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Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes

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ABSTRACT

To control postharvest decay, table grapes are commercially fumigated with sulfur dioxide. We evaluated ozone (O_3) fumigation with up to 10,000 $\mu L\,L^{-1}$ of ozone for up to 2 h to control postharvest gray mold of table grapes caused by *Botrytis cinerea*. Fumigation for 1 h with 2500 or 5000 $\mu L\,L^{-1}$ of ozone were equal in effectiveness. Both treatments reduced postharvest gray mold among inoculated 'Thompson Seedless' grapes by approximately 50% when the grapes were examined after storage for 7 d at 15 °C following fumigation. In a similar experiment, 'Redglobe' grapes were stored for 28 d at 0.5 °C following fumigation for 1 h with 2500 or 5000 $\mu L\,L^{-1}$ of ozone. Both treatments were equal in effectiveness, but inferior to fumigation with 10,000 $\mu L\,L^{-1}$. Ozone was effective when grapes were inoculated and incubated at 15 °C up to 24 h before fumigation. The cluster rachis sustained minor injuries in some tests, but berries were never harmed. Ozone was applied in three combinations of time and ozone concentration (10,000 $\mu L\,L^{-1}$ for 30 min, 5000 $\mu L\,L^{-1}$ for 1 h, and 2500 $\mu L\,L^{-1}$ for 2 h) where each had a constant concentration × time product ($c \times t$) of 5000 $\mu L\,L^{-1} \times h$. The effectiveness of each combination was similar. The incidence of gray mold was reduced by approximately 50% among naturally inoculated, organically grown 'Autumn Seedless' and 'Black Seedless' table grapes, and by 65% among 'Redglobe' table grapes, when they were fumigated with 5000 $\mu L\,L^{-1}$ ozone for 60 min in a commercial ozone chamber and stored for 6 weeks at 0.5 °C. Residues of fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin were reduced by 68.5, 75.4, 83.7, and 100.0%, respectively, after a single fumigation of table grapes with 10,000 $\mu L\,L^{-1}$ ozone for 1 h. Residues of iprodione and boscalid were not significantly reduced. Ozone is unlikely to replace sulfur dioxide treatments in conventional grape production unless its efficacy is improved, but it could be an acceptable technology to use with grapes marketed under "organic" classification, where the use of SO_2 is prohibited, or if SO_2 use were to be discontinued.

1. Introduction

Botrytis cinerea Pers. causes gray mold, the most important postharvest disease of table grapes (*Vitis vinifera* L.). It is controlled by sulfur dioxide (SO_2) fumigation and storage at −0.5 °C. During warehouse cold storage of grapes practiced in California, grapes are usually subjected to an initial fumigation with SO_2 during forced-air cooling within hours of harvest, which is followed 1 week later by a 2–6 h-long SO_2 fumigation that is repeated weekly during cold storage (Harvey and Uota, 1978; Luvisi et al., 1992). SO_2 is an effective means to control decay of table grapes, but there are reasons to find alternatives to it. Safe, effective, and economical alternative strategies to control gray mold are needed because of issues associated with sulfite residues, SO_2 emissions, and its negative impact on grape quality (Lichter et al., 2006). While the residue tolerance is low and rarely exceeded in commercial practice (Austin et al., 1997), excessive residues of SO_2 can occur when it accumulates in wounded or detached berries (Smilanick et al., 1990b). SO_2 can cause unacceptable bleaching injuries to berries (Crisosto and Mitchell, 2002), and organoleptic quality may be compromised as well (Chervin et al., 2005). In the USA, other issues associated with SO_2 include the prohibition of its use on certified organic grapes. Furthermore, some regulatory agencies do not allow the discharge of SO_2 to the air after fumigation and require that worker maximum exposure limits do not exceed 2 $\mu L\,L^{-1}$. Also, storage facilities that use SO_2 fumigation must be able to resist damage due to the corrosiveness of the gas.

Alternatives to SO_2 fumigation that require additional processing of table grapes are unlikely to be implemented by California growers, who normally pack their fruit into their final commercial

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packages in vineyards (Crisosto and Mitchell, 2002). The principle advantage of fumigation to control postharvest decay, compared with other approaches, is that it does not require processing or manual handling of the grapes.

