual postharvest handling procedures include degreening of early season fruit with continuous exposure to gaseous ethylene at about 30ºC and high RH for more than 48 h. As shown by early work by Brown (1973), this degreening process approached a curing treatment and partially controlled green mold. However, these environmental conditions and especially the presence of ethylene typically increase the incidence of other diseases, such as anthracnose (Brown and Barmore 1977) and stem-end rots (Brown and Lee 1993), probably by both direct effects on pathogen development and indirect effects on fruit tissues that increase their natural susceptibility to these particular diseases. From their work with 'Valencia' oranges from Florida, Zhang and Swingle (2005) reported that while a curing treatment of 35ºC for 48 h satisfactorily reduced the incidence of green mold, it was not effective against stem-end rot caused by *L. theobromae*. The optimal temperatures for *in vitro* mycelial growth of *P. digitatum* and *L. theobromae* were about 25 and 30°C, respectively.

The inhibitory effect of curing treatments on disease development primarily depends on the treatment conditions, namely temperature, RH, and duration, but the type of fruit and their physical and physiological condition when the treatment is applied are also very important factors to account for. According to Lanza and Di Martino Aleppo (1996), a temperature of 32°C applied for 3 days was a severe enough curing treatment to reduce the incidence of green mold on artificially inoculated 'Femminello' lemons and 'Valencia' oranges to 0 and 2%, respectively. On the other hand, temperatures of 36°C and longer exposure times were required to reach similar disease control levels on 'Tarocco' oranges, a cultivar that is less resistant to infection by *P. digitatum*. In spite of their good efficacy against citrus green and blue molds, commercial implementation of curing treatments for decay control is rare, firstly because of the expense of heating and immobilizing large amounts of fruit for relatively long periods and, secondly, because excessive or uncontrolled treatments may harm or adversely affect the fruit. Fruit weight loss and heat phytotoxicity are major potential risks associated to the use of curing treatments. Their incidence, similarly to treatment effectiveness, depends not only on treatment conditions but also on the type of fruit and their initial condition. Other risks are the inhibition of pigment synthesis in the peel and the loss of flavor quality. Besides the loss of fruit value caused by apparent heat damage (rind pitting or irregular brownish staining), injured fruit are more susceptible to the attack by contaminating microorganisms and show a decreased shelf or storage life as a consequence of undesirable increments in fruit respiration or ethylene production rates (Ben-Yehoshua *et al.* 1990; Barkai-Golan and Phillips 1991; Mulas *et al.* 2008). Therefore, curing treatments should be particularly designed for each citrus species and cultivar, taking also into account the particular initial characteristics of each set of fruit and their more appropriate postharvest handling. In general, they would be better tolerated by less mature citrus fruit with excellent rind physical condition that are transported to the packinghouse immediately after harvest and are not intended for long-term cold storage.

Besides combination with other control methods, which will be discussed in another section, new technological approaches for curing treatment include intermittent curing (two 18-h cycles at 38°C; Pérez *et al.* 2005), curing at higher temperatures for reduced periods of time (18 h at 40°C; Nunes *et al.* 2007), or, in the case of low rainfall areas where early season mandarins are degreened with 5-10 µL/L ethylene at about 20°C for 2-3 days, the integration of curing treatment in the degreening process (Plaza *et al.* 2004a). On the other hand, it has been recently determined that exposure to hot air at 50°C and RH higher than 75% for 1 day effectively killed spores of *P. digitatum* and it could be used as a good sanitation practice for empty storage rooms (Smilanick and Mansour 2007).

## Hot water dips

Similarly to curing, immersion in hot water was first evaluated in California citrus packinghouses to control brown rot (Fawcett 1922). During the 1960s, it was observed that 5 min of immersion in water heated to temperatures of about 50°C in packinghouse washing tanks was effective against green mold on oranges (Smoot and Melvin 1963) and lemons (Houck 1967). Likewise, relatively brief immersions (2-5 min) in water at 45-55°C have repeatedly shown value in reducing citrus postharvest diseases, especially green and blue molds, in a wide variety of citrus species and cultivars (Spalding and Reeder 1985; Couey 1989; Rodov *et al.* 1995a; Tuset *et al.* 1996; Schirra and D'hallewin 1997; Schirra *et al.* 2004; Erkan *et al.* 2005; Hong *et al.* 2007). Research conducted in Catalonia with various artificially

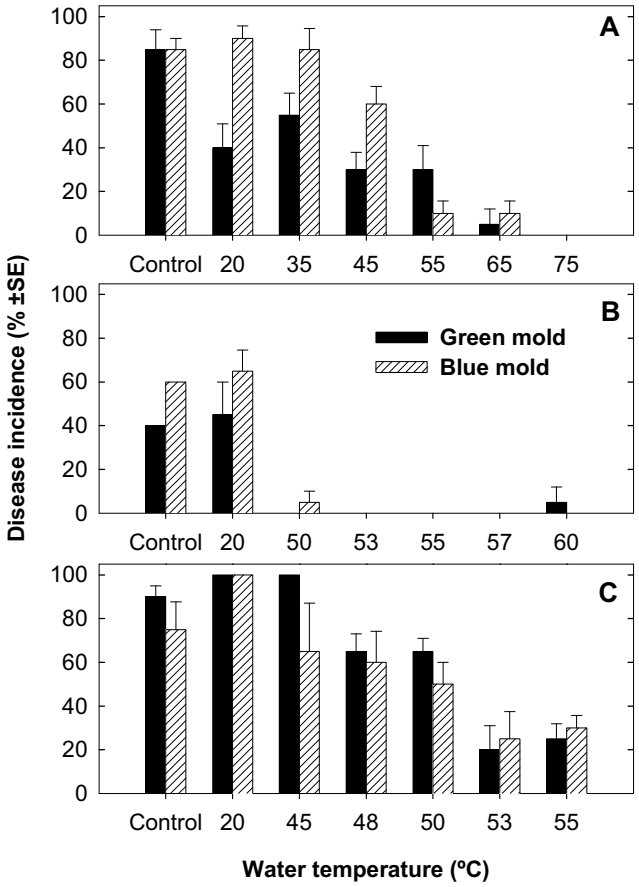


**Fig. 1** Incidence of green and blue molds on artificially inoculated 'Navelate' (**A**, **B**) and 'Valencia' (**C**) oranges immersed for 150 s in water at various temperatures and incubated at 20°C and 90% RH for 7 days. Control fruit were inoculated but not treated.

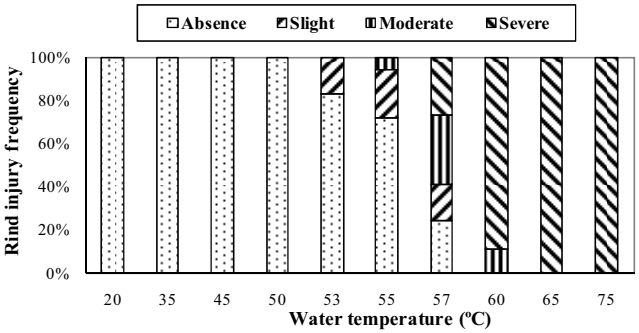


**Fig. 2 Influence of water temperature on the incidence of rind injuries on 'Navelate' oranges immersed for 150 s and incubated at 20°C and 90% RH for 7 days.**

inoculated orange cultivars (Palou *et al.* 1999, 2001b) showed that among the wide range of water temperatures tested, those from 50 to 55°C were the most effective to reduce both green and blue molds on oranges dipped for 150 s and incubated at 20°C for 7 days. The effectiveness of the treatments depended not only on the cultivar but also on the particular lot of fruit (**Fig. 1**). In general, lower and higher temperatures were ineffective and phytotoxic, respectively. Specifically, after 7 days of incubation at 20ºC, slight rind blemishes were present on about 15 and 30% of 'Navelate' oranges treated at 53 and 55°C, respectively, while severe heat injury was observed on the rind of 30 and 90% of the oranges treated at 57 and 60°C, respectively (**Fig. 2**). These results were in agreement with those reported by other authors, who noticed injury to the peel of a variety of citrus fruit after 1-3 min dips in water at temperatures higher than 53°C (Houck 1967; Barkai-Golan and Apelbaum 1991; Schirra and D'hallewin 1997; Schirra *et al.* 2000). In similar experiments with 'Clemenules' clementine mandarins (Palou *et al.* 2002a), the effectiveness of hot water against *P. digitatum* and *P. italicum* was lower than on oranges, showing again the great influence of the fruit host and its condition on the curative activity of hot water. Further, Schirra *et al.* (1998) demonstrated that the incidence of postharvest decay on hot water-treated oranges significantly varied with fruit maturity at harvest.

Hot water dips are a technology that is easier, cheaper, and more feasible for heat application than curing. Since water is a more efficient heat-transfer medium than air (Wang *et al.* 2001), temperature equilibrium in the tissues of treated fruit is reached faster and the duration of the treatments might be reduced. Current technological approaches for the application of dips in hot water or heated aqueous solutions include the batch and the continuous systems. In the former, the produce is loaded onto a platform that is lowered into the hot water container, while in the latter the produce moves slowly from one end of the water tank to the other (Fallik 2007). This latter system can generally be found in citrus packinghouses, although it is very rarely used for the application of hot water alone. In fact, commercial application of hot water as a stand-alone treatment for citrus decay control is limited to small fruit like kumquat, whose peel is also eaten, or some organically-grown fruit (Ben-Yehoshua and Porat 2005). This is primarily due to the lack of the treatment to provide persistent protection from subsequent infections. Another important reason is that the range of effective yet nonphytotoxic temperatures is very narrow. The mode of action of hot water dips at nonphytotoxic temperatures is not fungicidal and their curative activity against preexisting fungal infections is limited. According to Schirra and D'hallewin (1997), the temperature in the albedo of mandarins dipped for 3 min in water at 50 and 58°C decreased to 29 and 33°C, respectively, during a 2-min period following the treatment, and it reached an ambient temperature of 18°C after 90 min. It has been also noted that the curative effect of hot water is also greatly influenced by the period of time between fungal infection and treatment. For example, immersion of grapefruits in hot water was not effective against *P. italicum* when the time between the inoculation of the pathogen and the treatment was 48 or 72 h (Dettori *et al.* 1996). Finally, other limitations to the commercial use of hot water dips in citrus packinghouses are related to technological aspects of treatment application. Immersion in water for several minutes requires the use of large high-volume tanks to avoid delays in packing operations during production peaks in the season and both the implementation and maintenance of this equipment are expensive. Further, energy costs needed to heat very large volumes of water are also high. Nevertheless, the commercial use of large tanks is common in production areas like California, although they rarely use hot water alone.

## Hot water rinsing and brushing (HWRB)

Some of the technological limitations associated with the use of hot water dips could be overcome by the implementation of this system. It was developed in 1996 in Israel by Fallik and coworkers, initially for the postharvest treatment of bell peppers (Fallik 2007). HWRB consists basically in packingline machinery that cleans and disinfects fresh produce by the application of hot water over rotating brushes at a relatively high temperature (55-65°C) for a very short time (10-30 s). When this technology was evaluated with citrus fruit, Rodov *et al.* (2000) found that the application of water at 56 or 60°C for 10 s effectively reduced postharvest diseases, especially penicillium molds, of the pummelo grapefruit hybrid 'Oroblanco'. Porat *et al.* (2000a) determined that HWRB at 56°C for 20 s reduced decay by 45-55% on organically-grown tangerines, oranges, and red grapefruits, with no rind injuries or adverse influence on fruit weight loss or internal quality parameters. These researchers observed that an indirect mode of action of

HWRB in grapefruits involved the induction of fruit resistance against *P. digitatum* (Porat *et al.* 2000c). Likewise, satisfactory control of green mold was obtained on artificially inoculated oranges and lemons with HWRB at 62.8°C for 30 s. Nevertheless, the incidence of sour rot, caused by *G. citri-aurantii*, was not significantly reduced under similar conditions (Smilanick *et al.* 2003). Similarly, significant control of incipient (24-h old) infections of *P. digitatum* on lemons and oranges was observed by Lanza *et al.* (2004) after HWRB treatment at 62°C for 20 s. On kumquat fruit, the best HWRB treatment conditions for decay control while maintaining fruit quality were 55°C for 20 s (Ben-Yehoshua and Porat 2005).

HWRB technology is commercially available and used in Israel, Spain, or some countries in South America for treatment of produce, such as peppers, tomatoes, mangoes, or melons. The use with citrus fruit is restricted to kumquats and organically-grown citrus cultivars in Israel (Ben-Yehoshua and Porat 2005). A complete description of the machinery can be found in Fallik (2004).

## IRRADIATION TREATMENTS

Harvested horticultural produce can be treated with radiation of energy higher [shorter wavelength (λ)] than that of visible light. Depending on the region in the electromagnetic spectrum, irradiation of fresh fruits and vegetables include the application of far ultraviolet light (UV-C, $100 < \lambda < 280$ nm) and ionizing radiation ($\lambda < 100$ nm). Obviously, the technological needs for the application of these irradiation treatments radically differ one from each other, and also from those needed for the application of heat. However, postharvest treatments based on each of these physical technologies are designed to provoke very similar mechanisms in fruit physiology and to obtain similar beneficial fruit responses, not only for pathogenic decay control but also for delay of fruit senescence and overall extension of storage potential and shelf life. Therefore, similarly to heat, citrus fruit responses to irradiation are strongly conditioned by the type of fruit and its initial condition, especially maturity at harvest.

