Use of Phosphite Salts in Laboratory and Semicommercial Tests to Control Citrus Postharvest Decay

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Abstract

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Potassium phosphite (KP) concentrations that inhibited the germination of 50% of *Penicillium digitatum* conidia were 229, 334, 360, 469, 498, or 580 mg/liter at pH 3, 4, 5, 6, 7, or 8, respectively. Increasing phosphate content in media reduced phosphite toxicity. To control green or blue mold, fruit were inoculated with *P. digitatum* or *P. italicum,* then immersed 24 h later in KP, calcium phosphite (CaP), sodium carbonate, sodium bicarbonate, or potassium sorbate for 1 min at 20 g/liter for each at 25 or 50°C. Mold incidence was lowest after potassium sorbate, CaP, or KP treatments at 50°C. CaP was often more effective than KP but left a white residue on fruit. KP was significantly more effective when fruit were stored at 10 or 15°C after treatment compared with 20°C. Acceptable levels of control were achieved only when KP was used in heated solutions or with fungicides. KP was compatible with imazalil (IMZ) and other fungicides and improved their effectiveness. KP increased thiabendazole or IMZ residues slightly. Phosphite residues did not change during storage for 3 weeks, except they declined when KP was applied with IMZ. KP caused no visible injuries or alteration in the rate of color change of citrus fruit in air or ethylene at 5 µl/liter.

Significant losses occur after harvest, during storage and marketing of citrus fruit, primarily due to green mold, caused by *Penicillium digitatum*. Blue mold, caused by *P. italicum,* also causes fruit losses but is of lesser importance (16,45). Currently, both diseases are commonly controlled by postharvest applications of sodium bicarbonate (SBC), imazalil (IMZ), thiabendazole (TBZ), pyrimethanil (PYR), azoxystrobin (AZO), fludioxonil (FLUD), or sodium o-phenylphenate (27,45,46,49,50). Concerns about how fungicides may impact human and environmental health and the widespread occurrence of fungicide-resistant isolates of *P. digitatum* (26,28) have stimulated the search for alternative treatments.

SBC partially controls green and blue molds of citrus fruit (6,36,46), and its addition to IMZ (45), PYR (50), or TBZ (47) improved their performance. The addition of SBC facilitates the use of reduced fungicide rates, which decreases costs and improves control of fungicide-resistant isolates of *P. digitatum*. SBC can be used in sequence with other treatments, such as biological control or hot water, to improve their efficacy (37,39). SBC is relatively inexpensive and approved for use for "organic" growers, and its residues are exempt from regulation. However, disposal of SBC raises regulatory issues in some locations, because of its high electrical conductivity, high pH, and sodium content. All three factors can make disposal of used solutions difficult. Therefore, compounds that could improve fungicide performance as SBC does yet avoid or minimize these disposal problems would be valuable. In prior work, we found that potassium sorbate (48) and calcium polysulfide (51) were effective replacements for SBC that solved disposal problems of spent solutions. The purpose for this work was to find a similar alternative.

Phosphorous acid and its phosphite salts merit evaluation as substitutes for conventional postharvest fungicides. Phosphite solutions can be manufactured to be devoid of sodium and have a neutral pH, which eliminates two important water-quality problems of SBC or sodium carbonate. Soil disposal of used phosphite solutions is a feasible route of disposal because many phosphite products are registered as fertilizers. Phosphites oxidize slowly to phosphates in soil (1,30). They are exempt from residue tolerances in the United States (55), and two commercial potassium phosphite formulations are registered for postharvest use in the United States (KPhos; Pace International, and Fungi-Phite; Plant Protectants Inc.). Phosphites offer another significant advantage over other alternative treatments in that they control postharvest brown rot, caused by several *Phytophthora* spp. (11,19). Brown rot is not controlled by the currently registered fungicides or their alternatives and, in some wet seasons in California, citrus fruit losses from brown rot become significant. The compatibility of phosphites with fungicides needs evaluation, to determine whether they can be used with fungicides now in use and if there are useful interactions present. Rosenberger et al. (42) reported that control of flyspeck of apple, caused by *Zygophiala jamaicensis*, by captan or thiophanate methyl was significantly improved by the addition of phosphite, although it did not improve control of fruit rot due to black rot caused by *Botryospheria* sp. or sooty blotch. This report indicates the potential synergism between phosphite salts and fungicides.

Phosphorous acid (H_3PO_3) in solution is in equilibrium with its tautomeric form, phosphonic acid. Phosphonates or phosphites, anionic forms of phosphonic or phosphorous acids, respectively, are used in formulations containing either the aluminum salt of ethyl-phosphonate (fosetyl-aluminum) or the potassium or calcium salt of phosphite (12,22,23,33). Many phosphite products are available today as fungicides, while others are marketed as fertilizers or "defense stimulators". Although foliar phosphite applications

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increased flower numbers and yields on 'Valencia' orange, controversy still exists as to their use as fertilizers, because benefits from phosphite applications may result from the control of pathogens, among other mechanisms (4). Phosphites do not supply phosphorus directly to plants. Although the phosphite cation is mobile and available, it requires oxidation to phosphate prior to use by plants and this process is mediated by microbes (31,32,54). Phosphites are effective for the control of diseases caused by oomycetes (*Phytophthora*, *Plasmopara*, and *Pythium* spp. and others) that are particularly susceptible to inhibition by phosphite (9,10,14, 24,33,34). Few reports describe control of true fungi by phosphites. Bassay Blum et al. (7) reported that immersion of apple fruit in phosphite solutions controlled blue mold, caused by *P. expansum*. Amiri and Bompeix (5) reported that potassium phosphite inhibited conidial germination and mycelial growth of *P. expansum* and it was more effective when applied in heated solutions. Treatment with potassium phosphite at 2 g/liter and 50°C for 3 min completely suppressed blue mold in 'Elstar' apple fruit after 7 days of storage at 20°C. This regime is commercially feasible for the processing of many kinds of fruit.

The objectives of this study were to (i) determine the effectiveness of phosphite salts for controlling citrus green and blue molds; (ii) quantify the effect of heat, phosphite concentrations, pH, and storage temperature on the efficacy of the treatments; (iii) evaluate the influence of phosphite salts on the performance of other citrus postharvest fungicides; (iv) evaluate the influence of the interval on the performance of IMZ when mixed with potassium or calcium salts of phosphite; and (v) determine how the addition of potassium phosphite to the most common commercial fungicides (IMZ or TBZ) influences the levels and persistence of fungicide residues on fruit.

Materials and Methods

Chemical sources and phosphite formulations. Unless stated otherwise, all reagents were obtained from Sigma-Aldrich. Four sources of phosphite were used: (i) phosphorous acid (99.9%); (ii) commercial formulation A, potassium phosphite (54.5% potassium phosphite, KPhos; Pace International); (iii) commercial formulation B (45.5% potassium phosphite, Fungi-Phite; Plant Protectants Inc.); and (iv) commercial formulation C (42.5% calcium phosphite, Calci-Phite; Biagro Western Sales, Inc.).

Pathogen culture. Two isolates of *P. digitatum* (IMZ-sensitive PD90 and IMZ-resistant D201) and one isolate of *P. italicum* (PI105) were cultured for 1 to 2 weeks on potato dextrose agar (Difco Laboratories) at 25°C. Both pathogens were isolated from infected lemon from citrus packinghouses in California. Isolates PD90 and PI105 can be controlled by typical commercial IMZ applications in California packinghouses, whereas isolate D201 has approximately a 10-fold resistance to IMZ and is only controlled by IMZ application rates that are so high that the resulting residues may exceed Environmental Protection Agency-approved tolerances (28). Conidia were harvested by adding 5 ml of sterile reverse-osmosis water (roH_2O) containing 0.05% (vol/vol) Triton X-100 into the petri dish. Colonies were rubbed with a sterile glass rod, and the resulting conidial suspension was passed through two layers of cheesecloth. The suspension was diluted with roH_2O to an absorbance of 0.1 at 425 nm as measured with a spectrophotometer and a density containing 1×10^6 conidia/ml (15), unless stated otherwise.

Conidial germination assays. Conidial germination of *P. digitatum* (isolate PD90) in media containing several concentrations of H_3PO_3 at different pH values was determined. The germination medium, buffered potato-dextrose broth (PDB-b), was prepared by placing 96 g of PDB (Difco Laboratories) and 20 mM each of glycylglycine and disodium phosphate in 1 liter of roH_2O. The pH of the medium was adjusted to 3, 4, 5, 6, 7, or 8, with 1 N KOH or HCl. The phosphate concentration in this medium greatly exceeded what is needed for the growth of *P. digitatum* (8) and other fungi (21). Sterile dishes with six macrowells (Corning Glass Works) with a capacity of 10 ml/well were used. In each well, 2.5 ml of PDB-b, H_3PO_3 at various concentrations, 0.1 ml of conidial suspension, and roH_2O were added to a final volume of 5.0 ml. Actual H_3PO_3 concentrations were 0, 125, 250, 500, 750, 1,000, 1,250, or 1,500 mg/liter. After 24 h of incubation at 25°C, 100 to 150 conidia were counted in each well by observation (×200) using an inverted compound microscope, and the percentage of germinated conidia was calculated. Phosphite concentrations that inhibited germination of 50% (EC_{50}) and 99% (EC_{99}) of the conidia were determined. All experiments were performed twice.

