Evaluation of Food Additives and Low-Toxicity Compounds as Non-polluting Means to Control the Main Postharvest Diseases of California Peaches

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Abstract

More than twenty food additives, GRAS (Generally Regarded as Safe), and low-toxicity compounds were evaluated as nonpolluting means to control postharvest decay. The chemicals were tested at three concentrations in in vivo primary screenings with California-grown 'Flavorcrest', 'O'Henry', or 'Last Chance' peaches 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 potassium sorbate, sodium benzoate, and sodium sorbate at 200 mM, 2-deoxy-D-glucose at 100 mM, sodium carbonate at 400 mM, and potassium carbonate at 250 mM. Sodium and ammonium molybdates, acid lactic, and hydrogen peroxide were somewhat effective but phytotoxic to fruit skin tissues. The selected compounds, however, lacked effectiveness and persistence when tested against brown rot, caused by *M. fructicola*, in small-scale trials as 60 s dips in aqueous solutions at ambient temperatures. Heating the solutions to 55 or 60°C significantly increased treatment efficacy and brown rot incidence and severity were reduced by 35 and 25%, respectively, after 7 days of incubation at 20°C on peaches treated with potassium sorbate. However, treatment efficacy was not superior to water alone at these temperatures. Therefore, the potential for use of common food additives or GRAS compounds as alternative chemicals to conventional fungicides for the control of brown rot of California peaches is rather limited and heat treatments appear more suitable than these chemicals to be combined with other environmentally-friendly antifungal treatments for integrated disease control. The control by these means of other important peach postharvest diseases such as gray mold and sour rot, caused by *B. cinerea* and *G. candidum*, respectively, deserves further study.

INTRODUCTION

Fruit losses caused by postharvest diseases are among the main concerns of peach growers in California, Spain, and other important producing areas. Brown rot, caused by *Monilinia fructicola*, *M. laxa*, or *M. fructigena*, is the most important postharvest disease

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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*; sour rot, caused by *Geotrichum candidum*; rhizopus rot, caused by *Rhizopus stolonifer*; mucor rot, caused by *Mucor piriformis*; alternaria rot, caused by *Alternaria alternata*; or blue mold, caused by *Penicillium expansum* (Ogawa and English, 1991; Narayanasamy, 2006). 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 (typically synthetic chemical fungicides), and maintenance of the proper environments during fruit storage and transportation. Alternatives to the use of conventional fungicides are needed because of concerns about human health risks and the protection of the environment associated with fungicide residues. In fact, postharvest application of conventional fungicides to stone fruits is prohibited in the European Union and other countries. Furthermore, the widespread use of these chemicals has led repeatedly in the past to the proliferation of resistant strains of the pathogens (Ma et al., 2003). Aqueous solutions of some common food additives and low-toxicity compounds have been evaluated as alternative nonpolluting treatments for the control of postharvest diseases of fruits and vegetables (Palou et al., 2008), but very little research has been conducted on peaches or other stone fruits (Gregori et al., 2008).

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 California peaches. Promising chemicals were identified by testing their effectiveness in in vivo primary screenings. Selected compounds were tested as heated aqueous solutions in small-scale trials.

MATERIALS AND METHODS

Fruit Inoculation

Peaches [*Prunus persica* (L.) Batsch.], 'Flavorcrest', 'O'Henry', and 'Last Chance', commercially grown in orchards in the San Joaquin Valley (California, USA) were surface disinfected and wound inoculated once on the equator with 20 µl of a suspension containing $5x10^4$ spores ml^{-1} of *M. fructicola*, *B. cinerea*, *A. alternata*, *P. expansum*, *M. piriformis*, or *R. stolonifer*, or $1x10^8$ arthrospores ml^{-1} of *G. candidum*.

