

# Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides

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## Abstract

Potassium sorbate (KS), a common food preservative, was evaluated to control postharvest decay of citrus fruit. Significant advantages of KS over the commonly used sodium bicarbonate, which similarly improved fungicide performance, are the relatively low salt concentration of KS, the absence of sodium, and its lower pH, so disposal of used KS solutions would raise fewer regulatory issues. The influence of KS concentration and pH (pH 4–9) on the germination of spores of *Penicillium digitatum* was determined alone or in combination with four postharvest citrus fungicides, imazalil (IMZ), thiabendazole (TBZ), pyrimethanil, and fludioxonil. The EC<sub>95</sub> concentrations of KS to inhibit spore germination were lowest from pH 4–6. To control green mold on inoculated fruit, KS was compatible with these fungicides and consistently improved their performance. KS alone or fungicide–KS solutions were more effective when heated. The combination of KS and sodium bicarbonate was only moderately better than either alone. Green mold caused by an isolate of *P. digitatum* resistant to IMZ and TBZ was effectively controlled when KS was added to a heated IMZ or TBZ solutions. Heat, but not KS, increased residues of all of the fungicides in oranges. Sour rot, caused by *Geotrichum citri-aurantii*, was reduced from 94.5% among control lemons, to 49.1 and 47.2%, respectively, by 30 s immersion in KS or sodium bicarbonate at 1% (wt/vol) at 25 °C, and to 37.0 and 15.7%, respectively, when these solutions were at 50 °C.

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## 1. Introduction

Significant losses can occur after the harvest during the storage and marketing of citrus fruit in California primarily due to green mold, caused by *Penicillium digitatum*, and secondarily by blue mold and sour rot, caused by *P. italicum* and *Geotrichum citri-aurantii*, respectively (Eckert and Eaks, 1989). Applications of sodium bicarbonate, imazalil (IMZ), thiabendazole (TBZ), pyrimethanil (PYR), fludioxonil (FLUD), and sodium *o*-phenylphenate are used to manage postharvest green and blue molds of citrus in California (Smilanick et al., 2006a, 1997, 1999, 2005; Ismail and Zhang, 2004). Sodium bicarbonate partially controls green mold, blue mold, and sour rot of

citrus fruit (Larrigaudière et al., 2002; Smilanick et al., 1999; Palou et al., 2001, 2002a). The addition of sodium bicarbonate to IMZ (Smilanick et al., 2005), TBZ (Smilanick et al., 2006c), or PYR (Smilanick et al., 2006b) improved their performance. Heating these fungicides also increased their effectiveness without causing excessive residues in citrus fruit. Addition of sodium bicarbonate is useful to reduce rates of the fungicides used, which would reduce fungicide costs, to obtain some control of fungicide-resistant isolates of *P. digitatum*, and to partially control sour rot, which is not controlled by the other fungicides with the exception of sodium *o*-phenylphenate. Sodium bicarbonate can be used in sequences with other treatments, such as biological control or hot water, to improve their performance (Larrigaudière et al., 2002; Palou et al., 2001; Porat et al., 2002). However, disposal of sodium bicarbonate raises regulatory issues in some locations, because the solution has a high electrical conductivity and pH, and it contains sodium, which

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make disposal of the used solution difficult. Therefore, compounds that could improve fungicide performance as sodium bicarbonate does, yet avoid or minimize these disposal problems, would be valuable.

Sorbic acid and its water-soluble salts, especially potassium sorbate (KS), are common food preservatives. Sorbates are the best characterized of all food antimicrobials as to their spectrum of action. They inhibit certain bacteria and food-related yeasts and mold species (Sofos and Busta, 1993). However, inhibition of microorganisms by sorbates varies, depending on species and strain differences, extent of contamination, type and composition of the substrate, concentration and pH of sorbate, water activity, presence of other additives, temperature of storage, and type of packaging (Sofos, 1989). KS was used commercially to retard citrus postharvest decay, but its use did not become popular because its efficacy was sometimes low, and it was reported to delay, rather than stop, green mold infections in some reports (Gutter, 1981; Palou et al., 2002b).

Low sorbate levels can act synergistically with heat to inactivate yeasts, molds, and bacteria. Increased thermal inactivation of *Aspergillus flavus*, *A. niger*, *Geotrichum candidum*, *Penicillium puberulum* in the presence of sorbate was reported (Sofos, 1989). KS acted synergistically with heat to inactivate conidia of *A. flavus* and *P. puberulum*, ascospores of *Byssoschlamys nivea*, vegetative cells of *G. candidum*, and to inhibit colony formation by *A. flavus* (Beauchat, 1981). Kitagawa and Kawada (1984) and Wild (1987) showed heating sorbate solutions improved their performance to control postharvest sour rot and green mold of citrus fruit. Wild (1987) and Nelson et al. (1981) reported KS improved TBZ effectiveness to control citrus green mold. In preliminary tests, we found KS at 0.5% improved the performance of IMZ. Since KS has no sodium, a lower pH than sodium bicarbonate, and it was effective as a fungicide additive at only 0.5%, it merits thorough evaluation as a substitute for sodium bicarbonate.

The objectives of this study were to: (1) determine the effectiveness of KS alone to control citrus green mold and sour rot; (2) evaluate the influence of KS on the performance of the citrus postharvest fungicides IMZ, TBZ, PYR, and FLUD; (3) determine if the addition of potassium sorbate to the fungicide solution would influence residue levels of these fungicides in treated fruit; (4) quantify the effect of heat on the efficacy of the treatments; and (5) evaluate the influence of the interval between inoculation and the application of treatments on the performance of these fungicides when mixed with KS.

## 2. Materials and methods

### 2.1. Pathogen culture

Two *P. digitatum* isolates (fungicide-sensitive PD90 and fungicide-resistant D201) were cultured for 1–2 weeks on potato dextrose agar (PDA, Difco Laboratories, Detroit) at 25 °C. Both were isolated from infected lemon fruit from citrus packinghouses in California. The EC<sub>50</sub> of conidia of isolate PD90 on fungicide-amended PDA containing IMZ, TBZ, or PYR was 0.05, 0.1, or 0.35 mg L<sup>-1</sup>, respectively. Fungicide-resistant iso-

late D201 was resistant to IMZ and TBZ, but not to PYR. The EC<sub>50</sub> of conidia of isolate D201 on fungicide-amended PDA containing IMZ, TBZ, or PYR was 1.0, 10.0, or 0.35 mg L<sup>-1</sup>, respectively. The level of IMZ resistance of this isolate is similar to the level of IMZ resistance observed among 50 isolates collected in 2004 from six packinghouses (Kinay et al., 2007). Isolate PD90 is controlled by typical commercial IMZ applications in California packinghouses, whereas isolate D201 is not controlled. The sensitivity of both isolates to FLUD had not been determined, but they were obtained before this fungicide was introduced and presumably these isolates were not resistant to the fungicide. Kanetis et al. (2004) reported the baseline EC<sub>50</sub> of *P. digitatum* isolates collected in California was 0.03 mg L<sup>-1</sup> of FLUD. Conidia were harvested by adding 5 mL of sterile, de-ionized water (diH<sub>2</sub>O) containing 0.05% Triton X-100 to the petri dish. Conidia then were rubbed with a sterile glass rod, and conidia suspension was passed through two layers of cheese cloth. The suspension was diluted with water to an absorbance of 0.1 at 425 nm as measured with a spectrophotometer; a density that contained about 1 × 10<sup>9</sup> conidia L<sup>-1</sup> (Eckert and Brown, 1986) unless stated otherwise. An isolate of *G. citri-aurantii* obtained from an infected lemon was cultured at 25 °C on PDA for 2 weeks. Arthroconidia were harvested by adding 5 mL of sterile, de-ionized water (diH<sub>2</sub>O) containing 0.05% Triton X-100 to the petri dish. Arthroconidia then were rubbed with a sterile glass rod, and conidia suspension was passed through two layers of cheese cloth. The suspension was diluted with water to an absorbance of 2.0 at 425 nm as measured with a spectrophotometer; a density that contained about 1 × 10<sup>10</sup> arthroconidia L<sup>-1</sup> (Eckert and Brown, 1986).

