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Application of low concentrations of ozone during the cold storage of table grapes☆

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ABSTRACT

The control by ozone of postharvest decay of table grapes, caused by *Botrytis cinerea* and other pathogens, was evaluated in chambers and commercial storage facilities. Ozone at 0.100 μL/L or higher inhibited the spread of gray mold among stored grapes. Ozone diffusion into many types of commercial packaging was measured. Boxes made of uncoated paper corrugate inhibited diffusion more than those composed of coated paper corrugate, plastic corrugate, hard plastic, or expanded polystyrene. Internal packaging of hard plastic clamshell containers inhibited diffusion less than low density polyethylene cluster bags. Atmospheres of 0.100 μL/L ozone in the day and 0.300 μL/L at night reduced the natural incidence of gray mold by approximately 65% after 5–8 weeks of storage. Its effectiveness to control postharvest decay was compared to sulfur dioxide fumigation. After 68 days at 1 °C the incidence of gray mold among grapes stored in air, ozone, or with weekly sulfur dioxide fumigation was 38.8%, 2.1%, and 0.1%, respectively. However, decay by other fungi, such as *Alternaria* spp. and *Penicillium* spp., was controlled by sulfur dioxide, but not by ozone. In some tests, rachis appearance was moderately harmed by ozone. The combination of ozone use in storage following a single initial sulfur dioxide fumigation, or its use in between biweekly sulfur dioxide fumigations, controlled both gray mold and other pathogens and matched the commercial practice of initial and weekly sulfur dioxide fumigation. The use of both gases in this way reduced sulfur dioxide use greatly. Differences in flavor of grapes treated with ozone were not detectable compared to those stored in air, and grapes treated with ozone were preferred over those treated with sulfur dioxide.

1. Introduction

Botrytis cinerea causes gray mold, the most destructive postharvest disease of table grapes, primarily because it grows at very low temperatures and spreads rapidly by aerial mycelial growth among stored products (Snowdon, 1990). Although in many controlled laboratory studies, ozone gas inhibited gray mold spread among stored grapes (Palou et al., 2002; Tzortzakis et al., 2007; Cayuela et al., 2009; Sharpe et al., 2009), little has been published about ozone use under commercial conditions. Inhibition of aerial mycelial growth of *B. cinerea*, and not the inactivation of its conidia, seems to be the primary inhibitory action of low concentrations of ozone on gray mold (Rubio Ames et al., 2013), and a contact between the gas and the fungal hyphae should be assured in order to make the ozone effective in controlling postharvest fruit spoilage (Palou et al., 2003). In addition to direct action on the pathogens, when ozone was tested *in vivo* on fruit, its effectiveness in controlling pathogens could be due, to some extent, to resistance induced in host tissues by ozone (Minas et al., 2010; Tzortzakis et al., 2011, 2013; Boonkorn et al., 2012), such as the increased production of bioactive phenolics in grapes after ozone exposure (Sarig et al., 1996; Artés-Hernández et al., 2003, 2007; González-Barrio et al., 2006; Cayuela et al., 2009).

The use of continuous low concentrations of ozone, rather than high concentrations, is preferred to minimize exposure of workers to hazardous concentrations of the gas, to reduce the risk of injury to the fruit and refrigeration equipment, and to minimize the cost of ozone generation equipment. Developing the best practices for use of ozone is particularly valuable because it does not deposit residues, unlike the commercial practice of sulfur dioxide fumigation used for many years (Romanazzi et al., 2012), most regulatory issues associated with its use are resolved (USFDA, 2001), it

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is classified as "organic" by the USDA National Organic Program, and high quality, reliable ozone generation equipment is now widely available. Compliance with the maximum decay tolerance rules is challenging for all producers of table grapes because they are very low; in the USA, the incidence of decayed berries must not exceed 0.5% incidence when grapes are shipped (USDA, 2009). This is especially challenging because "organic" production rules state neither vineyard fungicides nor sulfur dioxide fumigation can be used. For conventional growers, sulfur dioxide is very effective and inexpensive, but it causes bleaching injuries (Luvisi et al., 1992) and a hairline cracking disorder (Zoffoli et al., 2008) after repeated fumigations, and it can harm the flavor of the berries (Fernández-Trujillo et al., 2008). Although preharvest fungicide applications (Franck et al., 2005; Smilanick et al., 2010) and cultural practices (Molitor et al., 2011; Schilder et al., 2011) can significantly reduce subsequent postharvest decay, they are not effective enough to eliminate the need for postharvest fumigation with sulfur dioxide.

Because of its highly reactive nature and oxidizing power, applications of ozone gas in packinghouses can cause physiological changes that can lead to modifications in external or internal quality of harvested fresh fruit and vegetables, although it does not bleach pigments in grapes or other fruit, even at relatively high rates (Palou et al., 2006; Karaca and Velioglu, 2014). In previous work, some rachis injuries appeared on stem of grapes cluster after ozone storage (Mlikota Gabler et al., 2010), in other work, rachis injuries did not occur (Sarig et al., 1996; Palou et al., 2002). Similarly, some authors stated that trained panelists reported the flavor of table grapes was harmed by ozone exposure (Cayuela et al., 2009), while in another study (Artés-Hernández et al., 2004), the flavor of table grapes was unaltered by ozone.

Our objectives in the present work were to: (i) compare different low ozone concentrations to find the lowest active dose needed to inhibit aerial mycelial spread from infected berries; (ii) measure ozone diffusion into various combinations of commercial external and internal grape packaging; (iii) determine the effectiveness of continuous and discontinuous low ozone concentrations in commercial facilities; (iv) combine the use of sulfur dioxide and ozone in sequences to minimize the deleterious effects of sulfur dioxide on berry quality and control fungi that ozone alone did not; and (v) assess the consumer acceptability of both for ozone- and sulfur dioxide-treated grapes through difference and preference sensory tests.

2. Materials and methods

2.1. Lowest continuous ozone concentrations to control postharvest decay

To determine the effective ozone concentrations to control postharvest decay, an 8 chamber system to generate and monitor ozone and control humidity was assembled. Freshly harvested, organic 'Crimson Seedless' table grapes (*Vitis vinifera*) were purchased from a local grower. About 2 kg of grape clusters were placed into each hard plastic clamshell container (40 cm in length, 20 cm high, and 20 cm wide) and 6 replicate containers were placed in 117 L stainless steel chambers containing air or ozone at 0.075, 0.100, 0.150, 0.200, 0.250, 0.300 or 0.500 μL/L at 2 °C for three weeks. When closed, the lids of clamshell containers left a gap of 0.5 cm and did not impede ozone diffusion into the grapes. Ozone was produced by passing compressed air through a 1.2 m × 3.7 cm wide column containing desiccant (Drierite, Sigma–Aldrich, St. Louis, MO) into a corona discharge ozone generator followed by a flow meter board with two flow meters per chamber, and finally the ozonated air was passed through a water solution to humidify it before is passed into the loaded chambers. Relative humidity was high (*ca.* 95%) and confirmed with a relative humidity monitor. Ozone concentration inside each chamber was continuously monitored and recorded every 20 min using two, six-channel UV ozone monitors (Model 465, API Inc., San Diego, CA) where the output was recorded on a laptop. Two single berries were inoculated by the injection at a depth of 5 mm of 20 μL of a suspension containing 10^6 spores of *B. cinerea* (isolate 1440) per mL before placement inside two clusters inside each clamshell. After storage, berries in each clamshell were examined to determine the incidences of natural gray mold and other rots. Observations included the spread of gray mold from the single artificially inoculated berry expressed as the number of berries near it that became infected, and the number of naturally detached berries (shatter). Indices for visible aerial mycelial growth on the surface of the original inoculated berry and the appearance of the grape cluster rachis were recorded. The index of aerial mycelium used a scale of 1–5, where: 0, no aerial mycelium present; 1, aerial mycelium visible but not more than 5% of berry surface; 2, >5–15% of the berry surface covered with aerial mycelium; 3, >15–30% of the berry surface covered with aerial mycelium; 4, >30–60% of the berry surface covered with aerial mycelium; or 5, >60% of the berry surface covered with aerial mycelium. The index describing rachis appearance used a 1–5 scale, where: 0, the entire rachis is fresh looking and green in color; 1, most pedicels of the rachis are brown; 2, all pedicels and less than 50% of lateral branches of the rachis are brown; 3, all pedicels and most laterals rachis branches are brown; 4, all pedicels and laterals branches are brown, and the main stem of the rachis exhibits some browning; or 5, the entire rachis is brown.

