Fumigation of Table Grapes with Acetic Acid to Prevent Postharvest Decay

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ABSTRACT

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Acetic acid (AA) fumigation controlled decay of Summerland Selection 494 and Selection 651 table grapes in repeated trials over a 3-year period when grapes were fumigated with 0.27% vol/vol AA and stored for 6 weeks at 2 or 5°C. Fumigation of Selection 651 at approximately 2week intervals with AA or SO₂ controlled both Botrytis and Penicillium decay and reduced berry shatter equally in two separate years. No significant difference occurred between SO₂ and AA treatments in yield components (cluster and berry weight), fruit composition (°Brix, titratable acidity, pH, and color), and degree of rachis drying.

Additional keywords: blue mold, Botrytis cinerea, Penicillium spp., vapor

Botrytis cinerea Pers.:Fr. is the main decay problem worldwide in late-harvested table grapes (Vitis vinifera L.) that have been exposed in the field to high humidity, dews, and rainfall (4). Blue mold rot of grapes caused by Penicillium spp. is a problem on stored table grapes in British Columbia and occurs in all grape-producing countries, developing slowly in refrigerated storage (20). Table grapes can be stored several months if refrigerated and periodically fumigated with sulfur dioxide (SO₂) to control gray and blue mold (12). Repeated applications are required because SO₂ only kills fungi on the grape berry surface (11). Alternatives to SO₂ are required because of concern with sulfite residues (7), damage to berries by bleaching and browning of the rachis (13), and poor decay control (2).

Thompson Seedless grapes inoculated with B. cinerea conidia, and various apple cultivars inoculated with P. expansum conidia, did not decay when fumigated with acetic acid (AA) at 2.0 and 4.0 mg per liter, respectively (17). Moyls et al. (15) found that fumigation with AA at 8.0 mg per liter followed by use of modified atmosphere packaging for 74 days at 0°C reduced the percentage of decayed grapes from 94% in the control to 2%. At these rates the grapes did not show any signs of phytotoxicity. These preliminary studies

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suggested that AA vapor could serve as an alternative to SO₂ for postharvest decay control in table grapes. The objectives of this study were threefold: (i) to determine if AA could control decay of table grapes in lugs comparable to those used commercially: (ii) to evaluate the effects of rates and fumigation frequency on grapes stored for 6 weeks at 2 or 5°C; and (iii) to study and compare the effect of AA and SO2 fumigation on decay, berry composition, external appearance, and sensory quality of table grapes.

MATERIALS AND METHODS

Harvest and inoculation of table grapes. Green table grapes Summerland Selection 494 (Vitis spp.; Vineland 37034 × cv. Romulus) of the Agriculture and Agrifood Canada selection series were harvested from six five-vine plots within a table grape evaluation trial at the Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada on 6 October 1993. Selection 651 (Vitis spp.; cv. Dattier × Selection 83 [cv. Bath × cv. Pearl of Casaba]) red table grapes were harvested from this trial on 5 October 1994 and 9 October 1995. Clusters were harvested into plastic picking lugs ($25 \times 30 \times 45$ cm) and immediately stored at 5°C. Loose berries, small clusters, and bird-damaged or rotten berries were removed. Fruit mass per box was adjusted to 11 kg.

For Selection 494, each lug was separated into two halves with a fiberboard divider allowing inoculation by random assignment to only one side of the lug. Grapes were thereafter inoculated with conidial suspensions of B. cinerea originally isolated from infected grapes, by misting the exposed surfaces of the clusters 24 h prior to fumigation. The Selection 494 grapes were inoculated with 5.0×10^4 conidia per ml. In the case of Selection 651, all of the grapes were inoculated as above; inoculum concentrations used were 1.5×10^6 and 1.0×10^4 conidia per ml in 1994 and 1995, respectively. The number of conidia in each suspension was counted with a hemacytometer. Approximately 20 ml was applied to each lug for both selec-

Experimental design. Experimental design for Selection 494 was a randomized complete block with a 2 × 4 factorial treatment arrangement, containing two inoculation treatments (inoculated versus noninoculated) and four fumigation levels: nonfumigated (control); one fumigation; biweekly fumigation (four fumigations); and weekly fumigation (seven fumigations). Fumigation treatments were assigned randomly to the 16 lugs. There were four lugs for each fumigation level. In 1994 and 1995, the experimental design for Selection 651 was a randomized complete block with four treatments: nonfumigated (control); 0.18% AA vol/vol; 0.27% AA vol/vol; and 0.6% SO₂. There were four lugs per treatment in 1994 and three lugs per treatment in 1995. In all cases, each lug represented one treatment replicate.