### 2.2. Spore germination assay

To determine the influence of pH on EC<sub>95</sub> values of KS, the germination of conidia of *P. digitatum* (isolate PD90) was evaluated in six concentrations up to 0.2% (wt/vol) of KS (EMD Chemicals, Inc. Gibbstown NJ) in buffered potato-dextrose broth (PDB; Difco Laboratories) at a pH 4, 5, 6, 7, 8, or 9. Sterile dishes with 24 micro-wells (Nunclon Surface; Nalge Nunc, Int., Roskilde, Denmark) with a capacity of 4 mL per well were used. A germination medium was prepared by placing 96 g of PDB and 12.14 g of Tris buffer (hydroxymethyl aminomethane, Polysciences, Inc. Warrington, PA) in 1 L of diH<sub>2</sub>O and adjusting the pH of the medium to 4, 5, 6, 7, 8, or 9 with concentrated H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, or NaOH. In each well, 0.5 mL of PDB–Tris, 0.1 mL of conidial suspension, and KS were added to obtain final concentrations of 0, 0.01, 0.02, 0.04, 0.08, 0.1, or 0.2% (wt/vol), and diH<sub>2</sub>O was added to a final volume of 2.0 mL and mixed. After 18 h of incubation at 24 °C, germinated and ungerminated conidia in each well were counted by observation using an inverted compound microscope (×200). Within each replicate, 100–150 conidia were examined and the percentage of germinated conidia was calculated. The test was conducted two times.

To determine the influence of pH on EC<sub>95</sub> values of FLUD, the germination of *P. digitatum* (isolate PD90) conidia was evaluated in six concentrations (0, 0.005, 0.01, 0.025, 0.05, 0.1, or 0.25 mg L<sup>-1</sup>) of FLUD in PDB–Tris at a pH 4, 5, 6, 7, 8, or 9.

FLUD is a recently registered fungicide for citrus postharvest use (Scholar, 50.0% fludioxonil, Syngenta Crop Protection, Inc., Greensboro, NC). Conidial germination was assessed as previously described. Each treatment included three replicates, and the test was conducted three times.

To determine the effectiveness of IMZ, TBZ, PYR, and FLUD when sorbate was added on conidia germination of *P. digitatum* (isolate PD90), similar tests were conducted with KS at the same concentrations mentioned previously. The solutions were PDB–Tris adjusted to pH 6.0. IMZ concentrations were 0, 0.005, 0.01, 0.025, 0.05, or 0.1 mg L<sup>-1</sup>. PYR concentrations were 0, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, or 0.5 mg L<sup>-1</sup>. TBZ concentrations were 0, 0.05, 0.1, 0.25, 0.5, 1.0, or 2.0 mg L<sup>-1</sup>. FLUD concentrations were 0, 0.005, 0.01, 0.025, 0.05, 0.1, or 0.25 mg L<sup>-1</sup>. Conidial germination was assessed as previously described. Each treatment included three replicates, and each test was conducted three times.

### 2.3. Inoculation and treatment procedures

Commercially harvested lemons (cv. Eureka), mandarins (cv. Murcott), Valencia, or navel oranges (cv. Atwood) were randomized before use in these experiments. The fruit were inoculated with *P. digitatum* 12, 24, or 36 h before treatments, or inoculated with *G. citri-aurantii* 24 h before treatments. The inoculum density of *P. digitatum* was 1 × 10<sup>9</sup> conidia L<sup>-1</sup> unless otherwise stated. The inoculum density of *G. citri-aurantii* was 1 × 10<sup>10</sup> arthroconidia L<sup>-1</sup> and contained 10% (vol/vol) fresh lemon juice to stimulate fungal growth, 30 mg L<sup>-1</sup> TBZ to inhibit other pathogens, and 5 mg L<sup>-1</sup> cyclohexamide to retard fruit wound healing. Inoculation was done by dipping a steel rod with a 1-mm-wide and 2-mm-long tip into the inoculum suspension and making a single puncture in each fruit with the rod (Eckert and Brown, 1986).

In laboratory experiments, fruit were immersed in 15 L of each solution contained within 22-L capacity stainless steel tanks with a computer-controlled thermostat. The temperature of the solutions was maintained at 25 or 50 °C (±0.5 °C) and constantly stirred with a 5-cm diameter propeller. After treatment, the fruit were not rinsed, packed into cavity trays, stored for 2 weeks at 20 °C and 95% relative humidity (RH), and then the number of decayed fruit was counted.

In semi-commercial experiments, a high-volume, low-pressure system that drenched fruit over a bed of rotating brushes for 20 s on a packing line was used (Smilanick et al., 2003). After treatment, the fruit were not rinsed or waxed but dried in a high-velocity, heated air tunnel at 50 °C for 90 s, packed into cartons, stored for 2 weeks at 20 °C and 95% RH, and then the number of decayed fruit was counted.

### 2.4. Comparison of KS, alone or combined with other postharvest fungicides, to control green mold and sour rot of citrus fruit

The effectiveness of KS alone to control green mold was determined in a laboratory experiment. Valencia oranges were inoculated with *P. digitatum* 24 h before treatment. Fruit were

inoculated with isolate PD90 or D201. Treatments were applied by immersing the fruit into the solutions for 30 s either at 25 °C or heated to 50 °C. Solutions contained 0, 0.5, 2.0, or 3.0% KS at native pH. Fruit infected with green mold were counted after storage for 2 weeks at 20 °C. Each treatment was applied to four replicates of 20 fruit each and the experiment was conducted twice with isolate PD90 and three times with isolate D201.

The effectiveness of KS and sodium bicarbonate to control green mold was determined in both laboratory and semi-commercial experiments. Valencia oranges were inoculated with *P. digitatum* PD90 24 h before they were immersed for 30 s in one of the treatment solutions at either 25 or 50 °C. The first laboratory experiment included: (1) water; (2) KS at 1%; (3) sodium bicarbonate at 1%; or (4) KS at 1% plus sodium bicarbonate at 1%. Infected fruit with green mold were counted after storage for 2 weeks at 20 °C. Each treatment included three replicates with 27 fruit each. The experiment was done once. In a semi-commercial experiment, navel oranges, lemons, and mandarins were inoculated with *P. digitatum* PD90 24 h before treatment. Inoculum densities were 1 × 10<sup>9</sup> conidia L<sup>-1</sup> for oranges and lemons, and reduced to 1 × 10<sup>8</sup> conidia L<sup>-1</sup> for mandarins because of their greater susceptibility to infection. Treatments were: (1) water; (2) sodium bicarbonate at 1.5%; (3) KS at 0.5%; (4) KS at 2%; (5) sodium bicarbonate at 1.5% plus KS at 0.5%; or (6) sodium bicarbonate at 1.5% plus KS at 2%. Treatment solutions were applied by drenching the fruit on roller brushes for about 20 s at 49 °C. Infected fruit with green mold were counted after storage for 3 weeks at 10 °C. Each treatment included four replicates (cartons) with 75 fruit per carton. The experiment was done once.

In a first series of laboratory experiments to determine the influence of KS addition to fungicide solutions to control green mold, the KS concentration was fixed at 0.5%, while the fungicides were used at several lower than recommended concentrations, so the nature of KS influence on their performance could be seen. Navel or Valencia oranges were inoculated with *P. digitatum* 24 h before treatment, with the exception of experiments with FLUD, where the interval between inoculation and treatment was 12–18 h. Fruit were inoculated with isolate PD90 or D201. Treatments were applied by immersing the fruit into the solutions for 30 s either at 25 °C or heated to 50 °C. Solutions contained 0 or 0.5% KS at native pH co-mixed with IMZ (0, 10, 25, or 50 mg L<sup>-1</sup> for fruit inoculated with isolate PD 90 and 0, 100, 250, or 500 mg L<sup>-1</sup> for fruit inoculated with D201), TBZ (0, 10, 25, or 50 mg L<sup>-1</sup> for fruit inoculated with isolate PD 90 and 0, 100, 250, or 500 mg L<sup>-1</sup> for fruit inoculated with D201), PYR (0, 25, 50, or 100 mg L<sup>-1</sup> for fruit inoculated with isolate D201), or FLUD (0, 60, 150, or 600 mg L<sup>-1</sup> for fruit inoculated with isolate D201). Fruit infected with green mold were counted after storage for 2 weeks at 20 °C. Each treatment was applied to four replicates of 20 fruit each and the experiment was conducted twice, once with isolate PD90 and once with isolate D201.

