Minimum Constant Sulfur Dioxide Emission Rates to Control Gray Mold of Cold-Stored Table Grapes

Lluís Palou,[1]* Carlos H. Crisosto,[2] David Garner,[3]
Lisa M. Basinal,[4] Joseph L. Smilanick,[5] and Juan P. Zoffoli[6]

Sulfur dioxide generating pads are used worldwide for the control of gray mold, caused by *Botrytis cinerea*, during long-term cold storage and/or export shipment of table grapes. We propose using an emission rate (measured in µmol SO_2 per kilogram of fruit exposed per hour of exposure) to assess the amount of sulfur dioxide that a generating pad should emit during storage/shipment. Inoculated berries were weighed and placed inside gas-tight containers attached to a flow-through fumigation system and continuously exposed to 0.00, 0.25, 0.50, 1.00, 2.00, or 3.00 µL/L sulfur dioxide (inlet concentrations) at 0°C for 6 weeks. These low concentrations simulated continuous emission of sulfur dioxide from a hypothetical slow-release generating pad. Grids with spray-inoculated Redglobe berries and open petri dishes with a central syringe-inoculated berry in contact with surrounding healthy berries were used for evaluating gray mold incidence and gray mold nesting, respectively. None of these sulfur dioxide emission rates completely controlled berry decay, while nesting was effectively prevented by sulfur dioxide emission rates of 3.6 and 5.5 µmol/kg hr (inlet concentrations of 2.0 and 3.0 µL/L). Both gray mold incidence and nesting were higher among control fruit at 95 to 98% relative humidity than at 65 to 75%, but no significant differences were observed when an inlet sulfur dioxide concentration of 3.0 µL/L was applied. Sulfur dioxide was continuously sorbed by the grapes during exposure and did not noticeably injure any fruit in these tests.

Key words: Botrytis cinerea, box liners, sulfur dioxide generating pads, postharvest decay, table grape export markets, *Vitis vinifera*

Table grapes can be stored for several months at about 0°C and high humidity. Gray mold, caused by *Botrytis cinerea* Pers.:Fr., is the most important disease limiting the duration of storage [3,16]. Gray mold during cold storage of table grapes is controlled by fumigations with sulfur dioxide, a practice used in California since the 1920s [16]. An initial sulfur dioxide treatment kills fungal inoculum present on the fruit surface but not those within the berry tissue, so subsequent fumigations are needed to prevent gray mold nesting, caused by mycelial spread from infected berries to adjacent healthy berries. Exported produce is often shipped to markets located at least 10 transit days from production areas. As periodic sulfur dioxide fumigations cannot be applied during shipment, gray mold control is achieved by the use of in-package sulfur dioxide generating pads combined with polyethylene box liners. This technology, first developed in California in the late 1960s [11], is now used worldwide. Gaseous sulfur dioxide is released after reaction of sodium metabisulfite with environmental moisture. Different types of sulfur dioxide pads, where the rate of sulfur dioxide release is controlled (one or two different phases with quick- and/or slow-release devices), have been developed according to industry needs.

Sulfite residues and phytotoxicity (bleaching of fruit color and hairline splits) are the main problems associated with both sulfur dioxide fumigation and generating pads. In 1986, a tolerance of 10 µL/L for sulfite residues in table grapes was established by the U.S. Environmental Protection Agency (EPA) [25]. Bleaching occurs when the gas is released in excessive concentrations and penetrates into the stem end or through lenticels or skin wounds, causing bleached or sunken areas [18]. Hairline splits in the berry surface appear to be related to excessive sulfur dioxide doses; symptoms are microscopic, and longitudinal splits are often followed by an exudation of berry juice [21,26]. The incidence of these injuries greatly depends on the cultivar, fruit condition, type of sulfur dioxide pad, postharvest handling, and storage environmental conditions. When water condensation occurs inside cluster bags or box liners as a consequence of temperature fluctuations, the rapid hydration of sodium metabisulfite in the generator pad can result in excessive release of sulfur dioxide, causing severe berry injury, especially when quick-release or two-phase pads are used [15,27].

These problems have motivated the development of alternatives to the use of sulfur dioxide for postharvest decay control, but viable commercial alternatives are not available to date [8]. Therefore, it would be useful to determine the minimum sulfur

[1]Postharvest Pathologist, [2]Postharvest Physiologist, [3]Staff Research Associate, [4]Postgraduate Researcher, Dept. of Pomology, University of California, Davis, Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier, CA 93648; [5]Postharvest Pathologist, San Joaquin Valley Agricultural Sciences Center, USDA-ARS, 9611 South Riverbend Ave., Parlier, CA 93648; and [6]Postharvest Physiologist, Dept. de Fruticultura. Pontificia Universidad Católica, Casilla 306-22, Santiago, Chile.

