Short Exposure to High CO₂ and O₂ at Curing Temperature to Control Postharvest Diseases of Citrus Fruit

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Abstract

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Curing of citrus fruit at 30 to 37°C and 90 to 98% relative humidity for 65 to 72 h is an effective alternative to fungicides to control postharvest green and blue molds caused by *Penicillium digitatum* and *P. italicum*, respectively. However, commercial adoption is limited because treatment is long and it may harm fruit quality. In order to improve the feasibility of curing, short CO₂ or O₂ exposures at curing temperature were evaluated on 'Nadorcott', 'Clemenules', and 'Ortanique' mandarin fruit and 'Valencia' orange. Fruit were artificially inoculated, exposed 24 h later to air (control); CO₂ at 15, 30, 50, or 95 kPa; or O₂ at 30 or 45 kPa at 20 or 33°C for 8, 24, or 48 h and incubated at 20°C for

4, 7, or 15 days. Exposure at 33°C with CO₂ at 15 kPa for 24 h or O₂ at 30 kPa for 48 h effectively controlled both green and blue molds after 7 days of incubation at 20°C; however, control of both diseases was minimal after 15 days. To assess potential induction of disease resistance, fruit were treated as described above, then inoculated after 1, 2, or 5 days at 20°C and evaluated after 3 and 6 more days at 20°C. All of the treatments were ineffective in inducing fruit resistance. Short exposures of citrus fruit to high CO₂ or O₂ at curing temperatures may be part of a control program alternative to synthetic fungicides, especially for organic fruit markets.

Fungicides such as imazalil, sodium *ortho*-phenylphenate, or thiabendazole have been widely used for treatment of citrus fruit against postharvest diseases. Green and blue molds caused by *Penicillium digitatum* (Pers.) Sacc. and *P. italicum* Wehmer, respectively, cause the majority of economic losses during storage and marketing of citrus fruit (13). The number of synthetic postharvest fungicides is declining because of their reduced effectiveness due to the proliferation of fungicide-resistant isolates of decay pathogens and concerns about human and environmental safety of their residues. Because the control of postharvest diseases is an imperative for the marketing of citrus fruit, new and nontoxic methods are required to substitute for or complement the current use of synthetic fungicides (25).

Studies to find alternatives to conventional fungicides have quantified the effect of heat on postharvest treatments of fruit (26,28,38). Heat treatments have a direct effect on reducing the effective inoculum size by inactivating or killing spores, and an indirect effect via physiological responses of the fruit tissue that inhibit fungal development (38). Curing is a heat treatment that consists of holding the fruit at 30 to 37°C and 90 to 98% relative humidity (RH) for 65 to 72 h. Methods based on curing treatments have been evaluated to control green or blue molds on citrus fruit (6,7,22,31,47). For instance, curing at 33°C and high RH for 65 h has been reported as an effective method to control green mold on orange fruit (31). Moreover, curing at 36°C for 72 h reduced chilling injury on 'Star Ruby' grapefruit (32). Although effective, its use commercially is inconvenient because it is time consuming, requires energy for heating, and may harm fruit quality. Nevertheless, although the use of curing as a stand-alone method is not feasible, some modifications of the method and combination with other treatments may overcome the drawbacks of curing alone. In this regard, short exposures to modified atmospheres created by elevating the concentration of CO₂ or O₂ may be complementary

