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Evaluation of Food Additives as Alternative or Complementary Chemicals to Conventional Fungicides for the Control of Major Postharvest Diseases of Stone Fruit

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ABSTRACT

To evaluate potential alternatives to conventional fungicides to control decay, more than 20 food additives and generally regarded as safe compounds were tested at three concentrations in in vivo primary screenings with several cultivars of California peaches, nectarines, and plums that had been artificially inoculated with seven major postharvest pathogens: *Monilinia fructicola, Botrytis cinerea, Geotrichum candidum, Alternaria alternata, Penicillium expansum, Mucor piriformis,* and *Rhizopus stolonifer.* Overall, the best compounds were 200 mM potassium sorbate (PS), 200 mM sodium benzoate (SB), 200 mM sodium sorbate, 100 mM 2-deoxy-D-glucose, 400 mM sodium carbonate, and 250 mM potassium carbonate. Sodium and ammonium molybdates, acid lactic, and hydrogen peroxide were somewhat effective but were phytotoxic to fruit skin tissues. However, the best compounds lacked effectiveness and persistence when tested against brown rot in small-scale trials of 60-s dips in aqueous solutions at ambient temperatures; PS and SB reduced brown rot incidence by less than 40%. Rinsing treated fruit with tap water reduced the efficacy of the compounds by up to 30%. In contrast, heating the solutions to 55 or 60°C significantly increased treatment efficacy. Brown rot incidence and severity were reduced by 35 and 25%, respectively, on PS-treated peaches after 7 days of incubation at 20°C. However, treatment efficacy was not superior to that with water alone at these temperatures. In semicommercial trials, mixtures of fludioxonil with PS, SB, or 2-deoxy-D-glucose applied as fruit coatings on a packing line were not synergistic in their effect on brown rot, gray mold, and sour rot.

Stone fruits such as peach, nectarine, and plum are major crops in California, Spain, and other fruit-producing areas. Fruit losses caused by postharvest diseases are among the main concerns of the stone fruit growers and marketers. Brown rot, which is caused by several species of the fungal genus *Monilinia* (*M. fructicola* (G. Wint.) Honey, *M. laxa* (Aderh. & Ruhl.) Honey, and *M. fructigena* (Aderh. & Ruhl.) Honey), is the most important postharvest disease of stone fruit worldwide. Depending on weather conditions and postharvest handling, other high-incidence postharvest diseases of stone fruit are gray mold (caused by *Botrytis cinerea* Pers.:Fr.), sour rot (caused by *Geotrichum candidum* Link), rhizopus rot (caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill.), mucor rot (caused by *Mucor piriformis* E. Fischer), alternaria rot (caused by *Alternaria alternata* (Fr.: Fr.) Keissler), and blue mold (caused by *Penicillium expansum* Link) *(16, 17).* Effective postharvest decay control depends on an integrated management approach based on appropriate preharvest fungicide treatments, adequate harvest and handling practices, effective sanitation of fruit and facilities in the packinghouses, appropriate postharvest antifungal treatments, and maintenance of the proper environments during fruit storage and transportation.

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Postharvest antifungal treatments usually are needed to effectively control decay on stone fruits for distant markets. These treatments typically have consisted of the application of synthetic chemical fungicides such as iprodione or more recently fludioxonil and fenhexamid *(3, 9).* However, concerns about human health risks and environmental problems associated with fungicide residues have increased the need to find and develop alternatives to fungicides *(10).* Compliance with pesticide regulations can be costly, and the presence of fungicide residues on the fruit can prevent their export to some foreign markets. Postharvest application of conventional fungicides to stone fruits is prohibited in the European Union (EU) and other countries, and the widespread use of these chemicals has led repeatedly to the proliferation of resistant strains of the pathogens *(13).* Alternatives to conventional fungicides should be natural or synthetic compounds whose toxicity to humans and wildlife has been extensively evaluated and proven to be very low, even at relatively high dosages. Food additives, preservatives, or generally recognized as safe (GRAS) compounds that are allowed with very few restrictions for many industrial and agricultural applications by regulations worldwide meet these criteria. Legislation and lists published by the U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition or the European Food Safety Authority can be found online *(7, 24).* The requirements for

effective postharvest treatments based on the use of low-toxicity chemicals will encourage development of new integrated pest management programs for stone fruits so that global pesticide usage can be effectively reduced. Many of these substances have been proposed as exempt from residue tolerances on agricultural commodities by the U.S. Environmental Protection Agency (EPA), and some of them are included in the U.S. National List of substances allowed as ingredients in or on products labeled as organic.

During the last few years, postharvest research groups worldwide have evaluated aqueous solutions of some of these common food additives, GRAS compounds, and low-toxicity compounds as alternative treatments for the control of postharvest diseases of fruits and vegetables *(16, 19, 20).* The most practical combination of solution temperature, chemical concentration, and treatment duration for optimal decay control must be determined for each chemical and each host-pathogen system *(8, 19).* Although heat and the integration of certain physical, chemical, or biological treatments have been evaluated for postharvest decay control of peaches and nectarines *(12, 14, 15, 25),* very little research has been conducted to assess the antimicrobial activity of food additives and GRAS compounds against postharvest pathogens of stone fruit *(11).*

The objective of the present work was to evaluate the effectiveness of a wide range of low-toxicity chemicals, mostly common food additives, for the control of the main postharvest pathogens of peach, nectarine, and plum. Promising chemicals were identified by testing their effectiveness in in vivo primary screenings. Selected compounds then were tested as heated aqueous solutions in small-scale trials. Integrated treatments combining selected alternative chemicals with reduced doses of the conventional synthetic fungicide fludioxonil also were evaluated in semicommercial trials.

MATERIALS AND METHODS

Fruit. Peach (*Prunus persica* (L.) Batsch.) cultivars Flavorcrest, O'Henry, Rich Lady, Ryan Sun, and Last Chance; nectarine (*P. persica* (L.) Batsch. var. *nucipersica* (Suckow) Schneid) cultivars Summer Fire and Spring Bright; and plum (*Prunus salicina* Lindl.) cultivars Fortune and Royal Diamond commercially grown in orchards in the San Joaquin Valley (California) were hand harvested at commercial maturity, selected, randomized, and used in the experiments before any postharvest treatments were applied. Fruit were used immediately or were packed in cardboard boxes and stored at 1°C for up to 3 days before use. In general, fruits were surface disinfected, rinsed with fresh water, and left to air dry at room temperature before fungal inoculation. Fruit surfaces were disinfected with diluted bleach (100 μg/ml free sodium hypochlorite) applied over rolling brushes on an experimental packing line. The experiments were conducted during three consecutive seasons.