*Corresponding author [email: lluis@uckac.edu]

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dioxide emission rate able to control gray mold and eliminate or minimize injury to the berries under commercial long-term storage/shipment conditions. Sulfur dioxide concentrations in the environment are easily and reliably measured with commercially available colorimetric dosimeter tubes [14,24]. However, atmospheric sulfur dioxide concentrations may not correctly estimate the amount of gas needed for control of gray mold under all conditions of storage. Emission of sulfur dioxide by a one-phase slow-release pad in a commercial package of table grapes is a dynamic system that to some extent could be compared to a continuous fumigation system. Smilanick et al. [22] demonstrated that sulfur dioxide residues in berries had no role in the protection of the berries from gray mold because even at high concentrations, sulfur dioxide could not eradicate the fungus when it was growing internally in the grape tissue. Doubling the dose of gas approximately doubled the surface residues but only slightly increased those in the pulp. Thus, only low fumigation doses were needed, enough to kill surface-borne spores in the first fumigation and periodically eliminate aerial mycelial growth in subsequent fumigations during storage. This approach contrasted with the suggestion of Peiser and Yang [20] that high doses were needed to leave sufficient residues to impart 5 to 7 days of fungal inhibition. As a result of these findings, a new protocol for sulfur dioxide fumigation that allowed the application of considerably lower amounts of gas was developed for California table grapes [14,24]. Similarly, effective decay control in a grape box with an in-package generator in storage for relatively long periods occurs when the amount of sulfur dioxide generated is enough to inhibit the growth of the pathogen. Measuring the concentration of sulfur dioxide within the air of grape packages alone during storage may not properly estimate the sulfur dioxide dose, because such measurements are unable to account for the proportion of gas that has been sorbed by the fruit but that may have a role in inhibiting decay. A proportion of the gas released by the pad will escape out of the box, particularly in packages without liners or with highly perforated liners; another proportion of the gas will be sorbed by the packaging materials before reaching the air spaces surrounding the berries; but the most important proportion of the gas will reach those spaces and will come into contact with the grapes. Sulfur dioxide can react with surface materials on the skin, including fungal structures (surface-borne spores and growing mycelia), or can penetrate through natural openings or wounds to react with constituents of the epidermal cells, or be diluted in the tissue liquids. All these processes are usually designated as sorption of the gas by the grapes. Depending on the gas concentration and other factors, fungal inhibition, phytotoxicity, and/or accumulation of sulfite residues can occur.

We propose using an emission rate (measured in µmol SO_2 per kilogram of fruit exposed per hour of exposure) to assess the amount of sulfur dioxide that a generating pad should emit during storage/shipment. We simulated continuous emission of sulfur dioxide at low concentrations from a hypothetical slow-release generating pad (one-phase) during long-term storage at 0°C and high relative humidity (RH). Our goal was to determine, under these conditions, the minimum sulfur dioxide emission rate effective in controlling both berry decay and nesting of *B. cinerea* on artificially inoculated Redglobe table grapes. The influence of relative humidity on the effectiveness of sulfur dioxide was also investigated.

Materials and Methods

Fruit. Table grapes (*Vitis vinifera* L.) cv. Redglobe from commercial vineyards in the San Joaquin Valley were harvested at commercial maturity and used before receiving any sulfur dioxide treatment. Individual berries were cut with the pedicels attached, randomized, superficially disinfected by immersion for 1 min in diluted bleach (0.5% sodium hypochlorite), rinsed with fresh water, and allowed to dry in air at room temperature (20 ± 2°C).

Inoculations. *B. cinerea* (isolate 28E7, from T.J. Michailides) was incubated on potato dextrose agar (PDA) in petri dishes at 20°C for 10 to 14 days. Five milliliters of 0.05% (w/v) Triton X-100 in sterile water was added to each dish and spores were rubbed from the agar surface with a sterile glass rod. This high-density spore suspension was passed through two layers of cheesecloth, measured with a hemacytometer, and diluted with sterile water to achieve the desired inoculum density.

To evaluate effects on the incidence of gray mold from surface-borne inoculum, 35 individual berries were weighed, placed on plastic grids in 7.8-L polypropylene containers, and uniformly sprayed with a suspension containing $2x10^4$ spores/mL of *B. cinerea*.

To evaluate control of gray mold nesting from aerial mycelial growth, 10 µL of a suspension of $2x10^6$ spores/mL of *B. cinerea* were injected 10 mm deep into the flesh of individual berries using a Hamilton syringe (needle of 1 mm external diameter) and incubated at 20°C for 4 days until mycelium was visible. One of these previously inoculated berries was placed in the center of a petri dish in contact with six surrounding healthy berries. Net weight of the fruit in each dish was determined. The petri dishes were placed in 7.8-L polypropylene containers (six petri dishes per container).