with curing. Exposure to CO₂-enriched atmospheres has been reported to provide fungistatic effects and, in some cases, induce resistance in fresh fruit against major postharvest diseases (28,33). Cold storage in high-CO₂ atmospheres is a common commercial practice with some commodities and suppression of decay has been widely assessed (2,5,39,45). According to El-Goorani and Sommer (14), the CO₂ concentrations required to inhibit growth or spore germination vary with fungal species but CO₂ levels above 10% are generally needed to significantly suppress fungal growth. However, very few studies have tested the effect of short-term exposures to high levels of CO₂ on postharvest diseases of fresh produce. Exposure of harvested avocados to 30% CO₂ for 24 h at 20 to 25°C delayed the development of anthracnose, caused by *Colletotrichum gloeosporioides* (33). Furthermore, table grape berries pretreated with CO₂ at 20 kPa for 3 days at 0°C had less natural decay during subsequent cold storage (37). On the other hand, superatmospheric O₂ has been shown to prevent growth of microbes in some fruit and vegetable crops during cold storage (1,40) and, specifically, inhibit the development of fungal diseases (9,19,44,48,49). Moreover, high-O₂ atmospheres may be advantageous for product quality (11,48) and prevent anaerobic fermentation and off-flavor development (1,41). Nevertheless, the levels of O₂ that were most effective for decay control were those close to 80% or higher, which could be difficult and expensive to maintain on a large scale as well as a fire hazard in a commercial situation.

Although controlled or modified atmospheres are commonly used for the storage or packaging of some commodities, this technology has not been used in citrus production because the potential benefits do not compensate for the high costs (18). Therefore, there is a lack of information about the effects of altered concentrations of CO₂ or O₂ on the control of decay in citrus fruit, especially in the case of exposure for short periods at high temperatures. Presumably, by using an atmosphere that inhibits fungal growth, curing time could be reduced, preserving fruit quality and maintaining antifungal effectiveness. In the present study, exposure of several citrus fruit cultivars to high CO₂ or O₂ treatments during short periods at curing temperature was evaluated as a method to either control established infections of *P. digitatum* or *P. italicum* or induce physical or biochemical changes in the fruit that could maintain or increase its resistance to infection. Some preliminary results have been presented (24,29).

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Materials and Methods

Fruit. Fruit used in these experiments were orange (*Citrus sinensis* (L.) Osbeck) 'Valencia', clementine mandarin (*C. reticulata* Blanco) 'Clemenules' (synonyms: 'Nules' and 'Clementina de Nules'), and hybrid mandarin 'Nadorcott' (*C. reticulata* × *C. sinensis*) (synonyms: 'Afourer' and 'W. Murcott') and 'Ortanique' (*C. reticulata* × (*C. sinensis* × *C. reticulata*)) from commercial orchards in Valencia (Spain). Fruit had not been treated with fungicides or artificially coated. The fruit were stored at 5°C and 90% RH until their use in the experiments within the next week. Prior to each assay, the fruit were selected, randomized, rinsed with fresh water, and allowed to air dry at room temperature.

Inoculum. Isolates NAV-7 of *P. digitatum* and MAV-1 of *P. italicum* used in the experiments are included in the fungal culture collection of the Pathology Laboratory of the Postharvest Technology Center of the Institut Valencià d'Investigacions Agràries (IVIA). They had been obtained from decayed orange or mandarin fruit from the Valencia area, purified, identified, and selected for their pathogenicity and aggressiveness among a high amount of autochthonous isolates. The two pathogens were cultured on potato dextrose agar (PDA) in petri dishes at 25°C for 7 to 14 days. Conidial suspensions of the pathogens were prepared in sterile water with Tween 80 (0.05% [wt/vol]) and adjusted to a concentration of 10⁵ or 10⁶ spores/ml after counting spores with a hemacytometer. Fruit were inoculated with *P. digitatum* by immersing a stainless-steel rod with a probe tip 1 mm wide and 2 mm long into the required spore suspension and then wounding each fruit with this inoculum-carrying tip once in the equator. The opposite side of the same fruit was inoculated with *P. italicum* using the same procedure.

