Mini-review: Heat treatments for the control of citrus postharvest green mold caused by *Penicillium digitatum*

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Postharvest green mold, caused by the pathogen *Penicillium digitatum* (Pers.:Fr.) Sacc., is the most economically important postharvest disease of citrus fruits in Spain, areas with Mediterranean climate, and citrus production areas worldwide characterized by low summer rainfall. Economic losses due to this disease have been typically reduced through the application of synthetic chemical fungicides such as imazalil or thiabendazole. However, human health risks and environmental contamination associated with chemical residues and the proliferation of resistant strains of the pathogen are major problems associated with the use of these chemicals. There is, therefore, an increasing need to find and implement alternative antifungal postharvest treatments as part of integrated management programs for disease control. Among alternative physical decay control methods, heat treatments are the most common and popular because they are relatively effective, simple, cheap, and easy to apply and combine with other control systems. In this article, research work based on the evaluation of heat treatments used alone or in combination with other physical, chemical, or biological methods for the control of citrus green mold is reviewed.

The most important postharvest heat treatments that have been tested against *P. digitatum* on fresh citrus fruits are curing, hot water dips (HWD), and hot water rinsing and brushing (HWRB). Typical citrus curing employs exposure of fruit for 2-3 days to an air atmosphere heated to temperatures higher than 30°C at relative humidity higher than 90%. HWD are generally applied as relatively brief immersions (1-5 min) in water heated to 40-55 °C. HWRB consists basically in packingline machinery that treats the fruit by the application of hot water over rotating brushes at a relatively high temperature (55-65 °C) for a short time (10-60 s). Efficacy results, general performance, modes of action, limitations, advantages and disadvantages, and commercial feasibility of these heat treatments are discussed.

Keywords non-polluting decay control; curing; hot water

1. Introduction

Green mold, caused by the pathogen *Penicillium digitatum* (Pers.:Fr.) Sacc., is the most economically important postharvest disease of citrus fruits in all production areas that, like Spain and other Mediterranean countries, are characterized by low summer rainfall [1]. Actual losses due to green mold are quite variable and, beyond postharvest factors, depend upon the area of production, citrus cultivar, weather and orchard conditions, and especially the extent of physical or mechanical injury to the fruit during harvest and subsequent handling. *P. digitatum* is a strict wound pathogen that can infect the fruit in the grove, the packinghouse, and during distribution and marketing. It reproduces very rapidly and the spores are ubiquitous in the atmosphere and on fruit surfaces and are readily disseminated by air currents [2]. Furthermore, citrus fruit can become "soiled" with conidia that are loosened in handling of decayed fruit. For these reasons, any successful cost-effective postharvest disease management program for citrus fruit is primarily based on the control of green mold.

The disease has been primarily controlled worldwide for many years by the application of conventional fungicides such as imazalil, sodium ortho-phenyl phenate, thiabendazole or, more recently, new active ingredients like pyrimethanil, azoxystrobin or fludioxonil [2-6]. Different mixtures of these compounds are also commercially available. Postharvest treatments with these synthetic chemicals typically are relatively inexpensive, easy to apply, have curative action against pre-existing or established infections and persistent preventive action against potential new infections, and many also inhibit the sporulation from lesions on decaying fruit [7]. However, concerns about environmental contamination and human health risks associated with fungicide residues periodically led to regulatory reviews and restrictions or cancellations, and export markets are increasingly more sensitive to the use of chemicals for disease control. Further, the widespread and continuous use of these synthetic fungicides has led to the proliferation of resistant biotypes of *P. digitatum* in commercial packinghouses that seriously compromises the effectiveness of these treatments [8, 9]. There is, therefore, a clear need to find and implement methods alternative to conventional fungicides as part of integrated disease management (IDM) programs for the control of postharvest green mold of citrus fruits [10].

According to their nature, these alternative decay control methods can be physical, chemical or biological. Among them, physical methods are advisable because they are absolutely residue-free. Among physical methods, heat treatments are the most important and popular because they are relatively effective, simple, cheap, easy to apply and easy to combine with other disease control methods [10]. The purpose for this mini-review is to describe significant research work focused on the evaluation of heat treatments for the control of citrus postharvest green mold, either alone or in combination with other antifungal treatments. Efficacy results, general performance, modes of action, limitations, advantages and disadvantages, and commercial feasibility of thermal curing and hot water applications are discussed.