Ozone (O_3) is a natural substance in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadre et al., 2001). Ozone is generated by the passage of air or oxygen gas through a high voltage electrical discharge or by ultraviolet light irradiation (Mahapatra et al., 2005). It can be applied either as a gas or dissolved in water. For commercial use, ozone must be produced on site and it is classified as GRAS (generally recognized as safe) for food contact applications in the USA (U.S. Food and Drug Administration, 2001). The ozone threshold concentration for continuous human exposure (8 h standard) is 0.075 $\mu L\,L^{-1}$ (US Environmental Protection Agency, 2008). The product of ozone degradation is oxygen; therefore it leaves no residues on treated commodities. There are other conceivable benefits of ozone, such as depuration of mycotoxins (Karaca and Velioglu, 2007) and pesticide residues (Ikehata and El-Din, 2005), and control of microbes of food safety concern (Selma et al., 2008). Fumigation of grapes with high doses of ozone has been used to control populations of black widow spiders, because their presence is of concern in grape export packages from California (Leesch and Tebbets, 2005).

Ozone increased berry stilbene content (Sarig et al., 1996; Artes-Hernandez et al., 2004; Gonzalez-Barrio et al., 2006). Ozone fumigation has been applied to table grapes to control postharvest decay. A single fumigation with 200 $\mu L\,L^{-1}$ ozone for 4 h, or overnight fumigation with 500 $\mu L\,L^{-1}$ ozone, reduced postharvest gray mold among table grapes (Shimizu et al., 1982; Mlikota Gabler et al., 2002). A single application of 0.1 mg ozone gas per g of grapes for 20–80 min controlled decay by *Rhizopus stolonifer* and reduced berry microflora populations (Sarig et al., 1996). Continuous fumigation during storage with a low concentration of ozone (0.1–0.3 $\mu L\,L^{-1}$) for 7 weeks at 5 °C prevented aerial mycelial growth (nesting) of *B. cinerea* among 'Thompson Seedless' grapes, but did not decrease the number of gray mold infections (Palou et al., 2002), even when used in combination with modified atmosphere packaging (Artes-Hernandez et al., 2004, 2007).

Our objective was to evaluate a novel technology to apply a single, short-term fumigation with a very high dose (up to 10,000 $\mu L\,L^{-1} \times h$) of ozone during pre-cooling to control postharvest decay of grapes. We determined effective rates of ozone, its impact on fungicide residues on grapes, and the influence of treatments on grape appearance.

2. Materials and methods

2.1. Inoculum preparation

A *B. cinerea* isolate from grape (isolate 1440 obtained from T. J. Michailides, UC Kearney Agricultural Center, Parlier, CA) was grown on potato dextrose agar (PDA) for 2 weeks at 23 °C. Conidia were dislodged from the colony surface with a glass rod after the addition of a small volume of sterile water with 0.05% (wt vol^{-1}) Triton X-100 surfactant. The conidial suspension was filtered through four layers of cheesecloth and diluted with sterile water to an absorbance of 0.25 at 425 nm as determined by a spectrophotometer. This density contained 1×10^6 conidia mL^{-1} and was diluted with sterile deionized water to obtain the desired concentrations of conidia.

2.2. Fruit

Freshly harvested 'Thompson Seedless', 'Autumn Seedless', 'Redglobe', 'Black Seedless', and 'Ruby Seedless' were used in these experiments. In small scale experiments, grape clusters were divided into small clusters of approximately 100 g each and randomized so that a portion of each cluster was represented in each treatment.