Gas exposure. Chambers used for gas exposure consisted of hermetically sealed, transparent polymethyl methacrylate cabinets (82 by 62 by 87 cm) fitted with outlet and inlet ports through which O₂ or CO₂ (Alphagaz, Air Liquide España S.A., Madrid) were injected until the desired concentrations of O₂ (30 or 45 kPa) or CO₂ (15, 30, 50, or 95 kPa) were achieved. The cabinets were also fitted with internal basal water trays that allowed achieving a high RH (95 ± 5%). Levels of O₂, CO₂, temperature, and RH were continuously monitored by means of the computer-controlled system (Control-Tec; Tecnidex S.A., Paterna, Valencia, Spain). This system activated the injection of O₂ or CO₂ when the concentration of the gases was below the settings and stopped when the target level was measured, with an oscillation of O₂ or CO₂ at approxi-

mately ± 2 kPa. Depending on the experiment, the treatment duration was 8, 24, or 48 h. Cabinets were installed in the postharvest research facilities of the IVIA in a 40-m³ standard cold storage/curing room that was set to each experimental temperature (20 or 33°C). A curing treatment of 33°C was selected because it is the lowest temperature that has been reported to be effective for the control of citrus green and blue molds (31). Not all the combinations of treatment conditions were tested on all cultivars; treatments that provided better disease-control responses were selected for further tests with the same or other citrus cultivars. The treatments applied to different cultivars are listed in Table 1.

Effect on green and blue molds. Fruit were inoculated with a suspension of *P. digitatum* and *P. italicum* at 10⁶ spores/ml and kept at 20°C for 24 h before treatment. This inoculum density is recommended for evaluation of curative postharvest treatments to control green and blue molds (12). Fruit were then placed in the gas cabinets and treated according to the specifications for each experiment (Table 1). Treated fruit were arranged in plastic trays and incubated at 20°C and 85 ± 5% RH. Green mold incidence (number of decayed fruit) was recorded after 4, 7, 14, or 15 days of incubation, depending on the experiment. Disease incidence is the most important parameter to evaluate the efficacy of treatments but severity (lesion diameter) and sporulation (percentage of sporulated lesions) were also measured at the same time to be able to detect more subtle differences between treatments in case disease incidences were similar. Fruit treated with air at 20°C were considered as controls. Each treatment was applied to three replicates each with 25 fruit.

Induced resistance to green mold. Noninoculated citrus fruit from the same four cultivars were treated as described above; stored at 20°C for 1, 2, or 5 days; and subsequently inoculated with *P. digitatum* at a concentration of 10⁵ spores/ml. The incidence, severity, and sporulation of green mold were evaluated after 3 and 6 days of incubation at 20°C. Each treatment was applied to three replicates each with 12 fruit.

Statistical analysis. Data from countings (disease incidence and pathogen sporulation) was arcsine transformed. The percentage of disease reduction with respect to the control treatments was calculated. Multifactor analyses of variance (ANOVAs) were performed for each evaluation date (after 4 to 15 days at 20°C) using Statgraphics Plus 4.1 (Manugistics, Inc., Rockville, MD) with disease incidence, reduction, and severity and pathogen sporulation as dependent variables and gas treatment composition, temperature, or time of exposure as factors, depending on the experiment.

Table 1. Summary of the gas treatments applied on mandarin and orange fruit

Fruit, gas	Treatments ^a			
	Concentration (kPa)	Temperature (°C)	Time (h)	Incubation (days) ^b
'Ortanique' mandarin				
Air	...	20, 33	8, 24	14
CO ₂	15, 30, 50	20, 33	8, 24	7
CO ₂	95	20	8, 24	7
CO ₂	95	33	24	7
O ₂	30, 45	20, 33	8, 24	7
O ₂	30	33	24, 48	14
'Nadorcott' mandarin				
Air	...	20, 33	8, 24	7
CO ₂	15, 30, 50	20, 33	8, 24	7
CO ₂	95	33	8, 24	7
N ₂	95	33	8, 24	7
'Clemenules' mandarin				
Air	...	33	24, 48	14
O ₂	30	20	48	14
O ₂	30	33	24, 48	14
'Valencia' orange				
Air	...	33	24, 48	15
O ₂	30	33	48	15
CO ₂	15	33	24	15

^a Air treatments were used as controls. Relative humidity was 95 ± 5% during treatments and 85 ± 5% during incubation.

^b Incubation time at 20°C.