2. Curing

The exposure of citrus fruit for 2-3 days to an air atmosphere heated to temperatures higher than 30°C at high relative humidity (RH >90%) is known as curing. This term was adopted after evidencing that a significant amount of rind wounds healed (cured) following exposure to this treatment [11]. The first report on the use of curing against citrus green mold was published in the late 1940s in Florida [12]. Since then, numerous workers have reported satisfactory disease reductions in a variety of citrus species and cultivars [13-19].

In general, high curative activity has been observed when citrus fruit artificially inoculated with *P. digitatum* between 2 and 24 h before the treatment were cured at 35°C for 72 h. In such trials, green mold reductions higher than 90% with respect to non-cured controls have been reported after 7-20 days of incubation at 20°C, simulating immediate fruit commercialization. Likewise, similar results were obtained by Plaza et al. [20] when previously inoculated oranges were cured for 65 h at 33°C, showing that curing conditions could be relatively reduced. Moreover, this treatment reduced total decay incidence by more than 90% on naturally infected oranges. It was noticed in this work, however, that curing treatments were not as effective on citrus fruit cold-stored for long periods. This fact was attributed to the higher incidence of citrus postharvest blue mold, caused by the pathogen *Penicillium italicum* Whemer, which is known to be better adapted to grow at temperatures lower than 10°C [21]. Another application of hot air is the sanitation of empty storage rooms. According to Smilanick and Mansour [22], a 24-h exposure to air atmospheres heated to 50°C with high RH effectively killed spores of *P. digitatum*.

In spite of their good efficacy, commercial implementation of curing treatments is scarce, firstly because of the costs of heating and immobilizing large amounts of fruit for relatively long periods and, secondly, because excessive or uncontrolled treatments may harm the fruit. Fruit weight loss and heat phytotoxicity are major potential risks that depend not only on treatment conditions but also on the type of fruit and their initial condition. Excessive curing exposures can damage the rind, producing apparent rind staining (pitting or browning) and/or increasing water loss and respiration [23, 24]. In general, fruit responses to heat depend on the cultivar, the fruit condition prior to treatment, the temperature and duration of treatment, and the mode of heat application. The fruit physiological responses can vary by season and growing location, and can be due to differences in climate, soil type, season, production practices, maturity at harvest, and fruit size [25, 26].

Attempts that have been conducted to reduce the risks associated with curing include combination of curing with other disease control methods, which will be discussed later in this review; curing at higher temperatures for reduced periods of time (18 h at 40°C) [27], intermittent curing (two 18-h cycles at 38°C) [28] and, in the case of early season mandarins or oranges, integration of curing in the degreening process [29].

3. Hot water dips (HWD)

Hot water was first assayed against green mold on oranges in the USA in the 1960s [30]. Since then, HWD applied at 45-55°C for 2-5 min have repeatedly shown value in research tests to reduce green mold in several citrus species and cultivars [18, 31-36]. In general, temperatures from 50 to 55°C were the most effective in reducing the disease on oranges and mandarins artificially inoculated with *P. digitatum*, dipped for 150 s, and incubated at 20°C for 7 days. Lower and higher temperatures were ineffective and phytotoxic, respectively. The effectiveness of HWD depended not only on the fruit species, but also on the cultivar and the fruit maturity at harvest, and it was lower on mandarins than on oranges ([37-39].

HWD are a technology easier, cheaper, and more feasible for heat application than curing. However, commercial application of HWD as stand-alone treatments for green mold control is limited to some organically-grown citrus fruit or fruit like kumquat, whose peel is eaten [11]. The reasons for this include the lack of persistence and preventive activity of the treatment, the limited curative activity, the very narrow range of effective yet non-phytotoxic temperatures, the influence of host genotype and condition, and limitations related to technological aspects of commercial treatment application, i.e. implementation and maintenance of large high-volume tanks and associated energy costs [40, 41].