Fungal inoculum. Isolates 79-1 of *M. fructicola,* 93-58 of *B. cinerea,* LP-2 of *G. candidum,* 12-27 of *A. alternata,* PES-1 of *P. expansum,* LP-7 of *M. piriformis,* and 72-2 of *R. stolonifer* were obtained from decayed peaches or nectarines from local packinghouses in the San Joaquin Valley. These fungal strains had been isolated, purified, identified, and maintained in a culture collection of postharvest pathogens kept at the F. Gordon Mitchell Postharvest Center (University of California Kearney Agricultural Center, Parlier) and were selected for their virulence on the most commercially important California stone fruit cultivars. Before the experiments, the *M. fructicola, B. cinerea, A. alternata,* and *M. piriformis* isolates were incubated on potato dextrose agar (PDA; Difco, Becton Dickinson, Sparks, MD) in petri dishes at 20°C for 14 to 21 days, and the *G. candidum, P. expansum,* and *R. stolonifer* isolates had been incubated on PDA at 25°C for 4 to 10 days. For inocula preparation, spores were rubbed from the agar surface with a sterile glass rod after adding 5 ml of 0.05% (wt/vol) Triton X-100 in sterile water. The high-density spore suspension was passed through two layers of cheesecloth, the number of spores was counted with a hemacytometer, and the suspension was then diluted with sterile water to the desired inoculum density.

Fruit inoculation. Unless otherwise stated, fruit inoculation procedure was conducted as described by Palou et al. *(18).* Peaches, nectarines, and plums were wounded once on the equator of the fruit with a probe tip (1 mm wide by 2 mm long), and a micropipette was used to inoculate the fruits with 20 μl of a suspension of *M. fructicola, B. cinerea, A. alternata, P. expansum, M. piriformis,* or *R. stolonifer* containing 5×10^4 spores per ml or 20 μl of a suspension of *G. candidum* containing 1×10^8 arthrospores per ml. Inoculated fruits were held at room temperature for 18 to 24 h before application of the antifungal treatments for spore germination to occur within the wound sites to simulate infections that occur during harvest.

Chemicals. Twenty-four chemicals, mostly mineral salts and organic acid salts classified as food additives or as GRAS according to U.S. and EU regulations *(7, 24),* were tested in in vivo primary screenings at different concentrations of active ingredients (Table 1). All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Some were selected to be evaluated as aqueous dip treatments in small-scale trials or as complementary treatments in semicommercial trials. In these semicommercial tests, the conventional fungicide fludioxonil (Scholar 50 WP, Syngenta Crop Protection, Inc., Greensboro, NC) also was used at different doses. Fludioxonil is an agrochemical classified by the EPA as a reduced-risk fungicide *(9).* It has been fully registered for postharvest use on stone fruit in California and elsewhere in the United States since 2003, and its tolerance for residues in or on apricots, nectarines, peaches, and plums is 5.0 mg/kg *(23).*

In vivo primary screenings. The effectiveness of 24 low-toxicity chemicals, usually at three different concentrations, against seven postharvest pathogens (*M. fructicola, B. cinerea, G. candidum, A. alternata, P. expansum, M. piriformis,* and *R. stolonifer*) was tested in three species of stone fruit. Three peach cultivars (Flavorcrest, O'Henry, and Last Chance), one nectarine cultivar (Summer Fire), and two plum cultivars (Fortune and Royal Diamond) were used in this set of experiments. Sterile equimolar aqueous solutions at the desired concentrations were prepared from a 1 M stock solution of each chemical by diluting with sterile water. All solutions were filter sterilized with a syringe by passing the solution through a 0.45-μm-pore-size membrane filter.

Forty microliters of the sterile chemical solution at the desired concentration was applied with a micropipette in the same pathogen inoculation site of peaches, nectarines, and plums that had been wound inoculated with the pathogens, and fruits were incubated at room temperature as described above. Control fruits were treated with 40 μl of sterile distilled water. Treated fruits were incubated at 20 ± 1°C and 90 + 5% relative humidity, and disease incidence (number of infected fruits) and severity (lesion

diameter measured with an electronic caliper) were determined after 3 and 5 days of incubation. For each combination of fruit species, chemical, concentration, and fungal pathogen, three replicates of four fruits each were used. For each fruit species, each test was repeated at least once, sometimes with the same cultivar and other times with another cultivar.

A qualitative 4-point scale was established to assess the effectiveness of the treatments or their decay control ability in all tested fruit species: 0, no control; +, slight control (less than 50% reduction of disease incidence and/or severity with respect to control fruits); ++, moderate control (greater than 50% but less than 80% decay reduction); +++, good control (greater than 80% decay reduction). Fruit skin damage caused by the application of the droplet of each chemical solution also was visually assessed at this time. Each treatment was classified into one of three categories in the following qualitative scale: 0, no skin injury; 1, slight to moderate skin injury; 2, severe skin injury. Injury symptoms were variable, but in general phytotoxic droplets caused staining, inking, or in the worst cases, degradation of peel tissues. According to their overall performance in all three fruit species in terms of decay control ability and induction of external phytotoxicities, screened chemicals and concentrations were discarded or selected for further testing in small-scale trials. To make this decision, higher relative importance was generally given to the activity against the postharvest pathogens typically causing the greatest economical losses in stone fruits: *M. fructicola, B. cinerea,* and *G. candidum.*

Small-scale trials. Compounds selected according to the results of the in vivo primary screenings were assayed at selected concentrations and applied as aqueous dip treatments in small-scale trials. Peaches and nectarines were wound inoculated with *M. fructicola* or *G. candidum,* incubated at room temperature for about 24 h as previously described, placed into plastic baskets, and immersed for 60 s in aqueous solutions of the selected food additives at their natural pH. Control fruits were dipped in water alone. In all experiments, each treatment was applied to three replicates of 20 to 22 fruits each. The treatment equipment was located in the facilities of the U.S. Department of Agriculture Agricultural Research Service at the San Joaquin Valley Agricultural Sciences Center (Parlier, CA) and consisted of twelve 22-liter stainless steel tanks, each one individually fitted with a computer-controlled electrical heater, a temperature sensor, and a mechanical agitation system. After treatment, fruits were placed in plastic cavity trays in open fiberboard boxes and stored at 20 ± 1°C and 90% ± 5% relative humidity. After 3 and 7 days of incubation, disease incidence and severity and phytotoxicity were recorded. Skin injury was assessed as both incidence (number of fruits with skin damage) and severity (none, slight, moderate, or severe skin damage).