ANOVAs for different levels of the factors were performed because of significant interactions. Where appropriate, means were separated by Fisher's Protected least significant difference test ($P = 0.05$). When exposure to high CO_2 or O_2 was tested at both 20 and 33°C, Limpel's formula (34) was applied to determine whether the effectiveness of the combination of curing temperature and high CO_2 or O_2 was synergistic.

Results

On Nadorcott mandarin treated with high CO_2 at 20°C, disease reduction was not significant regardless of gas composition and treatment duration (*data not shown*). When applied at 33°C for 8 h, the reduction in the incidence of green and blue molds was lower than 10 and 40%, respectively, after 4 days of incubation at 20°C. In contrast, CO_2 treatments at 33°C for 24 h greatly reduced green and blue mold incidence after 4 days compared with air-treated fruit (Fig. 1). However, reductions were very low after 7 days of incubation, when 100% of control fruit (exposed to air at 20°C for 8 h) was decayed. In general, most of the tested CO_2 concentrations showed a similar ability to control the diseases. Green mold reduction significantly differed from 55% (CO_2 at 50 kPa) to 80% (CO_2 at 15 kPa) but it was statistically similar and intermediate on mandarin treated with the rest of the CO_2 concentrations. Blue mold reduction reached 99% after exposure to CO_2 at 95 kPa at 4 days of incubation, and results for the rest of the CO_2 treatments

were statistically similar. High levels of N_2 (95 kPa) applied to Nadorcott mandarin at 33°C for 8 or 24 h only reduced the incidence of green and blue molds by 5 and 40%, respectively. In virtually all cases, the combination of curing temperature and high CO_2 or O_2 was synergistic according to Limpel's formula (Fig. 1).

Exposure of Ortanique mandarin to high CO_2 or O_2 at 30 or 45 kPa for 8 or 24 h at 20°C did not significantly control blue mold (*data not shown*). At 20°C, green mold was significantly reduced only by CO_2 at 95 kPa (Fig. 2). However, these treatments applied at 33°C effectively controlled both molds (Fig. 2). The average green mold reduction after 4 days of incubation was 71, 70, and 60%, and 81, 87, and 92% for 8- and 24-h exposures to CO_2 at 50, 30, and 15 kPa, respectively. The effectiveness declined after 7 days of incubation but Ortanique mandarin treated with CO_2 at 15 kPa still had 74% less green mold incidence than control fruit (100% disease incidence). Exposure of Ortanique mandarin to O_2 at 30 or 45 kPa at 33°C for 8 h marginally controlled green and blue molds after 4 days of incubation at 20°C, whereas exposure for 24 h totally controlled both diseases. Once more, the effectiveness declined after 7 days. Nonetheless, when the treatment with O_2 at 30 kPa was extended to 48 h, the incidence reductions of green and blue molds after 7 days of incubation were 96 and 92%, respectively (Fig. 2), although they dropped to 24 and 8%, respectively, after 14 days (*data not shown*). Again, the application

Fig. 1. Reduction in green and blue mold incidence with respect to the control treatment (air at 20°C for 8 h; 100% incidence of both molds after 7 days of incubation) on 'Nadorcott' mandarin fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, respectively, treated 24 h later with air; CO_2 at 15, 30, 50, or 95 kPa; or N_2 at 95 kPa at 33°C for 8 or 24 h, and incubated at 20°C for 4 and 7 days. For each individual graph, values within columns with the same letters did not differ significantly according to Fisher's protected least significant difference test ($P < 0.05$). Asterisks indicate that synergistic activity was present between temperature and gas treatment according to Limpel's formula.

of high temperature with high CO₂ or O₂ was synergistic in most of the cases according to Limpel's formula.

