Synergism between potassium sorbate dips and brief exposure to high CO_2 or O_2 at curing temperature for the control of citrus postharvest green and blue molds

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Abstract

Synergistic effects and very effective control of citrus postharvest green and blue molds, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, were observed on artificially inoculated 'Valencia' oranges and 'Clemenules' and 'Ortanique' mandarins after a potassium sorbate (PS) treatment was followed by 2 days of storage in atmospheres of elevated CO_2 or O_2 at a curing temperature. A combined treatment consisting of 60-s dips in aqueous solutions of 3% PS heated to 62 ºC was followed by 48-h exposure to air, 15 kPa CO_2 or 30 kPa O_2 at 33 ºC. Control treatment was a 60-s water dip at 20 ºC followed by 24-h exposure to air at 20 ºC. Synergism was observed on citrus fruit either incubated at 20 ºC for up to 22 days, simulating direct commercialization, or stored at 5 ºC for up to 45 days, simulating commercial cold storage. This research offers potential new tools to the citrus industry for implementation of nonpolluting integrated postharvest disease management programs, especially devoted to high added value organic markets or export markets with zero residue tolerance.

Keywords: Postharvest decay; *Penicillium digitatum*; *P. italicum;* food preservatives; potassium sorbate; antifungal controlled atmospheres; integrated disease control

1. Introduction

Green mold, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and blue mold, caused by *Penicillium italicum* Wehmer, are the cause of the most important economic losses due to postharvest decay in the citrus industry in Spain and other countries with Mediterranean-type climate (Palou, 2014). For many years, these diseases have been controlled by the application of conventional fungicides such as imazalil (IMZ), sodium

orthophenylphenate (SOPP) or thiabendazole (TBZ), but now there are important general restrictions to the use of these products. Concerns about the effect of chemical residues on human health and the environment have led major European and worldwide markets to demand fruit that comply with zero or very low chemical residue tolerances. Moreover, the continuous and in some cases uncontrolled use of these fungicides in the past has caused the rise of resistant strains of the pathogens in many citrus packinghouses. Since effective decay control is imperative for the citrus industry, new low toxicity control methods are needed to replace or reduce the present use of synthetic fungicides (Palou et al., 2008).

Food additives or GRAS (generally regarded as safe) substances such as carbonates, sorbates, parabens, benzoates, etc. (Palou et al., 2001, 2002; Smilanick et al., 2008; Moscoso-Ramírez et al., 2013) and some brief exposures to high O_2 or CO_2 tested in our laboratory in recent years showed a good potential as alternative means for the control of citrus postharvest decay. Particularly, 60-s dips in aqueous solutions of 3% potassium sorbate (PS) heated to 62 ºC (Montesinos-Herrero et al., 2009) and 24- or 48-h exposures to 15 kPa CO_2 or 30 kPa O_2 at the curing temperature of 33 ºC (Montesinos-Herrero et al., 2012) effectively controlled green and blue molds in several citrus cultivars and were selected among the most promising nonpolluting alternative treatments. The objective of this work was to evaluate the compatibility and possible synergistic effects between these two types of treatments on oranges and mandarins incubated at 20 ºC or cold-stored at 5 ºC.

2. Materials and methods

Fruit used in the trials were oranges (*Citrus sinensis* (L.) Osbeck) cv. 'Valencia', clementine mandarins (*Citrus clementina* Hort. ex Tanaka) cv. 'Clemenules', and

hybrid mandarins cv. 'Ortanique' [*Citrus reticulata* Blanco x (*C. sinensis* x *C. reticulata*)]. Fruit were harvested from commercial orchards and no postharvest treatments were applied. Inoculum was prepared from isolated local strains of *P. digitatum* and *P. italicum* included in the IVIA CTP fungal culture collection of postharvest pathogens. The two pathogens were grown on potato dextrose agar (PDA) petri dishes at 25 ºC for 7-14 days. Spore suspensions of 10^6 spores/mL were prepared and fruit were inoculated by dipping the tip (1 mm wide, 2 mm long) of a sterile rod in the spore suspension and inserting it once in the equatorial area of the fruit rind. The two pathogens were inoculated in opposite sides of each fruit.