The influence of phosphorus on phosphite toxicity was determined by utilizing the methods as described above, except that modified Pratt's medium (53) was used with final H_3PO_3 concentrations of 0.5, 0.75, 1, 1.25, or 1.5 g/liter and final phosphate concentrations of 0, 0.1, or 10 mM. Phosphate was prepared from KH_2PO_3 and the final pH of the medium was 6.0.

Fruit inoculation. Freshly harvested lemon ('Eureka'), mandarin orange ('WMurcott', 'Marisol', or 'Clementina de Nule'), navel orange ('Atwood', 'Barnfield', or 'Fukimoto'), or seeded orange (Valencia) were used. They were selected because they are popular cultivars that were mature and available at the time the tests were conducted. Postharvest decay can differ greatly among cultivars (38). Control of decay on mandarin oranges is generally more difficult than other citrus cultivars (37). The fruit were randomized by dividing them into groups equal to the number of treatments being evaluated; equal numbers of fruit from each harvest box were assigned to every treatment. Conidial suspensions of *P. digitatum* and *P. italicum* were prepared as described previously and a concentration of 1×10^6conidia/ml was used to inoculate the fruit 24 h before treatments were applied (15). The tip of a stainless steel rod, 1 mm wide and 2 mm in length, was immersed in a conidial suspension of the corresponding pathogen and inserted afterward at the equatorial position in the fruit rind. Inoculated fruit were maintained at 20°C and 95% relative humidity (RH) until treatment. In most tests, fruit were inoculated once at a single site with *P. digitatum* or *P. italicum.* In some tests, fruit were inoculated at one end with *P. digitatum* and at the opposite end with *P. italicum*.

Influence of heat, pH, and phosphite concentration on decay control. To examine the effect of heat on the effectiveness of several salt solutions, *P. digitatum* (isolate D201)- and *P. italicum*-inoculated Eureka lemon or *P. digitatum* (isolate D201)-inoculated WMurcott mandarin orange were immersed for 1 min in 20 liters of water (control) or potassium phosphite (KP, formulation B), calcium phosphite (CaP, formulation C), sodium carbonate (SC), SBC, or potassium sorbate (KS) solutions at 20 g/liter in 22-liter-capacity stainless steel tanks with a computer-controlled thermostat. The temperature of the solutions was 25 or 50°C (±0.5°C) and each was constantly stirred with a 5-cm-diameter propeller. Each treatment was applied to four replicates of 27 fruit each. The test was done twice. In the first test, the lemon were not rinsed after treatment whereas, in the second experiment, WMurcott mandarin orange were either not rinsed or briefly immersed in fresh water at 20°C after every treatment. A treatment with PYR (Penbotec 400SC; Janssen PMP) at 1 g/liter was included and these fruit were not rinsed after treatment.

To examine the effect of pH on phosphite decay control efficacy, *P. digitatum* (isolate PD90)- and *P. italicum*-inoculated Eureka lemon fruit were immersed for 1 min in water (control) or buffered solutions that contained KP (formulation A) at 10 g/liter adjusted to pH 3, 4, 5, 6, 7, or 8. The buffer was 100 mM K_2HPO_4 and 10 mM citric acid for the pH 3, 4, or 5 KP solutions, and 100 mM K_3PO_4 and 10 mM citric acid for pH 6, 7, or 8 KP solutions. The pH was adjusted with concentrated H_2SO_4, H_3PO_4, or KOH. Each treatment was applied to five replicates of 20 fruit each. A similar experiment was done with Marisol mandarin orange, with the differences that 20 mM potassium phosphate buffer was used, only *P. digitatum* (isolate PD 90) was used to inoculate the fruit, the KP (formulation A) concentration was 15 g/liter, and the contact time of the fruit in the solution was 2 min. Each treatment was applied to four replicates of 27 fruit each.

To examine the effect of temperature and phosphite salt (CaP or KP, formulations B or C) concentration on effectiveness, *P. digitatum* (isolate D201)-inoculated Barnfield navel orange were immersed for 15 s in water (control) or CaP or KP solutions at 5 or 15 g/liter and 21, 41, 46, 52, or 57°C (±0.5°C). The fruit were not rinsed after treatment. Each treatment was applied to three replicates of 20 fruit each with two inoculations sites per fruit. In a second experiment evaluating both KP concentration and temperature, *P. digitatum* (isolate PD90)- and *P. italicum*-inoculated Eureka lemon fruit were immersed for 1 min in water (control) or in 20 liters of KP (formulation A) at 5, 10, or 20 g/liter and 25 or 50°C (±0.5°C). In this test, a treatment with IMZ (Fungaflor 500 EC, 44.6% IMZ; Janssen PMP) at0.2 g/liter and 50°C during 15 s was included. After treatment, unrinsed fruit were placed into cavity trays and stored for 1 or 2 weeks at 20°C and 95% RH, and then the number of infected fruit was counted. Each treatment was applied to five replicates of 20 fruit each.

Influence of storage temperature on effectiveness of phosphites salts. Clementina de Nule mandarin orange or Fukimoto navel orange fruit were inoculated with *P. digitatum* (isolate PD90) and *P. italicum* as previously described, and were then immersed in KP (formulation A) at 20 g/liter and 25 or 50°C (±0.5°C) for 60 s. The fruit were not rinsed after treatment. Each treatment was applied to three replicates of 20 fruit each. After treatment, the fruit were packed into cavity trays and stored for 3 weeks at 10, 15, or 20°C and 95% RH. Prior preliminary tests indicated that the number of fruit that became infected after inoculation did not increase significantly after 3 weeks of storage at these temperatures.

Evaluation of phosphite salts combined with conventional postharvest fungicides. Eureka lemon fruit were inoculated with *P. digitatum* (isolate D201), and immersed for 30 s in 25°C solutions containing either IMZ at 0.5 g/liter, TBZ (Sealbrite, 98.5% thiabendazole; EcoScience Corp.) at 25 mg/liter, PYR at 25 mg/liter, or a mixture of FLUD + AZO that contained each (Graduate A+, 20.6% fludioxonil and 20.6% azoxystrobin; Syngenta Crop Protection, Inc.) at 20 mg/liter. They were applied alone or mixed with KP (formulation A) at 4 g/liter. The fruit were not rinsed after treatment. For FLUD + AZO treatment, fruit were inoculated 12 h before treatments were applied. After treatment, the fruit were packed into cavity trays, stored for 17 days at 10°C and 95% RH, and then evaluated for decay. Each treatment was applied to four replicates of 27 lemon fruit each. In a second test, Eureka lemon fruit inoculated with *P. digitatum* (isolate D201) were immersed for 2 min at 20 or 40°C (±0.5°C) in either IMZ at 0.2 g/liter, KP (formulation B) at 10 g/liter, SBC at 20 g/liter, a mixture of KP + IMZ, or a mixture of KP + SBC. After treatment, unrinsed fruit were stored for 21 days at 20°C, and then the number of infected fruit was counted. Each treatment was applied to four replicates of 27 fruit each.

In a large commercial test using commercial packing equipment, Eureka lemon, WMurcott mandarin orange, and Atwood navel orange were inoculated with *P. digitatum* (isolate D201 or PD90) at 1×10^5 conidia/ml. After 18 to 20 h at 20°C, the fruit were treated with either KP (formulation B) at 15 g/liter, CaP (formulation C) at 15 g/liter, or IMZ alone by passing the fruit down a packing line where they were drenched for 15 s by 16 solid cone nozzles, each delivering 2.1 liter/min over a 1.5-m-long bed of 25 brushes (Tufted Polycor; Industrial Brush Co.) rotating at 80 revolutions/min. The IMZ concentration applied was 0.05 g/liter for the IMZ-sensitive isolate and 0.5 g/liter for the IMZ-resistant isolate. The solution temperature was 46°C. After treatment, unrinsed fruit were dried for 2 min at 60°C in a commercial fruit dryer, packed into cartons with cavity trays to prevent fruit-to-fruit contact, and stored for 3 weeks at 10°C. Each treatment was applied to three or four replicates of approximately 50 to 75 fruit each.

Influence of inoculation time on KP and IMZ effectiveness. To evaluate the curative and protective activities of KP and IMZ, Eureka lemon fruit were inoculated with *P. digitatum* (isolate PD90) near the stem attachment point and again on the distal end with *P. italicum*. Inoculations were done either 48 or 24 h before treatments were applied or 24, 48, 72, 96, or 120 h after treatments were applied. The treatments included 30 s of immersion at 25°C in water alone, IMZ at 0.5 g/liter, KP (formulation A) at 5 g/liter, or IMZ at 0.5 g/liter plus KP at 5 g/liter. After treatment, unrinsed fruit were stored for 14 days at 20°C, before the number of infected fruit was counted. Each treatment was applied to five replicates of 20 lemon each. In a second test, Eureka lemon or Valencia orange fruit were dipped for 30 s at 25°C in IMZ at 0.5 g/liter, KP (formulation A) at 5 g/liter, or a mixture of both. After 5 days at 10°C, the lemon fruit were all inoculated with 1×10^4 conidia/ml and the orange with 1×10^5 conidia/ml using *P. digitatum* isolate D201. The fruit were not rinsed after treatment and stored for 14 days at 20°C; then, the number of infected fruit was counted. Each treatment was applied to five replicates of 20 orange fruit or four replicates of 27 lemon fruit each.