In a second series of laboratory experiments to determine the influence of KS addition to fungicide solutions to control green mold, the KS concentration was fixed at 0.5%, while the fungicides were used at several lower than recommended concentrations, so the nature of KS influence on their perfor-

mance could be seen. Rather than one inoculation interval of 24 h that was used in the first series of experiments, two inoculation intervals were used in the second series of experiments. Navel or Valencia oranges were inoculated with *P. digitatum* either 12 or 36 h before treatment. Fruit were inoculated with isolate PD90 or D201. Treatments were applied by immersing the fruit into the solutions for 30 s either at 25 °C or heated to 50 °C. Solutions contained 0 or 0.5% KS at native pH co-mixed with IMZ (0, or 100 mg L<sup>-1</sup> for fruit inoculated with isolate PD 90 and 0, or 500 mg L<sup>-1</sup> for fruit inoculated with D201), TBZ (0, or 100 mg L<sup>-1</sup> for fruit inoculated with isolate PD 90 and 0, 500 mg L<sup>-1</sup> for fruit inoculated with D201), PYR (0, or 50 mg L<sup>-1</sup> for fruit inoculated with isolate D201), or FLUD (0, or 300 mg L<sup>-1</sup> for fruit inoculated with isolate D201). Fruit infected with green mold were counted after storage for 2 weeks at 20 °C. Each treatment was applied to four replicates of 20 fruit each and the experiment was conducted twice, once with isolate PD90 and once with isolate D201.

The effectiveness of KS and sodium bicarbonate to control sour rot on lemons was determined in a laboratory experiment. Treatments were (1) water; (2) sodium bicarbonate at 1.0%; (3) sodium bicarbonate at 2.0%; (4) KS at 0.5%; and (5) KS at 1%. Treatments were applied by immersing the lemons in the solutions for 30 s at 25 or 50 °C. Infected fruit with sour rot were counted after storage for 2 weeks at 20 °C. Each treatment included four replicates of 27 fruit each. The experiment was done once.

### 2.5. Fungicide residues

To determine if fungicide residues were affected by KS, it was added to solutions of IMZ (100 mg L<sup>-1</sup>), TBZ (100 mg L<sup>-1</sup>), PYR (50 mg L<sup>-1</sup>), and FLUD (300 mg L<sup>-1</sup>). Valencia oranges were immersed for 30 s in the fungicide solutions at 25 or 50 °C alone or with 0.5% KS. After treatment, the fruit were not rinsed and the residues of each fungicide were determined within 4 days using three replicate determinations of five fruit each. Each of the five fruit was cut into eight portions, 300 g of the portions were placed in a blender with 150 mL water, blended at high speed for several minutes, a 50-g portion of the macerate was removed and 2 mL of 50% sodium hydroxide was added to it. The organic phase was filtered over 2 g of anhydrous sodium sulfate, then the TBZ, PYR, and FLUD concentrations were determined by gas chromatography using a nitrogen phosphorus detector, or an electron capture detector for IMZ only. Residues are reported as mg kg<sup>-1</sup> fresh weight. The detection limit was 0.05 mg kg<sup>-1</sup> and the efficiency of recovery was approximately 95% for all of the fungicides.

### 2.6. Statistical analyses

The percentage of germination of conidia was calculated and mean values and their standard deviations are reported. The incidence of green mold was analyzed by ANOVA applied to the arcsin of the square root of the proportion of infected fruit, which was followed by Fisher's protected least significant difference test ( $P \leq 0.05$ ) to separate means. Actual values are shown. Pro-

bit analysis (SPSS 14.5, Chicago IL) was applied to estimate the concentrations, with 95% confidence intervals, when germination decreased by 95% (EC<sub>95</sub>) compared to that of the initial germinability of the conidia or arthrospores. Limpel's formula, as described by Richter (1987), was applied to determine if synergy was present in the effectiveness of the KS and fungicides mixtures. Limpel's formula is  $E_e = X + Y - (XY/100)$ , in which  $E_e$  is the expected effect from additive responses of two treatments and  $X$  and  $Y$  are the percentages of decay reduction relative to each agent used alone. Thus, if the combination of the two agents produces any value of decay reduction greater than  $E_e$ , then synergism exists.

## 3. Results

### 3.1. Spore germination assays

Germination of conidia was inhibited at pH 8, and irregular and often very low at pH 9 in all of the germination tests, even when no fungicide was present. The inhibitory activity of KS increased with decreasing pH (Table 1). It was similar between pH 4 and 6, about three-fold less toxic at pH 7, and 10-fold less toxic at pH 8. KS toxicity at pH 9 was similar to that at pH 8, although germination of conidia was low at pH 9. The EC<sub>95</sub> of FLUD was about 0.25 mg L<sup>-1</sup> and similar from pH 4 to 7 (Table 1). The EC<sub>95</sub> of FLUD declined at pH 8 and 9, when germination was also inhibited by pH alone. KS was compatible with and increased the inhibitory activity of IMZ, TBZ, PYR, and FLUD (Fig. 1). IMZ effectiveness was particularly improved, for example, the addition of 0.04% KS reduced the germination of conidia in 0.010 mg L<sup>-1</sup> IMZ from 78 to 5%. The magnitude of inhibition that KS contributed to the other fungicides, particularly FLUD, was lower.

### 3.2. Control of green mold

The effectiveness of KS to control postharvest green mold on Valencia oranges at 25 °C was modest, particularly with isolate D201 (Fig. 2). Concentrations of 2 and 3% were similar

Table 1  
EC<sub>95</sub> concentrations of potassium sorbate (PS) or of fludioxonil (FLUD) that inhibited germination of conidia of *Penicillium digitatum* in potato dextrose broth adjusted to pH 4–9

Solution pH	EC <sub>95</sub> <sup>a</sup>	
	PS (%)	FLUD (mg L <sup>-1</sup> )
4	0.065 (0.061; 0.070)	0.251 (0.232; 0.276)
5	0.094 (0.088; 0.102)	0.239 (0.216; 0.269)
6	0.066 (0.061; 0.073)	0.247 (0.228; 0.270)
7	0.188 (0.174; 0.206)	0.249 (0.231; 0.271)
8	0.678 (0.442; 1.216)	0.216 (0.181; 0.273)
9	0.697 (0.509; 1.167)	0.028 (0.000; 0.068)

Germination of conidia in potato dextrose broth alone was above 95% at pH 4–7, 65.4% at pH 8, and 9.0% at pH 9.

<sup>a</sup> EC<sub>95</sub> = concentration that inhibited the germination of 95% of the conidia determined by probit analysis. Values in parenthesis are the upper and lower 95% fiducial limits.

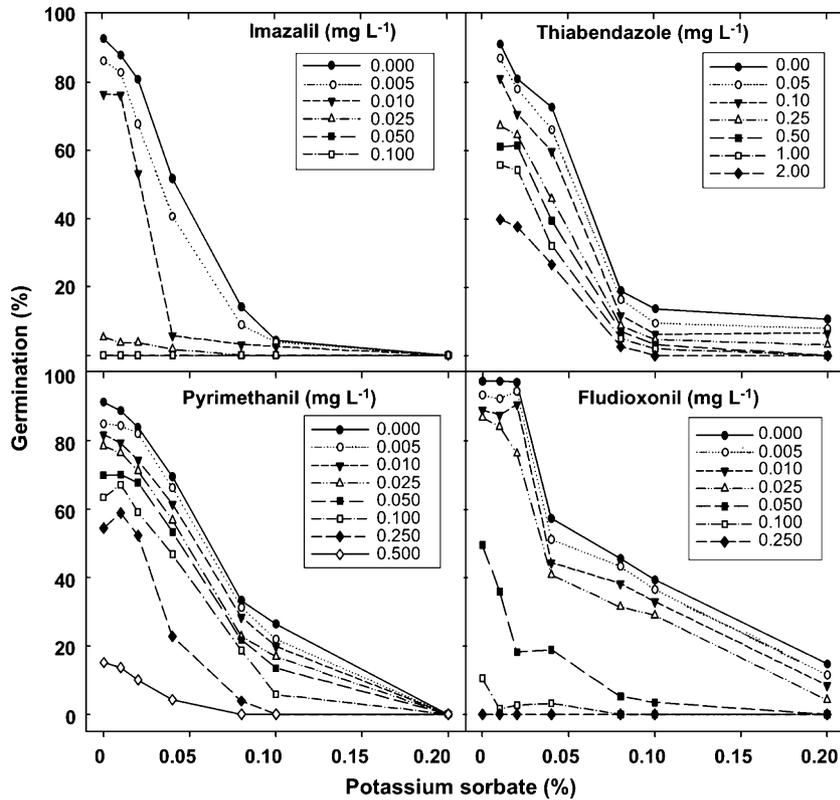


Fig. 1. Germination of conidia of *Penicillium digitatum* in buffered potato dextrose broth at pH 6 containing potassium sorbate amended with the fungicides imazalil, pyrimethanil, thiabendazole, or fludioxonil. Germination was determined after 18 h at 25 °C. Each value is the mean from three experiments of three replicates of 100–150 conidia each.

in effectiveness, while 0.5% was inferior. Heated water controlled isolate PD90 more than D201. Heating the KS solutions to 50 °C improved their effectiveness consistently and markedly, and in some cases the increase in performance was synergistic according to Limpel’s formula.

