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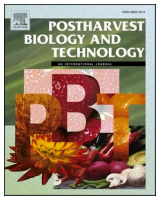

Review

Heat treatments for the control of postharvest decay of fresh fruit: Case studies of peach brown rot, kiwifruit gray mold and citrus green and blue molds

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ABSTRACT

The increase in restrictions on the use of synthetic fungicides has led to the adoption of new eco-friendly strategies that guarantee high quality and food safety standards, such as heat treatment (HT). This review focuses on the main HTs used to preserve peach, kiwi and citrus fruits from fungal infections during the postharvest phase. The mechanisms of action of HTs on fruits rely on induced resistance through genes regulation, and with a direct inhibition of fungal pathogens. Among the HTs, hot water (HW) and air treatments are mainly considered alongside 'curing' to manage postharvest infections caused by *Monilinia* spp., *Botrytis cinerea*, and *Penicillium* spp. The HTs were effective in controlling fungal diseases and improving fruit quality, nutritional value and shelf life. Consumer safety is ensured by HT, but this strategy should always be considered as part of an integrated management of postharvest fungal diseases, since HT alone does not provide complete decay control. The development of time × temperature combination remains the main challenge of HTs, to keep fruit quality and resistance during postharvest stage, even considering the evolution of the effects of agronomical approaches and of the phytosanitary management.

1. Introduction

The risk of fungal diseases occurring during the postharvest phase is addressed mainly through pre harvest fungicide treatments (Ippolito and Nigro, 2000). However, recent regulatory policies and the consumer demand are aiming for healthier and more sustainable foods, potentially reducing the synthetic product use, both in pre and postharvest stage. The European Green Deal, which includes the proposal to reduce the use and the risk related to the application of synthetic pesticides by 50 % until 2030 will further promote this trend, together with the increasing request of retailers of limit of pesticide maximum residue level (MLR), considerably lower than the legal threshold (Romanazzi et al., 2022). These actions have resulted in an increasing demand and use of alternative pre and postharvest disease control means and a change in disease management, that tend to prevent the infection and also to increase the host resistance (Prusky and Romanazzi, 2023). In postharvest stage, physical means and in particular heat treatments (HTs) may represent a valid approach due to the versatility with which they can be applied: hot water (HW) dips, rinses or brushing, vapour, and hot air (HA) (Di Francesco et al., 2018; Lurie at al., 1998). In addition, HTs can have a twofold effectiveness against fungal diseases: preventative and curative (Chen et al., 2015). HTs can stimulate defense responses in fruit tissue (Romanazzi et al., 2016) by the production of antifungal substances, the enhancement of wound healing, the induction of PR proteins, or the synthesis of cell wall hydrolytic enzyme inhibitors (Schirra et al., 2000; Di Francesco et al., 2018). The heat can directly inhibit the pathogen by killing or inactivating the spores or slowing germ tube growth, consequently reducing disease development (Liu et al., 2012). HTs can also inhibit fruit ripening, induce resistance to chilling injuries, and extend product storability (Fallik, 2004). A partial melting of the wax of fruit cuticle may be caused by HTs, so a mechanical barrier is provided at the sites where pathogens can penetrate, such as microcracks (Lurie, 2006). Further, HTs may stimulate lignification of the cell wall (Lurie, 2006) and induce production of phenolic compounds (Chen et al., 2015). However, among the methods, heat applied as forced HA or by dipping in HW appears to be the most promising (Di Francesco et al., 2021,

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2022). In the early 20th century, postharvest HTs were used commercially to control fungal diseases and insect infestations in horticultural crops (Schirra et al., 2000). Several types of HT machines are already operating, bringing several substantial benefits in particular in citrus fruits storage (Ben-Yehoshua et al., 2000).

The aim of this review was to summarize the available information on the use of HTs to control postharvest peach brown rot, kiwifruit gray mold and citrus green and blue molds, among the most prevalent and economically damaging diseases during the postharvest phase. The causal agents of these three diseases (*Monilinia* spp., *Botrytis cinerea*, *Penicillium* spp.) also share a latent infection period, making them particularly dangerous and difficult to manage during the cold storage.