Stainless steel 10-L buckets were used for 60-s dip treatments with 3% (w/v) PS. Salt solutions were heated to 62 ºC inside a 250-L water tank provided with two electric resistances (4.5 kW each), a thermostat, and an automatic water-recirculating system. About 24 h after inoculation, fruit were placed into 18 L multi-perforated wall stainless steel containers, exactly fitting in the previously mentioned buckets and completely immersed in the corresponding solution for 60 s. Control fruit were immersed in water at 20 ºC for the same time. After treatment, fruit were rinsed with tap water at low pressure, dried in a packingline air tunnel at 45 ºC for 15 s, and exposed to the different gas treatments. Chambers used for gas exposure consisted of hermetically sealed, transparent polymethyl methacrylate cabinets (82 × 62 × 87 cm) fitted with outlet and inlet ports through which O_2 or CO_2 (Alphagaz, Air Liquide España S.A., Madrid, Spain) were injected to the desired concentrations. The cabinets were also fitted with internal basal water trays that allowed achieving a high relative humidity (RH) of about 95±5%. Cabinets were inside a 40 m^3 chamber conditioned at the experimental temperature (20 or 33 ºC for 24 or 48 h). Control fruit were exposed to air at 20 ºC for 24 h. Levels of CO_2, O_2, temperature and RH were set and continuously monitored by means of a

computer-controlled system (Control-Tec®, Tecnidex S.A., Paterna, València, Spain). For each combination of treatments, 3 replicates of 25 fruit each were used. Depending on the trial, treated fruit were incubated at 20 ºC for up to 22 days, or cold-stored at 5 ºC and 90% RH for up to 42 days. During these periods, disease incidence (number of infected wounds) and visible skin damage were periodically assessed. Every experiment was conducted twice.

For each cultivar, pathogen, treatment and evaluation date, disease reduction compared to control fruit was calculated by means of the equation: (% infected wounds in control fruit - % infected wounds in treated fruit / % infected wounds in control fruit) x 100. On 'Valencia' oranges, average green and blue mold incidence in control fruit was 89, 98 and 100% and 65, 93 and 100%, respectively, after 4, 8, and 14 days of incubation at 20 ºC. On 'Clemenules' mandarins, incidence of both green and blue molds in control fruit was 100% after 8 days of incubation at 20 ºC and also 100% after 14 days of storage at 5 ºC. On 'Ortanique' mandarins, incidence of both green and blue molds in control fruit was 100% after 8 days of incubation at 20 ºC, while it was 96 and 97% and 100 and 100%, respectively, after 14 and 28 days of storage at 5 ºC. Data from repeated experiments were statistically analyzed using analysis of variance (ANOVA) applied to percentages previously subjected to the arc-sine transformation in order to assure the homogeneity of variances. When appropriate, Fisher's Protected LSD test ($P < 0.05$) was used for means separation. The term synergy was applied as defined by Richer (1987), where the effectiveness of a combination of treatments exceeds the prediction of the effectiveness of their additive action estimated by Limpel's formula ($Ee = X + Y - (XY/100)$).

3. Results and discussion

The reduction of disease incidence obtained with different treatments and combinations applied to 'Valencia' oranges and 'Clemenules' and 'Ortanique' mandarins incubated at 20 ºC is shown in Fig. 1. On 'Valencia' oranges treated and incubated at 20 ºC for 8 or 14 days, dip treatments with 3% PS at 62 ºC for 60 s followed by 24-h exposure to either air at 20 or 33 ºC or 15 kPa CO_2 at 33 ºC, resulted in general high efficacy of PS treatments and general low efficacy of 24-h gaseous treatments. Furthermore, synergism between PS treatment and exposures to 33 °C was observed, although exposure to 15 kPa CO_2 did not significantly improve the efficacy of treatments with air at that temperature. These combined treatments were further assayed with mandarins, but using gas treatments of 30 kPa O_2 and testing also longer exposures of 48 h. In general, efficacy was higher on 'Ortanique' than on 'Clemenules' mandarins, and higher against green mold than blue mold. The poor effectiveness of gaseous exposures as brief as 24 h and the synergism between PS dip treatments and exposures to 33 ºC were confirmed, especially when high O_2 was applied.

In mandarins stored at 5 ºC (Fig. 2), treatment efficacy was also higher on 'Ortanique' than 'Clemenules' mandarins and against green mold than blue mold. Synergism between treatments was also marked. Green mold reduction on 'Ortanique' mandarins treated with PS and 30 kPa O_2 for 48 h after 14, 28, and 42 days of cold storage was 100, 97, and 79%, respectively. In all cases, a fungistatic rather than fungicidal effect of the treatments was observed, since their disease control ability decreased as incubation or cold storage periods increased, and it was dependent on the cultivar. Irrespective of the combination of treatments and cultivar, no external damage was observed on the rind of treated fruit.