Fumigation. The aforementioned plastic interlocking picking lugs containing 11 kg of grapes each were placed one upon the other in a 1 m³ gas-tight fumigation chamber (10). The chamber and grapes were equilibrated to 10°C for 24 h and humidified by vaporizing water for 1 h before fumigation. Within the chamber an electric coil heater in a glass reservoir was filled with, depending upon treatment, either 5.0 (0.18% vol/vol) or 7.5 ml (0.27% vol/vol) laboratory grade glacial AA. Immediately thereafter the chamber was sealed, exhaust damper closed, circulation fan turned on, and voltage to the electric coil heater adjusted to 10 V, causing the coil to boil and vaporize the AA in approximately 30 s. Vaporization of the AA was observed through a glass viewing port located on the top of the fumigation chamber. Fruit were fumigated for 30 min, then the exhaust port was opened, the air-tight seal broken to allow outside air into the chamber, and the exhaust fan was turned on to blow out any remaining gas. Aeration continued for at least 30 min before the fruit were removed from the chamber and stored at 2.0°C in 1993 (Selection 494) and 5.0°C in 1994 and 1995 (Selection 651), SO₂ was produced in the chamber by placing 25 g of sodium bisulfite (NaS₂O₅) in a glass petri dish and injecting 50 ml of 10 N sulfuric acid (H₂SO₄) through a 1-ml-diameter plastic tube into the sealed chamber. Reaction time was approximately 2 min before all the NaS_2O_5 had reacted with the acid. As with AA, fumigation was continued for 30 min before the chamber was exhausted.

Consistent with experimental design, Selection 494 grapes were fumigated with 0.27% vol/vol AA on 8, 15, 22, and 28 October, and 5 and 15 November 1993; Selection 651 grapes were fumigated with 0.18 and 0.27% vol/vol AA and 0.6% vol/vol SO₂ on 13 and 27 October, and 14 November 1994, and on 13 and 27 October, and 9 and 23 November 1995. Concentrations of AA and SO₂ were monitored within the sealed chamber 1 min before the end of fumigation with Kitagawa precision gas detector tubes (Matheson Safety Products, East Rutherford, NJ) on 9 November 1995.

Evaluation of external fruit quality. Selection 494 grapes (stored for 42 days at 2°C) and 651 grapes (stored for 43 days at 5°C) were evaluated for external fruit quality by randomly selecting 10 clusters per treatment replicate and recording indi-

vidual cluster weights. Berry shatter was thereafter determined by holding the peduncle and gently shaking in a uniform manner (14). Botrytis and Penicillium decay were estimated by counting the number of berries displaying discoloration or presence of fungal sporulation or mycelia. Rachis drying was based on a 1 to 5 subjective scale in which 1 = 0 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100% rachis dried per cluster.

Five randomly selected healthy grapes from each cluster (50 per treatment replicate) were used to determine mean berry weight and fruit composition. Each berry sample was juiced, and percent soluble solids (°Brix) and pH were measured on settled juice with an Abbé refractometer (AO Instruments, Buffalo, NY) and pH meter (Model 825 MP; Fisher Scientific, Vancouver, Canada), respectively. Titratable acidity (TA) was determined by titration to 8.30 pH endpoint on diluted 10 ml of fresh juice with a Brinkmann Titroprocessor ensemble (Metrohm, Herisau, Switzerland). Color values (L*a*b*) were de-

Table 1. Effect of acetic acid fumigation (0.27% vol/vol) on selection 494 grapes stored for 6 weeks at $2^{\circ}C$

Factor	Cluster wt. (g)	Berry wt. (g)	°Brix	Titrat- able acidity (g/liter)	pН	Decay	Shatter	Rachis drying ^w
Fumigation frequency (FUM)								
None (CTL)	290	3.07	17.6	9.8	3.10	10.1	6.6	1.2
One fumigation (1) ^x	313	3.18	17.5	9.9	3.09	6.0	4.2	1.6
Biweekly fumigation (3)x	307	3.10	17.6	10.1	3.08	6.8	3.6	2.0
Weekly fumigation (6) ^x	288	3.25	18.5	9.7	3.16	5.8	5.6	2.8
Inoculation (INOC)								
Not inoculated	292	3.13	17.7	10.0	3.09	6.6	4.4	1.9
Inoculated	307	3.18	18.0	9.7	3.12	7.9	5.5	1.9
Significance FUM (1,3,6) ^{y,z}	NS	NS	*L	NS	NS	NS	*L, O	***L
Significance FUM (1,3,6 vs CTL) ²	NS	NS	NS	NS	NS	***	***	***
Significance INOC ^z	NS	NS	NS	NS	NS	*	**	NS
Interaction FUM × INOC ^z	NS	NS	NS	NS	NS	NS	NS	NS

