



Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage

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Abstract

Continuous ozone exposure at 0.3 ppm (v/v) (US-OSHA Threshold Limit Value for short term exposure) inhibited aerial mycelial growth and sporulation on 'Elegant Lady' peaches wound inoculated with *Monilinia fructicola*, *Botrytis cinerea*, *Mucor piriformis*, or *Penicillium expansum* and stored for 4 weeks at 5 °C and 90% relative humidity (RH). Aerial growth and sporulation, however, resumed afterward in ambient atmospheres. Ozone exposure did not significantly reduce the incidence and severity of decay caused by these fungi with the exception of brown rot. Gray mold nesting among 'Thompson Seedless' table grapes was completely inhibited under 0.3 ppm ozone when fruit were stored for 7 weeks at 5 °C. Gray mold incidence, however, was not significantly reduced in spray inoculated fruit. Continuous ozone exposure at 0.3 ppm increased water loss after 5 weeks of storage at 5 °C and 90% RH in 'Zee Lady' peaches but not after 4 weeks of storage in 'Flame Seedless' grapes. Respiration and ethylene production rates of 'O'Henry' peaches were not affected by previous exposure to 0.3 ppm ozone. In every test, no phytotoxic injuries of fruit tissues were observed in ozonated or ambient atmosphere treatments. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ozonation; Postharvest decay control; Decay management; Alternative postharvest treatments

1. Introduction

Peaches and table grapes are major crops in the San Joaquin Valley (California, USA). Internal breakdown and fruit decay are the main causes of peach postharvest losses. Brown rot, caused by

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Monilinia fructicola (G. Wint.) Honey, is the most important postharvest disease of stone fruit in California. Depending on weather conditions and postharvest handling, other high-incidence postharvest diseases of stone fruit are gray mold, caused by *Botrytis cinerea* Pers.:Fr., Mucor and Rhizopus rots, caused by *Mucor piriformis* E. Fischer and *Rhizopus stolonifer* (Ehrenb.) Lind., respectively, and blue mold, caused by *Penicillium expansum* Link. (Ogawa and English, 1991). Integrated pest management practices including the use of both preharvest and postharvest fungicides are currently required to effectively control postharvest diseases. Problems associated with the use of synthetic fungicides such as the proliferation of resistant strains of the pathogens or concerns about public health and environmental contamination have increased the need for development of alternative treatments.

In California, table grapes may be held for as long as 4 months under refrigerated storage at $-1-0$ °C and 90–95% RH, but gray mold caused by *B. cinerea* and stem browning due to water loss can become significant problems limiting prolonged storage (Capellini et al., 1986; Nelson, 1991). Control of gray mold during cold storage of table grapes is achieved by periodic fumigation with sulfur dioxide. However, sulfite residues on fumigated produce and associated injuries to the berries, such as bleaching, are the main concerns related to this practice (Smilanick et al., 1990; Luvisi et al., 1992).

In 1997, an expert panel reviewed the safety and potential for food processing use of ozone, the triatomic form of oxygen (O_3), and declared ozone to be generally recognized as safe (GRAS) for food contact applications in the United States (Graham et al., 1997; US-FDA, 1997). Since that time, interest in developing ozone applications in the food industry has increased. Currently, an Electric Power Research Institute supported committee is submitting a Food Additive Petition to the United States Food and Drug Administration (US-FDA) that would allow ozone to be used in food contact applications. For the postharvest treatment of fresh fruit and vegetables, ozone can be used as a relatively brief pre-storage or storage treatment in air or water, or as a continuous or

intermittent component of the atmosphere throughout storage or transportation. Both procedures have recently received considerable commercial interest, especially because of the lack of residues on the produce and the new regulatory issues.

The current Threshold Limit Value — Short Term Exposure Limit (TLV-STEL) established by the United States Occupational Safety and Health Administration (US-OSHA) for gaseous ozone is 0.3 ppm. This is the level to which individuals can be exposed for 15 min without suffering from irritation or other acute effects. The US-OSHA Threshold Limit Value — Time Weighted Average (TLV-TWA) is 0.1 ppm and it is defined as the ozone concentration to which individuals can be repeatedly exposed for a normal 8 h workday.