In the present study, the high efficacy of PS aqueous dips (Smilanick et al., 2008; Montesinos-Herrero et al., 2009) and brief exposures to high CO_2 or O_2 at curing

temperature (Montesinos-Herrero et al., 2012) was confirmed and an important synergistic effect between these treatments was observed. Therefore, the combination of these treatments could be an alternative for the reduction of the long curing times (65 to 72 h) that are currently required for effective control of citrus green and blue molds (Plaza et al., 2003), thus facilitating the commercial implementation of curing treatments. Future research should focus on the evaluation of these treatments in commercial scale trials with naturally infected citrus fruit and on the assessment in these trials of the effect of the combined treatments on the quality of long-term cold stored fruit.

This research offers potential new tools to the citrus industry for implementation of nonpolluting integrated postharvest disease management programs, especially devoted to high added value organic markets or export markets with zero residue tolerance. In spite of the highly satisfactory results obtained in these tests, the general commercial implementation of this combination of alternative treatments in citrus packinghouses working with fruit for conventional markets is currently hindered by the present availability of convenient conventional fungicides and the high implementation and maintenance costs of gaseous treatments.

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Figure captions

Fig. 1. Reduction of the incidence of green and blue molds with respect to control fruit (60-s water dip at 20 °C + exposure to air at 20 ºC for 24 h) on 'Valencia' oranges and 'Clemenules' and 'Ortanique' mandarins artificially inoculated with *Penicillium digitatum* or *P. italicum*, respectively, treated 24 h later with the indicated combinations of potassium sorbate (PS) dips and gaseous treatments, and incubated at 20 ºC for up to 22 days. For each cultivar and incubation time, treatment means are significantly (no symbol) or not significantly (ns) different according to Fisher's protected LSD ($P < 0.05$) test applied after an ANOVA. (*) Synergistic effect between PS and gaseous treatments according to Limpel's formula.

Fig. 2. Reduction of the incidence of green and blue molds with respect to control fruit (60-s water dip at 20 °C + exposure to air at 20 ºC for 24 h) on 'Clemenules' and 'Ortanique' mandarins artificially inoculated with *Penicillium digitatum* or *P. italicum*, respectively, treated 24 h later with the indicated combinations of potassium sorbate (PS) dips and gaseous treatments, and stored at 5 ºC for up to 42 days. For each cultivar and cold storage time, treatment means are significantly (no symbol) or not significantly (ns) different according to Fisher's Protected LSD ($P < 0.05$) test applied after an ANOVA. (*) Synergistic effect between PS and gaseous treatments according to Limpel's formula.