After 8 days of incubation at 20°C, the incidence of green and blue mold on Clemenules mandarin fruit exposed to O₂ at 30 kPa at 33°C for 24 h was significantly lower than that on air-treated or control mandarin fruit (100% disease incidence) but the reduction was poor and no reduction was observed in the case of blue mold (Fig. 3). However, extending the treatment time to a period of 48 h resulted in green and blue mold incidence reductions of 97 and 74% after 8 days of incubation, and 40 and 20% after 14 days, respectively. When high O₂ treatments for 48 h were tested at 20°C, no significant reduction in both molds was obtained (*data not shown*). A synergistic effect was observed with the combination

of curing temperature and high O₂ according to Limpel's formula (Fig. 3).

After 4 days of incubation at 20°C, treatments with CO₂ at 15 kPa at 33°C for 24 h reduced the incidence of green and blue molds on Valencia orange fruit by 74 and 98%, respectively (Fig. 4). However, after 7 days of incubation, with 100% decay on control fruit, reduction in green and blue mold incidence dropped to 20 and 35%, respectively. The incidence reductions on cured (air-treated) orange fruit subjected to the same incubation conditions were significantly lower. Extending fruit exposure to air at 33°C from 24 to 48 h reduced the incidence of green mold by more than 95 and 85% after 4 and 7 days of incubation, respectively, but blue mold incidence was 55% lower than on control fruit after 7 days.

Fig. 2. Reduction in green and blue mold incidence with respect to the control treatment (§, air at 20°C for 8 h; 100% incidence of both molds after 7 days of incubation) on 'Ortanique' mandarin fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, respectively, treated 24 h later with air; CO₂ at 15, 30, 50, or 95 kPa; or O₂ at 30 or 45 kPa at 20 or 33°C for 8, 24, or 48 h, and incubated at 20°C for 4 and 7 days. For each individual graph, values within columns with the same letters did not differ significantly according to Fisher's protected least significant difference test ($P < 0.05$). Where no letters are shown, treatment conditions were not tested or readings were not performed. Asterisks indicate that synergistic activity was present between temperature and gas treatment according to Limpel's formula.

Exposing orange fruit to O₂ at 30 kPa for this period of time reduced both diseases by 100 and 95% after 4 and 7 days of incubation, respectively. Treatments at 20°C were not performed with Valencia orange (Table 1).

In general, data on disease severity followed trends similar to that of disease incidence. Lesion diameter was significantly reduced by high CO₂ or O₂ applied at 33°C for 24 or 48 h after 4 days of incubation at 20°C but the reduction considerably decreased after 7 or more days of incubation. Treatments applied at 20°C or for 8 h did not influence severity (*data not shown*). Similarly, the sporulation of *P. digitatum* and *P. italicum* was significantly inhibited by gas treatments at curing temperature during the first 4 or 7 days of incubation at 20°C in an air environment but, in general, the inhibitory effect did not persist. The most important

effect of the treatments on pathogen sporulation was observed in Valencia orange in which, for instance, exposure to CO₂ at 15 kPa at 33°C for 24 h reduced the sporulation of green and blue molds by 81 and 70%, respectively, after 7 days of incubation, and 43 and 24%, respectively, after 14 days (*data not shown*). Based on the disease incidence and severity data, none of the treatments induced resistance to the pathogens when applied before inoculation of fruit, regardless of cultivar, treatment conditions, and time between treatment and inoculation (*data not shown*).

Discussion

In this study, artificially inoculated citrus fruit treated at a curing temperature with CO₂ or O₂ at high concentrations showed lower green and blue mold incidence than those treated by curing in air

Fig. 3. Reduction in green and blue mold incidence with respect to the control treatment (air at 20°C for 24 h; 99 and 100% incidence of green and blue molds, respectively, after 7 days of incubation) on 'Clemenules' mandarin fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, respectively, treated 24 h later with air or O₂ at 30 kPa at 33°C for 24 or 48 h, and incubated at 20°C for 8 and 14 days. For each individual graph, values within columns with the same letters (a-c and m-n for 8 and 14 days, respectively) did not differ significantly according to Fisher's protected least significant difference test ($P < 0.05$). Asterisks indicate that synergistic activity was present between temperature and gas treatment according to Limpel's formula.