2. Heat treatments (HTs)

2.1. Hot water (HW)

The HTs should be used as an immersion in Hot Water (HW) or as a spraying on fruits. The HW treatment was originally used to manage fungal diseases (Lu et al., 2007), sanitising the surface of fruit and removing or inhibiting fungal spores and latent infections. Immersion in HW often consisted of few min dip at different temperatures depending on the commodity. Usually, the temperature is between 45 and 60 °C for up to 10 min (Gonzalez Lopez et al., 2019). However, the treatment must consider different variables to be effective, such as the type of fruit, temperature, time of exposure to the heat source and the disease to be controlled. HW could also be applied by spraying, which is a treatment consisting of a pressurised spray of HW, often part of a production line where fruit is moved by brush rollers (Fallik, 2004).

2.2. Hot air (HA)

HA is one of the most effective HTs for controlling fruit fungal diseases. As Lurie (1998) describes, HA has a beneficial effect on fruit physiology, preventing attacks by pathogens. Usually, the HA treatments are long, ranging between 12 and 96 h and 38–46 °C (Lu et al., 2007). The heating treatments for the HA are subject to change depending on factors such as heat transfer, fruit size, and sensitivity. HA has a slow heat transfer rate, so it could be more effective when applied to small fruits. Indeed, if not uniform, HTs can damage the quality of fruit in terms of firmness and colour (Wang et al., 2001).

2.3. Curing

Curing is a postharvest approach that reduces decay and maintains quality during the long-term storage of fruit. It involves placing the fruit in picking bins and exposing them to specific temperature and humidity conditions for a few days immediately after harvest. Curing was applied in various horticultural products such as citrus fruit, peaches, carrots, sweet potatoes, kiwifruit, and onions (Ben-Yehoshua, 2005). The curing process for citrus lasts between two and three days when exposed to temperature of 30 °C and a relative humidity (RH) higher than 90 % (Palou, 2013). Although with temperatures not very high, worth of mention is the curing of kiwifruit before storage, which consists in a delay between harvest time and cold storage (Pennycook and Manning, 1992). Holding kiwifruit for 3 d at temperatures between 10 and 20 °C and RH around 95 % is the best combination for optimal control of gray mold and also chilling injury (Bautista-Baños et al., 1997). The global kiwi industry uses this technique, which is very simple to apply, to control grey mould. It involves leaving bins containing fruit under a shelter for the required time (Mari et al., 2015).

3. Mechanisms of action of HTs

All HTs exert different effects, involving fungal pathogens and hosts. In this section, some of the main mechanism of action of HTs, firstly on the pathogen and later on the host are described.

3.1. Fungal pathogen inhibition

The effect of HTs on fungal pathogens was reported as a direct means to inhibit fungal spore germination and mycelial growth on fruit surface (Karabulut et al., 2002), so reducing the microbial epiphytic population (Fallik, 2004). HTs may affect the initial growth of pathogen in the superficial host tissue (Couey and Alvarez, 1984). HTs can act directly on fungi by inhibiting spore germination (Di Francesco et al., 2018, 2021; Liu et al., 2012), or accumulating reactive oxygen species (ROS) causing oxidative damage of proteins and lipids (Zhao et al., 2014). Usall et al. (2016) reported that thermal death points for spores of many fungi range between 40 and 60 °C for an exposure time of 1–10 min. Nevertheless, the inactivation of some pathogens can also need the use of higher temperatures and longer exposure time. Despite this, fungal pathogens respond to heat stress by activating protective mechanisms such as increasing the production of the Heat Shock Proteins (HSPs), altering their growth, cellular composition, and initiating alternative metabolic pathways to maintain homeostasis (Tiwari et al., 2015; Dunayevich et al., 2018; Francisco et al., 2023). In citrus fruit, air temperature of 50 °C for 24 h with high RH are needed to kill the spores of *P. digitatum* (Smilanick and Mansour, 2007). The wet spores are in a hydrated state, then are more sensitive than dry ones to HTs (Porat et al., 2000; Karabulut et al., 2002; Usall et al. 2016), as well as the germinated spores with respect to non-germinated ones.

Table 1 included the most significant cases of successful use of HTs in the postharvest treatment of fruit against fungal pathogens.