2.2. Ozone concentrations inside commercial packages under controlled conditions

The concentrations of ozone that diffused into 'Thompson Seedless' grape packages in five kinds of boxes with two types of internal packaging was measured using two six-channel ozone monitors (Model 465L, Teledyne API, Inc., San Diego, CA). Ozone was produced and controlled by a PurFresh-Cold Storage system (PurFresh, Inc., 47211 Bayside Parkway, Fremont, CA), inside a stainless steel environmental chamber 3 m wide, 2.7 m in length, and 3 m tall with two fans behind cooling coils. The air is mixed relatively uniformly in the room by these fans and air speed measurements taken on the exposed sides of the boxes were 8.9 ± 2.8 m/s measured with a hot wire air-speed meter (Model 9870, Alnor TSI Inc., Shoreview, MN). The ozone concentration within the room was constantly 0.300 μL/L. The boxes were arranged in a six-down pattern three boxes high, and the only boxes in the middle layer were monitored. Sampling lines were placed inside the packages within grape clusters. Three replicates of each combination of box and internal packaging were done. The five kinds of boxes included returnable plastic container of hard plastic, expanded polystyrene, plastic corrugate, paper corrugate with a water resistant coating, or uncoated paper corrugate. In all tests, when ozone diffusion was evaluated, the boxes contained 9 kg of grapes within internal packaging of either nine vented, low density polyethylene plastic cluster bags or eight polystyrene plastic clamshell containers filled with table grapes. The box vent area percentage respect to the entire box surface area was 6.3%, 5.6%, 5.9%, 7.6%, and 4.6%, respectively for returnable plastic container, expanded polystyrene, plastic corrugate, coated paper corrugate, and uncoated paper corrugate. The vent area of the clamshell containers, with many slots and circular slots and a 3 mm wide open gap when closed, was approximately 10%, while that of cluster bags, with the vents composed of approximately 90 circular holes 4 mm in diameter, was approximately 3.5% when closed, but these bags are always used in an open position so their vent area is variable.

2.3. Ozone concentrations inside commercial packages under commercial conditions

Freshly harvested, organic 'Princess Seedless' table grapes in about 9 kg boxes were placed in commercial storage at 1 °C with sampling lines inside packages to monitor their internal ozone concentration. Three kinds of boxes, expanded polystyrene, coated paper corrugate, and uncoated paper corrugate, and three kinds of internal packaging, naked or plain packed grapes, clamshell containers, or open plastic cluster bags, as previously described, were used. The boxes were arranged on a pallet in a five-down pattern with three layers for each combination of box and internal packaging. During precooling, the grapes were cooled in an atmosphere of 0.300 μL/L ozone, then, during long cold storage, they were exposed to 0.100 μL/L ozone from 5 AM to 12 AM and 0.300 μL/L from 12 AM to 5 AM every day. Ozone was generated using the (PurFresh) equipment previously described. The average ozone concentrations was recorded by multiple channel monitors (Teledyne API) as previously described with sampling lines 15.2 m in length placed in the room and within the various table grape packages over repeated 3-day periods for 4 consecutive weeks. Two samples of each type of package were monitored and each value is the mean of the ozone concentrations measured at intervals of 20 min inside three packages over each of the measurement periods.

2.4. Ozone effectiveness under commercial conditions

Freshly harvested, organic 'Princess Seedless', 'Flame Seedless', and 'Thompson Seedless' table grapes were used for the experimental trials carried out in commercial facilities. The grapes were packed in approximately 9 kg commercial uncoated paper corrugate boxes (with a vented area of 5%) with internal packaging of: (1) four clamshell containers containing clusters with 400–600 berries. When closed, the lids of clamshell containers had a gap of 0.5 cm with a vent area of approximately 10%; or (2) nine cluster bags containing 150–250 berries each, each ventilated with 50 round holes approximately 0.3 cm in diameter, with a vented area of approximately 1.1% when closed, but these bags are always used in an open position so their vent area was variable. Boxes were arranged in pallets with all sides of the pallets exposed to the room atmosphere. After initial pre-cooling of grapes in air, during long cold storage at 2 °C, they were exposed to 0.100 μL/L ozone from 5 AM to 12 AM and 0.300 μL/L from 12 AM to 5 AM every day, or stored in air alone for up to 8 weeks. Ozone was generated using the (PurFresh) equipment previously described. Prior to storage, single berries were inoculated and placed inside the clusters within each clamshell container or cluster bag as previously described. After storage, the incidences of natural gray mold and that of other rots, the spread of gray mold from the single artificially inoculated berry, and indices for visible aerial mycelial growth on the original inoculated berries and the appearance of the grape cluster rachis were recorded as previously described. Five or six replicate boxes of each treatment were examined.

2.5. Effectiveness of continuous or discontinuous low concentrations of ozone

Freshly harvested 'Autumn King' table grapes were used to evaluate the effectiveness of continuous or discontinuous low concentrations of ozone to control postharvest decay. The grapes were stored in air, with weekly 350 μL/L sulfur dioxide fumigation at a commercial grape storage facility, or with continuous or discontinuous 0.300 μL/L ozone in a laboratory chamber for 5 weeks at 1 °C. When ozone was applied discontinuously, the exposure to ozone atmosphere was: (1) once per week for 15 h; (2) four times per week for 15 h, in total 60 h per week; (3) once per week for 60 h; or (4) four times per week for 15 h plus once per week for 60 h, for a total 120 h per week. The exposure to ozone of these periods simulated scenarios of a cold storage that uses ozone fumigation at night or weekends when few workers are present. Ozone was generated using the (PurFresh) equipment previously described. Eight replicate plastic clamshell containers with a vented area of approximately 10% containing about 1 kg of grapes in an expanded polystyrene box per each treatment were prepared. Prior to storage, single berries were inoculated and placed inside the clusters within each clamshell container as previously described. After storage, the incidences of natural gray mold and that of other rots, the spread of gray mold from the single artificially inoculated berry, and indices for visible aerial mycelial growth on the original inoculated berries and the appearance of the grape cluster rachis were recorded as previously described.