### Far ultraviolet (UV-C) illumination

Depending on radiation wavelength, the UV spectrum has been divided into three regions: near UV (UV-A, $320 < \lambda < 400$ nm), mid-range UV (UV-B, $280 < \lambda < 320$ nm), and far UV (UV-C). Among these regions, only UV-C is suitable for effective postharvest treatment of fresh produce. Specifically, UV irradiation at 254 nm is used because this is the most efficient wavelength for this purpose. However, depending on UV dose (product of irradiance or radiation intensity in W $m^{-2}$ by exposure time in seconds and expressed in J $m^{-2}$), these treatments may also deleteriously affect plant tissues. Thus, UV-C exposure conditions should be optimized for each specific commodity and application case in order to maximize effectiveness without causing phytotoxicity (Ben-Yehoshua and Mercier 2005). Furthermore, these treatments may also cause significant harmful effects on exposed humans, so appropriate safety measures must be implemented whenever UV-C illumination devices are operated. The sources for UV-C are low- or medium-pressure mercury discharge lamps that consist of quartz tubes filled with an inert gas, such as argon, and small quantities of mercury. For practical commercial implementation of this technology in fresh produce packinghouses, banks of these lamps should be integrated in the packinglines. The lamps should be shielded and correctly located to avoid direct radiation exposure to packinghouse workers and personnel. An additional hazard is the release by UV irradiation at $\lambda < 260$ nm of gaseous ozone, which should be monitored and adequately vented if necessary (Civello *et al.* 2007).

Exposure of citrus fruit to UV-C doses ranging from 0.5 to 8 kJ $m^{-2}$ has repeatedly reduced postharvest decay losses in different citrus species and cultivars. As it will be discussed in detail later, both direct and indirect effects of the treatment have been described. In general, the prevalent mode of action of UV-C light for the control of citrus postharvest diseases is the induction of beneficial responses in the fruit host (Ben-Yehoshua *et al.* 1992; Droby *et al.* 1993). Droby *et al.* (1993) reported that the incidence of green mold was significantly reduced in 'Marsh Seedless' grapefruits exposed to UV-C light and artificially inoculated with *P. digitatum* 24 h later. The induction of resistance by UV-C irradiation reached a maximum after 24-48 h and was significantly influenced by treatment dose, harvest date, and the temperature at which the fruit was stored following the treatment. The UV-C dose required for development of maximum resistance increased as the season progressed. Biochemical resistance mechanisms were actively induced in grapefruits kept at 11, 17, 20, or 25°C for 24 h between treatment and fungal inoculation, but similar responses were not observed in fruit maintained at 6°C. The development of only sporadic fungal mycelium and marked inhibition of sporulation were observed in irradiated fruit. Similar findings regarding the influence of harvest date were reported by D'hallewin *et al.* (1999b, 2000) working with 'Washington Navel', 'Biondo Comune', 'Tarocco', and 'Valencia' oranges and 'Star Ruby' grapefruits. Moreover, D'hallewin *et al.* (2000) noted that the critical threshold for effective control of green mold on 'Star Ruby' grapefruits was a dose as low as 0.5 kJ $m^{-2}$. Higher doses did not further improve decay control and caused rind injuries. The impact of the period of time following irradiation that is needed before fungal infection for effective induction of resistance was also studied in kumquat fruit by Rodov *et al.* (1992). They determined that a period of 48 h between irradiation and fungal inoculation was sufficient to accumulate considerable amounts of phytoalexins and consequently improve fruit resistance. Stevens *et al.* (1996) found that the application of UV-C at doses of 1.3 kJ $m^{-2}$ significantly reduced postharvest green mold of 'Marsh Seedless' grapefruits and 'Dancy' tangerines and also alternaria stem end rot and sour rot of tangerines. On the other hand, recent research work with 'Satsuma' mandarins showed that a 10-min exposure to UV-C light at 3.38 kJ $m^{-2}$ was phytotoxic (Kinay *et al.* 2005).

Although an on-line UV-C apparatus to treat harvested fresh fruit was developed (Wilson *et al.* 1997) and currently there is increasing commercial interest to design suitable prototypes for either intact or fresh-cut produce, a number of issues will have to be addressed before realizing the practical implementation of UV-C systems in citrus packinghouses. Illumination devices should be appropriately integrated in the packinglines to provide continuous effective treatment of the entire area of the fruit rapidly enough for commercial purposes. At the same time, the system should be flexible enough to change treatment conditions as a function of particular fruit attributes and destination. Currently, considerable attention is on pulsed light (synonyms: pulsed UV light, pulse white light), that uses short time pulses of intense broad spectrum, rich in UV-C light, and is touted as an improved technology compared to classic continuous-wave UV-C light delivering (Gómez-López *et al.* 2007). To our knowledge, though, this technique has not been specifically tested against citrus postharvest diseases. In any case, besides scaling-up efficacy trials, additional research on the effects of UV-C on fruit physiology, sensory quality, and consumer acceptance is also needed before attempting to use this technology on a commercial scale.

### Ionizing radiation

Ionizing radiation sources for food treatment include radioactive ($^{60}Co$ or $^{137}Cs$, γ-rays) and machine sources [electron beams (β particles) and X-rays (bremsstrahlung)]. Irradiation of fresh foods, including fruits and vegetables at doses not to exceed 1,000 Gy (100 krad), was approved by the United States Food and Drug Administration (US FDA) (21

CFR Part 179.26) and amended to include an energy level of ≤ 7.5 MeV for X-rays generated from machine sources using tantalum or gold as the target material (US FDA 1986, 2004). As a machine source, electron beams are more cost-effective treatments and have the advantage of not requiring the use of radioactive isotopes. However, high-energy electrons can only penetrate a few centimeters into fresh produce and this is a handicap for their commercial application, especially for treatment of pallet-loads of produce. This problem could be overcome with the use of X-rays, which are produced when electrons are directed toward a metallic target, and they have much higher penetration power (McLaughlin 1999). Therefore, in terms of their effects on fruit, X-rays are very similar to γ-rays. A major difference is that X-irradiation is concentrated in the same direction as the electron beam, while γ-rays are emitted uniformly in all directions and do not need to be applied in a conveyor system.

Uses of ionizing radiation of fruits and vegetables include insect disinfestation, sprout inhibition, control of human pathogens, maintenance of quality during storage, improvement of nutritional or functional components, and control of postharvest diseases (Kader 1999; Patil 2004). Barkai-Golan (1992) published an excellent overview of the applications of irradiation with γ-rays and electron beams to control decay and extend the postharvest life of fruits and vegetables. Since then, new research has mainly focused on the use of X-rays as postharvest quarantine treatments and, regarding control of postharvest diseases, on the combination of X-rays with other nonpolluting control means for integrated decay control. In the case of postharvest diseases of citrus fruit, early extensive research was conducted with both γ-ray and electron irradiation in the United States (USA) (Beraha *et al.* 1959; Sommer *et al.* 1964; Bramlage and Couey 1965; Grierson and Dennison 1965), Israel (Barkai-Golan and Kahan 1966; Barkai-Golan and Padova 1981), and Japan (Umeda *et al.* 1969; Ojima *et al.* 1974). In general, effective control of established fungal infections (curative activity) required irradiation doses higher than 1,000 Gy and these doses often injured the fruit, causing rind pitting and browning. Because of this negative impact on fruit quality, ionizing radiation as a single treatment for decay suppression cannot be commercially adopted and lower doses should be evaluated in combination with other physical or chemical treatments. The combinations evaluated to date will be described in the last section of this article, which focuses on the integration of treatments. In general, the effects of ionizing radiation on matter depend on the type of radiation and its energy level, as well as the composition, physical state, temperature and atmospheric environment of the absorbing material (Morehouse and Komolprasert 2004). In the case of fresh horticultural perishables and particularly citrus fruit, the response is affected by factors related to the produce itself (e.g. species, cultivar, fruit physical and physiological condition) and postharvest handling (e.g. postharvest treatments, length and environmental conditions of storage) (Kader 1999). As a function of all these factors, variable effects of irradiation on citrus fruit quality have been reported. In a study on the effects of γ-rays at doses ranging from 150 to 450 Gy on the quality attributes of selected mandarin cultivars, Miller *et al.* (2000) found a wide range of tolerance to irradiation, with damage from 1.7 to 100% peel pitting in 'Minneola' and 'Sunburst' mandarins, respectively. Other work showed that 'Clemenules' (Alonso *et al.* 2007; Palou *et al.* 2007a) and 'Nagpur' (Ladaniya *et al.* 2003) mandarins can both be classified as highly tolerant cultivars, while 'Fortune' mandarins were considerably more susceptible (Alonso *et al.* 2002). Provided that the use of nonphytotoxic doses in combination with other control means becomes an effective strategy for integrated postharvest decay control of selected citrus cultivars, another major handicap for commercial use of ionizing radiation is that the potential benefits from this treatment, which include not only decay control but also the stimulation of the synthesis of bioactive or functional phenolic compounds and the extension of shelf life by delaying ripening and senescence (Mahrouz *et al.* 2002; Patil 2004), are not economically important enough for the citrus industry to justify for the high initial investment and operation costs required for the implementation and operation of radiation treatment plants. Therefore, these treatments will be limited to citrus production areas (e.g. Hawaii) where radiation plants for fresh fruits and vegetables are available for other cost-effective purposes like quarantine treatments to control economically important common pests. Limited consumer acceptance is an additional handicap for the widespread use of ionizing radiation technologies to treat food products. Although the safety of irradiated food has been unanimously endorsed by the most prominent health international organizations and numerous regulatory agencies worldwide after extensive scientific research, public awareness of this information has been limited and there is a general lack of consumer education (Morehouse and Komolprasert 2004). Nevertheless, it has been noticed that in countries like the USA an unprecedented and rapidly growing level of acceptance of ionizing radiation has occurred across a broad cross-section of society that includes food processors, industry associations, retailers, foodservice operators, investors, and consumers. Actually, acceptance of ionizing radiation is driven forward in the food industry by two fundamental needs, to control microbes of food safety concern and to control quarantine pests on agricultural products in national and international trade (Borsa 2004).

## MODE OF ACTION OF POSTHARVEST ANTIFUNGAL PHYSICAL TREATMENTS

The mode of action of physical treatments that are specifically applied after harvest to control fungal diseases of citrus fruit can be direct effects on the pathogen by killing or damaging the infecting fungal structures and consequently inhibiting or retarding spore germination, germ tube elongation, or mycelial growth, or indirect effects on the fruit host by inducing mechanisms of resistance in the infection sites in the fruit rind. Therefore, the efficacy and effects of an antifungal physical treatment may considerably vary for each pathosystem.

### Direct effects on the pathogen

Direct effects of heat on postharvest pathogens have not been studied very extensively, but they may consist of changes in nuclei and cell walls, protein denaturation, destruction of mitochondria or outer membranes, disruption of vacuolar membranes, formation of gaps in the cytoplasm, lipid liberation, destruction of hormones, asphyxiation of tissue, depletion of food reserves, or metabolic injury with or without accumulation of toxic intermediates, and some of these mechanisms may be in action simultaneously (Barkai-Golan and Phillips 1991; Schirra *et al.* 2000). Heat treatments applied for citrus decay control are generally fungitoxic, but too mild to be lethal. Fungicidal treatments would often require phytotoxic temperatures. Besides genetic traits that define the sensitivity of each pathogen to heat and extrinsic factors like treatment temperature and duration, the response of the pathogen to heat treatment is influenced by its metabolic activity, the age of inoculum, the moisture content of spores, chemical composition and water activity of the treatment medium, and location of the pathogen upon the host (Barkai-Golan and Phillips 1991; Ben-Yehoshua and Porat 2005). In general, latent fungal structures and inactive or nongerminated spores are markedly more resistant to heat than germinating spores, which are more sensitive than growing mycelium. Hence, the effectiveness of some heat treatments may vary with the length of the period between fungal infection and treatment application and, further, the inhibitory effect of some thermal treatments on the pathogen may be either permanent or transitory (Stange and Eckert 1994; Schirra *et al.* 2000). For instance, Nafussi *et al.* (2001) observed in *in vitro* tests that a 2 min hot water dip

at 52-53°C had only a transient inhibitory effect on *P. digitatum*, arresting its growth for 24 to 48 h. It is also known that moist spores are considerably more sensitive to heat than dry spores. Barkai-Golan *et al.* (1969) reported that only 10% of previously hydrated conidia of *P. digitatum* survived after being immersed for 30 min in water at 70°C; contrastingly, about 90% of dry conidia survived after this treatment. Additional research is needed to better elucidate the direct mechanisms of action of heat treatments against postharvest pathogens. Otherwise, the general adoption of inadequate treatments could lead to the acquisition of thermotolerance by important target pathogens. The development of resistance to heat treatments is already an important issue in heat-related control of insects on fruit (Ferguson *et al.* 2000).

Exposure to UV-C light at 254 nm, the most efficient wavelength for damaging deoxyribonucleic acid (DNA), is a very effective surface disinfection treatment because it easily kills or severely injures pathogenic microorganisms by contact. UV-C technologies could therefore be used in citrus packinghouses as commercial sanitizing agents to disinfect either fruit or equipment surfaces, but much simpler, cheaper, and more cost-effective chemical treatments are still available for this task. Although sanitation is an important part of any postharvest IDM program, satisfactory control of major postharvest diseases of fresh citrus fruit requires indeed the inhibition of the fungal pathogens present under the fruit surface. Typically, disease inhibition by UV-C illumination has been attributed to a larger extent to indirect effects on the fruit host than to direct effects on the pathogens. The direct germicidal action of UV-C light is due to the absorption of radiation by the microorganisms followed by damage to their membrane structures, nucleic acids, and other cell components. Besides treatment parameters, such as the dose, the sensitivity of target microorganisms to UV-C exposure is affected by cell size and structure, pigmentation, and the activity of the radiation-damage repairing systems. As a general rule, complex microorganisms are more resistant than simple microorganisms; thus, molds are much more resistant to UV-C damage than yeasts and bacteria (Civello *et al.* 2007). In *in vitro* experiments (Asthana and Tuveson 1992), both UV-A and UV-B light alone were ineffective in causing inactivation of conidia of the pathogens *P. digitatum* and *P. italicum* that were suspended in liquid media and directly exposed to the UV source. Only UV-C radiation significantly damaged the spores of these fungi, although it was noticed that the pigments of both *Penicillium* species were able to protect the conidia to some extend. According to Fernández and Hall (2004), *P. digitatum* growing in potato dextrose agar (PDA) medium plates was affected (slight spore and mycelial inhibition) by exposure to UV-C light at 254 nm at the rate of 40 mws $cm^{-2}$ (milliwatt seconds per square centimeter) and totally killed at about 400 mws $cm^{-2}$. These values were 80 and 200 mws $cm^{-2}$, respectively, for the pathogenic fungus *G. candidum* under these conditions.