3.2. Host structural and physiological responses

Host responses to HTs involve principally epicuticular waxes, which seal fruit entry points (stomata, lenticels, and microcracks) to pathogens (Schirra et al., 2000). The HTs cause the wax to melt, which induced a rearrangement of the fruit epicuticular layer.

Another structural response to HTs is the induction of lignification, which acts as a strong barrier to fungal pathogen invasion (Bhuiyan et al., 2013). Host tissue lignification strength cell wall preventing pathogen penetration and block partially infiltrated hyphae (Sui et al., 2016).

The HTs were found to increase the level of phenolic and flavonoid compounds in fruits such as kiwifruit and melon (Chen et al., 2015; Sui et al., 2014; Ippolito et al., 1995). Many studies have shown that heat-treated fruits have a higher level of polyphenols than untreated fruits, displaying increased resistance to fungal pathogens and mycotoxin build-up (Sanzani et al., 2009). The build-up of phenolic compounds was both very early and rapid, which led to the slowing or

Table 1
Heat treatments (HTs) for postharvest fungal diseases management.

Fruit	Disease	HT	T × Time	References
Apple	Green mold	HW*	55 °C× 15 s	Fallik et al., 2001
	Bull's eye rot	HW	45 °C× 10 min	Di Francesco et al., 2018
Stone fruit	Brown rot	HW	60 °C× 20 s /1 min	Spadoni et al., 2013
Mango	Anthracnose	HW	53 °C× 20 min	Alvindia and Acda, 2015
Sweet cherry	Green mold	HA*	44 °C× 114 min	Wang et al., 2015
Loquat	Anthracnose	HA	38 °C× 36 h	Liu et al., 2010
Kiwifruit	Gray mold	HW	55 °C× 1.5 min	Koukounaras et al., 2008
		HW	45 °C× 10 min	Chen et al., 2015
Citrus fruit	Green and Blue mold	HWB*	56 °C× 20 sec 53 °C× 2 min	Porat et al., 2000 Zhou et al., 2014
		HW		

*HW=Hot Water; *HA= Hot Air; *HWB=Hot Water Brushing

arresting of pathogen infection (Ruiz-García and Gómez-Plaza, 2013).

3.3. Host genes regulation

One of the most studied mechanisms of action of HTs is related to plant or fruit gene expression changes that can induce defence responses. Among the genes/proteins upregulated by HTs, the HSPs have been reported to be the most affected in different hosts, such as apple and peaches (Spadoni et al., 2015; Ritenour et al., 2001). This family of proteins is regulated by the heat shock transcription factors (HSTFs) that perceive abiotic stresses by activating a protection to different biotic and abiotic events (Sui et al., 2016). The HSPs confer protection against different abiotic and biotic stresses, mainly by functioning as molecular chaperones, so assist in protein folding, assembly and transport, and preventing protein aggregation (Bakthisaran et al., 2015). Spadoni et al. (2015) reported that different HSPs and HSTFs were upregulated after a HWT of 45 °C for 10 min in apple exocarp and mesocarp.

Other group of proteins associated to the response to HTs are the Pathogenesis Related (PR) proteins. These proteins can be significantly detectable in response to several stress factors (Sui et al., 2016), in particular in response to fungal pathogen infection. PR proteins seemed to play a major role in plant defense responses against different pathogens. Among these proteins, the better known are those that have β-1, 3-glucanase and chitinase activity often evaluated in fruit in response to HTs (Sui et al., 2016; Romanazzi et al., 2016).

Phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway, was also found to increase in response to HTs (Chen et al., 2008). Various studies have reported that this increase is associated with resistance to postharvest fruit disease (Zhou et al., 2014; Spadoni et al., 2014).

The host antioxidant system is positively affected by HTs. Gonzalez-Aguilar et al. (2010) showed that HTs have a significant role in activating host catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), directly involved in disease inhibition. Also, genes involved in fruit metabolism of sugars, polyphenols, and acids were associated as response to HTs (Luria et al., 2014).

