Influence of Sulfur Dioxide Fumigant Dose on Residues and Control of Postharvest Decay of Grapes

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ABSTRACT

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To determine if sulfite residues in grapes (*Vitis vinifera*) after sulfur dioxide (SO_2) fumigation were proportional to the product of the SO_2 dose and fumigation period, grapes were fumigated with 1,250 ppm SO_2 for 2 hr, 2,500 ppm for 1 hr, 5,000 ppm for 0.5 hr, or 10,000 ppm for 0.25 hr. The product of SO_2 dose and fumigation period in each test was 2,500 ppm/hr. Sulfite residues after fumigation were not significantly different among the first three time × dose combinations. Grape cultivars Flame Seedless and Thompson Seedless contained an average of 9.6 and 6.1 μg of sulfite per gram of fresh weight, respectively. Fumigation with 10,000 ppm for 0.25 hr left residues about 30% higher than the lower doses and bleached Flame Seedless grapes. To determine if SO_2 fumigant doses recommended for commercial use could be reduced to minimize sulfite residues and still control postharvest decay by *Botrytis cinerea*, three commercially packaged *V. vinifera* cultivars in storage at 0 C were fumigated 30 min weekly for 2 mo with 312, 625, 1,250, or 2,500 ppm SO_2. In three seasons of tests, decay control was not significantly different among the doses. Sulfite residues in grapes fumigated weekly with 312 or 625 ppm did not exceed the 10 μg of sulfite per gram of fresh weight residue tolerance, whereas those with higher fumigation doses occasionally did. Commonly used commercial doses (5,000–10,000 ppm for the first fumigation followed by 1,000–2,500 ppm weekly) were higher than needed for decay control. Before a low-dose fumigation recommendation can be made, however, the influence of other factors, such as temperature management, package design, load factor, SO_2 sorption, and venting, must also be examined.

Additional keywords: ion chromatography, pararosaniline, sulfur dioxide residues, table grapes, tetrachloromercurate

Table grapes are periodically fumigated with sulfur dioxide (SO_2) during refrigerated storage to control decay (9,14). A typical commercial practice is to fumigate with 5,000–10,000 ppm SO_2 for 20–30 min immediately after the grapes enter storage, followed by weekly fumigations with 1,000–2,500 ppm SO_2 for 30 min (15,20). Grapes can remain in cold storage for as long as 5 mo. Residues 1–2 days after fumigation are usually less than 3 μg/g (8,22) but can occasionally exceed 10 μg/g (H. Shorey, *personal communication*). Many dietary applications of SO_2 have been reduced or eliminated since 1985, principally because of human health problems associated with the ingestion of food containing 1,000 μg of sulfite per gram of fresh weight or more (6). Recently, the FDA revoked its "generally recognized as safe" status of SO_2 (1), and a 10 ppm (or μg/g) tolerance for sulfite in table grapes was established by the Environmental Protection Agency (2). Since this tolerance may be exceeded in commercial practice, investigations to characterize and minimize residues were needed.

Botrytis cinerea Pers., the principal postharvest pathogen of table grapes in California (15), can be killed by SO_2 concentrations far lower than those applied commercially in weekly fumigations. Death of conidia and mycelium of *B. cinerea* occurs in vitro after about 20–30 min of exposure to 100 ppm SO_2 under the high-humidity conditions in which grapes are stored (5,13). Only 5–10 ppm SO_2 is required to inhibit decay during cold storage in a constant atmosphere (4,18). Fumigation with SO_2 concentrations as low as 200 ppm applied at 3-day intervals without venting controlled decay as well as weekly commercial-rate (2,500 ppm) fumigations (12). The atmosphere inside grape packages with in-package SO_2 generators contains 250 ppm SO_2 or less, even with quick-release pads (11,14). Since little work with periodic fumigation has been done to compare SO_2 concentrations within packages, decay control, and residues, we sought to quantify this relationship in hopes of modifying SO_2 fumigation schedules and thereby minimize residues.