^v Expressed in terms of berries per cluster. Means of 10 to 20 clusters per treatment replicate.

termined on Selection 651 grapes in 1994 with a Minolta Chroma meter (Minolta Canada, Mississauga, Ontario, Canada).

Experimental data were analyzed with the general linear models procedure (SAS Institute, Inc., Cary, NC). The Waller-Duncan k-ratio t test was used at k = 100, which approximates P = 0.05, for multiple comparison of means and estimation of the minimum significant differences between means.

RESULTS

Selection 494. Berry and cluster weights of Selection 494, as well as TA or pH, were not affected by AA fumigation, but there was a slight linear increase in °Brix with increasing fumigation frequency (Table 1). AA-fumigated grapes had less Botrytis decay than the control. Increasing the frequency of fumigations did not generally increase the effectiveness of AA (Table 1). Both a single fumigation and biweekly fumigations with AA produced less berry shatter than weekly fumigation. Rachis drying increased linearly with fumigation frequency, although the rachis remained green for both control and treated grapes.

Selection 651. Both cluster and berry weights of Selection 651 grapes were unaffected by treatments in 1994 and 1995 (Table 2). Mean berry weight in 1995 was 2.62 g (due to lack of cluster thinning), compared with 4.95 g in 1994. The smaller berry size in 1995 was less likely to shatter.

AA- or SO₂-fumigated 651 grapes had less Botrytis decay in both years of the trial (Table 2; Fig. 1). Botrytis decay was reduced from 40.9 to 4.4 berries per cluster by fumigation with 0.27% AA in 1994. Penicillium decay was also reduced. Rachis drying was not affected by fumigation, but was severe on both control and treated grapes in both years of the trial. Berry shatter was reduced in 1994 from 29.6 to less than 4 berries per cluster in 1994, but there were no treatment effects in 1995. AA fumigation did not differ from SO₂ in terms of any of the external fruit quality variables, although SO₂ was ap-

Table 2. Effect of SO₂ and acetic acid (AA) fumigation on yield components and external quality variables of selection 651 grapes stored for 6 weeks at 5°C in 1994 and 1995

Treatment	Cluster wt. (g)		Berry wt. (g)		Botrytis decayw		Penicillium decay ^w		Shatterw		Rachis drying ^x	
	1994	1995	1994	1995	1994	1995	1994	1995	1994	1995	1994	1995
Control	285.7	289.3	4.95	2.62	40.9a ^y	25.7a	7.2a	6.2a	29.6a	7.7	4.7	4.1
0.60% SO ₂	263.8	281.6	4.68	2.75	5.9b	8.4b	1.7b	0.3b	2.6b	4.5	4.2	4.1
0.18% AA	310.2	286.6	4.56	2.63	6.7b	11.4b	1.8b	1.2b	2.9b	7.2	4.2	4.5
0.27% AA	299.5	286.5	4.71	3.28	4.4b	12.7b	1.0b	0.4b	3.2b	5.8	4.5	4.0
Orthogonal contrasts												
Fumigants vs control ^z	NS	NS	NS	NS	***	***	***	***	***	NS	NS	NS
SO2 vs AA ^z	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.18 vs 0.27% AAz	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

wExpressed in terms of berries per cluster. Means of 10 clusters per treatment replicate.

^{*}Based on 1 to 5 subjective scale in which 1 = 0 to 20% and 5 = 81 to 100% rachis dried.

^{*} Number in parentheses = number of times grapes were fumigated.

yL, Q,: linear or quadratic, respectively.

 $z^*, **, ***$, NS: Significant at $P \le 0.05, 0.01, 0.001$, or not significant, respectively.

^x Based on 1 to 5 subjective scale in which 1 = 0 to 20% rachis and 5 = 81 to 100% rachis dried.

y Means within a column followed by the same letter are not significantly different at P = 0.05 according to the Waller-Duncan k-ratio t test.

