Evaluation of Heated Solutions of Sulfur Dioxide, Ethanol, and Hydrogen Peroxide to Control Postharvest Green Mold of Lemons

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ABSTRACT

Smilanick, J. L., Margosan, D. A., and Henson, D. J. 1995. Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. Plant Dis. 79:742-747.

Lemon fruit were inoculated with spores of *Penicillium digitatum* and immersed in solutions of ethanol, sulfur dioxide, or hydrogen peroxide to control postharvest green mold. Green mold incidence and fruit injury were assessed after treatments employing various combinations of concentration, duration of treatment, temperature, and post-treatment rinses. Heating of the solutions was needed to attain acceptable efficacy. Sulfur dioxide and ethanol controlled green mold without injury to fruit, whereas hydrogen peroxide did not effectively control green mold and caused unacceptable injury to fruit. Treatments selected for extensive evaluation were immersion in 10% ethanol at 45°C for 150 s without rinsing, or in 2% sulfur dioxide at 45°C for 150 s followed by two fresh water rinses. These treatments were compared with two existing decay control methods: immersion in 3% sodium carbonate at 45°C for 150 s followed by two fresh water rinses, or in 1,000 µg/ml imazalil at 25°C for 60 s. Lemons were inoculated at 20°C then incubated for 12, 24, 48, or 60 h before treatments were applied. Efficacy of sulfur dioxide and ethanol treatments was comparable to that of sodium carbonate and imazalil. Sulfur dioxide and ethanol did not injure the fruit and their residues were low. The sulfur dioxide content of lemons immediately after treatment was less than 1 µg/ml. The ethanol content of lemons analyzed immediately after ethanol treatment was 58.6 (±9.6) µg/ml and 24.4 (±11.7) µg/ml after storage for 7 days at 20 C. The ethanol content of untreated fruit was 3.3 µg/ml.

Green mold of citrus, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., is one of the most economically important postharvest diseases of citrus worldwide (16). Powell (36) established that the primary infection courts of *P. digitatum* are wounds on fruit inflicted during harvest and subsequent handling. Eradication of these infections is required to achieve acceptable levels of control (15,16). Currently, green mold is controlled in the United States by applications of the fungicides ortho-phenyl phenate, imazalil, and thiabendazole. New methods are needed, because pathogen resistance to these chemicals has developed (8,17), and regulatory issues and public concerns about health risks of ingesting fungicide residues threaten the continued use of fungicides in the future (33). We evaluated a combination of heat and chemical control, employing compounds that have well-studied environmental and animal toxicological properties and extensive precedents as additives or natural components in foods. By selecting these compounds, we hope to facilitate their approval by minimizing health, environmental, and disposal issues posed by regulators. A cleaning process that meets these criteria is the immersion of fruit in heated solutions of sodium carbonate or bicarbonate, a practice described about seventy years ago (4,5,31). Our objectives were to evaluate heat combined with sulfur dioxide, ethanol, and hydrogen peroxide and to compare the efficacy of these treatments with that of treatments already available. Although all these compounds are produced in food grade quality, are approved as conditioners or antimicrobial additives for some foods, and occur as natural constituents in many foods (2,3, 45), they have not been approved for use on fresh citrus.

Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Accepted for publication 18 April 1995.

MATERIALS AND METHODS

Fruit. Lemons (*Citrus limon* (L.) N. L. Burm.) used in all experiments were California grown (cv. Eureka) and selected by hand from field bins after harvest, before any commercial postharvest treatments were applied. Lemons selected were light green to pale yellow in color and were stored no longer than 2 weeks after harvest at 10°C before use. Before each experiment, the lemons were washed with water on commercial processing equipment and randomized.

Inoculum. Petri dishes of potato-dextrose agar were inoculated with *P. digitatum* isolate M6R (from J. W. Eckert, University of California, Riverside) and incubated at 20°C for 1 to 2 weeks. Spores were rubbed from the agar surface with a glass rod after a small volume of sterile 0.05% Triton X-100 was added. The spore suspension was passed through two layers of cheesecloth and diluted with sterile water to an absorbance of 0.1 at 420 nm. This density, approximately equivalent to 1×10^6 spores per ml, is recommended for evaluation of postharvest treatments to control green mold (15). Each fruit was wounded and inoculated once with a saw-inoculation device (38), in which a saw blade spun continuously through the inoculum solution and made an injury 2 mm deep by 2 mm wide by 1 cm long on each fruit. The shallow wounds penetrated the albedo tissue but not the juice sacs and simulated natural inoculation (15).

Green mold incidence. The number of infected fruit was counted after 1 to 3 weeks of storage at 20°C. Little change in disease incidence occurred if storage extended beyond 3 weeks. This temperature was selected because green mold develops rapidly at this temperature and it represents a mean value among storage and transit temperatures encountered commercially.