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MATERIALS AND METHODS

Freshly harvested, nonfumigated *Vitis vinifera* L. table grape cultivars Flame Seedless, Thompson Seedless, Ribier, and Emperor, grown in the San Joaquin Valley of California, were used. Fumigations were conducted in epoxy-coated 120- or 230-L steel chambers with a 10-cm-diameter fan moving 50 m/sec of the atmosphere inside to ensure homogeneous distribution of SO_2. Fumigations were conducted at ambient temperature (20 ± 5 C), 20 ± 1 C, or 0 ± 1 C. Fumigation was terminated by 5 min of forced-air ventilation of the chambers with fresh air.

Influence of fumigation period and dose on residues. The effect of fumigation period and SO_2 dose on residues was determined by the fumigation of Flame Seedless and Thompson Seedless grapes with 1,250, 2,500, 5,000, and 10,000 ppm SO_2 for 2.0, 1.0, 0.5, and 0.25 hr, respectively, where the product of SO_2 dose delivered and fumigation time (D × T) was constant. Flame Seedless and Thompson Seedless grapes were fumigated at 20 ± 1 C and 0 ± 1 C, respectively. A single expanded polystyrene box containing 1 kg of grapes was used. The amount of SO_2 lost during fumigation was minimized by the use of polystyrene boxes that sorb little SO_2 (8) and by using a small quantity of fruit. Residues were determined on quadruplicate samples by tetrachloromercurate pararosaniline colorimetry. Residue data were analyzed by a one-way analysis of variance, and means were separated by LSD ($P = 0.05$).

SO_2 application and decay assessment using commercial packages. SO_2 doses less than the commercial recommendation were applied to expanded polystyrene boxes containing 10.4 kg of grapes each, with nine *B. cinerea*-inoculated berries placed among the clusters. The inoculated berries were present to ensure sufficient decay potential. Polystyrene boxes were selected because they sorb little SO_2, unlike other packaging employed by industry (8). Initial doses of 0, 625, 1,250, 2,500, and 5,000 ppm SO_2 were applied for 30 min in the initial fumigation, followed by 2 mo of weekly applications of half these rates for 30 min. The rates were calculated on the volume of an empty chamber. Two boxes

per treatment were used in 1986 and 1987 in a 120-L chamber that provided a load factor of 34%, whereas three boxes per treatment were used in 1988 in a 230-L chamber that provided a load factor of 26%. The load factor is the percentage of the chamber volume displaced by the grapes and packages. Grapes stored at 0–1 C were fumigated at ambient temperature (20 ± 5 C) and returned to 0–1 C storage after treatment in 1986 and 1987. In 1988, the grapes were stored and fumigated at 0–1 C.

In 1986, weekly samples were removed for residue analysis by headspace gas chromatography 1 or 2 days after fumigation. After 2 mo of storage, decay was assessed by determining the number of infected berries per box. PROC GLM (SAS Institute, Cary, NC) was used for statistical analysis of decay data. The nonfumigated control treatments were omitted before analysis to identify differences in decay rates among fumigant doses. Preliminary experiments showed that bleaching injury induced by these fumigations was minor and too irregular to quantify, although bleaching is often a significant defect associated with long-term storage and periodic SO_2 fumigation.

The penetration of SO_2 inside and outside the packages during fumigation was determined for all doses with Thompson Seedless grapes. The SO_2 concentration during the fumigations was monitored with two nondispersive, infrared analyzers (Horiba PIR-2000, Kyoto, Japan). The atmosphere was sampled 2, 15, and 30 min after the fumigation began, both inside the chamber and in the interior of the middle box in the 230-L chamber at 0–1 C or the interior of the bottom box in the 120-L chamber at 20 ± 5 C. The sampled atmosphere was pumped continuously through the analyzer and returned to the chamber to avoid creation of a vacuum or dilution of the SO_2. Three replicate treatments of each dose were applied and monitored, with different packages used for each replicate.