Sulfur dioxide exposure. About 5 to 6 hr after inoculation (once the inoculum droplets on the spray-inoculated berries were air dried), the two sets of plastic containers were attached to a flow-through fumigation system at 0°C and exposed to a continuous flow of 0.00 (control), 0.25, 0.50, 1.00, 2.00, or 3.00 µL/L SO_2 (inlet concentrations). Considering sulfur dioxide as an ideal gas and the average weight of grapes in the containers, these concentrations were equivalent to 0.0, 0.5, 1.1, 2.2, 4.4, and 6.6 µmol/kg hr SO_2 for the containers with 35 spray-inoculated berries, and 0.0, 0.4, 0.9, 1.8, 3.6, and 5.5 µmol/kg hr SO_2 for the containers with petri dishes that had the syringe-inoculated berry and the six healthy berries. To enhance the distribution of sulfur dioxide inside the containers, the gas was released into each container through a perforated and end-closed 1.5-cm diameter polyvinyl chloride tube 32 cm in length anchored inside the container about 4 cm above the berries. Perforations of 0.3 cm diameter in the tube were 9 cm apart.

Desired sulfur dioxide concentrations were obtained by mixing ethylene-free compressed air with gaseous sulfur dioxide (from a cylinder containing 45 µL/L gas; Praxair, Los Angeles,

CA) using micrometering needle valves to control the rate of each gas. Total flow rate through the containers was 24 L/h and was selected as a function of the volume of the containers to get an adequate air exchange rate that prevented either respiratory gas accumulation in the containers or excessive air speed. Flow rates were measured with a digital flowmeter (model ADM1000, J&W Scientific, Folsom, CA). RH inside the containers was elevated to 95 to 98% by circulating air through free water (through a closed water bottle) before mixing with sulfur dioxide. RH levels were monitored by chilled-mirror dew-point psychrometry (model 1100DP, General Eastern Instruments, Woburn, MA). Inlet and outlet sulfur dioxide concentrations were periodically monitored with a gas-sampling pump (model 8014-400A, SE certified model 42CFR84; Matheson Kitagawa, East Rutherford, NJ) using colorimetric dosimeter tubes (tube 103SE with detection limits of 0.25 to 10 µL/L SO_2, Matheson Kitagawa). Very low outlet sulfur dioxide concentrations (below the detection limit of the dosimeters) indicated that much of the gas was sorbed within the containers. No water from condensation was observed inside the containers; thus, the gas was not sorbed by free water. In preliminary tests, measurement of sulfur dioxide from the outlet of empty plastic containers attached to the fumigation system indicated that the experimental apparatus sorbed very little sulfur dioxide. Therefore, most of the gas was sorbed by the fruit.

For each sulfur dioxide emission rate, three containers (replicates) containing a plastic grid with 35 spray-inoculated berries each and three containers (replicates) each containing six petri dishes were used. The number of infected berries in each grid and the number of infected berries surrounding the central inoculated berry in each petri dish (nesting ability) were recorded weekly during a 42-day period. The experiment was conducted twice (once in two consecutive harvest seasons, in 1999 and 2000).

Influence of relative humidity. To assess the possible influence of environmental RH on the effectiveness of low emission rates of sulfur dioxide, an additional set of experiments were conducted in the second season. Two sulfur dioxide emission rates (inlet concentrations of 0.0 and 3.0 µL/L) were tested at two different humidity levels (65 to 75% and 95 to 98% RH) during storage at 0°C for control of gray mold on surface-inoculated berries (emission rates of 0.0 and 6.6 µmol/kg hr SO_2) and control of gray mold nesting (emission rates of 0.0 and 5.5 µmol/kg hr SO_2). Plastic containers with spray-inoculated berries or syringe-inoculated berries were prepared as previously described and attached to the sulfur dioxide flow-through fumigation system. In this test, however, syringe-inoculated berries were not incubated at 20°C. Differences in humidity inside the containers were obtained by adjusting the water content in the air going to the main flow board (sulfur dioxide mixing board). Two additional flow boards were used to mix in different proportions "humidified" air (air that had been circulating through free water) with normal "dry" air. Sulfur dioxide and RH levels inside the containers were periodically monitored as described above. Decay incidence among the berries on the grids and nesting ability in the petri dishes were evaluated weekly for 63 days of storage at 0°C. The test was conducted twice during the same season.

Statistical analysis. Linear regression lines were fitted to describe gray mold incidence on spray-inoculated berries as a function of the sulfur dioxide emission rate.

