Postharvest Control of Table Grape Gray Mold on Detached Berries with Carbonate and Bicarbonate Salts and Disinfectants

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The control of postharvest gray mold on detached table grape berries by treatment with carbonate and bicarbonate salt solutions, alone or with chlorine, ozone, or ethanol, was evaluated. Sodium carbonate (SC), potassium carbonate (PC), sodium bicarbonate (SBC), potassium bicarbonate (PBC), and ammonium bicarbonate (ABC) were tested without control of pH for their toxicity to spores of *Botrytis cinerea* in vitro, and the concentrations that stopped germination of 95% (EC_{95}) of the spores were 16, 17, 36, 58, and 163 mM, respectively. When bicarbonate solutions were adjusted to pH 7.2 (±0.2), the mean EC_{95} concentrations for two *B. cinerea* strains of ABC, SBC, and PBC were 48, 102, and 112 mM, respectively. In 1.5 µg/ml of ozone in water, 50% and 95% mortality of spores of *B. cinerea* occurred after 21.3 and 35.6 sec, respectively. In tests to control gray mold on grapes, among the bicarbonates, each applied at 500 mM, ABC was significantly more effective than SBC and PBC. It was also superior to PC (100 mM) and chlorine (200 µg/ml) and equal in effectiveness to SC (100 mM) and ethanol (70% wt/vol). The addition of 200 µg/ml chlorine to the bicarbonate salts significantly decreased gray mold incidence. Ozone in water at 10 µg/ml significantly controlled gray mold, although its efficacy was irregular and dependent on grape condition. Among all the treatments, berry condition was an important factor; for example, there was significant decrease in control when wounded berries were treated compared to unwounded berries. The quality of grapes after treatment with ABC, SBC, ethanol, and chlorine was acceptable; ozone in water caused minor rachis injury; while severe injuries, mostly brown spots on berries, occurred after SC, PC, and PBC treatments.

Gray mold, caused by the fungus *Botrytis cinerea* Pers., is the most economically important postharvest disease of table grapes [2]. *B. cinerea* is especially troublesome because of its vigorous growth rate and ability to spread among berries even at cold temperatures (-0.5°C). Infections that cause postharvest losses can originate from spores on the surface of the berries, microscopic latent infections that occurred before harvest during the growing season, or visibly infected berries that escaped removal during packaging [12]. The fungus produces abundant white surface mycelia, which spread from infected to healthy berries, so that an uncontrolled infection from a single berry can infect an entire package of grapes. Postharvest gray mold is usually controlled by an initial sulfur dioxide fumigation, followed by weekly fumigations during cold storage [35].

Recently, there has been commercial interest in packaging detached berries in transparent boxes rather than the conventional packages of whole grape clusters. Advantages of these packages are that they are conveniently sized to suit a single consumer purchase, the producer is identified on the label, the hard box protects the berries, and shattered berries, often lost from loose bulk displays of grapes, can be placed inside the boxes and retained for sale. The removal of berries from the clusters increases distribution of the inoculum of *B. cinerea*, and the detachment of berries from pedicels creates large wounds. This additional handling offers an opportunity to apply a postharvest treatment; postharvest treatments are now difficult to incorporate commercially because most growers pack dry grapes at harvest into their final commercial packages in the vineyard. Sulfur dioxide fumigation of packages of single detached berries is less feasible because the berries are more tightly packed together and penetration of sulfur dioxide would be reduced. Furthermore, the pedicel-removal scar provides an entry point for the accumulation of excessive sulfur dioxide residues, probably above the tolerance of 10 mg/kg, that cause unsightly bleaching injuries [5,35]. Sulfur dioxide injuries on berries also make them more susceptible to subsequent infection by *B. cinerea* [40]. Because of the issues associated with sulfite residues and its negative impact on the quality of detached berries, alternative strategies to control gray mold are needed that are safe, effective, and economical.

Sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, and ammonium bicarbonate are common food additives [3,13] for leavening, pH control, taste, texture modification, and spoilage control, and they inhibit various plant pathogens [19,17,25]. They also successfully control powdery mildew on roses [11], cucumbers [10,45], and euonymus [44]. The postharvest pathogen *Rhizoctonia carotae*

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[31] was controlled in vitro; however, these salts did not control crater rot, caused by the same pathogen. Black root rot of carrot caused by *Chalara elegans* was successfully suppressed by sodium bicarbonate and potassium bicarbonate [28], as was silver scurf of potato caused by *Helminthosporium solani* [23]. Sodium bicarbonate controls postharvest green mold, caused by *Penicillium digitatum* on citrus fruit [36], and is in common commercial use. The addition of surfactants improved effectiveness of sodium bicarbonate against green mold on citrus [9]. Preharvest application of 2% potassium bicarbonate on bell peppers significantly reduced postharvest gray mold, caused by *B. cinerea* [4], but concentrations of 3% were phytotoxic, causing shriveling, weight loss, and increased gray mold incidence. Preliminary research demonstrated the potential of carbonate and bicarbonate salts for the control of postharvest gray mold on grapes [18].

In 1997, an expert panel reviewed the safety and potential for food processing use of ozone and declared ozone to be generally recognized as safe (GRAS) for food contact applications [8,41]. Since that time, the interest of developing ozone applications in the food industry has increased, although some of the regulatory issues are not yet resolved [30]. Ozone in water is often described as an alternative to chlorine as a disinfectant or sanitizer [34]. Significant advantages of ozone in water are that it quickly decomposes to oxygen, it leaves no residues, and it has more potency against bacteria, protozoa cysts, viruses, and fungal spores than chlorine [42]. Ozone can have a role in reducing pesticide residues [20,24] and mycotoxins [16]. Ozone controlled *Rhizopus stolonifer* on table grapes and, furthermore, induced the reservatol and pterostilbene phytoalexins in grape berries, making them more resistant to subsequent infections [32]. Ozone in water inactivated the spores of *B. cinerea* on the surface of tomatoes, but it did not control the pathogen inoculated in wounds like *B. cinerea* on tomato [21], *Penicillium digitatum* on citrus [34], and *Penicillium expansum* on pear [37].