There are numerous reports on both the benefits and the lack of benefits of ozone in air of storage rooms. However, little research has been focused on the effect of ozone on either stone fruits or table grapes and the effects of continuously supplied ozone gas at 0.3 ppm, which would currently be the highest concentration to be used under commercial conditions, have not been previously evaluated. Early work by Spalding (1966, 1968) showed that ozone did not significantly retard the growth of both *M. fructicola* and *R. stolonifer* on artificially inoculated peaches until a concentration of at least 0.5 ppm was used; ozone at this concentration and higher inhibited the fungal surface growth. In contrast, Ridley and Sims (1967) found reductions in decay incidence and severity on peaches inoculated with *M. fructicola* or *Rhizopus* sp. and stored under 0.5 ppm ozone. High atmospheric ozone levels (0.1 ppm) during the growing season increased postharvest weight loss in plums, but did not affect internal fruit quality or the incidence of internal breakdown (Crisosto et al., 1993). Ozone at 0.3 ppm for two 6 h exposure periods significantly inhibited *in vitro* sporulation and germination of *B. cinerea* (Krause and Weidensaul, 1977). In the experiments by Spalding (1968), continuous exposure to 0.5 ppm ozone at 15 °C for 6 days did not control gray mold. Sarig et al. (1996) reported that ozone controlled *R. stolonifer* and induced resveratrol and pterostilbene phytoalexins in table grapes,

making the berries more resistant to subsequent infections.

The objectives of this work were to evaluate the effects of continuous gaseous ozone exposure at 0.3 ppm (US-OSHA TLV-STEL) on: (1) the development of the most important postharvest pathogens of stone fruits and table grapes on artificially inoculated fruit stored at low temperature; (2) the weight loss of peaches and table grapes; and (3) the respiration and ethylene production rates of peaches.

2. Materials and methods

2.1. Ozone exposure implementation

A 90 W corona discharge ozone generator (AgroCare™, Model Oxtomcav XEE-245, Agroquality International, LLC, Bridgewater, NJ, USA) was installed in a 66.6 m³ standard cold storage room and set to maintain a room concentration of ozone of 0.3 ± 0.05 ppm (v/v; 0.3 ± 0.05 $\mu\text{L L}^{-1}$) at 5 °C and 90% relative humidity (RH). Ozone was released into the room through a perforated PVC tube anchored to the ceiling in front of the cooler unit fans. The ozone concentration in the room was continuously monitored by circulating air from the room through an ultraviolet absorption ozone analyzer (Model IN-2000-1, INUSA Inc., Needham, MA) with a measurement range of 0.0–1.0 ppm (v/v) at 253.7 nm and a minimum detection limit of 0.01 ppm. In order to have a comparative control room with an ambient air atmosphere, the same environmental conditions of temperature and RH were set and continuously monitored in another similar standard cold storage room. Ozone levels in this control room were periodically assessed with either a heated metal oxide ozone sensor (Model 21-Z, Eco Sensors Inc., Santa Fe, NM, USA), with a minimum detection limit of 0.02 ppm, or a gas sampling pump (Sensidyne Model 800, Clearwater, FL) with detection tube no. 18L and a minimum detection limit of 0.025 ppm. No measurable ozone was detected by either method during the whole storage period. The desired RH was maintained in both rooms by an air-assisted

low pressure RH system, equipped with a computer-controlled humidity transmitter (Model HMD20VB, Vaisala, Helsinki, Finland).

2.2. Fruit

Peaches [*Prunus persica* (L.) Batsch.], cvs. 'Elegant Lady', 'Zee Lady', and 'O'Henry', grown in organic orchards in the San Joaquin Valley (California, USA) were selected, randomized, and used in the experiments before any postharvest treatments were applied. Table grapes (*Vitis vinifera* L.) cvs. 'Thompson Seedless' and 'Flame Seedless' from commercial vineyards in the San Joaquin Valley were harvested at commercial maturity and used before receiving any sulfur dioxide treatment. They were superficially disinfected by immersion for 1 min in diluted bleach (0.5% sodium hypochlorite), rinsed with fresh water, and allowed to air dry at room temperature.