4. How HTs affect fruit quality and tolerance to chilling injury

The desirable trait of HTs is the release of adequate firmness, color and biochemical traits, with the stopping or slowing of postharvest biotic and abiotic stress. The main problem with using HT techniques on a large scale is that the temperature tolerance of the fruit can vary. Sometimes, the difference between an effective treatment and a heat damage can be as little as a few degrees and a few minutes (Bollen and Dela Rue, 1999). The length of time that fresh produce is kept at these temperatures has an effect on it. Regarding kiwifruit, a HWT for 10 min at temperature of 55 °C prior to 3 months of storage at 0 °C displayed severe fruit injury (Ma et al., 2014). Conversely, 35 and 45 °C treatments per 10 min improved kiwifruit quality, decreasing malondialdehyde content, lipoxygenase activity and ethylene production (Ma et al., 2014). Holland et al. (2002) observed that increasing levels of sucrose by HTs were associated with an increase in the chilling tolerance of citrus fruit. As reported by den Ende and Valluru (2009) sugar signalling and sugar-modulated gene expression are often related to the control of oxidative stress. Soluble solids content (SSC) is an important indicator of fruit quality and can increase intracellular solute concentration, reducing the freezing point and enhancing chilling tolerance (Der Agopian et al., 2011).

SSC accumulation by HTs, such as HW and HA, has been reported in various fruits, including stone fruits and citrus fruits (Huan et al., 2017; Chen et al., 2012; Lara et al., 2009). Huan et al. (2017) demonstrated that HTs caused an increase in SSC on the first day of refrigerated storage, which probably stimulated a short-term effect on sugar metabolism in peaches. Other positive effects of HTs in mitigating chilling injuries have been attributed to enhancing membrane integrity and HSP gene expression, as well as altering PAL and polyphenol oxidase (PPO) enzyme activities (Aghdam and Bodbodak, 2014). However, it should always be considered that the inappropriate use of HTs can damage fruit externally and internally (Valero and Serrano, 2010), and that the success of the treatment is influenced by different variables (e.g. fruit species, cultivar, maturity, preharvest agronomic conditions, handling between harvest and treatment) (Fallik, 2004). Table 2 showed the most significant examples of the successful use of HTs to prevent quality and chilling injuries in fruit.

5. HTs effectiveness against *Monilinia* spp. of stone fruits

Brown rot is one of the major fungal disease affecting stone fruits worldwide (Mari et al., 2012). The disease can be caused by three *Monilinia* species: *M. laxa* (Aderhold and Ruhland) Honey, *M. fructicola* (Winter) Honey, and *M. fructigena* (Aderhold and Ruhland). However, *M. fructigena* predominantly infects pome fruit (Martini and Mari, 2014). All three pathogens can cause notable product losses, most often after harvest, during storage, transport, and commercialization. *Monilinia* species can cause visible rots on fruit starting from the field, when the environmental conditions result favourable for the infection (Garcia-Benitez et al., 2017). However, these fungi can remain latent until suitable conditions arise (Byrde and Willetts, 1977), making them very difficult to control in the postharvest stage. As reported by Garcia-Benitez et al. (2016), *Monilinia* spp. can remain latent in the form of subcuticular intercellular hyphae (Fig. 1).

In Europe, the importance of the losses caused by *Monilinia* spp. can reach high values (M€ 1.7/year) with dramatic impacts on the stone fruit market (Mari et al., 2012). Among control strategies, physical treatments represent a growing area of investigation because they are easy to implement, relatively economical and respectful for the environment and human health.

Conidial germination of the three *Monilinia* species is completely inhibited by HT at 55 °C for 1 min. Among pathogens that cause brown rot in stone fruits, *M. fructicola* is more heat-tolerant than *M. laxa* and *M. fructigena* (Spadoni et al., 2014). Heat treatment trials carried out by immersion in water at 60 °C for 20 and for 60 sec on commercial scale with naturally infected peaches showed a significant reduction of *Monilinia* spp. rot by more than 70 % (Spadoni et al., 2013).

6. HTs effectiveness on *Botrytis cinerea* of kiwifruit

Gray mold is the most important postharvest disease on a list of fruit (Romanazzi et al., 2016a PBT), however, like many storage fungal diseases, also in kiwifruit it has a preharvest component (Michailides and Elmer, 2000). Although, sepal infection does not induce disease symptoms on fruit in the field, it can cause latent infections that can develop during storage (Fig. 2).