2.6. Continuous low concentrations of ozone used alone or with weekly sulfur dioxide fumigation

A large test with 'Crimson Seedless' table grapes was conducted in cold chambers and sulfur dioxide chambers to evaluate the use of ozone in sequence with sulfur dioxide fumigation. The grapes were stored with sulfur dioxide fumigation and/or ozone fumigation (continuous 0.300 μL/L) in weekly or bi-weekly increments, or in air (control) for 68 days at 1 °C. Ozone was generated using the (PurFresh) equipment previously described. The sulfur dioxide concentration × time products measured using dosimeter tubes (model 5DH, Gastec Corporation, Kanagawa, Japan) applied in these fumigations was 400–600 μL/L h. For each treatment, four replicate uncoated paper corrugate boxes containing nine cluster bags that contained approximately 950 g of grapes each were prepared. The bags had vents composed of approximately 90 circular holes 4 mm in diameter, with a vent area of approximately 3.5% when closed, but these bags are always used in an open position so their vent area is variable. Prior to storage, single berries were inoculated and placed inside the clusters within each clamshell container as previously described. After storage, the incidences of natural gray mold and that of other rots, the spread of gray mold from the single artificially inoculated berry, and indices for visible aerial mycelial growth on the original inoculated berries and the appearance of the grape cluster rachis were recorded as previously described.

2.7. Continuous low concentrations of ozone used alone or in combination with sulfur dioxide fumigation conducted during initial precooling

Freshly harvested 'Autumn King' table grapes were used for the experimental trials to evaluate the effectiveness in controlling postharvest decay of continuous low ozone atmosphere used alone or in combination with sulfur dioxide fumigation conducted during precooling. Ozone was generated using the (PurFresh) equipment previously described. Three replicate expanded polystyrene boxes containing eight clamshell containers that contained approximately 1 kg of grapes each were prepared per each treatment. Prior to precooling or the beginning of storage in ozone, single berries were inoculated and placed inside the clusters within each clamshell container as previously described. Table grapes were precooled in air or fumigated with 350 μL/L sulfur dioxide. After the precooling, table grapes were stored in air, with weekly 350 μL/L sulfur dioxide fumigation at a commercial grape storage facility or with continuous 0.300 μL/L ozone in a laboratory chamber for 5 weeks at 1 °C. Evaluation of table grape decay was carried out twice, one at the end of the storage, and the second after 48 h at 20 °C to simulate temperatures during shelf-life. After storage, the incidence of natural gray mold and that of other rots, the spread of gray mold from the single artificially inoculated berry, and indices

for visible aerial mycelial growth on the original inoculated berries and the appearance of the grape cluster rachis were recorded as previously described.

2.8. Consumer sensory evaluation

Triangle discrimination testing (Meilgaard et al., 1999; Lawless and Hildegarde, 2010) was the technique employed to determine whether there is a detectable difference in the flavor of table grapes stored in air, in an atmosphere of ozone, or fumigated with sulfur dioxide. The test was carried out with 30 untrained panelists. Each panelist was presented with two sets of three berries each. One set was composed by three berries that were stored for 3 weeks at 1 °C in air or in atmosphere of 0.300 μL/L ozone. The other set was composed by three berries that were stored for 3 weeks at 1 °C in air or that were fumigated with 350 μL/L of sulfur dioxide weekly. In each set, two of three berries had the same storage condition, while one was different. Samples were presented in a random order and assigned three-digit codes to reduce influencing the decisions of panelists. Per each set, the panelists were asked to taste the berries and circle the sample number that they were determined was the different berry. This test was repeated on three different days, each test at 10 AM. For the air/ozone triangle test, 'Autumn King' and the selections 'B26-120' and 'Y151-142' were used. All of the grapes were green in color. For the air/sulfur dioxide triangle test, only the 'Autumn King' was used.

In the sensory analysis of table grapes, paired preference test was employed to establish whether there is a preference between two 'Autumn King' table grape samples stored in different conditions. Each panelist was presented with sets of two berries each. In each set, the berries were stored for 3 weeks at 1 °C in air, or in atmosphere of 0.300 μL/L ozone, or fumigated with 350 μL/L sulfur dioxide weekly, but both berries never had the same storage conditions. Samples were presented in a random order and assigned three-digit codes to reduce influencing the decisions of panelists. Per each set, the panelists were asked to taste the berries and circle the sample number that they preferred. This test was carried out with 30 untrained panelists, repeated in four different days, every day at 10 AM.

2.9. Statistical analysis

Data were analyzed by a paired *t*-test or a one-way analysis of variance with mean separation by Fisher's protected least significant difference or Tukey's honestly significant difference test at $P = 0.05$ (SPSS Statistics 17.0 Inc., IBM, Chicago, IL). Percentage data were arcsine transformed before analysis to improve homogeneity of variance when the range of percentages was greater than 40. Actual values are shown. To analyze the data obtained from the sensory evaluation panelists, the correct answers and the expressed preferences of the triangle and preference tests, respectively, were compared to tabulated critical values (Lawless and Hildegarde, 2010).

3. Results

3.1. Lowest continuous ozone concentrations to control postharvest decay

Storage in ozone at 0.100 μL/L or more significantly decreased the natural incidence of gray mold (Table 1). Increasing the ozone concentration above 0.100 μL/L did not significantly decrease the number of naturally infected berries. The incidence of other rots among grapes stored in ozone was irregular and not significantly reduced compared to the control. The other rots were mostly *Alternaria* spp., with some *Penicillium* spp. Ozone storage at all concentrations significantly reduced the aerial mycelial growth of *B. cinerea* on the surface of the inoculated berries. The spread of gray mold from the inoculated berry to adjacent healthy berries was significantly reduced by ozone concentrations of 0.100 μL/L and higher. Increasing the ozone concentration above 0.100 μL/L did not significantly decrease the number of berries that became infected that were adjacent to the inoculated berry. The number of shattered berries was significantly reduced by all the ozone concentrations, except with 0.075 or 0.200 μL/L in which it was lower, but not significantly so. The rachis appearance was little altered by any treatment, except at 0.150 μL/L, where the rachis was greener than the control. Ozone at 0.100 μL/L or more reduced the natural incidence and the spread of *B. cinerea* from an artificially infected berry, did little to influence decay by other pathogens, berry shatter or rachis appearance. None of the berries appeared harmed by any of the ozone concentrations used in this study (data not shown).

3.2. Ozone concentrations inside commercial packages under controlled conditions

The concentration of ozone into packages in chambers over a 4-day period was influenced and often greatly reduced by the external box and internal packaging. Ozone concentrations were highest inside reusable plastic containers or expanded polystyrene boxes, and lower in plastic corrugate or coated paper corrugate, and lowest inside uncoated paper corrugate boxes (Fig. 1). The effect of

Table 1
Influence of ozone on gray mold or other rots (mainly *Alternaria* spp. and *Penicillium* spp.) on 'Crimson Seedless' grapes after storage in air or different concentrations of ozone at 2 °C for 3 weeks. The grapes were placed in clamshell boxes and exposed to ozone in laboratory chambers.