2.3. Decay development experiments

B. cinerea (isolates 93-58 and 28E7) and *M. piriformis* (isolate LP-7) were incubated on potato dextrose agar (PDA) medium in Petri dishes at 20 °C for 7–14 days, *M. fructicola* (isolate 79-1) on V-8 agar medium at 20 °C for up to 15 days, and *P. expansum* (isolate PES-1) on PDA at 25 °C for 7–10 days. Spores were rubbed from the agar surface with a sterile glass rod after 5 ml of 0.05% (w/v) Triton X-100 in sterile water were added. The high density spore suspension was passed through two layers of cheese cloth and, after counting the number of spores with a hemacytometer, diluted with sterile water to the desired inoculum density.

'Elegant Lady' peaches were wounded once on the equator of the fruit with a probe tip 1 mm wide by 2 mm in length and inoculated, using a micropipet, with 20 μL of a suspension containing 2×10^4 spores mL^{-1} of *M. fructicola*, *B. cinerea* (isolate 93-58), *M. piriformis*, or 10^5 spores mL^{-1} of *P. expansum*. Inoculated fruit were held at room temperature in the laboratory for about 24 h, at which time peaches were stored for 4 weeks at 5 °C and 90% RH under 0.3 ppm ozone (ozone room) or in an ambient air atmosphere (control

room). Inoculated fruit were placed on cavity trays on wooden trays that assured adequate gas contact. For each pathogen and storage treatment, four 20-fruit replicates (trays) were used. Disease incidence and severity, as well as external disease appearance, were checked weekly. Severity was assessed as lesion area by measuring the two main lesion diameters. The following qualitative scale was used to characterize aerial mycelial growth: 0 = no visible mycelia; 1 = visible mycelia with abnormal characteristics; 2 = visible mycelia with normal appearance. Sporulation was recorded as present or absent. When no normal mycelial growth and/or lack of sporulation were noticed on decayed ozone treated fruit at the end of the cold storage period, recovery of the pathogen was checked by incubating fruit samples in an ambient atmosphere at 20 °C and 90% RH for 2 days.

For the evaluation of gray mold incidence on grapes, a conidial suspension containing 2×10^4 spores mL^{-1} of *B. cinerea* (isolate 28E7) was uniformly sprayed for about 5 s on clusters of 'Thompson Seedless' table grapes placed on open wooden trays. For the evaluation of *B. cinerea* nesting ability, 10 μL of a suspension of 2×10^6 spores mL^{-1} of *B. cinerea* were injected in the flesh of one central single berry per cluster using a Hamilton syringe (Hamilton Co., Reno, NV, USA). About 3–4 h after inoculation, when the inoculum droplets on the spray-inoculated clusters were dried, inoculated grapes were stored for 7 weeks at 5 °C and 90% RH in the ozone or the control room. In each storage treatment, there were six 2-cluster replicates (trays) for the spray inoculation method and six 4-cluster replicates for the syringe inoculation method. The number of infected berries per cluster and the number of infected berries surrounding the inoculated berry were recorded weekly.

Data on the peach severity (lesion area) and arcsine-transformed data on the incidence of decayed peaches or berries were evaluated by an analysis of variance and Fisher's Protected Least Significant Difference (LSD) test ($P = 0.05$) using SAS software.

2.4. Physiological response experiments

2.4.1. Weight loss

Ten wooden trays with 24 'Zee Lady' peaches each were weighed; five trays were stored under 0.3 ppm ozone and five trays under ambient air for 6 weeks at 5 °C and 90% RH. The weight of four trays from each room was recorded weekly. After 1 week of storage, 12 peaches from the fifth tray from each room were separated, individually marked, and held in ambient atmospheres at 20 °C and 90% RH for a 7-day ripening period. Those fruit were weighed daily. After 2 weeks of storage, the procedure was repeated with the other 12 fruit from the fifth tray in each room.