2.3. Small scale experiments employing ozone fumigation to control gray mold

Grapes were inoculated by briefly spraying them to run-off with 1×10^5 conidia mL^{-1} of *B. cinerea* 1 or 24 h prior to ozone fumigation. Inoculated grapes were kept in a humid box at 15 °C before initiation of treatments. Gray mold on grapes inoculated 48 h before ozone fumigation was not significantly controlled by ozone fumigation in preliminary tests, so this interval between inoculation and treatment was omitted from later tests. Grapes were fumigated in perforated cluster bags ventilated with holes in the back and front (2.7% vented area). Ozone was applied at a constant concentration of up to 10,000 $\mu L\,L^{-1}$ ozone for various periods. The temperature during fumigation was 5 ± 2 °C. To facilitate penetration of the gas, this equipment applied ozone under a moderate vacuum (33 kPa). The ozone concentration was monitored continuously and did not change during fumigation. Ozone was applied within a small fumigation chamber (Tahoe Foods Technology, Inc., Sparks, NV) with an internal volume of 31.6 L, equipped with a corona discharge ozone gas generator (Model CD12 Clearwater Tech L.L.C., San Luis Obispo, CA), and ozone gas analyzer (Model HA-100-GTP-12, Ozocan Corp., Scarborough, Ontario, Canada). The $c \times t$ (concentration × time) product ($\mu L\,L^{-1} \times h$) that provided the best control of gray mold was determined. The influence of time between inoculation and treatment (1 or 24 h) on gray mold incidence among inoculated grape clusters was determined. The effectiveness of fumigation with 0, 2500, 5000, or 10,000 $\mu L\,L^{-1}$ of ozone delivered over 1 h to control gray mold among inoculated grape clusters was evaluated. Total of four replicates were treated and each replicate contained 1 cluster bag with 600–1000 g of grapes. In the first experiment, 'Thompson Seedless' grapes were placed after fumigation in boxes and stored for 1 week at 15 °C to simulate conditions during the marketing of grapes. This experiment was repeated twice, each time with four replicates. In the second experiment, 'Redglobe' grapes were stored after fumigation for 28 d at 0.5 °C to simulate commercial cold storage. This experiment was done once. After storage, the number of berries that developed gray mold was determined and the incidence (%) was calculated.

To evaluate if the same dose of ozone delivered over different period of time would influence effectiveness, an experiment was conducted where the effectiveness of a 5000 $\mu L\,L^{-1} \times h$ ozone $c \times t$ product, delivered over 30 min to 2 h, was evaluated to control gray mold among inoculated 'Autumn Seedless' grape clusters. A total of four replicates were treated and each replicate contained 1 cluster bag with approximately 900 g of grapes. The experiment was done twice.

2.4. Semi-commercial tests employing ozone fumigation to control postharvest gray mold

Organically grown, naturally inoculated grapes were packaged in corrugated fiberboard grape boxes with 9 perforated cluster bags ventilated with holes in the back and front (2.7% vented area) containing approximately 1000 g each. The average weight of each box was approximately 9 kg. The boxes of grapes were fumigated with 5000 $\mu L\,L^{-1}$ ozone for 1 h at 0 °C under moderate vacuum (33 kPa) in a large capacity (5 m long, 3 m high, 3 m wide; 45 m^3 internal volume) commercial prototype ozone chamber (OzoFresh™, Sterilization and Fumigation Services, Jamestown, RI), that could accommodate several pallets of

grapes. After fumigation, the grapes were stored 1 month at 0.5 °C when the number of gray mold infected berries was counted. Each treatment consisted of 4 replicate boxes and each replicate box contained 9 cluster bags of grapes for each cultivar. The experiment was conducted with 'Thompson Seedless', 'Redglobe', and 'Black Seedless' grapes. The ozone concentration did not change during fumigation.