Ozone (μL/L)	Natural decay (%)		Gray mold on inoculated berries		Shatter[d]	Rachis[e]
	Gray mold	Other	Spread[b]	Aerial mycelium[c]		
0	6.7 a [a]	3.4	4.6 a	4.4 a	10.5 a	1.6 ab
0.075	7.3 a	3.1	4.7 a	2.3 bc	9.6 ab	1.6 ab
0.100	1.8 b	1.3	0.7 b	1.7 cde	4.1 cd	1.1 bc
0.150	1.1 b	1.6	0.6 b	1.7 cde	3.6 d	0.6 c
0.200	1.5 b	2.7	2.2 b	2.4 b	8.8 abc	1.6 ab
0.250	1.8 b	1.7	1.1 b	1.2 de	5.7 bcd	2.2 a
0.300	1.4 b	0.9	1.0 b	1.8 bcd	4.9 bcd	1.3 bc
0.500	2.3 b	1.2	1.7 b	1.1 e	6.3 bcd	1.5 ab
Sig. (*P*)	*0.002*	*0.212*	*0.000*	*0.000*	*0.011*	*0.021*

[a] Values within columns followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).
[b] The number at the end of storage of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters.
[c] Aerial mycelium on the inoculated berry at the end of storage: 0, none; 1, just visible to 5% of berry surface; 2, >5–15%; 3, >15–30%; 4, >30–60%; or 5, >60%.
[d] The percentage of naturally detached berries at the end of storage.
[e] Rachis appearance rating: 0, entire perfect fresh and green; 1, pedicels only are brown; 2, pedicels and <50% of laterals brown; 3, pedicels and most laterals brown; 4, pedicels and laterals brown and main stem some browning; or 5, entire rachis brown.

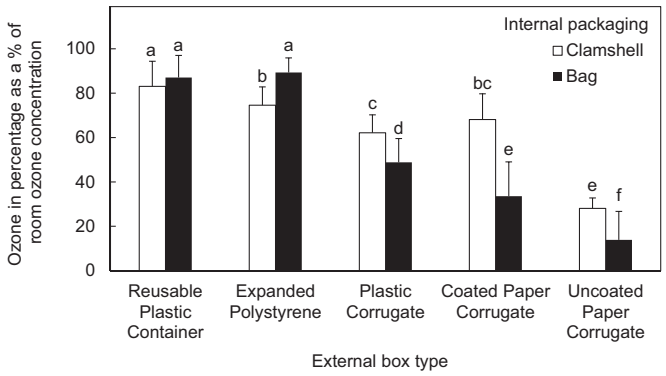

Fig. 1. Ozone concentrations within packages of 'Thompson Seedless' table grapes. Values were recorded at the end of a 96 h period in 0.300 μL/L ozone at 1 °C in a 3 × 2.4 × 2.4 m chamber. The external boxes and internal packaging used are in common commercial use.

internal packaging with clamshell containers or cluster bags on the concentration of ozone within packages was variable. Inside reusable plastic containers, ozone concentrations were not significantly different inside cluster bags and clamshell containers. Inside expanded polystyrene boxes, ozone concentrations were significantly higher inside cluster bags than clamshell containers. Inside plastic corrugate, coated paper corrugate, or uncoated paper corrugate boxes, the concentration of ozone was significantly lower inside internal packaging of plastic bags than clamshell containers.

3.3. Ozone concentrations inside commercial packages under commercial conditions

When the ozone concentration was measured inside packages in a commercial cold storage, it was greatly influenced by packaging (Fig. 2). Uncoated paper corrugate boxes had less ozone inside them than the coated paper corrugate or expanded polystyrene boxes. Prolonged periods of ozone exposure did not alter the concentration of ozone within the packages; the concentration of ozone inside the packages on the first week of monitoring was nearly identical and not significantly different than that measured on the final week, more than 28 days later.

3.4. Ozone effectiveness under commercial conditions

In commercial facilities, ozone reduced the natural incidence of gray mold among grapes in uncoated paper corrugate boxes that had grapes in either clamshell containers or cluster bags by

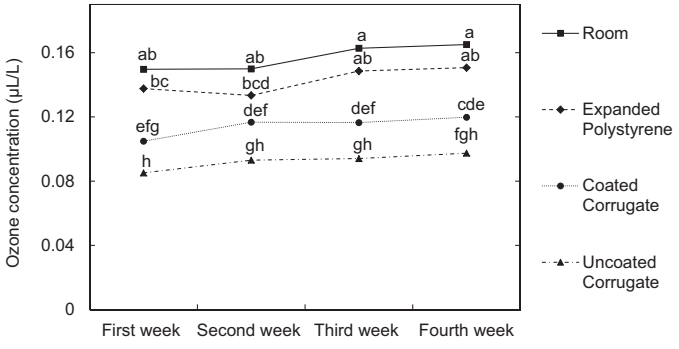

Fig. 2. Average ozone concentrations within 'Princess Seedless' table grape packages about 9 kg in weight in a commercial cold storage room over repeated 3-day periods for 4 consecutive weeks. The ozone concentration within the room was 0.100 μL/L ozone from 5 AM to 12 AM and 0.300 μL/L from 12 AM to 5 AM every day. Each value is the mean of the ozone concentrations measured at intervals of 20 min inside three packages over each of the measurement periods.

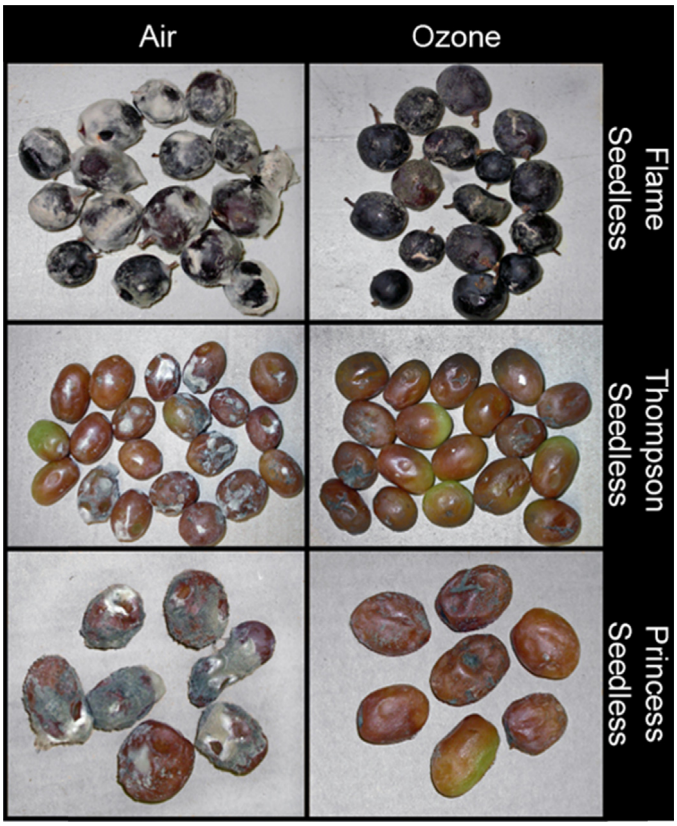

Fig. 3. Appearance of 'Flame Seedless', 'Thompson Seedless', and 'Princess Seedless' table grapes berries inoculated with a suspension of 10^6 spores of *B. cinerea* per ml and stored at 2 °C in air or in an ozone atmosphere for 7 weeks, 5 weeks, or 8 weeks, respectively. The concentration of ozone was 0.100 μL/L from 5 AM to 12 AM and 0.300 μL/L from 12 AM to 5 AM.

approximately 65% after 5–8 weeks of storage (Table 2). Control of the natural incidence of gray mold was always significantly reduced by the ozone treatment, while the incidence of decay caused by other pathogens was low and often not significantly reduced. Ozone effectiveness to control gray mold incidence and the growth of aerial mycelium of this pathogen on inoculated berries was significant and relatively high in all tests with the exception of 'Thompson Seedless' grapes in cluster bags, where control of natural decay and aerial mycelial growth were significant but the magnitude of the control was low. The appearance of the inoculated berries after storage in air or ozone was markedly different (Fig. 3). In addition to reduced aerial mycelial growth, some sporulation was present on the surface of the inoculated berries from the packages stored in ozone. Ozone reduced the spread of gray mold from artificially inoculated berries, placed within packages before storage, from 3.1 additional infected berries in air to 0.8 infected berries in ozone. Rachis quality was not significantly altered by ozone, except among 'Flame Seedless' grapes stored in ozone for 7 weeks, where the ratings in air and ozone were 3.3 and 3.7, respectively.