A recent study conducted by Zuniga et al. (2023) reported that conidial germination of *Botrytis* strains was totally inhibited at 44 °C

Table 2
Heat treatments (HTs) to prevent quality and chilling injuries in fruit.

Fruit	HT	T°×Time	Effect	References
Banana	HW*	53 °C × 9 min	Delayed fruit ripening	Amin and Hossain, 2012
Papaya	HW	54 °C× 4 min	Decreased ethylene production and fruit softening	Li et al., 2013
Strawberry	HW	45 °C× 5 min	Maintained a high level of antioxidant capacity and fruit quality	Caleb et al., 2016
Citrus fruit	HA*	37°C× 3 d	Prevented sucrose decline	Holland et al., 2002
Avocado	HW	42 °C× 25 min	Improved peel color	Hofman et al., 2002

*HW=Hot Water; HA= Hot Air

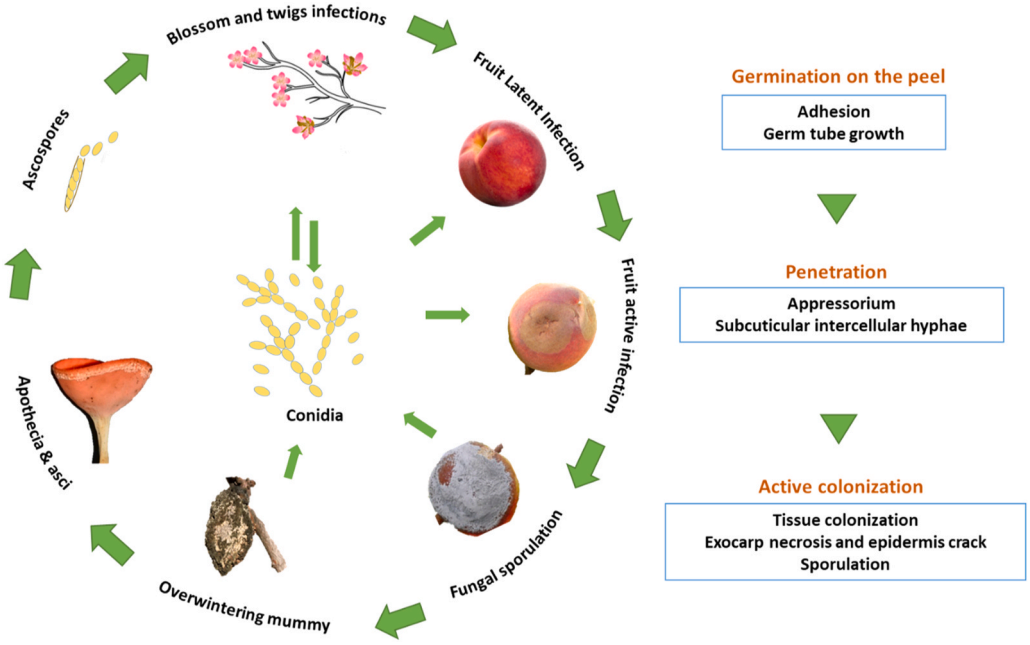

Fig. 1. *Monilinia* spp. epidemiological cycle on peach.

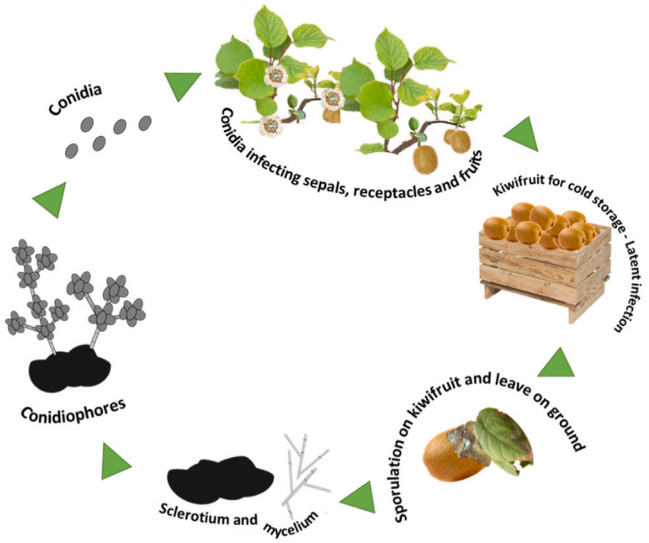

Fig. 2. *Botrytis cinerea* epidemiological cycle on kiwifruit.