Fruit quality. In every test, rind injuries were visually observed and rated for severity on a 0 to 3 scale of injury classes: 0 = none, no injury or not different from the control, 1 = minor, slight to 10% of rind surface injured; 2 = moderate, 11 to 30% of rind surface injured; and 3 = severe, more than 30% of rind surface injured. Green lemons are selected for storage because they tolerate long storage better than yellow lemons. During storage, lemons change from green to a marketable yellow color. Therefore, the influence of treatments on color change during subsequent storage is important. The surface color of lemons was analyzed with a tristimulus colorimeter with a 8 mm aperture (Minolta Model DP301). L*a*b* CIELAB chromaticity values (32) were recorded by triplicate determinations on lemons that had not been inoculated from each treatment at repeated intervals during storage.

Sulfur dioxide treatments. In preliminary experiments, a 1-min immersion of inoculated lemons in 1.5 to 2% (wt/wt) sulfur dioxide solutions without rinsing at 20 or 30°C reduced decay incidence 95% or more. However, the regulatory status of sulfur dioxide in those applications for which it is approved prevents its use on raw agricultural products when residues exceed 10 µg/g unless content labels are attached to the product (9). Therefore, to minimize residues we rinsed the fruit twice by brief immersion in cool (20°C) fresh water after sulfur dioxide treatment. In preliminary tests with two post-treatment rinses, acceptable control of green mold required heated solutions containing 1.5 to 2.5% sulfur dioxide. Reducing the pH of sulfur dioxide solutions enhances their toxicity, but unacceptable off-gassing can occur at low pH. Sulfur dioxide solutions were adjusted to pH 5 so as to minimize off-gassing, retain sufficient potency and buffer capacity, and minimize potential changes in solution pH that might occur during prolonged use of the solution for washing lemons. Because the pH of lemon rind tissue is 5.0 to 5.5 (7), minimal change in the solution pH should occur during repeated use. A 2% sulfur dioxide solution was prepared by adding 513 g of $NaHSO_3$, adjusting the pH to 5 with 5N HCl, and adjusting the volume to 15 liters. The solution was placed in a 30-liter glass tank suspended in a 300-liter temperature-controlled water bath and heated. Sulfur dioxide gas concentration in air above the treatment tank was measured by colorimetric dosimeters (Gastec Corp., Ayase, Japan). The sulfur dioxide concentration in the tanks before and after the treatments were applied was determined by titration with standardized iodine (45). Several drops of a starch indicator were added to a 10-ml aliquot of sulfur dioxide solution before titration with 100 mM iodine solution.

The sulfur dioxide content of lemons was determined by a colorimetric method (1). Four replicates, each composed of three fruit extracted together, were immersed for 1, 2, or 16 h in 50 mM sodium tetrachloromercurate (1 g of lemon fresh weight to 1 ml of extractant) on a shaker rotating at 75 rpm. This reagent retards oxidation of sulfur dioxide to sulfate. Aliquots of the extract were removed and added to acid-bleached pararosaniline hydrochloride and formaldehyde. The optical absorbance at 560 nm of each aliquot was recorded after 30 min incubation at 25°C. This value was compared with a standard curve. Limits of detection were about 0.5 µg of sulfur dioxide per gram of fresh weight of the fruit.

In initial experiments to determine optimal sulfur dioxide rates, lemons inoculated 5 h before treatment were immersed in 15 liters of a 2% solution of sulfur dioxide (pH 5) at 20, 30, 40, or 47°C (±1 C°) for 1 to 10 min. Control treatments included untreated inoculated fruit and inoculated fruit immersed for 1 to 10 min in 15 liters of water at 47°C (±1°C). Water control treatments were not included at each temperature, because only water at 47°C controlled green mold significantly in preliminary tests. Each replicate of the treated fruit was rinsed twice by sequential immersion for 5 s in two 10-liter volumes of fresh water immediately after treatment and stored at 20°C for 1 week. Each treatment was applied to four replicates of 25 lemons each. Experiments were repeated at least twice.

Ethanol treatments. Ethanol solutions were prepared by dilution of 95% ethanol with distilled water in 15-liter volumes in 30-liter capacity glass tanks suspended in a 300-liter temperature-controlled water bath as described previously. Ethanol concentration in the tanks throughout the tests was determined with a hydrometer.