Ethanol was reported as an effective antifungal agent for controlling some postharvest pathogens [6,22,43], especially if heated [14]. Its postharvest application did not affect fruit quality [27] and even enhanced certain quality parameters of some fruit [14,26]. Chlorine (Cl_2) is a potent disinfectant with powerful oxidizing properties. It is soluble in water, either by injection of chlorine gas or by addition of hypochlorite salts. Chlorine in aqueous solutions consists of a mixture of chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions ($OC1^-$) in ratios controlled by pH. The concentrations generally used in postharvest applications are 50 to 200 µg/mL [39]. At concentration of 50 µg/ml, chlorine significantly inhibited germination of *B. cinerea*, but did not control disease in previously inoculated pear fruit [38].

Our objectives were to evaluate carbonate, bicarbonate, and ozone solutions for the inhibition of germination of spores of *B. cinerea* and for their ability to control postharvest gray mold on detached grape berries, to compare the efficacy of these solutions alone or in combination with a surfactant or the sanitizers ethanol and chlorine, and to determine the impact of these treatments on berry quality.

Materials and Methods

Inoculum preparation. Spores were harvested from two-week-old potato dextrose agar (PDA) cultures of *B. cinerea* isolate 93-58, BCG5, or BCG8 grown at 25°C. Five mL of sterile water, containing 0.05% (vol/vol) Triton X-100, was added to a petri dish culture, the spores were gently dislodged from the surface with a sterile glass rod, and the suspension was filtered through three layers of cheesecloth to remove mycelial fragments. The suspension was diluted with water to an absorbance of 0.25 at 425 nm as determined by a spectrophotometer. This density contains approximately 1 x 10^6 spores/mL.

Inhibition of spore germination by carbonate and bicarbonate solutions. To determine the influence of bicarbonate and carbonate solutions on spore germination of *B. cinerea*, a 10 µL aliquot containing 1 x 10^5 spores of isolate 93-58 was added to potato dextrose broth (PDB) that contained 0, 10, 20, 30, 40, 50, 70, or 100 mM of sodium carbonate (Na_2CO_3), potassium carbonate (K_2CO_3), sodium bicarbonate ($NaHCO_3$), potassium bicarbonate ($KHCO_3$), or ammonium bicarbonate (NH_4HCO_3). Three replicates each were prepared from anhydrous salts (Sigma Chemical Co., St. Louis, MO) to a final volume of 4 mL. To determine whether pH contributed to spore mortality, in the first test, pH of the solutions was not controlled, increased with increased salt concentration, and ranged from 8.5 to 9.5. In the second test, pH of the bicarbonate solutions was constant and adjusted to 7.2 ± 0.2 by the addition of 0.5 mL of 40 mM KH_2PO_4 and of 0.5 mL of 40 mM K_2HPO_4 to 3 mL of PDB. Two isolates (BCG5 and BCG8) were tested to determine if differences in susceptibility between isolates were present. After an 18 hr incubation at 22°C, 100 µL of acid fuchsin (0.2% wt/vol acid fuchsin in 50% vol/vol acetic acid and 50% vol/vol of 95% ethanol) was added to each well to stop germination. Germinated and nongerminated spores were counted by observation with an inverted compound microscope (200x). Within each replicate, 100 to 150 spores were examined and the percentage of germinated spores calculated.

Ozone in water equipment. Ozonated water was prepared from ozone gas that was generated from pure oxygen gas that flowed through a water-cooled, corona discharge unit (Technozone, Inc., Corona del Mar, CA). Ozone gas was dissolved in water in two 300 L ozone gas contactor tanks and the ozonated water pumped continuously through a 1000 L tank and returned to the contactor tanks at an exchange rate of one tank volume every 4 min. Ozone concentration in water was continuously monitored with an ozone selective electrode (Rosemont Engineering, Inc., Irvine, CA) and did not change significantly during the treatments. The concentration of ozone in water was measured colorimetrically (indigo blue test) with a Hach DR 890 colorimeter to calibrate the ozone selective electrode and periodically verify the ozone concentration.

Spore mortality in ozonated water. A spore suspension of *B. cinerea* (isolate 93-58) was prepared as previously described. A 0.2 mL aliquot of the spore suspension was placed on a 20 mm-diameter sterile filter with 3-µm pores and clamped onto a porous glass support. The water was removed from the suspension by the brief application of a low-pressure vaccum to the supported filter. Water containing 1.5 µg/mL ozone flowed through the filter at a rate of 1.6 mL/s. At the end of each exposure period, 3 mL of 1000 µg/mL calcium thiosulfate was added to destroy any remaining ozone, followed by 5 mL of sterile water, and the excess water was then removed by low-pressure vacuum. The filter was removed from the support, inverted, and placed on PDA, where most of the spores were deposited. After 18 hr incubation at 25°C, the proportion of germinated spores was determined by microscopy as previously described.

Fruit inoculation. Table grape cultivars Thompson Seedless, Flame Seedless, Ruby Seedless, and Perlette of recent harvest were obtained from a commercial storage. We harvested Crimson Seedless grapes from vines just before use. Single berries were cut from the rachis with the pedicel attached or detached, depending on the experiment, and placed on a metal rack. In most tests, the berries were fresh and undamaged; in some tests the berries looked aged and had obvious damage. On damaged berries, the pedicels were loose and often the tissue around the pedicel was torn, exposing the berry flesh, and cracks were present on the berry surface. Two inoculation methods were employed: (1) a 15 µL droplet containing ~250 spores of *B. cinerea* was placed on various parts of the berry, including randomly on the berry surface, near the pedicel, or within a wound on the berry ("droplet inoculation"); and (2) suspensions that contained 12.5×10^3 spores/mL were sprayed on the berry surfaces for a few seconds to each replicate until they were evenly coated ("spray inoculation"). The temperature of the fruit at the time of inoculation was 20°C (±1°C). Approximately 45 min after inoculation, the spore suspension had dried and the treatments were applied.