Residue analysis. Each residue value reported for any treatment is the mean of three or four replicates of 20–50 g of grapes. Residues were determined by headspace gas chromatography or tetrachloromercurate pararosaniline colorimetry. Headspace gas chromatography with flame photometric detection was employed by the method of Hamano et al (7) for total sulfite with modifications described by Harvey et al (8). Sulfite residues in grapes were quantified by tetrachloromercurate pararosaniline colorimetry by a method derived from Nury et al (17) and Peiser and Yang (18). A tetrachloromercurate sulfite trapping solution was prepared by adding 27.2 g of $MgCl_2$ and 11.7 g of NaCl in a final volume of 100 ml of distilled water. Pararosaniline-HCl was prepared by placing 100 mg of pararosaniline in 200 ml of distilled water in a 1-L volumetric flask, adding 80 ml of concentrated HCl, and bringing the solution to volume. Frozen grape tissues (50 g) were purged with nitrogen for 15 sec, then blended at high speed in a Waring blender with 50 ml of 300 mM *N*-glycylglycine and 5% (v/v) isopropanol, pH 8.9, and 50 ml of 100 mM tetrachloromercurate. The maceration was collected in 1.5-ml volume tubes and centrifuged at 14,000 *g*

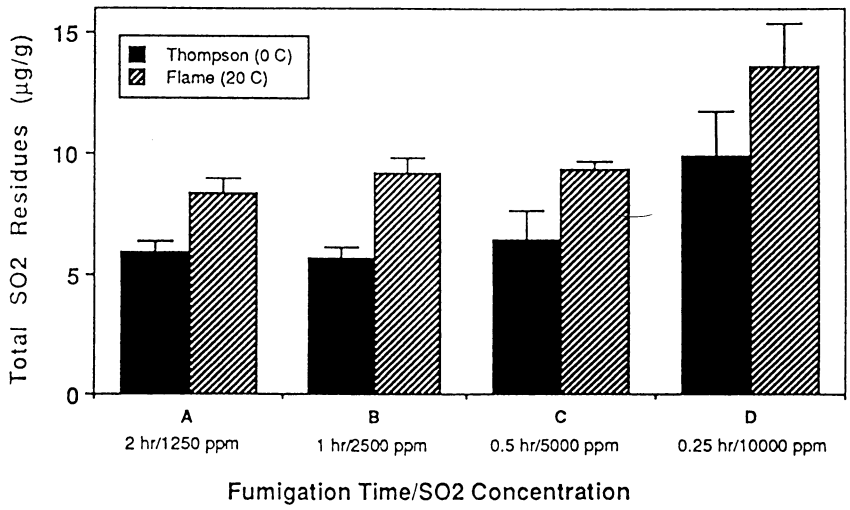

Fig. 1. SO_2 residues in Flame Seedless and Thompson Seedless grapes after fumigation with 1,250, 2,500, 5,000, or 10,000 ppm SO_2 for 2.0, 1, 0.5, or 0.25 hr, respectively, where the dose × time product was constant. Residues were determined by pararosaniline colorimetry on quadruplicate samples.

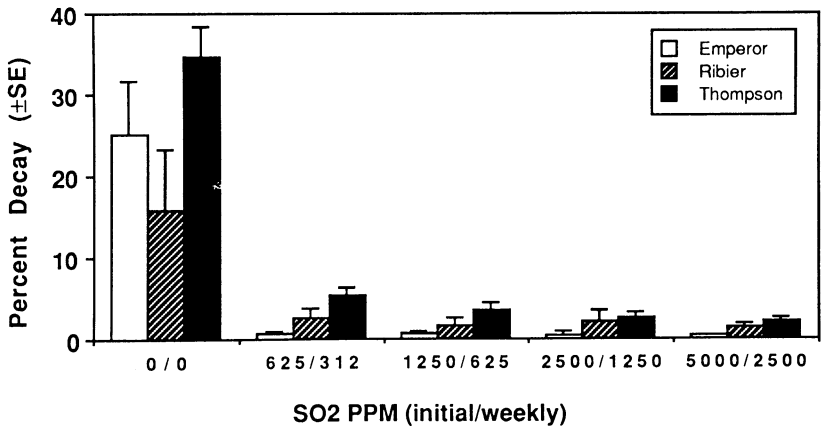

Fig. 2. Decay after 60 days of storage at 0 C of grapes fumigated with SO_2 for 30 min weekly. Means are combined for 3 yr of tests of Emperor and Ribier grapes and 2 yr of tests of Thompson Seedless grapes.