In a laboratory experiment, KS was inferior to sodium bicarbonate to control green mold, however, when combined, control of green mold was synergistic (Fig. 3). Synergy was evident when the solutions were applied at 25 or 50 °C. In a semi-commercial experiment, where the fruit were drenched over

rotating brushes rather than immersed in the solutions, control of green mold was generally less than that in the laboratory experiment (Fig. 4), particularly on mandarins, where disease control was very poor. Sodium bicarbonate at 1.5% and KS at 2.0% were approximately equal in efficacy on oranges and lemons, but their combination was only slightly superior to either alone and not

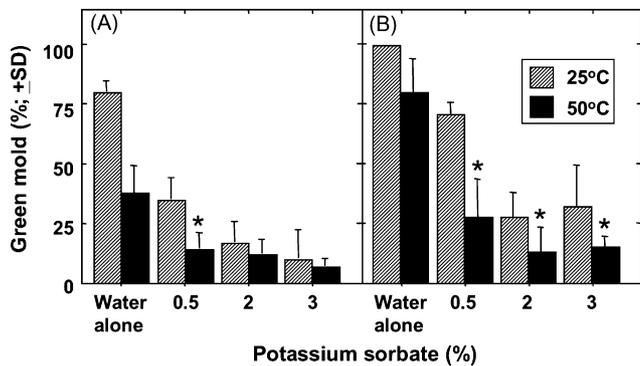


Fig. 2. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on Valencia oranges after immersion for 30 s in potassium sorbate solutions at 25 or 50 °C followed by storage for 2 weeks at 20 °C. Two isolates of the pathogen were used; fungicide-sensitive isolate PD90 (A) or imazalil and thiabendazole-resistant isolate D201 (B). Fruit were inoculated 24 h before treatments. Asterisk indicates synergistic activity was present according to Limpel’s formula.

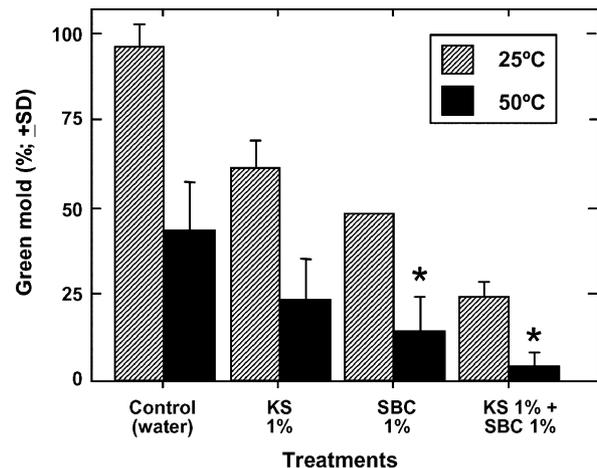


Fig. 3. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on Valencia oranges after immersion for 30 s in potassium sorbate (KS) or sodium bicarbonate (SBC) solutions at 25 or 50 °C followed by storage for 2 weeks at 20 °C. The fruit were inoculated 24 h before treatment with fungicide-sensitive isolate PD90. Asterisk indicates synergistic activity was present according to Limpel’s formula.

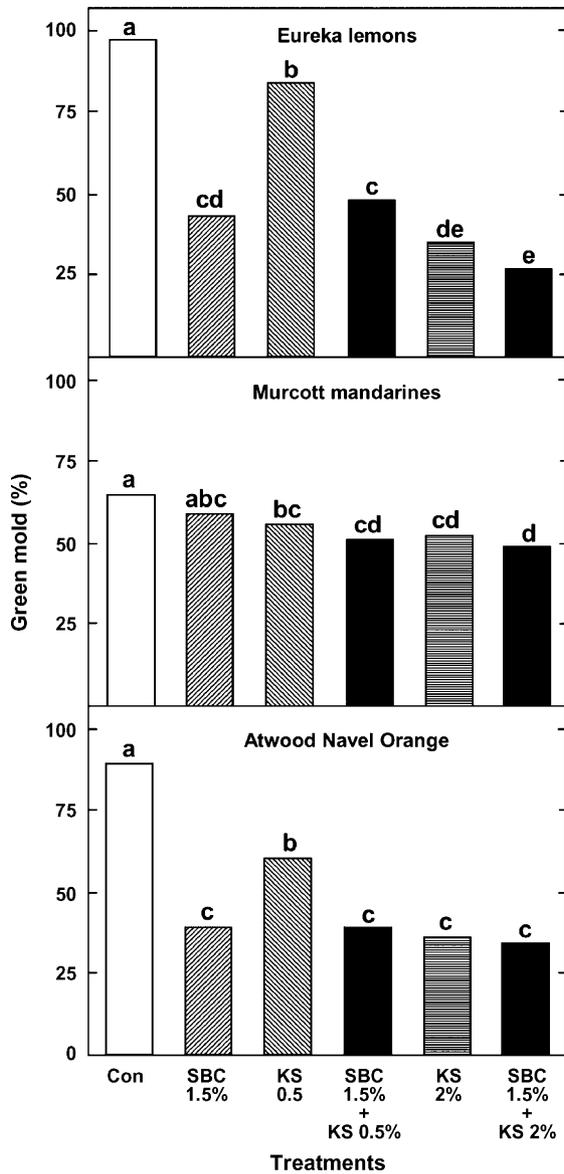


Fig. 4. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on lemons, mandarin oranges, and navel oranges after they were drenched in a packing line by a high-volume, low-pressure system over a bed of rotating brushes for 20 s with potassium sorbate (KS) or sodium bicarbonate (SBC) solutions at 49 °C followed by storage for 3 weeks at 10 °C. The fruit were inoculated 24 h before treatment with fungicide-sensitive isolate PD90. The treatments were applied with commercial drenching equipment.

synergistic. KS at 0.5% did not improve sodium bicarbonate performance.

The addition of KS and heat consistently improved the effectiveness of IMZ to control green mold (Figs. 5 and 6). On oranges that were inoculated with either an IMZ-sensitive (PD90) or -resistant (D201) isolate of *P. digitatum* 24 h before treatment, the enhancement in effectiveness that occurred when KS was added was synergistic, with one exception (Fig. 5). Control of green mold caused by either isolate was better when the treatments were applied 12 h after inoculation than when they were applied 36 h after inoculation (Fig. 6). Treatment with IMZ, at a higher but approved rate of 500 mg L<sup>-1</sup>, combined with KS and heated

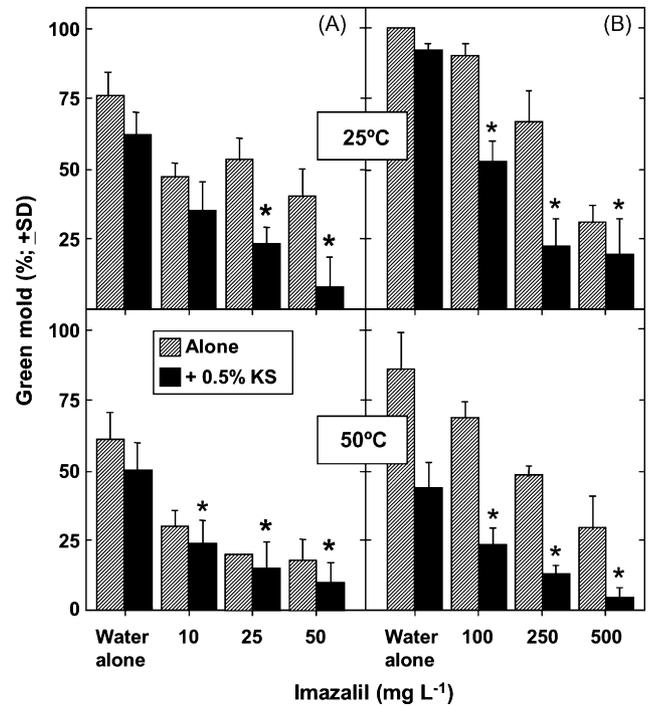


Fig. 5. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on navel oranges after they were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or imazalil (IMZ), alone or in combination at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Two isolates of the pathogen were used; IMZ-sensitive isolate PD90 (A) or IMZ-resistant isolate D201 (B). Fruit were inoculated 24 h before treatments. Asterisk indicates synergistic activity was present according to Limpel's formula.

to 50 °C, effectively controlled green mold on oranges caused by an IMZ-resistant isolate inoculated 36 h before treatment.