^z **,***, NS: Significant at $P \le 0.01$, 0.001, or not significant, respectively.

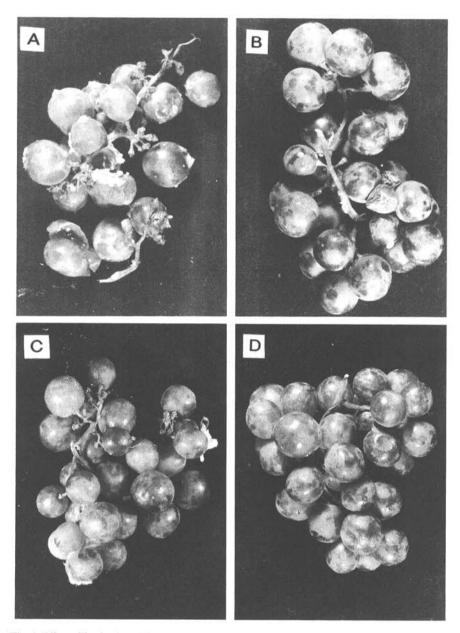


Fig. 1. Effect of fumigation with SO₂ or acetic acid (AA) on decay of Selection 651 table grapes after storage for 6 weeks at 5°C: (A) Selection 651 grapes not fumigated; (B) fumigated with 0.60% SO₂; (C) fumigated with 0.18% AA; and (D) fumigated with 0.27% AA.

Table 3. Effect of SO₂ and acetic acid (AA) fumigation on berry composition of selection 651 grapes stored for 6 weeks at 5°C in 1994 and 1995

	°В	rix	Titratable (g/lit		рН		
Treatment	1994	1995	1994	1995	1994	1995	
Control	18.2	15.7	7.1 ab ^y	6.7	3.40	3.20	
0.60% SO ₂	18.7	15.4	6.1 b	6.1	3.48	3.25	
0.18% AA	18.5	14.5	6.8 ab	6.7	3.45	3.22	
0.27% AA	19.3	15.6	7.3 a	6.0	3.46	3.27	
Orthogonal contrasts							
Fumigants vs control ^z	NS	NS	*	NS	NS	NS	
SO ₂ vs AA ^z	NS	NS	NS	NS	NS	NS	
0.18 vs 0.27% AAz	NS	NS	NS	NS	NS	NS	

y Means within a column followed by the same letter are not significantly different at P=0.05 according to the Waller-Duncan k-ratio t test.

plied at a much higher concentration. Concentration of AA present in the fumigation chamber just before aeration was 0.02% vol/vol, compared with 0.3% vol/vol SO₂. This was after three lugs (33 kg of grapes) had been fumigated with 0.27% AA or 0.6% vol/vol SO₂. Hence, only 6% of the AA remained after fumigation, compared with 50% of the SO₂.

°Brix and pH were unaffected by fumigation in both years, but TA was higher in grapes treated with 0.27% AA than in SO₂-treated grapes in 1994, and fumigation in general tended to reduce TA as well (Table 3). The positive a* and b* tristimulus color readings indicated that Selection 651 grape skins were primarily within the red-yellow color quadrant. Skins of fumigated grapes had less red/more green color (lower tristimulus a* values) and less yellow/more blue color (lower b* values) than control grapes (Table 4). However, there was no difference in color between grapes fumigated with SO₂ or AA.

DISCUSSION

AA fumigation of table grapes controlled decay in each of 3 years, was used without producing any serious quality deficiencies, and was equally as effective as SO₂ fumigations applied at commercial rates.

AA fumigation of table grapes is an alternative to SO2 that could provide the table grape industry with many benefits. The U.S. Environmental Protection Agency has established a 10 mg per kg tolerance for sulfite in table grapes (8). AA vapor does not produce sulfite and likely does not produce any toxic residues on grapes. Thus, grapes fumigated with AA could be advertised as sulfite free. Furthermore, wine grapes could benefit from fumigation with AA. In a preliminary experiment with Riesling grapes, we found that SO2 used at crush, or after pressing (plus additions common to all treatments after racking and bottling) led to higher wine TA than if AA was initially used (data not shown). AA could thus potentially replace SO2 at crush to allow the production of low sulfite wines. Further research will be necessary

Table 4. Effect of SO₂ and acetic acid (AA) fumigation on tristimulus color parameters of selection 651 grapes stored for 6 weeks at 5°C

	Color functions							
Treatment	L	а	b					
Control	32.56	5.28	3.06					
0.60% SO ₂	31.23	3.89	0.79					
0.18% AA	30.52	4.04	0.56					
0.27% AA	30.55	4.43	1.07					
Orthogonal contrasts								
Fumigants vs control ^z	NS	*	*					
SO ₂ vs AA ^z	NS	NS	NS					
0.18 vs 0.27% AAz	NS	NS	NS					

z *, NS: Significant at P ≥ 0.05, or not significant, respectively.

