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Short communication

Ozone gas penetration and control of the sporulation of *Penicillium digitatum* and *Penicillium italicum* within commercial packages of oranges during cold storage

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Abstract

Ozone gas penetration through packaging materials and its effectiveness in controlling sporulation of *Penicillium digitatum* and *P. italicum* were evaluated on artificially inoculated and commercially packed 'Lanelate' oranges stored at 12.8°C and exposed to an average ozone concentration of 0.72 ppm (v/v) for 14 days. Inoculated control fruit were stored in a non-ozonated room with the same environmental conditions. Oranges were packed naked in California standard citrus cartons, naked or bagged (in polyethylene bags) in vented RPCs (returnable plastic containers), or bagged in fiberboard Master cartons. Ozone penetration was strongly dependent on the vented area of each type of package, and while it was very low through fiberboard cartons or polyethylene bags (9–17%), it was acceptable through RPCs (82%). Sporulation inhibition of both *P. digitatum* and *P. italicum* was clearly related to ozone penetration and it was satisfactory only on oranges packed naked in RPCs. Since the gas was not able to penetrate through fiberboard cartons or plastic bags, which are commonly used in California and worldwide for commercial packaging of not only citrus but a large variety of fruits and vegetables, the practical use of ozone gas exposure during storage for the treatment of fresh produce is limited to highly vented packages or open-top containers.

Keywords: Citrus; Green mold; Blue mold; Ozonation; Postharvest decay management

1. Introduction

In 2001, ozone, the triatomic form of oxygen (O_3), was approved as a food additive by the United States Food and Drug Administration (US-FDA, 2001). For the postharvest treatment of fresh fruits and vegetables, ozone can be used as a relatively brief pre-storage treatment in air or water, or it can be added continuously or intermittently to the storage room atmosphere throughout the storage period. These procedures have recently attracted considerable commercial interest for the development of new applications now feasible because of the new regulatory approvals, especially because ozone does not deposit a persistent residue on the produce, and it is accepted by many organic grower organizations.

In recent work we studied the effects of gaseous ozone continuously released into cold storage rooms at low doses (0.3 or 1.0 ppm, v/v; 0.3 or 1.0 $\mu l\,l^{-1}$) on the development of the most important postharvest diseases of table grapes, stone fruit, and citrus fruit (Palou et al., 2001, 2002). We observed that exposure to gaseous ozone did not reduce final incidence of postharvest green mold, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mold, caused by *P. italicum* Wehmer, on artificially wound-inoculated and cold-stored oranges or lemons, although infections developed more slowly on fruits stored in an ozonated atmosphere than on fruits stored in an ambient air atmosphere (Palou et al., 2001). Because of this inability of ozone to control pathogens in wounds, the gas could not be a substitute for the synthetic fungicides that are currently

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applied on citrus fruit packing lines. In our tests, however, ozone gas inhibited the normal aerial growth of the mycelia and greatly prevented sporulation of *P. digitatum* and *P. italicum* from lesions among infected fruit once lesions developed. Aerial mycelial growth and sporulation, however, resumed afterward in ambient atmospheres (Palou et al., 2001). In prior trials, however, inoculated citrus fruit were placed on cavity trays that assured adequate gas contact and the effectiveness of ozone applied to commercially packed citrus fruit was not evaluated. Presumably, some materials used for fruit packaging could reduce exposure of the fruit to ozone gas. The objectives of this work were to quantify the penetration of gaseous ozone into different commercial orange fruit packages and evaluate the effectiveness of the gas in controlling sporulation on fruit within these packages during cold storage.

2. Materials and methods

2.1. Fruit inoculation

'Lanelate' navel oranges (*Citrus sinensis* (L.) Osbeck) from commercial orchards in the San Joaquin Valley (California) were taken for use in these experiments before any commercial postharvest treatments were applied. Oranges were inoculated 10-mm deep into the flesh in the equator of two opposite faces with 0.25 ml of a 10^6 spores ml^{-1} suspension of *P. digitatum* or *P. italicum* using a plastic syringe with a 20-mm-long needle. This inoculation system is recommended for evaluation of postharvest treatments to prevent *Penicillium* sporulation on citrus fruit (Eckert and Brown, 1986).