For each evaluation date, arcsine-transformed data on the proportion of infected berries surrounding the syringe-inoculated berry were evaluated by an analysis of variance. Fisher's protected least significant difference test (LSD; $p = 0.05$) was used to separate means. Analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

Results

Gray mold incidence on spray-inoculated berries. Results were similar for both seasons and the regression lines presented are based on the average data from the two experiments (Figure 1). Gray mold symptoms among control fruit of the spray-inoculated berries were first observed following 28 days of storage at 0°C. In general, decay incidence decreased when sulfur dioxide emission rate increased. The highest rate applied (6.6 µmol/kg hr) controlled berry decay by 60 and 45% after 28 and 42 days of storage at 0°C, respectively. According to the regression equations, complete decay control (gray mold incidence = 0) would have been obtained with sulfur dioxide emission rates of 13.7 and 17.8 µmol/kg hr (inlet concentrations of 6.3 and 8.2 µL/L) after 28 and 42 days of storage at 0°C, respectively.

Gray mold nesting. After 42 days of storage at 0°C, gray mold nesting was effectively prevented by sulfur dioxide emission rates of 3.6 and 5.5 µmol/kg hr (inlet concentrations of 2.0 or 3.0 µL/L) (Figure 2). The percentage of infected berries surrounding the central inoculated berry after 42 days of exposure to sulfur dioxide emission rates of 0.0, 0.4, 0.9, 1.8, 3.6, and 5.5 µmol/kg hr was 98.0, 81.7, 74.9, 43.6, 15.6, and 3.9%, respectively. After 28 and 42 days of storage at 0°C, there was significantly less nesting in berries exposed to sulfur dioxide rates higher than 0.9 µmol/kg hr (0.5 µL/L, Figure 2).

Influence of relative humidity. In this set of experiments, decay development on inoculated grapes was considerably delayed compared to other experiments and the evaluation period

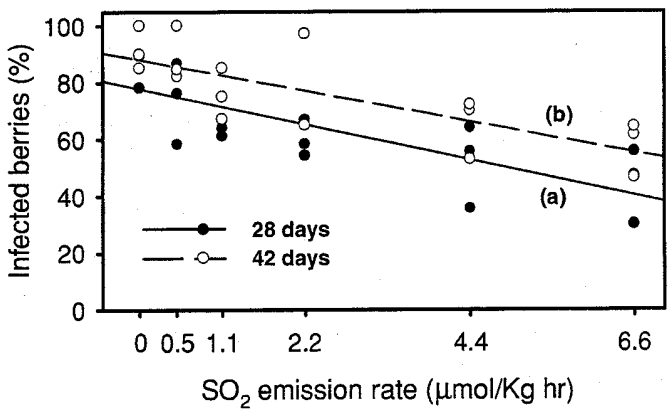

Figure 1 Regression lines for the incidence of gray mold on spray-inoculated Redglobe table grapes continuously exposed to different sulfur dioxide emission rates during storage at 0°C and 95 to 98% RH for 28 and 42 days. (**a**) $y = 77.77 - 5.67\ x$, $R^2 = 0.589$; (**b**) $y = 87.94 - 4.95\ x$, $R^2 = 0.572$.

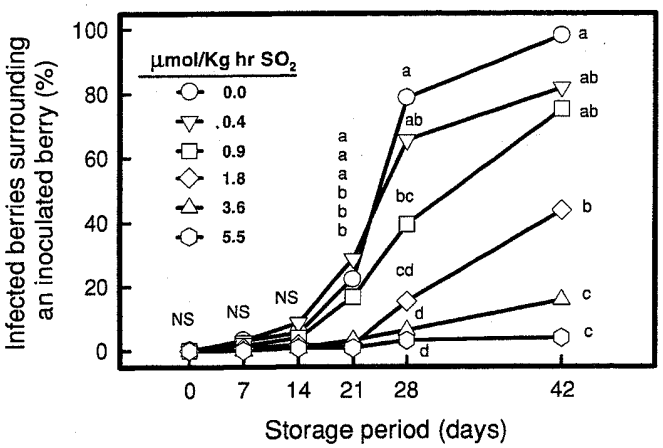

Figure 2 Gray mold nesting on syringe-inoculated Redglobe table grapes continuously exposed to different sulfur dioxide emission rates during storage at 0°C and 95 to 98% RH. NS or different letters indicate a non-significant or a significant difference, respectively, between treatments on that evaluation date according to Fisher's protected LSD test (p = 0.05) applied after ANOVA to the arcsine of the square root of the proportion of infected berries.

was longer. Among all evaluation dates, berry decay was significantly lower on berries exposed to 6.6 µmol/kg hr sulfur dioxide than on control berries (Table 1). After 35 days of storage at 0°C, decay incidence was not significantly different between the RH levels on spray-inoculated berries. After 42 days, the interaction between RH and sulfur dioxide emission rate became significant (Table 1). Therefore, a new one-way analysis of variance was applied to determine the interaction between main factors for all evaluation dates. RH did not influence berry decay when fruit were exposed to 6.6 µmol/kg hr SO_2, but, in contrast, when fruit were exposed to 0.0 µmol/kg hr SO_2 (control treatment), decay incidence was significantly higher at 95 to 98% RH than at 65 to 75% RH (Table 2).