As is true with UV-C exposure, ionizing radiation treatments are very effective for disinfection of produce surfaces by contact since they directly harm the genetic material of living cells (Barkai-Golan 1992). However, their ability to control postharvest diseases cannot be predicted by their activity against free spores or other fungal structures. Results from early *in vitro* experiments with the major citrus postharvest pathogens showed that the most sensitive spores to γ-irradiation were those of *T. viride* followed in order of decreasing sensitivity by those of *P. citri*, *P. italicum*, *P. digitatum*, *G. candidum*, *B. cinerea*, *L. theobromae*, *R. stolonifer*, and *A. citri* (Sommer *et al.* 1964; Maxie *et al.* 1969). In these tests, an irradiation dose of 1,000 Gy killed a high percentage (> 90%) of conidia of *T. viride*, *P. citri*, *P. italicum*, *P. digitatum*, and *G. candidum* , while doses of about 1,500 and 2,500 Gy were needed to kill 90% of spores of *B. cinerea* and *L. theobromae*, and *R. stolonifer* and *A. citri*, respectively. It was suggested that spore radiation resistance may be related to multicellularity and the presence of multiple nuclei within single-cell spores. Another finding from this interesting *in vitro* research was that the γ-ray dose required to inactivate all the spores in a fungal population increased markedly as the population size increased. The same authors further corroborated this fact in *in vivo* experiments with California-grown 'Washington Navel' and 'Valencia' oranges. They noted that irradiation was more effective against citrus postharvest diseases when applied before extensive fungal development in the infected fruit. They also determined that, in spite of the relatively high level of sensitivity of spores of *P. italicum* and *P. digitatum* to irradiation, infections by these fungi on oranges were not effectively controlled by nonphytotoxic γ-ray doses. Similar results were obtained with electron irradiation in *in vitro* tests with spores of *Penicillium* spp. and in *in vivo* tests with artificially inoculated oranges and 'Satsuma' mandarins (Umeda *et al.* 1969; Barkai-Golan and Padova 1981). Besides genetic resistance, other factors that may influence the sensitivity of fungal pathogens to ionizing radiation include the presence of oxygen in the atmosphere (higher irradiation doses are required to reduce fungal survival under anoxia), the water content of the cells (spores are drier and more resistant to irradiation than vegetative cells), and all environmental parameters encouraging fungal growth and population size (Barkai-Golan 1992, 2001). One of the latter, for instance, is the time between fruit infection and irradiation. Spalding and Reeder (1985) reported that the incidence of green mold was lower on grapefruits irradiated with γ-rays 2 h after artificial inoculation with *P. digitatum* than on fruits irradiated 24-72 h after inoculation.

## Indirect effects on citrus fruit

Depending on the characteristics of both the infected host and the heat treatment, the application of heat can induce several indirect mechanisms to inhibit postharvest decay of citrus fruit. In general, more than one of these mechanisms will be triggered at the same time to different extents and their partial contribution to total disease reduction will depend on a variety of intrinsic (fruit species, cultivar, and physical and physiological condition; pathogenic strain and infection determinants) and extrinsic (treatment conditions, primarily temperature and duration) factors in the pathosystem. One of the most evident effects of heat treatments, especially of those based on the application of hot water, is the induction of physical changes on the surface of the fruit rind. Structural changes of epicuticular wax have been observed on the rind of oranges, mandarins, and grapefruits after immersion in hot water at 50-54°C for 2-3 min (Schirra and D'hallewin 1997; Schirra *et al.* 2000; D'hallewin and Schirra 2001; Sen *et al.* 2008). Similar or even more pronounced effects were noted by Porat *et al.* (2000a) after HWRB of organically-grown citrus fruit. Schirra *et al.* (2000) proposed that as a consequence of these changes in wax structure, cuticular microcracks and stomata are partially or completely plugged by melting wax, thereby providing a mechanical barrier against wound pathogens such as *Penicillium* spp. or *G. citri-aurantii*. As other causes of stress, heat treatment may promote fruit resistance to disease in citrus fruit by the induction of certain biochemical responses. Comprehensive reviews of this phenomena have been published (Schirra *et al.* 2000; Ben-Yehoshua and Porat 2005). According to them, constitutive antifungal compounds present in the peel of young immature citrus fruit, such as the terpenoid citral (3,7-dimethyl 2,6-octadienal) in lemons, act as a first line of defense against pathogens. The ageing-associated decline of the concentration of these compounds may be inhibited by some heat treatments and the natural fruit resistance to infection is maintained (Ben-Yehoshua *et al.* 1995). Citral has been artificially synthesized, but its application failed as an *in vivo* antifungal postharvest treatment because this compound was phytotoxic on citrus fruit (Rodov *et al.* 1995b). In contrast, 7-geranoxycoumarin, another preformed antifungal material in citrus fruit that was artificially synthesized, successfully con-

trolled penicillium molds in both *in vitro* and *in vivo* tests (Angioni *et al.* 1998). A second line of defense comprises induced resistance mechanisms that are elicited by fungal infection. Those described within citrus fruit exposed to heat treatments include the biosynthesis of lignin-like materials, the production of phytoalexins, and the accumulation of certain proteins. The accumulation of lignin or lignin-like polymers in the cell walls at sites of pathogen inoculation in the fruit rind has been observed in several citrus species after both curing (Brown *et al.* 1978; Brown and Barmore 1983; Ben-Yehoshua *et al.* 1987, 1989) and hot water treatments (Nafussi *et al.* 2001). The process, catalyzed by the enzyme phenylalanine ammonia lyase (PAL), began within 24 h of heat treatment and, depending on the kind of treatment, continued for up to 7 days (Nafussi *et al.* 2001). Decay reduction was achieved because the lignification creates a physical barrier in rind wounds that hamper the penetration or development of the pathogens. Numerous studies reported that the biosynthesis of phytoalexins, which are secondary metabolites with antifungal activity, may be triggered by the application of heat or other stress-promoting postharvest treatments to wounded or wound-infected citrus fruit. The best known citrus phytoalexins are the coumarins scoparone (6,7-dimethoxy coumarin) and scopoletin (7-hydroxy, 6-dimethoxy coumarin). These phenolic compounds are effectively induced by both curing and hot water treatment by enhanced PAL activity and their mode of action is primarily based on the inhibition of spore germination and germ tube elongation (Kim *et al.* 1991; Ben-Yehoshua *et al.* 1992; Rodov *et al.* 1996; Venditti *et al.* 2005). Nafussi *et al.* (2001) found that 2 min dips in hot water at 52-53°C increased the concentration of scoparone and scopoletin in lemons to levels high enough to completely control green mold within 2 days of the treatment. Finally, another fruit host defense mechanism elicited by heat treatments that has been extensively documented in citrus fruit is the production of pathogenesis-related proteins (PRP). Chitinases or β-1,3-glucanases are well-characterized proteins that inhibit mycelial growth by damaging fungal cell walls. Increased levels of these PRP have been found in citrus fruit artificially inoculated with *P. digitatum* and dipped in hot water (Rodov *et al.* 1996) or treated with HWRB (Pavoncello *et al.* 2001; Porat *et al.* 2002b). Heat shock proteins (HSP) are other proteins whose synthesis and accumulation in rind tissues of citrus fruit is triggered by heat treatments. Proteins in this group are produced by the fruit in response to exposure to high temperatures in order to protect itself from severe heat damage (irreversible protein denaturation and breakdown). Hence, HSP play a key role in the induction of produce thermotolerance, but it is still not clear if their accumulation leads to improved disease resistance (Ferguson *et al.* 2000; Pavoncello *et al.* 2001).

As it was previously discussed, the prevalent mode of action of UV-C illumination for control of citrus postharvest diseases is the stimulation of beneficial responses in the host. This phenomenon is known as hormesis when it is achieved by the application of sublethal doses of the agent (hormetin) (Luckey 1991). UV-C light is thus a hormetic agent because it is harmful at high doses. Similarly to heat treatment, there are different defensive reactions that can lead to the induction of fruit resistance to fungal development after the application of UV-C light. One of these responses is an alteration in the levels of preformed antifungal compounds naturally present in the fruit rind. Several studies have shown that the synthesis and/or accumulation of flavonoids, such as some polymethoxyflavones or flavanones, substantially increased after exposure to UV-C irradiation. For instance, changes in the levels of the polymethoxyflavone, tangeretin, and the flavanone, naringin, in the peel of bitter oranges (*Citrus aurantium* L.) reduced the growth of *P. digitatum* by up to 45% on previously irradiated fruit (Arcas *et al.* 2000). These researchers suggested that these two constitutive secondary metabolites of *C. aurantium* may act as fungitoxins in the resistance mechanism against fungal attack, acting as first and second defense barriers, respectively, since polymethoxylated flavones are mainly localized in the flavedo of the peel while flavanones are located in the albedo. Another response that has been related to the application of UV-C light to citrus fruit is the accumulation of PRP, such as chitinase or β-1,3-endoglucanase (Porat *et al.* 1999a). Porat *et al.* (2002b) isolated the gene "gns1" that encodes the production of β-1,3-endoglucanase and observed that its expression is markedly induced by wounding and inoculation with *P. digitatum* and following UV-C treatments. Nevertheless, the most documented defense mechanism induced by UV-C exposure is perhaps the elicitation of the biosynthesis of phytoalexins. Enhanced levels of scoparone or scopoletin have been detected in several UV-C-irradiated citrus species and cultivars and correlated with the inhibition of fungal development (Ben-Yehoshua *et al.* 1992; Rodov *et al.* 1992; D'hallewin *et al.* 1999b, 2000). Likewise, inhibition has also been related to the UV-C-induced increase in synthesis of lignin-like compounds in the fruit rind that act as mechanical barriers and impede the penetration and invasion of fungal pathogens (Ben-Yehoshua and Mercier 2005). The activity of enzymes, such as PAL or peroxidase, has been found to considerably increase in the peel of UV-C-treated citrus fruit and this fact has been related to the activation of some of these indirect antifungal mechanisms (Droby *et al.* 1993). Besides the reduction in the number of infected fruits, other evidence of the induction of disease resistance by UV-C illumination in citrus fruit artificially inoculated with *P. digitatum* included irregular mycelium growth and marked inhibition of sporulation (Droby *et al.* 1993; Porat *et al.* 1999a).

As other postharvest treatments that cause oxidative stress, ionizing radiation can directly affect the content of bioactive phytochemicals in citrus fruit (Patil 2004). According to Oufedjikh *et al.* (2000), the content of the major phenolic compounds present in the peel of clementine mandarins [flavanones, such as hesperidin, narirutin, or didymin; polymethoxylated flavones, such as nobiletin, heptamethoxyflavone, or sinensetin; and *p*-coumaric acid, a precursor of coumarins] significantly increased in fruit that had been previously irradiated with γ-rays at 300 Gy. This increase was correlated with an enhancement of the activity of the enzyme PAL. These authors discussed the possibility that the enhanced synthesis of these constitutive flavonoids could extend citrus storage life, but there is also evidence that in some cases it may induce some degree of resistance to the development of pathogenic fungi (Del Río and Ortuño 2004). Furthermore, another line of fruit defense that may be triggered by exposure to ionizing radiation is the synthesis of phytoalexins in the fruit rind as a response to fungal challenge. Riov *et al.* (1972) reported an accumulation of coumarins, such as scopoletin, scopolin, or scoparone, in the peel of γ-irradiated grapefruits. Likewise, Dubery *et al.* (1988) isolated a novel non-coumarin antifungal metabolite from irradiated citrus fruit that was identified as 4-(3-methyl-2-butenoxy) isonitrosoacetophenone. In our recent work with 'Clemenules' mandarins (Palou *et al.* 2007a), X-irradiation at doses ranging from 195 to 875 Gy did not induce any resistance to green or blue molds in the fruit. To the contrary, green mold development was slightly favored in fruit treated at 875 Gy when *P. digitatum* was inoculated 6 days after irradiation (**Fig. 3**). This might be related to a negative effect of X-rays at this dose on the physical and/or physiological condition of the fruit rind that would facilitate the fungal mycelial growth through the albedo and flavedo cells. We could not conclude from these results that X-irradiation did not promote the synthesis in the peel of treated mandarins of constitutive or induced antifungal compounds potentially involved with an increase in fruit resistance to green or blue mold because the levels of these compounds were not measured. In case they were actually synthesized, their concentrations were insufficient to significantly affect disease resistance under our experimental conditions. Further, accumulation to reach significant levels was not influenced by either X-ray dose, time between irradiation and

