# Near-Harvest Applications of *Metschnikowia fructicola*, Ethanol, and Sodium Bicarbonate to Control Postharvest Diseases of Grape in Central California

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### **ABSTRACT**

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The yeast *Metschnikowia fructicola*, ethanol, and sodium bicarbonate (SBC), alone or in combinations, were applied to table grapes on vines 24 h before harvest to control the incidence of postharvest diseases. In four experiments, all significantly reduced the total number of decayed berries caused by *Botrytis cinerea*, *Alternaria* spp., or *Aspergillus niger* after storage for 30 days at 1°C followed by 2 days at 20°C. In three experiments, a mean gray mold incidence (caused by *B. cinerea*) of 34.2 infected berries per kilogram among untreated grape was reduced by *Metschnikowia fructicola* at 2 × 10<sup>7</sup> CFU/ml, ethanol at 50% (vol/vol), or SBC at 2% (wt/vol) to 12.9, 8.1, or 10.6 infected berries per kilogram, respectively. Ethanol, SBC, and SO<sub>2</sub> generator pads were similarly effective. *M. fructicola* effectiveness was not improved when combined with ethanol or SBC treatments. Ethanol and yeast treatments did not harm the appearance of the grapes. *M. fructicola* and SBC left noticeable residues, and SBC caused some visible phytotoxicity to the rachis and berries. Ethanol applied at 50% (vol/vol) reduced epiphytic fungal and bacterial populations by about 50% compared with controls. *M. fructicola* populations persisted on berries during storage when applied alone or after ethanol treatments, whereas SBC reduced its population significantly.

Additional keywords: biological control

Botrytis cinerea Pers.:Fr., Aspergillus niger Tiegh., and Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. are common postharvest pathogens of table grapes in most regions of the world (6). Currently, postharvest diseases of table grapes are controlled by the postharvest application of SO2 gas, either by weekly fumigation in storage rooms or by packing grapes in polyethylene-lined boxes with SO<sub>2</sub> generator pads. Problems associated with SO<sub>2</sub> use include (22) the following: (i) SO<sub>2</sub> residues that exceed the tolerance of 10 mg/kg of most countries can occur if the gas dosage is too high; (ii) unsightly bleaching injuries can occur after numerous or high dosage fumigations; (iii) SO<sub>2</sub> cannot be used on or-

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ganically certified grapes; and (iv) because of sulfite hypersensitivity in some people, the dietary hazard of SO<sub>2</sub> was recognized and it was removed from the United States Food and Drug Administration "generally regarded as safe" classification in 1986 (30). Therefore, the development of alternative strategies to control postharvest decay of table grapes that are safe, effective, economical, and compatible with commercial handling is of interest.

Most table grape growers pack their grapes in the vineyard at harvest into their final commercial packages. Field packing, rather than packinghouse packing, has become popular because it minimizes postharvest handling, which incurs substantial labor costs, causes abrasion of the berry surface that removes the bloom, and the time to accomplish handling can delay cooling. To incorporate a process after harvest, such as the application of a fungicide solution by spraying or immersing the berries in it, would require additional postharvest handling and would be perceived by growers as less practical. A replacement treatment for SO<sub>2</sub> that is applied in the vineyard before harvest would not require changes in the current harvest and packing practices and would be more feasible to incorporate into grower operations than one applied after harvest.

Alternatives to SO<sub>2</sub> could be developed from food additives of proven safety or biological control agents. Selected organic and inorganic salts are active antimicrobial agents and are widely used food additives (21). Among these, sodium bicarbonate is used for controlling pH, taste, and texture. It also has broad-spectrum antifungal activity (7). Bicarbonate salts applied after harvest controlled a variety of postharvest pathogens in citrus (4,28), carrot (25), bell pepper (10), melon (2), sweet cherry (12), and table grapes (22,23). Ethanol is a common natural food component and occasional additive that possesses potent antimicrobial activity (16,20). Immersion in ethanol or exposure to its vapors controlled postharvest diseases of peach, citrus fruit, and table grapes (11,17,18,22,24,27,29). Biological control of postharvest diseases of table grapes has been pursued actively in several laboratories (5,19,26,30). Recently, preharvest applications of the biocontrol agent, the yeast Metschnikowia fructicola Kurtzman & Droby, sp. Nov., isolated from the surface of table grape (cv. "Superior") by Droby and coworkers (15), was shown to be effective for controlling postharvest diseases of strawberry (13).