4. Hot water rinsing and brushing (HWRB)

The first prototype for this system was developed in the 1990s in Israel. It 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) [25]. Trials with different citrus species including tangerines, oranges, lemons and grapefruits showed that applications of water at 56-60ºC for 10-20 s effectively reduced green mold and other diseases with no rind injuries or adverse influence on fruit weight loss and internal quality [42, 43]. It was also observed that HWRB induced fruit resistance against infection by *P. digitatum* [44]. On kumquat fruit, the best HWRB treatment conditions for decay control while maintaining fruit quality were 55°C for 20 s [11].

Other prototypes based on the same principle have been developed in other countries with similar results against citrus green mold [16, 45]. However, the commercial use of HWRB technology is higher with other commodities that, in contrast to citrus, cannot be currently treated with conventional fungicides.

5. Combination with other control methods

Because of the limitations associated with heat treatments, considerable attention has been devoted to their integration with other non-polluting antifungal treatments for the control of citrus green mold without the use of chemical fungicides. In general, combination of treatments can pursue three different objectives [10]: 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.

In general, heat has often been used as a component of integrated control strategies for the control of citrus postharvest diseases. Hot water is relatively cheap and easy to apply and heating often provides complementary effects to other treatments of different nature. In the case of curing, other methods have been assayed as complements to reduce its length, risks and costs without diminishing its efficacy. Comprehensive descriptions of different approaches that have been considered for integration of heat treatments with other control methods in the context of IDM programs for citrus green mold control are available [10, 41].

5.1. With other physical treatments

Both curing treatments and HWD have been combined with other physical treatments such as UV-C illumination [46, 47] or ionizing radiation treatments [48]. Heat applied prior to UV-C exposure reduced the risk of rind phytotoxicity caused by the light. Likewise, the use of HWD allowed reducing the dose and consequently the rind damage risks associated with exposures to irradiation by γ-rays, electron beams or X-rays. Furthermore, in some cases, the combined treatments were synergistic to suppress spore germination and mycelial growth of *P. digitatum*.

In order to reduce the length and risks of curing, short treatments (24-48 h) with CO_2 or O_2 at curing temperature (33°C) were tested on mandarins and oranges artificially inoculated with *P. digitatum* and it was found a synergistic effect for decay control [49]. Moreover, the combined treatments induced a positive effect for quality retention of treated and long-term cold-stored citrus fruit [50].

In early research, curing was also combined with individual plastic sealing of citrus fruit and it was observed that weight loss and rind condition were significantly improved [13]. When plastic seals and liners were combined with curing, HWD or HWBR, green mold control and fruit quality were favorably affected [43].

5.2. With chemical treatments

The most important thermal application for integration of treatments against citrus green mold is heating aqueous solutions of antifungal chemicals, including conventional fungicides at low doses [3, 6, 51-55]. It was recently found, nevertheless, that the new fungicide propiconazole was an exception and the use of solutions heated to 48ºC did not improve the efficacy of ambient-temperature solutions (22ºC) [56].

The activity of low-toxicity alternative chemicals such as food additives or ‘generally regarded as safe’ (GRAS) compounds (e.g. sodium carbonate, sodium bicarbonate, potassium sorbate, sodium benzoate, …) [38, 57-62], phosphite salts [63], calcium polysulfide [64] or other chemicals has been repeatedly enhanced by the use of solutions heated to non-phytotoxic temperatures (40-50ºC). Optimal solution temperature will depend on the treatment duration and it should be particularly determined for each combination of active ingredient and fruit species and cultivar. Heat probably facilitates the uptake of the active ingredient through the fruit cuticle [40]. Exceptions to this general rule were sodium parabens, whose application at 50ºC did not enhance the control of green mold obtained with solutions at 20ºC [65, 66].

The combination of curing treatments with the application of conventional synthetic fungicides at low doses [17, 67], GRAS compounds like sodium carbonate [16, 68], ethanol [16] or acetic acid [19] also resulted in improved control of citrus green mold.