Fruit tissue phosphite, phosphate, and fungicide analysis. Analyses were conducted to determine the influence of phosphite treatment on the phosphite and phosphate residues on fruit, and whether fungicides residues were altered when applied in mixtures with phosphite. Atwood orange fruit were immersed for 30 s in IMZ (0.4 g/liter) or TBZ (0.4 g/liter) solutions at 25 or 50°C, either alone or with KP (formulation B) at 20 g/liter. After treatment, the fruit were not rinsed and the residues of each fungicide and contents of phosphite and phosphate were determined within 2 days and again after 3 weeks of storage at 5°C. Each treatment analyzed included four replicate of five fruit each. Subsamples from the five fruit were pooled to create a single sample for analysis from each replicate. The test was done once. Fungicide residues were reported as milligrams per kilogram fresh weight. For IMZ and TBZ analysis, the whole fruit were extracted for 1 h with ethyl acetate followed by gas chromatography by the method of Yamazaki and Ninomiya (56). The detection limit was less than 0.1 mg/kg and the efficiency of recovery exceeded 95% for both fungicides. The phosphate and phosphite concentrations in the orange rind tissue were determined using the high-performance ion chromatographic method of Roos et al. (41), with some modifications. Phosphite and phosphate contamination was minimized by wiping all tools and surfaces with dilute HCl. The top 2 mm of the rind of the fruit was scraped to produce tissue fragments approximately 1 mm in size. Only flavedo tissue was collected. A 5-g portion of the flavedo tissue fragments was placed in a glass jar and frozen at –16°C for 8 h. Then, 50 ml of 40 mM HCl was added to the jar and the sample and extract were shaken vigorously 50 times. The jar was placed at 5°C for 24 h so particulates could condense and precipitate; then, 2 ml of the extract was collected and passed through a 0.2-µm pore size Teflon filter and injected into an HPLC (Shimadzu) with a column recommended for anion chromatography (STAR-ION-A 300, 100 by 4.6 mm; Phenomenex), using a mobile phase of 100 mM succinic acid with 0.02% (wt/vol) sodium azide, and a conductivity detector. Standards containing phosphorous acid and potassium dihydrogen orthophosphate were prepared. Tissue phosphite and fungicide contents were expressed as milligrams per kilogram fresh weight.

Influence of postharvest treatments with KP on fruit rind color. The test was done with freshly harvested, non-waxed, Eureka lemon, WMurcott mandarin orange, and Atwood navel orange from the University of California Lindcove Research and Extension Center, Exeter. Fruit that were dark green in color were either not treated (dry control), immersed for 60 s in water at 25 or 50°C (wet control), or immersed for 60 s in KP (formulation A) at 20 g/liter and 25 or 50°C. Fruit were not rinsed after treatment and immediately placed at 20ºC and 90 to 95% relative humidity in either air or air adjusted to contain ethylene at 5 µl/liter. The test was done three times, each time with four replicates of 10 fruit each The rate of color change during ethylene degreening was measured with a colorimeter (CR400; Minolta Corp.). Measurements were made at the time of treatments and weekly thereafter until most all green color was absent. L* and calculated hue angle values were used for statistical analysis (35).

Statistical analysis. The concentrations of phosphite in PDB-b that resulted in 50 or 99% reductions in conidial germination (EC_{50} or EC_{99}, respectively) were estimated by probit analysis (SPSS Statistics 17.0). Disease incidence, color values, fungicide residues, and phosphite and phosphate contents were analyzed by using a one- or two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test, or Fisher' protected least significant difference was calculated at $P = 0.05$ (SPSS Statistics 17.0). Statistical significance values indicated in the prose may refer to an ANOVA applied but not shown. Disease incidence values among citrus fruit were arcsin transformed and residue contents were $\log_{10}$ transformed before analysis to improve homogeneity of variance. Actual values are shown.

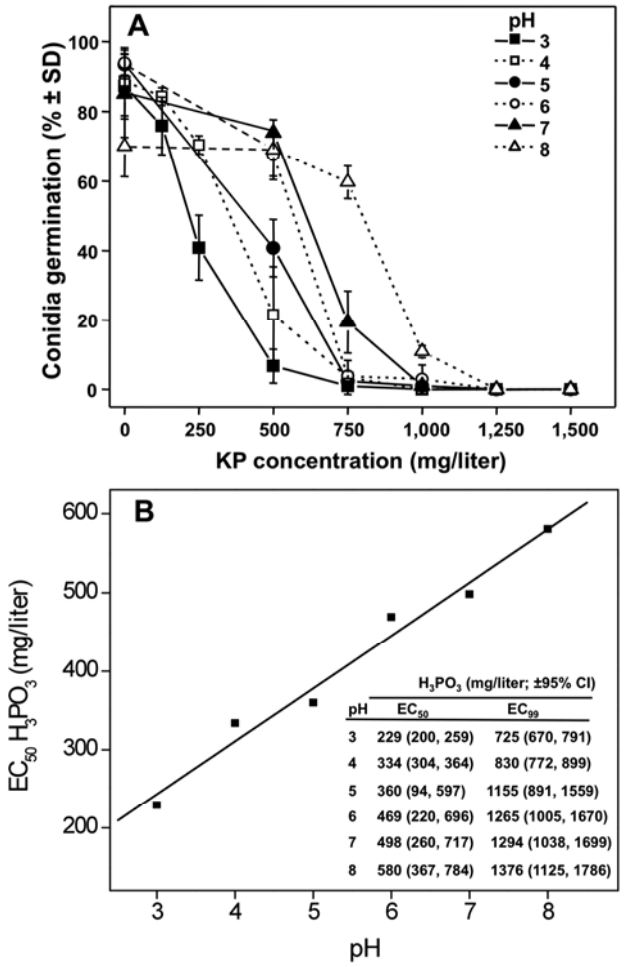

Fig. 1. Germination of conidia of *Penicillium digitatum* (isolate PD90) in buffered potato dextrose broth (PDB-b) containing different concentrations of phosphorous acid at pH 3, 4, 5, 6, 7, or 8. **A,** Germination was determined after 24 h at 25°C. Each value is the mean of two replicates of 100 to 150 conidia each. **B,** Effective concentrations of phosphorous acid in PDB-b at pH 3, 4, 5, 6, 7, or 8 to inhibit germination of 50% (EC_{50}) or 99% (EC_{99}) of conidia of *P. digitatum* exposed to phosphorous acid for 24 h at 25°C. Values in parenthesis indicate upper and lower 95% fiducial limits.

Results

Evaluation of phosphite salts on conidial germination. Germination of conidia of *P. digitatum* (isolate PD90) was more than 90% in PDB-b without phosphite amendment from pH 3 to 7 whereas, at pH 8, the germination declined to 70%. Phosphite inhibitory activity was greater at lower pH, being approximately twice as toxic at pH 3 as at pH 7 (Fig. 1A). The EC_{50} values of H_3PO_3 were 229 mg/liter at pH 3 and 498 mg/liter at pH 7. The EC_{99} values of H_3PO_3 were 725 mg/liter at pH 3 and 1,290 mg/liter at pH 7 (Fig 1B).

Phosphorus content greatly influenced phosphite toxicity to conidia of *P. digitatum.* In Pratt's medium at pH 6.0, conidia did not germinate without phosphorus. When this medium was amended with 0.1 mM phosphorus, more than 90% of conidia germinated in the control well without phosphite but conidia did not germinate when phosphite was present, even at the lowest concentration of 500 mg/liter. When the medium was amended with 10 mM phosphorus, conidia germinated in the presence of phosphorous acid at 500 or 750 mg/liter but not at 1,000 mg/liter or higher. Among conidia that germinated, the germ tubes were shorter and thicker and more branched than those in this medium without phosphite.

Effect of salt solution temperature on control of citrus green and blue mold. Increasing the temperature of the treatment solutions increased their effectiveness significantly ($P \leq 0.05$; Table 1). Control of green and blue mold on Eureka lemon by CaP and KP solutions was better than sodium carbonate or SBC, particularly when used at 50°C. Water alone at 50°C and KP at 25°C moderately controlled decay in this test (Table 1). ANOVA applied to these data indicated that increasing the temperature of KP and CaP solutions increased their effectiveness significantly ($P \leq 0.001$).

On WMurcott mandarin orange, green mold incidence was lower after KP or CaP treatment at either 25 or 50°C than potassium sorbate, SBC, or sodium carbonate but it was higher than after PYR treatment (Fig. 2). Water treatment alone at 50°C reduced green mold incidence significantly in this experiment. CaP but none of the other salts left white deposits on the fruit that were not rinsed after treatment. ANOVA applied to these data indicated that increasing the temperature of the treatment solutions increased their effectiveness significantly ($P \leq 0.001$), whereas the brief rinse after treatment did not ($P = 0.486$).