3.5. Effectiveness of continuous or discontinuous low concentrations of ozone

The natural incidence of gray mold among grapes stored with weekly conventional sulfur dioxide fumigation or with continuous ozone at 0.300 μL/L for 5 weeks at 1 °C was reduced compared to the control (Table 3). When the exposure to ozone was discontinuous, regardless the duration of the treatment, it was insufficient to effective control of the natural incidence of gray

Table 2
Influence of ozone on gray mold or other rots (mainly *Alternaria* spp. and *Penicillium* spp.) on 'Thompson Seedless', 'Flame Seedless', or 'Princess Seedless' grapes after storage in air or ozone at 2 °C for 5 weeks, 7 weeks, or 8 weeks, respectively. The grapes were stored in commercial storage facilities within uncoated paper corrugate boxes with internal packaging of four clamshell containers or nine cluster bags. When stored in ozone, the concentration was 0.300 μL/L nightly (12 AM to 5 AM) with 0.100 μL/L in the day (5 AM to 12 AM).

	Storage atmosphere	Packaging	Natural decay (%)		Gray mold on inoculated berries		Rachis[d]
			Gray mold	Other	Spread[b]	Aerial mycelium[c]	
Thompson	Air	Bag	10.0[a]	1.6	3.9	4.7	0.9
Seedless	Ozone	Bag	6.9	1.8	2.6	4.1	1.2
5 week storage	Sig. (*P*)		*0.018*	*0.625*	*0.026*	*0.003*	*0.232*
	Air	Clamshell	1.1	0.4	3.1	5.0	2.4
	Ozone	Clamshell	0.3	0.5	0.5	2.8	2.2
	Sig. (*P*)		*0.003*	*0.482*	*0.000*	*0.000*	*0.373*
Flame	Air	Bag	11.8	0.9	4.6	3.4	3.2
Seedless	Ozone	Bag	3.0	0.2	0.5	0.0	3.4
7 week storage	Sig. (*P*)		*0.000*	*0.000*	*0.000*	*0.000*	*0.071*
	Air	Clamshell	4.9	0.4	3.5	2.8	3.3
	Ozone	Clamshell	1.8	0.5	0.0	0.7	3.7
	Sig. (*P*)		*0.005*	*0.685*	*0.000*	*0.000*	*0.003*
Princess	Air	Bag	1.0	1.7	0.4	3.4	4.9
Seedless	Ozone	Bag	0.2	0.5	0.1	1.2	5.0
8 week storage	Sig. (*P*)		*0.000*	*0.000*	*0.030*	*0.000*	*0.081*

[a] Values for each cultivar within columns are significantly different or not according to a paired *t*-test ($P = 0.05$).
[b] The number at the end of storage of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters.
[c] Aerial mycelium on the inoculated berry at the end of storage: 0, none; 1, just visible to 5% of berry surface; 2, >5–15%; 3, >15–30%; 4, >30–60%; or 5, >60%.
[d] Rachis appearance rating: 0, entire perfect fresh and green; 1, pedicels only are brown; 2, pedicels and <50% of laterals brown; 3, pedicels and most laterals brown; 4, pedicels and laterals brown and main stem some browning; or 5, entire rachis brown.

mold. The natural incidence of decay by other pathogens, mostly *Alternaria* spp., was very effectively controlled by sulfur dioxide fumigation, while ozone treatment only partially reduced their incidence when the exposure to the gas was present the entire week (168 h), applied once for 60 h each week, or applied five times for a total of 120 h each week. The spread of gray mold from the inoculated berry and the aerial mycelial growth of *B. cinerea* on inoculated berries were most effectively controlled by the sulfur dioxide weekly fumigation. Continuous ozone (168 h) or five exposures to ozone (a total of 120 h each week) reduced the spread of gray mold from the inoculated berry and the aerial mycelial growth of *B. cinerea* on inoculated berries, while the briefer ozone regimes were ineffective. Rachis appearance was better than the control after the sulfur dioxide weekly fumigations or continuous ozone exposure. Except for the incidence of decay by other pathogens, there was no difference in ozone effectiveness between the 60 h/week 0.300 μL/L ozone exposure applied in 4 times for 15 h each week compared to a single exposure of 60 h each week.

3.6. Continuous low concentrations of ozone used alone or with weekly sulfur dioxide fumigation

The natural incidence of gray mold among grapes stored in an ozone concentration of 0.300 μL/L for 68 days at 1 °C was reduced by 94% compared to the control (Table 4). However, it was significantly less effective than sulfur dioxide applied once weekly, the conventional industry practice, or sulfur dioxide applied once every two weeks. The natural incidence of gray mold was nearly totally inhibited by all of the sulfur dioxide treatments. When sulfur dioxide was applied once every two weeks, with storage in ozone at 0.300 μL/L in between fumigations, the natural incidence of gray mold was also totally inhibited and not different than either of the sulfur dioxide treatments alone. The natural incidence of decay by other pathogens, mostly *Alternaria* and *Penicillium* spp., was very effectively controlled by all of the sulfur dioxide fumigations, while the ozone treatment did not reduce their incidence. The spread of gray mold from an inoculated berry was most effectively controlled by the sulfur dioxide weekly fumigation,

Table 3
Influence of weekly ozonation period on gray mold or other rots (mainly *Alternaria* spp. and *Penicillium* spp.) on 'Autumn King' grapes. The grapes were packaged in expanded polystyrene boxes with internal packaging of clamshell containers and stored for 5 weeks at 1 °C.

Treatment (weekly)[a]	Natural decay (%)		Gray mold on inoculated berries		
	Gray mold	Other	Spread[c]	Aerial mycelium[d]	Rachis[e]
Air continuous	2.4 a[b]	9.6 ab	5.7 a	4.5 a	2.5 a
O_3 (once 15 h)	2.5 a	6.3 bc	5.3 a	4.6 a	2.0 ab
O_3 (4 times 15 h = 60 h)	1.5 abc	11.5 a	5.9 a	4.5 a	2.8 a
O_3 (once 60 h)	2.0 ab	6.0 c	5.5 a	4.3 a	2.5 a
O_3 (4 times 15 h + once 60 h = 120 h)	0.9 abc	3.6 cd	3.1 b	2.6 b	2.1 ab
O_3 (continuous 168 h)	0.7 bc	3.9 c	2.8 b	1.9 b	1.6 b
SO_2 fumigation once weekly	0.1 c	0.7 d	0.0 c	1.0 c	0.3 c
Sig. (*P*)	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*

[a] When stored in ozone, the concentration was 0.300 μL/L. Storage regimes were: air, fumigated weekly with 350 μL/L of sulfur dioxide and stored in air, or exposed to ozone for various periods. During each week of storage, the grapes were exposed to ozone: (1) continuously (168 h); (2) once for 15 h per week; (3) four times per week for 15 h (60 h); (4) once for 60 h per week; and 5) four times for 15 h and once for 60 h per week (120 h).
[b] Values within columns followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).
[c] The number at the end of storage of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters.
[d] Aerial mycelium on the inoculated berry at the end of storage: 0, none; 1, just visible to 5% of berry surface; 2, >5–15%; 3, >15–30%; 4, >30–60%; or 5, >60%.
[e] Rachis appearance rating: 0, entire perfect fresh and green; 1, pedicels only are brown; 2, pedicels and <50% of laterals brown; 3, pedicels and most laterals brown; 4, pedicels and laterals brown and main stem some browning; or 5, entire rachis brown.