Control of gray mold on table grapes. Forty-five minutes after the berries were spray inoculated, as previously described, 0, 100, and 500 mM of sodium carbonate, potassium carbonate, sodium bicarbonate, potassium bicarbonate, or ammonium bicarbonate solutions were sprayed on single grape berries placed on a metal rack (n = 50) until run-off. The solutions were applied alone or in combination with 200 µg/mL of NaOCl. In ozone experiments, inoculated berries were immersed for 1 to 6 min in the stream of ozonated water containing 10 µg/mL of O_3; some treatments were followed by application of sodium or potassium bicarbonate. In some experiments, berries were inoculated (as described previously) 24 hr before ozone treatments to determine whether ozone could eradicate infections of this age. To determine whether treatment with the stream of water alone would influence the incidence of gray mold by removing the spores, inoculated berries were immersed for 1 to 4 min in the stream of water without ozone. Some treatments were compared to those of 200 µg/mL NaOCl alone, or 60 or 70% (vol/vol) ethanol, or fumigation with sulfur dioxide gas. In each test the control treatments were inoculated fruit treated with water.

Two methods of bicarbonate application were compared: (1) spraying until run-off and (2) immersion of the berries in the solution for 15 sec. The influence of surfactants on the effectiveness of carbonate and bicarbonate solutions, applied by immersion or by spraying the berries, was assessed by their application alone or with surfactant (0.1% vol/vol Triton X-100). Single table grape berries on wire racks were fumigated for 45 min with sulfur dioxide gas. Sulfur dioxide was dispensed from a low-pressure cylinder (Snowden Enterprises, Malaga, CA) into a 30 m^3 chamber at a concentration of 2000 µL/L for 45 min at 5°C.

After treatment, berries were placed on a metal rack inside plastic boxes humidified with paper tissue soaked with 150 mL of water on the bottom of each box. Berries were incubated for 7 days at 15°C, then berries that developed gray mold were counted and the incidence (%) was calculated.

Grape berry quality. The impact of all treatments on berry quality was assessed on Crimson Seedless grapes harvested the same day the treatments were applied. Large commercial-sized clusters were cut into small clusters of 15 to 20 berries, each weighing about 100 grams, and then they were randomized. Five small clusters, which comprised each replicate, were dipped into: (1) 7 µg/mL of ozonated water for 4 min; (2) 20 or 200 µg/mL of NaOCl for 1 min; (3) 70% ethanol for 1 min; (4) water alone for 1 min; or (5) 500 mM of Na_2CO_3, K_2CO_3, $NaHCO_3$, $KHCO_3$ or NH_4HCO_3 for 15 sec. Grapes were put into open-topped plastic bags, stored at 3°C for 8 days, and then their quality was assessed. Quality observations included: (1) berry appearance (visual injury index 0 to 5, where 0 = no injury); (2) rachis appearance (visual injury index 0 to 8, where 0 = no injury); (3) weight loss; (4) color change (color recorded as CIE Lab color space determined with a Minolta CR-200 surface colorimeter [Minolta Corp. Ramsey, NJ]) of 20 randomly selected berries per replicate, followed by calculation of hue angles [15]; and (5) firmness (displacement in mm of individual berries under 0.68 kg force applied by Instron Model 4201 materials tester [Instron, Canton, MA] of a randomly selected sample of 20 berries per replicate). After 30 days storage at 3°C (±1°C) the same grapes were checked again for weight loss and firmness.

Statistical analysis. Finney's Probit analysis was used to calculate concentrations and estimate upper and lower fiducial limits of carbonate and bicarbonate salts that stopped germination of 50 and 95% of the spores [7]. The same analysis was applied to spore mortality in ozone tests, except the dosage was calculated from exposure periods in ozonated water that prevented subsequent spore germination. The incidence of gray mold was analyzed by an analysis of variance applied to the arcsine of the square root of the proportion of infected berries, followed by Fisher's Protected LSD ($p = 0.05$) to separate means (SuperANOVA, Abacus Concepts, Inc., Berkeley, CA). Actual values are shown.

Results

Inhibition of spore germination by carbonate and bicarbonate solutions. At native pH, the carbonate salt solutions were significantly more toxic than bicarbonate solutions (Table 1). Ammonium bicarbonate was toxic at lower pH than the other salts. The toxicity of ammonium bicarbonate (EC_{50} = 20 mM) to spores was higher than that of sodium bicarbonate (EC_{50} = 26 mM) or potassium bicarbonate (EC_{50} = 40 mM). No significant (p = 0.05) differences in germination were found between two isolates of *B. cinerea* (BCG5 and BCG8) (Fig. 1, Table 2). When bicarbonate solutions were tested at pH 7.2 (±0.2), they were less toxic than at higher pH. The EC_{50} concentrations of ammonium bicarbonate, sodium bicarbonate, and potassium bicarbonate at pH 7.2 were 26, 46, and 48 mM, respectively. The ammonium salt was significantly more toxic, with about twice the potency of the other bicarbonate salts (Table 2).

Table 1 Concentrations (mM) of bicarbonate or carbonate salts in potato dextrose broth that inhibited the germination of spores of *B. cinerea* isolate BCG5.