Fig. 4. Reduction in green and blue mold incidence with respect to the control treatment (air at 20°C for 24 h; 100% incidence of both molds after 7 days of incubation) on 'Valencia' orange fruit artificially inoculated with *Penicillium digitatum* and *P. italicum*, respectively, treated 24 h later at 33°C with air, CO₂ at 15 kPa for 24 h, or O₂ at 30 kPa for 48 h, and incubated at 20°C for 4, 7, and 15 days. For each individual graph, values within columns with the same letters (a-c, m-p, and x-y for 4, 7, and 15 days, respectively) did not differ significantly according to Fisher's protected least significant difference test ($P < 0.05$). Where no letters are shown, treatment conditions were not tested.

alone, regardless of cultivar and length of treatment. In studies using conventional curing treatments in which citrus diseases were effectively controlled, fruit were held for 3 days at up to 36 to 37°C (4,22). However, these exposure conditions may adversely affect weight loss, rind color, taste, and flavor of fruit (38). In some cases, curing at milder conditions of 33°C for 72, 65, or even 48 h controlled green and blue molds on inoculated 'Navelina' or 'Salustiana' orange fruit (31). Similar results were observed in our study after curing Valencia orange fruit for 48 h; however, none of the other cultivars treated with air at 33°C and high RH for 48 h or briefer periods were protected by this treatment against the inoculated pathogens. Thus, we found an evident effect of the fruit variety on the efficacy of the treatments. The mode of action of heat in general (26,28,38), and curing treatments in particular (6,7,22,31,47), as methods to control postharvest diseases of citrus fruit have been extensively discussed. Heat treatments may induce several changes in the nucleus and cell wall of fungi, destroy their mitochondria and vacuoles, and cause loss of spore cytoplasm (3). In addition to these direct effects on fungi, indirect effects of heat on the interaction between fruit and pathogen have been described. Heat can induce biosynthesis of lignin or lignin-like polymers in injured fruit rind, which may act as a physical barrier to hyphae penetration (6), and the production of antifungal compounds (phytoalexins such as scoparone or pathogen-related proteins such as chitinase or β -1,3-glucanase) in response to pathogen infection (21,38). Furthermore, some studies suggest that proteins biosynthesized by fruit to prevent denaturalization by heat (heat shock proteins), or melting of natural rind waxes, that would close potential infection sites, could act as defense mechanisms against molds (38). These indirect effects are dependent on fruit species and cultivar and also on fruit physical and physiological condition. This may be the reason why, in the present study, the same treatments with air at 33°C applied on different citrus fruit cultivars resulted in different degrees of decay inhibition: lack of control on Nadorcott and Clemenules mandarin and reductions of up to 50% on Ortanique hybrids.

Treatments applied at the same temperature and duration but with CO₂- or O₂-enriched atmospheres consistently improved the initial inhibition of green and blue molds on all citrus cultivars. Comparably, exposures of CO₂ at 15 kPa were more effective against green and blue molds than those of CO₂ at 30, 50, or 95 kPa. The use of high CO₂ concentrations during cold storage of fresh fruit and vegetables to control postharvest pathogens is based on both their direct toxicity to fungi and their indirect effect on delaying fruit ripening and senescence, therefore reducing fruit susceptibility to decay (17). The direct toxicity of high CO₂ levels has been assessed in *in vitro* studies, where CO₂ at 13 kPa prevented spores of *P. expansum* from germinating after exposure for 21 days (10). Short treatments tested in the present study may not exert much delay on fruit senescence; however, they may trigger the production of high levels of antifungal volatile compounds, which increase fruit resistance to rot (43). On the other hand, according to Kader (17), atmospheres with excessively elevated concentrations of CO₂ may harm some commodities and induce physiological breakdown and render it more susceptible to pathogens. CO₂ concentrations higher than 15 kPa that would result in higher direct toxicity to *P. digitatum* and *P. italicum* were apparently injurious to fruit and resulted in poorer disease control. Accordingly, we observed in previous studies that treatments with CO₂ at 15 kPa and 33°C for 48 h were significantly better than those with higher CO₂ concentrations to maintain the quality of citrus fruit during long cold storage (36). Treatments with a CO₂ concentration as high as 95 kPa increased the subsequent production of acetaldehyde and ethyl acetate during cold storage of Ortanique and Nadorcott mandarin fruit, which may be very toxic to pathogenic fungi but caused an important loss of fruit quality, including elevated water loss during storage, decrease in acidity, and increase in maturity index (36). These alterations led to an increased susceptibility of fruit to fungal infection and colonization. Inoculated Nadorcott mandarin fruit exposed to hypoxia by