Our objective was to evaluate near-harvest vineyard applications of sodium bicarbonate and ethanol solutions, either alone or in combination with a biocontrol agent, *M. fructicola*, to control postharvest diseases of table grapes. This approach could accomplish control of table grape postharvest diseases without the need of additional harvest or postharvest handling procedures, and it could reduce or eliminate the need for SO<sub>2</sub> fumigation.

# MATERIALS AND METHODS

Preharvest treatments of table grape.

Experiments evaluated the control of postharvest table grape diseases by near-harvest applications of ethanol (Pharma, Brookfield, CT) at 35 and 50% (vol/vol), sodium bicarbonate (Fisher Chemicals, Fair Lawn, NJ) at 1 and 2% (wt/vol), and the yeast antagonist *M. fructicola* (Proyeast, Minrav Agrogreen, The Biological Division, Jerusalem, Israel). All work was conducted at the United States Department of Agriculture-Agricultural Research Service San Joaquin Valley Agricultural Sciences Center in Parlier, Fresno

County, CA. The efficacy of these treatments was compared with water-treated grapes (control) packed with and without SO<sub>2</sub> generators. Four experiments were conducted with three table grape cultivars. Mid-season organically grown 'Thompson Seedless' grapes were used in experiments 1 and 2 (in different vineyards), and conventionally grown late-ripening 'Princess' and 'Crimson Seedless' grapes were used in experiments 3 and 4, respectively. A randomized complete block design with four replications was used. Each replicate consisted of a row 8 m in length and included five vines. A volume of 1 liter of solution was applied to the grape clusters in each replicate with a 6-liter-capacity, lowpressure sprayer with an adjustable solid cone nozzle (Model 70192; H. D. Hudson Manufacturing Co., Hastings, MN).

The yeast antagonist was applied alone or in combination with ethanol or sodium bicarbonate. Dustless, water-dispersible granules containing M. fructicola at  $2 \times$ 10<sup>10</sup> CFU/g were mixed with water and applied at a rate of 2 g/liter. The solution contained about  $2 \times 10^7$  CFU/ml. When M. fructicola was used in combination with ethanol, the clusters were first sprayed with ethanol and the yeast was applied 45 min later. When M. fructicola was used in combination with sodium bicarbonate, the veast and sodium bicarbonate were mixed together and immediately applied. All of the treatments were applied 24 h before harvest. From each replication, 7 to 9 kg of fruit were harvested and placed in ventilated polyethylene bags, each containing 600 to 700 g of fruit. In all, 10 or 12 bags of grape clusters were placed in a single fiberboard box. SO<sub>2</sub> treatment consisted of dual-release generator pads containing 7 g of sodium metabisulfite (Uvas Quality, Santiago, Chile). Two generator pads were placed on top and two beneath the grapes within each box, after which boxes were enclosed within plastic bags just prior to storage. The boxes were stored at 0 to 1°C, relative humidity > 90%, for 24 h, then wrapped with polyethylene stretch film (20 um) to minimize weight loss and stem desiccation, and stored under the same conditions for about 4 weeks. The film was applied 24 h after the fruit were first placed in cold storage so as not to retard initial cooling. After cold storage, the polyethylene stretch film was removed and the fruit were stored at 20°C for an additional 48 h to simulate the environmental conditions that occur during market display. In one experiment, the grapes were placed at 20°C immediately after harvest, no polyethylene film was used, and they were examined after 6 days to simulate direct marketing of the grapes without storage. Observations were made visually by counting the number of decayed berries that showed typical symptoms of B. cinerea, Alternaria spp., or Aspergillus spp. infections per kilogram of fruit. Incidence data were transformed

(arcsin of the square root of the proportion of infected fruit) before an analysis of variance was applied. Means were separated using Fisher's least significant difference  $(P \le 0.05)$ . Results from each experiment were analyzed and are shown independently.

Influence of ethanol treatment on natural microbe populations on berries. The effect of vineyard ethanol applications on populations of epiphytic fungi on the surface of berries was determined in three experiments. One hour after ethanol or water (control fruit) was applied as previously described, 40 single berries (including pedicels) were collected using sterile scissors from 20 clusters within each of four replicate plots. Using scissors sterilized by brief immersion in 10% (vol/vol) laundry bleach (5.25% sodium hypochlorite; Clorox Company, Oakland CA), two berries were clipped from opposite sides of the sampled clusters and dropped into sterile plastic bags (16.8 by 14.9 cm). In the laboratory, 200 ml of sterile distilled water was added, and the bags were shaken on a rotary shaker at 200 rpm for 30 min. After 10-fold serial dilutions in water, five aliquots of 10 µl from each dilution were plated in petri dishes containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) amended with chloramphenicol (Sigma-Aldrich, St. Louis, MO) at 250 ug/liter to inhibit bacterial growth, and cultures were observed after 48 h of incubation at 24°C. Three replicate determina-

tions were made from the extract prepared from each plot. Population of fungi was expressed as CFU per berry.

