CENTRAL VALLEY POSTHARVEST NEWSLETTER

COOPERATIVE EXTENSION **UNIVERSITY OF CALIFORNIA**
Kearney Agricultural Center
9240 S. Riverbend Avenue, Parlier, CA 93648 USA
(559) 646-6500

April 2005
Vol. 14, No. 1

Carlos H. Crisosto
Editor

Contents:

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EVALUATION OF OZONE GAS PENETRATION THROUGH CITRUS COMMERCIAL PACKAGES AND CONTROL OF GREEN AND BLUE MOLDS SPORULATION DURING COLD STORAGE

Lluís Palou, Joseph L. Smilanick, Monir Mansour, Carlos H. Crisosto, and **Thomas J. Clark**

Introduction

In recent work we reported the ability of gaseous ozone continuously released at low doses (0.3 or 1 ppm, v/v) to inhibit the sporulation of several important postharvest pathogens of table grapes, stone fruit, and citrus fruit (Palou et al., 2001a, 2001b, 2002). Sporulation of *Penicillium digitatum* and *P. italicum* on cold-stored oranges or lemons was suppressed without injuring the fruit. In those trials, however, exposure of the fruit to the gas was unimpeded and we did not evaluate the effectiveness of ozone applied to commercially-packed citrus fruit.

The objectives of this work were to test the ability of ozone gas to penetrate into different commercial citrus fruit packages and to

evaluate the effectiveness of the gas in controlling sporulation on commercially-packed citrus fruit.

Materials and methods

Fruit inoculation. Lane late navel oranges (*Citrus sinensis* (L.) Osbeck) from commercial orchards in the San Joaquin Valley (California), were used in the experiments before any commercial postharvest treatments were applied. *P. digitatum* and *P. italicum* were grown on PDA in petri dishes at 25ºC for 7 to 10 days. Spores were rubbed from the agar surface and a high-density spore suspension (approximately 10^6 spores ml^{-1}) was prepared. Oranges were inoculated 1-cm deep into the flesh in the equator of two opposite faces with a plastic syringe with a 20-mm needle. Approximately 0.25 ml of the spore suspension was applied at each inoculation point.

Fruit packaging. The following types of packages were prepared separately with fruit inoculated with each pathogen. See Table 1 for characteristics of each package.

Table 1. Characteristics of the different packages used in the experiments.

Package	Dimensions (inches) (long x wide x tall)	Volume (in^3)	Lid	Box vented area (%)
Carton	17.3 x 11.9 x 11.7	2,408.7	Yes	2.6
RPC	23.5 x 15.5 x 10.1	3,678.9	No	35.9
Master carton	19.5 x 13 x 14.5	3,675.7	Yes	2.9
Plastic bag	21 x 10.5	-	-	0.7

1) Carton (naked): standard corrugated fiberboard citrus cartons with vents were filled with 60-70 oranges. 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. Ten inoculated oranges per carton were used. Cartons were stored with the lids on.

2) RPC (naked): returnable plastic boxes were filled with approximately 50 oranges. Inoculated fruit were placed in the four corners and at the center of the box at both the bottom and top levels of the box. Ten inoculated oranges per box were used.

3) RPC (bagged): 5 lb polyethylene bags with small vents were filled with oranges, 4 were inoculated and 10 were not inoculated. Eight bags were placed in each RPC.

4) Master carton (bagged): polyethylene bags were filled with inoculated and non-inoculated oranges as previously described and placed in Master cartons. Ten bags were placed in each carton. 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. Packed fruit was held at 55 ± 2ºF for 24 h before ozone exposure.

Continuous exposure to gaseous ozone. A water-cooled corona discharge ozone generator (Model Genesis CD-25G, Del Industries, San Luis Obispo, CA) was installed in an adjacent non-ozonated room and set to produce 2.5 g h^{-1} ozone. The gas was continuously released to a 23,940 ft^3 cold storage room with a constant temperature of 55 ± 2ºF (12.8 ± 1ºC) through a 0.2-inch diameter Teflon tube anchored to the wall of the room. The room was aerated through 105 ceiling cones (with a 6 inch outlet) spaced 5 ft 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 13 days. 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 (air atmosphere, control room).