The effect of ozone exposure on table grape weight loss was also investigated. Eight wooden trays with three clusters of 'Flame Seedless' table grapes each were weighed. Four trays each were stored at 5 °C and 90% RH for 4 weeks in both the ozone and control rooms. Ozone concentration in the ozone room was 0.3 ppm. The weight of three trays from each room was recorded weekly. After 1 week of storage, the clusters from the fourth tray in each room were individually marked, placed under ambient air at 20 °C and 90% RH, and weighed daily for 7 days.

Data on weekly weight loss during cold storage or daily weight loss during ripening were analyzed using analysis of variance procedures of SAS. Fisher's Protected LSD test ($P = 0.05$) was used to separate means.

2.4.2. Peach respiration and ethylene production

'O'Henry' peaches were individually marked, weighed, placed on wooden trays, and stored for 3 weeks at 5 °C and 90% RH under 0.3 ppm ozone or in the control room. After 1, 2, and 3 weeks of exposure, ten fruit were removed from each room and each one was weighed and placed in a 750 mL plastic container. The containers, connected to a compressed air flow-through system, were placed in an ambient atmosphere room at 20 °C and 90% RH for a 5-day ripening period. Natural ethylene was removed from the air by circulating through a potassium permanganate filter. The total airflow rate delivered to each container was adjusted so that carbon dioxide

accumulation from respiration remained below 0.2% (Crisosto et al., 1993). Air samples were taken daily from each container and carbon dioxide and ethylene concentrations measured with an infrared gas analyzer (Horiba PIR-2000R, Horiba Instruments Inc., Irvine, CA, USA) and a gas chromatograph equipped with a flame ionization detector (Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK, USA), respectively. Fruit carbon dioxide and ethylene production rates were calculated from the gas concentrations, fruit mass, and flow rates. Airflow rates were measured with a digital flowmeter (Model ADM1000, J&W Scientific, Folsom, CA, USA). An analysis of variance was applied to the daily data and means were separated using Fisher's Protected LSD test ($P = 0.05$).

3. Results

3.1. Decay development experiments

3.1.1. Peaches

The incidence of brown rot caused by *M. fructicola* on inoculated 'Elegant Lady' peaches was

significantly lower under 0.3 ppm ozone than under ambient air after 14 days of storage at 5 °C, but not after 21 or 28 days (Fig. 1A). Brown rot severity as lesion area was significantly lower under ozone after 21 days storage. The difference after 21 and 28 days reached about 550 and 1650 mm², respectively. There were no significant differences between ozone and control treatments for either disease incidence or disease severity of gray mold (Fig. 1B), Mucor rot (Fig. 1C), and blue mold (Fig. 1D). Mucor rot incidence among control fruit reached only 2.5 and 21% after 21 and 28 days, respectively. Continuous ozone exposure at 0.3 ppm strongly affected external mycelial growth and sporulation of all the pathogens tested. Aerial mycelia were not developed or were abnormally developed on decayed fruit under ozone, while normal disease symptoms at 5 °C were observed on decayed control fruit (Table 1). After visual assessment, *M. fructicola* did not develop aerial mycelia under ozone during the entire 28-day cold storage period. Toward the end of the storage period under ozone, *B. cinerea* developed a short, compact, and dark mycelium that did not develop conidia.