^z *, NS: Significant at $P \le 0.05$, or not significant, respectively.

before the use of AA can be recommended for wine production.

Previous studies have been conducted on alternatives to SO2. Most recently, hydrogen peroxide (H2O2) vapor was evaluated. Unfortunately, H2O2 only inhibits postharvest decay of table grapes (9). Vapor phase H₂O₂ reduced germination of B. cinerea conidia from 83 to 16%. Alternatively, fumigation with AA killed all B. cinerea conidia dried onto Thompson seedless grapes (17). The incidence of decay on inoculated Thompson Seedless and Red Globe grapes fumigated with H2O2 was reduced 33 and 16%, respectively, after 8 days of storage at 10°C, compared with control fruit (9). H2O2 application caused drying of the grape pedicels and rachis in the Turkish grape cv. Muskule (5). In contrast, in the present study, AA reduced the number of berries decayed due to B. cinerea on Selection 494 (43%) and Selection 651 (89%) grapes after 6 weeks storage at 2 or 5°C, respectively. AA fumigation did not affect rachis drying on Selection 651 grapes, although it did cause slight rachis drying in Selection 494 grapes.

Acetaldehyde vapors have also been considered as a possible alternative to SO2 for controlling decay in stored grapes. Avissar et al. (1) reported that 0.7% acetaldehyde vapor was lethal to B. cinerea and Rhizopus stolonifer and that a sublethal concentration inhibits their growth. AA vapor is much more toxic than acetaldehyde to B. cinerea conidia; it is lethal to B. cinerea at only 0.1% vol/vol (17). Furthermore, AA vapor at concentrations as low as 0.18% controlled decay caused by B. cinerea and Penicillium spp. on grapes of Selection 651 in this study. Acetaldehyde can negatively affect grape composition and have an adverse effect on sensory quality. Pesis and Frenkel (16), for instance, found that treatment with 0.2 to 0.9% acetaldehyde vapors for 24 h damaged berries of Sultanina (Thompson Seedless) grapes and left some off-flavor. In contrast, AA fumigation did not negatively affect fruit composition (°Brix, TA, and pH) when compared with SO₂. Selection 494 grapes were also evaluated for several sensory descriptors but the results were inconclusive (data not shown). AA fumigation could prevent off-flavors by inhibiting significant populations of fungi and bacteria on the berry surface that produce both ethanol and AA. Although Selection 651 grapes were not subjected to sensory evaluation, casual tasting during evaluation of these grapes did not suggest any offflavors. Further studies on the use of AA fumigation should include rigorous sensory

Problems with current fumigation methodology exist with regard to SO2 residues and disposal of excess fumigant. SO2 residues of 10 ppm are considered safe (21); however, this value may be exceeded (18) if grapes held in storage for a long period are gassed repeatedly at concentrations of 1,250 ppm or more (12). Residues of AA necessary to control mold on table grapes are not likely to be of concern because AA is commonly found in many food products at concentrations ranging from 0.25 (baked goods) to 9.0% (relishes) (3) and the Food and Agriculture Organization, recognizing that AA is a normal constituent of foods, has set no limit on the acceptable daily intake for humans (6). In many parts of California, the release of SO2 into the outside atmosphere is prohibited, requiring commercial fumigation operators to use scrubbers to dispose of excess SO2 after fumigation (12). To our knowledge there are no restrictions on the release of AA vapor into the outside atmosphere.

In conclusion, AA fumigation of table grapes is a viable alternative to SO₂ for preserving grapes in cold storage. Methods to accurately monitor AA vapor in the air similar to those available for SO2 need to be developed (19). This will allow use of the minimum concentration for optimum control with least chance of fruit injury. In the current study concentrations were estimated with methods adapted from those used with apples and these techniques require further refinement. Additional research is necessary on procedures to maximize decay control during normal storage and marketing of table grapes.

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