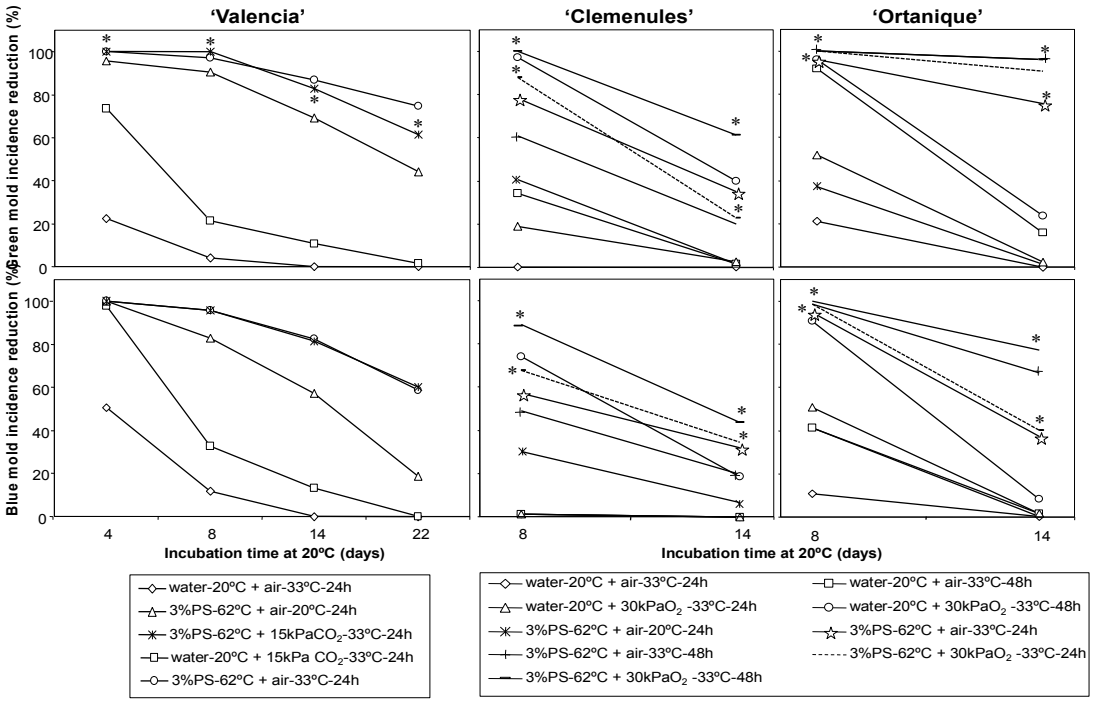
'Valencia'
'Clemenules'
'Ortanique'
Green mold incidence reduction (%)
Blue mold incidence reduction (%)
Incubation time at 20ºC (days)
water-20ºC + air-33ºC-24h
3%PS-62ºC + air-20ºC-24h
3%PS-62ºC + 15kPaCO2-33ºC-24h
water-20ºC + 15kPa CO2-33ºC-24h
3%PS-62ºC + air-33ºC-24h
water-20ºC + air-33ºC-48h
water-20ºC + 30kPaO2 -33ºC-24h
water-20ºC + 30kPaO2 -33ºC-48h
3%PS-62ºC + air-20ºC-24h
3%PS-62ºC + air-33ºC-24h
3%PS-62ºC + air-33ºC-48h
3%PS-62ºC + 30kPaO2 -33ºC-24h
3%PS-62ºC + 30kPaO2 -33ºC-48h

Fig. 1

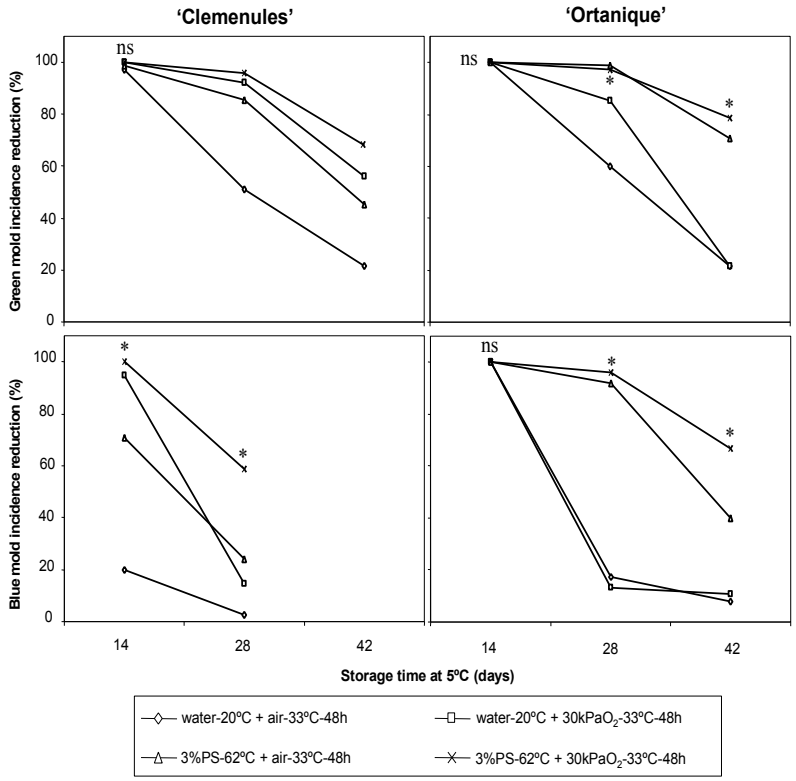
'Clemenules'
'Ortanique'
Green mold incidence reduction (%)
Blue mold incidence reduction (%)
ns
*
0
20
40
60
80
100
14
28
42
Storage time at 5ºC (days)
water-20ºC + air-33ºC-48h
water-20ºC + 30kPaO2-33ºC-48h
3%PS-62ºC + air-33ºC-48h
3%PS-62ºC + 30kPaO2-33ºC-48h

Fig. 2