Survival of M. fructicola on grapes during storage. Survival of the yeast antagonist M. fructicola on berries within clusters that had been treated with the yeast alone or in combination with sodium bicarbonate or ethanol was determined soon after application and during storage for 4 weeks at 1°C. Samples were collected three times; 1 h after application in the vineyard and after 2 and 4 weeks of storage. The experiment was done twice with 'Thompson Seedless' grapes. Extracts were cultured on PDA amended with chloramphenicol at 250 µg/liter; the sample size, method of collection, and population determination procedures were as previously described to determine the influence of ethanol treatment on natural fungal populations. M. fructicola colonies were distinguishable from the other yeasts and fungi because this yeast has characteristic pinkcolored colonies after 48 h of incubation on PDA.

#### RESULTS

Preharvest treatments with ethanol (50%), sodium bicarbonate (2%), M. fructicola, or their combinations reduced the number of decayed 'Thompson Seedless' berries significantly after storage at 20°C for 6 days (Fig. 1). These treatments were comparable in efficacy to the SO<sub>2</sub> treatment. In all of the experiments, decayed

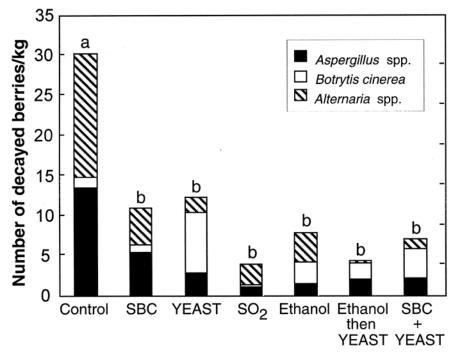


Fig. 1. Postharvest decay of 'Thompson Seedless' table grapes treated 24 h before harvest and stored for 6 days at 20°C. Preharvest treatments were water (control), the yeast Metschnikowia fructicola, ethanol (50% vol/vol), ethanol followed by the yeast, or a combination of sodium bicarbonate (2% wt/vol) and the yeast. Clusters treated with water and packaged at harvest with sulfur dioxide generator sheets (SO<sub>2</sub>) that were present throughout storage represented SO<sub>2</sub> treatment. Clusters treated with water and packed without SO<sub>2</sub> generator sheets represented control fruit. Within each experiment, columns with unlike letters differ significantly by Fisher's least significant difference ( $P \le 0.05$ ).

berries on which more than one pathogen was present on a single berry were rare. Dark brown *Alternaria* spp. and *Aspergillus niger* infections typically appeared on infected berries near the pedicel. Lightbrown infections of *B. cinerea* typically appeared elsewhere and the surface of the discolored flesh detached easily ("slip skin") when touched (6).

In experiment 1, all of the near-harvest treatments applied to 'Thompson Seedless' grapes, except sodium bicarbonate at 1% (wt/vol), reduced both the total number of decayed berries and the number with symptoms caused by B. cinerea, Alternaria spp., or Aspergillus niger (Table 1). The effectiveness of ethanol (50% vol/vol) and sodium bicarbonate (2% wt/vol) treatments was not significantly different than that of the SO<sub>2</sub> treatments against each of three pathogens or their total. In contrast, the effectiveness of ethanol (35%) and sodium bicarbonate (1%) was inferior to SO<sub>2</sub> treatment. Neither application of ethanol before M. fructicola nor the combination of M. fructicola with sodium bicarbonate improved efficacy of the yeast.

The results of a second experiment with 'Thompson Seedless' grapes were similar to those of experiment 1, with a few excep-

tions (Table 1). All of the near-harvest treatments (experiment 2) significantly reduced the total number of decayed berries, as well as decay caused individually by B. cinerea or Alternaria spp.. Ethanol (50% vol/vol) applied alone or followed by M. fructicola most effectively reduced the total decay observed or that caused by each of the three pathogens alone. When either was used alone or combined, sodium bicarbonate (2% wt/vol) and M. fructicola reduced decay caused by B. cinerea, Alternaria spp., and Aspergillus niger about 50%. Application of ethanol before M. fructicola or the combination of M. fructicola with sodium bicarbonate did not improve the efficacy of the yeast. SO2 treatment reduced the number of decayed berries by about 80% in experiment 2 compared with 50% in experiment 1.