The ozone concentration in the room and inside some of the packages on the pallet was continuously monitored by a 6-channel UV absorption ozone analyzer (Model 450 Nema, API Inc., San Diego, CA) with a minimum detection limit of 0.001 ppm. Air from the sampling points in the ozonated room was pumped through 0.15 inch internal diameter tubes to the analyzer, which was located in the adjacent room near the generator. The sampling points are specified in Table 2.

Sporulation assessment. Green and blue mold sporulation on Lane late navel oranges packed and stored in both ozonated and control rooms were recorded for each inoculated fruit after 13 days of storage at 55ºF. A sporulation index was used where numbers 0, 0.5, 1, 2, 3, 4, and 5, respectively, indicated soft lesion but no spores or mycelium present, mycelium but no spores present, < 5%, 6 to 30%, 31 to 60%, 61 to 90%, and > 91% of the fruit surface covered with spores.

Statistical analysis. Scores in the sporulation index were considered as a quantitative variable. Each value in the data set was transformed to the square root of the value plus 0.5. An analysis of variance was applied to the transformed data and means were separated by Fisher's Protected Least Significant Difference test (LSD, $P = 0.05$).

Results and discussion

Average levels of the ozone concentration for the entire storage period are given for each sample point (Table 2) and type of package (Table 3). Ozone penetration in each type of package, calculated as a percentage of the ozone concentration in the room ambient, is also presented (Table 3).

Table 2. Average ozone levels for the entire storage period at the different sampling points.

Analyzer channel	Sampling point	Position in the pallet	Ozone levels (ppm, v/v)
Channel 1	Inside a plastic bag in a RPC box	Middle	0.12
Channel 2	Inside a RPC box (naked fruit)	Middle	0.59
Channel 3	Inside a carton (naked fruit)	Middle	0.03
Channel 4	Inside a plastic bag in a Master carton	Middle	0.07
Channel 5	Inside a carton (naked fruit)	Top	0.11
Channel 6	In the room ambient	-	0.72

A comparison between ozone concentrations inside the different packages indicated that the gas penetrated more easily into RPC boxes than into cartons or Master cartons. Nevertheless, ozone concentration in RPC boxes was significantly higher in the spaces surrounding the naked fruit than inside plastic bags (Table 3).

Table 3. Average ozone levels and percentage of ozone penetration (based on the average level in the room) for the entire storage period inside the different types of packages.

Packaging system	Ozone levels (ppm, v/v)	Ozone penetration (%)
Carton (naked)	0.07	9.7
RPC (naked)	0.59	81.9
RPC (bagged)	0.12	16.7
Master (bagged)	0.07	9.7

Ozone penetration was related to the vented area of each package (Table 1), indicating that the gas was not able to go through corrugated fiberboard carton or polyethylene bags. Ozone penetration was acceptable only in RPC boxes with naked fruit (82%, Table 3). On the other hand, the position of the box on the pallet also influenced the ozone concentration; ozone

levels inside a mid-placed carton were in general lower than inside a top-placed carton (channel 3 vs. channel 5, Table 2).

Sporulation of both *P. digitatum* and *P. italicum* was significantly inhibited by ozone exposure on oranges packed naked in RPC boxes, but it was not on oranges packed following the other packaging methods (Fig. 1). According to the percentages of ozone penetration inside the packages (Table 3), this result confirmed the need for good penetration and full contact to the decayed area on the fruit for ozone gas to be effective in controlling sporulation.

Conclusions

- Gaseous ozone continuously generated in a cold storage room at rates ranging 0.5 to 1 ppm (v/v) effectively penetrated and controlled sporulation of both *P. digitatum* and *P. italicum* on oranges packed naked in RPC boxes.

- The gas was not able to penetrate properly through corrugated fiberboard carton or polyethylene bags. Therefore, it was not able to control sporulation on oranges packed in standard cartons, Master cartons, or plastic bags. Effective control of sporulation relied on actual physical contact between the gas and the decayed area of the fruit.