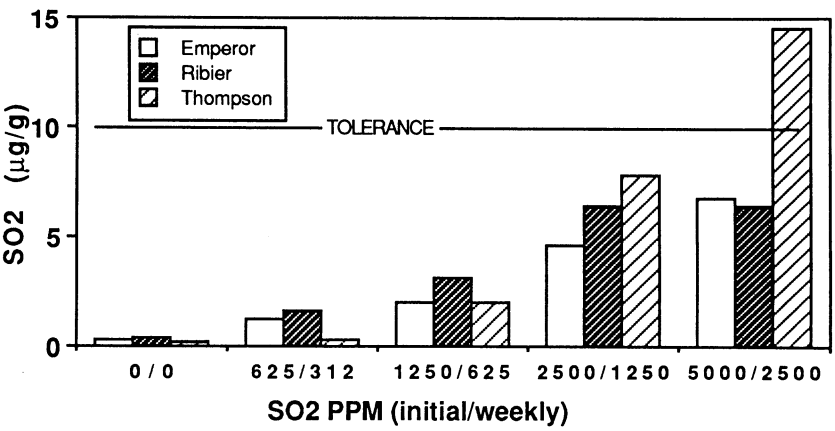

Fig. 3. SO_2 residues in grapes fumigated initially with the SO_2 concentration shown for 30 min, then weekly with a dose one-half that concentration. The values are the overall mean of two residue determinations each week during 2 mo of storage 1–2 days after fumigation by headspace chromatography. Residues were determined during only 1 yr of the fumigation tests.

for 2 min. Then, 0.5 ml of the extract was added to 5 ml of 100 mM tetrachloromercurate and mixed, followed by 0.5 ml of 0.2% formaldehyde in distilled water, and finally 0.5 ml of pararosaniline HCl solution. After 30 min incubation at 20–25 C, the absorbance at 560 nm was recorded. After correction for the background absorbance of extracts from nonfumigated grapes, the values were compared against a standard curve. The minimum sensitivity of this method was about 2 μg sulfite per gram of residue.

RESULTS AND DISCUSSION

Influence of fumigation period and dose on residues. Total sulfite residues in Flame Seedless and Thompson Seedless grapes fumigated with 1,250, 2,500, or 5,000 ppm SO_2 for 2, 1, or 0.5, respectively, where the product of SO_2 dose and fumigation time (D × T) was constant, were 9.6 and 6.1 μg of sulfite per gram of fresh weight, respectively (Fig. 1). Fumigation with 10,000 ppm SO_2 for 0.25 hr left residues significantly ($P < 0.05$) higher (about 30%) than predicted from the lower doses, and the Flame Seedless grapes were visibly bleached. The SO_2 sorption by the grapes, expanded polystyrene boxes, and fumigation chamber was minor, since the SO_2 concentration in the chambers was not lower than 90% of the initial value over the fumigation period.

The fumigation period can be lengthened or shortened without changing the SO_2 residues in the grapes if the product of SO_2 dose and fumigation period is equivalent. Since SO_2 doses of 10,000 ppm left residues higher than that predicted by their D × T product, the practice of using this dose in initial fumigations may lead to high residues. Application of equivalent D × T products in industrial situations, however, may not leave equivalent residues, since many other factors (such as package sorption, poor circulation, or incomplete venting) may modify the SO_2 dose (8,15).

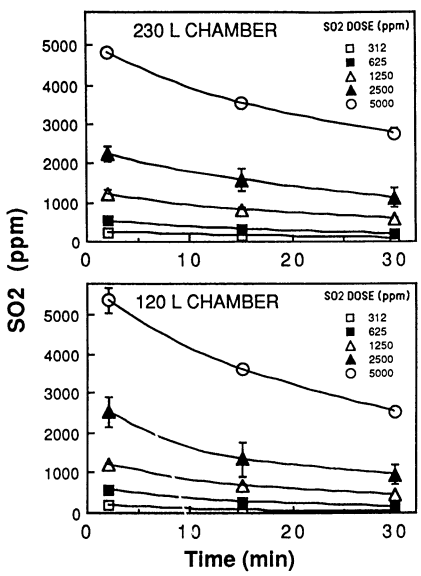

Fig. 4. SO_2 concentration in the center of expanded polystyrene boxes containing 10 kg of Thompson Seedless grapes during fumigation at 0–1 C in a 230-L chamber or at 20 ± 5 C in a 120-L chamber.