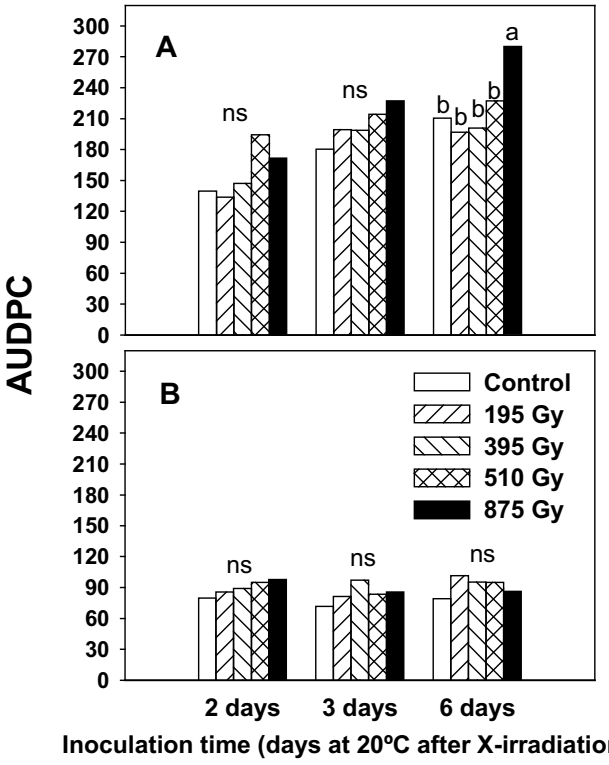


**Fig. 3** Area under the disease progress curve (AUDPC) of green (**A**) and blue (**B**) molds on 'Clemenules' clementine mandarins irradiated with X-rays at 0 (control), 195, 395, 510, or 875 Gy, artificially inoculated with the pathogen after 2, 3, or 6 days of storage at 20°C following irradiation, and incubated after fungal inoculation at 20°C for 7 days. Lesion diameters to determine the AUDPC were measured after 3, 5, and 7 days of incubation at 20°C following fungal inoculation. For each pathogen and evaluation, different letters and 'ns' indicate significant and no significant differences, respectively, according to Fisher's Protected LSD test ($P$ = 0.05). Reproduced from **Palou L, Marcilla A, Rojas-Argudo C, Alonso M, Jacas J, del Río MA** (2007a) Effects of X-ray irradiation and sodium carbonate treatments on postharvest *Penicillium* decay and quality attributes of clementine mandarins. *Postharvest Biology and Technology* **46**, 252-261, ©2007, with kind permission from Elsevier Ltd.

pathogen inoculation, or incubation time after inoculation. The cultivar and the fruit physical and physiological condition at the time of irradiation are other factors that could reasonably account for the lack of resistance induction. Since in these trials X-irradiation markedly inhibited the sporulation of both *P. digitatum* and *P. italicum* on decayed mandarins, we suggested that the direct effects of irradiation on the fungal structures growing in the rind were more important for disease reduction than a possible indirect effect on the fruit mechanisms of defense.

## COMPLEMENTARY PHYSICAL METHODS

In general, conventional cold storage or storage in controlled or modified atmospheres can be considered complementary physical tools for postharvest decay control of fresh fruits and vegetables. These systems cannot be used as stand-alone antifungal treatments because typically they only provide fungistatic activity by inhibiting or delaying the growth and development of the pathogens. In addition, they considerably reduce the metabolic activity of the host, delay its senescence, and therefore contribute to the maintenance of fruit resistance to fungal infection.

### Conventional cold storage

Optimal storage temperature for harvested citrus fruit clearly varies with fruit susceptibility to chilling injury. While most of the commercially important orange and mandarin cultivars can be stored at temperatures of 3 to 5°C, lemons, limes, and grapefruits are better maintained at temperatures from 10 to 14°C (Kader and Arpaia 2002). RH should be 90 to 95% in all cases. The following are ambient temperatures below which the growth of the most common citrus postharvest pathogens is effectively inhibited (Tuset 1987; Snowdon 1990; Brown and Eckert 2000): -3°C for *A. citri*; -2°C for *B. cinerea*; 0°C for *P. italicum*; 3°C for *P. digitatum*; 4°C for *Phytophthora* spp.; 5°C for *G. citri-aurantii*, *R. stolonifer*, and *T. viride*; 8°C for *L. theobromae*, 9°C for *C. gloeosporioides*; 10°C for *P. citri*; and 15°C for *A. niger*.

### Storage in controlled atmospheres

Cold storage of citrus fruit in conventional CA (5-10% $O_2$ + 0-5% $CO_2$ for oranges and mandarins and 5-10% $O_2$ + 0-10% $CO_2$ for lemons, limes, and grapefruits; Kader and Arpaia 2002) has not been generally adopted because potential benefits do not compensate for the high installation and operation costs. Results of early research work are contradictory and both positive (Smoot 1969) and negative (Chace 1969; Aharoni and Lattar 1972) effects of CA on the incidence of postharvest decay were reported. Other technological options involving CA such as modified atmosphere packaging (MAP), storage in either carbon monoxide CA (5% $O_2$ + 5-10% CO; Kader and Arpaia 2002), low-pressure (hypobaric) CA (Spalding and Reeder 1976), or ethylene removal from storage rooms (McGlasson and Eaks 1972; Wild *et al.* 1976), may have beneficial effects on decay suppression, but they are not economically viable for fresh citrus fruit.

### Storage in ozonated atmospheres

Storage in ozonated atmospheres and general ozone applications for sanitation and control of postharvest diseases of fresh fruits and vegetables have been recently reviewed (Palou *et al.* 2007b). Ozone ($O_3$) is a highly reactive, potent biocide that has recently received regulatory approval for many food contact applications. It is a residue-free effective sanitizer, but its efficacy in controlling postharvest diseases cannot be predicted by its toxicity against free fungal spores and hyphae. It was determined in *in vitro* tests that about 200 µL/L ozone gas was required to kill spores of major citrus postharvest pathogens such as *P. digitatum*, *P. italicum*, and *G. citri-aurantii* in humid air (about 95% RH) at 5°C within 1 h (Margosan and Smilanick 1998). If the air was dry (35% RH), a dose 5 to 10 times higher was required. Spores of *R. stolonifer* were, by far, more resistant and doses of about 500 and 7,500 µL/L gaseous ozone were needed to kill 99% of the spores in humid and dry air, respectively. Because of interactions of the gas with fruit tissues, higher ozone gas concentrations may be required to kill fungal spores on fruit surfaces and there is a very high risk of phytotoxicity associated to such fruit sanitation treatments. When fruit are not present in storage rooms, higher doses of gaseous ozone and shorter exposure periods could presumably be of use for surface sanitation of rooms, mobile equipment, or packages. The effectiveness of these treatments with ozone, however, will not only rely on dose and exposure time, but also on environmental conditions (temperature, humidity, and air circulation) and especially on the presence of fruit residues, dirt, or any organic matter that can protect the inoculum from the action of the gas. It is therefore important to perform a prior effective cleaning of surfaces before disinfection with ozone. On the other hand, resistance of materials and facilities to corrosion, measures to scrub ozone from vented air, and other safety measures are also important issues to consider before the application of ozone gas to empty citrus storage rooms (Palou *et al.* 2007b).

In work to assess the efficacy of ozone gas as a postharvest fungicide against citrus penicillium molds, continuous or intermittent exposure to ozone gas at nonphytotoxic con-

centrations of 0.3-1.0 µL/L did not control infection of fruit by *P. digitatum* and *P. italicum* in wounds and consequently did not reduce final disease incidence after storage (Palou *et al.* 2001a). We discussed that following wound or latent infection, the development of the pathogen after harvest occurs at a subepidermical level where, presumably, fungal structures remain protected from the oxidizing effect of ozone because of limited ozone penetration, reduced ozone concentration as it reacts with fruit tissue or extracellular biochemicals, and/or the presence of antioxidants in the fruit. In these tests, however, gaseous ozone inhibited aerial mycelial growth and sporulation of these fungi, which can help to reduce the proliferation of fungicide-resistant strains of the pathogens. Nevertheless, these effects were transitory and both *P. digitatum* and *P. italicum* resumed normal surface growth and sporulated on fruit when removed from the ozone room and incubated at 20°C for 2 days (Palou *et al.* 2001a). Similarly, Klotz (1936) and Harding (1968) noticed a significant suppression of the sporulation of *Penicillium* spp. on citrus fruit under ozone only as long as the gas was present. In additional trials conducted in California, we observed that the beneficial effects of ozone exposure in reducing mycelial growth and sporulation were limited to infected citrus fruit stored in highly vented packages or open-top containers that allowed direct contact to the gas (Palou *et al.* 2003). Ozone penetration through different citrus packaging materials was strongly dependent on the vented area of each type of package and the inhibition of the sporulation of both *P. digitatum* and *P. italicum* on decayed oranges was clearly related to ozone penetration ability.

Like all oxidizing agents, ozone can harm humans if exposure occurs to high concentrations for a sufficient duration. Therefore, issues related to the safety of workers and personnel must be addressed before the installation of ozone application systems in citrus packinghouses. Regulations in the European Union (EU) and the USA establish that the ozone concentration to which individuals doing light work can be repeatedly exposed for a normal 8-h workday is 0.1 µL/L (ppm) and that for workload shorter than 2 h the limit is 0.2 µL/L. The concentration that is Immediately Dangerous to Life and Health (IDLH) is 5.0 µL/L. This is the maximum concentration for which there are approved respirators; higher rates than this are dangerous and require self-contained breathing equipment.

## INTEGRATION OF PHYSICAL MEANS WITH OTHER CONTROL MEANS

Successful commercial control of citrus postharvest diseases must be extremely effective and reliable and such levels of control cannot consistently be achieved by the physical treatments tested to date as stand-alone treatments. Therefore, researchers have devoted considerable attention to the integration of different nonpolluting treatments in order to overcome the variable performance and augment the efficacy of existing approaches alternative to the use of chemical pesticides. In general, three objectives may be pursued by the integration of two or more treatments (Palou *et al.* 2008b): additive and/or synergistic effects to increase the effectiveness and/or the persistence of individual treatments, complementary effects to combine preventive and curative activities, and potential commercial implementation of effective treatments that are too impractical, costly, or risky as single treatments. For example, combinations of treatments can be made to reduce the length and cost of curing treatments or reduce the dose and phytotoxicity risk of irradiation treatments.

### Combination of different physical treatments

Heat has been as typical component of integrated strategies designed to substitute the use of chemical fungicides for the control of citrus postharvest diseases. Some heat treatments are cheap and easy to apply and often provide synergistic effects with other complementary postharvest decay control

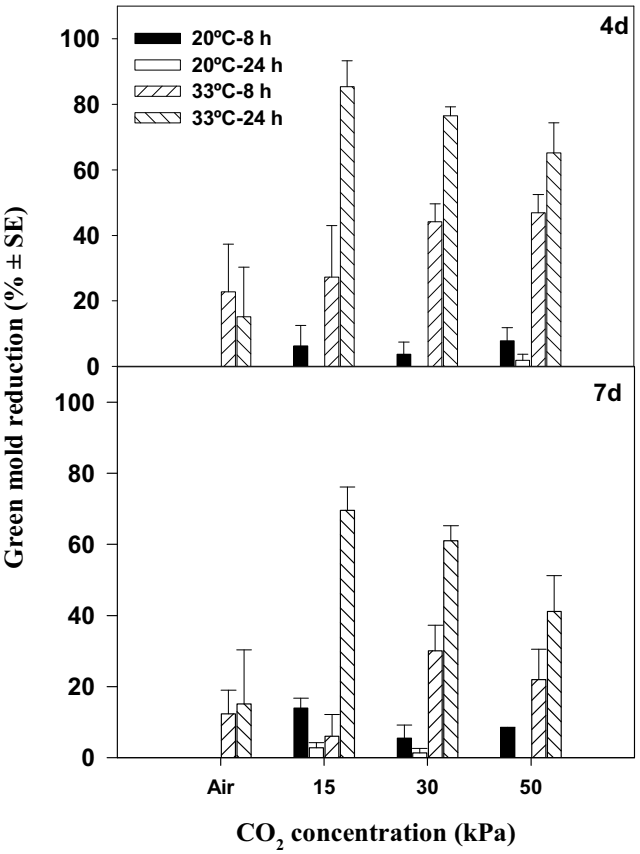


**Fig. 4** Reduction in the incidence of green mold with respect to the control treatment (air at 20°C for 8 h) on 'Ortanique' mandarins artificially inoculated with *Penicillium digitatum*, treated 24 h later with 0.03 (air), 15, 30, or 50 kPa $CO_2$ at 20 or 33°C for 8 or 24 h, and incubated at 20°C for 4 or 7 days. Reproduced from **Palou L, Montesinos-Herrero C, del Río MA** (2008a) Short-term $CO_2$ exposure at curing temperature to control postharvest green mold of mandarins. *Acta Horticulturae* **768**, 257-263, ©2008, with kind permission from the International Society for Horticultural Science, ISHS.

treatments. Indeed, treatments based on the application of either air or hot water have also been combined with other physical control means.

Several studies with mandarins (D'hallewin *et al.* 1994), kumquats, and oranges (Ben-Yehoshua *et al.* 2005) showed that the integration of curing treatments (35°C for 72 h) or hot water dips (50-55°C for 2 min) with UV-C illumination was superior to either treatment alone in reducing decay and maintaining fruit quality. Previous application of heat significantly reduced the risk of rind damage occurrence due to UV-C exposure. When UV-C treatment preceded heat treatment, the elicitation of phytoalexins in the fruit rind was inhibited.