Several small-scale tests were performed. In the first set of experiments, room temperature (nonheated) dips in solutions of the following compounds and concentrations were assayed on Spring Bright nectarines and Flavorcrest peaches previously inoculated with *M. fructicola* and on Spring Bright nectarines previously inoculated with *G. candidum*: 46 mM (10 g/liter) D-glucosamine hydrochloride (this chemical was used instead of 2-deoxy-D-glucose because glucosamine is a much less expensive precursor of this sugar), 400 mM (42.4 g/liter) sodium carbonate, 250 mM (34.5 g/liter) potassium carbonate, 200 mM (26.8 g/liter) sodium sorbate, 200 mM (30.0 g/liter) potassium sorbate, and 200 mM (28.8 g/liter) sodium benzoate. These compounds and concentrations were selected based on the results of the in vivo primary screenings. For nectarines inoculated with *M. fructicola,* some of the treated fruits were rinsed with tap water at low pressure in a spray for about 5 s to evaluate the effect of rinsing on treatment efficacy. In another set of experiments, the effect of temperature on control of brown rot was determined on O'Henry peaches inoculated with *M. fructicola* and dipped in either water alone or 200 mM (30.0 g/liter) potassium sorbate solutions at temperatures of 24, 55, and 60°C.

Semicommercial trials. Based on the results of the previous experiments, potentially commercial postharvest integrated treatments were selected and evaluated for control of brown rot, gray mold, and sour rot by treating artificially inoculated Summer Fire nectarines in an experimental packing line that realistically simulated commercial fungicide applications. The integrated treatments combined antifungal food additives with conventional synthetic fungicides such as fludioxonil to study the feasibility of new fungicidal mixtures containing reduced doses of the conventional fungicide. Commercial treatments with fludioxonil at the recommended dose for postharvest treatment of stone fruit were included for comparison purposes.

In tests to control brown rot and gray mold, nectarines were wound inoculated once on the equator as described previously with 20 μl of a 3×10^4 spores per ml conidial suspension of either *M. fructicola* or *B. cinerea* and incubated at room temperature for 14 to 18 h before application of the treatments. Two artificial inoculation procedures were used in tests to control sour rot: wound inoculation to assess the effect of the treatments on established infections and surface inoculation to evaluate the efficacy of the treatments for eliminating sour rot contamination from the surface of the fruit. For wound inoculation, the nectarines were wounded and inoculated as previously described with 20 μl of a *G. candidum* suspension containing 1×10^6 arthroconidia per ml in two different sites on the cheek of each fruit. To retard wound healing and facilitate infection, 10 mg/liter cycloheximide were added to each inoculation site. The fruits were incubated at room temperature for 14 to 18 h before application of the treatments. For surface inoculation, two circular areas (1 to 2 cm in diameter) were marked on the cheek of each nectarine with a permanent marker, and a 20-μl droplet of a *G. candidum* conidial suspension at 1×10^6 spores per ml was deposited on the surface of each marked area. The droplets were allowed to dry in air at room temperature for 14 to 18 h until the application of the treatments. After treatment and before incubation of treated fruits, the marked inoculation sites were puncture wounded once about 2 to 3 mm deep with toothpicks. In these trials, fruit were not surface disinfected or rinsed with water before fungal inoculation.

The treatments were conducted on an experimental packing line located in the facilities of the F. Gordon Mitchell Postharvest Center. The small scale (90 cm wide by 1,140 cm long) of this packing line is designed to simulate commercial handling processes, especially fruit washing and waxing processes typically conducted in stone fruit packinghouses in this area. The packing line includes a brush wash bed, a drying area with sponge rollers, and high- and low-volume agrochemical application equipment over brush and roller beds (90 cm wide and 90 to 120 cm long). For low-volume application of fungicides, a controlled droplet applicator (CDA) unit is used.

The inoculated fruits were rinsed with water before the application of the treatments. Solutions were applied at the commercial rate of 10.4 liters/10,000 kg of fruit (25 gal per 200,000 lb). To correctly apply this rate, the nectarines were weighed before treatment (average weight of 4.1 kg per tray of 16 or 17 fruits; 240 to 255 g per fruit), and the applicator pump was calibrated to release the treatment solution at 0.8 ml/s. The fruit entered into

TABLE 1. *Activity of low-toxicity chemicals to control seven major postharvest pathogens of stone fruit in in vivo laboratory screenings with Flavorcrest, O'Henry, or Last Chance peaches, Summer Fire nectarines, and Fortune or Royal Diamond plums*