In Vivo Primary Screenings

The effectiveness of 24 low-toxicity chemicals, usually at three different concentrations was tested in three peach cultivars against the above seven postharvest pathogens. About 24 h after fungal inoculation, 40 µl of sterile water (control) or a sterile solution of the food additive at the desired concentration were applied with a micropipette in the same pathogen inoculation site of the fruit. Treated fruit were incubated at 20°C and 90% RH and disease incidence (number of infected fruit) and severity (lesion diameter) were determined after 3 and 5 days of incubation. For each combination of chemical, concentration and fungal pathogen, 3 replicates of 4 fruit each were used. Each test was repeated at least once, sometimes with the same cultivar and other times with another cultivar. Qualitative 4- and 3-point scales were established to assess the effectiveness of the treatments and fruit skin damage, respectively.

Small-Scale Trials

'Flavorcrest' peaches wound inoculated with *M. fructicola* and incubated at room temperature for about 24 h were immersed for 60 s in water (control) or aqueous solutions of compounds selected according to the results of the in vivo primary screenings. In another set of experiments, wound inoculated 'O'Henry' peaches were dipped in either water alone or 200 mM (30.0 g L^{-1}) potassium sorbate solutions at temperatures of 24, 55, and 60°C. Each treatment was applied to three replicates of 20-22 fruit each. Brown rot

incidence and severity and phytotoxicity occurrence were recorded after 3 and 7 days of incubation at 20°C and 90% RH.

Statistical Analysis

Mean differences were determined by Fisher's protected least significant difference test (LSD, $P<0.05$) applied after an analysis of variance (ANOVA). For disease incidence data, the ANOVA was applied to arcsine transformed values.

RESULTS AND DISCUSSION

In Vivo Primary Screenings

Among the screened chemicals, 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. The following compounds and concentrations were selected for further testing in small-scale trials (Table 1): 2-deoxy-D-glucose at 100 mM very effectively controlled gray mold, sour rot, black rot, and blue mold, and was moderately effective against brown rot, rhizopus and mucor rots; sodium carbonate at 200 mM and potassium carbonate at 250 mM were moderately effective for controlling brown rot, gray mold, and sour rot; sodium sorbate at 200 mM had good activity against gray mold and partially inhibited brown rot and sour rot; potassium sorbate and sodium benzoate, both at 200 mM, 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: 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 'Flavorcrest' peaches previously inoculated with *M. fructicola*, none of the six compounds showed acceptable activity against *M. fructicola* after 3 or 7 days of incubation at 20°C. Brown rot incidence and severity were higher on peaches dipped in chemical solutions such as glucosamine hydrochloride or sodium sorbate than on peaches dipped in water (Fig. 1). Therefore, although glucosamine is considerably less expensive, it was not an effective substitute for 2-deoxy-D-glucose for control of brown rot and it even increased the severity of this disease.

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 treatments compared with dips applied at room temperature (24°C) in 'O'Henry' peaches previously wound inoculated with *M. fructicola*. The percentages of infected fruit 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. 2A). 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 nearly as effective as dips in hot potassium sorbate and the effectiveness of the treatments was mostly due to the effect of heat (Fig. 2B). In these tests, no skin injuries were observed on fruit treated for 60 s at 55 or 60°C.

This is the first study in which a wide cultivar of food additives and low-toxicity

compounds were tested to assess their antifungal activity against the most important fungal pathogens causing postharvest decay of peaches. Most of the chemicals assayed during this selection process had no in vivo inhibitory activity on artificially inoculated fruit at the wide range of concentrations tested. Other 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.