2.5. Influence of ozone fumigation on fungicide residues

Fungicide residues were evaluated on freshly harvested 'Ruby Seedless' grapes. The grapes were not previously treated with any of the tested fungicides before harvest. About 400 g of grape berries were sprayed in the laboratory to run-off with a fungicide solution that contained 0.27 g L^{-1} of cyprodinil (Vangard 75WG, Syngenta Corp., Wilmington, DE, 75% cyprodinil), 37.1 g L^{-1} pyrimethanil (Scala SC, Bayer CropScience, Research Triangle Park, NC, 54.6% pyrimethanil), a mixture of 0.11 g L^{-1} of boscalid and 0.06 g L^{-1} of pyraclostrobin (Pristine WG, BASF, Florham Park, NJ, 25.2% boscalid and 12.8% pyraclostrobin), 0.29 g L^{-1} of fenhexamid (Elevate 50WDG, Arysta LifeScience, Cary, NC, 50% fenhexamid), and 0.5 mL L^{-1} iprodione (Rovral, Bayer CropScience, 41.6% iprodione). Fungicide rates were calculated based on their maximum recommended label rate and a water volume of 1700 L ha^{-1}. After fungicide application, the berries were dried in air for 24 h; then, one half of the berries was fumigated with 10000 μL L^{-1} ozone for 1 h. Residues of the fungicides, all currently registered for use in California to control gray mold on table grapes, were quantified according to Lee et al. (1991). Fifty grams of a homogenized sample of the grapes was extracted with 100 mL of acetonitrile for 3 min using a blender. Three replicates were prepared. The liquid of acetonitrile phase was filtered; then, aliquots were removed for organochlorine or organonitrogen fungicide analysis. Organoclorine extracts were further purified using Florisil™ solid phase extraction columns and analyzed by gas chromatograph (Varian 3800, Varian Inc., Palo Alto, CA, USA) equipped with electrolytic conductivity detector with dual column confirmation. Organonitrogen extracts were exchanged into acetone and analyzed by gas chromatograph (Varian 3400, Varian Inc., Palo Alto, CA, USA) equipped with flame photometric detector and thermionic specific detector without further purification. Residue analyses were conducted 3–5 d after fumigation with ozone. This experiment was done once.

2.6. Statistical analyses

Homogeneity of variances was determined using Levene's test. The incidence of gray mold was analyzed by ANOVA applied to the arcsin of the square root of the proportion of infected berries (SPSS 15.0, SPSS Inc., Chicago, IL). Means were separated by Fisher's protected least significant difference or paired *t*-test ($P \leq 0.05$). Fungicide residues were analyzed by an independent *t*-test ($P \leq 0.05$).

3. Results

3.1. Small scale experiments employing ozone fumigation to control gray mold

Short-term fumigations with 2500 or 5000 μL L^{-1} × h ozone were equal in effectiveness. They reduced postharvest gray mold of 'Thompson Seedless' grapes in storage by approximately 50% when the grapes were examined after storage for 7 d at 15 °C following ozone fumigation (Fig. 1). In a similar second experiment, where the 'Redglobe' grapes were stored for 28 d at 0.5 °C following fumigation, 2500 or 5000 μL L^{-1} × h ozone were equal in effectiveness, but

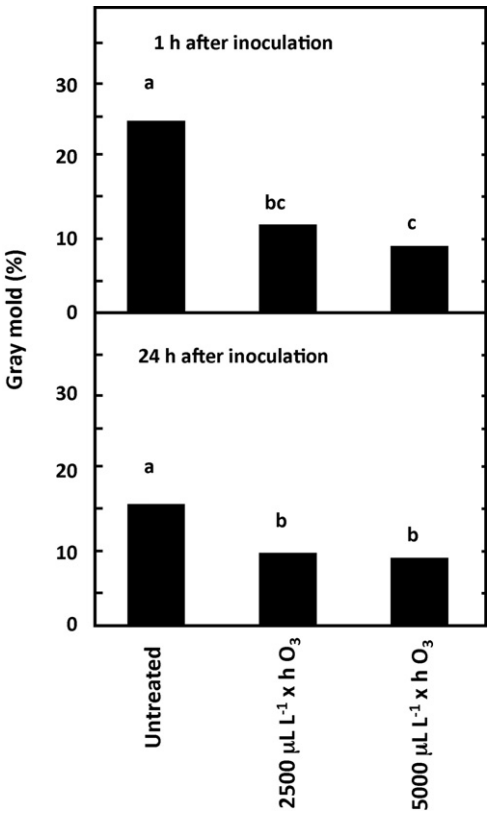

Fig. 1. Gray mold incidence among inoculated 'Thompson Seedless' grapes that were fumigated in perforated cluster bags for 1 h with different ozone (ozone) concentrations. Grapes were inoculated either 1 h or 24 h before treatment by spraying them with a conidial suspension of *Botrytis cinerea*. Inoculated grapes were kept at 15 °C and high relative humidity before treatments. After treatments, grapes were stored for 7 d at 15 °C. Each value is the mean of four replicates per treatment and each replicate contained 9 cluster bags with 600 g of grapes each. Before ANOVA, arcsin of the square root transformation was applied to the proportion of infected berries. Actual values are shown. For each inoculation period, columns with unlike letters differ significantly at $P = 0.05$, according to Fisher's Protected LSD test.

inferior to fumigation with 10,000 μL L^{-1} × h ozone (Fig. 2). Ozone controlled *B. cinerea* up to 24 h after inoculation. Ozone fumigation did not control *B. cinerea* when grapes were inoculated 48 h prior (data not shown).