Chemical	Formula	Tested concn		Activity against[a]:							Skin injury[b]
		mM	g/liter	MF	BC	GC	AA	PE	MP	RS	
Mineral salts											
Sodium carbonate	Na_2CO_3	100	10.6	0	+	++	0	+	0	0	0
		200	21.2	+	+	ND	ND	ND	ND	ND	0
		400	42.4	++	++	ND	ND	ND	ND	ND	0
Potassium carbonate	K_2CO_3	100	13.8	0	++	++	0	0	+	0	0
		200	27.6	+	+	+	ND	ND	ND	+	0
		250	34.5	+	+	0	ND	ND	ND	ND	0
Ammonium carbonate	$(NH_4)_2CO_3$	100	9.6	0	+	+++	+	+	0	0	0
		200	19.2	ND	+	ND	ND	ND	ND	ND	0
		400	38.4	ND	+	ND	ND	ND	ND	ND	0
Sodium bicarbonate	$NaHCO_3$	100	8.4	0	0	+	0	0	+	0	0
		200	16.8	0	0	0	ND	ND	ND	0	0
		400	33.6	0	+	0	ND	ND	ND	0	0
Potassium bicarbonate	$KHCO_3$	100	10.0	0	0	++	0	+	0	0	0
		200	20.0	ND	0	ND	ND	ND	ND	ND	0
		400	40.0	ND	0	ND	ND	ND	ND	ND	0
Ammonium bicarbonate	$(NH_4)HCO_3$	100	7.9	0	0	++	+	+	0	0	0
		200	15.8	0	0	++	ND	ND	ND	0	0
		400	31.6	0	0	+	ND	ND	ND	ND	0
Sodium molybdate	Na_2MoO_4	12.5	2.6	0	0	ND	++	ND	ND	0	1
		50	10.3	0	0	ND	++	ND	ND	ND	2
		100	20.6	0	++	+	++	+++	+++	+++	2
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	8	9.9	0	0	ND	ND	ND	ND	ND	0
		16	19.8	0	+	ND	ND	ND	ND	ND	0
		100	123.6	0	+	++	++	++	+	++	2
Organic acids and salts											
Lactic acid	$C_3H_6O_3$	8	0.7	0	+	+	++	+	0	0	2
L-Ascorbic acid	$C_6H_8O_6$	100	17.6	+	+	++	+	+	0	0	0
Sodium acetate	$C_2H_3O_2Na\cdot 4H_2O$	100	15.4	0	0	++	0	+	0	0	0
Potassium acetate	$C_2H_3O_2K$	30	2.9	0	0	ND	ND	ND	ND	ND	0
		100	9.8	0	0	++	0	+	0	0	0
		300	29.4	0	0	ND	ND	ND	ND	ND	0
Sodium propionate	$C_3H_5O_2Na$	30	2.9	0	0	ND	0	ND	ND	0	0
		100	9.6	0	+	+	0	+	0	0	0
		300	28.8	ND	++	ND	ND	ND	ND	ND	0
Potassium propionate	$C_3H_5O_2K$	20	2.2	ND	0	ND	ND	ND	ND	ND	0
		100	11.2	0	0	+	0	0	0	+	0
		200	22.4	ND	+	ND	ND	ND	ND	ND	0
Sodium sorbate	$C_6H_7O_2Na$	20	2.7	0	+	ND	0	ND	ND	0	0
		100	13.4	+	+	++	0	+	0	0	0
		200	26.8	++	++	+	ND	ND	0	0	0
Potassium sorbate	$C_6H_7O_2K$	20	3.0	0	+	0	0	0	0	0	0
		100	15.0	+	++	+	++	+	+	0	0
		200	30.0	++	++	+	ND	ND	ND	0	0
Sodium benzoate	$C_7H_5O_2Na$	20	2.9	0	0	0	0	0	0	0	0
		100	14.4	++	++	++	+	+	+	0	0
		200	28.8	+	++	0	ND	ND	ND	+	0
Potassium benzoate	$C_7H_5O_2K$	20	3.2	0	0	ND	ND	ND	ND	ND	0
		100	16.0	0	+	++	+	+	0	0	0
		200	32.0	ND	++	ND	ND	ND	ND	ND	0
Sodium citrate	$C_6H_5O_7Na_3\cdot 2H_2O$	100	29.4	0	0	++	0	0	0	0	0
Sodium lactate	$C_3H_5O_3Na$	100	11.2	0	0	0	0	0	0	0	2
Sodium L-tartrate	$C_4H_4O_6Na_2\cdot 2H_2O$	100	23.0	0	0	0	0	0	0	0	0

TABLE 1. *Continued*

Chemical	Formula	Tested concn		Activity against[a]:							Skin injury[b]
		mM	g/liter	MF	BC	GC	AA	PE	MP	RS	
Other compounds											
Hydrogen peroxide	H_2O_2	30	1.0	0	0	0	0	0	0	0	1
		170	5.8	0	0	0	0	0	0	0	2
		340	11.6	+	+	++	++	+	0	0	2
Deoxy-D-glucose	$C_6H_{12}O_5$	25	4.1	0	+	ND	0	ND	ND	0	0
		50	8.2	0	++	+	0	ND	ND	0	0
		100	16.4	+	+++	++	+++	+++	++	++	0
Deoxy-D-ribose	$C_5H_{10}O_4$	25	3.3	0	0	ND	ND	ND	ND	ND	0
		50	6.7	0	0	ND	ND	ND	ND	ND	0
		100	13.4	0	0	+++	0	0	0	0	0

[a] MF, *Monilinia fructicola*; BC, *Botrytis cinerea*; GC, *Geotrichum candidum*; AA, *Alternaria alternata*; PE, *Penicillium expansum*; MP, *Mucor piriformis*; RS, *Rhizopus stolonifer*. 0, no control; +, slight control; ++, moderate control; +++, good control; ND, not determined. With the exception of ND combinations, each concentration of each chemical was tested with each fruit species in at least two screenings of three replicates of four fruits each. The repeated screenings were performed with the same or different fruit cultivars.

[b] 0, no skin injury; 1, slight to moderate skin injury; 2, severe skin injury.