Nonheated solutions of food additives such as sodium and potassium carbonates and sorbates or sodium benzoate at selected concentrations were ineffective against brown rot caused by *M. fructicola* in small scale trials. In contrast, in recent research conducted in Italy, 2-min dips in aqueous solutions of potassium sorbate, sodium carbonate, sodium bicarbonate, or potassium bicarbonate at ambient temperature satisfactorily controlled brown rot on 'Springbelle' peaches and 'Big Top' nectarines naturally infected with *Monilinia* spp. These treatments were all superior to sodium benzoate treatment. However, potassium sorbate at 15 g L^{-1} adversely affected fruit quality: fruit firmness, soluble solids content, and titratable acidity were significantly reduced on treated and unrinsed peaches and nectarines incubated at 20°C for 5 days (Gregori et al., 2008). It can therefore be concluded that, in contrast to previous results with other fresh fruit such as citrus (Palou et al., 2002, 2008), the potential for use of common food additives and GRAS compounds as alternative chemicals to conventional fungicides for the control of major peach postharvest diseases is currently limited. In this research, 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 and, in agreement with extensive previous research with food additives or GRAS compounds (Narayanasamy, 2006; Palou et al., 2008), decay control by potassium sorbate was considerably enhanced by heating the solutions to nonphytotoxic temperatures. Nevertheless, because results were comparable to those obtained by immersion in hot water alone, heat probably was more responsible for decay reduction than was the low toxicity chemical. Furthermore, 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. According to this and other research (Karabulut et al., 2002; Mari et al., 2007), 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, 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. Likewise, alternative treatments could be adopted in production areas like Spain, Italy, or Turkey, where currently the application of conventional postharvest fungicides, even those classified as 'reduced risk', is entirely banned.

ACKNOWLEDGEMENTS

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Literature Cited

Gregori, R., Borsetti, F., Fantuzzi, E., Neri, F., Mari, M. and Bertolini, P. 2008. Effects of food additives on postharvest brown rot of stone fruit. p.438-442. In: P. Bertolini (ed.), Proc. COST Action 924 Int. Congr., Novel Approaches for the Control of Postharvest Diseases and Disorders. CRIOF, University of Bologna, Bologna, Italy.

Karabulut, O.A., Cohen, L., Wiess, B., Daus, A., Lurie, S. and Droby, S. 2002. Control of brown rot and blue mold of peach and nectarine by short hot water brushing and yeast

antagonists. Postharvest Biol. Technol. 24:103-111.
Ma, Z., Michael, A., Yoshimura, M.A. and Michailides, T.J. 2003. Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. Appl. Environ. Microbiol. 69:7145-7152.
Mari, M., Torres, R., Casalini, L., Lamarca, N., Mandrin, J.F., Lichou, J., Larena, I., De Cal, M.A., Melgarejo, P. and Usall, J. 2007. Control of post-harvest brown rot on nectarine by *Epicoccum nigrum* and physico-chemical treatments. J. Sci. Food Agric. 87:1271-1277.
Narayanasamy, P. 2006. Postharvest Pathogens and Disease Management. John Wiley and Sons Inc. Wiley-Interscience, Hoboken, NJ, USA.
Ogawa, J.M. and English, H. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. Pub. 3345. University of California, Division of Agriculture and Natural Resources, Oakland, CA, USA.
Palou, L., Smilanick, J.L. and Droby, S. 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue molds. Stewart Postharv. Rev. 2:2.
Palou, L., Usall, J., Smilanick, J.L., Aguilar, M.J. and Viñas, I. 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest Manag. Sci. 58:459-466.

Tables

Table 1. Activity of low-toxicity chemicals to control seven major postharvest pathogens in in vivo laboratory screenings with 'Flavorcrest', 'O'Henry', or 'Last Chance' peaches.

Chemical	Formula	Tested concentrations		Activity against[a,b,c]							Skin injury[d]
		(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

[a]MF = *Monilinia fructicola*, BC = *Botrytis cinerea*, GC = *Geotrichum candidum*, AA = *Alternaria alternata*, PE = *Penicillium expansum*, MP = *Mucor piriformis*, RS = *Rhizopus stolonifer*.

[b]0 = no control, + = slight control, ++ = moderate control, +++ = good control, ND = not determined.

[c]With the exception of ND combinations, each concentration of each chemical was tested at least in two screenings of three replicates of four fruit each. The repeated screenings were performed with the same or different fruit cultivars.