To determine the ethanol content of lemons, four replicates of five lemons each were macerated in a centrifugal juicer immediately after treatment or after 7 days of storage at 13°C. The macerate was centrifuged for 10 min at 13,000 × *g* and passed through a 0.45 µm pore size filter to clarify

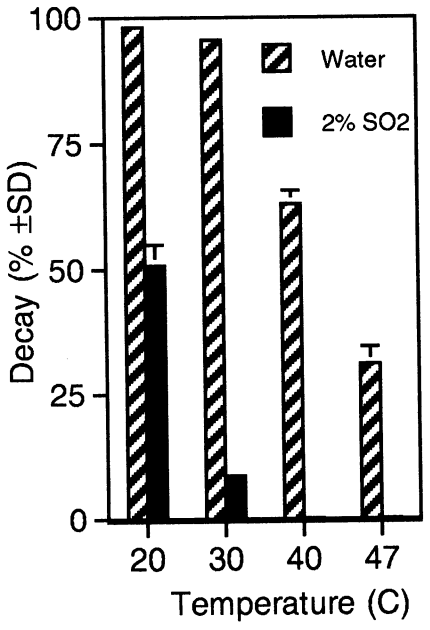

Fig. 1. Incidence of postharvest green mold of lemons after inoculation followed 5 h later by immersion for 10 min in water alone at 47°C or 2% sulfur dioxide solutions at 47°C followed by two fresh water rinses and storage for 1 week at 20°C. Each value is the mean of two experiments; the treatments in each experiment were applied to four replicates of 25 fruit.

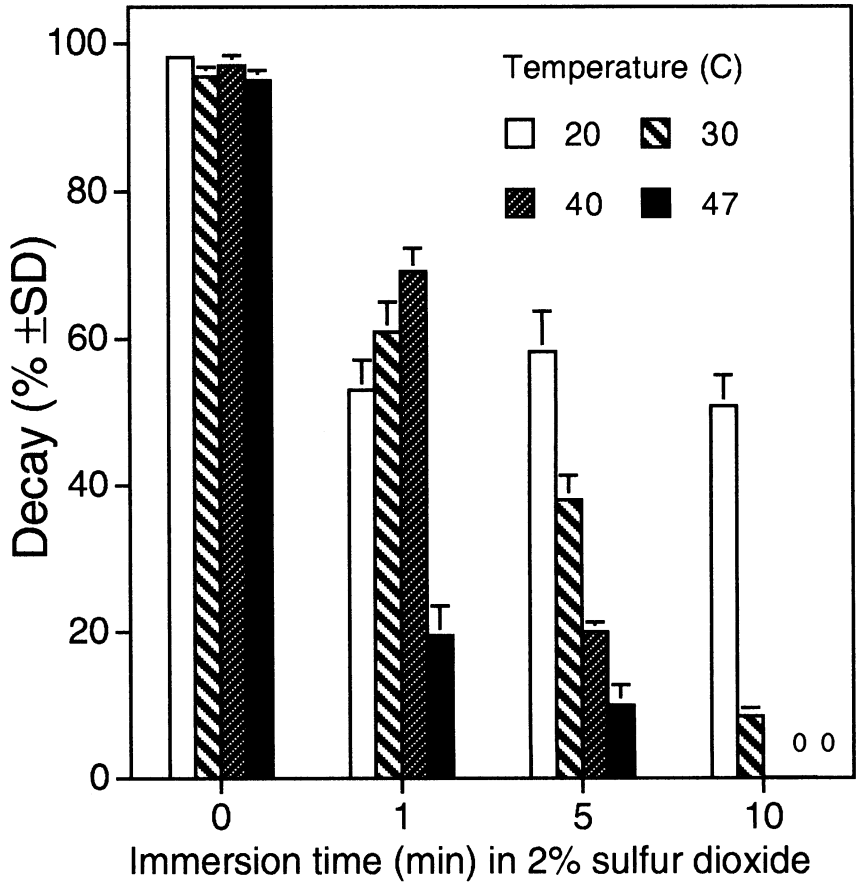

Fig. 2. Incidence of postharvest green mold of lemons after inoculation followed 5 h later by immersion for 1 to 10 min in 2% sulfur dioxide solutions at 20 to 47°C followed by two fresh water rinses and storage for 1 week at 20°C. Control fruit were inoculated but not treated (immersion time = 0). Each value is the mean of two experiments; the treatments in each experiment were applied to four replicates of 25 fruit.

it. Ethanol was measured by alcohol dehydrogenase conversion of ethanol to acetaldehyde in 500 mM glycine buffer at pH 9 with the simultaneous reduction of NAD to NADH. The consequent increase in optical absorbance at 340 nm was directly proportional to the ethanol concentration in the sample. The limit of detection was about 3 µg of ethanol per gram of fresh weight.

In initial experiments to determine optimal ethanol rates, lemons inoculated 18 h before treatment were immersed for 150 s in 15 liters of water alone (control) or in water containing 2.5, 5, 10, 20, or 40% ethanol at 32, 38, 44, or 49°C and stored at 20°C for 3 weeks. Each treatment was applied to four replicates of 25 lemons each. The experiment was repeated three times.