In order to reduce the dose and phytotoxicity risk of ionizing radiation treatments, they were combined in early research with certain heat treatments. Dipping fruit in hot water at 52°C for 5 min followed by γ-irradiation at low dose (500 Gy) delayed the appearance of green mold by up to 40 days (Barkai-Golan *et al.* 1969). Exposure to electron beam radiation at low doses exhibited synergistic effects with hot water in both reducing the viability of spores of *P. digitatum* in *in vitro* tests and suppressing the development of green mold in artificially inoculated oranges (Barkai-Golan and Padova 1981). In contrast, no benefits from similar combinations were observed in experiments with grapefruits (Spalding and Reeder 1985).

We recently tested whether short treatments with $CO_2$ at a curing temperature exhibited synergistic effects against green mold in different mandarin cultivars to facilitate a decrease in the curing time that is usually required for effective disease control (65 to 72 h). On 'Ortanique' hybrid mandarins artificially inoculated with *P. digitatum* and ex-

posed 24 h later to air (control) or 15, 30, and 50 kPa $CO_2$ at 20 or 33°C for 8 or 24 h, only treatments at 33°C for 24 h reduced the incidence of disease significantly after 4 or 7 days incubation at 20°C, with 15 kPa $CO_2$ slightly superior to other gas concentrations tested (**Fig. 4**). Similar results were obtained on 'Nadorcott' mandarins after 4 days of incubation, but in this case the effect of brief $CO_2$ shocks was less persistent and no disease reduction was observed after 7 days at 20°C. We proposed that the ability of the combined treatments to control green mold was cultivar-dependent and it was higher on 'Ortanique' than on 'Nadorcott' mandarins because the latter were more susceptible to disease (Palou *et al.* 2008a).

In order to maintain fruit quality, heat treatments have also been combined, with variable results, with plastic packaging of citrus fruit. Individual sealing of citrus fruit in high density plastic films reduced the potential adverse effects of curing treatments by reducing fruit transpiration and maintaining rind firmness (Ben-Yehoshua *et al.* 1987, 1989). In interesting research with the hybrid 'Oroblanco', the use of individual polyolefin seals or polyethylene liners in combination with curing at 36°C for 72 h, hot water dip at 52°C for 2 min, or HWBR at 60°C for 10 s reduced fruit weight loss and slowed fruit softening, while controlling the development of postharvest pathogens, especially that of penicillium molds (Rodov *et al.* 2000).

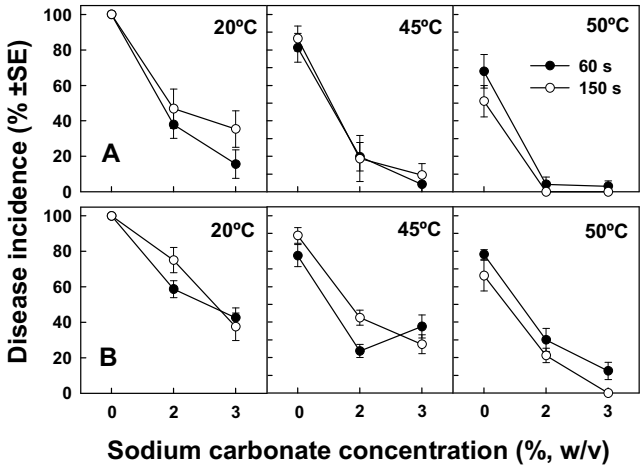


**Fig. 5** Influence of solution temperature (20, 45, or 50°C), sodium carbonate concentration (0, 2, or 3%), and immersion period (60 or 150 s) on the incidence of green (**A**) and blue (**B**) molds on artificially inoculated 'Clemenules' mandarins stored at 20°C and 90% RH for 7 days. Reproduced from **Palou L, Usall J, Muñoz JA, Smilanick JL, Viñas I** (2002a) Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of clementine mandarins. *Postharvest Biology and Technology* **24**, 93-96, ©2002, with kind permission from Elsevier Ltd.

## Combination with chemical treatments

It has been repeatedly reported that heating aqueous solutions of either conventional chemical fungicides (Barkai-Golan and Apelbaum 1991; Schirra and Mulas 1995; Smilanick *et al.* 1997; Schirra *et al.* 1998, 2005; Smilanick *et al.* 2006b; Cunningham and Taverner 2007) or low-toxicity alternative chemicals [food additives or GRAS (generally regarded as safe) compounds] such as sodium carbonate, sodium bicarbonate (Smilanick *et al.* 1995, 1999, Palou *et al.* 2001b, 2002a; Porat *et al.* 2002a; Cunningham and Taverner 2007; Lesar 2008; Usall *et al.* 2008), potassium sorbate (Wild 1987; Brown and Baraka 1996; Palou *et al.* 2002b; Smilanick *et al.* 2008), sodium benzoate, sodium and ammonium molybdates (Palou *et al.* 2002b), ethanol, sulfur dioxide (Smilanick *et al.* 1995), or calcium polysulfide (Smilanick and Sorenson 2001) significantly enhanced their effectiveness against penicillium molds and other citrus postharvest diseases. For instance, we observed enhanced control of both green and blue molds on artificially inoculated

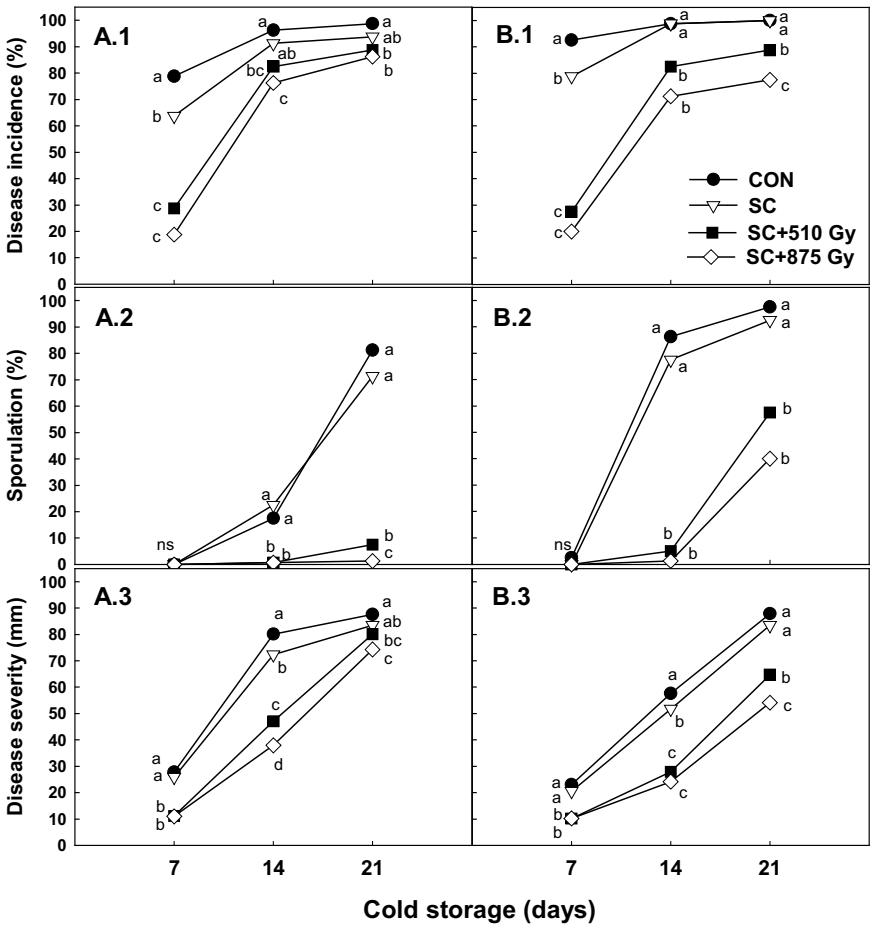


**Fig. 6** Percentage of infected (1) and sporulated (2) fruit and lesion size (3) on 'Clemenules' clementine mandarins artificially inoculated with the pathogens *Penicillium digitatum* (**A**) or *Penicillium italicum* (**B**) and dipped for 150 s in water at 20°C (CON) or 3% sodium carbonate at 20°C (SC). About 36 h later, part of SC-treated fruit were irradiated with X-rays at 510 (SC+510 Gy) or 875 Gy (SC+875 Gy), kept at 20°C for 24 h, and stored at 5°C for 21 days. For each parameter, pathogen, and evaluation date, different letters and 'ns' indicate significant and no significant differences, respectively, according to Fisher's Protected LSD test ($P = 0.05$). Percentage data were arcsine transformed previous to the analysis of variance. Non-transformed means are shown. Reproduced from **Palou L, Marcilla A, Rojas-Argudo C, Alonso M, Jacas J, del Río MA** (2007a) Effects of X-ray irradiation and sodium carbonate treatments on postharvest *Penicillium* decay and quality attributes of clementine mandarins. *Postharvest Biology and Technology* **46**, 252-261, ©2007, with kind permission from Elsevier Ltd.

'Clemenules' clementine mandarins when 2 or 3% sodium carbonate solutions were heated to 45 or 50°C (**Fig. 5**). No visible rind injury occurred in any test (Palou *et al.* 2002a). Heat probably facilitates the uptake of the active ingredient through the fruit cuticle (Schirra *et al.* 2000) in a similar way that it is facilitated by dip treatments in comparison to spray or drench applications (Brown and Dezman 1990; Smilanick *et al.* 1997). The most appropriate solution temperature should be specifically determined for each combination of active ingredient and fruit species and cultivar, but in general, if compared to hot water alone, similar effectiveness is obtained at much lower solution temperatures, which considerably reduces the risk of heat injury to the fruit.

The combination of curing treatments with the application of conventional synthetic fungicides at low doses (Zhang and Swingle 2005; Kinay *et al.* 2005), GRAS compounds like sodium carbonate (Lanza *et al.* 2004; Plaza *et al.* 2004c) or ethanol (Lanza *et al.* 2004), or postharvest surfactants, such as dodecylbenzenesulfonate (Stange and Eckert 1994), also resulted in improved control of citrus green or blue molds.

Treatments with conventional chemical fungicides (e.g. SOPP or diphenyl) were successfully applied in combination with ionizing radiation to reduce both the irradiation dose and the chemical concentration (Barkai-Golan and Kahan 1967; Kahan and Barkai-Golan 1968). According to Barkai-Golan (1992), since the effects of irradiation and chemical fungicides may differ greatly among fungal species, their integration might considerably broaden the spectrum of pathogens controlled. We recently investigated the performance of the integration of sodium carbonate dips and X-irradiation for penicillium decay control on 'Clemenules' mandarins and observed that the combined treatments, especially at the highest X-ray dose of 875 Gy, significantly reduced disease incidence and severity of both green and blue molds on mandarins stored at 5°C (**Fig. 6**). However, these reductions were not high enough for satisfactory disease control under hypothetical commercial conditions and we concluded that under our experimental conditions this combination of treatments could not be a substitute for the synthetic fungicides that are currently applied on citrus fruit packinglines. In contrast to fungal growth, pathogen sporulation, especially that of *P. digitatum* (**Fig. 6A2**), was clearly inhibited on inoculated clementines by the combined treatments. Since sodium carbonate does not exert antisporulant activity, this effect was attributed to X-irradiation (Palou *et al.* 2007a).

### Combination with biological control agents

Heat treatments and the application of microorganisms with antagonistic activity against postharvest pathogens (biocontrol microbial antagonists) are complementary treatments that often show synergistic effects on the control of citrus postharvest diseases. Heat typically offers some curative activity against existing or incipient pathogenic infections but does not adequately protect the fruit. Biocontrol agents are yeasts, bacteria, or other filamentous fungi able to colonize rind infection sites and offer effective preventive activity against pathogens that may reach the treated fruit during storage or commercialization (El-Ghaouth *et al.* 2002; Janisiewicz and Korsten 2002).

Thermal curing treatments are good candidates to be combined with the application of microbial antagonists because this combination might allow the reduction of the length, costs, and risks of curing and the implementation of more flexible and practical treatment procedures (Arras and Maltoni 2004). Considerable reduction in the curing period and improved control of green mold on oranges were obtained by the application of the bacterium *Pseudomonas glathei* before curing the fruit at 30°C for 24 h. The curing period enhanced the establishment of the antagonist in rind wounds and delayed the germination and subsequent development of *P. digitatum* (Huang *et al.* 1995). Work by D'hallewin *et al.* (1999a) evidenced synergistic effects on the control of green mold on artificially inoculated grapefruits by combining exposure to curing conditions (37°C for 72 h) with treatment with the antagonistic yeast *Candida famata*. Significantly higher levels of penicillium decay control on lemons was achieved after the integration of curing at 33°C for 65 h with the application of the bacterium *Pantoea agglomerans* than after each treatment alone (Plaza *et al.* 2004b). The most effective integrated treatment sequence was first the application of the antagonist followed by exposure to curing temperatures. Such a sequence, however, requires the use of heat-tolerant antagonist strains. Similar synergistic activity against citrus postharvest diseases was observed after integration of curing treatments with other biocontrol agents like *Candida oleophila* (Lanza *et al.* 2004) or *Metschnikowia mulcherrima* (Yildiz *et al.* 2005). Hot water dips and HWBR treatments have also been found to be treatments that may complement the antifungal activity of microbial antagonists. Obagwu and Korsten (2003) reported that the biocontrol activity of several strains of *Bacillus subtilis* against the pathogens *P. digitatum* and *P. italicum* was significantly enhanced by previous dips in water at 45°C. Likewise, the application of HWBR (62°C for 20 s) and the yeast *C. oleophila* in both *P. digitatum*-inoculated and naturally infected oranges and grapefruits reduced decay development to a greater extend than did both stand-alone treatments. In these trials, the combined treatments were almost as effective as commercial treatment with imazalil (Porat *et al.* 2002a).