[d]0 = no skin injury, 1 = slight to moderate skin injury, 2 = severe skin injury.

Table 1 (continued). Activity of low-toxicity chemicals to control seven major postharvest pathogens in in vivo laboratory screenings with 'Flavorcrest', 'O'Henry', or 'Last Chance' peaches.

Chemical	Formula	Tested concentrations		Activity against[a,b,c]							Skin injury[d]
		(mM)	(g/liter)	MF	BC	GC	AA	PE	MP	RS	
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	$C_6H_5O_7Na_3.2H_2O$	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

[a]MF = *Monilinia fructicola*, BC = *Botrytis cinerea*, GC = *Geotrichum candidum*, AA = *Alternaria alternata*, PE = *Penicillium expansum*, MP = *Mucor piriformis*, RS = *Rhizopus stolonifer*.

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Table 1 (continued). Activity of low-toxicity chemicals to control seven major postharvest pathogens in in vivo laboratory screenings with 'Flavorcrest', 'O'Henry', or 'Last Chance' peaches.

Chemical	Formula	Tested concentrations		Activity against[a,b,c]							Skin injury[d]
		(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*.

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Figures

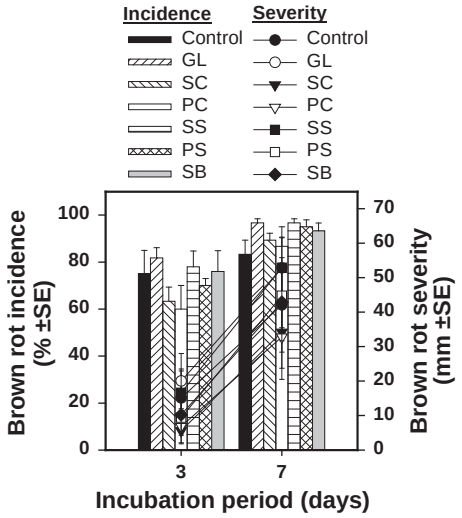

Fig. 1. Incidence (bars) and severity (lines) of brown rot on 'Flavorcrest' peaches wound inoculated with *Monilinia fructicola*, dipped 24 h later for 60 s in water (Control) or aqueous solutions at room temperature of 46 mM glucosamine hydrochloride (GL), 400 mM sodium carbonate (SC), 250 mM potassium carbonate (PC), 200 mM sodium sorbate (SS), 200 mM potassium sorbate (PS), or 200 mM sodium benzoate (SB), and incubated at 20°C and 90% RH for 3 or 7 days.

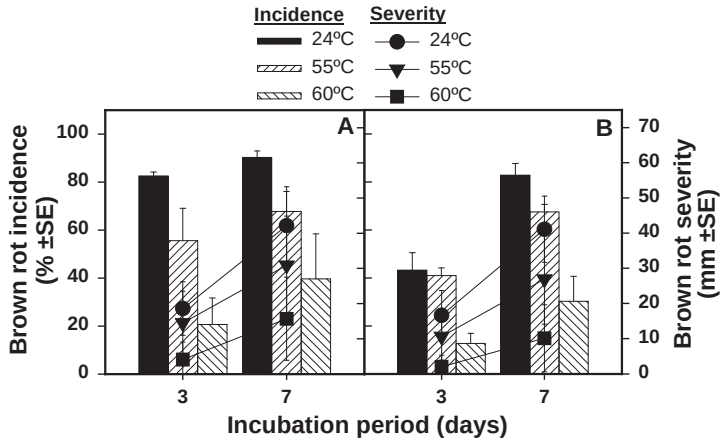

Fig. 2. Incidence (bars) and severity (lines) of brown rot on 'O'Henry' peaches wound inoculated with *Monilinia fructicola*, dipped 24 h later for 60 s in water alone (A) or aqueous solutions of 200 mM potassium sorbate (B) at 24, 55, or 60°C, and incubated at 20°C and 90% RH for 3 or 7 days.