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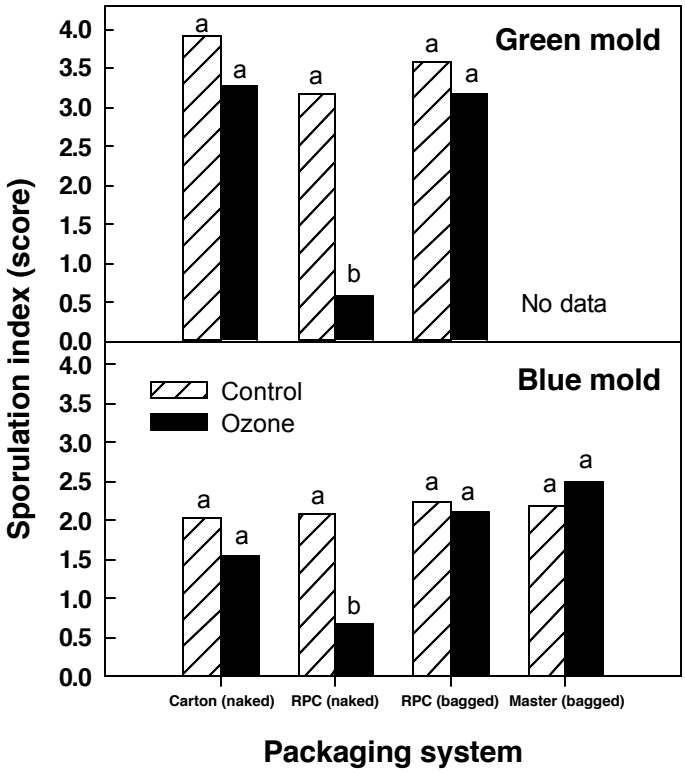

Fig. 1. Sporulation index on Lane late oranges artificially inoculated with *Penicillium digitatum* or *P. italicum*, packed following different packaging systems, and stored at 55ºF for 13 days in an air atmosphere (control) or in an ozonated atmosphere (0.5-1 ppm O_3 v/v). Within packaging systems, 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 to the square root transformed data. Non-transformed means are shown.

EVALUATION OF THE EFFECT OF OZONE EXPOSURE ON DECAY DEVELOPMENT AND FRUIT PHYSIOLOGICAL BEHAVIOR

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Proc. 4th Int. Conf. on Postharvest
Eds. R. Ben-Arie & S. Philosoph-Hadas
Acta Hort. 553, ISHS 2001 429

Keywords: orange, peach, grape, ethylene, respiration

Introduction

Since the declaration of GRAS status (Generally Recognized As Safe) for ozone in the United States in 1997, interest in developing ozone applications in the food industry has increased.

Materials and methods

Artificially inoculated oranges, peaches, and table grapes were stored at 5ºC and 90% RH under 0 (control room) or 0.3 ppm ozone (OSHA short-term exposure limit). Decay incidence and severity, and external disease appearance, as well as the number of infected berries around the inoculated berry were checked weekly. Respiration and ethylene production of 'O'Henry' peaches, previously stored for 1, 2, and 3 weeks at 5ºC and 90% RH under ozone free or 0.3 ppm ozone, were measured daily during a 5-day softening period at 20ºC and 90% RH. Individually weighed 'Zee Lady' peaches were stored for 6 weeks at 5ºC and 90% RH under 0 or 0.3 ppm ozone. Weight loss was recorded weekly.

Results and discussion

Ozone at 0.3 ppm did not affect decay incidence or severity on artificially inoculated 'Elegant Lady' peaches during 4 weeks storage at 5ºC, except for brown rot, caused by *Monilinia fructicola* (Table 1). Ozone exposure at 5ºC altered normal mycelium growth and inhibited sporulation. Normal mycelium growth resumed after ozone exposure. The incidence of citrus green and blue molds, caused by *Penicillium digitatum* and *P. italicum*, respectively, on inoculated 'Valencia' oranges was significantly delayed by 1 week under 0.3 ppm ozone at 5ºC (Fig. 1). Ozone exposure prevented the sporulation of both pathogens. Ozone at 0.3 ppm eliminated gray mold (*Botrytis cinerea*) nest formation on 'Thompson Seedless' table grapes stored for 7 weeks at 5ºC (Fig. 2). Peach respiration and ethylene production were not affected by 3 weeks ozone exposure (0.3 ppm). After 4 weeks exposure to 0.3 ppm ozone, peaches became more susceptible to water loss (data not shown).

Conclusions

The lack of nesting and 'soilage' could have an economic impact on bin stored fruit. Ozone treatment could reduce the proliferation of fungicide-resistant strains. Ozone at 0.3 ppm did not prevent decay of artificially inoculated fruits. Diseases developing on fruit infected by wound pathogens in the field would be difficult to control using ozone in air during storage.

Table 1. Influence of ozone exposure (0.3 ppm) on disease incidence and severity on artificially inoculated 'Elegant Lady' peaches stored at 5ºC and 90% RH.