Besides heat treatments, other physical control means that have been combined with the application of antagonistic microorganisms to control citrus postharvest decay include UV-C illumination and storage in controlled atmospheres. The application of UV-C in combination with the yeast antagonist *Debaryomyces hansenii* completely inhibited the development of *P. digitatum* on 'Dancy' tangerines (Stevens *et al.* 1997). While similar results were obtained on navel oranges with the combination of UV-C and the yeast *C. oleophila*, no synergistic effects were observed when UV-C was combined with the bacterium *B. subtilis* (D'hallewin *et al.* 2005). Satisfactory decay control was found on clementine mandarins previously treated with the bacterium *P. agglomerans* and stored for 60 days at 3.5°C in 5 kPa $O_2$ + 3 kPa $CO_2$ (Palou, Usall, and Viñas, unpublished). These storage conditions did not adversely affect the viability of the antagonist on fruit surface wounds.

## CONCLUSIONS

Before the increasing need to implement cost-effective antifungal nonpolluting treatments as alternatives to conventional chemical fungicides for the control of postharvest diseases of citrus fruit, extensive research work has been conducted worldwide for many years and continues today to identify and evaluate different physical control means. The most important benefits from the use of physical methods, such as heat and irradiation, as direct postharvest antifungal treatments are undoubtedly the total absence of residues of any kind on/in treated produce and their minimal environmental impact. In addition, most of these treatments are not only compatible but also synergistic to other antifungal means of the same or different nature and have shown improved effectiveness and persistence when used in combination with complementary treatments as part of integrated disease management strategies. Moreover, treatments such as the application of hot water or UV-C illumination are simple, fast, and inexpensive. Other physical procedures such as conventional cold storage or storage in controlled atmospheres are excellent complementary tools for postharvest decay control because they inhibit or delay the development of the pathogens and contribute to maintain the resistance of citrus fruit to fungal infection.

Despite substantial progress in research, the commercial use of physical control methods by the citrus industry has been rather limited. The lack of preventive activity, low persistence, and high variability are general limitations associ-

ated to the nature and mode of action of these treatments that restrict their use as stand-alone treatments. All heat and irradiation treatments have repeatedly shown both direct effects against fungal pathogens and indirect effects on citrus fruit hosts. In fact, some treatments are very effective sanitizers but their efficacy in controlling postharvest diseases cannot be predicted by their toxicity against free fungal spores or hyphae because fungi infecting fruit tissues remain somehow protected and more severe and effective treatments might be often phytotoxic. Hence, direct effects, although fungitoxic, are not usually fungicidal. Indirect effects are based on the induction in the fruit peel of a variety of defense mechanisms against disease development that, depending on treatment characteristics and fruit condition, might be triggered alone or in combination at different intensity levels. Typically, such effects include melting of rind waxes and biosynthesis of lignin-like materials, constitutive and/or induced (phytoalexins) antifungal compounds, PRP, or HSP. The dependence of the elicitation of these mechanisms on species, cultivar, and fruit physical and physiological condition may greatly explain the high variability and inconsistent performance frequently associated with the use of some physical treatments. These limitations and other factors, like the availability of new conventional fungicides for traditional markets, are additional reasons that may hinder the broad commercial use of these treatments. Likewise, the risk of adverse effects on fruit quality or technological problems for cost-effective application have impeded the implementation of some other physical treatments like thermal curing or ionizing radiation as commercial decay control means.

As we learn more about the fundamental basis underlying host-pathogen interactions and the physiology, biochemistry, and molecular biology of treated citrus fruit, more precise and effective new physical control methods will emerge to be used in combination with other alternative treatments in a multifaceted approach to successfully manage postharvest decay of citrus fruit without the application of synthetic chemical fungicides. Research should provide appropriate tools to tailor the application of these integrated treatments to particular produce and specific handling and market situations.

## ACKNOWLEDGEMENTS

The author thanks the Spanish 'Ministerio de Ciencia e Innovación' and the EU FEDER program for their financial support. The researchers Inmaculada Viñas and Josep Usall (UdL-IRTA Center, Lleida, Catalonia, Spain), Joseph L. Smilanick (USDA-ARS, Parlier, CA, USA) and Carlos H. Crisosto (UC Davis, Parlier, CA, USA) are acknowledged for their collaboration and guidance in conducting research in this topic.

## REFERENCES

**Aharoni Y, Lattar FS** (1972) The effect of various storage atmospheres on the occurrence of rots and blemishes on Shamouti oranges. *Phytopathologische Zeitschrift* **7**, 371-374

**Alonso M, del Río MA, Jacas JA** (2002) Ionización con electrones acelerados como tratamiento de cuarentena contra *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) en cítricos. *Boletín de Sanidad Vegetal y Plagas* **28**, 419-426 (in Spanish with English summary)

**Alonso M, Palou L, del Río MA, Jacas JA** (2007) Effect of X-ray irradiation on fruit quality of clementine mandarin cv. 'Clemenules'. *Radiation Physics and Chemistry* **76**, 1631-1635

**Angioni A, Cabras P, D'hallewin G, Pirisi FM, Reniero F, Schirra M** (1998) Synthesis and inhibitory activity of 7-geranoxycoumarin against *Penicillium* species in citrus fruit. *Phytochemistry* **47**, 1521-1525

**Arcas MC, Botía JM, Ortuño A, Del Río JA** (2000) UV irradiation alters the levels of flavonoids involved in the defence mechanism of *Citrus aurantium* against *Penicillium digitatum*. *European Journal of Plant Pathology* **106**, 617-622

**Arras G, Maltoni SL** (2004) Postharvest biological and integrated control of fungal pathogens on fruit. In: Dris R, Niskanen R, Mohan Jain S (Eds) *Crop Management and Postharvest Handling of Horticultural Products (Vol 4) Diseases and Disorders of Fruits and Vegetables*, Science Publishers Inc, Enfield, NH, USA, pp 115-183

**Asthana A, Tuveson RW** (1992) Effects of UV and phototoxins on selected fungal pathogens of citrus. *International Journal of Plant Science* **153**, 442-452

**Barkai-Golan R** (1992) Suppression of postharvest pathogens of fresh fruits and vegetables by ionizing radiation. In: Rosenthal I (Ed) *Electromagnetic Radiations in Fruit Science*, Springer-Verlag, Berlin, Germany, pp 155-193

**Barkai-Golan R** (2001) *Postharvest Diseases of Fruits and Vegetables. Development and Control*, Elsevier Science BV, Amsterdam, The Netherlands, 418 pp

**Barkai-Golan R, Apelbaum A** (1991) Synergistic effects of heat and sodium o-phenyl phenate treatments to inactivate *Penicillium* spores and suppress decay in citrus fruits. *Tropical Science* **31**, 229-233

**Barkai-Golan R, Kahan RS** (1966) Effect of gamma radiation on extending the storage life of oranges. *Plant Disease Reporter* **50**, 874-877

**Barkai-Golan R, Kahan RS** (1967) Combined action of diphenyl and gamma radiation on the *in vitro* development of fungi pathogenic to citrus fruit. *Phytopathology* **7**, 696-698

**Barkai-Golan R, Kahan RS, Padova R** (1969) Synergistic effects of gamma radiation and heat on the development of *Penicillium digitatum* in vitro and in stored citrus fruit. *Phytopathology* **59**, 922-924

**Barkai-Golan R, Padova R** (1981) Eradication of *Penicillium* on citrus fruits by electron radiation. *Proceedings of the International Society of Citriculture* **2**, 799-801

**Barkai-Golan R, Phillips DJ** (1991) Postharvest heat treatment of fresh fruits and vegetables for decay control. *Plant Disease* **75**, 1085-1089

**Ben-Yehoshua S, Barak E, Shapiro B** (1987) Postharvest curing at high temperatures reduces decay of individually sealed lemons, pomelos, and other citrus fruit. *Journal of the American Society for Horticultural Science* **112**, 658-663

**Ben-Yehoshua S, Kim JJ, Shapiro B** (1989) Elicitation of resistance to the development of decay in sealed citrus fruit by curing. *Acta Horticulturae* **258**, 623-630

**Ben-Yehoshua S, Mercier J** (2005) UV irradiation, biological agents, and natural compounds for controlling postharvest decay in fresh fruits and vegetables. In: Ben-Yehoshua S (Ed) *Environmentally Friendly Technologies for Agricultural Produce Quality*, CRC Press, Taylor and Francis Group, Boca Raton, FL, USA, pp 265-299

**Ben-Yehoshua S, Porat R** (2005) Heat treatments to reduce decay. In: Ben-Yehoshua S (Ed) *Environmentally Friendly Technologies for Agricultural Produce Quality*, CRC Press, Taylor and Francis Group, Boca Raton, FL, USA, pp 11-42

**Ben-Yehoshua S, Rodov V, D'hallewin G, Dore A** (2005) Elicitation of resistance against pathogens in citrus fruits by combined UV illumination and heat treatments. *Acta Horticulturae* **682**, 2013-2019

**Ben-Yehoshua S, Rodov V, Fang DQ, Kim JJ** (1995) Preformed antifungal compounds of citrus fruit: effect of postharvest treatments with heat and growth regulators. *Journal of Agricultural and Food Chemistry* **43**, 1062-1066

**Ben-Yehoshua S, Rodov V, Kim JJ, Carmeli S** (1992) Preformed and induced antifungal materials of citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. *Journal of Agricultural and Food Chemistry* **40**, 1217-1221

**Ben-Yehoshua S, Shapiro B, Shomer M** (1990) Ethylene enhanced heat damage to flavedo tissue of cured citrus fruit. *HortScience* **25**, 122-124

**Beraha L, Ramsey GB, Smith MA, Wright WD** (1959) Factors influencing the use of gamma radiation to control decay of lemons and oranges. *Phytopathology* **49**, 91-96

**Borsa J** (2004) Outlook for food irradiation in the 21st century. In: Komolprasert V, Morehouse KM (Eds) *Irradiation of Food and Packaging. Recent Developments*, American Chemical Society Symposium Series **875**, Washington, DC, USA, pp 326-339

**Bramlage WJ, Couey HM** (1965) Gamma radiation of fruit to extend market life. *ARS-USDA Marketing Research Report* **717**, 27 pp

**Brown GE** (1973) Development of green mold in degreened oranges. *Phytopathology* **63**, 1104-1107

**Brown GE, Baraka MA** (1996) Effect of washing sequence and heated solutions to degreened Hamlin oranges on Diplodia stem-end rot, fruit colour and phytotoxicity. *Proceedings of the International Society of Citriculture* **2**, 1164-1170

**Brown GE, Barmore CR** (1977) The effect of ethylene on susceptibility of 'Robinson' tangerines to anthracnose. *Phytopathology* **67**, 120-123

**Brown GE, Barmore CR** (1983) Resistance of healed citrus exocarp to penetration by *Penicillium digitatum*. *Phytopathology* **73**, 691-694

**Brown GE, Dezman DJ** (1990) Uptake of imazalil by citrus fruit after postharvest application and the effect of residue distribution on sporulation of *Penicillium digitatum*. *Plant Disease* **74**, 927-930

**Brown GE, Eckert JW** (2000) Postharvest fungal diseases. In: Timmer LW, Garnsey SM, Graham JH (Eds) *Compendium of Citrus Diseases* (2nd Edn), APS Press, St. Paul, MN, USA, pp 37-45

**Brown GE, Ismail MA, Barmore CR** (1978) Lignification of injuries to citrus fruit and susceptibility to green mold. *Proceedings of the Florida State Horticultural Society* **91**, 124-126