Influence of pH, heat, and rates on phosphite salts effectiveness. The influence of pH on the effectiveness of KP to control green mold was small and not significant. The incidence of green mold among Eureka lemon fruit after immersion in buffered water was 100%, while decay after immersion in KP at 10 g/liter and pH 3, 4, 5, 6, 7, or 8 was 100, 91.7, 93.3, 93.3, 92.3, or 81.7%, respectively, and these percentages were not significantly different from each other or the water treatment. The mean incidence of green mold among Marisol mandarin orange fruit after immersion in buffered water was 99.1%, while decay after immersion in KP at15 g/liter and pH 3, 5, 7, or 9 was 66.3, 65, 57.5, or 60%, respectively,

Table 1. Green mold and blue mold incidence among 'Eureka' lemon immersed for 1 min in 25 or 50°C salt solutions at 20 g/liter followed by storage for 1 week at 20°C[z]

	Green mold incidence (% ± SD)		Blue mold incidence (% ± SD)	
Treatment	**25°C**	**50°C**	**25°C**	**50°C**
Water control	100.0 a	58.3 a	32.1 ab	23.8 a
Sodium carbonate	53.3 cd	35.7 ab	21.9 bc	7.1 bc
Sodium bicarbonate	38.1 cde	32.1 ab	22.6 bc	15.5 abc
Potassium phosphite	59.5 c	16.7 bc	15.5 bc	2.4 bc
Potassium sorbate	23.8 e	6.0 c	7.1 c	3.6 bc
Calcium phosphite	35.7 de	4.8 c	6.0 c	1.0 c

[z] Lemon fruit were inoculated with *Penicillium digitatum* (isolate D201) or *P. italicum* (isolate PI105) 24 h before treatment. Each treatment was applied to four replicates of 27 fruit each. Values within columns followed by the same letter are not significantly different according to Tukey's honestly significant difference ($P = 0.05$). SD = standard deviation.

and these percentages were significantly different from the control but not from each other. The influence of pH on the effectiveness of KP to control blue mold was also small but some differences were significant. The incidence of blue mold among Eureka lemon fruit after immersion in buffered water was 97.5%, whereas decay after immersion in KP at 10 g/liter and pH 3, 4, 5, 6, 7, or 8 was 73.3, 65, 63.3, 80, 55, or 55%, respectively, and decay among the KP treatments was significantly ($P \leq 0.05$) lower at pH 7 and 8.

The control of green mold on Barnfield navel orange by CaP and KP was improved by heating the solutions and by increasing the phosphite concentration. KP or CaP at 15 g/liter was more effective than 5 g/liter in controlling green mold (Table 2). In a second experiment, KP treatments at 25 or 50°C significantly controlled blue mold on Eureka lemon, particularly at 10 or 20 g/liter, and were equal in effectiveness to IMZ when heated (Fig. 3A). Green mold incidence on Eureka lemon after treatment with KP at 25°C was high at all rates tested. Heating the solution to 50°C resulted in improved control with rates of 10 or 20 g/liter, and were as effective as IMZ (Fig. 3B). In this test, the influence of temperature was greater than that of concentration in reducing the two molds.

Influence of storage temperature on phosphite effectiveness. Post-treatment storage temperature and the temperature of the solution used to treat the fruit had a large impact the incidence of green and blue mold (Table 3). Incidences after 3 weeks of storage are shown. To control green mold on Fukimoto navel orange, decreasing the storage temperature from 20 to 15 to 10°C significantly ($P \leq 0.000$) improved the control of green mold. Increasing the temperature of the KP solution from 25 to 50°C significantly ($P \leq$

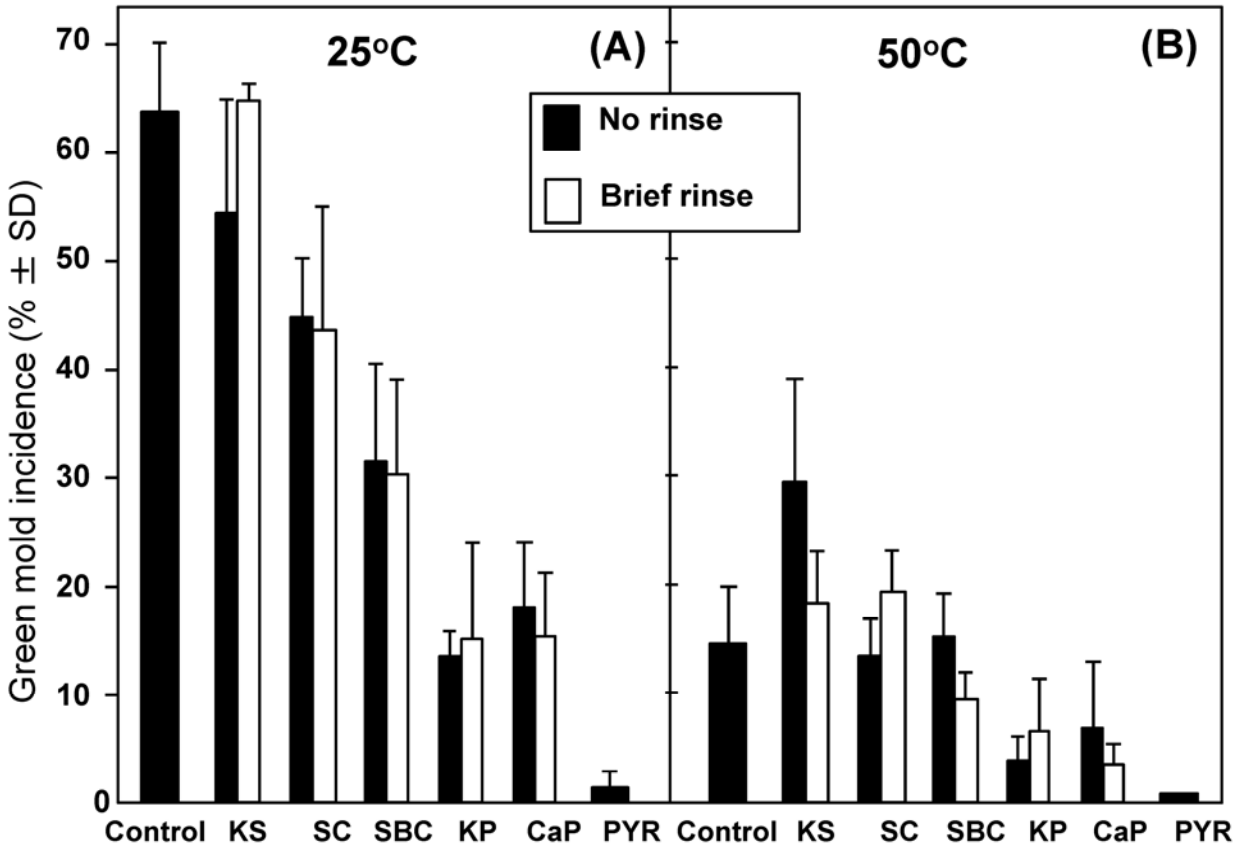

Fig. 2. Green mold incidence among 'WMurcott' mandarin orange fruit inoculated with *Penicillium digitatum* (imazalil-resistant isolate D201) and 24 h before they were immersed for 1 min in (i) water alone (Control); potassium sorbate (KS) at 20 g/liter; (iii) sodium carbonate (SC); (iv) sodium bicarbonate (SBC); (v) potassium phosphate (KP), (vi) calcium phosphite (CaP), or (vii) pyrimethanil (PYR) at 1 /liter. The temperature of the solutions was **A,** 25°C or **B,** 50°C. All were stored 1 week at 20°C before the incidence of green mold was determined.

Table 2. Green mold incidence among 'Barnfield' orange after treatment with calcium phosphite or potassium phosphite at different temperatures

	Green mold incidence (%)[z]					
	Calcium phosphite (g/liter)		Potassium phosphite (g/liter)			
Treatment temperature (°C)	5	15	5	15	$LSD_{0.05}$	*P*
21	22.5	5.0	18.3	10.0	4.98	0.017
41	27.5	5.8	10.8	5.8	4.39	0.003
46	32.5	10.0	16.7	11.7	6.28	0.015
52	10.0	1.7	6.7	1.7	2.70	0.05
57	13.3	4.2	6.7	0.8	nsd	0.06
$LSD_{0.05}$	4.8	nsd	nsd	nsd	…	…
P	0.002	0.279	0.116	0.076	…	…

[z] Each fruit was inoculated twice with *Penicillium digitatum* (isolate D201) 24 h before they were immersed for 15 s in water at room temperature (untreated control) or phosphite salt solutions at 5 or 15 g/liter and 21, 41, 46, 52, or 57°C (±0.5°C). Each treatment was applied to three replicates of 20 fruit each. Fruit were examined after storage for 2 weeks at 20°C. Fisher's protected least significant difference (LSD) is shown for values within columns and rows; the untreated control is excluded; nsd = no significant difference. Incidence among untreated control fruit was 98.3%.

0.000) improved control of this disease (Table 3). Similarly, on Clementina de Nule mandarin orange, decreasing the storage temperature from 20 to 15 to 10°C ($P \leq 0.000$) or increasing the temperature of the KP solution from 25 to 50°C significantly ($P \leq 0.000$) improved the control of green mold. Water alone at 50°C controlled green mold substantially on both Fukimoto fruit and Clementina de Nule fruit, particularly when the storage temperature after treatment was 10°C.

