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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.