the roller bed at 40 fruits per run and passed under the CDA for approximately 12 s. The following treatments were applied to control all three postharvest diseases: (i) untreated control, (ii) fludioxonil at half-strength (568.75 mg/liter [ppm], i.e., 0.118 liter [4 oz] of commercial product Scholar 50 WP per 10.4 liters of treatment solution; this rate is half the recommended rate for low-volume postharvest application of this fungicide on peaches, nectarines, and plums), (iii) fludioxonil at half-strength (568.75 mg/liter) plus an aqueous mixture of GRAS compounds consisting of 200 mM potassium sorbate and 200 mM sodium benzoate at (M1), (iv) fludioxonil at half-strength (568.75 mg/liter) plus another aqueous mixture of GRAS compounds consisting of 200 mM potassium sorbate, 200 mM sodium benzoate, and 60 mM 2-deoxy-D-glucose (M2), (v) fludioxonil at full strength (1,137.5 mg/liter, i.e., 0.236 liter [8 oz] of commercial product Scholar 50 WP per 10.4 liters of treatment solution; label recommended rate for postharvest low-volume applications) plus M1, and (vi) fludioxonil at full strength (1,137.5 mg/liter) plus M2. The solutions were applied in a vegetable oil-based stone fruit coating (20% PrimaFresh 50-V, Pace International LLC, Seattle, WA) at 1:1. Between treatments, all the equipment in the packing line was washed with a commercial alkaline detergent (PacFoam Plus, Pace International) and extensively rinsed with water. In all experiments, each treatment was applied to four replicates of 16 or 17 fruits each. Treated fruit were packed in cavity trays in single-layer open fiberboard boxes with the inoculated side up and incubated for 5 days at 20°C and 90% relative humidity. After incubation, disease incidence and severity were recorded. For severity of brown rot and gray mold, lesion diameters were measured; for severity of sour rot, each fruit was scored according to the following 5-point quantitative scale: 0, no visible lesion; 1, sunken lesion; 2, sunken lesion with slight sporulation; 3, sunken lesion with sporulation; and 4, expanding lesion with extensive sporulation. A sour rot severity rating (0 to 4 scale) was calculated for each treatment.

Statistical analysis. Depending on the experiment, one-, two-, or three-way analyses of variance were applied to disease incidence and severity data using SAS software (SAS Institute Inc., Cary, NC). Data from counts such as disease incidence were transformed to the arcsine of the square root of the proportion of decayed fruit. When appropriate, means were separated by Fisher's protected least significant difference test with a significance level of $P = 0.05$.

RESULTS

In vivo primary screenings. Among the chemicals screened in this set of experiments, only nonphytotoxic compounds with the best overall performance against the tested diseases, especially brown rot, gray mold, and sour rot, were used for the next research stage (Table 1). The following compounds and concentrations were selected for further testing in small-scale trials: 100 mM 2-deoxy-D-glucose very effectively controlled gray mold, sour rot, black rot, and blue mold and was moderately effective against brown rot, rhizopus, and mucor rots; 200 mM sodium carbonate and 250 mM potassium carbonate were moderately effective for controlling brown rot, gray mold, and sour rot; 200 mM sodium sorbate had good activity against gray mold and partially inhibited brown rot and sour rot; 200 mM potassium sorbate and 200 mM sodium benzoate effectively controlled gray mold and had acceptable activity against brown rot, sour rot, and most of the rest of tested diseases.

The following chemicals had good decay control ability but were unacceptably phytotoxic to the skin of peaches, nectarines, or plums: sodium and ammonium molybdates (which caused moderate to severe dark staining or inking where the droplet of the compound solution was applied), lactic acid (which appeared to digest plant tissues, causing cellular breakdown in the application point), and hydrogen peroxide (which also was highly corrosive to skin tissues).

Small-scale trials. In the first test with selected food additives or GRAS compounds applied as 60-s dips at room temperature to Spring Bright nectarines previously inoculated with *M. fructicola,* the incidence of brown rot after 3 days of incubation at 20°C was significantly lower on fruits treated with potassium sorbate and sodium benzoate than on control fruits or fruits treated with the remaining com-

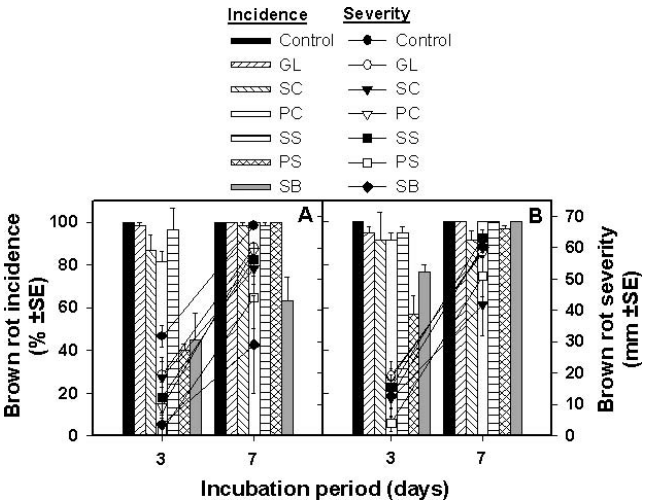

FIGURE 1. *Incidence (bars) and severity (lines) of brown rot on Spring Bright nectarines wound inoculated with* Monilinia fructicola *and dipped 24 h later for 60 s in water (control) or aqueous solutions at room temperature of 46 mM glucosamine hydrochloride (GL), 400 mM sodium carbonate (SC), 250 mM potassium carbonate (PC), 200 mM sodium sorbate (SS), 200 mM potassium sorbate (PS), or 200 mM sodium benzoate (SB). Fruit was either not rinsed (A) or rinsed (B) with tap water for 5 s and incubated at 20°C and 90% relative humidity for 3 or 7 days.*

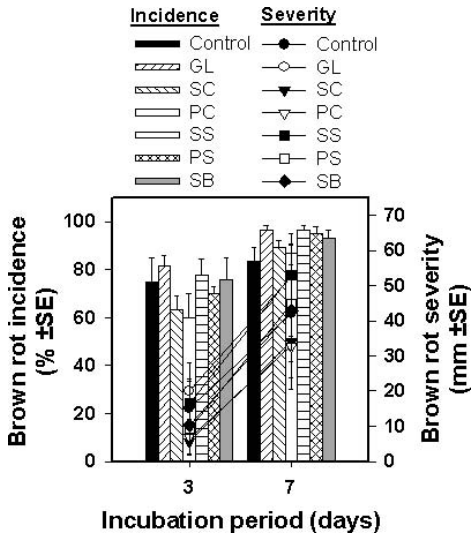

FIGURE 2. *Incidence (bars) and severity (lines) of brown rot on Flavorcrest peaches wound inoculated with* Monilinia fructicola *and dipped 24 h later for 60 s in water (control) or aqueous solutions at room temperature of 46 mM glucosamine hydrochloride (GL), 400 mM sodium carbonate (SC), 250 mM potassium carbonate (PC), 200 mM sodium sorbate (SS), 200 mM potassium sorbate (PS), or 200 mM sodium benzoate (SB). Treated fruit was incubated at 20°C and 90% relative humidity for 3 or 7 days.*

