# Gaseous ozone-enrichment for the preservation of fresh produce

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# Gaseous Ozone-Enrichment for the Preservation of Fresh Produce

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**Keywords:** modified atmosphere storage, ozone-enrichment, spoilage, postharvest fungal pathogens

**Abstract**

**Ozone is considered the most economically important air pollutant worldwide. Experimental studies reveal contrasting effects of ozone on foliar pathogens with several successful applications in postharvest level. Oranges, grapes and tomatoes were infected with green mould (*Penicillium digitatum*) and grey mould (*Botrytis cinerea*), then fruit incubated in 'clean air' or an ozone-enriched atmosphere (concentrations ranging from 0.05 to 1.0 ppm). In vivo and in vitro experimentation revealed lesion development and spore production to be markedly reduced in fruit maintained in an ozone-enriched atmosphere. Higher concentrations/duration of exposure resulted in greater impacts on lesion development/spore production, with considerable benefits resulting from exposure to low levels of ozone (i.e., below the 0.2 ppm threshold set for the protection of human health). In vitro studies performed on fungi raised on potato dextrose agar (PDA) for 6-8 days at 13°C and 95% relative humidity (RH) revealed no direct effects of ozone on fungal development, implying that suppression of pathogen development was due in a large part to the impacts of ozone on fruit-pathogen interactions.**

## INTRODUCTION

Postharvest storage of naturally-ripened fruit is limited by both fruit softening and disease development (Barkai-Golan et al., 1989). Microbial spoilage is most commonly-controlled by decontaminating fruit (by washing in the presence of chlorine- or bromine-based disinfectants) or modification of the storage and transit environment (i.e., fungicide application, temperature, T, and relative humidity, RH) (Snowdon, 1990). Due to commercial losses of fresh produce, postharvest sanitation techniques are still ineffective.

Thus, there is growing interest in safe but effective residue-free disease control strategies including the application of gaseous or aqueous ozone (Rice, 1999). Ozone is one of the most powerful oxidants known to man (Lide, 1991), and leaves no detectable residues in/on treated produce (Graham et al., 1997; Rice, 1999). However, ozone is considered the most economically important air pollutant worldwide with contradictions on experimental studies regarding the effects of ozone on foliar pathogens with several successful applications in postharvest level.

Several studies revealed that ozone-treatment prevents spore production and germination (Palou et al., 2002, 2003; Aguayo et al., 2006; Karaca and Velioglu, 2007; Tzortzakis et al., 2007, 2008), though spore production is only retarded when the gas is present and resumes when the fruit are removed from the ozone-enriched atmosphere (Smilanick, 2003). The inhibitory effects of ozone on sporulation have considerable commercial potential, because the treatment breaks the infection cycle. Sanitation of equipment and fruit surfaces with high concentrations of gaseous ozone has also been attempted. These studies reveal inactivation of spores of green mold, blue mold and sour rot (*Penicillium digitatum*, *Penicillium italicum* and *Geotrichum citri-auranatii* respectively) within 1 h at 200 µmol $mol^{-1}$ (5°C, 95% RH) (Smilanick, 2003). However, the adoption of such high ozone levels to inactivate fungal spores runs the risk of

damaging treated produce and has significant health and safety/human toxicological implications.

The principal aim of this study was to investigate the efficacy of low-level considered as safe levels, atmospheric ozone-enrichment for the control of fungal growth in stored orange, grape and tomato fruit.

## MATERIAL AND METHODS

### Inocula

*Penicillium digitatum* and *Botrytis cinerea* were incubated on PDA medium at 20-23°C for up 15 and 8 days, respectively. To harvest conidia, plates were flooded with sterile water containing a wetting agent [0.01% (v/v) Tween80] and rubbed with a sterile L-shaped spreader. Spore concentration was determined with a haemocytometer and adjusted to obtain $10^6$ spores $ml^{-1}$.

### Impacts of Ozone on *P. digitatum* and *B. cinerea* In Vitro

The effect of ozone enrichment on colony development and spore production was examined in *P. digitatum* (Pers.: Fr. Sacc.) and *B. cinerea* (Pers.: ex Fr.). Spore suspensions were taken (as described previously) and transferred using aseptic culture to the centre of petri dishes containing PDA medium. Five partially open dishes per pathogen were incubated in chambers ventilated with "clean air" (i.e., CFA) or CFA plus ozone (0.05, 0.2 and 1.0 ppm) at 13°C and 95% RH. Growth rates for each pathogen were obtained by recording the colony diameter (cm) at regular intervals. After 10 days, spores were harvested by adding 5 ml of sterile water containing 0.01% (v/v) Tween80 to each petri dish and rubbing the surface with a sterile L-shaped spreader (3 times). The spore concentration was determined with a haemocytometer.