increasing the concentration of N₂ in the chamber to 95 kPa showed slightly less disease development than air-exposed fruit but this treatment was less effective than those with elevated CO₂. The toxicity of low O₂ on fungi was assessed in early studies, in which it was observed that the germination of five pathogenic fungi decreased linearly with O₂ concentrations decreasing below 4% (42). Later, *in vivo* studies showed that storage at 5°C for up to 4 weeks in atmospheres with O₂ at 1 kPa reduced decay incidence on Clemenules clementine mandarin fruit but increased decay incidence on Nadorcott mandarin fruit (23). Hypoxia, either alone or with high concentrations of CO₂, typically has a direct fungistatic effect (10,42) that, nevertheless, is not constantly or proportionally related to lower fruit decay (23). The effect of these treatments on citrus fruit quality, which varies from neutral to negative (23,36), may influence the host-pathogen interaction and impair the natural resistance of fruit to disease, therefore facilitating the infection by spores that might have escaped the direct toxicity of the treatments. In another set of experiments in our work, treatments at 33°C for 48 h with high levels of O₂ controlled green and blue molds to a greater extent and more persistently than those with high levels of CO₂.

High O₂ probably reduces decay by its toxic effect on microbial cells that lack mechanisms to protect themselves from damage (45). Amanatidou et al. (1) found that atmospheres with high concentrations of O₂ imposed oxidative stress that led to an increase of intracellularly generated reactive oxygen species (mainly O₂⁻, H₂O₂, and free hydroxyl radicals), which disturbed the cellular homeostasis due to catabolic imbalance and resulted in the inhibition of the growth of certain microorganisms. To our knowledge, there are no studies assessing the direct toxicity of high O₂ or CO₂ atmospheres on *P. digitatum* or *P. italicum*. In early work, bacteria and fungi exposed to O₂ at 10 atmospheres (1,013.25 kPa) for 8 h (8) or 7 and 14 days (35) completely halted their growth. On return to air, the fungi treated for 8 h began to grow after a variable delay period, whereas those treated for 7 or 14 days recovered after longer delay periods or never recovered. Although *P. digitatum* and *P. italicum* were not tested, several *Penicillium* spp. were among those fungi killed by hyperbaric O₂ (35). Other pathogenic imperfect fungi such as *Botrytis cinerea* or *Aspergillus flavus* were inhibited by high levels of O₂ (70, 80, and 95 kPa; 16) and, therefore, it is reasonable to hypothesize that *P. digitatum* and *P. italicum* might be similarly affected. However, it has been reported that direct toxicity of high O₂ treatments on microorganisms is generally lower than that of high CO₂ treatments (1,15,44). Therefore, an indirect effect by increasing fruit resistance to pathogens may have contributed, as in other pathosystems (44,46), to the higher control of green and blue molds observed in fruit after exposure to high O₂. On citrus fruit, Kader and Ben-Yehoshua (19) observed that O₂ at 80 kPa effectively reduced *Penicillium* decay on grapefruit. We also observed (36) that exposure to O₂ at 30 or 45 kPa at 33°C for 24 or 48 h positively affected citrus quality. Clemenules and Ortanique mandarin fruit exposed to O₂ for 48 h lost less weight after 4 weeks of storage at 5°C followed by 1 week of shelf life at 20°C than air-exposed fruit. Clemenules mandarin fruit exposed to O₂ were firmer and had a reduced ethanol content in the juice compared with control fruit. Similarly, Valencia orange fruit exposed to O₂ at 30 kPa for 48 h at 20 or 33°C lost less weight than air-exposed fruit, while the rest of external and internal fruit quality parameters were not affected (36). One of the positive effects of high O₂ treatments on citrus fruit quality might be an increase in their natural resistance to pathogens.

