Control of *Botrytis cinerea* on Grape Berries During Postharvest Storage with Reduced Levels of Sulfur Dioxide

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ABSTRACT

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Mature berries of *Vitis vinifera* 'Thompson Seedless,' 'Flame Seedless,' or 'Emperor' were inoculated with *Botrytis cinerea* and stored at 0 C for 6 wk. The berries were treated three times a week with 0, 50, 100, 200, 800, or 3,200 ppm of sulfur dioxide. The 200-ppm sulfur dioxide treatment controlled the development and spread of disease in the inoculated berries, but complete control on naturally infected berries was not obtained until 800 ppm of sulfur dioxide was used. Bleaching was not observed on berries treated with 200 ppm sulfur dioxide or less. The 200-ppm rate applied three times a week was tested under commercial storage conditions and compared with the standard practice of 2,500 ppm of sulfur dioxide applied every 7 days. After 10 wk in storage, 30% of the inoculated berries had mycelium of the pathogen on them in the 200-ppm treatment compared with 71.2% in the standard control treatment. Spread from inoculated berries to neighboring berries was 0.65 and 2.35 berries per inoculated berry in the 200-ppm treatment and the standard control, respectively. The 75% reduction in total sulfur dioxide used in the 200-ppm treatment, compared with the standard treatment, reduced bleaching of berries.

Use of sulfur dioxide to control postharvest diseases in fruit was developed early in this century. In 1925, Winkler and Jacob (7) reported on the use of sulfur dioxide to control *Botrytis cinerea* Pers. during storage of table grapes (*Vitis vinifera* L.). The present practice is to initially treat the harvested berries with 5,000 ppm of sulfur dioxide for 30 min and then repeat applications of 2,500 ppm of sulfur dioxide every 7–10 days for 45 min (2). The repeated applications are required because the sulfur dioxide treatment does not kill the fungus inside the berry but only that on the surface (1).

Use of sulfur dioxide fumigation in the storage of table grapes is an important commercial practice, but several problems with the procedure exist. First, the sulfur dioxide can damage the berries by bleaching; this is especially critical with the nonwhite cultivars. The sulfur dioxide also can cause premature browning of the stems, which decreases the marketability of the berries and increases the rate of water loss. A delicate balance must be reached between the beneficial disease control properties of sulfur dioxide and the detrimental effects it may have on the fruit. The objective of this research was to determine if a better sulfur dioxide fumigation procedure could be developed that would increase disease control and decrease adverse effects of the fumigation on grape berries. Rate and frequency of sulfur dioxide application were investigated.

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MATERIALS AND METHODS

Mature berries of the table grape cultivars Thompson Seedless, Flame Seedless, or Emperor were stored in commercial boxes that held 10.4 kg of berries. The inside dimensions of the boxes were 30 × 50 × 18 cm. One box was placed in a 220-L container and stored at 0 C. Five berries from each box were removed and inoculated with conidia obtained from 2-wk-old cultures of *B. cinerea* grown on Difco potato-dextrose agar at room temperature and light. The berries were inoculated by injecting 0.10 ml of a conidial suspension containing 5×10^4/ml into the berry with a hypodermic

syringe. After inoculation, the berries were incubated at 23 C for 48 hr before they were returned to the boxes. The berries were placed 7.5 cm in from each corner and one in the middle of the box at a depth of 3 cm covered by two berries.

Sulfur dioxide was injected into the containers to obtain 0, 50, 200, 800, or 3,200 ppm three times a week (Monday, Wednesday, and Friday). After 6 wk, the berries were examined for the development of *B. cinerea* and bleaching resulting from the detrimental effects of the sulfur dioxide.

Disease was rated using three methods. Development of disease on the inoculated berries was rated as 0 = no mycelium visible, 1 = mycelium visible, 2 = actively growing mycelium, 3 = leaking berry caused by enzymatic activity of the pathogen and visible mycelium, and 4 = leaking berry plus entire surface of berry covered with mycelium. The number of berries infected by spread of *B. cinerea* from the inoculated berries also was determined by examining the adjacent berries for disease symptoms. Berries that developed disease but were not artificially inoculated or obviously contaminated by inoculated berries were considered naturally infected. The degree of bleaching of the berries resulting from the sulfur dioxide treatments was recorded by estimating the percentage of bleached area compared with the entire berry for 20 randomly chosen berries per box. The bleached areas of the berry began at the stem end and advanced down the berry because of the ability of the sulfur dioxide to enter the berry at the juncture of the stem and the berry skin (2). The experiment was repeated five times, with one container used for each treatment each time.