### Impacts of Ozone on Disease Development in Wound-Inoculated Fruits

Trials were performed on orange (*Citrus sinensis* L. Osbeck 'Valencia'), table grape (*Vitis vinifera* L. 'Thompson Seedless'), and tomato (*Solanum lycopercicum* Mill. 'Mareta') purchased at a local market. Fruit ($n$=6) were selected for uniformity in size and appearance, and the absence of physical defects prior experimentation.

Two wounds (4 mm diameter and 3 mm deep) for oranges and tomatoes and 1 wound for grapes were made in opposing sides of each fruit using a sterilised scalpel. Spore suspensions were introduced on fruit via superficial wound made on the equator. In case of oranges, 100 µl of spore suspension of *P. digitatum* were inoculated, in tomatoes 50 µl of *B. cinerea*, and in table grapes 20 µl of *B. cinerea*. Inoculated fruit were held in CFA at RT for 24 h, then transferred to fumigation chambers maintained at 13°C and 95% RH and exposed to CFA or ozone (0.05, 0.2 or 1.0 ppm). Decay progression was quantified by measuring lesion diameter. After 10 days, fruit were immersed in distilled water containing Tween80 and shaken for 30 min (at 120 rpm) to determination of spore concentration with a haemocytometer.

### Statistical Analysis

Data were first tested for normality, and then subjected to analysis of variance (ANOVA). Sources of variation were time of storage and treatments. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, USA).

## RESULTS AND DISCUSSION

Ozone-enrichment resulted in no significant effects on the colony diameter of *P. digitatum* and *B. cinerea* in vitro (i.e., fungi grown and exposed to ozone on PDA) (see Figure 1) being in accordance with previous studies on *B. cinerea*, *Alternaria alternata* and *Colletotrichum coccodes* (Tzortzakis et al., 2007, 2008). Microscopic observations of fungal colonies on PDA indicated that fungal spore production was inhibited in the presence of ozone, and these effects were quantified in the current work. Thus

atmospheric ozone concentration (i.e., 0.05 ppm) reduced up 67% spore production for both fungi, while higher ozone concentration eliminates spore production. Exposure of agar-based colonies to higher ozone concentrations than those employed in the present study (>15 ppm) reduce the diameter of colonies of *B. cinerea* by up to 50% (Roberts, 2005), but at these concentrations marked shifts in the growth of fungi on plates pre-exposed to ozone were observed which may be attributable to effects of ozone on the culture medium.

The impact of ozone-enrichment on lesion development in fruit artificially-inoculated with *P. digitatum* or *B. cinerea* is illustrated in Figure 2. ANOVA revealed lesion development to be significantly ($P<0.05$) reduced in fruit maintained in an ozone-enriched atmosphere. Low-level ozone exposure (<0.2 ppm) reduced lesion development to 23 and 31% of that observed in control orange and tomato fruit (i.e., fruit maintained in 'clean air' throughout), respectively. This finding is consistent with the reported suppression of surface fungal diseases and moulds on produce exposed to ozone under experimental conditions (Sarig et al., 1996; Forney et al., 2001; Palou et al., 2001), although effects were observed at lower concentrations than previously examined.

Maintaining wound-inoculated fruit in an ozone-enriched atmosphere inhibited spore production by 19-99% ($P<0.001$) compared with equivalent fruit stored in 'clean air' (Fig. 2). This finding is consistent with the reported effects of gaseous ozone (0.3 to 1.0 ppm) on the sporulation of *Penicillium* spp. on citrus fruit (Palou et al., 2002, 2003). The suppression of spore production by ozone treatment could make a major contribution to limiting the spread of the pathogen by lowering the spore load in the storage atmosphere and on surfaces. The mechanism underlying the action of ozone-enrichment on the switch between vegetative and reproductive phases of fungal development remains to be understood, while further experimentation is needed. The impacts of ozone on sporulation may reflect effects of the gas on mycelial development and thus the basis to support spore production or the perception/transduction of signals involved in the switch from vegetative to reproductive development.