Salt	EC_{50}[a]		pH at EC_{50}	EC_{95}		pH at EC_{95}
Na_2CO_3	11	(10, 12)	9.2	16	(14, 22)	9.5
K_2CO_3	12	(11, 16)	9.2	17	(14, 35)	9.5
NH_4HCO_3	20	(15, 26)	8.5	36	(27, 79)	8.6
$NaHCO_3$	26	(23, 30)	9.1	58	(50, 70)	9.4
$KHCO_3$	40	(20, 85)	9.3	163	(78, 1443)	9.5

[a]Each value was calculated from three observations. Values in parentheses are upper and lower 95% fiducial limits.

Table 2 Concentrations (mM) of bicarbonate salts in potato dextrose broth at pH 7.2 (±0.2) that inhibited the germination of spores of two isolates of *B. cinerea*.

	Isolate BCG5				Isolate BCG8			
Salt	EC_{50}[a]		EC_{95}		EC_{50}		EC_{95}	
NH_4HCO_3	26	(24, 27)	45	(42, 50)	34	(32, 37)	51	(48, 55)
$KHCO_3$	46	(34, 65)	102	(71, 387)	57	(55, 60)	85	(79, 92)
$NaHCO_3$	48	(37, 64)	121	(83, 368)	57	(55, 60)	101	(93, 114)

[a]Each value was calculated from three observations. Values in parentheses are upper and lower 95% fiducial limits.

Spore mortality in ozonated water. The inhibition of germination of *B. cinerea* spores after exposure to 1.5 µg/mL of ozone in water for different contact times is shown in Fig. 2. LD_{50} and LD_{95} mortality, estimated by probit analysis, occurred after 21.3 and 35.6 seconds exposure in 1.5 µg/mL ozone, respectively.

Control of gray mold on table grapes. In repeated tests where bicarbonates at 500 mM were compared, the reduction in gray mold incidence following ammonium and sodium bicarbonate treatments was similar, while potassium bicarbonate was usually significantly inferior (p = 0.05, Fig. 3, Fig. 4). Among the bicarbonates, each applied at 500 mM, ammonium bicarbonate was significantly more effective than sodium and potassium bicarbonate in controlling gray mold on table grapes (Fig. 4). Ammonium bicarbonate was also superior to potassium carbonate (100 mM) and chlorine (200 µg/mL) and equal in effectiveness to sodium carbonate (100 mM) and ethanol (70% vol/vol). Because the carbonates caused immediate darkening of pedicels and dark brown spots on berries, we did not include them in subsequent tests. Injuries caused by the other treatments were none or very minor.

Ozone in water at 10 µg/mL significantly controlled gray mold, although its efficacy was irregular and very dependent on grape condition (Fig. 5). When undamaged grape berries were treated with ozone 45 min after inoculation (tests 1, 3, and 4, Fig. 5), gray mold was reduced up to 70% compared to the controls in tests 3 and 4. Prolonged exposure to ozone did not improve its effectiveness. Water treatment alone did not influence gray mold incidence. There was no reduction in gray mold incidence when grape berries were inoculated 18 hr before

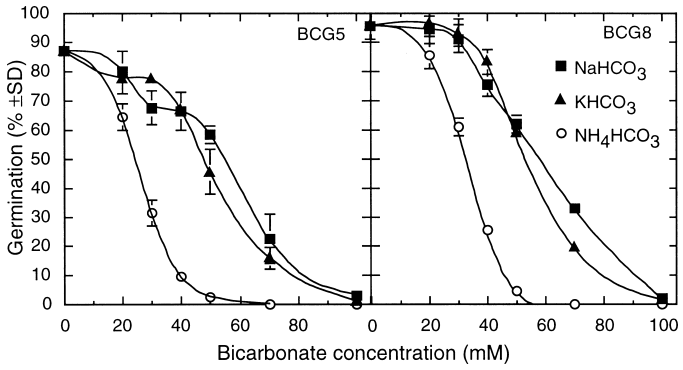

Fig. 1 Germination of the spores of two isolates of *B. cinerea* after 18 hr incubation at 22°C at pH 7.2 (± 0.2) in potato dextrose broth containing bicarbonate salts.

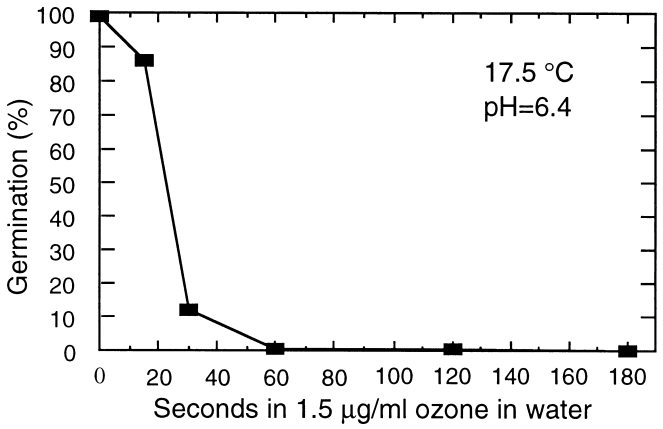

Fig. 2 Germination of spores of *B. cinerea* after exposure to water containing ozone at 1.5 µg/mL followed by incubation on potato dextrose agar at 20°C for 18 hr.

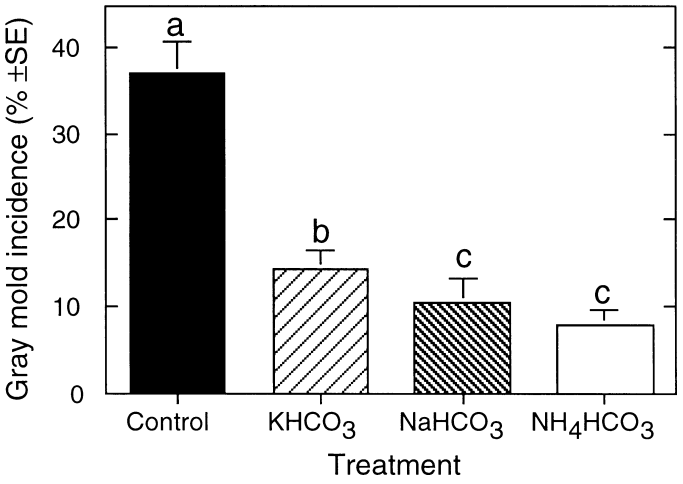

Fig. 3 Gray mold incidence on inoculated table grape berries of Thompson Seedless, Flame Seedless, or Perlette, after treatment with water alone (control) or 500 mM of bicarbonate solutions, followed by 7 days storage at 15°C. Average of six experiments.