		Storage period (days)					
		14		21		28	
Pathogen	Treatment[x]	Incidence[y] (%)	Severity[z] (mm^2)	Incidence (%)	Severity (mm^2)	Incidence (%)	Severity (mm^2)
Monilinia fructicola	Control	80.0 a	55.3 a	98.7 a	818.0 a	100.0 a	2955.1 a
	Ozone	28.7 b	14.9 b	95.0 a	267.8 b	100.0 a	1305.9 b
Botrytis cinerea	Control	56.2 a	22.8 a	93.7 a	477.0 a	100.0 a	2828.6 a
	Ozone	63.7 a	32.4 a	97.5 a	443.2 a	100.0 a	2326.3 a
Mucor piriformis	Control	2.5 a	40.5 a	2.5 a	384.8 a	21.2 a	606.9 a
	Ozone	2.5 a	15.3 a	2.5 a	133.0 a	5.0 b	389.8 a
Penicillium expansum	Control	0.0	0.0	73.7 a	69.0 a	100.0 a	353.0 a
	Ozone	0.0	0.0	58.7 a	48.7 a	93.7 a	296.6 a

[x] For each pathogen, values within columns followed by unlike letters are different according to Fisher's protected LSD test ($P = 0.05$).

[y] Incidence data were transformed to the arcsine of the square root of the proportion of infected fruits before the analysis of variance. Actual data are shown.

[z] Lesion area.

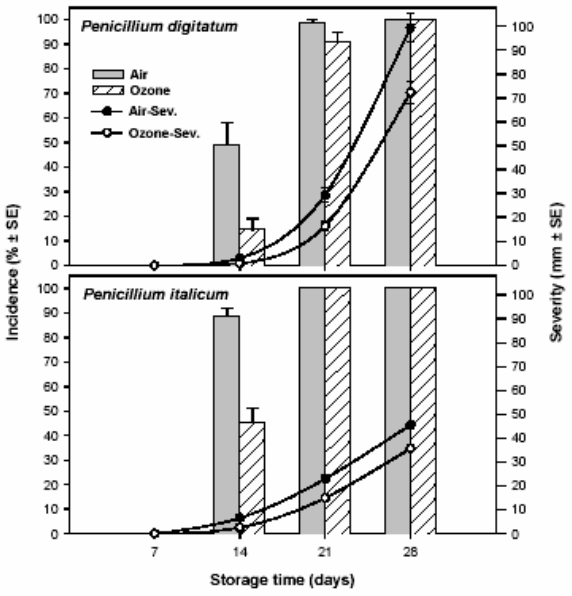

Fig. 1. Citrus green and blue mold incidence (bars) and severity (lines) on artificially inoculated 'Valencia' oranges stored for 4 weeks at 5ºC and 90% RH under 0 (air) or 0.3 ppm ozone.

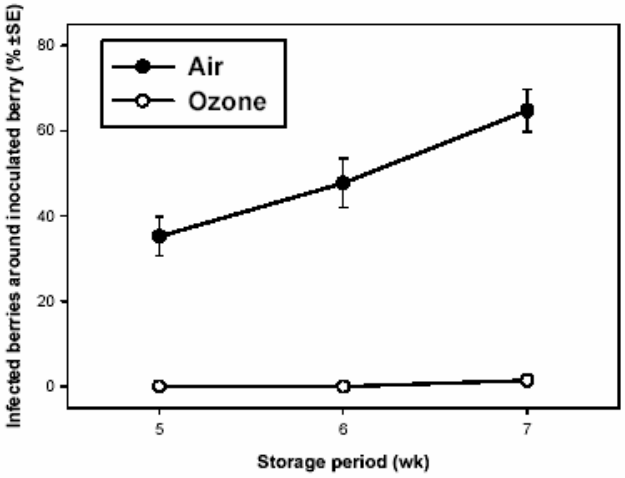

Fig. 2. Proportion of infected berries surrounding one berry inoculated with *B. cinerea* in clusters of 'Thompson Seedless' table grapes stored at 5ºC and 90% RH under 0 (air) or 0.3 ppm ozone.

Abstracts

From *Postharvest Biology and Technology*. 2002. Vol. 24: 39-48.

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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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.

From *Plant Disease*. 2001. Vol. 85(6): 632-638.