with an exposure of 30 min, or after only 5 min if exposed to 48 °C. A significant difference in germination was found between different isolates in response to heat treatment for sclerotia. Cetiz et al. (2007) showed the antifungal effect of steam treatments at the stem end abscission zone of kiwifruit. Different temperatures from 45 to 50 °C for 15 min were tested on kiwifruit against *B. cinerea*. The treatment at 47.5 °C showed a significant reduction of fungal growth, while treatment at 50 °C showed a complete inhibition. Conversely, infection by wounds confirmed that none of the treatments completely inhibited the growth of *B. cinerea* on kiwifruits (Cetiz et al., 2007). By HW dipping, treatments applied at 50, 55, and 60 °C for 1.5 and 3 min completely inhibited gray mold at the stem end of kiwifruit (Koukounaras et al., 2008). The authors reported that only the treatment at 55 °C for 1.5 min was effective in extending kiwifruit storage, preserving the quality (firmness, vitamin C, color, and sugar) (Koukounaras et al., 2008).

The treatment appeared to affect some biochemical aspects of the kiwifruit, determining an increase in the degree of esterification of pectins in the pulp of treated fruits (Le Cam et al., 1994; Marty et al., 1997; Wydra and Beri, 2006), and a reduction in the content of superficial waxes in the peel, data closely related to a probable influence on the susceptibility of the host to a fungal attack. Physiological change may in part explain the beneficial effect of curing on kiwifruit with an intensification of the activity of chitinase, PAL, and PPO, as well as increases in concentrations of antifungal phenolic compounds and suberin in stem plugs and pericarp (Ippolito et al., 1995; Wurms, 2005). Ippolito et al. (1994) reported that 48h-cured kiwifruits at 15 °C showed the lowest grey mould incidence in comparison with the 24h-cured ones. Longer curing treatments (72–96 h) did not induce any further significant reduction of infection.

7. HTs effectiveness on *Penicillium* spp. of citrus fruits

Penicillium digitatum and *Penicillium italicum* represent the most important and impactful species affecting citrus fruit during the postharvest stage. *Penicillium* spp. conidia can be introduced into fruit in different phases: in the orchard, during the harvest or in the packinghouse facilitated by the presence of wounds or injuries on fruit surface (Kanashiro et al., 2020). Once inside the fruit, *Penicillium* spp. grow and colonize citrus tissues. The germination process was enhanced by the nutrients present in the wounded fruit such as sugars and organic acids (Eckert and Ratnayake, 1994). Subsequently, the produced conidia could land and infect nearby healthy fruits (Barkai-Golan, 2001) (Fig. 3).

The use of physical treatments against *Penicillium* spp. on citrus fruits has increased in importance due to their effectiveness, lack of residue release and minimal environmental impact (Usall et al., 2016; Bhatta, 2022).

The most effective HTs on citrus fruit before the storage consisted of curing, HW dips or rinsing and brushing (Palou, 2013). Regarding citrus curing, fruits are exposed to a heated atmosphere (30 °C) with elevated relative humidity (90 %) for a period of 2–3 days. Unappropriate HTs, such as with excessive temperatures and/or prolonged exposure, can damage fruit (Bhatta, 2022). As reported by several authors (Schirra et al., 1997; Porat et al., 2000; Palou et al., 2001), treatments at 53–55 °C × 2–3 min or 60 °C × 20 s caused oranges surface injury and browning. Nunes et al. (2007) placed citrus fruits in a room conditioned at 40 °C or 50 °C for 18 h, after which they were suddenly moved at 5 °C