Fungal growth and spore production fluctuated in the present study, even if similar effects followed. Palou and colleagues (2002) suggest the reason for this variation in treatment efficacy is due to differences in the degree of protection afforded by the nature and morphology of contrasting fruit surfaces. Alternatively, it is possible that ozone-enrichment results in differing degrees of inducible resistance in different fruit. As a consequence, it is important that the period of exposure and the concentration of ozone is determined (i.e., optimised) for specific commodities.

Ozone produces many toxic molecular species and it has been identified that it acts as a phytotoxic agent, which can elicit plant defence reactions (Perez et al., 1999) and influence the ripening process (Rice et al., 1982). Sarig and colleagues (1996), for example, report the induction of the stilbenes, resveratrol and pterostilbene, in table grapes treated with ozone; a strong correlation between the presence of these compounds and the degree of resistance to fungal decay was reported (Sarig et al., 1996).

## CONCLUSIONS

This study revealed that atmospheric ozone-enrichment markedly reduces spoilage by green mould and grey mould with the protection afforded by ozone dependent on the commodity and concentration of the gas with considerable benefits resulting from exposure to low levels of ozone below the 0.2 ppm threshold set for the protection of human health. The benefits associated with atmospheric ozone-enrichment were similar for different kinds of produce, i.e., effects were not commodity-specific.

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**Figures**

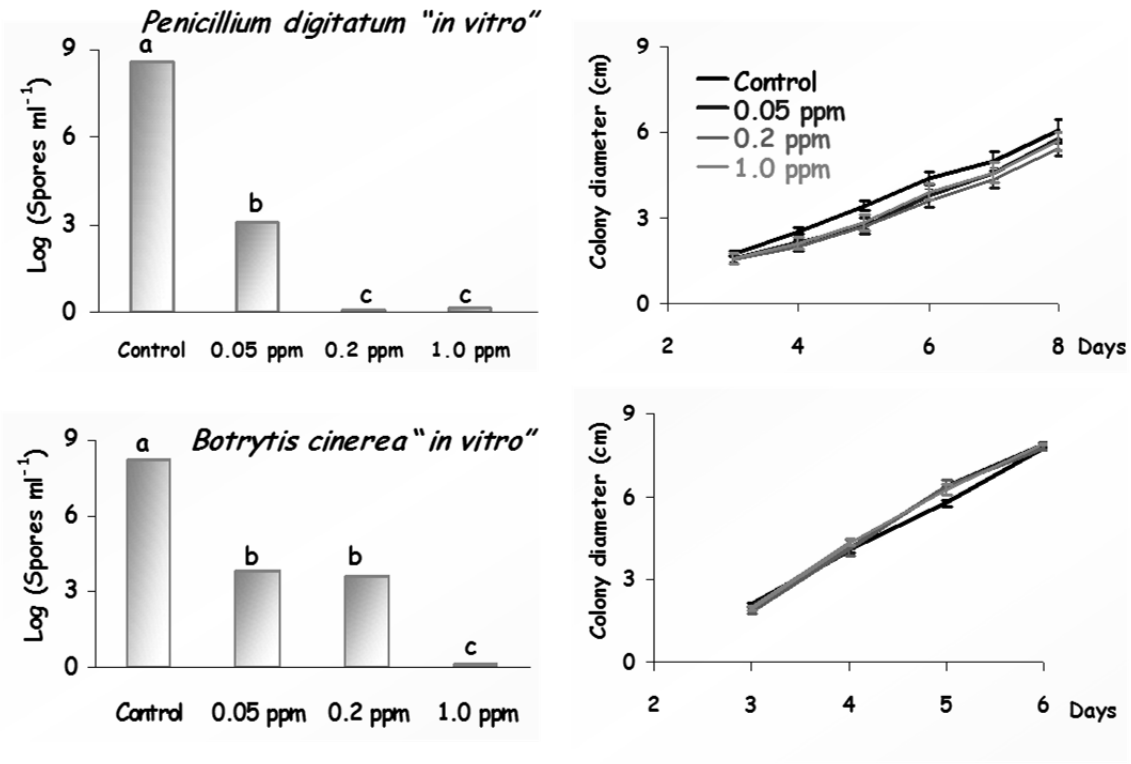


Fig. 1. Impacts of ozone-enrichment (0.05, 0.2, 1.0 ppm) on colony development (cm) and spore production (spores $ml^{-1}$) of green mould (*Penicillium digitatum*) and grey mould (*Botrytis cinerea*) raised and exposed to ozone on PDA at 13°C and 95% RH. Values represent mean of measurements made on five independent plates per treatment.