Gray mold nesting was completely controlled by a sulfur dioxide emission rate of 5.5 µmol/kg hr (Table 3). Although nesting was slightly increased by high RH, it did not significantly affect the percentage of infected berries surrounding the central inoculated berry. Interactions between RH and sulfur dioxide emission rate were not significant for gray mold nesting during the entire storage period at 0°C (Table 3).

In every test, sulfur dioxide exposure did not noticeably injure the fruit.

Discussion

Conidia and mycelia of *B. cinerea* contaminating the surface of the berries and latent infections established before harvest are the causes of gray mold infections during long-term cold storage or export shipment of table grapes. The fungus is able to grow, although slowly, at a temperature of –1°C [2,16]. Both types of infections were simulated in our experiments. While gray mold nesting was controlled effectively by sulfur dioxide emission rates of 3.6 and 5.5 µmol/kg hr (inlet concentrations of 2.0 and 3.0 µL/L), the highest emission rate tested (6.6 µmol/kg hr; inlet concentration of 3.0 µL/L) failed to control gray mold completely on spray-inoculated berries. An atmospheric sulfur dioxide concentration of 5 µL/L has been reported to inhibit *B. cinerea* spore germination by 50% [20]. Under South African postharvest handling conditions, concentrations of 7 to 10 µL/L and lower than 20 µL/L are considered to be adequate sulfur dioxide levels in the atmosphere surrounding the berries to control decay and avoid bleaching, respectively [5]. In our experimental conditions, an emission rate of 13.7 µmol/kg hr (inlet concentration of 6.3 µL/L) would have been required to obtain a complete control on spray-inoculated berries after 28 days of storage at 0°C. Spores were not germinated at the beginning of gas exposure because inoculated fruit had remained at room temperature only for a period of 5 to 6 hr. Other workers have shown that gaseous sulfur dioxide kills mycelium more readily than spores of *B. cinerea*. In their

Table 1 Percentage of infected Redglobe table grape berries after spray inoculation with *Botrytis cinerea* and storage under 0.0 or 6.6 µmol/kg hr (0.0 or 3.0 µL/L) of continuously supplied sulfur dioxide at 0°C and 65 to 75% or 95 to 98% RH.

		Infected berries (%)[a,b]					
		Storage period (days)					
Factor	Level	28	35	42	49	56	63
SO_2 emission rate	0.0	17.7	22.2	24.2	34.3	68.2	83.8
(µmol/kg hr)	6.6	4.0	10.1	16.7	22.2	34.8	41.9
	p-value	0.0051	0.0322	0.0999	0.0353	<0.0001	<0.0001
RH (%)	65-75	8.1	13.6	20.2	24.2	42.9	58.6
	95-98	13.6	18.7	20.7	32.3	60.1	67.2
	p-value	0.1584	0.3123	0.9043	0.1302	0.0022	0.0262
SO_2 x RH	p-value	0.0667	0.0882	0.0214	0.0681	0.0004	0.0062

[a]ANOVA was applied to the arcsine of the square root of the proportion of infected berries. Values are the nontransformed means of two tests of three replicates of 35 berries each.
[b]Berries were inoculated with a 2x10⁴ spores/mL suspension of *B. cinerea*.

Table 2 Percentage of infected Redglobe table grape berries after spray inoculation with *Botrytis cinerea* and storage under 0.0 or 6.6 µmol/kg hr (0.0 or 3.0 µL/L) of continuously supplied sulfur dioxide at 0°C and 65 to 75% or 95 to 98% RH. Analysis of the interaction between RH and sulfur dioxide emission rate.

SO_2			Infected berries (%)[a,b]			
			Storage period (days)			
Concn (µL/L)	Emission rate (µmol/kg hr)	RH (%)	42	49	56	63
0.0	0.0	65-75	18.2 b	25.2 b	48.5 b	73.7 b
0.0	0.0	95-98	30.3 a	43.4 a	87.9 a	93.9 a
3.0	6.6	65-75	22.2 b	23.2 b	37.4 bc	43.4 c
3.0	6.6	95-98	11.1 c	21.2 b	32.3 c	40.4 c
		p-value	0.0319	0.0349	<0.0001	<0.0001

[a]ANOVA was applied to the arcsine of the square root of the proportion of infected berries. Values are the nontransformed means of two tests of three replicates of 35 berries each.
[b]Berries were inoculated with a 2x10⁴ spores/mL suspension of *B. cinerea*.