The effectiveness to control gray mold of 'Autumn Seedless' grapes of the three combinations of time and ozone concentration was similar, where each had a constant concentration × time product of 5000 μL L^{-1} × h ozone, although fumigation with 5000 μL L^{-1} ozone for 60 min was slightly more effective than 2500 μL L^{-1} ozone for 120 min (Fig. 3). Ozone fumigation was particularly effective in this experiment.

The rachis of 'Thompson Seedless' grapes fumigated with ozone was sometimes harmed with the development of thin longitudinal darkened lesions (Fig. 4). Rachis injury appeared irregularly, and was not always associated with a particular ozone dose or cultivar.

3.2. Semi-commercial experiments employing ozone fumigation to control gray mold

The incidence of gray mold was reduced by approximately 50% among 'Autumn Seedless' and 'Black Seedless', and by 65% among 'Redglobe' grapes, when table grapes were fumigated with 5000 μL L^{-1} ozone for 60 min in a commercial ozone chamber and stored for 6 weeks at 0.5 °C (Fig. 5). Decay caused by *Alternaria* and *Penicillium* spp. was poorly controlled (data not shown).

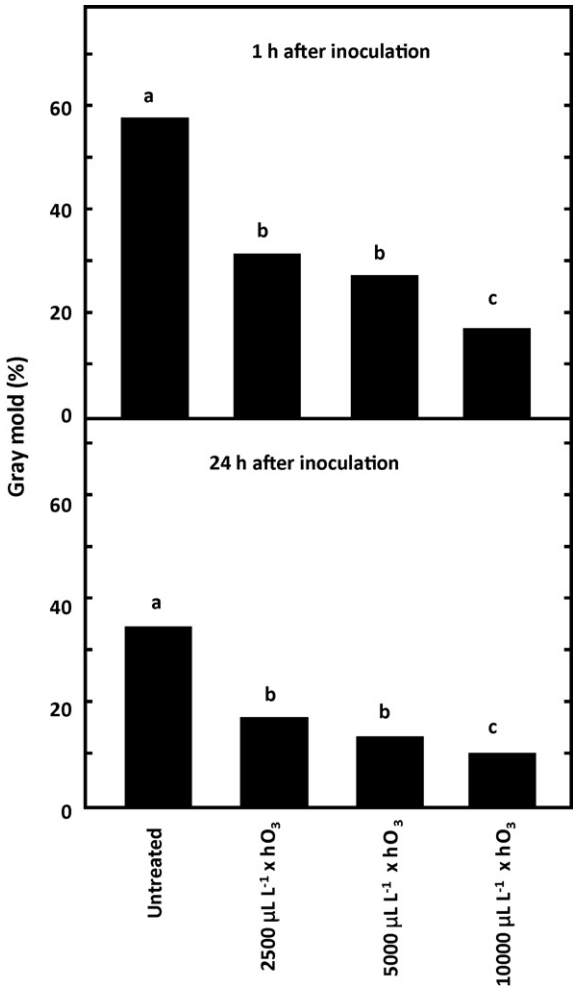

Fig. 2. Gray mold incidence on inoculated 'Redglobe' grapes that were fumigated in perforated cluster bags for 1 h with different ozone (ozone) concentrations. Grapes were inoculated either 1 h or 24 h before treatment by spraying them with a conidial suspension of *Botrytis cinerea*. Inoculated grapes were kept at 15 °C and high relative humidity before treatments. After treatments grapes were incubated for 28 d at 0.5 °C. Each value is the mean of four replicates per treatment and each replicate contained 9 cluster bags with 900 g of grapes each. Before ANOVA, arcsin of the square root transformation was applied to the proportion of infected berries. Actual values are shown. For each inoculation period, columns with unlike letters differ significantly at $P = 0.05$, according to Fisher's Protected LSD test.