Hydrogen peroxide treatments. Solutions of hydrogen peroxide were prepared by dilution of concentrated food-grade hydrogen peroxide (35% a.i. wt/wt, Durox, Food Machinery Corp., PN) with distilled water to concentrations of 5, 10, and 15% in 15-liter volumes in 30-liter capacity glass tanks suspended in a 300-liter temperature-controlled water bath. The concentration of hydrogen peroxide throughout the test was determined by titration with potassium permanganate (40). A 1-ml sample of each hydrogen peroxide solution was added to 50 ml of 6N sulfuric acid and titrated with 200 mM potassium permanganate.

In initial experiments to determine optimal hydrogen peroxide rates, lemons inoculated 18 h before treatment were immersed for 10, 30, or 90 s in water alone (controls) or in 5, 10, or 15% hydrogen peroxide at 25°C and stored at 20°C for 1 week. This experiment was done once.

Comparative tests. Optimal treatments of ethanol and sulfur dioxide were compared with immersion in imazalil, heated water alone, or heated 3% sodium carbonate. Incidence of green mold, rind injuries, surface color, and ethanol and sulfur dioxide residues were determined. Treatments included 1) control, no treatment, 2) 150 s immersion in water at 45°C, 3) 150 s immersion in 10% ethanol at 45°C, 4) 150 s immersion in 2% sulfur dioxide at 45°C followed by two fresh water rinses, 5) 150 s immersion in 3% sodium carbonate at 45°C, followed by two fresh water rinses, and 6) 1 min dip in 1000 µg/ml imazalil (44% a.i., Bromazil 50, Brogdex Co., Pomona, Calif.). The ethanol and sulfur dioxide treatments were selected from our initial tests and consist of regimes predicted to produce acceptable efficacy with minimal risk of injury. The sodium carbonate treatment is within regimes described by Klotz (26), who recommended 3 to 4 min immersion in 1.5 to 3.0% sodium carbonate at 37.7 to 43.3°C for green mold, 46.1 to 47.8°C for sour rot caused by *Geotrichum candidum*, and 46.1 to 48.9°C for brown rot caused by *Phytophthora* spp. The imazalil treatment approximates typical recommended commercial rates for citrus. Lemons were inoculated and incubated at 20°C for 12, 24, 48, or 60 h before the treatments were applied. The number of fruit infected with green mold was counted after storage at 20°C for 1 and 3 weeks. Residues of ethanol and sulfur dioxide were determined in one experiment while decay control and rind injury were assessed in three experiments. In a separate experiment, long-term rind injury observations and L*a*b* CIELAB chromaticity values (32) were recorded on 15 noninoculated lemons at repeated intervals during storage at 13°C for 10 weeks. The temperature and the long duration of storage selected for this test simulate commercial storage practices in California (16). The experiment reported was done once; however, two experiments of shorter duration with similar results were done but are not reported.

Statistical analysis. In tests to control green mold, the replicate boxes were placed in a randomized pattern in temperature-controlled storage rooms and green

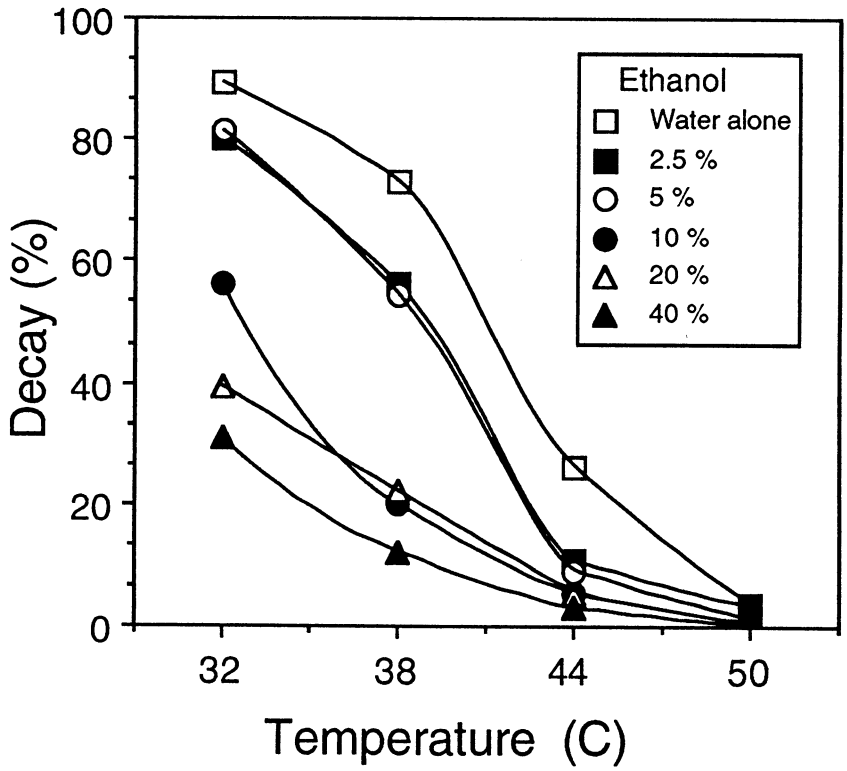

Fig. 3. Incidence of postharvest green mold of lemons after inoculation followed 18 h later by immersion in water alone or ethanol solutions for 150 s and storage for 1 week at 20°C. Each value is the mean of three experiments; the treatments in each experiment were applied to four replicates of 25 fruit.