pounds. These chemicals, both applied at 200 mM, reduced brown rot incidence by 55 to 60% with respect to the control treatment. However, the activity of these treatments was not persistent, and after 7 days of incubation at 20°C only sodium benzoate reduced disease incidence by about 40% (Fig. 1A). In contrast, brown rot severity, measured as lesion size, was significantly reduced after 3 days of incubation at 20°C by all chemical treatments, especially potassium sorbate, sodium benzoate, potassium carbonate, and sodium sorbate. These reductions, however, diminished markedly after 7 days of incubation, and only sodium benzoate reduced disease severity by more than 50% (from lesion diameter of 67 mm on control fruits to lesion diameter of 30 mm; Fig. 1A). In this trial, rinsing the nectarines with fresh water after treatment slightly reduced the efficacy of the chemicals, although this reduction was largest (up to 30%) with the two most effective compounds, potassium sorbate and sodium benzoate. After 7 days of incubation at 20°C, none of the solutions significantly reduced the incidence of brown rot on rinsed nectarines (Fig. 1B).

Similarly, none of the six compounds had acceptable activity against *M. fructicola* on wound-inoculated Flavorcrest peaches after 3 or 7 days of incubation at 20°C. In this test, brown rot incidence and severity were higher on peaches dipped in chemical solutions such as glucosamine or sodium sorbate than on peaches dipped in water (Fig. 2). Therefore, although glucosamine is considerably less expensive, it was not an effective substitute for 2-deoxy-D-glucose for control of brown rot; it increased the severity of this disease.

In contrast to the results with brown rot, all six chemical treatments significantly reduced the incidence of sour rot (caused by *G. candidum*) in Spring Bright nectarines dipped for 60 s, not rinsed, and incubated at 20°C for 7 days. Sodium and potassium sorbates were the most effective chemicals in this trial, reducing disease incidence from about 85% on control fruits to 6 and 13%, respectively (Fig. 3).

In tests to assess the effect of the temperature of the dip solutions on control of brown rot, heating water alone or an aqueous solution of 200 mM potassium sorbate to 55 or 60°C increased the efficacy of these dips compared with dips applied at room temperature (24°C) in O'Henry peaches previously wound inoculated with *M. fructicola.* The

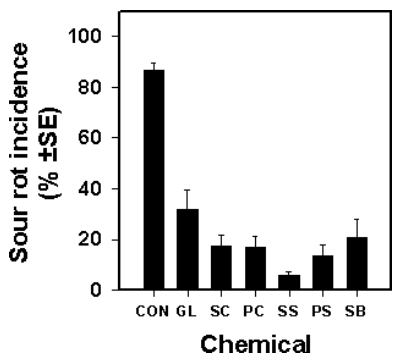

FIGURE 3. *Incidence of sour rot on Spring Bright nectarines wound inoculated with* Geotrichum candidum *and dipped 24 h later for 60 s in water (CON) or aqueous solutions at room temperature of 46 mM glucosamine hydrochloride (GL), 400 mM sodium carbonate (SC), 250 mM potassium carbonate (PC), 200 mM sodium sorbate (SS), 200 mM potassium sorbate (PS), or 200 mM sodium benzoate (SB). Treated fruit was incubated at 20°C and 90% relative humidity for 7 days.*

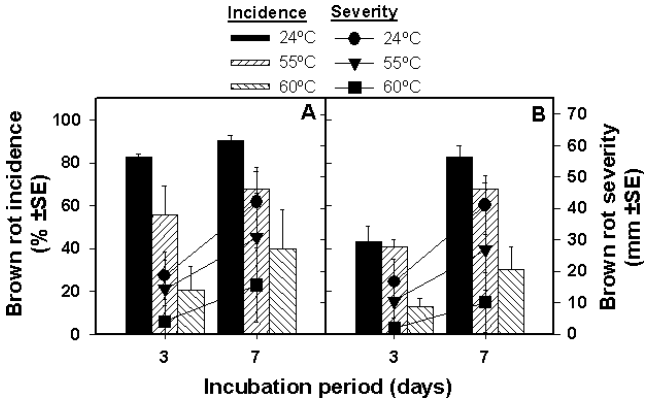

FIGURE 4. *Incidence (bars) and severity (lines) of brown rot on O'Henry peaches wound inoculated with* Monilinia fructicola *and dipped 24 h later for 60 s in water alone (A) or aqueous solutions of 200 mM potassium sorbate (B) at 24, 55, or 60°C. Treated fruit was incubated at 20°C and 90% relative humidity for 3 or 7 days.*

proportions of infected fruits after treatment with hot water at 24, 55, and 60°C for 60 s were approximately 83, 55, and 20%, respectively, after 3 days of incubation at 20°C and were about 90, 70, and 40%, respectively, after 7 days of incubation. The beneficial effect of heating also was observed on disease development, and after 7 days of incubation, brown rot severity was reduced from 42 mm after dipping fruit at 24°C to 31 and 16 mm after treatment at 55 and 60°C, respectively (Fig. 4A). Similar results were obtained when 200 mM potassium sorbate was heated to these temperatures. The use of this food additive considerably improved the performance of hot water alone against brown rot in peaches after 3 days of incubation at 20°C but not after 7 days of incubation; thus, hot water dips were

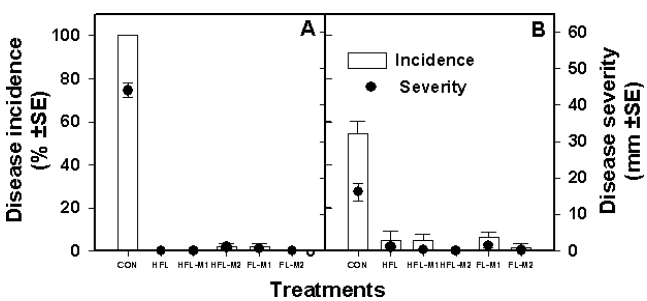

FIGURE 5. *Incidence (bars) and severity (points) of brown rot (A) and gray mold (B) on Summer Fire nectarines wound inoculated with* Monilinia fructicola *or* Botrytis cinerea, *respectively. Control (CON) fruits were not treated. Other fruits were treated 18 h after inoculation with a commercial controlled drop applicator in a packing line with 20% diluted commercial fruit coating mixed 1:1 with aqueous solutions of fludioxonil at half-strength (568.75 mg/liter; HFL), HFL plus 200 mM potassium sorbate plus 200 mM sodium benzoate (HFL-M1), HFL plus 200 mM potassium sorbate plus 200 mM sodium benzoate plus 60 mM 2-deoxy-D-glucose (HFL-M2), fludioxonil at full strength (1,137.5 mg/liter; FL) plus 200 mM potassium sorbate plus 200 mM sodium benzoate (FL-M1), or FL plus 200 mM potassium sorbate plus 200 mM sodium benzoate plus 60 mM 2-deoxy-D-glucose (FL-M2). Treated fruit was incubated at 20°C and 90% relative humidity for 5 days.*