Influence of reduced doses on decay control and residues. Low doses applied to expanded polystyrene packages of Thompson Seedless, Ribier, and Emperor grapes reduced decay 85% or more after 2 mo of storage compared to the nonfumigated control (Fig. 2). Decay was not significantly different among the 3 yr. Emperor grapes decayed significantly less than the other cultivars. The recommended commercial SO_2 dose (5,000 ppm initially, 2,500 ppm weekly) was higher than necessary to stop nested decay. Although decay was not statistically different among the SO_2 doses, we believe a more rigorous experiment may identify minor differences in efficacy among the treatments, since the percent decay in the boxes fumigated with the commercial dose averaged 1–2% less than the lowest doses. These results are similar to those obtained by Nelson and Richardson (16), who found initial SO_2 doses could be decreased from 8,000 ppm to 1,000 ppm and weekly SO_2 doses could be decreased from 2,000 ppm to 500 ppm with very minor increases in decay, as long as storage temperatures were approximately 0 C.

Residue analysis 1–2 days after fumigation by headspace gas chromatography showed that residues were generally proportional to the dose applied. Residues exceeded 10 μ/g occasionally in all cultivars fumigated with the two highest doses but not with those receiving 1,250 ppm initially and 625 ppm weekly or less (Fig. 3). Decay control was obtained without the consequence of high residues, since fumigation with the efficacious lower doses left residues well below 10 ppm.

SO_2 concentrations inside and outside the packages approached or rose slightly above the calculated doses at the start of each fumigation because of the chamber volume displaced by the boxes and grapes. Thereafter, the SO_2 level dropped because of sorption by the grapes and packages (Fig. 4). The SO_2 concentration inside and outside the boxes was similar; outside the packages, the SO_2 concentration was 10–50 ppm higher after 2 min of fumigation but was not significantly different after 15 and 30 min of fumigation. Cant and Nelson (3) reported similar rapid and uniform penetration of wood boxes when chamber air was mixed, although SO_2 penetration was poor in static air.

Control of postharvest decay by SO_2 can be achieved with far lower doses than the industry now employs, although low doses should be applied to commercial situations with care because the industry does not use standardized fumigation facilities or product packages. Many variables not addressed in these experiments, such as package and pallet penetration, gas distribution, aeration, and cold storage temperature, must also be evaluated in commercial facilities to develop a general recommendation. Furthermore, varying load factors, chamber sorption and tightness, incomplete venting or scrubbing, and nonuniform circulation of the fumigant can modify the SO_2 dose (8,15). The expanded polystyrene boxes used in this study sorb very little gaseous SO_2, whereas the widely employed fiberboard and wood/kraft veneer boxes do sorb SO_2, lowering the fumigant doses applied to the grapes (8). Sorption of SO_2 by wood and fiber packages is determined in large part by their hydration, a variable characteristic that complicates estimates of SO_2 doses (3). The use of cluster wraps or bags, an increasingly popular industry practice (19), significantly retards gas penetration into packages (9). Temperature also influences SO_2 residues and minimal efficacious SO_2 dose. SO_2 residues approximately double between 0 and 20 C (J. L. Smilanick, *unpublished*; 10). Nelson and Richardson (16) reported that the SO_2 dose needed for adequate decay control at 0.5 C required doubling at 2.2 C and quadrupling at 4.4 C, because decay by *B. cinerea* is faster at these temperatures.

If low SO_2 doses are to be used by industry to help comply with the 10 ppm SO_2 residue tolerance, standardization of facilities, methods, and packaging to accommodate low SO_2 dose schedules should be developed. Adoption of packages that neither sorb nor impede penetration of SO_2 and the use of fumigant chamber coatings, such as epoxy paint, to reduce SO_2 sorption would simplify dose calculations. Furthermore, the development of procedural schedules for fumigation, such as those employed to standardize insect fumigations (21), would help provide predictable and effective decay control with minimal residues.

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