Table 1. Incidence of postharvest green mold of lemons after inoculation 12, 24, 48, or 60 h before treatment by immersion in water, ethanol, sulfur dioxide, sodium carbonate, or imazalil and storage for 1 week at 20°C

Treatment	Percentage of decayed lemons				Regression	r^2	P
	12 h[y]	24 h[y]	48 h[y]	60 h[y]			
Inoculated, untreated control	96.3 a[z]	97.4 a	98.3 a	97.9 a	...	...	...
Water 45°C, 150 s	17.0 b	18.7 b	35.0 b	62.0 b	$y = 0.012(\log x) + 1.04$	0.922	0.03
Ethanol (10%) 45°C, 150 s	6.3 c	2.7 cd	13.3 dc	58.7 b	$y = 0.022(\log x) + 0.241$	0.717	0.15
Sulfur dioxide (2%) 45°C, 150 s with rinse	6.0 c	5.0 c	2.3 d	20.0 c	$y = 0.006(\log x) + 0.572$	0.111	0.67
Sodium carbonate (3%) 45°C, 150 s with rinse	2.0 c	0.3 d	6.3 dc	53.3 b	$y = 0.032(\log x) - 0.562$	0.565	0.25
Imazalil (1,000 µg/ml) 25°C, 60 s	3.0 c	1.7 cd	14.0 c	30.3 c	$y = 0.024(\log x) - 0.044$	0.848	0.08

[y] Hours between inoculation and treatment.

[z] Each value is the mean of three experiments; in each experiment the treatments were applied to four replicates of 25 fruit. Means in columns followed by the same letter are not significantly different (Fisher's protected least significant difference; $P \leq 0.05$).

mold incidence per replicate was recorded after 1 to 3 weeks. Data were analyzed using regression or one-way analysis of variance (ANOVA) procedures using log or arcsine-transformed percentages followed by Fisher's protected least significant difference (P = 0.05) to separate means. Actual percentages are reported. Surface color L*a*b* CIELAB chromaticity values (32) were recorded by triplicate determinations on lemons at repeated intervals during storage. Color difference values ($\Delta E = \text{sqrt}[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]$) and hue angles (arctangent (b*/a*)) from each observation were calculated after pooling the three values per fruit and analyzed by a one-way ANOVA (24).

RESULTS

Sulfur dioxide treatments. A 10-min treatment with 2% sulfur dioxide followed by two fresh water rinses controlled green mold better than 10-min treatments with water alone at the same temperatures (Fig. 1). Immersion for only 1 min at 47°C in 2% sulfur dioxide reduced green mold incidence to 19%, compared with more than 95% among controls (Fig. 2). Green mold incidence was reduced to less than 10% after sulfur dioxide treatments applied for 10 min at 30, 40, and 47°C.

No injury was observed after any of these treatments except after the most severe treatment (immersion for 10 min in 2% sulfur dioxide at 47°C). This treatment caused injury in one test of four conducted. The percentage of lemons from this treatment after 1 week storage that were classified with no injury, or minor, moderate, or severe injuries was 47, 23, 20, and 10%, respectively. Less than 2% of the lemons immersed for 10 min in water at 47°C had any injury.

Ethanol treatments. Green mold was effectively controlled by ethanol at concentrations of 10 to 20%, and control was superior to that of water alone at 32, 38, and 44°C (Fig. 3). At 50°C, all treatments reduced green mold to less than 5%. Little additional enhancement occurred when ethanol concentrations exceeded 10%. No injury to the lemons was observed.

Hydrogen peroxide treatments. Green mold was not effectively controlled by hydrogen peroxide, even after using treatments that injured the lemons. Results with tests using 5, 10, or 15% hydrogen peroxide were not significantly different. An immersion period of 30 s was slightly but significantly superior to 10 and 90 s treatments. Green mold incidence after 10, 30, or 90 s of hydrogen peroxide treatment and storage for 1 week at 20°C was 65.3, 55.0, and 73.3%, respectively, while decay among water-treated controls was 98%. Lemons were not injured by 10 or 30 s of treatment in hydrogen peroxide at 25°C, whereas rind injury was moderate to severe on all lemons after 90 s of treatment in 5, 10, or 15% hydrogen peroxide. The injury was a red to bronze discoloration limited to the flavedo, with these areas becoming brown and necrotic during storage.