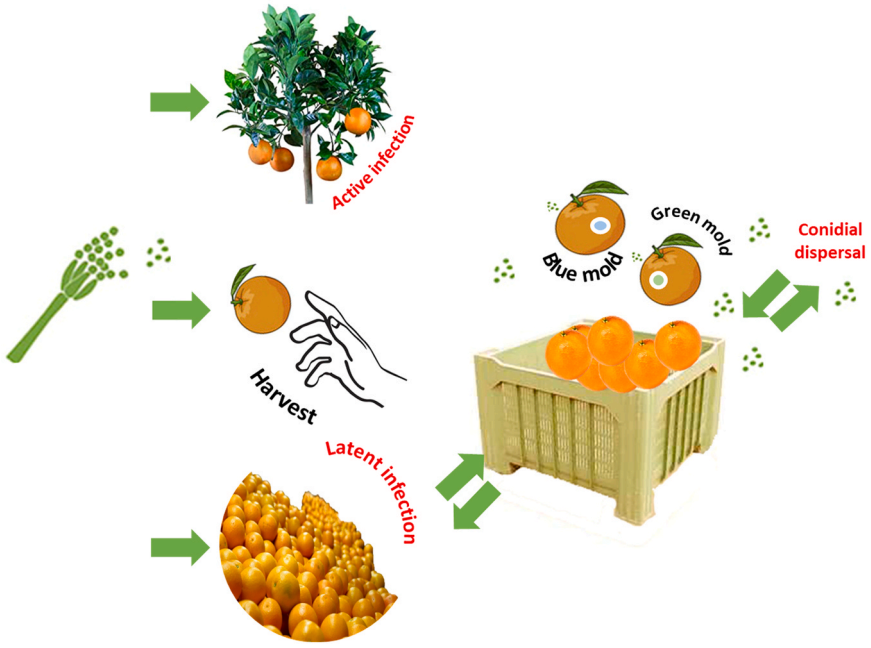

Fig. 3. *Penicillium* spp. epidemiological cycle on citrus fruit.

for five days, following a week shelf life at 20 °C. Only the curing treatment at 40 °C showed a total control *Penicillium* spp., while the temperature of 50 °C caused on fruit superficial scald and significant weight loss. The HW dips consisted of an immersion ranged between 2 and 5 min in water heated from 40 to 55 °C (Rodov et al., 1995). Usually, in packing lines the fruit were treated by the application of HW (55–65 °C) over rotating brushes for 10–60 sec to improve cleanliness, disinfection and fruit resistance against *Penicillium* spp. (Palou, 2013; Porat et al. 2000).

Also for citrus fruit, the effectiveness of HTs depends on the product condition prior to treatment, type of commodity, temperature, duration of treatment, and mode of heat application (Bhatta, 2022). Perotti et al. (2015) and Sui et al. (2016) showed that HTs could stimulate the production of secondary metabolites in citrus fruit like proteins (HSPs), phytoalexins (scoparone and scopoletin), lignins, so inducing resistance against the pathogens and at the same time interrupting *Penicillium* spp. conidial germination and mycelial growth.

8. Conclusions

Looking forward, HTs are set to play a key role in organic treatments, particularly given the limited availability of fungicide treatments. This is going to have a significant impact on the postharvest phase because HTs not only reduce infections, but could also have beneficial effects on fruit resistance to cold damage (Woolf et al., 1997; Abu-Kpawoh et al., 2002; Liu et al., 2012). However, it is essential to deepen knowledge on the effects of heat to avoid phytotoxic outcomes by maintaining fruit quality characteristics and at the same time effectively inhibiting pathogens and disease development. The activity of HTs depends at least on two components, as described above: the first consists of a direct inhibition on fungal inoculum (spores and/or mycelium) on fruit epicarp; the second is often considered an indirect action mediated by a stress response that induce disease resistance in fruits. To better manage fungal diseases with HTs, it is important to consider all components of the postharvest systems: the host and its microbiome, the pathogen, and the environment. The development of time × temperature combination remains the main challenge to keep fruit quality and resistance during postharvest stage. Regarding this, dedicated protocols should be developed for new varieties, for fruit exposed to different cultivation techniques as well as treatments with biostimulants and the latest plant protection products.

Author Contributions

A.D.F: conceptualization, writing original draft; A.I and G.R.: visualization, writing original draft; A.D.F., A.I., G.R.: writing - review and editing.

CRediT authorship contribution statement

Alessandra Di Francesco: Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **Antonio Ippolito:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization. **Gianfranco Romanazzi:** Writing – review & editing, Validation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no conflicts of interests.

Data availability

No data was used for the research described in the article.

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