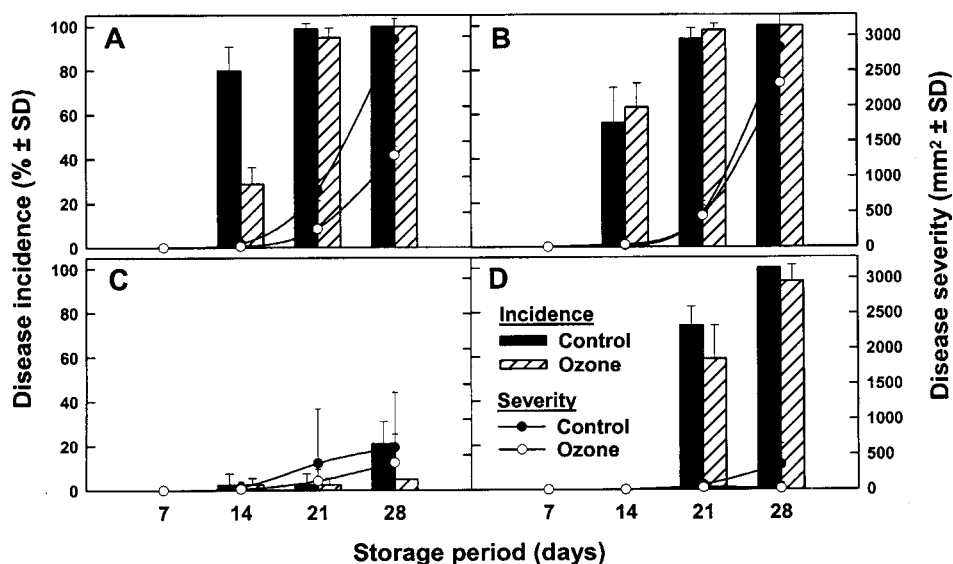


Fig. 1. Disease incidence (bars) and severity (lines) of postharvest brown rot (A); gray mold (B); Mucor rot (C); and blue mold (D) on wound inoculated 'Elegant Lady' peaches stored for 4 weeks at 5 °C and 90% RH under ambient air (control) or 0.3 ppm ozone.

Table 1

Influence of continuous 0.3 ppm ozone exposure on aerial mycelial growth and sporulation of postharvest decay fungi on wound inoculated 'Elegant Lady' peaches stored for 4 weeks at 5 °C and 90% RH

Pathogen	Treatment	Storage period (days)								R ^c
		7		14		21		28		
		AM ^a	S ^b	AM	S	AM	S	AM	S	
<i>M. fructicola</i>	Control	0	–	0	–	2	–	2	+	
	Ozone	0	–	0	–	0	–	0	–	+
<i>B. cinerea</i>	Control	0	–	0	–	2	–	2	+	
	Ozone	0	–	0	–	0	–	1	–	+
<i>M. piriformis</i>	Control	0	–	2	+	2	+	2	+	
	Ozone	0	–	1	–	1	–	1	–	+
<i>P. expansum</i>	Control	0	–	0	–	0	–	2	+	
	Ozone	0	–	0	–	0	–	1	–	+

^a Aerial mycelia rating (visible assessment): 0 = no visible mycelia; 1 = visible mycelia with abnormal characteristics; 2 = visible mycelia with normal appearance.

^b Sporulation rating: + = spores present; – = spores absent.

^c Recovery rating: + = normal disease symptoms after 2 days incubation at 20 °C and 90% RH in an ambient air atmosphere.

M. piriformis under ozone developed masses of short and yellowish stromatic bodies that did not develop sporangia. Appearance of external mycelia of *P. expansum* on ozone treated fruit was normal, although the area where aerial mycelia were present was substantially smaller than on control fruit and sporulation was prevented. All four pathogens resumed normal surface growth, developed typical mycelia and sporulated on samples of decayed fruit from the ozone room that were incubated in an ambient atmosphere at 20 °C and 90% RH for 2 days (Table 1).

3.1.2. Table grapes

No significant differences were observed for the incidence of gray mold between 0.3 ppm ozone and control treatments on 'Thompson Seedless' table grapes inoculated by spraying their surface and stored at 5 °C. After 7 weeks of storage, decay incidence on ozone and ambient air exposed clusters reached about 19 and 25%, respectively (Fig. 2A). Decay on clusters in the control room was apparent only after 4 weeks of storage. Disease symptoms on decayed berries treated with ozone were browning, softening, and shriveling. While typical gray mycelia developed on control

berries, decayed berries exposed to ozone showed a small amount of blackened mounds of stromatic tissue that did not develop conidiophores.