**Brown GE, Lee HS** (1993) Interactions of ethylene with citrus stem-end rot

caused by *Diplodia natalensis*. *Phytopathology* **83**, 1204-1208
**Chace WG Jr.** (1969) Controlled atmosphere storage of Florida citrus fruit. *Proceedings of the First International Citrus Symposium* **3**, 1365-1373
**Civello PM, Vicente AR, Martínez GA** (2007) UV-C technology to control postharvest diseases of fruits and vegetables. In: Troncoso-Rojas R, Tiznado-Hernández ME, González-León A (Eds) *Recent Advances in Alternative Postharvest Technologies to Control Fungal Diseases in Fruits and Vegetables*, Transworld Research Network, Trivandrum, Kerala, India, pp 71-102
**Coates LM, Johnson GI** (1993) Effective disease control in heat-disinfested fruit. *Postharvest News and Information* **4**, 35-40
**Couey HM** (1989) Heat treatment for control of postharvest diseases and insect pests of fruits. *HortScience* **24**, 198-202
**Cunningham NM, Taverner PD** (2007) Efficacy of integrated postharvest treatments against mixed inoculations of *Penicillium digitatum* and *Geotrichum citri-aurantii* in 'Leng' navel oranges (*Citrus sinensis). New Zealand Journal of Crop and Horticultural Science* **35**, 187-192
**D'hallewin G, Arras G, Castia T, Piga A** (1994) Reducing decay of 'Avena' mandarin fruit by the use of UV, heat and thiabendazole treatments. *Acta Horticulturae* **368**, 387-394
**D'hallewin G, Arras G, Dessi R, Dettori A, Schirra M** (1999a) Citrus green mould control in stored 'Star Ruby' grapefruits by the use of a biocontrol yeast under curing conditions. *Acta Horticulturae* **485**, 111-115
**D'hallewin G, Arras G, Venditti T, Rodov V, Ben-Yehoshua S** (2005) Combination of ultraviolet-C irradiation and biocontrol treatments to control decay caused by *Penicillium digitatum* in 'Washington navel' orange fruit. *Acta Horticulturae* **682**, 2007-2012
**D'hallewin G, Schirra M** (2001) Structural changes in epicuticular wax and storage response of 'Marsh Seedless' grapefruits after ethanol dips at 21 and 50°C. *Acta Horticulturae* **553**, 441-442
**D'hallewin G, Schirra M, Manueddu E, Piga A, Ben-Yehoshua S** (1999b) Scoparone and scopoletin accumulation and ultraviolet-C induced resistance to postharvest decay in oranges as influenced by harvest date. *Journal of the American Society for Horticultural Science* **124**, 702-707
**D'hallewin G, Schirra M, Pala M, Ben-Yehoshua S** (2000) Ultraviolet C irradiation at 0.5 $kJ \cdot m^{-2}$ reduces decay without causing damage or affecting postharvest quality of star ruby grapefruit (*C. paradisi* Macf.). *Journal of Agricultural and Food Chemistry* **48**, 4571-4575
**Del Río JA, Ortuño A** (2005) Biosynthesis of flavonoids in citrus and its involvement in the antifungal defense mechanisms. In: Dris R, Niskanen R, Mohan Jain S (Eds) *Crop Management and Postharvest Handling of Horticultural Products (Vol 4) Diseases and Disorders of Fruits and Vegetables*, Science Publishers Inc, Enfield, NH, USA, pp 185-220
**Dettori A, D'hallewin G, Aggabio M, Marceddu S, Schirra M** (1996) SEM studies on *Penicillium italicum* – 'Star Ruby' grapefruit interactions as affected by fruit hot water dipping. *Proceedings of the International Society of Citriculture* **2**, 1158-1163
**Droby S, Chalutz E, Horev B, Cohen L, Gaba V, Wilson CL, Wisniewski M** (1993) Factors affecting UV-induced resistance in grapefruit against the green mould decay caused by *Penicillium digitatum. Plant Pathology* **42**, 418-424
**Dubery IA, Holzapfel CW, Kruger GJ, Schabort JC, van Dyk M** (1988) Characterization of a γ-radiation-induced antifungal stress metabolite in citrus peel. *Phytochemistry* **27**, 2769-2772
**Eckert JW, Eaks IL** (1989) Postharvest disorders and diseases of citrus fruits. In: Reuter W, Calavan EC, Carman GE (Eds) *The Citrus Industry* (Vol V), University of California Press, Berkeley, CA, USA, pp 179-260
**El-Ghaouth A, Wilson CL, Wisniewski M, Droby S, Smilanick JL, Kortsten L** (2002) Biological control of postharvest diseases of citrus fruits. In: Gnanamanickam SS (Ed) *Biological Control of Crop Diseases*, Marcel Dekker Inc, New York, USA, pp 289-312
**Erkan M, Pekmezci M, Karasahin I, Uslu H** (2005) Reducing chilling injury and decay in stored 'Clementine' mandarins with hot water and curing treatments. *European Journal of Horticultural Science* **70**, 183-188
**Fallik E** (2004) Prestorage hot water treatments (immersion, rinsing and brushing). *Postharvest Biology and Technology* **32**, 125-134
**Fallik E** (2007) Heat treatments to control fungal diseases. In: Troncoso-Rojas R, Tiznado-Hernández ME, González-León A (Eds) *Recent Advances in Alternative Postharvest Technologies to Control Fungal Diseases in Fruits and Vegetables*, Transworld Research Network, Trivandrum, Kerala, India, pp 21-38
**Fawcett HS** (1922) Packing house control of brown rot. *Citograph* **7**, 232-234
**Ferguson IB, Ben-Yehoshua S, Mitcham EJ, McDonald RE, Lurie S** (2000) Postharvest heat treatments: introduction and workshop summary. *Postharvest Biology and Technology* **21**, 1-6
**Fernández YJ, Hall DJ** (2004) *In vitro* response of *Penicillium digitatum* and *Geotrichum candidum* to ultraviolet (UV-C) exposure. *Proceedings of the Florida State Horticultural Society* **117**, 380-381
**Gómez-López VM, Ragaert P, Debevere J, Devlieghere F** (2007) Pulsed light for food decontamination: a review. *Trends in Food Science and Technology* **18**, 464-473
**Grierson W, Dennison RA** (1965) Radiation treatment of 'Valencia' oranges and 'Marsh' grapefruit. *Proceedings of the Florida State Horticultural Society* **78**, 233-237
**Harding PR Jr.** (1968) Effect of ozone on *Penicillium* mold decay and sporulation. *Plant Disease Reporter* **52**, 245-247
**Holmes GJ, Eckert JW** (1999) Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology* **89**, 716-721
**Hong S, Lee H, Kim D** (2007) Effects of hot water treatment on the storage stability of satsuma mandarin as a postharvest decay control. *Postharvest Biology and Technology* **43**, 271-279
**Hopkins EF, Loucks KW** (1948) A curing procedure for the reduction of mold decay in citrus fruits. *Bulletin of the University of Florida Agricultural Experiment Station* **450**, 26 pp
**Houck LG** (1967) Hot water treatments for control of *Penicillium* green mold of Eureka lemons. *Phytopathology* **57**, 99 (abstract)
**Huang Y, Deverall BJ, Morris SC** (1995) Postharvest control of green mould on oranges by a strain of *Pseudomonas glathei* and enhancement of its biocontrol by heat treatment. *Postharvest Biology and Technology* **5**, 129-137
**Janisiewicz WJ, Korsten L** (2002) Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**, 411-441
**Kader AA** (1999) Current and potential applications of ionizing radiation in postharvest handling of fresh horticultural perishables. *International Produce Journal* **8**, 38-39
**Kader AA, Arpaia ML** (2002) Postharvest handling systems: subtropical fruits. In: Kader AA (Ed) *Postharvest Technology of Horticultural Crops* (3rd Edn), University of California Agriculture and Natural Resources, Publication **3311**, Oakland, CA, USA, pp 375-383
**Kahan RS, Barkai-Golan R** (1968) Combined action of sodium orthophenilphenate and gamma radiation on the *in vitro* development of fungi pathogenic to citrus fruit. *Phytopathology* **58**, 700-701
**Kanetis L, Förster H, Adaskaveg JE** (2007) Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. *Plant Disease* **91**, 1502-1511
**Kim JJ, Ben-Yehoshua S, Shapiro B, Henis Y, Carmeli S** (1991) Accumulation of scoparone in heat-treated lemon fruit inoculated with *Penicillium digitatum* Sacc. *Plant Physiology* **97**, 880-885
**Kinay P, Mansour MF, Mlikota Gabler F, Margosan DA, Smilanick JL** (2007) Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Protection* **26**, 647-656
**Kinay P, Yildiz F, Sen F, Yildiz M, Karacali I** (2005) Integration of pre- and postharvest treatments to minimize *Penicillium* decay of Satsuma mandarins. *Postharvest Biology and Technology* **37**, 31-36
**Klotz LJ** (1936) Nitrogen trichloride and other gases as fungicides. *Hilgardia* **10**, 27-52
**Ladaniya MS, Singh S, Wadhawan AK** (2003) Response of 'Nagpur' mandarin, 'Mosambi' sweet orange and 'Kagzi' acid lime to gamma radiation. *Radiation Physics and Chemistry* **67**, 665-675
**Lanza G, Di Martino Aleppo E** (1996) Control of green mould of oranges and lemons by curing at high temperatures. *Proceedings of the International Society of Citriculture* **2**, 1187-1191
**Lanza G, Di Martino Aleppo E, Strano MC** (2004) Evaluation of alternative treatments to control green mold in citrus fruit. *Acta Horticulturae* **632**, 343-349
**Lesar KH** (2008) The potential role of GRAS (Generally Regarded as Safe) chemicals, alone and in combination with the post-harvest fungicides, in the control of the major post-harvest citrus pathogens, *Penicillium digitatum* (citrus green mold) and *Geotrichum candidum* (sour rot). In: Bertolini P (Ed) *Proceedings of COST Action 924 International Congress, Novel Approaches for the Control of Postharvest Diseases and Disorders*, 3-5 May 2007, CRIOF, University of Bologna, Bologna, Italy, pp 322-326
**Luckey TD** (1991) *Radiation Hormesis*. CRC Press, Boca Raton, FL, USA, 239 pp
**Lurie S** (1998) Postharvest heat treatments. *Postharvest Biology and Technology* **14**, 257-269
**Mahrouz M, Lacroix M, D'Aprano G, Oufedjikh H, Boubekri C, Gagnon M** (2002) Effect of γ-irradiation combined with washing and waxing treatment on physicochemical properties, vitamin C, and organoleptic quality of *Citrus clementina* Hort. Ex. Tanaka. *Journal of Agricultural and Food Chemistry* **50**, 7271-7276
**Margosan DA, Smilanick JL** (1998) Mortality of spores of *Botrytis cinerea*, *Monilinia fructicola*, *Penicillium digitatum*, and *Rhizopus stolonifer*, after exposure to ozone in air under humid conditions. *Phytopathology* **88**, S58 (Abstract)
**Maxie EC, Sommer NF, Eaks IL** (1969) Effect of gamma radiation on citrus fruit. *Proceedings of the First International Citrus Symposium* **3**, 1375-1387
**McGlasson WB, Eaks IL** (1972) A role for ethylene in the development of wastage and off-flavors in stored "Valencia" oranges. *HortScience* **7**, 80-81
**McLaughlin WL** (1999) Gamma-ray sources vs. X-ray or electron beams for quarantine treatment. In: Moy JH, Wong L (Eds) *Proceedings of the Workshop The Use of Irradiation as Quarantine Treatment of Food and Agricultural Commodities*, Honolulu, HI, USA, pp 78-89
**Miller WR, McDonald RE, Chaparro J** (2000) Tolerance of selected orange and mandarin hybrid fruit to low-dose irradiation for quarantine purposes. *HortScience* **35**, 1288-1291
**Morehouse KM, Komolprasert V** (2004) Irradiation of food and packaging:

an overview. In: Komolprasert V, Morehouse KM (Eds) *Irradiation of Food and Packaging. Recent Developments*, American Chemical Society Symposium Series **875**, Washington, DC, USA, pp 1-11

**Mulas M, Mereu V, Ligios G, Schirra M** (2008) Heat treatments effects on respiration and ethylene production rates and on ethanol and acetaldehyde accumulation in the juice or their release by 'Valencia Late' oranges during storage. In: Bertolini P (Ed) *Proceedings of COST Action 924 International Congress, Novel Approaches for the Control of Postharvest Diseases and Disorders*, 3-5 May 2007, CRIOF, University of Bologna, Bologna, Italy, pp 206-215

**Nafussi B, Ben-Yehoshua S, Rodov V, Peretz J, Ozer BK, D'hallewin G** (2001) Mode of action of hot-water dip in reducing decay of lemon fruit. *Journal of Agricultural and Food Chemistry* **49**, 107-13

**Narayanasamy P** (2006) Integrated systems for the management of postharvest diseases. In: *Postharvest Pathogens and Disease Management*. John Wiley and Sons Inc, Wiley-Interscience, Hoboken, NJ, USA, pp 537-554

**Nunes C, Usall J, Manso T, Torres R, Olmo M, García JM** (2007) Effect of high temperature treatments on growth of *Penicillium* spp. and development on 'Valencia' oranges. *Food Science and Technology International* **13**, 63-68

**Obagwu J, Korsten L** (2003) Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. *Postharvest Biology and Technology* **28**, 187-194

**Ojima T, Hori S, Ueno T, Toratani H, Katayama T, Fujimoto H, Kitoh S, Yoshizako F, Nishimura A, Shiomi N, Inoue M, Mori S** (1974) Effects of electron irradiation on the preservation of *Citrus unshiu*. Part III. Storage and browning of peel by irradiation. *Food Irradiation Japan* **9**, 1-12 (in Japanese with English summary)

**Oufedjikh H, Mahrouz M, Amiot MJ, Lacroix M** (2000) Effect of gamma-irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of *Citrus clementina* Hort. Ex. Tanaka. *Journal of Agricultural and Food Chemistry* **48**, 559-565

**Palou L, Marcilla A, Rojas-Argudo C, Alonso M, Jacas J, del Río MA** (2007a) Effects of X-ray irradiation and sodium carbonate treatments on postharvest *Penicillium* decay and quality attributes of clementine mandarins. *Postharvest Biology and Technology* **46**, 252-261

**Palou L, Montesinos-Herrero C, del Río MA** (2008a) Short-term $CO_2$ exposure at curing temperature to control postharvest green mold of mandarins. *Acta Horticulturae* **768**, 257-263

**Palou L, Smilanick JL, Crisosto CH, Mansour MF** (2001a) Effect of gaseous ozone exposure on the development of green and blue molds on cold stored citrus fruit. *Plant Disease* **85**, 632-638

**Palou L, Smilanick JL, Crisosto CH, Mansour MF, Plaza P** (2003) Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage. *Crop Protection* **22**, 1131-1134

**Palou L, Smilanick JL, Droby S** (2008b) Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Postharvest Review* **2:2**, 1-16

**Palou L, Smilanick JL, Margosan DA** (2007b) Ozone applications for sanitation and control of postharvest diseases of fresh fruits and vegetables. In: Troncoso-Rojas R, Tiznado-Hernández ME, González-León A (Eds) *Recent Advances in Alternative Postharvest Technologies to Control Fungal Diseases in Fruits and Vegetables*, Transworld Research Network, Trivandrum, Kerala, India, pp 39-70

**Palou L, Smilanick JL, Usall J, Viñas I** (2001b) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Disease* **85**, 371-376

**Palou L, Usall J, Aguilar MJ, Pons J, Viñas I** (1999) Control de la podredumbre verde de los cítricos mediante baños con agua caliente y carbonatos sódicos. *Levante Agrícola* **348**, 412-421 (in Spanish with English summary)