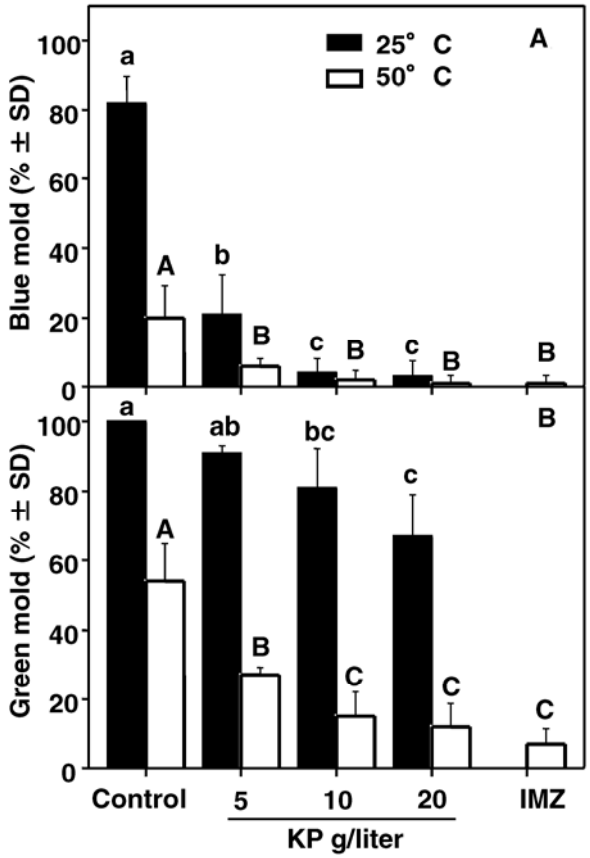

Fig. 3. A, Blue mold and **B,** green mold incidence among 'Eureka' lemon fruit inoculated with *Penicillium digitatum* (isolate PD90) or *P. italicum* 24 h before they were immersed for 1 min in water (control) or potassium phosphite (KP) at 5, 10, or 20 g/liter and 25 or 50°C, or immersion for 15 s in imazalil (IMZ) at 0.2 g/liter and 50°C followed by storage for 2 weeks at 20°C. Each treatment was applied to five replicates of 20 fruit each. Columns with the same letters (uppercase letters for 50°C and lowercase letters for 25°C) are not significantly different according to Tukey's honestly significant difference ($P = 0.05$).

Increasing the temperature of the KP solution from 25 to 50°C significantly ($P \leq 0.000$) improved control of blue mold on Fukimoto navel orange (Table 3). Decreasing the storage temperature from 20 or 15 to 10°C significantly ($P = 0.012$) improved the control of blue mold. Similarly, on Clementina de Nule mandarin orange, increasing the temperature of the KP solution from 25 to 50°C significantly ($P = 0.007$) improved the control of blue mold. However, in contrast to the results with green mold, decreasing the storage temperature did not significantly ($P = 0.119$) improve the control of blue mold on Clementina de Nule mandarin orange. Water alone at 50°C controlled blue mold substantially on Fukimoto fruit but was not very effective on Clementina de Nule fruit.

Evaluation of KP combined with conventional postharvest fungicides to control green mold. Control of green mold on Eureka lemon by all the fungicides tested was significantly improved when KP at 4 g/liter was added to the tank solutions (Fig. 4). Decay incidence was high after KP treatment. The IMZ-resistant isolate of *P. digitatum* used in this test (D201) was controlled by IMZ at 0.5 g/liter. In a second experiment, this isolate was inadequately controlled on Eureka lemon by IMZ at 0.2 g/liter. SBC treatment alone reduced green mold incidence moderately but significantly at the two temperatures assayed and KP effectiveness alone was again poor. However, the combination of KP and IMZ or KP and SBC improved in effectiveness to control green mold in lemon when they were used at 40°C (Fig. 5). The combination of KP with SBC was particularly effective to control green mold in lemon, even when used at 20°C.

In the large-scale test that employed commercial pack line equipment, results with the two isolates of *P. digitatum* were not significantly ($P = 0.120$) different; therefore, they were combined (Table 4). Control of green mold on Eureka lemon, WMurcott mandarin orange, and Atwood navel orange by KP alone at 46°C was insufficient for commercial purposes although significantly different of the control, whereas CaP alone or IMZ alone much more effective (Table 4).

Influence of the time of inoculation on KP and IMZ effectiveness. The combination of KP with IMZ was more effective than either IMZ or KP alone to prevent Eureka lemon or Valencia orange from developing green mold when inoculation with *P. digitatum* was made 5 days after treatment (Table 5). Protection from infection by treatments with either KP or IMZ alone was inadequate on Eureka lemon and Valencia orange. In a second experiment, both post-infection control and protection from blue mold were insufficient for fruit treated by KP. Blue mold incidence among Eureka lemon fruit inoculated 72 h or longer after KP treatment was similar to the control (Fig. 6A). Both eradication and protection from blue mold or green mold infections were excellent by IMZ alone or IMZ combined with KP (Fig. 6A and B). IMZ

Table 3. Green mold and blue mold incidence among 'Fukimoto' navel orange and 'Clementina de Nule' mandarin orange after treatment with potassium phosphite at 25 or 50°C followed by storage for 3 weeks at 10, 15, or 20°C

		Storage temperature (°C)[z]					
		Green mold (%)			Blue mold (%)		
Variety, treatment	Solution (°C)	10	15	20	10	15	20
Fukimoto							
Water	25	46.7 a	70.0 a	95.0 a	88.3 a	90.0 a	75.0 a
Potassium phosphite	25	1.7 b	16.7 b	61.7 b	25.0 b	63.3 b	70.0 a
Water	50	0.0 b	16.7b	24.4 c	26.7 b	25.0 c	30.2 b
Potassium phosphite	50	0.0 b	3.3 c	15.0 c	0.0 c	1.7 d	6.7 c
Clementina de Nule							
Water	25	41.7 a	65.0 a	80.0 a	86.7 a	95.0 a	85.0 a
Potassium phosphite	25	10.0 b	21.7 b	50.2 bc	28.3 c	66.7 b	51.7 b
Water	50	6.7 b	31.7 b	64.8 b	75.0 a	83.3 a	53.7 b
Potassium phosphite	50	0.0 c	8.3 c	42.2 c	43.3 b	26.7 c	55.5 b

[z] Fruit were inoculated with *Penicillium digitatum* (isolate PD90) or *P. italicum* (isolate PI105). After 24 h at 20°C, they were immersed for 1 min in water or potassium phosphite (20 g/liter) at 25 or 50°C, then stored 3 weeks at 10, 15, or 20°C. Each value is the mean of three replicates of 20 fruit each. Values within columns for each cultivar followed by the same letters are not significantly different according an analysis of variance applied to arcsin transformed values followed by Tukey's honestly significant difference ($P = 0.05$). Actual values are shown.

alone controlled green mold infections from inoculations made 48 or 24 h before treatments and significantly improved when KP was added (Fig. 6B). The combination of KP with IMZ was more effective than either IMZ or KP alone to protect Eureka lemon inoculated 120 h after treatment with *P. digitatum* from developing green mold. KP at 5 g/liter alone at 25°C did not control green mold in this test.

Fungicide residues. Initial residues of phosphite in Atwood orange were not influenced by the temperature of the KP treatment or presence of IMZ or TBZ (Table 6). Phosphite content in untreated control fruit was low but measureable and declined during cold storage. After 21 days, residues of phosphite were unchanged, except those after the KP + IMZ treatment, which were significantly lower (Table 6). IMZ and TBZ residues when measured initially generally increased when the treatment temperature increased from 25 to 50°C, although the increase was modest and often not significant. The addition of KP slightly but significantly increased IMZ residues when IMZ was applied at 50°C and approximately doubled TBZ residues when TBZ was applied at 25°C. After 21 days of storage at 5°C, the increase in IMZ residues associated with the heated KP + IMZ treatment remained significant whereas the increase in TBZ residues did not.

Influence of postharvest treatments with KP on fruit surface color during ethylene degreening. The change in the rind color, expressed as hue angle (Fig. 7), was not influenced by KP treatment at either 25 or 50°C in ethylene. The change in hue angle in air was slightly but significantly delayed in WMurcott mandarin orange treated with water at 50°C and in Eureka lemon treated with water or KP at 50°C.