Gray mold nesting was nearly prevented under 0.3 ppm ozone. The percentage of decayed berries surrounding the inoculated berry in clusters from the ozone and the control rooms after 7 weeks of storage at 5 °C was 1 and 65%, respectively (Fig. 2B). *Botrytis* nests quickly developed on ozone treated clusters that were placed under ambient air at 20 °C after the 7-week exposure period.

3.2. Physiological response experiments

3.2.1. Weight loss

'Zee Lady' peaches exposed to 0.3 ppm ozone at 5 °C and 90% RH for 6 weeks lost more weight than peaches stored in a non-ozonated environment from the second week of storage. However, significant differences were observed only after 5 weeks of storage (Fig. 3). Cold storage for 1 or 2 weeks under 0.3 ppm ozone did not affect weight loss of 'Zee Lady' peaches during a subsequent 7-day ripening period at 20 °C and 90% RH (data not shown).

No differences in weight loss were found between 0.3 ppm ozone and control treatments on 'Flame Seedless' table grapes stored for 4 weeks at 5 °C and 90% RH. Weight loss was also not different during a 7-day ripening period in an ambient atmosphere at 20 °C and 90% RH following 1 week of storage at 5 °C and 90% RH in the ozone or control rooms (data not shown).

In every test with both peaches and table grapes, no visible injuries on the fruit tissues were noticed during or after ozone exposure.

3.2.2. Peach respiration and ethylene production

Respiration of 'O'Henry' peaches during a 5-day ripening period under ambient air at 20 °C following 1, 2 or 3 weeks ozone exposure at 5 °C was not significantly different from that following ambient air exposure (Fig. 4). Likewise, although daily fruit ethylene production rate was slightly higher after ozone exposure, the differences were not significant. Both fruit respiration rate (CO₂,

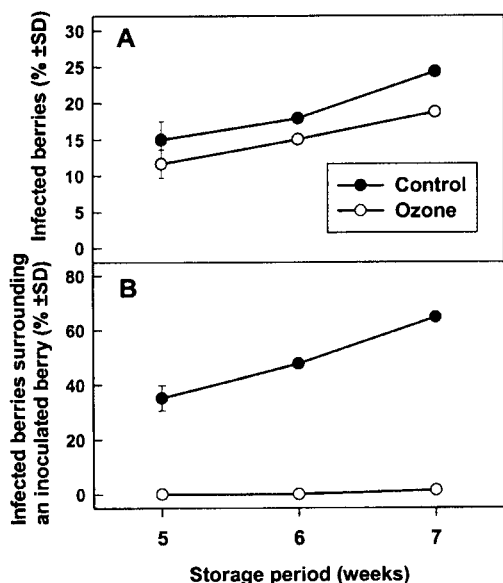


Fig. 2. Incidence of *Botrytis cinerea* on clusters of 'Thompson Seedless' table grapes stored for 7 weeks at 5 °C and 90% RH under ambient air (control) or 0.3 ppm ozone. (A) Infected berries in spray inoculated clusters; and (B) infected berries surrounding a central berry inoculated with a syringe.

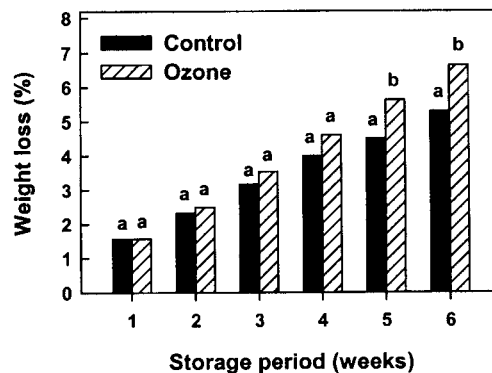


Fig. 3. Weekly percent of weight loss on 'Zee Lady' peaches stored for 6 weeks at 5 °C and 90% RH under ambient air (control) or 0.3 ppm ozone. Within each week, bars with different letters are significantly different based on Fisher's Protected Least Significant Difference test applied after an analysis of variance ($P = 0.05$).