Table 4
Influence of ozone and sulfur dioxide fumigation on gray mold or other rots (mainly *Alternaria* spp. and *Penicillium* spp.) on 'Crimson Seedless' grapes. The grapes were packaged in expanded polystyrene boxes with internal packaging of clamshell containers and stored for 68 days at 1 °C.

Treatment (weekly)[a]	Natural decay (%)		Gray mold on inoculated berries		
	Gray mold	Other	Spread[c]	Aerial mycelium[d]	Rachis[e]
Control	38.8 a[b]	30.5 a	4.32 a	4.2 a	3.8 b
Sulfur dioxide weekly	0.1 c	0.4 b	0.15 d	0.6 c	3.2 c
Sulfur dioxide bi-weekly	0.1 c	0.7 b	0.62 bc	1.5 b	3.6 c
Sulfur dioxide bi-weekly + ozone	0.2 c	1.2 b	0.21 cd	0.4 c	4.2 a
Continuous ozone	2.1 b	30.3 a	0.65 b	1.6 b	4.9 a
Sig. (*P*)	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*

[a] They were stored: (1) in air (control); (2) with weekly 350 μL/L sulfur dioxide fumigation; (3) with bi-weekly 350 μL/L sulfur dioxide fumigation; (4) with bi-weekly 350 μL/L sulfur dioxide fumigation with continuous 0.300 μL/L ozone between sulfur dioxide fumigations; or 5) in continuous 0.300 μL/L ozone. This test was conducted in laboratory chambers.
[b] Values within columns followed by the same letter are not significantly different according to Tukey's HSD ($P = 0.05$).
[c] The number at the end of storage of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters.
[d] Aerial mycelium on the inoculated berry at the end of storage: 0, none; 1, just visible to 5% of berry surface; 2, >5–15%; 3, >15–30%; 4, >30–60%; or 5, >60%.
[e] Rachis appearance rating: 0, entire perfect fresh and green; 1, pedicels only are brown; 2, pedicels and <50% of laterals brown; 3, pedicels and most laterals brown; 4, pedicels and laterals brown and main stem some browning; or 5, entire rachis brown.

while the bi-weekly sulfur dioxide or ozone alone treatments were similar to each other and less effective. When sulfur dioxide was applied once every two weeks, with storage in ozone at 0.300 μL/L in between fumigations, the spread of gray mold was as highly effective as the weekly sulfur dioxide treatment. Aerial mycelial growth of *B. cinerea* on inoculated berries was most effectively controlled by the sulfur dioxide weekly fumigation, while the bi-weekly sulfur dioxide or ozone alone treatments were similar to each other and less effective. When sulfur dioxide was applied once every two weeks, with storage in ozone at 0.300 μL/L in between fumigations, the suppression of aerial mycelial growth was as effective as the weekly sulfur dioxide treatment. Rachis appearance was best after the sulfur dioxide weekly or bi-weekly fumigations, and significantly injured by the treatments where ozone was applied.

3.7. Continuous low concentrations of ozone used alone or in combination with sulfur dioxide fumigation conducted during initial precooling

The natural incidence of gray mold was reduced when the grapes were stored in a continuous atmosphere of 0.300 μL/L ozone or when precooled with sulfur dioxide fumigation and then stored for 5 weeks at 1 °C in air, 0.300 μL/L ozone atmosphere, or with weekly sulfur dioxide fumigation (Table 5). In the second decay evaluation, conducted after 48 h at 20 °C, the initial sulfur dioxide fumigation during precooling was the treatment that caused the greatest reduction in the natural incidence of gray mold, regardless the subsequent storage in air, ozone, or weekly sulfur dioxide fumigation. However, to a lesser extent, storage in the ozone atmosphere alone also reduced the natural incidence of gray mold. Concerning the control of the natural incidence of pathogens other than *B. cinerea*, in both evaluations, the initial precooling with sulfur dioxide fumigation was the treatment with the greatest effectiveness regardless the subsequent storage in air, ozone, or weekly sulfur dioxide fumigation. However, as with the control of gray mold, storage in the ozone atmosphere alone also reduced the natural incidence of decay by other pathogens, but its effectiveness was poor. The spread of gray mold from an inoculated berry at the end of the storage and two additional days at 20 °C, was lowest among the grapes stored with weekly sulfur dioxide fumigation and low among grapes stored in a continuous atmosphere of 0.300 μL/L ozone regardless if they were precooled or not with sulfur dioxide fumigation. Similar results were observed at the end of the storage regarding the extent of aerial mycelial growth of *B. cinerea* on inoculated berries, and the rachis appearance. After two additional days at 20 °C, the aerial mycelial growth of *B. cinerea* on inoculated berry increase markedly, and was most effectively controlled by weekly sulfur dioxide fumigation. It was also controlled by initial sulfur dioxide fumigation during precooling followed by storage in ozone, while aerial mycelial growth on the berries stored in ozone alone was not significantly different than that of the control in air. Rachis appearance was best retained during storage by weekly sulfur dioxide fumigations, followed by precooling with sulfur dioxide fumigation and storage in ozone, and lastly by storage in ozone alone.

Table 5
Influence of ozone and sulfur dioxide fumigation on the gray mold or other rots (mainly *Alternaria* spp.) of 'Autumn King' grapes. The grapes were packaged in expanded polystyrene boxes with internal packaging of clamshell containers for 5 weeks at 1 °C and then exposed to 48 h of shelf life at 20 °C. The grapes were precooled in either air or with 350 μL/L sulfur dioxide fumigation, and then stored in air, with weekly 350 μL/L sulfur dioxide fumigation, or in 0.300 μL/L ozone atmosphere.