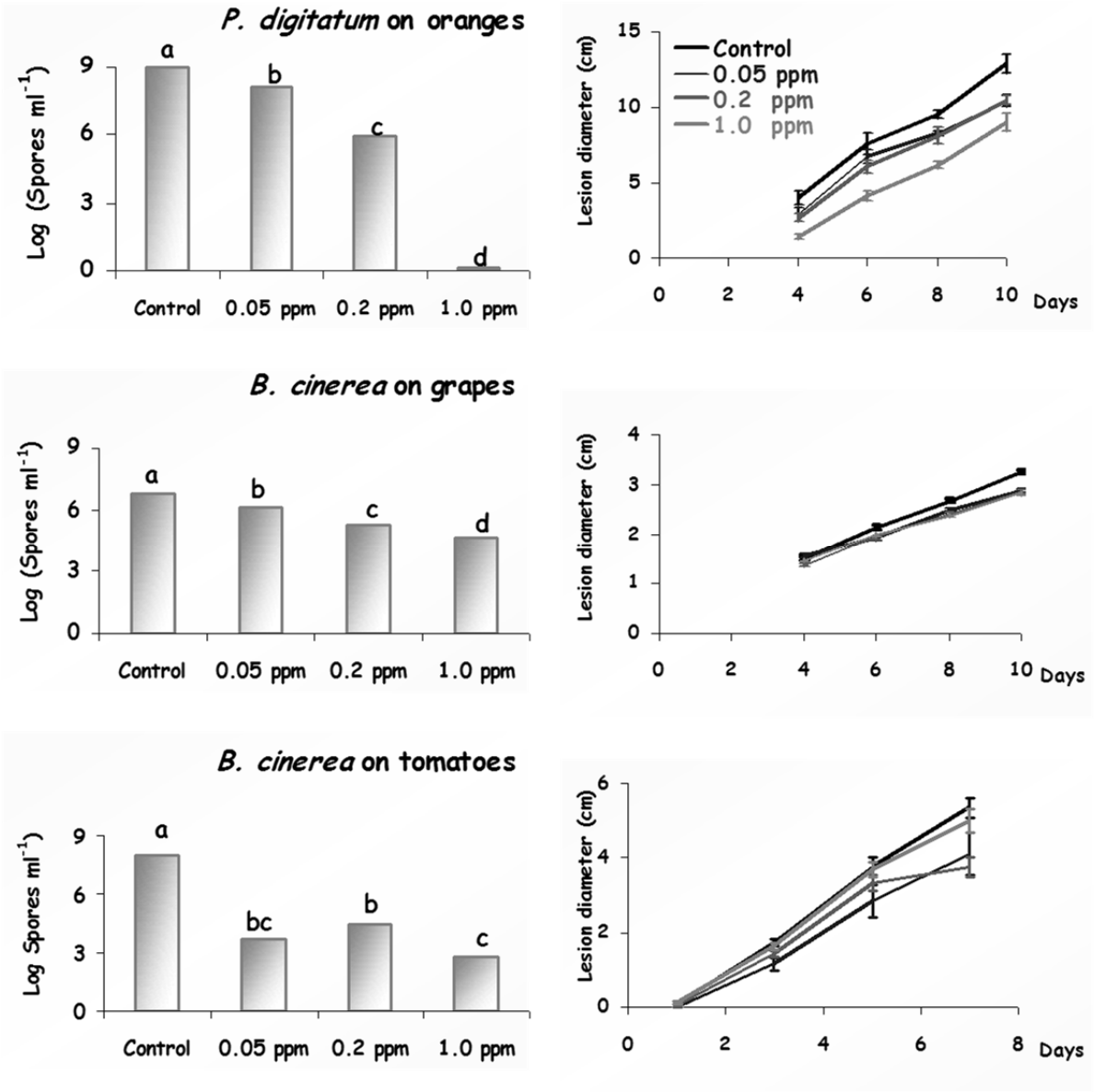


Fig. 2. Impacts of ozone-enrichment (0.05, 0.2, 1.0 ppm) on lesion development (cm) and spore production (spores $ml^{-1}$) of green mould (*Penicillium digitatum*) and grey mould (*Botrytis cinerea*) in wound-inoculated oranges, grapes and tomatoes at 13°C and 95% RH. Values represent mean of measurements made on six independent fruits per treatment.