1.3–1.5 mmol kg⁻¹ h⁻¹) and ethylene production rate (0.6–1.6 μmol kg⁻¹ h⁻¹) after 3 weeks of cold storage were higher than after 1 or 2 weeks (CO₂, 1.0–1.3 mmol kg⁻¹ h⁻¹; C₂H₄, 0.2–1.3 μmol kg⁻¹ h⁻¹) (Fig. 4).

4. Discussion

Our results on decay incidence and severity on wound-inoculated peaches agree with those of Spalding (1968), who found that gaseous ozone at 0.5 ppm did not reduce either brown rot or Rhizopus rot incidence, but disagree with those of Ridley and Sims (1967). The inability of either gaseous ozone or ozone in water to control pathogens in wounds or under the fruit surface (field infections) have been observed on table grapes (Shimizu et al., 1982), apples (Schomer and McColloch, 1948), pears (Spotts and Cervantes, 1992), citrus (Hopkins and Loucks, 1949; Smilanick et al., 1999) and other commodities (Spalding, 1966, 1968; Ogawa et al., 1990). Apparently, fungal structures in wounds remain protected from the oxidant effect of ozone possibly because of reduced ozone penetration, an interaction of ozone with fruit tissue or fruit surface compo-

nents that reduce the effective ozone concentration, and/or the presence of antioxidants. Some of these factors could also explain the failure of other strong oxidants such as chlorine and chlorine dioxide to control infections within wounds (Spotts and Peters, 1980; Adaskaveg, 1995). Since most of the economically important postharvest fruit diseases are initiated by infections in wounds on the fruit surface or by latent infections, the efficacy of ozone in controlling diseases cannot be predicted by the toxicity of ozone against free spores and hyphae, and ozone could not be a substitute for the fungicidal treatments that are currently applied on stone, pome, and citrus fruit packinglines.

In our tests, ozone exposure at 0.3 ppm inhibited the normal aerial growth of the mycelia and prevented sporulation on peaches wound inoculated with *M. fructicola*, *B. cinerea*, *M. piriformis*,

and *P. expansum*. Spalding (1966, 1968) and Ridley and Sims (1967) reported similar observations. Therefore, ozone treatment would prevent the nesting that occurs when *Mucor* rot, *Rhizopus* rot, gray mold, or brown rot spread from decayed fruit to adjacent healthy fruit. Ozone would also decrease the load of airborne pathogenic spores in the storage rooms and inhibit the surface growth of mold on packages, walls, and floors of the rooms, with a subsequent reduction in the amount of inoculum available for reinfections of the stored produce. Such a reduction has been previously reported (Schomer and McColloch, 1948; Song et al., 2000). Furthermore, the lack of sporulation on decayed fruit stored under ozone could particularly be useful to prevent the proliferation of fungicide-resistant strains of the pathogens. Usually, cold stored fruit have been previously treated with fungicides in the packingline and, therefore, many spores produced during storage are from resistant strains. Any practice that reduces inoculum production on decayed fruit after fungicide treatment can be effective for preventing or managing resistance in target pathogen populations.

Ozone exposure at 0.3 ppm did not reduce gray mold incidence during cold storage of table grapes when inoculum was sprayed on the cluster surface. The grapes were surface disinfected, inoculated, and exposed to ozone without an incubation period. Therefore, the lack of control could result either from development of internal inoculum or from spores that were located where effective contact with the gas was limited. Ozone exposure, however, prevented surface mycelial growth and inhibited gray mold nesting. We did not notice any phytotoxic injuries of fruit tissues or rachis in grapes stored under 0.3 ppm ozone. Gaseous ozone exposure did not control gray mold on grapes inoculated on the surface by immersion in a spore suspension (Spalding, 1968), but effectively controlled *Rhizopus* rot on grapes inoculated in a similar way (Sarig et al., 1996). Shimizu et al. (1982) reported the incapability of ozone gas to control wound infections of *B. cinerea* and serious rachis damage on 'Kyoho' grapes exposed to gaseous ozone at 500 ppm.