Table 3 Gray mold nesting on Redglobe table grapes during storage at 0°C under 0.0 or 5.5 µmol/kg hr (0.0 or 3.0 µL/L) of continuously supplied sulfur dioxide and under relative humidity of 65 to 75% or 95 to 98%.

		Infected berries surrounding a syringe-inoculated berry (%)[a]					
		Storage period (days)					
Factor	Level	28	35	42	49	56	63
SO_2 emission rate	0.0	7.4	12.0	16.7	20.8	27.3	32.9
(µmol/kg hr)	5.5	0	0	0	0	0	0
	p-value	0.0061	0.0030	0.0010	0.0003	<0.0001	<0.0001
RH (%)	65-75	1.4	3.2	6.0	7.4	11.1	14.4
	95-98	6.0	8.8	10.6	13.4	16.2	18.5
	p-value	0.0810	0.1595	0.3468	0.2682	0.4164	0.5370
SO_2 x RH	*p*-value	0.0810	0.1595	0.3468	0.2682	0.4164	0.5370

[a]ANOVA was applied to the arcsine of the square root of the proportion of infected berries. Values are the nontransformed means of the proportion of infected berries out of six berries placed in a petri dish. Two tests with three replicates of six dishes each were conducted.

evaluation of sulfur dioxide fumigations, Smilanick and Henson [24] found that the CT product (gas concentration x exposure time) required to control spore germination both in vitro and in vivo at 0°C (100 µL/L - hr or 25 µL/L x 4 hr) was about 2-fold higher than that required to kill mycelium (50 µL/L - hr or 25 µL/L x 2 hr); mycelium was injured but not killed with a CT of 10 µL/L - hr. Under high-humidity conditions, death of conidia in vitro occurred with a CT of 20 to 30 µL/L - hr [6]. Opperman et al. [19] reported that polymer discs containing sodium metabisulfite were, under commercial conditions, more effective in controlling nesting than in inhibiting latent infections. Auger et al. [1] found that, after exposure to sulfur dioxide, decay incidence on grapes inoculated with ungerminated conidia of *B. cinerea* was lower than on grapes in which the conidia had germinated and the germ tube was twice as long as the conidium or on grapes in which the germ tube had penetrated the berry skin.

Because the gas was applied at low concentrations, the fruit sorbed much of it, resulting in outlet concentrations in the containers being below detectable levels (0.25 µL/L). These observations confirmed the assumption that the amount of sulfur dioxide that a generating pad should provide to control decay effectively cannot be assessed by measuring the concentration of sulfur dioxide in the atmosphere around the fruit. For instance, sulfur dioxide emission rates of 3.6 and 5.5 µmol/kg hr reduced gray mold nesting by about 75 and 95%, respectively, but in both cases the concentration in the containers was always below 0.25 µL/L (detection limit of the dosimeters). Sorption of sulfur dioxide by fruit depends on the amount and the physical condition of the fruit and on the environmental conditions (basically temperature and humidity). Harvey et al. [12] reported that 55% of the sulfur dioxide applied was sorbed by the berries when an initial dose of 0.5% sulfur dioxide (based on the volume of an empty chamber) was applied at ambient temperature for 30 min. Because sulfur dioxide is highly soluble in water, sorption and accumulation of residues is more important in split or crushed berries and in berries infected with *B. cinerea* than in intact berries [14,23]. In three of four cultivars evaluated, immature grapes accumulated more sulfur dioxide than mature grapes [23].

In previous work [7], we determined by titration the sulfur dioxide content of several brands of slow-release pads (one-phase) commercially available in California and the sulfur dioxide content remaining after 42 days of commercial storage at 0°C and 95% RH. Palletized foam boxes were used that contained approximately 8.2 kg of Redglobe table grapes in cluster bags, without box liners, and with one sulfur dioxide pad placed on top of each box. Under these conditions, the actual sulfur dioxide emission rates averaged from 0.9 to 2.8 µmol/kg hr, depending on the pad—values that are lower than those we have now found to control gray mold nesting effectively (3.6 and 5.5 µmol/kg hr). After 42 days of storage, however, the pads had only delivered from 6 to 35% of their sulfur dioxide potential. The trial, however, was performed without plastic liners in the boxes. Polyethylene box liners enclosing the cluster bags are used to moderate the rate of water loss from the fruit and, thus, to prevent stem browning and desiccation [10,15]. Liners can be solid, but usually some venting is necessary to allow postharvest fumigants to penetrate and be purged from the packed fruit. The presence or absence and the type of box liner greatly influence the performance of a sulfur dioxide generating pad. Nelson [15] reported that in boxes with a quick-release generator held at 25°C for 10 hr, the concentration of sulfur dioxide around the grapes was about 190 µL/L inside unvented liners, but was about 20 µL/L inside liners with 0.23% of vented area. Further, relative humidity or water condensation can influence the toxicity of gaseous sulfur dioxide. In in-vitro tests, the gas was more than 20 times as effective in suppressing germination of dry spores of *B. cinerea* in an atmosphere of 96% RH as in an atmosphere of 75% RH [6]. In our tests, berry decay and gray mold nesting were higher at 95 to 98% RH than at 65 to 75% RH, but environmental humidity did not significantly influence decay on grapes exposed to 6.6 µmol/kg hr SO_2.