5.3. With biocontrol microbial antagonists

The application of biological control agents with antagonistic activity against *P. digitatum* in combination with heat treatments often resulted in synergistic activity for decay reduction. Biocontrol microorganisms typically colonize rind infection sites and protect the fruit from further fungal infections that can occur during storage or commercialization [69]. This mode of action can effectively complement the relative curative activity that heat offers against incipient *P. digitatum* infections present in the fruit when they arrive to the packinghouse from the orchard [10].

Several researchers have reported benefits from the integration of curing treatments with biocontrol antagonists, mostly yeasts or bacteria [70-73]. Main benefits were the reduction of the length and risks of curing and the consequent increase of the potential for implementation of more practical postharvest treatments. In some cases the most appropriate treatment application sequence was first the microbial antagonist followed by curing exposure. It is clear that in these cases a heat-tolerant antagonistic strain was used.

Treatments with HWD [73, 74] and HWBR [75] have also been reported as complementary to the application of biocontrol agents for green mold control. In this case, it has been usually recommended to apply first the heated water prior to the treatment with the microbial antagonists.

6. Mode of action

Heat treatments reduce postharvest green mold of citrus fruit by the combination of direct effects on the pathogen *P. digitatum* and indirect effects on the citrus fruit host. Heat can affect the spores or hyphae of *P. digitatum* present on rind wounds depending on the temperature and treatment duration. In this sense, higher temperatures are more effective and the limiting factor is the fruit susceptibility to phytotoxicity by heat. On the other hand, heat can induce different mechanisms of resistance in the rind infection sites. Besides the heat treatment nature and characteristics, this induction depends greatly on the fruit genotype and its physiological condition when the treatment is applied.

General direct effects of heat on pathogenic structures that have been described include 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. Some of these mechanisms may be simultaneously involved [40, 76]. Heat treatments applied against citrus green mold are generally fungitoxic but, because of phytotoxicity risks, too mild to be fungicidal, i.e. lethal. An issue to consider when including heat treatments on postharvest decay management programs is the potential acquisition of thermotolerance by *P. digitatum*. The development of resistance to heat treatments is already an important issue in heat-related control of insects on fruit [77].

Indirect mode of action of heat treatments is based on the induction on the fruit host of several resistance mechanisms against the infection process and the development of the pathogen. In general, more than one of these mechanisms can be triggered at the same time to different extend. One of these mechanisms is the induction of physical changes on the fruit rind surface. Structural changes of epicuticular rind wax have been observed after immersion in hot water or treatment with HWRB that led to the formation of a mechanical barrier by melting wax in rind wounds [40, 42]. Other mechanisms are the promotion of resistance to disease by the inhibition of the decline of the concentration of certain constitutive antifungal compounds (e.g. citral) present in the peel of immature fruit [78], or the induction of the biosynthesis of new antifungals as a response to the stress induced by heat and fungal infection. This is the case of lignin-like materials, phytoalexins such as the coumarins scoparone and scopoletin, pathogenesis-related proteins (PRP) such as chitinases or β-1,3-glucanases, or heat shock proteins (HSP) [13, 79-84]. Besides biochemical analyses, proteomic and metabolomic studies are also been conducted to characterize the host responses and the induction of resistance at a molecular level [85-87].

7. Concluding remarks

Major advantages from the use of heat treatments for the control of postharvest green mold of citrus fruit, caused by *P. digitatum*, are the total absence of chemical residues on/in treated fruit and their minimal environmental impact. This is currently important because of the increasing need to implement non-polluting antifungal treatments as an answer to regulatory and consumer demands, especially for EU export markets. Major disadvantages are the limited effectiveness at non-phytotoxic temperatures, lack of preventive activity, low persistence, and inconsistency associated to the nature and mode of action of these treatments. The fungicidal activity is limited and the efficacy typically varies according to the physical and physiological condition of the fruit host. Therefore, their use as stand-alone treatments is restrained. Furthermore, in the case of thermal curing, the risks of adverse effects on fruit quality or technological problems for cost-effective application are hindering the broad commercial use of these treatments, in spite of an excellent disease control efficacy. However, some heat treatments are simple, fast, and inexpensive and, in some cases, compatible and complementary with other postharvest disease control methods, so they can be used as part of IDM strategies in a multifaceted approach to successfully control green mold without the application of synthetic chemical fungicides.

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