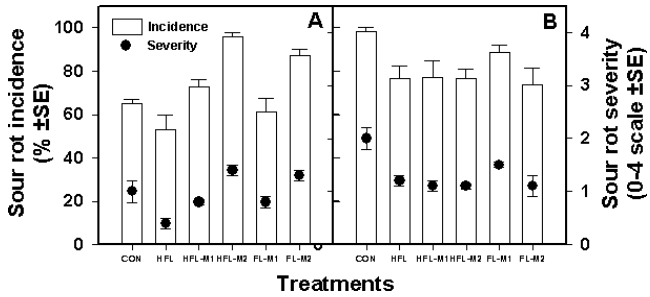

FIGURE 6. *Incidence (bars) and severity (points) of sour rot on Summer Fire nectarines wound (A) or surface (B) inoculated with* Geotrichum candidum. *Control (CON) fruits were not treated. Other fruits were treated 18 h later with a commercial controlled drop applicator in a packing line with 20% diluted commercial fruit coating mixed 1:1 with aqueous solutions of fludioxonil at half-strength (568.75 mg/liter; HFL), HFL plus 200 mM potassium sorbate plus 200 mM sodium benzoate (HFL-M1), HFL plus 200 mM potassium sorbate plus 200 mM sodium benzoate plus 60 mM 2-deoxy-D-glucose (HFL-M2), fludioxonil at full strength (1,137.5 mg/liter; FL) plus 200 mM potassium sorbate plus 200 mM sodium benzoate (FL-M1), or FL plus 200 mM potassium sorbate plus 200 mM sodium benzoate plus 60 mM 2-deoxy-D-glucose (FL-M2). Treated fruit was incubated at 20°C and 90% relative humidity for 5 days. Surface-inoculated fruit was punctured at the inoculation site after treatment and before incubation.*

nearly as effective as dips in hot potassium sorbate and the effectiveness of the treatments was mostly due to the effect of heat (Fig. 4B). In these tests, no skin injuries were observed on fruit treated for 60 s at 55 or 60°C.

Semicommercial trials. Both incidence and severity of brown rot on Summer Fire nectarines wound inoculated with *M. fructicola* and incubated at 20°C for 5 days were reduced by about 100% after packing line application with a commercial CDA containing 20% fruit coating amended with half or full doses of fludioxonil and different mixtures of potassium sorbate, sodium benzoate, or 2-deoxy-D-glucose (Fig. 5A). Similarly, all treatments greatly controlled gray mold on nectarines wound inoculated with *B. cinerea* (Fig. 5B). It was not possible in these trials to determine the effect on both diseases of the addition of different mixtures of GRAS compounds to the commercial fludioxonil because of the excellent inhibitory activity of the fungicide applied alone, even at half of the recommended commercial dose (treatment HFL, Fig. 5).

In contrast, fludioxonil at either half- or full strength was completely ineffective against sour rot on Summer Fire nectarines either wound (Fig. 6A) or surface (Fig. 6B) inoculated with *G. candidum.* In these tests, the addition of the food additives potassium sorbate and sodium benzoate to the fruit coating containing the fungicide fludioxonil did not improve control of sour rot. The use of 2-deoxy-D-glucose increased both incidence and severity of sour rot with respect to the control treatment on wound-inoculated fruits (Fig. 6A).

DISCUSSION

This is the first study in which a wide variety of food additives and GRAS and low-toxicity compounds were test-

ed to assess their antifungal activity against the most important fungal pathogens causing postharvest decay of stone fruit. Most of the chemicals assayed during this selection process had no in vivo inhibitory activity on artificially inoculated peaches, nectarines, or plums at the wide range of concentrations tested. Others chemicals were phytotoxic at effective concentrations and thus were also discarded.

The assessment of skin injury caused by the treatment was one of the main reasons for using in vivo primary screenings instead of in vitro tests. Another reason was that the ability of low-toxicity chemicals such as some GRAS compounds or natural plant-origin preservatives (e.g., essential oils) to control postharvest diseases of fresh fruit cannot be predicted by their inhibitory activity in vitro against fungal pathogens growing in artificial culture media *(5, 22, 26).* Disease development is a result of complex interactions among the host, pathogen, and environment. In contrast to conventional synthetic fungicides, the inhibitory activity of these low-toxicity chemicals is rather modest and depends on the presence of residues of the compound within the fruit infection areas occupied by the fungus and on multiple interactions between these residues and constituents of the fruit tissues. These interactions, such as a decrease in pH or water activity, alter the original toxicity of the antifungal compound to the target pathogen, and therefore results from in vivo and in vitro efficacy tests are often notably different. Because the nature of those interactions may be different in different fruit hosts as a consequence of different peel characteristics or the presence of different peel constituents, the level of disease reduction by low-toxicity chemicals is strongly dependent on fruit species, fruit cultivar, and fruit physical and physiological condition.