Recent studies [8,9,10] showed that, for California handling conditions, the best packaging alternative for export table grapes was the use of a polyethylene box liner with a 0.3 to 1.2% vented area combined with a slow-release sulfur dioxide generating pad, after an initial sulfur dioxide fumigation. The lack of control we observed in our tests with spray-inoculated berries confirms the importance of the initial fumigation to kill surface-borne spores and inoculum in fresh wounds made during handling. When an appropriate initial fumigation is applied, the use of two-phase generating pads, which can potentially cause more phytotoxicities because of the quick-release phase, could not be necessary [10]. Commercial one-phase slow-release pads with minimum sulfur dioxide emission rates of 3.6 or 5.5 µmol/kg hr, that would inhibit aerial mycelial growth from latent infections during cold storage/shipment, could be developed by adjusting the sodium metabisulfite doses, the size of salt granules, and/or the amount and/or nature of the barriers between salt and environmental

moisture. The emission rates should be higher when box liners are not included. To ensure an adequate contact between sulfur dioxide and fruit, packed clusters should not be too tight [4] and packages should not be too wide. Nelson and Ahmedullah [17] estimated that sulfur dioxide concentration drops by one-sixth with every 10-cm move away from the generators. Commercially, proper postharvest handling of the fruit is critical. After the grapes have been packed in the field or in the packing line, they should be cooled as soon as possible. Deleterious effects such as high incidence of berry bleaching [13] and berry hairline split [26], as well as premature exhaustion of the sodium metabisulfite in the pad [13], have been directly associated with delays in cooling packed grapes containing sulfur dioxide pads. In addition, delayed cooling also caused cluster water loss and stem browning [9]. Cooling within 6 hr after picking has been recommended [13]. The implementation of a forced-air precooling room would have the additional advantage of allowing shippers to perform the essential initial fumigation under the total utilization system, which uses 75% less sulfur dioxide (700 µL/L) than traditional initial fumigation (3,000 to 5,000 µL/L) and consistently provides an in-box CT of at least 100 µL/L - hr in all package types [14].

Literature Cited

1. Auger, J., M. Esterio, E. Vega, R. Ulloa, and M. Davanxo. Control of three growth stages of *Botrytis cinerea* Pers. in six table grape cultivars by means of a sulfur dioxide controlled atmosphere. Fitopatología 26:86-91 (1991).

2. Bulit, J., and B. Dubos. Botrytis bunch rot and blight. *In* Compendium of Grape Diseases. R. C. Pearson and O. Goheen (Eds.), pp. 13-15. American Phytopathological Society, St. Paul, MN (1994).

3. Capellini, R.A., M.J. Ceponis, and G.W. Lightner. Disorders in table grape shipments to the New York market, 1972-1984. Plant Dis. 70:1075-1079 (1986).

4. Combrink, J.C., and L. Ginsburg. Methods to prevent post-harvest decay of table grapes. Decid. Fruit Grow. 22:186-189 (1972).

5. Combrink, J.C., and A.B. Truter. The control of post-harvest decay of table grapes: A review of developments in South Africa. Decid. Fruit Grow. 29:272-283 (1979).

6. Couey, H.M., and M. Uota. Effect of concentration, exposure time, temperature, and relative humidity on the toxicity of sulfur dioxide to the spores of *Botrytis cinerea*. Phytopathology 51:815-819 (1961).

7. Crisosto, C.H., D. Garner, L. Palou, P.D. Metheney, J L. Smilanick, D.A. Luvisi, D. Armson, and D. Corey. Assuring good arrivals of California table grapes in export markets. California Table Grape Commission, Research Report 28 (2000).

8. Crisosto, C.H., and J.L. Smilanick. New table grape postharvest technologies. *In* Proceedings of the Fourth International Symposium on Table Grape. J. Retamales et al. (Eds.), pp. 179-192. Instituto de Investigaciones Agropecuarias. November 28-December 1, La Serena, Chile (2000).

9. Crisosto, C.H., J.L. Smilanick, and N.K. Dokoozlian. Table grapes suffer water loss, stem browning during cooling delays. Calif. Agric. 55:39-42 (2001).