2.2. Fruit packaging

The following four types of packages were prepared separately with oranges inoculated with each pathogen: (i) Carton (with naked fruit): standard corrugated fiberboard citrus cartons ($43.9 \times 30.2 \times 29.7$ cm; 39.4 l) with a vented surface area of 2.6% were filled with 60–70 oranges, about 10 of which were artificially inoculated. Inoculated fruit were placed in the four corners and at the center of the carton at both the bottom and top levels of the carton. Cartons were stored with the lids on. (ii) RPC (returnable plastic container, with naked fruit): polypropylene boxes ($59.7 \times 39.4 \times 25.6$ cm; 60.2 l) with a vented surface area of 35.9% were filled with approximately 50 oranges, about 10 of which were inoculated. Inoculated oranges were placed in the four corners and at the center of the box at both the bottom and top levels of the box. (iii) RPC (with bagged fruit): transparent 2.27-kg low-density polyethylene bags (53.3×26.7 cm) with a vented surface area of 0.7% were filled with 14 or 15 oranges, four of which were inoculated. Inoculated oranges were randomly distributed inside the bag. Eight bags were placed in each RPC. (iv) Master carton (with bagged fruit): polyethylene bags were filled with inoculated and non-inoculated oranges as previously described and placed in corrugated fiberboard Master cartons ($49.5 \times 33.0 \times 36.8$ cm; 60.1 l) with a vented surface area of 2.9%. Ten bags were placed in each carton. Master cartons were stored with the lids on.

Six packages of each type were prepared with fruit inoculated with *P. digitatum* and six with fruit inoculated with *P. italicum*. Master cartons were only prepared with fruit inoculated with *P. italicum*. For each pathogen, three of these six packages (replicates) were randomly stacked on one pallet and the other three on another pallet. Both pallets with packed fruit were held in an ambient air cold storage room at $12.8 \pm 1^{\circ}C$ for 24 h before ozone exposure.

2.3. Continuous exposure to gaseous ozone

A water-cooled corona discharge ozone generator (Model Genesis CD-25G, Del Industries, San Luis Obispo, CA, USA) was installed in an adjacent non-ozonated room and set to produce 2.5 g h^{-1} ozone. The gas was continuously released to an empty 678 m^3 commercial cold storage room with a constant temperature of $12.8 \pm 1^{\circ}C$ through a 5.1-mm diameter teflon tube anchored to the wall and ceiling of the room. The room was aerated through 105 ceiling cones (with a 15.2 cm outlet) spaced 1.5 m from each other. About 24 h after inoculation and packaging, the pallet containing one-half of the packed fruit was stored in this room for 14 days (ozonated room). No additional fruit load was placed in the room. This ozonated room was kept closed for the entire storage period. The pallet containing the other half of the packed fruit was stored at the same temperature and for the same time in an identical non-ozonated room (ambient air atmosphere, control room).

Ozone concentration in the ozonated room and inside some of the different packages on the pallet was continuously monitored by a 6-channel UV absorption ozone analyzer (Model 450 Nema, API Inc., San Diego, CA, USA) with a minimum detection limit of 0.001 ppm. Air from the sampling points was pumped through 3.8 mm internal diameter tubes to the analyzer, which was located in an adjacent room near the generator. Ozone levels in the control room were periodically assessed with 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. No measurable ozone was detected in the room during the entire storage period. Temperature was also continuously monitored in both rooms during the experiments.

2.4. Sporulation assessment

A quantitative sporulation index, adapted from Eckert and Brown (1986), was used in which the numbers 0, 0.5, 1, 2, 3, 4, and 5 indicated soft lesion but no spores or mycelium present, mycelium but no spores present, <5%, 5–30%, 31–60%, 61–90%, and >91% of the fruit surface covered with spores, respectively.

2.5. Statistical analysis

Scores in the sporulation index were considered as a quantitative variable. In order to homogenize variances, each value in the sporulation data set was transformed to the square root of the value plus 0.5. An analysis of variance (SAS Institute Inc., Cary, NC, USA) was applied to the transformed data and means were separated by Fisher's Protected Least Significant Difference test (LSD, $P = 0.05$).

3. Results and discussion

Average ozone concentrations for the entire storage period were calculated for each type of package and sample point from the ozone analyzer records. Ozone penetration in each type of package, expressed as a percentage of the average ozone concentration in the atmosphere of the ozonated storage room (0.72 ppm), was also calculated (Table 1). The actual ozone concentration in the atmosphere of the ozonated room during the 14-day storage period was 0.55–0.92 ppm.