The concentrations of sulfur dioxide, ethanol, and hydrogen peroxide in the tanks in which the lemons were immersed did not decline markedly during the treatments. No change in ethanol or hydrogen peroxide content was detected in the tanks after 200 lemons were treated. After 200 lemons had been treated at 47°C with 2% sulfur dioxide, 97% of the original sulfur dioxide content of the tank remained.

Comparative studies. Among untreated controls, decay exceeded 95% (Tables 1 and 2). Ethanol, sulfur dioxide, and sodium carbonate treatments were significantly superior to treatments using water alone at 45°C. After 1 week of storage, green mold incidence among lemons treated with ethanol, sulfur dioxide, sodium carbonate, and imazalil within 24 h

Table 2. Incidence of postharvest green mold of lemons after inoculation 12, 24, 48, or 60 h before treatment with water, ethanol, sulfur dioxide, sodium carbonate, or imazalil and incubation 3 weeks at 20°C

Treatment (min)	Percentage of decayed lemons				Regression	r^2	P
	12 h[y]	24 h[y]	48 h[y]	60 h[y]			
Inoculated, untreated control	98.4 a[z]	98.7 a	99.0 a	98.5 a	...	...	...
Water 45°C, 150 s	21.3 b	23.3 b	39.7 b	69.3 b	$y = 0.01(\log x) + 1.157$	0.937	0.03
Ethanol (10%) 45°C, 150 s	10.3 c	3.7 d	18.0 bc	67.0 b	$y = 0.011(\log x) + 1.026$	0.446	0.33
Sulfur dioxide (2%) 45°C, 150 s with rinse	11.3 c	13.7 c	24.3 bc	50.7 bc	$y = 0.013(\log x) + 0.854$	0.937	0.03
Sodium carbonate (3%) 45°C, 150 s with rinse	3.0 d	0.7 d	8.3 c	56.3 bc	$y = 0.03(\log x) - 0.338$	0.682	0.17
Imazalil (1,000 µg/ml) 25°C, 60 s	3.0 d	2.7 d	22.7 bc	46.0 c	$y = 0.027(\log x) - 0.007$	0.935	0.03

[y] Hours between inoculation and treatment.

[z] Each value is the mean of three experiments; in each experiment the treatments were applied to four replicates of 25 fruit. Means within columns followed by the same letter are not significantly different (Fisher's protected least significant difference; $P \leq 0.05$).

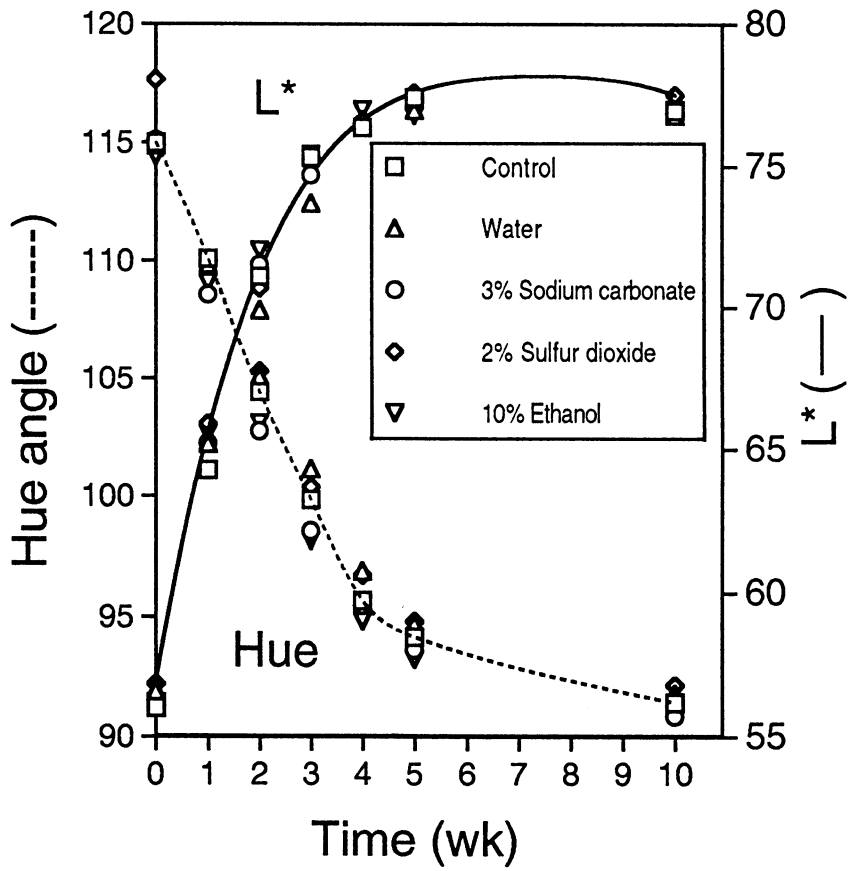

Fig. 4. Color (hue angle; 90 = yellow, 115 = green) and brightness (L*; low value = dark, high value = light) of lemons during 10 weeks of storage at 13°C after no treatment (control) or after immersion for 150 s in water or solutions of sodium carbonate, sulfur dioxide, or ethanol at 45°C. No significant (P = 0.05) color differences among treatments were present at any evaluation.