The addition of KS and heat consistently but modestly improved the effectiveness of TBZ to control green mold (Figs. 7 and 8). On oranges that were inoculated with either a TBZ-sensitive (PD90) or -resistant (D201) isolate of *P. digitatum* 24 h before treatment, the enhancement in effectiveness that occurred when KS was added was synergistic in only two cases (Fig. 7). Control of green mold caused by either isolate was better when the treatments were applied 12 h after inoculation than when they were applied 36 h after inoculation (Fig. 8). Treatment with TBZ, at a higher but approved rate of 500 mg L<sup>-1</sup>, combined with KS and heated to 50 °C, partially controlled green mold on oranges inoculated 36 h before treatment with a TBZ-resistant isolate.

The addition of KS and heat consistently but modestly improved the effectiveness of PYR to control green mold (Figs. 9 and 10). On oranges that were inoculated with isolate D201 of *P. digitatum* 24 h before treatment, the enhancement in effectiveness that occurred when KS was added was synergistic in three of six cases (Fig. 9). Control of green mold was better when the treatments were applied 12 h after inoculation than when they were applied 36 h after inoculation (Fig. 10). Treatment with PYR at a very low rate of 50 mg L<sup>-1</sup> (10% of the lowest recommended rate), combined with KS and heated to 50 °C, effectively controlled green mold on oranges inoculated 36 h before treatment. The improvement in effectiveness

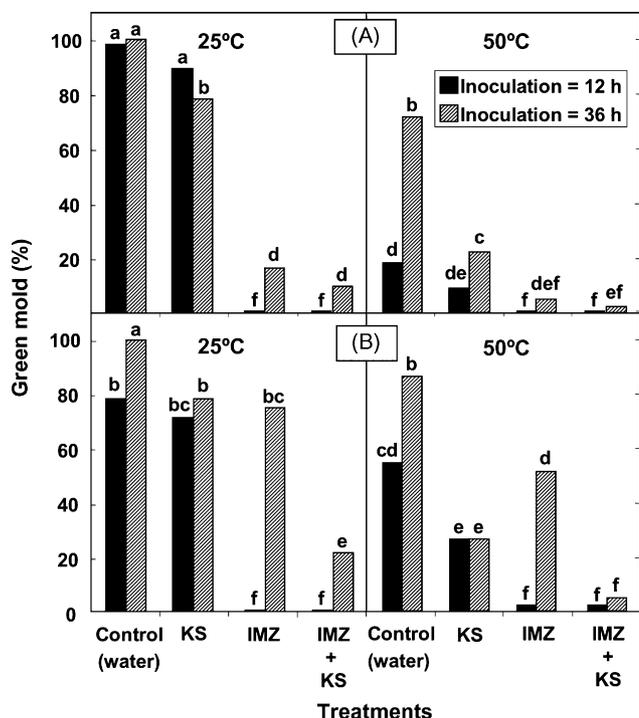


Fig. 6. Incidence of postharvest green mold on Valencia oranges, inoculated with *Penicillium digitatum* 12 or 36 h prior to treatments. Fruit were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or imazalil (IMZ), alone or in combination, at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Two isolates of the pathogen were used; (A) IMZ-sensitive isolate PD90 (IMZ concentration was 100 mg L<sup>-1</sup>); (B) IMZ-resistant isolate D201 (IMZ concentration was 500 mg L<sup>-1</sup>). Values within each isolate at 25 and 50 °C with unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

was synergistic twice at 25 and once at 50 °C, while KS did not improve PYR in some cases.

The addition of KS and heat consistently but modestly improved the effectiveness of FLUD to control green mold (Figs. 11 and 12). On oranges that were inoculated with isolate D201 of *P. digitatum* 24 h before treatment, the enhancement in effectiveness that occurred when KS was synergistic in three of six cases (Fig. 11). Control of green mold was better when the treatments were applied 12 h after inoculation than when they were applied 36 h after inoculation (Fig. 12). Treatment with FLUD at 300 mg L<sup>-1</sup> (50% of the lowest recommended rate), combined with KS and heated to 50 °C, effectively controlled green mold on oranges inoculated 36 h before treatment. KS effectiveness increased at 50 °C, while FLUD effectiveness was little influenced by heat. The performance of FLUD was slightly improved by heat or the addition of KS when used at 25 °C (Fig. 12). FLUD alone did not effectively control green mold at the low rate employed in this experiment (300 mg L<sup>-1</sup>), and the increase in its effectiveness by the addition of KS was modest.

In order to examine the influence of sorbate and heat on fungicide performance, the results of experiments depicted in Figs. 6, 8, 10 and 12 were subjected to ANOVA (Table 2). The fungicides, sorbate, and heat significantly reduced green mold incidence in every experiment. Significant interaction between sorbate and IMZ and sorbate and PYR was present, which

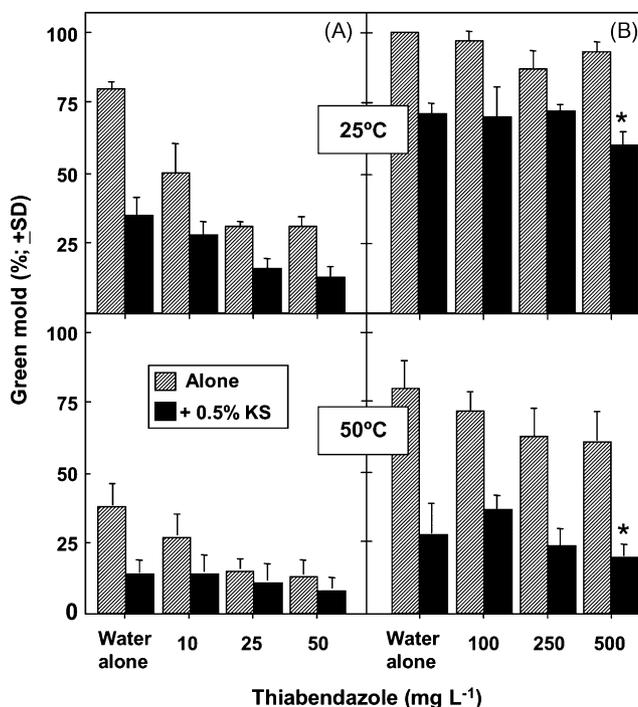


Fig. 7. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on Valencia oranges after they were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or thiabendazole (TBZ), alone or in combination at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Two isolates of the pathogen were used; TBZ-sensitive isolate PD90 (A) or TBZ-resistant isolate D201 (B). Fruit were inoculated 24 h before treatments. Asterisk indicates synergistic activity was present according to Limpel's formula.

indicated their performance together exceeded either alone. No significant interaction between sorbate and TBZ and sorbate and FLUD was present.

### 3.3. Control of sour rot

Sour rot, caused by *G. citri-aurantii*, was reduced moderately by KS, particularly when the solution was heated. Sodium bicarbonate at 1 or 2% (wt/vol) was similar in effectiveness to each other and to KS at 1% (wt/vol) (Fig. 13).

### 3.4. Fungicide residues

Fungicide residues on Valencia oranges after 30 s immersion in the fungicides (Table 3) were significantly affected by the solution temperature. Increasing the temperature from 25 to 50 °C greatly increased the residues of IMZ, TBZ, and PYR; however, the increase in FLUD residues was modest. The addition of KS did not affect the residues of IMZ, TBZ, PYR, or FLUD at either 25 or 50 °C.

## 4. Discussion

KS at concentrations of 0.05 to 0.2% inhibited germination of conidia of *P. digitatum*. The inhibitory activity of KS was higher at lower pH. The inhibitory activity of KS was similar from pH 4 to 6, and it was about 3- and 10-fold less toxic at pH 7

Table 2

ANOVA applied to the arcsin of the square root of the proportion of infected oranges that developed after immersion for 30 s at 25 or 50 °C in imazalil (IMZ), thiabendazole (TBZ), pyrimethanil (PYR), or fludioxonil (FLUD)

Source	IMZ <sup>a,b</sup>			TBZ <sup>a,b</sup>			PYR <sup>a,c</sup>			FLUD <sup>a,c</sup>		
	d.f.	MS	P	d.f.	MS	P	d.f.	MS	P	d.f.	MS	P
Fungicide (F) <sup>d</sup>	6	3221.6	0.000	6	1348.8	0.003	3	6381.4	0.000	3	3431.3	0.000
Sorbate (S)	1	4425.8	0.000	1	11914.1	0.000	1	1308.9	0.000	1	1104.6	0.001
Heat (H)	1	7031.5	0.000	1	13368.9	0.000	1	2278.5	0.000	1	1118.6	0.000
F × S	6	575.2	0.000	6	356.4	0.272	3	307.2	0.018	3	112.4	0.252
F × H	6	133.5	0.380	6	70.3	0.854	3	810.2	0.000	3	1055.0	0.000
S × H	1	453.9	0.058	1	125.3	0.498	1	105.2	0.268	1	17.6	0.640
F × H × S	6	575.2	0.000	6	25.8	0.962	3	23.5	0.839	3	169.3	0.110

Each fungicide was applied at three concentrations alone or with 0.5% potassium sorbate (KS). They were stored 2 weeks after treatment at 20 °C before examination.