Treatment		Natural decay (%)				Gray mold on inoculated berries				Rachis[d]	
SO_2 precooling	Storage	Gray mold		Other		Spread[b]		Aerial mycelium[c]			
		0 h	+48 h	0 h	+48 h	0 h	+48 h	0 h	+48 h	0 h	+48 h
No	Air	2.4 a[a]	7.0 a	9.6 a	12.8 a	5.7 a	9.6 a	4.5 a	4.7 ab	2.5 a	3.7 a
Yes	Air	0.5 b	0.8 c	2.2 c	2.5 c	6.4 a	8.9 a	4.9 a	5.0 a	2.4 a	3.7 a
No	O_3	0.7 b	4.6 b	3.9 b	9.7 b	2.8 b	5.0 b	1.9 b	4.4 bc	1.6 b	2.8 b
Yes	O_3	0.2 b	0.6 c	0.8 c	1.3 c	2.7 b	4.7 b	1.8 b	4.1 c	1.5 b	2.2 c
Yes	SO_2	0.1 b	0.1 c	0.7 c	0.6 c	0.0 c	0.6 c	1.0 c	2.8 d	0.3 c	0.6 d
Sig. (*P*)		*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*

[a] Values within columns followed by the same letter are not significantly different according to Fisher's Protected LSD ($P = 0.05$).
[b] The number at the end of storage of gray mold infected berries adjacent to an initial *B. cinerea*-inoculated berry placed within clusters.
[c] Aerial mycelium on the inoculated berry at the end of storage: 0, none; 1, just visible to 5% of berry surface; 2, >5–15%; 3, >15–30%; 4, >30–60%; or 5, >60%.
[d] Rachis appearance rating: 0, entire perfect fresh and green; 1, pedicels only are brown; 2, pedicels and <50% of laterals brown; 3, pedicels and most laterals brown; 4, pedicels and laterals brown and main stem some browning; or 5, entire rachis brown.

Table 6
Number of total and correct answers obtained from triangle discrimination tests using table grapes stored for 3 weeks at 1 °C in atmospheres of air, ozone at 0.300 μL/L ozone, or in air with weekly 350 μL/L sulfur dioxide fumigation.

Triangle comparison	Variety/selection	Total answers	Correct answers[a]	Significance[b]
Air/ozone	Autumn King	30	9	n.s.
Air/ozone	B26-120	30	10	n.s.
Air/ozone	Y151-142	30	12	n.s.
Air/sulfur dioxide	Autumn King	90	45	0.01

[a] Panelists were presented with three grapes, where two were from one storage regime and the third from a second regime, and asked to identify the different grape. An answer was correct when the single grape of the second regime was correctly identified. The tests were conducted with 30 untrained panelists and repeated on three different days.
[b] Chi^2, threshold number of correct answers to be significant ($P = 0.05$) for 30 or 90 panelists was 15 and 38, respectively.

Table 7
Preference of sensory panelists when presented with 'Autumn King' table grapes stored for 3 weeks at 1 °C in atmospheres of air, ozone at 0.300 μL/L ozone, or in air with weekly 350 μL/L sulfur dioxide fumigation.

Preference test	Chosen preference[a]			Significance[b]
	Air	Ozone	Sulfur dioxide	
Air/ozone	28	32	–	n.s.
Air/sulfur dioxide	27	–	33	n.s.
Ozone/sulfur dioxide	–	48	12	0.01

[a] Panelists were presented with two grapes, where one was from one storage regime and the second from a second regime, and asked to identify the preferred grape. This test was conducted with 30 untrained panelists, repeated in four different days.
[b] Chi^2, threshold number of preference answers to be significant ($P = 0.05$) for 60 panelists was 39.

3.8. Consumer sensory evaluation

In the triangle discrimination tests, regardless of the table grape selections or variety utilized, panelists did not correctly identify the different berry, indicating there were no significant sensory differences between the air and ozone stored grapes (Table 6). Conversely, when air and sulfur dioxide treated grapes were similarly utilized, of 90 answers, 45 were correct, which indicates a significant difference ($P = 0.01$) was detected by the panelists. Therefore, compared to table grapes stored in air, the presence of a continuous atmosphere of 0.300 μL/L ozone during 3 weeks cold storage did not influence the taste of table grapes, while weekly sulfur dioxide fumigation did. The preference tests that compared air and ozone or air and sulfur dioxide stored grapes were not significant, since an approximately equal number of panelists preferred table grapes stored in air or in atmosphere of 0.300 μL/L ozone or fumigated with 350 μL/L sulfur dioxide weekly (Table 7). In contrast, in the ozone and sulfur dioxide preference test, out of 60 answers, 48 preferred ozone over sulfur dioxide, which is a significantly ($P = 0.01$) higher number than the critical value for paired preference test.

4. Discussion

In our chamber studies, ozone concentrations of 0.100 μL/L or more reduced natural gray mold and the mycelial growth of the pathogen on infected fruit, which would inhibit the berry to berry spread characteristic of the disease. Rubio Ames et al. (2013) reported that mortality of many fungal conidia in ozone at 0.150 μL/L under humid conditions at 2 °C occurred very slowly; generally 3 weeks or more elapsed before mortality occurred. They stated that the mode of action of low concentrations of ozone applied to control fungi on stored products was probably not mortality of their conidia. In our report, aerial mycelial growth on *B. cinerea* inoculated berries was markedly reduced by ozone compared to air (Fig. 3). Palou et al. (2002) reported that when conidia of *B. cinerea* were applied to 'Thompson Seedless' grapes followed by their storage at 5 °C in ozone at 0.300 μL/L, mycelial spread from these berries was completely inhibited, although the incidence of gray mold on the inoculated berries was not reduced. The response of *B. cinerea* to ozone was similar to that reported by Hildebrand et al. (2008) on carrots in storage. They observed greatly reduced aerial mycelial growth on the infected carrots during storage in ozone at 0.05 μL/L, and a suppression of sporulation for the first two months followed by an increase in sporulation after storage for three months.

Concentrations of ozone that kill fungal conidia in brief periods are very high, even under high humidity conditions that enhance its potency, and greatly exceed those we evaluated in the present work (Foarde et al., 1997; Ozkan et al., 2011). Ozkan et al. (2011) showed mortality of *P. digitatum*, *P. italicum* and *B. cinerea* required more than 900 μL/L when ozone was applied for 1 h period at high relative humidity. Mlikota Gabler et al. (2010) showed that a 1 h fumigation with 10,000 μL/L, an exposure that would rapidly kill the conidia of these and probably many other fungi, reduced the development of gray mold in subsequent cold storage. Some rachis injury was observed in her work. We chose to use much lower concentrations because they are more feasible; a smaller and less costly ozone generator can be used, and there would be fewer fumigant containment requirements and worker safety considerations.

In our chamber studies with 'Crimson Seedless' grapes and in the subsequent commercial scale experiments with other cultivars, we observed that the control of gray mold by ozone was good while that of other fungi was poor. This could be due to the different habitus of colonization of the different pathogens. Visually the best known characteristic of gray mold is "slip skin". The term "slip skin" refers to a condition where the epidermis of a berry slips off easily from the interior pulp when rubbing lightly over the berry, because *B. cinerea* does not grow deeply into the berry tissue (Nelson, 1956). Infected berries later develop surface cracks on which dark gray spores and aerial mycelium grow (Snowdon, 1990). This symptom is not observed for other pathogens, such as *Alternaria* spp., that grow deeply into the berry. Since direct contact between the gas and the fungal mycelium is required to achieve ozone inhibition of the hyphae growth (Liew and Prange, 1994; Palou et al., 2006), we speculate that ozone would better control pathogens that have more superficial growth, such as *B. cinerea*, than others that grow into the berry, where ozone cannot penetrate. In other works, when table grapes were removed from the ozonated atmosphere, normal aerial mycelial growth and sporulation of *B. cinerea* resumed, probably because that portion of the pathogen growing within the fruit, where the ozone cannot

penetrate, emerged from the infected tissue and resumed growth (Liew and Prange, 1994; Palou et al., 2006; Tuffi et al., 2012).