Different results were obtained with selected chemicals in small-scale trials with nectarines and peaches. Although 200 mM potassium sorbate and 200 mM sodium benzoate were the most effective preservatives for reducing brown rot on Spring Bright nectarines dipped for 60 s in aqueous solutions at room temperature, none of the tested additives reduced this disease on Flavorcrest peaches. All of these chemicals were similarly effective against sour rot on Spring Bright nectarines. In recent studies conducted in Italy, 2-min dips in 15 g/liter potassium sorbate solutions at ambient temperature satisfactorily controlled brown rot on Springbelle peaches and Big Top nectarines naturally infected with *M. laxa (11).* However, these treatments adversely affected fruit quality; firmness, soluble solids content, and titratable acidity were significantly reduced on treated and unrinsed peaches and nectarines incubated at 20°C for 5 days. We rinsed the fruit at low pressure to avoid potential negative effects on fruit quality, but rinsing was associated with a significant loss of salt effectiveness. In contrast to the results reported here, Gregori et al. *(11)* observed that control of brown rot on peaches and nectarines was similar with sodium carbonate, sodium bicarbonate, potassium bicarbonate, and potassium sorbate. In their tests with peaches, these treatments were all superior to sodium benzoate treatment. Droby et al. *(5)* concluded that dips in 20 g/liter sodium bicarbonate significantly reduced natural decay by *M. fructicola* and *R. stolonifer* on Loring peaches. Because we found potassium sorbate and sodium benzoate to be superior to bicarbonates and other salts for the control of brown rot on nectarines and they are known to be compatible with conventional fungicides such us fludioxonil *(21),* we selected these two chemicals for testing in our semicommercial trials. Decay control by these substances was considerably enhanced by heating the solutions to nonphytotoxic temperatures. According to Smilanick et al. *(21),* additional significant advantages of potassium sorbate over sodium carbonate salts are the relatively low salt concentration of potassium sorbate, the absence of sodium, and the lower pH, meaning that disposal of used solutions in fruit packinghouses would raise fewer regulatory issues. Because of its performance in primary screenings, the sugar analog 2-deoxy-D-glucose also was added as a component of one of the mixtures of GRAS compounds tested in semicommercial trials in combination with fludioxonil against brown rot, gray mold, and sour rot. In a previous study, 2-deoxy-D-glucose had inhibitory activity both in vitro and in vivo against postharvest stone fruit pathogens such as *M. fructicola, B. cinerea, P. expansum,* and *R. stolonifer (6).* Unfortunately, our attempt to use D-glucosamine hydrochloride as a cheap precursor of 2-deoxy-D-glucose was not successful, and the use of this substance increased the severity of brown rot on peaches artificially inoculated with *M. fructicola.* Presumably, this chemical provided additional nutrients and/or enhanced environmental conditions for the development of the pathogen.

Fludioxonil is a broad-spectrum phenylpyrrole fungicide effective against some of the most destroying postharvest pathogens of stone fruit, including *Monilinia* spp., *B. cinerea, R. stolonifer,* and *P. expansum (3, 4, 9).* This fungicide was registered for postharvest use in California and throughout the United States in 2003 and since then has been an essential tool of the stone fruit industry for managing decays, especially on exports to long-distance markets. Mixtures or rotations of this fungicide with other active antifungal compounds with different modes of action may be important for developing and implementing rational fungicide resistance management programs *(9).* In our tests, a commercial low-volume CDA was used for fruit coating mixed with fludioxonil alone and was so effective in controlling both brown rot and gray mold of Summer Fire nectarines, even at half the recommended commercial dose of the fungicide (570 mg/liter), that potential beneficial effects from the addition of mixtures of GRAS compounds could not be determined. Commercial application of the mixtures of potassium sorbate and sodium benzoate or potassium sorbate, sodium benzoate, and 2-deoxy-D-glucose without fludioxonil was not considered in these laborious semicommercial trials because of the limited inhibitory activity of these preservatives when applied alone in the previous small-scale laboratory tests. D'Aquino et al. *(4)* reported effective decay control in a variety of stone fruits by 2-min dips in a 20°C solution containing fludioxonil at a concentration as low as 100 mg/liter. As expected, CDA coating with fludioxonil at both half and full commercial doses was ineffective against sour rot on nectarines either wound or

surface inoculated with *G. candidum.* The fungicide, therefore, failed at both controlling established infections of *G. candidum* and hindering infection from surface inoculation of the pathogen. This result was not surprising because sour rot is not effectively controlled by any registered postharvest fungicide, and significant decay reduction relies on proper culture practices, storage under appropriate conditions, and effective sanitation programs *(1).* We did not anticipate, based on the results of the small-scale trials, that the addition of the GRAS mixtures to the fruit coating containing fludioxonil would not improve the control of sour rot. We presumed that the effectiveness of the food additives against sour rot was determined by the mode of application of the treatment (aqueous dips or CDA coating), and future studies may be conducted in which these treatments are applied during the fruit washing operation instead of the waxing procedure in the packing line.

The results of this research suggest that, in contrast to previous results with other fresh fruit such as citrus *(19, 20),* the potential for use of common food additives and GRAS compounds as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruits in California is currently limited. Even after an accurate selection process, the best compounds applied alone at selected concentrations as aqueous solutions at ambient temperature lacked effectiveness, persistence, and consistency. More promising was the use of heated solutions, but because results were comparable to those obtained by immersion in hot water alone, heat probably was more responsible for decay reduction than were the low-toxicity chemicals. However, the application of heated solutions to stone fruits is greatly limited by the risks of fruit injury, and it is generally necessary to investigate damage thresholds for various species and cultivars. Additional work is in progress to characterize effective and safe postharvest hot water dips for California nectarines, peaches, and plums. According to this and other research *(12, 15),* heat treatments appear more suitable than treatments with food additives to be combined with other relatively environmentally benign antifungal treatments (e.g., modified atmospheres, natural compounds, and biocontrol agents) for integrated control of stone fruit postharvest diseases. Such integration of treatments may be especially useful in California for handling organic tree fruit or commodities destined for national or international markets that currently are rejecting pesticide-treated produce or demanding very low residue levels in and/or on the fruit. Alternative treatments could be adopted in production areas such as Spain, Italy, and Turkey where currently the application of conventional postharvest fungicides, even those classified as ''reduced risk,'' is entirely banned.

According to the results reported here, potassium sorbate, sodium benzoate, and other food additives should be evaluated in combination with low doses of the fungicide propiconazole (Menthor 45 WP, Syngenta) for their activity against sour rot as an additional strategy for managing postharvest application of this active ingredient and reducing the risk of proliferation of resistant strains of *G. candidum.* Propiconazole is highly active against sour rot and can be currently used as a postharvest treatment in California under a Section 18 emergency registration *(2).*

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