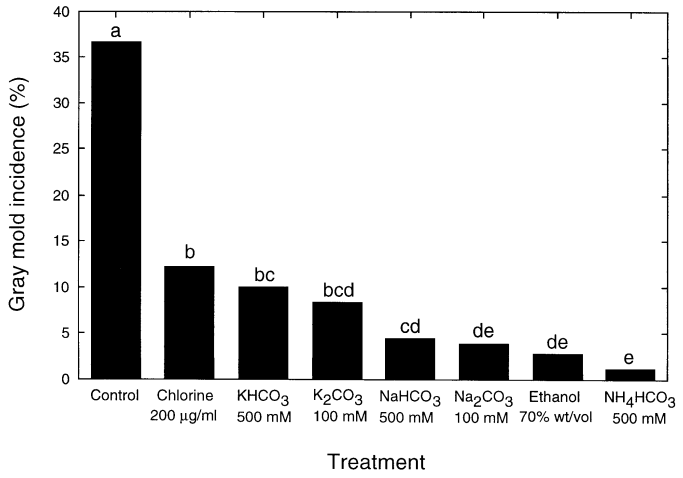

Fig. 4 Incidence of gray mold on inoculated Perlette berries after spray application of water (control) and various antifungal compounds, followed by storage for 7 days at 15°C.

ozone treatments were applied (data not shown). When damaged grape berries were treated with ozone (test 2, Fig. 5), the reduction in gray mold was not significant.

Similarly, the condition of the fruit greatly influenced the efficacy of the bicarbonates. When undamaged, inoculated berries were tested, bicarbonates applied at 500 mM significantly reduced gray mold incidence (Fig. 6; $p = 0.0078$). When bicarbonates were applied at 100 mM, no gray mold reduction occurred. When grape berries were wounded and inoculated within the wounds, the bicarbonates could not protect them against infection at either rate (Fig. 6).

Chlorine at 200 µg/mL significantly reduced gray mold incidence (Fig. 7). Furthermore, the effectiveness of the bicarbonates was significantly improved ($p = 0.0003$) when they were combined with 200 µg/mL of chlorine (Fig. 7) and was superior to either the bicarbonates or chlorine alone.

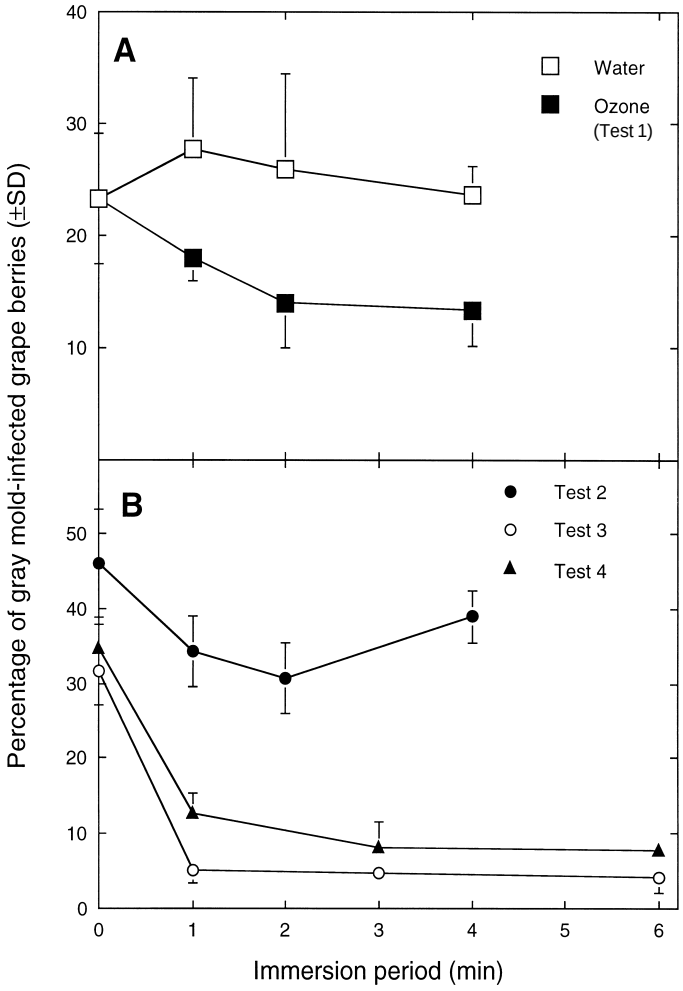

Fig. 5 Incidence of gray mold among inoculated table grape berries after immersion in water containing ozone at 10 µg/mL or water alone, followed by storage for 7 days at 15°C. Tests 1, 2, and 4 used Perlette grapes; test 3 used Flame Seedless grapes.