**Palou L, Usall J, Muñoz JA, Smilanick JL, Viñas I** (2002a) Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of clementine mandarins. *Postharvest Biology and Technology* **24**, 93-96

**Palou L, Usall J, Smilanick JL, Aguilar MJ, Viñas I** (2002b) Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. *Pest Management Science* **58**, 459-466

**Patil BS** (2004) Irradiation applications to improve functional components of fruits and vegetables. In: Komolprasert V, Morehouse KM (Eds) *Irradiation of Food and Packaging. Recent Developments*, American Chemical Society Symposium Series **875**, Washington, DC, USA, pp 117-137

**Pavoncello D, Lurie S, Droby S, Porat R** (2001) A hot water treatment induces resistance to *Penicillium digitatum* and promotes the accumulation of heat shock and pathogenesis-related proteins in grapefruit flavedo. *Physiologia Plantarum* **111**, 17-22

**Pérez AG, Luaces P, Olmo M, Sanz C, García JM** (2005) Effect of intermittent curing on mandarin quality. *Journal of Food Science* **70**, 64-68

**Plaza P, Sanbruno A, Usall J, Lamarca N, Torres R, Pons J, Viñas I** (2004a) Integration of curing treatments with degreening to control the main postharvest diseases of clementine mandarins. *Postharvest Biology and Technology* **34**, 29-37

**Plaza P, Usall J, Smilanick JL, Lamarca N, Viñas I** (2004b) Combining *Pantoea agglomerans* (CPA-2) and curing treatments to control established infections of *Penicillium digitatum* on lemons. *Journal of Food Protection* **67**, 781-786

**Plaza P, Usall J, Torres R, Abadias M, Smilanick JL, Viñas I** (2004c) The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. *Pest Management Science* **60**, 815-821

**Plaza P, Usall J, Torres R, Lamarca N, Asensio A, Viñas I** (2003) Control of green and blue mould by curing on oranges during ambient and cold storage. *Postharvest Biology and Technology* **28**, 195-198

**Porat R, Daus A, Weiss B, Cohen L, Droby S** (2002a) Effects of combining hot water, sodium bicarbonate and biocontrol on postharvest decay of citrus fruit. *Journal of Horticultural Science and Biotechnology* **77**, 441-445

**Porat R, Daus A, Weiss B, Cohen L, Fallik E, Droby S** (2000a) Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. *Postharvest Biology and Technology* **18**, 151-157

**Porat R, Lers A, Dori S, Cohen E, Weiss B, Daus A, Wilson CL, Droby S** (1999a) Induction of chitinase and β-1,3-endoglucanase proteins by UV irradiation and wounding in grapefruit peel tissue. *Phytoparasitica* **27**, 233-238

**Porat R, McCollum TG, Vinokur V, Droby S** (2002b) Effects of various elicitors on the transcription of a β-1,3-endoglucanase gene in citrus fruit. *Journal of Phytopathology* **150**, 70-75

**Porat R, Pavoncello D, Peretz J, Ben-Yehoshua S, Lurie S** (2000b) Effects of various heat treatments on the induction of cold tolerance and on the postharvest qualities of 'Star Ruby' grapefruit. *Postharvest Biology and Technology* **18**, 159-165

**Porat R, Pavoncello D, Peretz J, Weiss D, Daus A, Cohen E, Ben-Yehoshua S, Fallik A, Droby S, Lurie S** (2000c) Induction of resistance to *Penicillium digitatum* and chilling injury in 'Star Ruby' grapefruit by a short hot-water rinse and brushing treatment. *Journal of Horticultural Science and Biotechnology* **75**, 428-432

**Riov J, Monselise SP, Goren R,** (1972) Stimulation of phenolic biosynthesis in *Citrus* fruit peel by gamma radiation. *Radiation Research Reviews* **3**, 417-427

**Rodov V, Agar T, Peretz J, Nafussi B, Kim JJ, Ben-Yehoshua S** (2000) Effect of combined application of heat treatments and plastic packaging on keeping quality of 'Oroblanco' fruit (*Citrus grandis* L. × *C. paradisi* Macf.). *Postharvest Biology and Technology* **20**, 287-294

**Rodov V, Ben-Yehoshua S, Albagli R, Fang DQ** (1995a) Reducing chilling injury and decay of stored citrus fruit by hot water dips. *Postharvest Biology and Technology* **5**, 119-127

**Rodov V, Ben-Yehoshua S, Fang DQ, Kim JJ, Ashkenazi R** (1995b) Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. *Journal of Agricultural and Food Chemistry* **43**, 1057-1061

**Rodov V, Ben-Yehoshua S, Kim JJ, Shapiro B, Ittah Y** (1992) Ultraviolet illumination induces scoparone production in kumquat and orange fruit and improves decay resistance. *Journal of the American Society for Horticultural Science* **117**, 788-792

**Rodov V, Burns P, Ben-Yehoshua S, Fluhr R, Ben-Shalom N** (1996) Induced local disease resistance in citrus mesocarp (albedo): accumulation of phytoalexins and PR proteins. *Proceedings of the International Society of Citriculture* **2**, 1101-1104

**Schirra M, D'Aquino S, Palma A, Angioni A, Cabras P, Migheli Q** (2006) Residues of the quinone outside inhibitor fungicide trifloxystrobin after postharvest dip treatments to control *Penicillium* spp. on citrus fruit. *Journal of Food Protection* **69**, 1646-1652

**Schirra M, D'Aquino S, Palma A, Marceddu S, Angioni A, Cabras P, Scherm B, Migheli Q** (2005) Residue level, persistence and storage performance of citrus fruit treated with fludioxonil. *Journal of Agricultural and Food Chemistry* **53**, 6718-6724

**Schirra M, D'hallewin G** (1997) Storage performance of Fortune mandarins following hot water dips. *Postharvest Biology and Technology* **10**, 229-238

**Schirra M, D'hallewin G, Ben-Yehoshua S, Fallik E** (2000) Host-pathogen interactions modulated by heat treatment. *Postharvest Biology and Technology* **21**, 71-85

**Schirra M, D'hallewin G, Cabras P, Angioni A, Garau VL** (1998) Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. *Journal of Agricultural and Food Chemistry* **46**, 1177-1180

**Schirra M, Mulas M** (1995) Improving storability of "Tarocco" oranges by postharvest hot-dip fungicide treatments. *Postharvest Biology and Technology* **6**, 129-138

**Schirra M, Mulas M, Fadda A, Cauli E** (2004) Cold quarantine responses of blood oranges to postharvest hot water and hot air treatments. *Postharvest Biology and Technology* **31**, 191-200

**Sen F, Kinay P, Karacali I** (2008) Effects of chlorine and heat applications after harvest on the quality and resistance capacity of 'Satsuma' mandarins. In: Bertolini P (Ed) *Proceedings of COST Action 924 International Congress, Novel Approaches for the Control of Postharvest Diseases and Disorders*, 3-5 May 2007, CRIOF, University of Bologna, Bologna, Italy, pp 231-239

**Smilanick JL, Brown GE, Eckert JW** (2006a) Postharvest citrus diseases and their control. In: Wardowski WF, Miller WM, Hall DJ, Grierson W (Eds) *Fresh Citrus Fruits* (2nd Edn), Florida Science Source Inc, Longboat Key, FL, USA, pp 339-396

**Smilanick JL, Mansour MF** (2007) Influence of temperature and humidity on

survival of *Penicillium digitatum* and *Geotrichum citri-aurantii*. *Plant Disease* **91**, 990-996

**Smilanick JL, Mansour MF, Mlikota Gabler F, Goodwine WR** (2006b) The effectiveness of pyrimethanil to inhibit germination of *Penicillium digitatum* and to control citrus green mold after harvest. *Postharvest Biology and Technology* **42**, 75-85

**Smilanick JL, Mansour MF, Mlikota Gabler F, Sorenson D** (2008) Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Biology and Technology* **47**, 226-238

**Smilanick JL, Margosan DA, Henson DJ** (1995) Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. *Plant Disease* **79**, 742-747

**Smilanick JL, Margosan DA, Mlikota Gabler F, Usall J, Michael IF** (1999) Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Disease* **83**, 139-145

**Smilanick JL, Michael IF, Mansour MF, Mackey BE, Margosan DA, Flores D, Weist CF** (1997) Improved control of green mold of citrus with imazalil in warm water compared with its use in wax. *Plant Disease* **81**, 1299-1304

**Smilanick JL, Sorenson D** (2001) Control of postharvest decay of citrus fruit with calcium polysulfide. *Postharvest Biology and Technology* **21**, 157-168

**Smilanick JL, Sorenson D, Mansour M, Aiyabei J, Plaza P** (2003) Impact of a brief postharvest hot water drench treatment on decay, fruit appearance, and microbe populations of California lemons and oranges. *HortTechnology* **13**, 333-338

**Smoot JJ** (1969) Decay of Florida citrus fruits stored in controlled atmospheres and in air. *Proceedings of the First International Citrus Symposium* **3**, 1285-93

**Smoot JJ, Melvin CF** (1963) Hot water as a control for decay of oranges. *Proceedings of the Florida State Horticultural Society* **76**, 322-327

**Snowdon AL** (1990) *A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables (Vol 1) General Introduction and Fruits*, CRC Press Inc, Boca Raton, FL, USA, 302 pp

**Sommer NF, Maxie EC, Fortlage RJ, Eckert JW** (1964) Sensitivity of citrus fruit decay fungi to gamma irradiation. *Radiation Botany* **4**, 317-322

**Spalding DH, Reeder WF** (1976) Low pressure (hypobaric) storage of limes. *Journal of the American Society for Horticultural Science* **101**, 367-370

**Spalding DH, Reeder WF** (1985) Effect of hot water and gamma radiation on postharvest decay of grapefruit. *Proceedings of the Florida State Horticultural Society* **98**, 207-208

**Stange RR Jr, Eckert JW** (1994) Influence of postharvest handling and surfactants on control of green mold of lemons by curing. *Phytopathology* **84**, 612-616

**Stevens C, Khan VA, Lu JY, Wilson CL, Pusey PL, Igwegbe ECK, Kabwe K, Mafolo Y, Liu J, Chalutz E, Droby S** (1997) Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. *Biological Control* **10**, 98-103

**Stevens C, Wilson CL, Lu JY, Khan VA, Chalutz E, Droby S, Kabwe K, Haung Z, Adeyeye O, Pusey LP, Wisniewski ME, West M** (1996) Plant hormesis induced by ultraviolet light-C for controlling postharvest diseases of tree fruits. *Crop Protection* **15**, 129-134

**Tuset JJ** (1987) *Podredumbres de los Frutos Cítricos*. Conselleria d'Agricultura i Pesca, Generalitat Valenciana, Valencia, Spain, 206 pp (in Spanish)

**Tuset JJ, Hinarejos C, Mira JL, Martínez-Jávega JM** (1996) Tratamientos térmicos a los frutos cítricos para el control de las enfermedades de la post-recolección. *Levante Agrícola* **337**, 342-347 (in Spanish)

**Umeda K, Kawashima K, Sato T, Iba Y, Nishiura M** (1969) Shallow irradiation of *Citrus unshiu* by cathod ray. Part I. Effective pasteurizing dose of radiation to *Penicillium digitatum* and the effects of irradiation on the fruit quality. *Food Irradiation Japan* **4**, 91-100 (in Japanese with English summary)

**US FDA** (United States Food and Drug Administration) (1986) Irradiation in the production, processing and handling of food: final rule. *Federal Register* **51**, 13375-13399

**US FDA** (United States Food and Drug Administration) (2004) Irradiation in the production, processing and handling of food: final rule. *Federal Register* **69**, 76844-76847

**Usall J, Smilanick JL, Palou L, Denis-Arrue N, Teixidó N, Torres R, Viñas I** (2008) Preventive and curative activity of combined treatments of sodium carbonates and *Pantoea agglomerans* CPA-2 to control postharvest green mold of citrus fruit. *Postharvest Biology and Technology* **50**, 1-7

**Venditti T, Molinu MG, Dore A, Agabbio M, D'hallewin G** (2005) Sodium carbonate treatment induces scoparone accumulation, structural changes, and alkalinization in the albedo of wounded citrus fruits. *Journal of Agricultural and Food Chemistry* **53**, 3510-3518

**Wang S, Tang J, Cavalieri RP** (2001) Modeling fruit internal heating rates for hot air and hot water treatments. *Postharvest Biology and Technology* **22**, 257-270

**Wild BL** (1987) Fungicidal activity of potassium sorbate against *Penicillium digitatum* as affected by thiabendazole and dip temperature. *Scientia Horticulturae* **32**, 41-47

**Wild BL, McGlasson WB, Lee TH** (1976) Effect of reduced ethylene levels in storage atmospheres on lemon keeping quality. *HortScience* **11**, 114-115

**Wilson CL, Upchurch B, El-Ghaouth A, Stevens C, Khan V, Droby S, Chalutz E** (1997) Using an on-line UV-C apparatus to treat harvested fruit for controlling postharvest decay. *HortTechnology* **7**, 278-282

**Yildiz F, Kinay P, Yildiz M, Sen F, Karacali I** (2005) Effects of preharvest applications of $CaCl_2$, 2,4-D and benomyl and postharvest hot water, yeast and fungicide treatments on development of decay on Satsuma mandarins. *Journal of Phytopathology* **153**, 94-98

**Zhang J** (2007) The potential of a new fungicide fludioxonil for stem-end rot and green mold control on Florida citrus fruit. *Postharvest Biology and Technology* **46**, 262-270

**Zhang J, Swingle PP** (2005) Effects of curing on green mold and stem -end rot of citrus fruit and its potential application under Florida packing system. *Plant Disease* **89**, 834-840