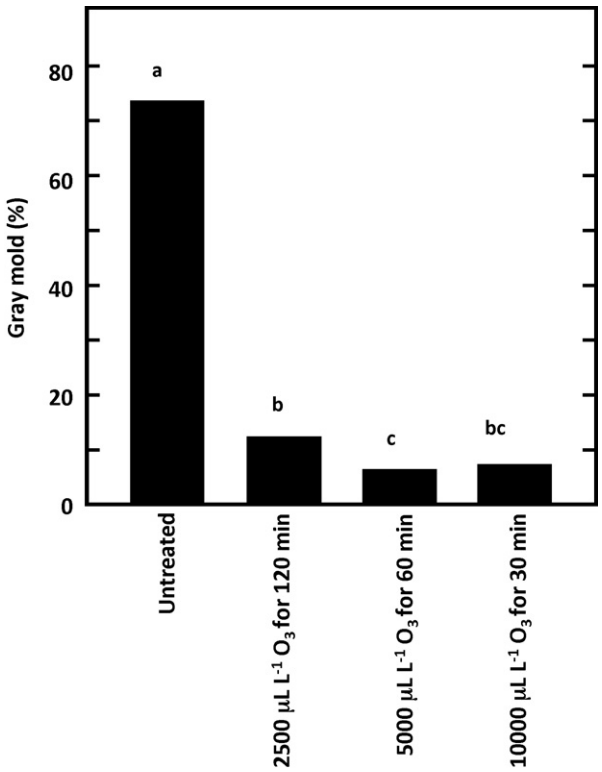

Fig. 3. Gray mold incidence on 'Autumn Seedless' table grapes after fumigation with different ozone concentration and treatment period combinations, all equal in doses to 5000 $\mu L\,L^{-1} \times$ h ozone concentration and time product, followed by 7-d storage at 15 °C. The fruit were inoculated by spraying with *Botrytis cinerea* conidial suspension and kept in humid boxes at 15 °C for 24 h prior to ozone fumigation. Each value is the mean of five replicates per treatment and each replicate contained 1 cluster bags with 900 g of grapes. Before ANOVA, arcsin of the square root transformation was applied to the proportion of infected berries. Actual values are shown. Columns with unlike letters differ significantly at $P = 0.05$, according to Fisher's Protected LSD test.

3.3. Influence of ozone fumigation on fungicide residues

Residues of fenhexamid, cyprodinil, pyrimethanil and pyraclostrobin were reduced significantly by ozone fumigation by 68.5, 75.4, 83.7, and 100.0%, respectively, after a single fumigation of table grapes with 10,000 $\mu L\,L^{-1}$ ozone for 1 h (Table 1). Residues of iprodione and boscalid were not significantly reduced.

4. Discussion

The effectiveness of a single ozone fumigation (5000 $\mu L\,L^{-1}$ of ozone for 60 min) varied among experiments and cultivars. Postharvest gray mold incidence in semi-commercial tests was usually reduced from 35 to 70%. We believe that variability in ozone effectiveness may somewhat be related to the cultivar fumigated as some cultivars are more resistant to gray mold than the others. The integrity of the berries may also be important, since conidia residing in wounds, such as those created by loose pedicels, would be pro-

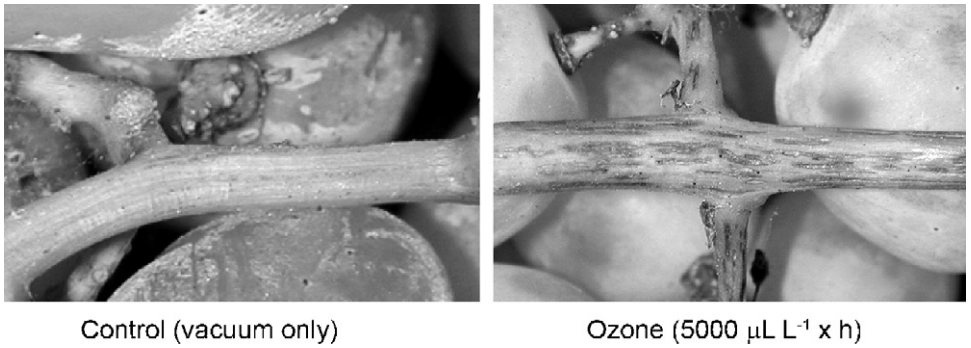

Fig. 4. Occasional injuries to 'Thompson Seedless' grape cluster rachis after grapes were fumigated once with 5000 $\mu L\,L^{-1}$ ozone for 1 h. Grapes were stored for 7 d at 15 °C.