In experiment 3, all treatments significantly reduced the number of decayed berries among 'Princess' grapes caused by *B. cinerea* and *Alternaria* spp. compared with the control (Table 1), and there were no significant differences among them.

In experiment 4, ethanol (50% vol/vol), sodium bicarbonate (2% wt/vol), and SO<sub>2</sub> similarly and significantly reduced the number of total decayed berries compared

with the control treatment among latematuring 'Crimson Seedless' grapes (Table 1). Decay incidence was low and *Alternaria* spp. caused most of the infections observed.

Ethanol significantly reduced fungal populations on surfaces of berries by about 60% in all of the experiments (Fig. 2). Epiphytic bacterial populations were very low (less than 2,000 CFU/berry) and were reduced similarly by preharvest ethanol treatments (*data not shown*).

M. fructicola populations changed little during storage (Fig. 3). Ethanol treatment had no influence on the population size or persistence of the yeast. In contrast, the yeast population on the berries treated with a combination of the yeast and sodium bicarbonate was significantly lower than after application of the yeast alone or the yeast in combination with ethanol.

## DISCUSSION

The results of this study demonstrated that near-harvest applications of ethanol, sodium bicarbonate, or the yeast antagonist *M. fructicola* significantly controlled post-harvest diseases of table grape.

Microflora populations on berry surfaces were significantly reduced by approxi-

Table 1. Effect of preharvest treatments on postharvest decay of table grapes after storage for 30 days at 1°C and 2 days at 20°C

Treatment	Number of decayed berries per kilogram <sup>z</sup>			
	Total decay	Botrytis cinerea	Alternaria spp.	Aspergillus niger
Thompson Seedless' (experiment 1)				
Ethanol (35%)	19.7 bcd	13.1 bc	5.8 ab	0.8 a
Ethanol (50%)	15.4 abc	6.8 ab	8.5 ab	0.0 a
Sodium bicarbonate (1%)	25.0 d	14.5 cd	9.7 bc	0.8 a
Sodium bicarbonate (2%)	14.3 abc	8.8 abc	4.8 ab	0.7 a
Yeast	22.5 bcd	13.2 bc	9.2 bc	0.0 a
Ethanol (35%)+ yeast	16.2 bcd	8.9 abc	7.1 ab	0.1 a
Ethanol (50%)+ yeast	17.6 bcd	10.5 abc	7.1 ab	0.0 a
Sodium bicarbonate (1%)+ yeast	24.2 cd	13.8 bc	9.8 bc	0.6 a
Sodium bicarbonate (2%)+ yeast	14.6 ab	5.0 a	9.5 bc	0.1 a
Sulfur dioxide	8.2 a	4.9 a	3.1 a	0.1 a
Water-treated control	42.6 e	24.3 d	15.0 с	3.3 b
Thomson Seedless' (experiment 2)				
Ethanol (35%)	30.4 bc	18.6 ab	11.7 ab	0.1 a
Ethanol (50%)	18.7 a	10.2 a	8.5 a	0.0 a
Sodium bicarbonate (1%)	37.6 c	21.7 b	15.8 b	0.1 a
Sodium bicarbonate (2%)	26.8 bc	13.2 ab	13.6 ab	0.0 a
Yeast	28.7 bc	15.5 ab	13.0 ab	0.2 a
Ethanol (35%)+ yeast	35.4 bc	19.7 b	15.5 b	0.2 a
Ethanol (50%)+ yeast	21.0 ab	12.4 ab	8.6 a	0.0 a
Sodium bicarbonate (1%)+ yeast	41.0 c	31.2 b	9.8 ab	0.0 a
Sodium bicarbonate (2%)+ yeast	27.4 bc	14.0 ab	13.3 ab	0.1 a
Sulfur dioxide	36.7 c	19.6 b	15.4 b	1.7 b
Water-treated control	73.1 d	36.5 c	35.4 c	1.2 b
Princess' (experiment 3)				
Ethanol (50%)	8.2 a	7.3 a	0.9 a	0.0 a
Sodium bicarbonate (2%)	11.1 a	9.7 a	1.4 a	0.0 a
Yeast	10.8 a	9.9 a	0.8 a	0.0 a
Ethanol (50%)+ yeast	6.1 a	5.5 a	0.5 a	0.0 a
Sodium bicarbonate (2%)+ yeast	10.9 a	9.8 a	1.1 a	0.0 a
Sulfur dioxide	7.0 a	6.0 a	0.9 a	0.0 a
Water-treated control	45.1 b	41.9 b	3.2 b	0.0 a
Crimson Seedless' (experiment 4)				
Ethanol (50%)	4.2 a	0.8 a	0.9 a	2.5 a
Sodium bicarbonate (2%)	4.0 a	0.4 a	1.0 a	2.6 a
Sulfur dioxide	3.1 a	0.5 a	0.5 a	2.1 a
Water-treated control	10.4 b	1.8 b	2.5 b	6.1 b