Discussion

Fenn and Coffee (18) reported that phosphate influenced phosphite toxicity, and our results confirm that increased phosphate content decreases phosphite inhibition of *P. digitatum*. Using

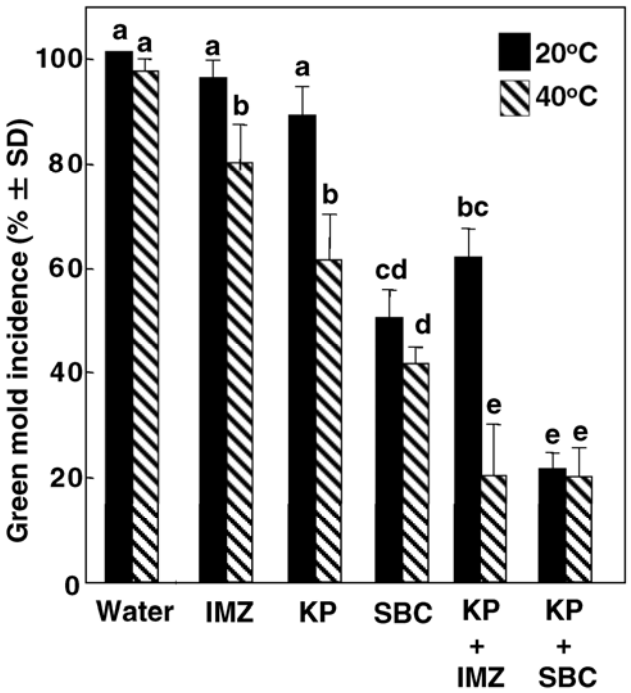

Fig. 5. Green mold incidence among 'Eureka' lemon fruit inoculated with *Penicillium digitatum* (isolate D201) 24 h before they were immersed for 2 min at 20 or 40°C (±0.5°C) in (i) imazalil (IMZ) at 0.2 g/liter, (ii) KP (formulation B) at 10 g/liter, (iii) sodium bicarbonate (SBC) at 20 g/liter, (iv) a mixture of KP + IMZ, or (v) a mixture of KP + SBC. Fruit were not rinsed after treatment and stored for 21 days at 20°C; then, the number of infected fruit was counted. Each treatment was applied to four replicates of 27 fruit each. Columns with the same letters are not significantly different according to Tukey's honestly significant difference (P = 0.05).

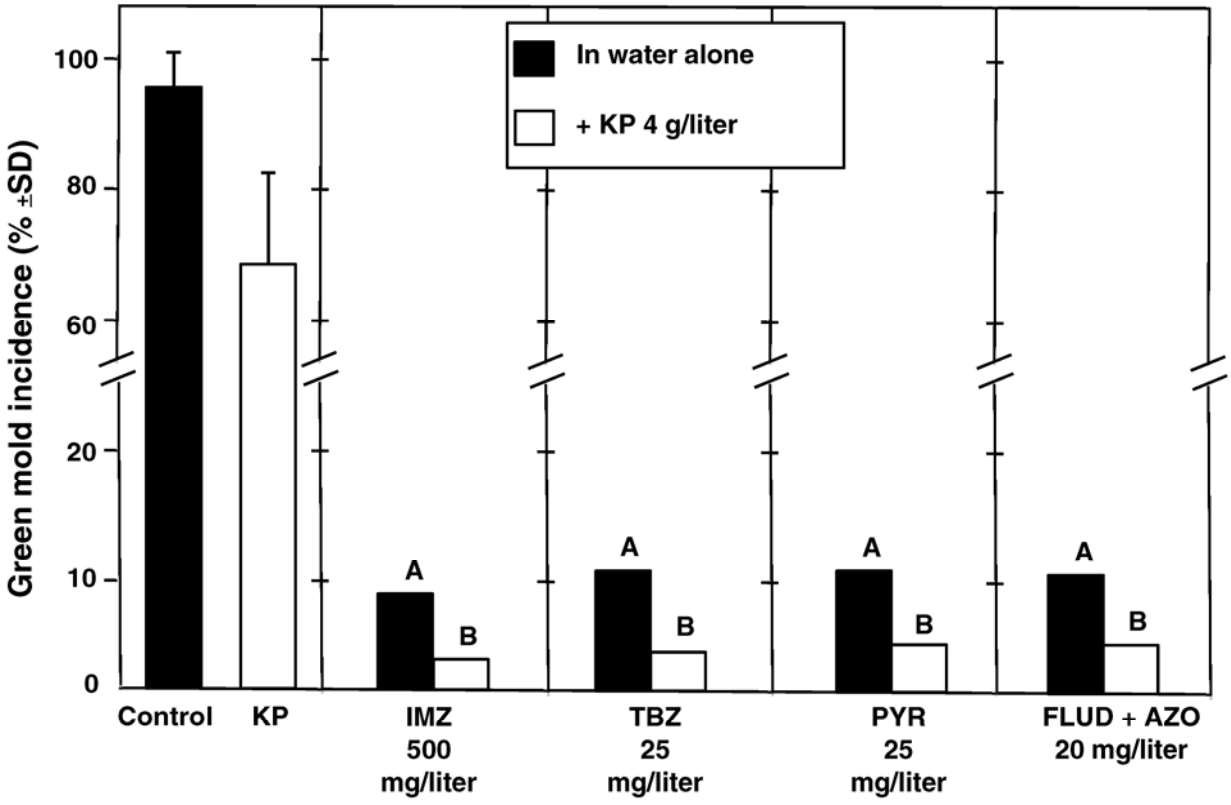

Fig. 4. Green mold incidence among 'Eureka' lemon fruit inoculated with *Penicillium digitatum* (isolate D201) 24 h before they were immersed for 30 s in 25°C solutions of (i) imazalil (IMZ) at 0.5 g/liter, (ii) thiabendazole (TBZ) at 0.025 g/liter, (iii) pyrimethanil (PYR) at 0.025 g/liter, or (iv) a mixture of fludioxonil (FLUD) + azoxystrobin (AZO) that contained each at 0.020 g/liter. They were applied alone or dissolved in potassium phosphite (KP) at 4 g/liter. Fruit were not rinsed after treatment and were stored for 17 days at 10°C and 95% relative humidity; then, the number of decayed fruit was counted. Each treatment was applied to four replicates of 27 lemon fruit each. Columns with the same letters are not significantly different according to Tukey's honestly significant difference (P = 0.05).

Pratt's medium (53) at pH 6 with phosphate in excess (10 mM), EC_{50} concentrations of H_3PO_3 that inhibited conidial germination were 469 mg/liter, which was similar to the phosphite concentration of 552 mg/liter reported by Fenn and Coffey (18) that reduced radial growth rate of *Alternaria alternata* by 59% and of *Rhizoctonia solani* by 38% on cornmeal agar at pH 6.2, a medium rich in phosphate. However, in a synthetic medium with a low phosphate concentration (0.084 mM), EC_{50} concentrations of H_3PO_3 at pH 6.2 that inhibited radial growth of *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Verticillium dahlia* were only 69 mg/liter, and the toxicity of phosphite to these fungi declined markedly as the phosphate content increased.

The pH of phosphite solutions influenced inhibition of conidial germination of *P. digitatum.* Phosphite was approximately twice as inhibitory to conidial germination at pH 3 as it was at pH 7. This is the first report that quantifies the influence of pH on phosphite toxicity in vitro. In preliminary tests using inoculated fruit, the effectiveness of KP solutions (2% wt/vol) adjusted to intervals of one pH unit from pH 3 to 8 to control green mold was similar and was not significantly influenced by solution pH (*data not shown*). It is likely that the pH of the albedo tissue in the rind wounds was not altered much by the pH of the phosphite solutions. In prior work, the pH of this tissue in lemon wounds 1 mm wide and 2 mm deep in size was measured with a 0.5-mm-diameter pH electrode at a depth of 1.5 mm after various treatments (46). The pH of albedo tissue was well buffered and resisted alteration; immersion of the fruit in high-pH bicarbonate solutions raised the pH of the rind tissue within the wounds approximately one unit and it returned to its natural pH (5.1 to 5.6) within 1 day (46).

Reports describing the postharvest use of phosphites to control diseases caused by true fungi are few. Gutter (25) reported that the phosphite-generating compound fosetyl-Al had modest activity on the control of postharvest green mold on orange at concentrations that effectively controlled brown rot. Furthermore, fosetyl-Al in vitro moderately inhibited the growth and sporulation of *P. digitatum* at 5, 10, or 20 g/liter but it was less effective when TBZ-resistant isolates were examined. More recently, Bassay Blum et al. (7) reported that immersion of apple fruit in KP and CaP solutions controlled blue mold caused by *P. expansum*. Amiri and Bompeix (5) reported that KP inhibited conidial germination and mycelia growth of *P. expansum* in vitro and it was more effective when applied in heated solutions. Treatment with KP at 20 g/liter and 50°C for 3 min completely suppressed blue mold in Elstar apple fruit after 7 days of storage at 20°C. Reuveni et al. (40) reported that postharvest treatment of apple fruit with KP at 0.5 g/liter controlled moldy-core decay of apple fruit, even when applied 48 h after inoculation with *A. alternata.*

KP and CaP at 10 or 20 g/liter were similar in effectiveness. If CaP was used under commercial conditions, a post-treatment rinse would be needed to eliminate the visible CaP residues. Unfortunately, if CaP was used with a fungicide, rinsing to remove visible CaP residue would also remove some portion of the fungicide residue. For CaP to be used with a fungicide, fruit would be treated with CaP first, then rinsed and partially dried before the fungicide would be applied. The effectiveness of this approach needs evaluation before it could be recommended. In the present work, phosphite treatments remained effective even with post-treatment water rinsing of the treated fruit (Fig. 2). In prior work with SBC, inoculated fruit were treated and the effectiveness of the treatment was little reduced by subsequent rinsing, unless it was very thorough (49).