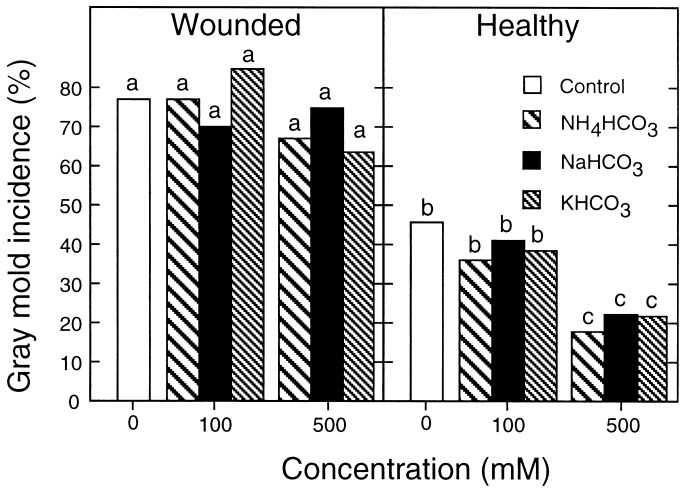

Fig. 6 Incidence of gray mold on inoculated wounded or nonwounded Thompson Seedless grape berries after spray application of water (control) or two concentrations of bicarbonate salts, followed by storage for 7 days at 15°C.

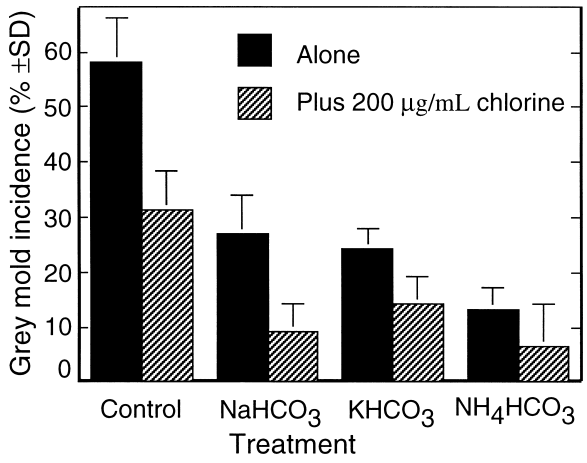

Fig. 7 Incidence of gray mold on inoculated Thompson Seedless berries after spray application of water alone, 500 mM of bicarbonate salts alone, or each with the addition of 200 µg/mL of chlorine, followed by storage for 7 days at 15°C.

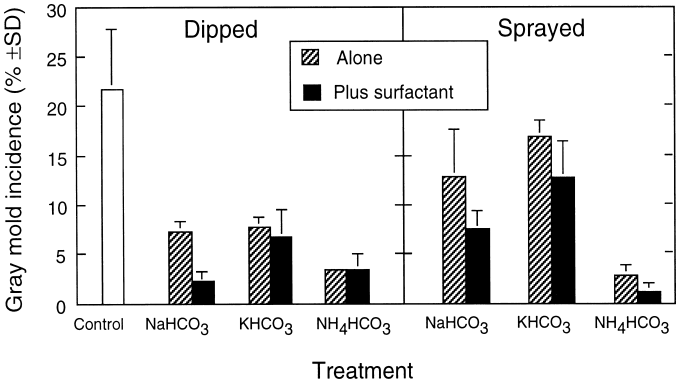

Fig. 8 Incidence of gray mold on inoculated Crimson Seedless grape berries after spray or dip application of water alone, 3% of bicarbonate salts alone, or with addition of 0.1% wt/vol Triton X-100 surfactant, followed by storage for 7 days at 15°C.

The bicarbonates were more effective when applied by immersion of the berries in the solutions rather than by spraying berries with the solutions (Fig. 8; $p = 0.0011$). The addition of the surfactant Triton X-100 (0.1% vol/vol) significantly improved the efficacy of bicarbonates when the berries were dipped ($p = 0.0298$) or sprayed ($p = 0.0111$) with the solutions. Ammonium bicarbonate was superior to sodium bicarbonate and both were superior to potassium bicarbonate ($p = 0.001$). There was no significant difference in gray mold reduction between ammonium bicarbonate and ethanol treatments alone. When ammonium bicarbonate (3% wt/vol) and ethanol (60% vol/vol) were combined, gray mold control was inferior to that obtained by these treatments applied alone (data not shown, $p = 0.05$).

Immersion for 15 sec in ammonium bicarbonate (3% wt/vol) or in ethanol (60% vol/vol) alone was as effective as sulfur dioxide fumigation for the control of gray mold on Thompson Seedless, Ruby Seedless, and Crimson Seedless grape berries (Fig. 9). Sodium bicarbonate (3% wt/vol) was equal to sulfur dioxide fumigation on Ruby Seedless and Crimson Seedless grape berries, but was inferior to it on Thompson Seedless grapes. Potassium bicarbonate (3% wt/vol) was equal to sulfur dioxide fumigation on Crimson Seedless grape berries, but was inferior to it on Thompson Seedless and Ruby Seedless varieties. Control of gray mold was significantly better on Crimson Seedless than on the other varieties.

Table grape quality. There was no significant impact on grape weight loss by the treatments (data not shown). The average weight loss among all the clusters after 8 and 30 days of storage was 0.67 and 1.07%, respectively. There were significant differences in rachis appearance, berry appearance, berry firmness, and berry color among the various treatments (Table 3). Rachis or cluster rachis appearance was not affected by treatment with chlorine, ammonium bicarbonate, or ethanol. However, rachis appearance was severely impacted by treatment with potassium carbonate, followed by a modest but significant impact by sodium carbonate, potassium bicarbonate, sodium bicarbonate, or ozone. Rachis injuries consisted of areas that turned dark brown to black. Injury after ozone treatment was different, with thin longitudinal, parallel light brown lines, approximately 3 mm in length and 0.5 mm in width, appearing in some areas on the rachis.