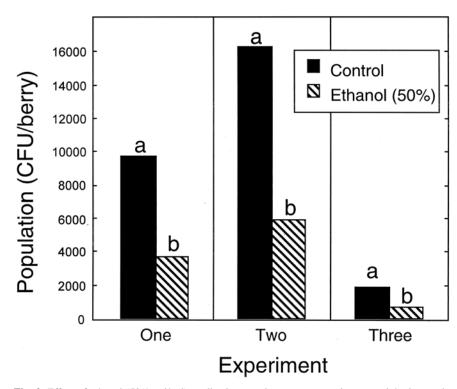
<sup>&</sup>lt;sup>z</sup> Means within columns within each experiment followed by unlike letters differ significantly according to Fisher's least significant difference ( $P \le 0.05$ ).

mately 50% compared with control treatments. Our results corroborate those of Lichter et al. (17), who reported that microbe populations were similarly reduced on grapes following their immersion in 70% ethanol. Relatively high concentrations of ethanol are required to kill fungal spores compared with vegetative bacteria (16). We found that spores of B. cinerea were more sensitive to ethanol than those of Alternaria alternata or Aspergillus niger (data not shown). The mode of action of ethanol on fungal physiology has been investigated in detail, primarily in the context of fermentation processes and under conditions of continuous and increasing stress from accumulating ethanol. The principal targets of ethanol action are membranes, but it has many other effects on fungal cells, including nonspecific denaturation of proteins and induction of water stress (16,20).

In four experiments with 'Thompson Seedless', 'Crimson Seedless', and 'Princess' grapes, near-harvest applications of ethanol significantly and consistently reduced their postharvest decay. The effectiveness of ethanol at 50% was typically superior to that at 35%, although the difference was not always significant (Table 1). In our study, 50% ethanol was superior or equal in efficacy to within-package sulfur dioxide generators in all experiments, but the efficacy of 35% ethanol was significantly less than that of sulfur dioxide in the first experiment. The rate at which ethanol evaporated may have influenced its efficacy in these tests. Probably, a portion of the ethanol evaporated during application, and we observed that clusters exposed to the sun usually were dry within a few minutes after treatment. We presume that vineyard applications of ethanol control these pathogens by inhibition of their spores, but this has not been determined empirically. Lichter et al. (17) reported that, when grapes were dipped into ethanol solutions, where little or no ethanol loss would occur during treatment, efficacy of 30 and 50% ethanol did not differ significantly.

Sodium bicarbonate at 2% was equal in effectiveness to within-package SO<sub>2</sub> generators in all of the experiments, whereas sodium bicarbonate at 1% was less effective. Previous studies demonstrated that increasing concentrations of sodium bicarbonate resulted in a corresponding increase in efficacy (9,10,13,22,23). The inhibitory effect of sodium bicarbonate on microorganisms may be due to a reduction of cell turgor pressure that causes a collapse and shrinkage of hyphae and spores, resulting in fungistasis (10).

Most observers believe the postharvest decay of grapes occurs primarily by infection of wounds made during harvest and packing; therefore, reducing pathogen inoculum on grapes at the time of harvest is important in managing postharvest decay



**Fig. 2.** Effect of ethanol (50% vol/vol) applications to clusters on grapevines on epiphytic populations of fungi on grape berries. In experiments 1 and 2, 'Thompson Seedless' grapes were used and, in experiment 3, 'Princess' grapes were used. Within each experiment, columns with unlike letters differ significantly by Fisher's least significant difference ( $P \le 0.05$ ).