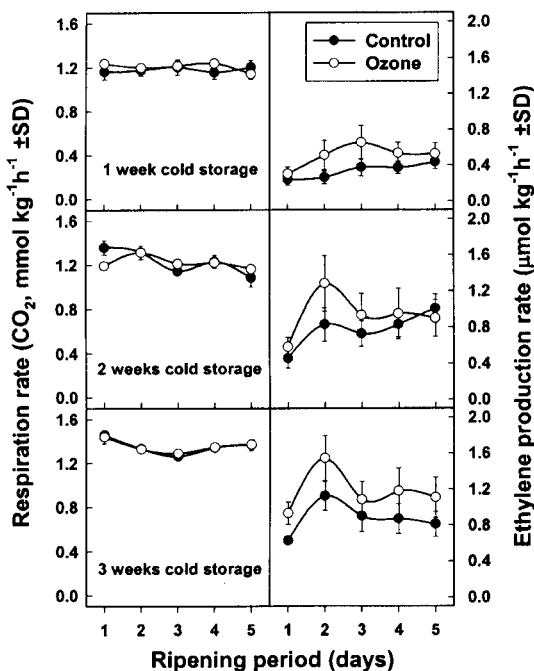


Fig. 4. Daily respiration rate (left) and ethylene production rate (right) of 'O'Henry' peaches held in an air flow-through system for a 5-day ripening period at 20 °C following a 1, 2, or 3-week storage period at 5 °C and 90% RH under ambient air (control) or 0.3 ppm ozone.

Although the differences became significant only after 5 weeks of storage and no shriveling or other injury symptoms were macroscopically visible, 'Zee Lady' peaches exposed to ozone lost more weight than control fruit. Therefore, the fruit cuticle and/or the epidermal tissue were probably damaged by the gas. Work by Crisosto et al. (1994) showed that shriveling symptoms on peach fruit were only visible when weight losses exceeded 10% of the initial fresh weight. Furthermore, high atmospheric ozone levels (about 0.1 ppm) during the growing season injured fruit cuticle in plums (Crisosto et al., 1993). However, neither peach respiration nor ethylene production rates during a ripening period following ozone exposure were significantly affected by gaseous ozone. Spalding (1966) reported that ozone injury to peaches (brown freckling) was evident at 0.9 ppm but not at 0.5 ppm. Smock and Watson (1941), and Ridley and Sims (1967) observed that peaches were injured at about 2 ppm ozone, showing sunken and browned tissue in the region of the stomata. Smock and Watson (1941) also found that ozone at low concentrations did not increase the respiration rate of apples. Jin et al. (1989) concluded that fruit senescence was delayed on tomatoes and mandarins exposed to ozone and negative ions, the respiratory intensity was lowered and the ethylene release rate decreased.

Considering that we optimized the exposure of fruit to the ozone in this work, the efficacy of gaseous ozone may decrease when fruit are packed under commercial conditions. Presumably, fiberboard boxes and/or polyethylene bags used for packaging could reduce fruit exposure to gaseous ozone treatments. In our study, ozone penetration through the plastic cavity trays used in some experiments was partially inhibited because we observed more mycelial growth on the surface of the fruit in contact with the plastic. Additional research is needed to evaluate the impact of current commercial packages on the efficacy of ozone gas during cold storage.

The ozone concentration in a storage room is greatly dependent on the environmental conditions and on the fruit load. Ozone concentration rapidly decreases when temperature, humidity, or

fruit load increase. Our results showed a synergistic effect between an ozonated atmosphere (0.3 ppm) and low temperature (5 °C) in preventing aerial mycelial growth, nesting, and sporulation, but it cannot be assumed that a similar benefit could be obtained at a higher temperature. Once produce are moved to low temperature storage, only minimal amounts of handling are required before transportation to market. The 0.3 ppm US-OSHA TLV-STEL concentration would ensure the safety of personnel and workmen. Another procedure that could be implemented is the generation of ozone in day-night cycles that allow the rooms to be free of gas during the work time (Shimizu et al., 1982). In this case, the impact of an intermittent exposure on the efficacy of ozone treatment should also be evaluated.

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