Comparisons among ozone concentrations within the different package types indicated that more gas penetrated into RPCs than into standard cartons or Master cartons. Nevertheless, ozone concentrations within RPCs were considerably higher in the spaces surrounding the naked fruit than among fruit inside plastic bags (RPC naked vs. RPC bagged, Table 1). The gas was not able to efficiently penetrate corrugated fiberboard carton or polyethylene bags. Ozone penetration was acceptable only in RPCs with naked fruit (82%) and it was clearly related to the vented area of each package.

Sporulation of both *P. digitatum* and *P. italicum* was significantly inhibited by ozone exposure only on oranges packed naked in RPCs (Fig. 1). This was the only type of package with an acceptable percentage of ozone penetration (82%, Table 1). For both pathogens, the sporulation index on oranges packed naked in RPCs and exposed to ozone did not reach 1.0, indicating that no spores were present. In contrast, the sporulation index on control fruit reached about 3.0 and 2.0 for *P. digitatum* and *P. italicum*, respectively, indicating that 5–60% of the fruit surface was covered with spores (Fig. 1). Similarly, Harding (1968) observed good control of *Penicillium* sporulation on citrus fruit placed in open boxes and continuously exposed to gaseous ozone at 1.0 ppm for 15 days, whereas control of sporulation was unsatisfactory on fruit placed in cartons

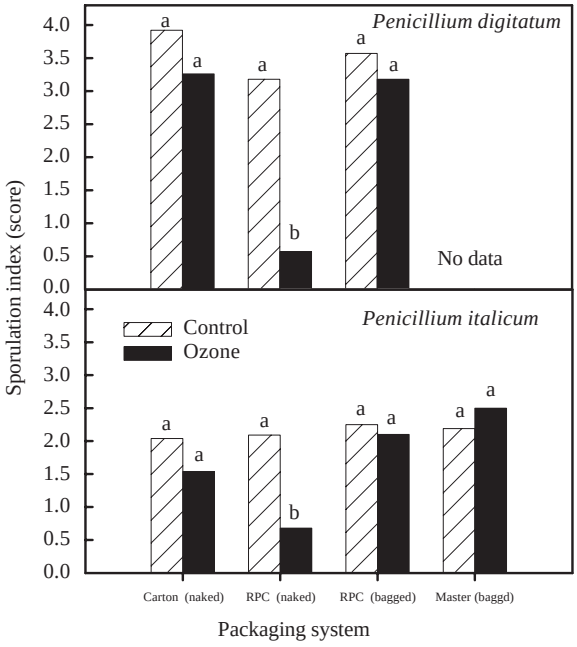

Fig. 1. Sporulation index on 'Lanelate' oranges artificially inoculated with *P. digitatum* or *P. italicum*, placed in different commercial packaging, and stored at 12.8°C for 14 days in an ambient air atmosphere (control) or in an ozonated atmosphere (average of 0.72 ppm O_3, v/v). Within packaging systems, columns with the same letter are not significantly different according to Fisher's Protected LSD test ($P<0.05$) applied after an analysis of variance of the square root transformed data. Non-transformed means are shown.

Table 1
Average ozone concentration and percentage of ozone penetration for the entire storage period inside the different types of packages

Packaging system	Sampling point	Ozone conc. (ppm, v/v)	Ozone penetration (%)
	In the atmosphere of the room	0.72	—
RPC (naked)	Inside a RPC	0.59	81.9
RPC (bagged)	Inside a plastic bag in a RPC	0.12	16.7
Master carton (bagged)	Inside a plastic bag in a Master carton	0.07	9.7
Carton (naked)	Inside a carton	0.07	9.7

with small vents. Furthermore, in other tests we obtained good suppression of sporulation over a 2-month period during a continuous exposure to 1.0 ppm ozone on oranges and lemons stored at low temperature in large, open-topped, plastic field bins with large side and bottom vents (J.L. Smilanick, unpublished data).

Our results confirm that ozone penetration inside the packages and unimpeded contact to the decayed area on the fruit are needed for ozone gas to be effective in controlling sporulation. Although the oxidizing power of ozone is considerably higher than that of other oxidants used for sanitation of fruits and vegetables, the efficacy of the gas in controlling postharvest fruit decay and sporulation cannot be predicted by its toxicity against free microbial cells or tissues. Due to the lack of ozone penetration, no benefit can be expected from ozone gas exposure of citrus fruit packed in corrugated fiberboard cartons (citrus standard and Master cartons) or plastic bags. Since these materials are commonly used in California and worldwide for commercial packaging of a large variety of fresh fruits and vegetables, the results obtained here with citrus packages may be easily extrapolated to other commodities such as stone fruits or table grapes.

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