



Short communication

Synergism between potassium sorbate dips and brief exposure to high CO₂ or O₂ 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 CO₂ or O₂ at a curing temperature. A combined treatment consisting of 60-s dips in aqueous solutions of 3% PS heated to 62 °C was followed by 48-h exposure to air, 15 kPa CO₂ or 30 kPa O₂ at 33 °C. Control treatment was a 60-s water dip at 20 °C followed by 24-h exposure to air at 20 °C. Synergism was observed on citrus fruit either incubated at 20 °C for up to 22 days, simulating direct commercialization, or stored at 5 °C 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.

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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 O₂ or CO₂ 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 °C (Montesinos-Herrero et al., 2009) and 24- or 48-h exposures to 15 kPa CO₂ or 30 kPa O₂ at the curing temperature of 33 °C (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 °C or cold-stored at 5 °C.

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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 °C for 7–14 days. Spore suspensions of 10⁶ 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 °C 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 °C for the same time. After treatment, fruit were rinsed with tap water at low pressure, dried in a packingline air tunnel at 45 °C 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 O₂ or CO₂ (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 m³ chamber conditioned at the experimental temperature (20 or 33 °C for 24 or 48 h). Control fruit were exposed to air at 20 °C for 24 h. Levels of CO₂, O₂, 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 °C for up to 22 days, or cold-stored at 5 °C 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) × 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 °C. On 'Clemenules' mandarins, incidence of both green and blue molds in control fruit was 100% after 8 days of incubation at 20 °C and also 100% after 14 days of storage at 5 °C. On 'Ortanique' mandarins,

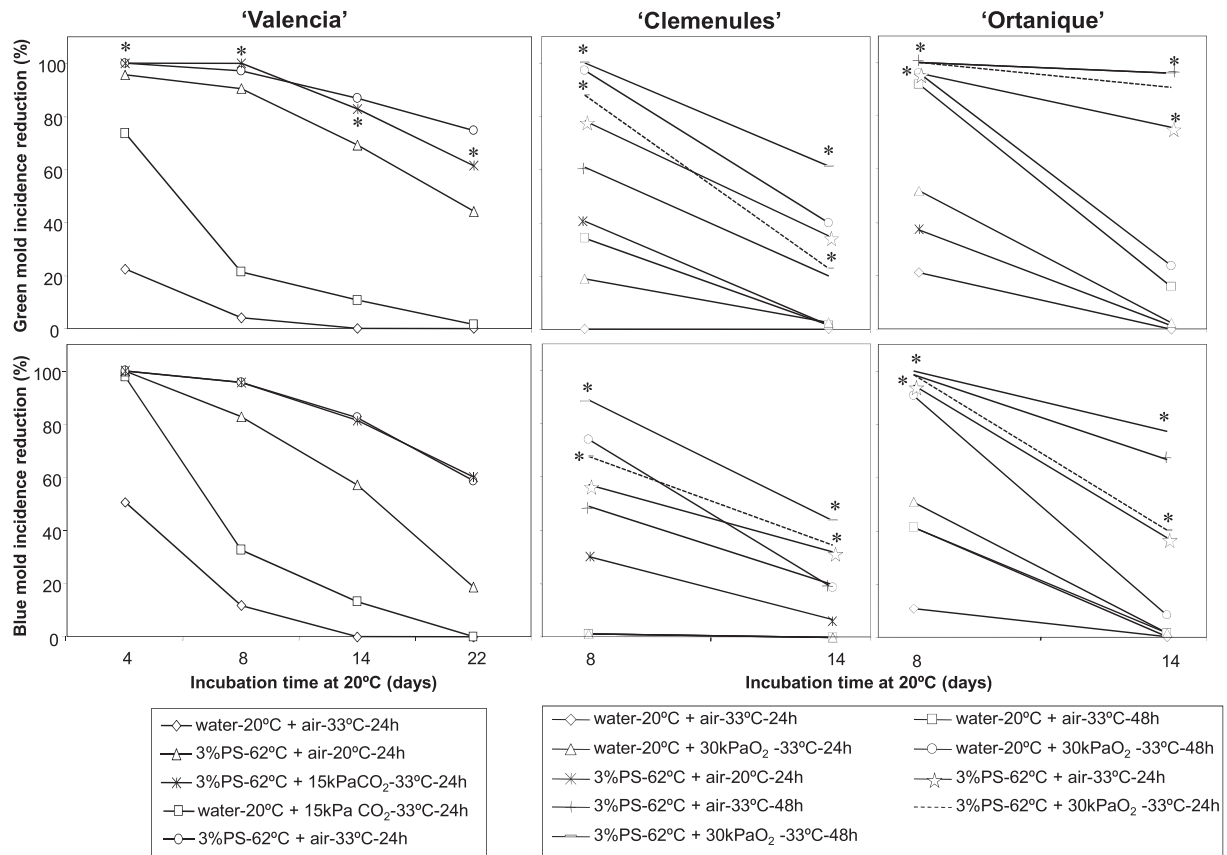


Fig. 1. Reduction of the incidence of green and blue molds with respect to control fruit (60-s water dip at 20 °C + exposure to air at 20 °C for 24 h) on 'Valencia' oranges and 'Clemenules' and 'Ortanique' mandarins artificially inoculated with *Penicillium digitatum* or *P. italicum*, respectively, treated 24 h later with the indicated combinations of potassium sorbate (PS) dips and gaseous treatments, and incubated at 20 °C 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.

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