of inoculation was approximately 5% or less; if applied 48 h after inoculation, the incidence of fruit with green mold was about 15% or less. Green mold was poorly controlled when treatments were applied 60 h after inoculation. After 3 weeks of storage, green mold incidence among lemons treated with ethanol, sodium carbonate, or imazalil within 24 h of inoculation was 3.7% or less, while among lemons treated with sulfur dioxide it was 13.7%. The incidence of green mold infections after sodium carbonate treatment was approximately equal to that of the imazalil treatment and lower than that of the ethanol and sulfur dioxide treated lemons.

The color of lemons after application of the treatments employed in the comparative tests changed from green to yellow during storage at 13°C for 10 weeks (Fig. 4). Hue and L* values changed at the same rate during storage; no significant (P = 0.05) color differences among treatments were present at any evaluation. No lemons were visibly injured by any of these treatments.

The ethanol content (± standard deviation) of lemons within 1 day or 7 days after ethanol treatment was 58.6 (±9.6) µg/ml and 24.4 (±11.7) µg/ml, respectively. The ethanol content of untreated fruit was 3.3 (± 1.1) µg/ml. The sulfur dioxide content of lemons immediately after treatment was less than 1 µg/ml.

DISCUSSION

Solutions of sulfur dioxide, ethanol, and sodium carbonate were superior to hot water alone for the control of green mold. They did not injure fruit when optimal rates were applied. Hydrogen peroxide injured the fruit and did not control decay effectively.

Sulfur dioxide was an excellent eradicant when green mold incidence was assessed after 7 days but was inferior to most of the other treatments when incidence was evaluated after storage for 3 weeks. Sulfur dioxide is a common and well-studied compound. It is an antimicrobial additive in dried fruit and fruit juices and it is used as a gas on table grapes to control gray mold caused by *Botrytis cinerea* (43,45). Evidence for the mode of action of sulfur dioxide indicates that its accumulation in the cytoplasm causes a disruptive decline in pH, and its reaction products within the cell deplete critical constituents (20,42,43). Sulfur dioxide employed as we tested it did not cause hazardous off-gassing. Brief rinsing with water was sufficient to reduce residues below the detection limits of the analysis we employed and below the regulatory action level of 10 µg/g established for other products (3).

Ethanol was equal in efficacy to the other treatments after 1 week of storage and only slightly inferior when its efficacy was assessed after 3 weeks. Ethanol is a common, naturally occurring substance in many foods and is an approved preservative for some (2). Its most probable mode of action is the denaturation of proteins, particularly those of mitochondrial membranes (30). The natural ethanol content of citrus fruit is within the range we report after ethanol treatment (13,25,39), and is below that generally of concern to regulators (2). Mature fruit contain more ethanol than less mature fruit; Davis (12) reported juice of Hamlin and Valencia oranges contained 5 to 40 µg/ml of ethanol early in the season and 254 to 480 µg/ml late in the harvest season.

Control of green mold by sodium carbonate was outstanding, particularly when green mold incidence was evaluated after 3 weeks of storage. Sodium carbonate is an approved food additive for many applications (4); however, little has been published to systematically quantify its ability to control postharvest decay. Using fruit inoculated 24 h before treatment, Houck (22) reported immersion of lemons in 3% sodium carbonate at approximately 45°C for 4 min reduced green mold more than 90% compared with controls. It was ineffective when applied at 25 to 27°C. Evidence describing the mode of action of sodium carbonate was first presented by Marloth (31) who reported that the germ tube elongation of *P. digitatum* was greatly inhibited above pH 7, and that a solution of sodium carbonate (pH 11 to 12) raises the pH of infection court tissue in the lemon rind to that inhibitory to *P. digitatum.* Pelser and Eckert (35) confirmed that hyphal elongation of *P. digitatum* was inhibited above pH 7.5. Marloth (31) observed that sodium bicarbonate and sodium carbonate had insufficient activity for a direct mode of action, leading to mortality of green and blue mold spores. Many spores survived severe treatments (5 min exposure to 6% sodium carbonate or sodium bicarbonate) and the fungus could germinate at pH 9 to 9.6, higher than the pH of the carbonate solutions that provide good decay control. Marloth's assays were conducted at ambient temperature, however, and he probably underestimated the influence of heat on action of the carbonates. Most biocides show a two- to threefold increase in potency for each 10°C increase in temperature (29). Homma and co-workers similarly concluded pH alone could not provide control of green mold, because other buffers of high pH were ineffective (21). Other workers stated similar conclusions that elevated pH alone is insufficient to constitute the mode of action of carbonates on fungi (11,14). Punja and Grogan (37) stated the proportion of carbonate and bicarbonate ions, rather than high pH alone, affected inhibition of *Sclerotium rolfsii,* although, like *P. digitatum,* growth of this fungus was inhibited at pH 8 and above. Carbon dioxide, a product of carbonate decomposition, is inhibitory at high concentrations to many fungi, particularly when accompanied by moderate positive pressure, and could conceivably have a role in the inhibition of these fungi (45).