The influence of post-treatment storage temperature on KP effectiveness for the control of green mold was large; storage at 20°C particularly reduced KP effectiveness compared with storage at 10 or 15°C. KP adequately controlled blue mold on Fukimoto navel orange but not Clementina de Nule mandarin orange, even when the KP solution was heated to 50°C and the storage temperature was low (10°C). In general, using the coldest feasible storage temperature is important to minimize green mold, whereas reducing storage temperature has less effect on blue mold (38). The effect of post-treatment storage temperature on the effectiveness of the KP treatments was very large and could, in part, explain the irregular effectiveness of KP reported to us by some industry personnel.

KP was compatible with all of the fungicides currently registered for postharvest use in the United States, and improved their performance. In work not reported here, we found that the combination of low-pH formulations of KP with IMZ was less effective than IMZ alone. When this occurred, IMZ residues were markedly lower when it was used with KP, which was likely a consequence of the effect of low pH reducing both residues (17) and potency (46) of IMZ. The improvement in IMZ performance we observed when adding phosphite was possibly related to an increase in IMZ residues in the fruit, because the addition of KP increased IMZ residues when treatment solutions were 50°C. The addition of KP increased TBZ residues in orange when treatments were applied at 25°C, and this difference could explain the improved effectiveness of this combination in comparison with the fungicide applied alone. The magnitude of this difference in residues was not evident after 3 weeks of storage at 5°C. The residues of both fungicides were increased when they were applied at 50°C, which has been reported previously for all the fungicides used in our work (44). SBC, which also markedly improved IMZ performance, was shown by Dore et al. (13) to cause a

Table 5. Green mold incidence among 'Eureka' lemon or 'Valencia' orange inoculated 5 days after immersion for 30 s at 25°C in imazalil (IMZ) at 0.5 g/liter, potassium phosphite at 50 g/liter, or a mixture of both, followed by storage for 1 week at 20°C

	Green mold incidence (%)[z]	
Treatment	**Lemon**	**Orange**
Control	100.0 a	78.8 a
IMZ	100.0 a	45.0 b
Potassium phosphite	99.1 a	37.5 b
IMZ + potassium phosphite	2.8 b	18.8 c

[z] After 5 days at 10°C, the lemon fruit were all inoculated with IMZ-resistant *Penicillium digitatum* (isolate D201). Values within columns followed by the same letters are not significantly different according an analysis of variance applied to arcsin-transformed values followed by Tukey's honestly significant difference (P = 0.05). Actual values are shown.

Table 4. Green mold incidence among 'Atwood' orange, 'Eureka' lemon, or 'WMurcott' mandarin orange after treatments with calcium phosphite (formulation C) at 15 g/liter, potassium phosphite (formulation B) at 15 g/liter, or imazalil (IMZ) after storage for 3 weeks at 20°C

	Green mold incidence (%) on fruit variety[z]			
Treatment	**Orange**	**Lemon**	**Mandarin orange**	**All**
Control	92.9 a	97.3 a	85.1 a	91.7 a
Potassium phosphite	20.2 b	47.0 b	40.7 b	36.7 b
Calcium phosphite	4.6 c	10.2 c	20.8 c	12.1 c
IMZ	0.0 d	0.5 d	4.1 d	1.7 d

[z] Fruit were inoculated with *Penicillium digitatum* (isolate PD90 or D201) 24 h before treatments and were treated by passage down a packing line, where they were drenched for 15 s. The IMZ concentration was 50 or 500 mg/liter for isolates PD90 or D201, respectively. The solution temperature was 46°C. Values within columns followed by the same letters are not significantly different according an analysis of variance applied to arcsin-transformed values followed by Tukey's honestly significant difference (P = 0.05). Actual values are shown.

displacement of intracuticular waxes that affected IMZ sorption, thereby allowing deeper diffusion into the rind, particularly into the albedo in rind wounds. It is conceivable that KP also alters the rind tissue and the distribution of the residues of these fungicides.

Residues of phosphite in orange peel were stable and not influenced by treatment temperature or the presence of IMZ or TBZ. However, after storage for 3 weeks, phosphite levels declined significantly in orange after IMZ + KP treatment. This may have occurred for several reasons. The phosphite residue may have reacted with IMZ so that less phosphite was present, although this seems unlikely because IMZ residues were not lower among orange fruit in which the phosphite content had declined. It is possible that the phosphite residue had diffused deeper into the rind and that this was caused by some aspect of the IMZ treatment, such as the surfactants in the IMZ formulation. The decline in phosphite residues associated with the KP + IMZ treatment may have a practical consequence. The phosphite-induced resistance of fruit to *Phytophthora* spp. (3,43,52) may be less persistent than when KP is used alone. Further investigation of the interactions, distribution, and persistence of these residues is needed.

Although not evaluated in this work, phosphites have long been known to control fruit rot caused by Phytophthora brown rot (10,19,20), a disease that caused significant losses in wet years in California. Adaskaveg (2) reported a brief immersion of orange fruit in KP at 0.27 g/liter had excellent preinfection and postinfection control of *Phytophthora citrophthora*, and it was the only fungicide of many tested with postinfection activity in that study. Thus, the phosphite treatments that controlled green mold and blue mold would be expected to control brown rot, because the phosphite rates that controlled them were far higher than those that

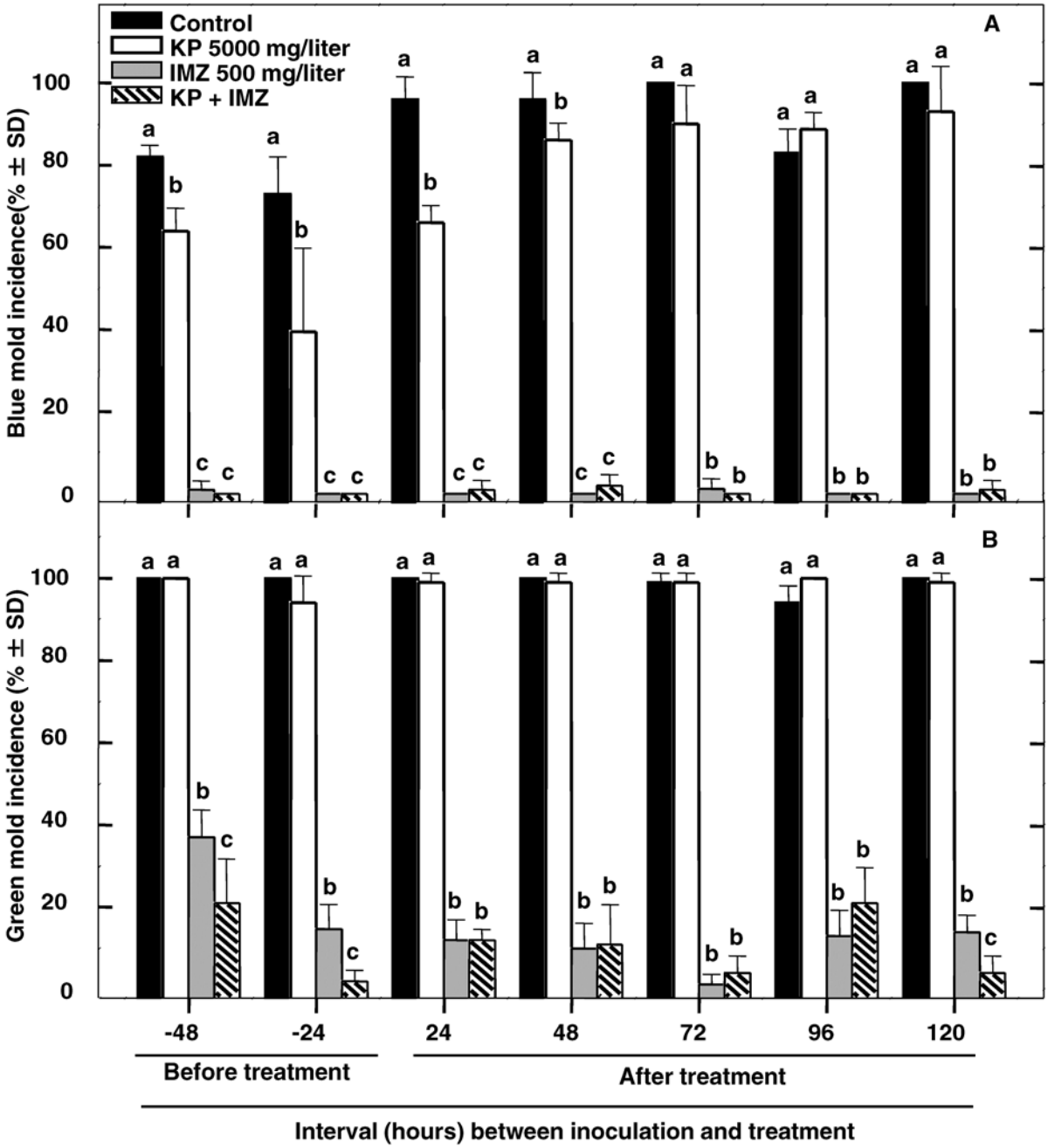

Fig. 6. Protectant and eradicant activity of potassium phosphite (KP) and imazalil (IMZ) treatments to control **A,** blue mold and **B,** green mold on 'Eureka' lemon. The interval between inoculation with *Penicillium digitatum* (isolate PD90) or *P. italicum* and treatment was varied; from inoculation 48 h before treatment (–48) to inoculation 120 h after treatment (120). Lemon fruit were immersed for 30 s at 25°C in (i) water alone (control), (ii) IMZ at 0.5 g/liter, (iii) KP at 5 g/liter, or (iv) a mixture of IMZ + KP. Fruit were not rinsed after treatment and were stored for 14 days at 20°C; then, the number of infected fruit was counted. Each treatment was applied to five replicates of 20 lemon fruit each. Columns with the same letters are not significantly different within in each time according to Tukey's honestly significant difference ($P = 0.05$).