Packaging obstructed ozone diffusion and prolonging ozone exposure did not overcome this phenomenon. Ozone inhibited decay in commercial tests in the uncoated paper corrugate boxes used in this study, which generally retarded ozone diffusion more so than other boxes, such as coated paper corrugate boxes, reusable plastic containers, expanded polystyrene, or corrugate plastic boxes. Prior work with citrus fruit packaging indicated uncoated paper corrugated boxes were more difficult for ozone to penetrate than other kinds of packaging (Palou et al., 2002). Ventilation area of packages was a crucial factor that affected ozone penetration (Karaca and Smilanick, 2011), but structural, physical, mechanical, and barrier properties of plastic packaging materials can be altered by ozone (Shanbhag and Sirkar, 1998; Ozen et al., 2002). In experiments where ozone gas diffusion and control of the sporulation of *Penicillium digitatum* and *P. italicum* within commercial packages of oranges during cold storage were measured, inhibition of the sporulation of both fungi was clearly related to ozone diffusion into commercial packaging (Palou et al., 2003). They found this was strongly dependent on the composition and vented area of each type of package; control of sporulation on infected oranges was satisfactory only in reusable plastic containers. Since contact between the gas and the fungal mycelium is required to achieve inhibition of the hyphal growth, the choice of type of package is important to control the spread of *B. cinerea* from decayed fruit to adjacent healthy fruit. Uncoated paper corrugate is very popular for produce packaging due to its low price and ready acceptance for recycling. However, when storage is prolonged, they absorb moisture, which exacerbates product moisture loss and weakens the boxes. In our tests, the added storage life that resulted from the ozone treatments may make the selection of other packaging worthwhile. Differences in ozone diffusion into cluster bags and clamshell containers are difficult to predict, because the number and size of holes present in the cluster bags is not uniform, they can be used open, zipped closed, or folded over, so that the ventilation area is variable and unpredictable. Furthermore, clamshell containers are produced in many dissimilar designs of shape and vented area.

It was necessary to adjust ozone concentrations upward to ensure sufficient ozone diffused into the packages to control gray mold. In commercial cold storages where our measurements were made, the ozone concentration in the rooms was approximately double that inside uncoated paper corrugate boxes. Therefore, a constant concentration of 0.200 μL/L would need to be applied in the room atmosphere to obtain the minimum 0.100 μL/L needed to inhibit the development of aerial mycelial growth of *B. cinerea*. Considering the variability we observed in the ozone concentrations within packages, it is probable a concentration of 0.300 μL/L would be effective in most facilities. However, in some facilities, we found diffusion even more inhibited than reported here (data not shown), so measurements to ensure adequate concentrations are present are recommended. Two methods to detect and quantify the obstruction of diffusion would be empirical measurements of ozone concentration or modeling diffusion of the gas. Currently, dosimeters that record sulfur dioxide concentration times time products are used to ensure adequate exposures of the gas occur within packages; it is conceivable a similar approach could be used for ozone (Smilanick and Henson, 1992). Recently a semi-quantitative method has been used to determine gaseous ozone diffusion through various packaging materials, commonly used by food industry, and through plastic films with different ventilation areas (Karaca and Smilanick, 2011). Modeling the diffusion of ozone is an important next step in its optimal use, as has been done for methyl bromide fumigation of fresh products (Walse et al., 2013). In our work, the ozone concentrations inside chamber were maintained by continuous generation; the weight of ozone applied was not recorded, so the ozone demand of the packaging, needed to modeling ozone consumption by the packaging, cannot be calculated from our experiments.

Feedback on consumer sensory responses provides important information to identify those sensory characteristics that are most important to consumer choice and which should therefore be rigorously controlled (Mason and Nottingham, 2008). In our consumer sensory tests ozone did not impart off-flavors in the variety and selections of table grapes tested, and, in preference tests, 'Autumn King' grapes stored in ozone were preferred over those fumigated weekly with sulfur dioxide. Our results in part corroborate those of Artés-Hernández et al. (2004) who reported the flavor of 'Autumn Seedless' table grapes was not influenced by storage in ozone at 0.100 μL/L. In strawberry fruit, ozone decreased aroma, which was a large part of the sensory quality of such fruit, unlike table grapes (Perez et al., 1999; Nadas et al., 2003).

The browning of the rachis could be the result of oxidation by ozone. In previous work, when high concentrations of ozone were used, some rachis injuries appeared on the stem of grapes cluster after ozone storage (Mlikota Gabler et al., 2010), in other works, with much lower concentrations of ozone, rachis injuries did not occur (Sarig et al., 1996; Palou et al., 2002). In addition, the variability in rachis responses to ozone could be due to the condition of the rachis at the beginning of the storage, the table grapes cultivar considered and the portion of the rachis exposed to the ozone atmosphere.

Ozone could be used in strategies with sulfur dioxide, with the aim to reduce sulfur dioxide use to minimize the injury to grapes caused by this gas. The first strategy was to reduce the sulfur dioxide fumigation frequency from weekly to biweekly, with storage in ozone between fumigations. The second was a single initial sulfur dioxide fumigation, followed by ozone alone in storage. During initial fumigation, usually done during pre-cooling, it is important to kill spores on the surface of the fruit, since these may later germinate and cause decay. Subsequent sulfur dioxide fumigation is typically applied weekly, because if used biweekly this is insufficiently frequent to inhibit aerial mycelial growth and spread of gray mold among grapes storage. However, a reduction in sulfur dioxide fumigation would reduce berry bleaching among cultivars that are sensitive to bleaching injuries by sulfur dioxide, particularly for red-colored berries such as 'Redglobe'. On the other side, a negative aspect of the ozone treatments has been their inability to control decay by pathogens other than *B. cinerea*. Excellent control of these pathogens was observed by the addition of initial or bi-weekly sulfur dioxide fumigation to the ozone treatment. The increase in effectiveness of the combination of these treatments was evident in the superior control of the spread of infected berries from an inoculated berry, and in the reduced aerial mycelial growth observed on the inoculated berry. A remaining negative aspect to the use of the two treatments in sequence, however, is the appearance of the rachis, where the ozone treatments caused some injury to them. In addition the reduction in sulfur dioxide fumigations would presumably reduce sulfite residues, responsible for decline in flavor quality and induction of adverse reactions in consumers (Montaño García, 1989). Fernández-Trujillo et al. (2008) stated that the benefits obtained in reducing total decay when 'Napoleon' grapes were stored with sulfur dioxide pads were accompanied by other detrimental effects in berry taste, both as regards to overall loss of taste and increased sulfur dioxide taste. Similarly, in our work the sensory evaluations revealed that consumers could detect the flavor differences existing between table grapes stored in air from those fumigated weekly with sulfur dioxide. Moreover consumers preferred the taste of table grapes when stored in an atmosphere of 0.300 μL/L ozone to those fumigated weekly with sulfur dioxide at 350 μL/L.

This work provides information to develop the rational and reliable use of ozone for the table grape industry to extend the shelf life of table grapes. Findings include the minimum concentrations of ozone and length of exposure required, the magnitude of control of decay pathogens, ozone diffusion into packaging, its effectiveness compared to sulfur dioxide fumigation and how the two treatments could be used together, and the impact of ozone on grape and sensory quality. If ozone is used alone, it appears particularly promising for "organic growers", because we estimate the storage life of grapes could be extended by two or three weeks, double that of the unprotected grapes. It could also be used in strategies to reduce the number of sulfur dioxide fumigations from the current practice of weekly fumigation.

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