The risk of injury to fruit deserves special attention in the application of the treatments we developed because sulfur dioxide, ethanol, and sodium carbonate are nonselective toxicants. Furthermore, in addition to the risk of chemical injury, injury by heat treatments to citrus fruit has been reported, although usually at temperatures higher than we evaluated. Citrus fruit can vary widely in their tolerance to heat, even within the same variety (18,19, 23). A subtle type of injury was described by Harding and Savage (19) when they compared sodium carbonate efficacy with naturally infected lemons at 32 and 48°C using more than 250,000 fruit. In their tests, fruit did not have any visible injury even after the most severe treatment (immersion for 2 min in 4% sodium carbonate at 48°C); however, the more mature of two lemon collections exhibited an increased, rather than reduced, incidence of green mold and blue mold (caused by *Penicillium italicum*) after 3 weeks storage. They (19) suggested the natural resistance of lemons to infection was reduced by sodium carbonate treatment and that the enhanced susceptibility to infection by airborne spores in storage resulted in increased decay. The influence of ethanol and sulfite treatments on fruit resistance to subsequent inoculation is unknown. In our work, all fruit were artificially inoculated, and some investigators warn that confirmatory studies with naturally inoculated fruit should be conducted to confirm conclusions obtained with artificially inoculated fruit (5,10,19,27). For example, Barger (5) stated lower concentrations of bicarbonate were needed to control green mold of naturally inoculated oranges than those artificially inoculated. Other workers who immersed lemons and other citrus in water without subsequent injury employed higher temperatures and longer duration treatments than we did in our work (6,23,41). To control green mold, Houck (23) recommended 5 to 10 min immersion of lemons in water at 51.7°C or 1 min at 54.4°C. This treatment was tolerated by yellow, winter-harvested lemons, the most sensitive fruit he tested, and readily tolerated by green, summer-harvested lemons, the most resistant fruit to heat injury. In addition to control of decay, other benefits have been reported for similar hot water treatments. No injury and a subsequent reduction in chilling injury during storage of grapefruit (39,44), Valencia oranges (44), and Washington navel oranges (44) occurred after immersion in water at 50°C for 2 min. Klotz and DeWolfe (27,28) cautioned that although lemons could routinely tolerate immersion in water for 4 min or longer at 46 to 49°C without injury, release of rind oils leading to oleocellosis could occur if

lemons were cold and turgid at the time of immersion. They (27,28) and others (18, 23,34) recommended fruit conditioning, which consists of a delaying immersion for 1 to 4 days after harvest to allow the rind to lose turgor. Without conditioning, turgid, freshly picked, winter-harvested lemons could be injured by temperatures as low as 38°C (18). As an additional precaution, Klotz and DeWolfe (27, 28) suggested using a soap in a post-treatment rinse to entrap released oils and terpenoids to further reduce chances of rind injury.

Sulfur dioxide, ethanol, and sodium carbonate could be valuable components in strategies of alternating treatments to manage fungicide resistance, particularly since they have modes of action different from those of the fungicides currently employed by this industry. Substitution of these treatments for fungicides on lemons that are entering long storage would reduce the selection of fungicide-resistant strains that occurs during storage within packinghouses. Sanitation in packinghouses after sulfur dioxide, ethanol, or sodium carbonate treatments would be of particular importance, since these treatments do not confer the persistent protection from decay and control of sporulation that fungicides usually provide. Although sulfur dioxide, ethanol, or sodium carbonate did not reduce sporulation in our work, control of sporulation during storage is of less importance than when fungicides are used because the spores produced would not be fungicide resistant and the cosmetic contamination of adjacent fruit by spores ("soilage") is removed when the stored fruit are re-processed for final packaging and marketing. The treatments could also be applied after storage before final packaging. The risk of fruit injury would presumably be minimized if the treatments were applied at this time since, after storage, fruit should lose turgor and become conditioned to better tolerate heat.

ACKNOWLEDGMENTS

We thank the California Citrus Research Board for financial assistance, Klink Citrus Association in Ivanhoe, Calif., for generously supplying fruit, and G. E. Brown, L. G. Houck, J. W. Eckert, P. Lewis, and R. G. McGuire for useful comments and suggestions.

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