<sup>a</sup> The fruit were inoculated with *Penicillium digitatum* 24 h before treatment with IMZ, TBZ, PYR, and 12–18 h before treatment with FLUD.

<sup>b</sup> Experiment was conducted with isolates PD90 and D201 of *P. digitatum*.

<sup>c</sup> Experiment was conducted with isolate D201 of *P. digitatum*.

<sup>d</sup> Rates of these fungicides are shown in Figs. 5, 7, 9 and 11.

or pH 8, respectively. Sofos (1989) reported that the antimicrobial activity of sorbate is dependent on the pH of the substrate; sorbate effectiveness increases when the pH of the environment approaches its dissociation constant (pKa). The antimicrobial activity of sorbate is greatest when it is in the undissociated state (sorbic acid). Therefore, with a pKa of 4.75, optimum effectiveness is at pH values below 6.0, while it becomes progressively less effective at pH values of 7.0 and above when the compound is dissociated and ionized. Above neutral pH, sorbate

is essentially completely dissociated and its toxicity is low; it may even provide a potential carbon source for some microorganisms (Piper et al., 2001). However, Stratford and Anslow (1996) stated that at neutral pH, high sorbate levels still could inhibit *Saccharomyces cerevisiae* and we found its toxicity to conidia of *P. digitatum* at neutral pH was still relatively high. The undissociated acid is 10–600 times more effective than the dissociated form (Eklund, 1983). The undissociated acid, being uncharged, readily diffuses across the cell membrane only to

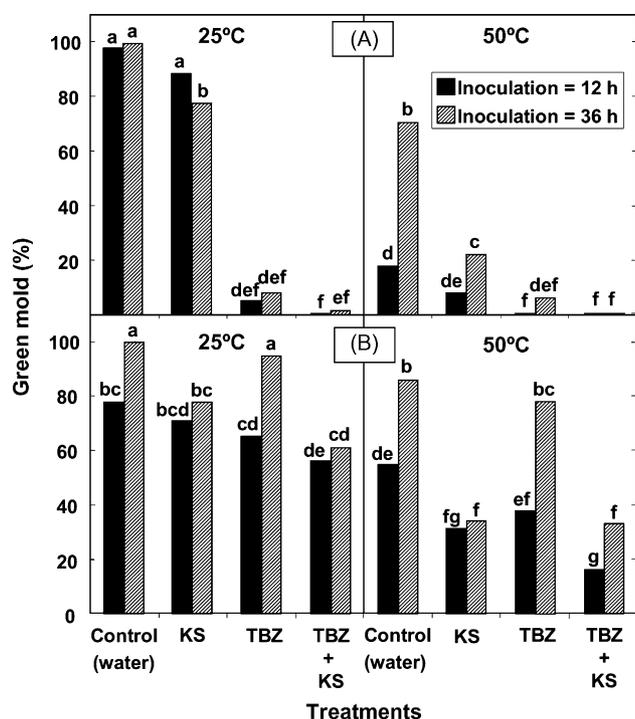


Fig. 8. Incidence of postharvest green mold on Valencia oranges, inoculated with *Penicillium digitatum* 12 or 36 h prior to treatments. Fruit were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or thiabendazole (TBZ), alone or in combination, at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Two isolates of the pathogen were used; (A) TBZ-sensitive isolate PD90 (TBZ concentration was 100 mg L<sup>-1</sup>); (B) TBZ-resistant isolate D201 (TBZ concentration was 500 mg L<sup>-1</sup>). Values within each isolate at 25 and 50 °C with unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

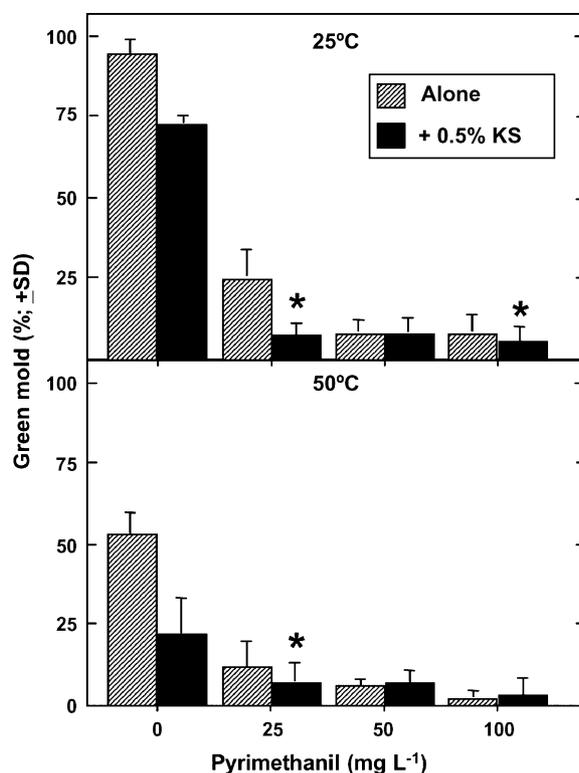


Fig. 9. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on Valencia oranges after they were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or pyrimethanil, alone or in combination at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Fruit were inoculated 24 h before treatments with isolate D201. Asterisk indicates synergistic activity was present according to Limpel's formula.

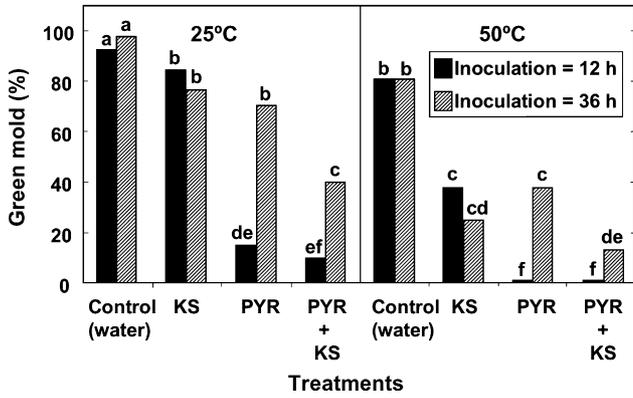


Fig. 10. Incidence of postharvest green mold on Valencia oranges, inoculated with isolate D201 of *Penicillium digitatum* 12 or 36 h prior to treatments. Fruit were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or pyrimethanil (PYR; 50 mg L<sup>-1</sup>), alone or in combination, at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Values at 25 and 50 °C with unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

dissociate in the higher pH environment of the cytosol (Piper et al., 2001). Such dissociation generates protons and the acid anion. The acid anion accumulates within cells to very high levels because, being charged, it cannot very readily diffuse

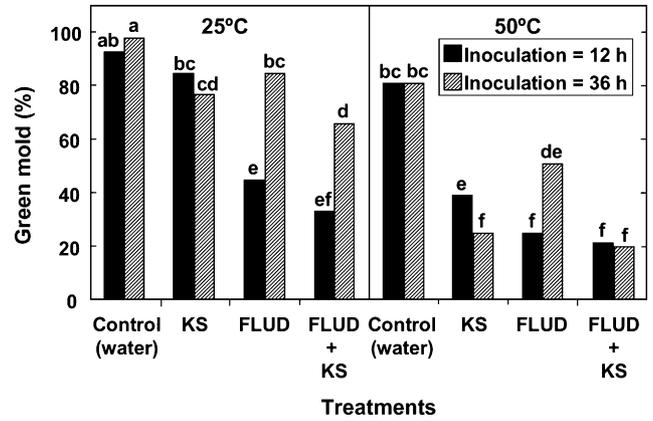


Fig. 12. Incidence of postharvest green mold on Valencia oranges, inoculated with isolate D201 of *Penicillium digitatum* 12 or 36 h prior to treatments. Fruit were dipped for 30 s in potassium sorbate (KS; 0.5%, wt/vol) or fludioxonil (FLUD; 300 mg L<sup>-1</sup>), alone or in combination, at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Values 25 and 50 °C with unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

from the cell. This high anion accumulation may generate an abnormally high turgor pressure (Piper et al., 2001). It can also influence free radical production, leading to the severe oxidative stress that is a major component of weak organic acid stress in aerobic *S. cerevisiae*. The proton release can potentially acidify the cytosol. This acidification, if it occurs, will inhibit many metabolic functions (Krebs et al., 1983). *P. digitatum* requires wounds in the fruit rind for infection to occur (Eckert and Eaks, 1989). Initial infections, but not subsequent infections, by *G. citri-aurantii* require wounds. The pH of the rind albedo tissue of citrus fruit is 4.5–5.5 (Prusky et al., 2004; Smilanick et al., 2005). The pH in the rind of lemons within wounds that were 2 mm deep and 1 mm wide was 5.1 on green lemons to 5.6 on yellow lemons (Smilanick et al., 2005). The pH within wounds was relatively well-buffered; the pH within the wounds was only elevated about 1 unit by immersion in 3% (wt/vol) sodium bicar-