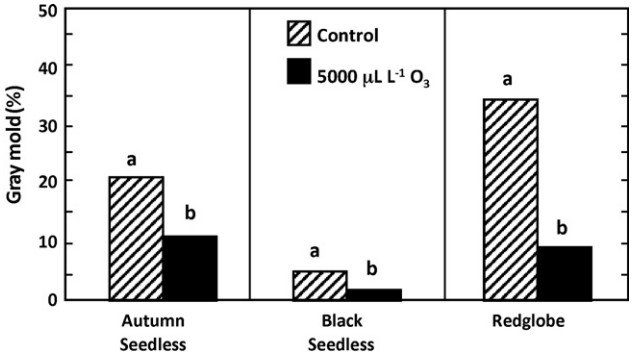

Fig. 5. Influence of postharvest ozone fumigation on the natural incidence of postharvest gray mold among several table grape cultivars. Freshly harvested, organically grown table grapes were fumigated with 5000 $\mu L\,L^{-1}$ ozone for 60 min in a commercial ozone chamber and stored for 6 weeks at 0.5 °C. Each value is the mean of four replicates per treatment and each replicate box contained 9 cluster bags with 1000 g of grapes each. Before ANOVA, arcsin of the square root transformation was applied to the proportion of infected berries. Actual values are shown. For each cultivar, columns with different letters differ significantly at $P \leq 0.05$ (paired *t*-test).

tected from ozone, which does not penetrate into berry flesh. Decay caused by *Alternaria* and *Penicillium* spp. was poorly controlled by ozone. Our results corroborate those of Shimizu et al. (1982), who reported that an overnight fumigation with 500 $\mu L\,L^{-1}$ ozone controlled postharvest gray mold, but did not control *Alternaria* spp. decay, and it caused brown spots on rachis and pedicel of 'Kyoho' grapes. In our tests ozone fumigation did not injure berries, but in some tests minor rachis injury occurred. Mlikota Gabler and Smilanick (2001) reported that injury to the rachis occurred after immersion of small grape clusters for up to 6 min in water that contained at 10 $\mu L\,L^{-1}$ ozone and described the injury as thin longitudinal, parallel light brown lines, approximately 3 mm in length and 0.5 mm wide, identical to what we sometimes observed after ozone fumigation with 5000 $\mu L\,L^{-1}$ of ozone for 60 min. The injuries we observed appeared in some tests, and not in other tests, even when the same doses of ozone were applied, which makes us believe that the condition or quality of rachis before harvest makes some of them more susceptible to injury than others. However, Palou et al. (2002) did not notice any phytotoxic injuries on fruit tissues or rachis in grapes stored under 0.3 $\mu L\,L^{-1}$ ozone for 7 weeks. Although ozone controlled gray mold, it seems that there is a relatively small difference between the lethal threshold to decay pathogens and ozone injury to rachis (Lichter et al., 2006).

The concentrations of SO_2 and ozone to kill conidia of *B. cinerea* on glass slides within 1 h are 78.3 and 236 $\mu L\,L^{-1}$, respectively, at cool temperatures (Smilanick and Henson, 1992; Palou et al., 2007). The concentrations we used in fumigation with ozone, up to 10,000 $\mu L\,L^{-1}$, far exceeded those required to kill exposed conidia. SO_2 is very soluble in aqueous solutions while ozone is not. Therefore penetration into fruit tissue, where the gas must first dissolve into an aqueous phase, is better with SO_2. In solution, SO_2 diffuses through membranes and accumulates in microbes by an ionization-entrapment mechanism (Smilanick et al., 1990a). In contrast to SO_2, ozone has limited water solubility and it leaves no residues. Ozone is highly reactive with organic and many inorganic constituents and is rapidly consumed by them. The effectiveness of ozone is greatly influenced by ozone demand of the medium (Karaca and Velioglu, 2007). When applied to fresh fruits and vegetables that are rich in organic matter, ozone demand is high and the amount of required ozone for microbial inactivation increases. We fumigated with ozone at rates that would kill conidia exposed to the gas, but it is likely that when the pathogen resided in latent infections or within wounds, it survived ozone fumigation. The high ozone demand of the grapes could explain the poor results to control postharvest gray mold in prior work with constant low doses of ozone (Palou et al., 2002; Artes-Hernandez et al., 2004, 2007).