Berry appearance was unaffected or improved compared to the control by treatment with chlorine, ammonium bicarbonate, ethanol, sodium bicarbonate, or ozone. The berry appearance of the control treatment was poorer than among some treatments, as small brown spots, symptoms of initial stages of gray mold infection, occurred after 8 days of storage in a few

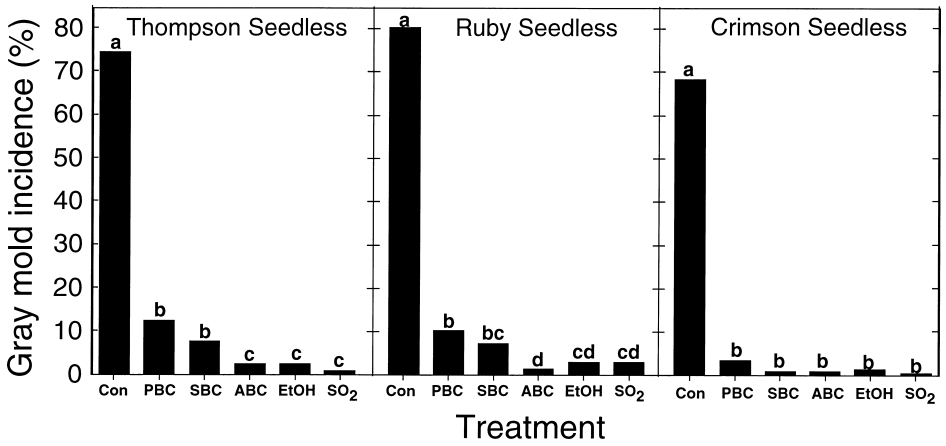

Fig. 9 Incidence of gray mold on inoculated grape berries after a dip application of water (Con), 3% potassium bicarbonate (PBC), 3% sodium bicarbonate (SBC), 3% ammonium bicarbonate (ABC), 60% ethanol (EtOH), or 45 min fumigation with sulfur dioxide (SO_2) with an initial dose of 2000 µL/L; followed by storage for 7 days at 15°C.

Table 3 Quality of Crimson Seedless table grapes after immersion in various solutions followed by storage at 3°C for 8 days.

Treatment	Conc.	Duration	Rachis appearance[a]	Berry appearance[b]	Firmness (mm) [c]	Firmness (mm) [c,d]	Berry color[e] L	Berry color[e] Hue angle
Ozone	7 µg/mL	4 min	1.63 bc	0.60 a	2.28 bc	2.27 bc	33.65 bc	65.99 cd
Chlorine	20 µg/mL	1 min	0.50 a	0.67 a	2.18 ab	2.30 cd	34.07 c	64.61 abcd
Chlorine	200 µg/mL	1 min	0.57 a	0.70 a	2.39 c	2.32 cd	34.25 c	60.92 a
$NaHCO_3$	500 mM	15 sec	1.40 b	1.43 b	2.20 ab	2.36 cd	32.50 ab	62.56 abc
$KHCO_3$	500 mM	15 sec	1.80 bc	1.83 c	2.37 c	2.26 bc	32.08 a	64.36 abcd
NH_4HCO_3	500 mM	15 sec	0.67 a	0.67 a	2.17 ab	2.16 ab	32.33 ab	68.87 d
Na_2CO3	500mM	15 sec	2.07 c	2.20 d	2.13 a	2.30 bcd	33.40 abc	61.37 ab
K_2CO3	500 mM	15 sec	4.87 d	3.93 e	2.21 ab	2.26 bc	32.13 a	63.03 abc
Ethanol	60% wt/vol	1 min	0.77 a	0.77 a	2.20 ab	2.11 a	33.39 abc	66.53 cd
Control	(water)	1 min	0.47 a	1.33 b	2.17 ab	2.40 d	33.76 bc	65.87 bcd
P-value			0.0001	0.0001	0.0006	0.0004	0.355	0.0345

[a]Rachis appearance is a visual injury index of 0 to 8, where 0 = no injury, 8 = severe injury.
[b]Berry appearance is a visual injury index 0 to 5, where 0 = no injury, 5 = severe injury.
[c]Firmness is displacement in mm of individual berries under 0.68 kg force.
[d]After 30 days of storage at 3°C.
[e]Color recorded as CIE Lab color space.

of these berries. Berry appearance was severely impacted by treatment with potassium carbonate, followed by less severe injury by sodium carbonate and potassium bicarbonate. These injuries were immediately visible after treatment. After potassium carbonate or sodium carbonate treatments, the berries were wet and salty tasting even after storage for 30 days. After ammonium bicarbonate and, particularly, ethanol treatments, the berries appeared drier than the control or other treatments after 8 and 30 days of storage.

After 8 days of storage, berries treated with potassium bicarbonate or chlorine at 200 µg/mL had reduced firmness. After 30 days of storage, berry firmness was the highest in the ethanol treatment, followed by ammonium bicarbonate, and similar among the other treatments. Berry color was significantly darker (L*) in the potassium bicarbonate and potassium carbonate treatments because of the injuries these treatments caused to the berry skin, than it was in the control, chlorine, or ozone treatments. Berries were lighter in color after chlorine treatment than after ammonium and sodium bicarbonate treatments. Berry hue angle was significantly lower, indicating more red color was present, in fruit treated with chlorine at 200 µg/mL than in fruit from control, ozone, ethanol, or ammonium bicarbonate treatments, which were similar to each other. Carbonate and bicarbonate treatments also did not differ in hue angle from the control.

Discussion

The in vitro toxicity of the carbonate and bicarbonate salts to spores of *B. cinerea* differed significantly and alone did not predict the in vivo treatment toxicity on table grape berries, where higher concentrations were needed to control postharvest gray mold. Because sodium and potassium carbonate inhibited spore germination at much lower concentrations than the bicarbonates (Table 1), they were used at a lower rate (100 mM) than the bicarbonates (500 mM) when their ability to control gray mold on grapes was assessed. They were very effective, even at this low rate, and similar to the bicarbonate salts (Fig. 4), but they darkened the pedicels and made dark brown spots on the berries. When bicarbonate solutions were tested in vitro the ammonium salt was significantly more toxic to *B. cinerea*, with about twice the potency of the other bicarbonate salts. In practical tests to control gray mold on table grape berries, ammonium bicarbonate was superior in effectiveness to sodium bicarbonate in some experiments and consistently superior to potassium bicarbonate. In addition to the inhibitory effect of the bicarbonate ions, ammonium bicarbonate has additional toxicity to microbes because it is a source of free ammonia gas, particularly at the pH (approximately 8) used in our tests [25,29]. The pH of ammonium bicarbonate was lower than that of sodium and potassium bicarbonate and did not injure the grapes.