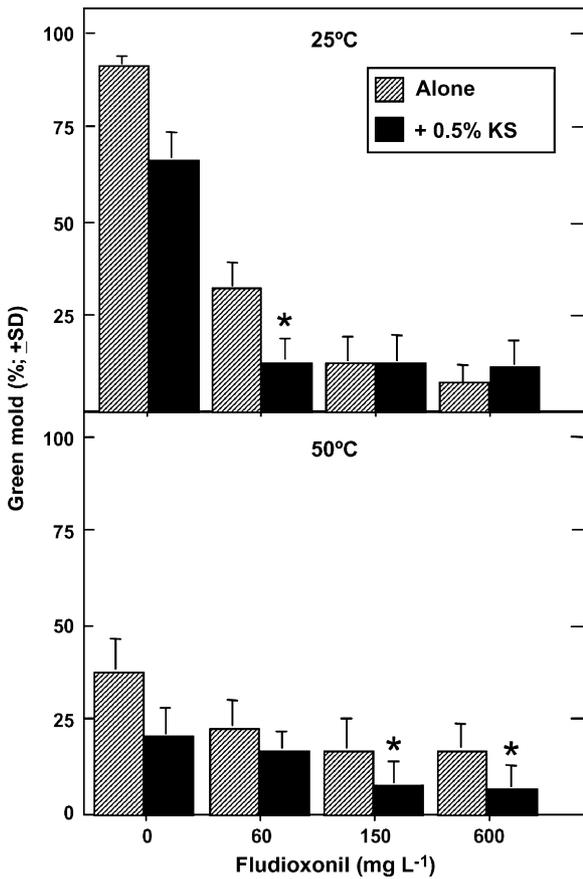


Fig. 11. Incidence of postharvest green mold, caused by *Penicillium digitatum*, on Valencia oranges after they were dipped for 30 s in fludioxonil alone or mixed with potassium sorbate (KS) at 25 or 50 °C, followed by storage for 2 weeks at 20 °C. Fruit were inoculated with isolate D201. Fruit were inoculated 24 h before treatments. Asterisk indicates synergistic activity was present according to Limpel's formula.

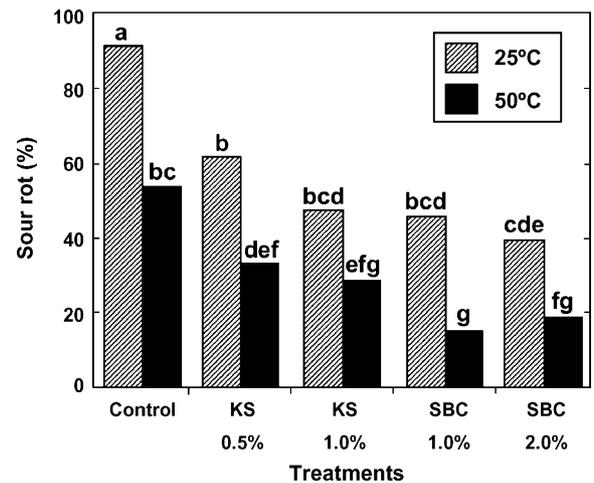


Fig. 13. Incidence of postharvest sour rot on lemons, inoculated with *Geotrichum citri-aurantii* 24 h prior to immersion for 30 s in potassium sorbate (KS) or sodium bicarbonate (SBC) solutions at 25 or 50 °C followed by storage for 2 weeks at 20 °C. Values with unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

Table 3

Residues in Valencia oranges after immersion for 30 s in aqueous solutions of imazalil (IMZ), thiabendazole (TBZ), pyrimethanil (PYR), or fludioxonil (FLUD) at either 25 or 50 °C, alone or with potassium sorbate (KS; 0.5%, wt/vol)

Treatment	Rate (mg L <sup>-1</sup> )	Temperature (°C)	Residues <sup>a</sup>
IMZ	100	25	0.77 (±0.12) a
IMZ	100	50	1.57 (±0.06) b
IMZ + KS	100	25	0.87 (±0.12) a
IMZ + KS	100	50	1.43 (±0.06) b
PYR	50	25	0.17 (±0.06) a
PYR	50	50	0.83 (±0.12) b
PYR + KS	50	25	0.17 (±0.06) a
PYR + KS	50	50	0.87 (±0.06) b
TBZ	100	25	0.60 (±0.10) a
TBZ	100	50	0.97 (±0.06) b
TBZ + KS	100	25	0.57 (±0.12) a
TBZ + KS	100	50	0.90 (±0.10) b
FLUD	300	25	0.63 (±0.12) a
FLUD	300	50	0.83 (±0.06) bc
FLUD + KS	300	25	0.77 (±0.06) ab
FLUD + KS	300	50	0.97 (±0.06) c

<sup>a</sup> Values (±S.D.) are mg kg<sup>-1</sup> fresh wt. Each value is the mean of three replicate determinations of five fruit each. Means within each of the fungicides followed by unlike letters are significantly different by Fisher's Protected LSD ( $P \leq 0.05$ ).

bonate and declined rapidly to nearly its original pH after one day (Smilanick et al., 2005). Therefore, the relatively low pH of wounds within rind tissue maximizes sorbate activity to inhibit these pathogens.

Sorbate treatment may also induce defensive responses in citrus fruit to pathogens, although nothing is known about this. Venditti et al. (2005) reported sodium carbonate treatment induced scoparone, caused structural changes, and increased the pH of rind tissue, all of which, in addition to the fungitoxicity of this compound, contributed to control of green mold by this treatment.

The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes, and inhibition of enzymes involved in metabolism of transport functions (Sofos, 1989). One of the primary targets of sorbic acid in vegetative cells appears to be the cytoplasmic membrane. It reduces the cytoplasmic membrane electrochemical gradient and consequently active transport, which in turn inhibits amino acid transport and could eventually result in the inhibition of many cellular enzyme systems (Ronning and Frank, 1987). York and Vaughn (1964) showed that sorbate reacts with the thiol group of cysteine and suggested that this is a mechanism of inactivation of sulfhydryl enzymes. Przybylski and Bullerman (1980) reported that a decrease in adenosine triphosphate (ATP) level in conidia of *Aspergillus parasiticus* was related to decreased viability after exposure to sorbic acid. Resistance to sorbate has been reported and innate resistance occurs in many microorganisms, while there is little evidence for acquired resistance (Davidson and Harrison, 2002). Schroeder and Bullerman (1985) found that there was no increase in tolerance to KS by two isolates of *P. digitatum* after repeated

and prolonged exposure, but that *P. italicum* developed a slight increase in tolerance.

Our results with KS used alone to control postharvest citrus decay are in agreement with previous work on this subject. Smoot and McCormack (1978) reported that a 2% aqueous solution of KS, applied as a dip, effectively reduced postharvest decay of several citrus fruit varieties. They also found that isolates of *P. digitatum* resistant to TBZ were sensitive to KS. Nelson et al. (1981) reported TBZ-resistant isolates could be controlled effectively by KS. Sorbate was used commercially to retard citrus postharvest decay, but its use did not become popular because its efficacy was sometimes low, particularly since its effect was often to delay, rather than stop, green mold infections (Gutter, 1981; Palou et al., 2002b). The effectiveness of KS and sodium bicarbonate was particularly poor on mandarin oranges in our work. Palou et al. (2002a) and Venditti et al. (2005) similarly reported sodium bicarbonate was not effective to control postharvest decay of mandarin fruit. In every test in our work, KS alone was more effective when it was heated to 50 °C. This result corroborates the report of Kitagawa and Kawada (1984) and Wild (1987), who showed that heat improved sorbate performance to control postharvest sour rot and green mold, respectively. Increasing the temperature of many microbial biocides enhances their potency by two- to three-fold for every 10 °C increase in temperature, until thermal destruction of the microbe occurs (Kostenbauder, 1991). We found KS was more effective when fruit were immersed in the solution rather than when it was applied by drenching the fruit with the solution over rotating brushes. This could be explained by shorter contact time we used with the overhead drench treatment (20 s versus 30 s), poorer infiltration of the chemical into the fruit by the overhead drench, and better transfer of heat into the fruit by the immersion treatment.