Commercial storage facilities were used to test the fumigation regimes under actual production conditions. Two cold storage rooms were used, each 760 m^3 and able to hold about 9,500 boxes, or 8 $\times$ 10^4 kg of berries. Four pallets containing 77 boxes each, stacked 11 layers high with seven boxes per layer, were broken down and five inoculated berries were placed in each of 10 boxes. The boxes with inoculated berries were stratified throughout the pallet so that layers 2, 4, and 6 from the floor had three inoculated boxes each. One box was positioned so that its long side faced the outside of the stack, one was positioned so that its short side faced the outside of the stack, and one was positioned so that none of its sides faced the outside of the stack. The 10th box was placed in the top corner of the stack so that its top and two sides faced the outside of the stack. Two pallets were placed in each cold storage room. One room was subjected to the standard commercial treatment, which is an application every 7 days of 2,500 ppm of sulfur dioxide for 45 min and then released by opening outside vents for about 30 min. The test room was treated with 200 ppm of sulfur dioxide every Monday, Wednesday, and Friday. After 10 wk, the inoculated berries were examined for development of mycelium of the fungus and spread of the pathogen to surrounding berries.

The rate of decline of sulfur dioxide in rooms that were empty or full of stored berries was determined by treating the rooms with the equivalent of 200 ppm of sulfur dioxide and monitoring at 0, 10, 20, 30, 40, 50, and 60 min after injection. A Kitigawa air sampler (Matheson Safety Products, East Rutherford, NJ) was used to monitor the sulfur dioxide by sampling 100 cm^3 of atmosphere over a 2-min period. The samples were taken near the middle of the room.

RESULTS

Inoculated berries stored at 0 C without sulfur dioxide applications had an average disease rating of 4.0. Inoculated berries treated every 3 days with 200 ppm or higher sulfur dioxide had an average disease rating of 0.0. An average of 12.8 berries were infected by *B. cinerea* originating from an inoculated berry when no sulfur dioxide was used. None of the adjacent berries became infected when 200 ppm or higher concentrations of sulfur dioxide were used. The sulfur dioxide applications also reduced the development of disease in naturally infected berries from 8.8 per box in the no-fumigation treatment to 0.8 per box in the 200- and 800-ppm treatments and to 0.0 per box in the 3,200-ppm treatment. Bleaching of berries was very light in the 200-ppm or less treatments (less than 10% of the berry was bleached) but severe in the 3,200 ppm treatment (more than 80% of the berry was bleached) (Table 1).

In the commercial storage tests, 30% of the inoculated berries developed mycelium of the fungus on the surface in the 200-ppm treatment compared with 71.2% of the inoculated berries in the standard sulfur dioxide treatment. The pathogen spread from the inoculated berries to the surrounding berries and infected 0.65 and 2.35 berries per inoculated berry in the 200-ppm and standard treatments, respectively. The differences were significant by the least significant difference test at $P = 0.05$.

In the commercial storage facilities, the initial concentration was increased slightly in the full rooms because of displacement of atmosphere by the boxes (Fig. 1). The rate of sulfur dioxide absorption was not significantly affected ($P = 0.01$) when the rooms were full or empty. After 60 min, the concentration of sulfur dioxide was about 3.5 ppm, similar to the ambient of 2 ppm in the storage facilities. The slopes of the log-transformed data were -0.43 for the empty room and -0.46 for the full room (Fig. 2). The $\log_{10}$ of the concentration of the sulfur dioxide was inversely proportional to time after fumigation ($r^2 = -0.98$).

DISCUSSION

Use of sulfur dioxide to control *B. cinerea* during storage of table grapes has been practiced for more than 50 yr (2). The major problem with the use of sulfur dioxide as a fumigant is that the concentration of sulfur dioxide required for control of the disease is very close to the levels that can be damaging to the fruit. In a series of papers (3–5), Nelson and co-workers determined that different levels of sulfur dioxide affected the fungus differently in and on the grape. The procedures developed in this research allow for the control of the disease with decreased levels of sulfur dioxide by reducing the amount applied at each fumigation but increasing the frequency of fumigation.

The actual mechanism by which sulfur dioxide controls *B. cinerea* has only recently been determined. When Thompson Seedless grape berries were exposed to 5,000 ppm of sulfur dioxide,

Table 1. Control of *Botrytis cinerea* on table grapes during storage at 0 C for 6 wk with sulfur dioxide applied every 3 days

Sulfur dioxide (ppm)	Disease severity of inoculated berries[a]	Berries infected by spread[b] (no.)	Naturally infected berries[c] (no.)	Severity of bleaching[d]
0	4.0	12.8	8.8	0
50	2.4	0.6	3.0	7
200	0.0	0.0	0.8	9
800	0.0	0.0	0.8	60
3,200	0.0	0.0	0.0	80
LSD[e]	2.3	0.9	3.6	15

[a] Numbers are means of five berries replicated five times (0 = no mycelium visible, berry appears sound; 1 = visible mycelium and water-soaking of berry; 2 = actively growing mycelium, lesions progressing; 3 = leaking berry with growing mycelium; and 4 = leaking berry plus entire surface of berry covered by mycelium.