Most treatments had little or no impact on berry quality parameters except the carbonate salts, which caused immediate and objectionable injuries to the berries, probably because of their high pH. Injury after ozone treatment was modest and probably acceptable. Additional evaluation of these treatments should include their impact on berry flavor.

The best treatments in terms of efficacy and impact on berry quality were ammonium bicarbonate and ethanol, followed by sodium bicarbonate. The addition of chlorine, a fast-acting general biocide, to the bicarbonate solutions significantly improved their effectiveness, and it also reduces the risk of introduction of other microbes to the grapes, which are undesirable from a food safety perspective [42]. Furthermore, postharvest rinses with ozone and chlorine can remove and degrade many pesticide residues [24]. However, ammonium bicarbonate and chlorine, when combined, can form nitrogen trichloride, a gas which can comprise a safety hazard [42].

A significant shortcoming of the bicarbonates, chlorine, and ozone was their inability to control gray mold when spores

were placed inside wounds. Wounds made by detaching berries from pedicels of the rachis cannot be avoided when packages of detached berries are prepared. Although they are initially free of spores, the wounds undoubtedly can become contaminated during subsequent handling. De Kock and Holz [12] stated that, because berries become infected primarily during harvest, packing operations, and storage, the necessity for reducing *B. cinerea* inoculum on harvested grapes should be emphasized. Good sanitation during the destemming operation to minimize contamination of these wounds is a necessity. To minimize the inoculum on the berries that could infect wounds during destemming, these treatments would most effectively be applied before removal of the berries from the rachis. When treated grapes were fresh (with green rachis and attached pedicel) and undamaged, all treatments successfully inhibited spores on berry surface and subsequent gray mold in storage was reduced. When we treated grapes that were damaged (with surface cracks or dry, loose pedicels that exposed berry flesh), the treatments could not effectively inhibit spores that were hidden in these wounds, and gray mold in storage was not reduced. Smilanick and coworkers [36] reported that when citrus fruit that had been inoculated in wounds with spores of *Penicillium digitatum* one day before treatment were immersed in sodium bicarbonate or carbonate solutions, it was very effective in preventing postharvest green mold. We were unable to control gray mold after inoculation of wounds on grapes; however, our experiments differed from those of Smilanick and coworkers [36]. When we tested bicarbonate salts for the control of gray mold on wound-inoculated grapes, we applied the treatments by spraying without surfactants and infiltration of the wounds was less complete than it would have been after immersion of the berries.

Control of gray mold by immersion in ozonated water was less effective than the other treatments, even after treatment for periods much longer and at concentrations much higher than those that controlled spore germination. Its efficacy was irregular and very dependent on grape condition. The results with sanitizers (chlorine and ozone) agree with the results of those who reported wounds on fruit protected fungal spores. Shimizu and coworkers [33] similarly reported that immersion of table grapes in ozonated water reduced postharvest decay, although long contact periods were needed and it was incapable of stopping infections in wounds.

Ogawa and coworkers [21] reported that *B. cinerea* spores placed in surface injuries of tomato fruit were not inactivated by chlorine or ozone at doses that killed free spores rapidly. Similarly, Spotts and coworkers reported decay of pears after the inoculation of wounds by *Pencillium expansum, B. cinerea,* or *Mucor piriformis* was not controlled by chlorine [38] or by ozone after inoculation by *P. expansum* [37] at doses far higher than those killing free spores. Controlling infections from wounds requires minimizing the creation of wounds, minimizing the inoculum present, and preventing contamination of the wounds. Shimizu and coworkers [33] reported ozone treatment was more effective when the grapes had fewer mechanical injuries at the time of treatment.

Ethanol at 60% (vol/vol) was particularly effective for postharvest use on grapes. It was effective and did not injure the cluster rachis or berries. Berry appearance was better among treated grapes than among the untreated control grapes. Similarly, Ben-Arie and coworkers [1] reported that ethanol treatment reduced postharvest decay losses, enhanced retention of berry firmness, and did not injure grapes. They reported that ethanol treated grapes were preferred by a taste panel. Margosan and coworkers [14] reported that combined heat and ethanol treatments reduced postharvest decay and enhanced the retention of firmness of stone fruit.

Immersion of grape berries in bicarbonate solutions was more effective than spraying with the same solutions. When berries are immersed, spores on the surface of the berry are in contact with an active agent for at least the duration of the treatment. When solutions are sprayed on berries, contact with the berry surface is less uniform, a significant portion of the surface is insufficiently protected, and spores within these areas might not have sufficient contact time with the fungistatic agents to prevent them from germinating. Surface tension reduces the effectiveness of both immersion and spray treatments; consequently, the addition of surfactant to the bicarbonate solutions significantly improved their effectiveness in most cases (Fig. 8). Similarly, Homma and coworkers [9] found that the effectiveness of sodium bicarbonate for the control of powdery mildew on cucumber and green mold of citrus was improved by the addition of some surfactants.

Brief immersion in ammonium bicarbonate, sodium bicarbonate, and ethanol were similar in effectiveness to sulfur dioxide fumigation and may be acceptable treatments to control postharvest gray mold on detached table grapes. They have minimal environmental or worker safety issues associated with their use, they pose a minimal ingestion hazard because of their low toxicity to animals, they are inexpensive, they do not injure berries, and their effectiveness against gray mold was reliable.

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