Control of citrus green mold and sour rot by KS was similar to that of sodium bicarbonate when they were used at similar concentrations. In our work, their combination resulted in an additive increase in their effectiveness, particularly when fruit were immersed in these solutions. It is conceivable the two compounds could be used together, although there is no benefit evident to us by doing this, particularly since sodium bicarbonate will raise the pH of the solution after several days of use (Smilanick et al., 1999), which could reduce KS effectiveness. A combination of potassium bicarbonate and KS could be used to eliminate sodium in the treatment solutions. Potassium bicarbonate, however, has been consistently less effective than sodium bicarbonate (Smilanick et al., 1999).

Sodium bicarbonate is compatible with chlorine which sanitizes both the treatment solution and treated fruit (Smilanick et al., 1999). In many applications, the solution is also heated. However, when chlorine (200 mg L<sup>-1</sup>) was added to a 0.5% KS solution, the chlorine content declined indicating they were not compatible (Smilanick et al., unpublished). It is unlikely sorbate solutions would carry the inoculum of decay pathogens, but other microbes may survive in it and contaminate it. To prevent this from happening, heat or other sanitizers could be employed, but the compatibility of sorbates with other sanitizers, such as peracetic acid, is not known.

The combination of sodium bicarbonate with citrus postharvest fungicides is a useful practice to improve fungicide performance, and in the present work we show KS can be similarly used. Purposes to use this combination are (1) to delay the development of fungicide-resistant isolates of *P. digitatum*; (2) to control fungicide-resistant isolates already present within packinghouses; (3) to facilitate a reduction in fungicide rates in order to minimize fruit residues and chemical costs; (4) to accomplish some control of sour rot, which is not controlled by any of the fungicides currently registered for use on citrus fruit in the USA; and (5) to reduce the presence of food-borne human or plant pathogens of regulatory concern on the fruit.

The most compelling reason to replace sodium bicarbonate with KS is to reduce water disposal issues associated with sodium bicarbonate, which includes its high pH, salinity, and sodium content. The pH of a fresh solution of sodium bicarbonate (2%, wt/vol) is about 8.3, and it increases during use to 9 or higher. The pH of a fresh solution of KS (0.5%, wt/vol) is 6.5, and it does not change during use. The electrical conductivity of the sodium bicarbonate and KS solutions at these concentrations were 16.8 and 2.9 mS cm<sup>-1</sup>, respectively.

When KS was added to fungicide solutions, it consistently increased their effectiveness without increasing the residues of any of the fungicides. KS particularly improved the effectiveness of IMZ. Schirra et al. (1996) and others reported that IMZ effectiveness was greatly improved by heat, and the improvement was not associated with excessive residues of the fungicide in the fruit (Smilanick et al., 1997). In the present work, the benefit of combining IMZ with KS was substantial and similar to the benefit we observed by combining this fungicide with sodium bicarbonate in earlier work (Smilanick et al., 2005), especially to control IMZ-resistant isolates of *P. digitatum*. The benefit of combining TBZ with KS was more modest than that obtained with IMZ. The control of a TBZ-resistant isolate was mainly due to the heated KS alone. In prior work, TBZ effectiveness was also enhanced by mild heating (41 °C), adding sodium bicarbonate, and immersing fruit, rather than drenching them, with the solution (Smilanick et al., 2006c). With these measures, an isolate of *P. digitatum* with a high level of TBZ resistance was significantly controlled. Smilanick et al. (2006c), in semi-commercial tests with naturally inoculated fruit, reported that TBZ and sodium bicarbonate treatment reduced green mold incidence from 11% among untreated oranges to 2%.

Combining PYR or FLUD with KS consistently improved their performance to control green mold. The influence of heat on their performance was modest. In earlier work, we found an increase in the temperature of the PYR solution improved its effectiveness to control green mold on lemons and oranges, but the improvement was often irregular and small (Smilanick et al., 2006b). With FLUD, we used a shorter interval between inoculation and treatment than the other fungicides, because effective use of this fungicide requires its prompt application after inoculation. FLUD applied 14–16 h after the inoculation of wounds on stone fruits was significantly more effective than when applied before inoculation, which indicates that it acts mainly as protectant that does not penetrate deeply enough into the fruit to prevent decay from wounds that extend below the fruit

epidermis (Förster et al., 2007). FLUD showed excellent activity as postinfection treatments to control brown rot (caused by *Monilinia fructicola* and *M. laxa*), gray mold (caused by *Botrytis cinerea*) on stone fruit (Adaskaveg et al., 2005), and blue mold (caused by *Penicillium expansum*) on apples (Errampalli et al., 2005). Zhang (personal communication) reported that FLUD at 250 mg L<sup>-1</sup> did not significantly reduce the green mold incidence on oranges; however, concentrations between 500 and 2000 mg L<sup>-1</sup> reduced green mold effectively, but not completely. Cochran et al. (2003) reported that FLUD significantly reduced green mold incidence on Valencia oranges but was not equivalent to IMZ in effectiveness. Schirra et al. (2005) reported FLUD was very effective and almost completely controlled postharvest green mold on Tarocco oranges when applied at 100 mg L<sup>-1</sup> at 50 °C or 400 mg L<sup>-1</sup> at 20 °C. In contrast to the results of Schirra et al. (2005), in our work we were not able to completely control green mold on Valencia oranges with FLUD, and the influence of heat on its performance was small. However, Schirra et al. (2005) employed a relatively long treatment time of 3 min while we used a period of 30 s.

Residues of IMZ, TBZ, PYR, and FLUD in Valencia oranges were not increased by the addition of KS to these fungicides. Often, the increase in effectiveness caused by KS was most apparent when the solutions were heated. The residues and effectiveness of IMZ (Cabras et al., 1999; D'Aquino et al., 2006; Smilanick et al., 1997), TBZ (Cabras et al., 1999; Smilanick et al., 2006c), PYR (D'Aquino et al., 2006; Smilanick et al., 2006b), and FLUD (Schirra et al., 2005) are enhanced by heating. Our work corroborates these reports. For example, Schirra et al. (2002) reported an increase of 4.2-fold in IMZ residue, on Star Ruby grapefruit, when treated at 50 °C compared to that at 20 °C, and the fungicide was much more effective when heated. Fungicide rates applied with heat are reduced compared to rates used at ambient temperatures to avoid excessive fungicide residues in the fruit (Smilanick et al., 1997). Heat increases fungicide residues, but in the case of IMZ, the increase in residues alone was insufficient to account for the magnitude of the improvement in its performance, so other aspects, such as the elicitation of host defensive responses, thermal inhibition of the pathogens, and improved fungicide infiltration may also important (Schirra, 2005; Smilanick et al., 1997; Venditti et al., 2005).

Sorbate residues in citrus fruit have not been studied. Sorbates leave a persistent antimicrobial residue in products they protect (Sofos, 1989) and it is possible in citrus fruit they provide a persistent residue on the fruit that sodium bicarbonate does not (Larrigaudière et al., 2002). In addition to protection of the fruit from infection by postharvest pathogens, the sorbate residue could reduce the risk that the fruit carry human pathogens of food safety concern or plant pathogens of quarantine concern. However, KS residues that remain after treatment may be an issue to some buyers. At one time registered as a fungicide in the US, KS was more recently listed as a “minimum risk pesticide” by the US EPA and exempt from residue tolerance requirements (40 CFR section 152.25(g)). The List of Allowed and Prohibited Substances of the USDA National Organic Program does not classify sorbate as an allowed substance, while it does allow sodium bicarbonate to be used.

We found the effectiveness of KS to control green mold or sour rot of citrus fruit was greatly enhanced when solutions were heated to 50 °C. Our results with KS treatment to control sour rot corroborate those of Kitagawa and Kawada (1984), who reported KS significantly reduced sour rot incidence on lemons and its effectiveness was improved by heat. We show both KS and sodium bicarbonate were about equal in effectiveness for this purpose; Kitagawa and Kawada (1984) showed KS was equal in effectiveness to the fungicide sodium *ortho*-phenyl phenate. However, while these treatments reduced sour rot incidence, none control it completely, and no fungicides registered in California control this disease adequately.

KS can be used instead of sodium bicarbonate to improve the effectiveness of fungicides, retard the development of fungicide-resistant isolates of *P. digitatum* in citrus packinghouses, control green mold caused by fungicide-resistant isolates of *P. digitatum*, and to obtain partial control of sour rot. Unlike sodium bicarbonate, KS has fewer environmental issues associated with its disposal.

### Acknowledgements

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