[b] Total number of disesased berries per box that were obviously infected by *B. cinerea* growing from the artificially inoculated berries.

[c] Total number of diseased berries per box that were not obviously infected by *B. cinerea* growing from artificially inoculated berries.

[d] Mean percent area of 20 berries bleached from sulfur dioxide treatments.

[e] Least significant difference at $P = 0.05$.

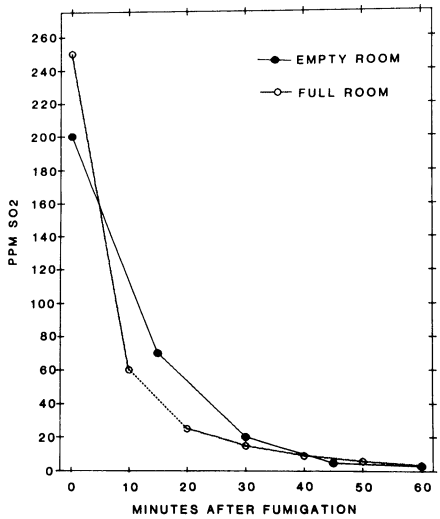

Fig. 1. Concentration of sulfur dioxide over time in storage room at 0 C either empty or filled with about 8×10^4 kg of grapes. Original injection was for equivalent of 200 ppm in the empty room.

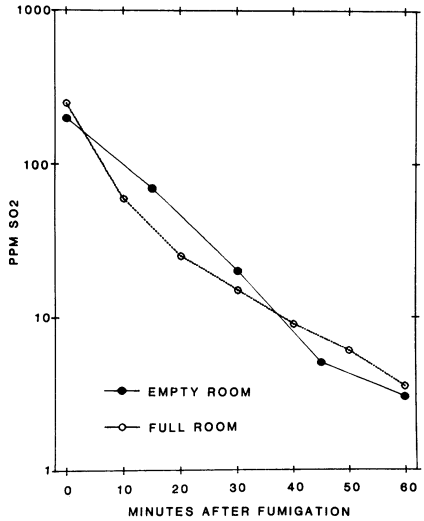

Fig. 2. Logarithmic transformation of the concentration of sulfur dioxide over time in storage room at 0 C either empty or filled with about 8×10^4 kg of grapes. Original injection was for equivalent of 200 ppm in the empty room.

more than 90% of the sulfite on the berries was oxidized to sulfate (6). The kinetics of the sulfite loss indicated that there were free and bound forms of sulfite on the grapes, representing about 70 and 30% of the total sulfite, respectively. The free form of sulfite would be released by the berries quickly, with only 15% remaining after 1 day; however, the bound form may last up to 5 days and possibly inhibit the development of the pathogen long after the fumigation is applied. Only 5 ppm of sulfur dioxide is required to inhibit germination of *B. cinerea* conidia (6).

In previous procedures for determining the amount of sulfur dioxide required to achieve a desired concentration, the room size and number of boxes within the room had to be considered because the boxes and the berries affected the final concentration of sulfur dioxide in the atmosphere (4). Because of the constant movement of fruit in a storage system, however, it is very difficult to properly use these formulas in production systems. In fact, the rooms are often fumigated as if they were full, resulting in overexposure of the berries to the fumigant and increased potential for bleaching. With the reduced 200-ppm method, the cooling systems and walls seemed to affect the rate of sulfur dioxide decrease more than the fruit and boxes, because full or empty rooms had similar slopes of decreasing sulfur dioxide concentration over time. The full rooms did have an increase of about 50 ppm during the first few minutes, but this was only temporary and still well below the concentrations berries are exposed to under standard storage fumigation. The increase in sulfur dioxide concentration was most likely due to the displacement of the air by the boxes and fruit.

In conclusion, 200 ppm of sulfur dioxide applied three times a week is more effective in controlling *B. cinerea* on grape berries during storage than the present procedure of 2,500 ppm every 7–10 days. It is also less damaging to the berries, uses only 25% of the sulfur dioxide that the present practice uses, and is easier to apply because the rooms do not require ventilation after fumigation.

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