The high ozone concentrations we employed significantly reduced the number of *B. cinerea* infections, most likely by reducing the number of viable conidia on the surface of the berry. In our work, high doses of ozone did not control *B. cinerea* infections already established in grape tissue because it was unable to control 48 h old infections on grapes that had been incubated at 15 °C before fumigation. Microorganisms embedded in fruit are more resistant to ozone than those exposed (Mahapatra et al., 2005). We suspect that the majority of postharvest gray mold in our work that developed after initial ozone fumigation was from older or latent infections.

Ozone applications reduced the content of many fungicides in water or on fruit surfaces. Captan and mancozeb residues on apples were degraded by ozonated water treatments (Ong et al., 1996; Hwang et al., 2001). Metzger et al. (2007) reported that imazalil residues on citrus fruits were reduced by approximately 40% after storage for 35 d in 0.18–0.20 $mL\,L^{-1}$ ozone enriched atmosphere. In our study, residues of four commonly used vineyard fungicides (fenhexamid, cyprodinil, pyrimethanil and pyraclostrobin) applied to control *B. cinerea* were significantly reduced by ozone fumigation. Iprodione and boscalid residues were not degraded by ozone. Residues of these fungicides, both with systemic activity, were either resistant to degradation or protected from ozone within the grape tissue. Hu et al. (2000) reported iprodione degradation in ozonated water was relatively slow. If the residues of the vineyard fungicides have some role in inhibiting gray mold development during storage, this aspect of ozone activity may be undesirable. Degradation of fungicide residues on grapes by ozone could have a practical application; namely, that grapes could be fumigated before shipping to reduce the residues. This approach may be feasible if the degradation products of the fungicides are known and are harmless.

In conclusion, fumigation with high doses of ozone gas during pre-cooling of grapes controlled postharvest decay and reduced residues of four commonly used fungicides. Ozone is unlikely to

Table 1
Influence of 10,000 $\mu L\,L^{-1}$ ozone fumigation for 1 h at 5 °C of 'Ruby Seedless' table grapes on residues (mg kg^{-1}) of fungicides applied before treatment. The fungicides were applied 1 d before ozone treatment. Residues were determined 3–5 d after treatment. Each value is the mean of three measurements.

Fungicide	Fungicide residue (mg $kg^{-1} \pm$ SD)				Reduction (%)
	Non-fumigated	Ozone fumigated	DL[a]	*t*-Test	
Iprodione	2.61 ± 0.24	2.48 ± 0.43	0.05	NSD[b]	5.0
Boscalid	0.48 ± 0.08	0.40 ± 0.04	0.02	NSD	16.7
Fenhexamid	1.11 ± 0.12	0.35 ± 0.05	0.05	<0.0001	68.5
Cyprodinil	5.05 ± 2.00	1.24 ± 0.26	0.05	0.03	75.4
Pyrimethanil	4.66 ± 0.76	0.76 ± 0.19	0.05	0.01	83.7
Pyraclostrobin	1.93 ± 0.18	0.0 ± 0.00	0.05	<0.0001	100.0

[a] DL = detection limit (mg kg^{-1}) of the method.
[b] NSD = not significantly different.

replace SO_2 treatments in conventional grape production unless their efficacy is improved, but it could be an acceptable technology to use with grapes marketed under "organic" classification, where the use of SO_2 is prohibited, or if SO_2 use were to be discontinued. The reactive nature of ozone, like that of SO_2, requires these chambers to be designed to resist damage caused by these corrosive gases.

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