10. Crisosto, C.H., J.L. Smilanick, N.K. Dokoozlian, and D.A. Luvisi. Maintaining table grape postharvest quality for long distance markets. *In* Proceedings of the International Symposium on Table Grape Production. J.A. Rantz (Ed.), pp. 195-199. American Society for Enology and Viticulture. June 28-29, Anaheim, CA (1994).

11. Gentry, J.P., and K.E. Nelson. Further studies on control of decay of table grapes by two-stage generation of sulfur dioxide within unvented containers. Am. J. Enol. Vitic. 19:70-81 (1968).

12. Harvey, J.M., C.M. Harris, T.A. Hanke, and P.L. Hartsell. Sulfur dioxide fumigation of table grapes: Relative sorption of SO_2 by fruit and packages, SO_2 residues, decay, and bleaching. Am. J. Enol. Vitic. 39:132-136 (1988).

13. László, J.C., J.C. Combrink, G.J. Eksteen, and A.B. Truter. Effect of temperature on the emission of sulphur dioxide from gas generators for grapes. Decid. Fruit Grow. 31:112-119 (1981).

14. Luvisi, D.A., H.H. Shorey, J.L. Smilanick, J.F. Thompson, B.H. Gump, and J. Knutson. Sulfur dioxide fumigation of table grapes. Bulletin 1932. University of California, Division of Agricultural and Natural Resources, Oakland, CA (1992).

15. Nelson, K.E. Effects of in-package sulfur dioxide generators, package liners, and temperature on decay and desiccation of table grapes. Am. J. Enol. Vitic. 34:10-16 (1983).

16. Nelson, K.E. Harvesting and handling California table grapes for market. Bulletin 1913. University of California, Division of Agricultural and Natural Resources, Oakland, CA (1985).

17. Nelson, K.E., and M. Ahmedullah. Effect of type of in-package sulfur dioxide generators and packaging materials on quality of stored table grapes. Am. J. Enol. Vitic. 23:78-85 (1972).

18. Nelson, K.E., and M. Ahmedullah. Effect of temperature change on the release rate of sulfur dioxide from two-stage sodium bisulfite generators. Am. J. Enol. Vitic. 24:75-80 (1973).

19. Opperman, W.J., J.F. Fourie, R.D. Sanderson, and T.J. Britz. A monolithic device for in-package SO_2 generation for the control of postharvest *Botrytis* decay of table grapes. J. Food Qual. 22:427-438 (1999).

20. Peiser, G.D., and S.F. Yang. Metabolism of sulfur dioxide in 'Thompson Seedless' grape berries. J. Amer. Soc. Hort. Sci. 110:224-226 (1985).

21. Santiago, M.S., and T.A. Hanke. Comparison between two different types of SO_2 pads on 'hairline' split level of table grapes cv. Thompson Seedless. *In* Proceedings of the Fourth International Symposium on Table Grape. J. Retamales et al. (Eds.), p. 97. Instituto de Investigaciones Agropecuarias. November 28-December 1, La Serena, Chile (2000).

22. Smilanick, J.L., P.L. Hartsell, D.J. Henson, D.C. Fouse, M. Assemi, and C.M. Harris. Inhibitory activity of sulfur dioxide on the germination of spores of *Botrytis cinerea*. Phytopathology 80:217-220 (1990).

23. Smilanick, J.L., J.M. Harvey, P.L. Hartsell, D.J. Henson, C.M. Harris, D.C. Fouse, and M. Assemi. Factors influencing sulfite residues in table grapes after sulfur dioxide fumigation. Am. J. Enol. Vitic. 41:131-136 (1990).

24. Smilanick, J.L., and D.J. Henson. Minimum gaseous sulphur dioxide concentration and exposure periods to control *Botrytis cinerea*. Crop Prot. 11:535-540 (1992).

25. U.S. Environmental Protection Agency. Interim policy for sulfiting agents on grapes. Fed. Regist. 51:47240-47241 (1986).

26. Zoffoli, J.P., J. Rodríguez, and C.H. Crisosto. Delayed cooling and sulfur dioxide generator pad induce skin splitting 'hairline' on Thompson Seedless table grape berries. *In* Proceedings of the Fourth International Symposium on Table Grape. J. Retamales et al. (Eds.), p. 23. Instituto de Investigaciones Agropecuarias. November 28-December 1, La Serena, Chile (2000).

27. Zoffoli, J.P., J. Rodríguez, and E. Koehler. Puntos críticos en el manejo de postcosecha de uva de mesa: Efecto en la condición del racimo. *In* Calidad y Condición de Llegada a los Mercados Extranjeros de la Uva de Mesa de Exportación Chilena. J. Pérez et al. (Eds.), pp. 135-150. Colección de Extensión, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile (2000).