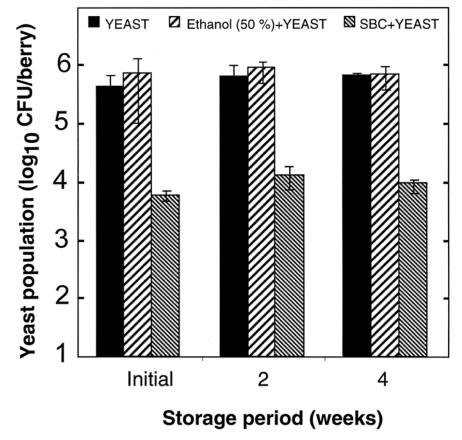


Fig. 3. Populations of the yeast Metschnikowia fructicola on 'Thompson Seedless' grape berries determined immediately after harvest (1 h after application in the vineyard) and after 2 and 4 weeks of storage at 1°C. The yeast was applied to clusters on vines alone, co-mixed with 3% (wt/vol) sodium bicarbonate, or 45 min after ethanol (50% vol/vol) was applied.

(8). Good sanitation before harvest and packing operations to minimize contamination of these wounds is a necessity. Treating the clusters 24 h before the harvest with ethanol or sodium bicarbonate reduced the number of infections, presumably by reducing inoculum and inhibiting germination of the spores of these pathogens, and this prolonged the storage and shelf life of the grapes. Careful handling also is important; the efficacy of sodium bicarbonate was significantly lower when it was applied to wounded berries (with cracks and detached pedicels) compared with its application to the berries without wounds (22).

Preharvest applications of *M. fructicola* reduced subsequent postharvest decay and were similar in efficacy to SO<sub>2</sub> treatment in experiments 2 and 3. Its effectiveness could be attributed to its persistence on berry surfaces under both field and postharvest conditions. Previous studies demonstrated that *M. fructicola* controlled postharvest diseases of table grape and strawberry when applied before harvest (13,14). This yeast apparently is quite tolerant of arid climates, where it has been effective in many environments in other disease control applications (1,3).

Combining M. fructicola with sodium bicarbonate or following ethanol treatment did not improve the effectiveness of the yeast. Application of ethanol before the yeast, although it did reduce the resident microbe populations on the berries, did not influence colonization of the berries by the yeast or its persistence. M. fructicola populations on fruit were much lower when it was co-mixed with sodium bicarbonate before application; therefore, no benefit in effectiveness by using them in combination would be expected. In another study on apple and peach, in contrast to our results, a solution of sodium bicarbonate at 2% (wt/vol) consistently enhanced performance of the yeast antagonist Candida oleophila (9).

Ethanol and yeast treatments had no impact on berry and rachis condition compared with the control treatment. We observed a slight rachis injury on clusters treated with sodium bicarbonate. These observations were in agreement with the findings of a previous study (22). Prior work showed that ethanol did not harm the appearance of table grapes, whereas sodium bicarbonate could leave a visible, topical residue and cause minor changes in rachis appearance (17,22,23). In our work, the yeast treatment also left a topical residue. Most growers prefer minimal visible residues on berries at harvest. Vineyard applications of sodium bicarbonate or yeasts made with longer intervals between treatment and harvest could minimize visible residues, although the influence of longer intervals on postharvest disease control efficacy is not known.

Ethanol and sodium bicarbonate are well-studied natural substances present in many food products. Issues associated with their use should be fewer than those associated with synthetic fungicides: they should pose a minimal ingestion hazard to humans because of their low mammalian toxicity, they are inexpensive, and their effectiveness was at commercially useful levels. Application before harvest of these treatments could be readily accomplished by many growers, who already possess vineyard sprayers, but who may not have packing line facilities with tanks or drenching equipment needed to apply postharvest treatments. A practical concern with sodium bicarbonate treatments is the presence of sodium, whose addition to agricultural soils usually is avoided; therefore, substitution by the more expensive and probably less effective potassium bicarbonate (22,23) may be needed in some locations. Ethanol residues in berries were not determined in our work, but they should be low because, in postharvest treatment studies, the residues on grapes and stone fruit were above natural levels but were relatively low and declined during storage (17,18). The flash point of ethanol, defined as the concentration at which ignition by flame is possible, is 24°C for a solution containing 50% (vol/vol) ethanol. Application of sodium bicarbonate or ethanol in vineyards to control postharvest decay has not been reviewed by regulatory agencies, and probably would need specific approval. Ethanol also is subjected to taxes and stringent regulation of its distribution in most countries.

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