Neither air at curing temperature, gaseous treatments at 20°C nor treatments for 8 h were effective to control green and blue molds on citrus fruit. However, when high CO₂ or O₂ were applied at 33°C, decay was inhibited to a greater extent than that expected by the mere addition of the two conditions. Thus, there was a synergistic effect between high levels of CO₂ or O₂ and curing temperatures, which was noticeable at all treatments lengths but was effective only against green and blue molds when treatments were applied for not less than 24 h.

Regarding the induced resistance assays, all citrus cultivars treated before wounding and fungal inoculation showed similar green mold incidence and severity compared with control fruit. Neither heat nor high CO₂ or O₂ exposure at the levels used in this study induced any noticeable defensive response in the fruit against green mold, which could be explained by the lack of any injury or pathogen that triggered the defense system of the fruit (21,27) or by insufficient temperature or treatment length, which were unable to induce any response in the nonwounded fruit. We have recently observed that superficial wounds in the rind of citrus fruit are not always necessary to induce phytoalexin accumulation as a response to a stressing postharvest treatment. Nonwounded Clementines mandarin fruit X-ray irradiated at 510 or 875 Gy and stored at 20°C for 7 or 14 days synthesized significant amounts of scoparone and scopoletin in the flavedo (C. Rojas-Argudo, *unpublished*). Nonetheless, the synthesis was substantially higher on mandarin fruit that had been wounded prior to irradiation and inoculated with *P. digitatum* afterward.

According to the findings in the present study, exposure of citrus fruit to CO₂ at 15 kPa for 24 h or to O₂ at 30 kPa for 48 h at 33°C can be a convenient measure to inhibit green and blue molds after harvest. Treatments were not persistent but a marked inhibition was observed on fruit incubated for up to 7 days at 20°C, which is a reasonable shelf-life period for domestic or, in the case of Spain, European export markets, especially taking into account that a nontoxic antifungal treatment was applied that could open opportunities for marketing of the fruit. New gaseous combinations should be tested on citrus to increase the effectiveness of the method. For instance, Amanatidou et al. (1) used atmospheres composed by high O₂ and high CO₂ and found that they were consistently more effective together than when used separately. Thus, superatmospheric O₂ concentrations appear to reduce the potential negative effects of high CO₂ and allow sensitive commodities to tolerate fungistatic CO₂ levels with minimal injury (19,20). The effect of such gaseous treatments on citrus fruit remains to be tested.

Exposure to CO₂ or O₂ at a relatively low curing temperature of 33°C for shorter time periods may be a suitable alternative to the existing effective curing times of 65 to 72 h and, thus, facilitate the commercial implementation of curing treatments on citrus. Although the commercial feasibility of heated gas treatments in the conventional citrus industry is questionable due to the treatment costs and the effectiveness, persistence, and availability of existing fungicides, these alternative treatments may be a useful tool to be considered. In an integrated approach, the effectiveness and persistence of these treatments might be enhanced by combining them with other nonpolluting treatments such as, for instance, sodium carbonate dips, which are known for improving the performance of curing (30). Exposure to high CO₂ or O₂ leaves no chemical residues on fruit, which enables treated citrus fruit to be potentially traded as organic, considerably increasing the value of the commodity. Higher prices of the final product achieved by compliance with organic fruit production or specific “green” markets may counteract the cost of establishing and using such postharvest treatments, especially when appropriate facilities are already available for citrus degreening or insecticidal quarantine treatments.

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