Table 6. Residues of phosphite, imazalil (IMZ), or thiabendazole (TBZ) in the rind of 'Atwood' orange after 30 s of immersion in IMZ (0.4 g/liter) or thiabendazole TBZ (0.4 g/liter) alone or in combination with potassium phosphite (KP; 20 g/liter) at 25 or 50°C[w]

		Residues (mg/kg fresh weight ± SD)[x]					
Temp (°C)[y]	Storage (days)[z]	Treatment	Phosphite	Treatment	IMZ	Treatment	TBZ
25	2	Control	28 ± 8 a	Control	0.09 ± 0.04 a	Control	0.08 ± 0.06 a
25	21	…	6 ± 4 b	…	0.09 ± 0.03 a	…	0.07 ± 0.02 a
50	2	…	36 ± 12 a	…	0.10 ± 0.06 a	…	0.08 ± 0.04 a
50	21	…	9 ± 4 b	…	0.10 ± 0.05 a	…	0.04 ± 0.03 a
25	2	KP alone	318 ± 86 de	IMZ alone	0.47 ± 0.12 b	TBZ alone	1.35 ± 0.21 b
25	21	…	298 ± 93 de	…	0.51 ± 0.20 b	…	1.88 ± 0.83 bc
50	2	…	319 ± 67 de	…	0.69 ± 0.10 c	…	2.55 ± 0.56 c
50	21	…	351 ± 57 de	…	0.60 ± 0.29 bc	…	2.38 ± 0.65 c
25	2	KP+IMZ	291 ± 75 de	KP+IMZ	0.49 ± 0.17 b	…	nd
25	21	…	173 ± 22 cd	…	0.58 ± 0.13 bc	…	nd
50	2	…	357 ± 98 de	…	0.91 ± 0.15 d	…	nd
50	21	…	117 ± 29 c	…	1.26 ± 0.19 d	…	nd
25	2	KP+TBZ	378 ± 21 de	…	nd	KP+TBZ	2.79 ± 0.48 c
25	21	…	357 ± 95 e	…	nd	…	1.88 ± 0.38 bc
50	2	…	259 ± 56 de	…	nd	…	2.18 ± 0.10 bc
50	21	…	259 ± 47 de	…	nd	…	3.07 ± 0.64 c

[w] Residues were determined within 2 days and again after storage 5°C for 21 days.
[x] Values were $\log_{10}$ transformed before analysis; those within columns followed by the same letters are not significantly different according to Fisher's Protected least significant difference ($P = 0.05$). SD = standard deviation. Actual values are shown; nd = not determined.
[y] Treatment temperature of the solution in which the fruit were immersed.
[z] Storage period at 5°C.

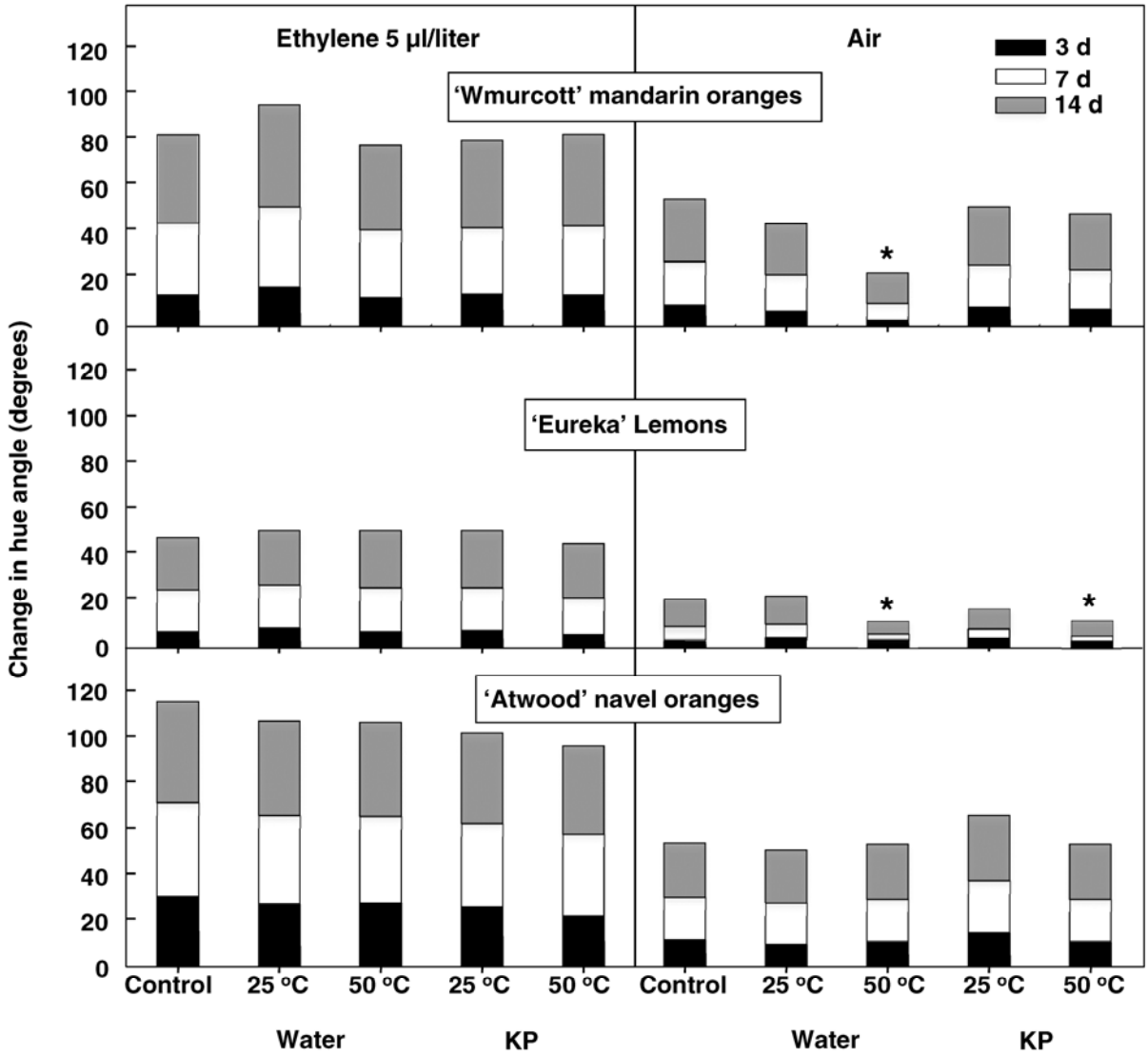

Fig. 7. Change in the surface color of the rind of 'Atwood' navel orange, 'WMurcott' mandarin orange, and 'Eureka' lemon fruit after immersion for 30 s in water or potassium phosphite (KP) at 25 or 50°C followed by continuous storage at 20°C in air or ethylene gas at 5 µl/liter. Asterisks indicate a significant difference from the control according to Tukey's honestly significant difference ($P = 0.05$).

control brown rot (2). Furthermore, brown rot is readily controlled by immersion in heated water (29) using thermal regimes similar to those we employed to improve the phosphite effectiveness.

In conclusion, the effectiveness of phosphite salts for controlling citrus green and blue molds in laboratory and semicommercial tests was demonstrated on mandarin orange, lemon, and orange. Control of decay on Clementina de Nule mandarin orange was more difficult than other citrus fruit, in agreement with Palou et al. (37), who reported that treatments of hot water, SBC, and sodium carbonate were less effective on this cultivar than on other citrus fruit. Phosphites improved fungicide performance when added to other labeled fungicides, particularly in heated solutions. As a general rule, as temperature of the treatment increased the decay incidence decreased. In particular, a heated phosphite + IMZ treatment effectively controlled IMZ-resistant isolates of *Penicillium digitatum*, which are common within packinghouses (28). The effectiveness of phosphite treatment was greater when post-treatment fruit storage temperatures were lower. Although generally more effective for the control of green mold than other fungicide alternatives used in packinghouses such as carbonates, bicarbonates, or sorbates, the most compelling advantage of phosphites is their ability to control Phytophthora brown rot. Phosphites are more costly than these alternatives but were compatible with SBC, and a phosphite-SBC mixture could be used to reduce costs. Moreover, the combination of KP with SBC was an effective treatment to control green mold in lemon, even when used at 20°C. Phosphite residues were persistent, and phosphite treatments did not influence rind color development in air or ethylene atmospheres and caused no visible rind injuries in any test.

Acknowledgments

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