EFFECT OF GASEOUS OZONE EXPOSURE ON THE DEVELOPMENT OF GREEN AND BLUE MOLDS ON COLD STORED CITRUS FRUIT

L. Palou, J. L. Smilanick, C. H. Crisosto, and **M. Mansour**

The effects of gaseous ozone exposure on in vitro growth of *Penicillium digitatum* and *Penicillium italicum* and development of postharvest green and blue molds on artificially inoculated citrus fruit were evaluated. Valencia oranges were continuously exposed to 0.3 ± 0.05 ppm (vol/vol) ozone at 5°C for 4 weeks. Eureka lemons were exposed to an intermittent day-night ozone cycle (0.3 ± 0.01 ppm ozone only at night) in a commercial cold storage room at 4.5°C for 9 weeks. Both oranges and lemons were continuously exposed to 1.0 ± 0.05 ppm ozone at 10°C in an export container for 2 weeks. Exposure to ozone did not reduce final incidence of green or blue mold, although incidence of both diseases was delayed about 1 week and infections developed more slowly under ozone. Sporulation was prevented or reduced by gaseous ozone without noticeable ozone phytotoxicity to the fruit. A synergistic effect between ozone exposure and low temperature was observed for prevention of sporulation. The proliferation of spores of fungicide-resistant strains of these pathogens, which often develop during storage, may be delayed, presumably prolonging the useful life of

postharvest fungicides. In vitro radial growth of *P. italicum*, but not of *P. digitatum*, during a 5-day incubation period at 20°C was significantly reduced by a previous 0.3 ± 0.05 ppm ozone exposure at 5°C for 4 days. Inoculum density did not influence the effect of gaseous ozone on decay incidence or severity on oranges exposed to 0.3 ± 0.05 ppm ozone at 20°C for 1 week. Susceptibility of oranges to decay was not affected by a previous continuous exposure to 0.3 ± 0.05 ppm ozone at 20°C for 1 week. A corona discharge ozone generator was effective in abating ethylene in an empty export container.

From *Levanta Agricola, Especial Postcosecha*. 2004.

COLD STORAGE OF CITRUS UNDER OZONE ENVIRONMENT: EFFECT ON POSTHARVEST DISEASES

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Before the growing interest of the Spanish citrus industry in the exposure of cold-stored fruit to ozone gas, results of recent research conducted in California are presented in this paper. Research focused on the effect of gaseous ozone exposure on the incidence and development of the most important citrus postharvest diseases in our conditions, namely green and blue molds, caused respectively by *Penicillium digitatum* and *P. italicum*. Ozone in air was not able to control infections established within the rind of the fruit. Therefore, it cannot substitute the treatments with synthetic fungicides that are currently applied in drenchers or packinglines. Continuous or intermittent exposure to ozone at 0.3 ppm (current Threshold Limit Value-Short Term Exposure Limit (TLV-STEL) established by the US Occupational Safety and Health Administration) was not phytotoxic to the fruit and greatly inhibited aerial mycelial growth and sporulation on articicially inoculated oranges and lemons stored at low temperature (4-5°C). As a consequence of this prevention of sporulation, pathogenical inoculum load in the packinghouses and proliferation of spores of fungicide-resistant strains of the pathogens could be reduced. However, since ozone gas was not able to penetrate through fiberboard cartons or plastic bags, its practical use during fruit storage is limited to highly vented packages such as field bins, open-top containers, or returnable plastic containers. Because of its extremely high oxidizing power, ozone may be harmful to humans, phytotoxic to fruit, and corrosive to numerous common materials. Therefore, when a generating system is implemented, adoption of safety means and monitoring and control of actual ozone concentrations inside the cold storage rooms are very important issues to consider.

From *Ozone Science and Engineering*. 2002. Vol. 24: 343-356.

IMPACT OF OZONATED WATER ON THE QUALITY AND SHELF-LIFE OF FRESH CITRUS FRUIT, STONE FRUIT AND TABLE GRAPES

J. L. Smilanick, D. M. Margosan, and **F. Mlikota-Gabler**

Interpretive Summary: Fungi that rot the fruit are a problem and must be controlled in order to effectively store and market the fruit. Ozone treatment did not control the most troublesome rot fungi on citrus fruit, peaches and nectarines, or table grapes. At very high doses, ozone did partially control rot on peaches and table grapes, but these doses caused injury to the

fruit. The brief immersion of these fruit in ozonated water does not appear to be a promising approach to manage rot problems.

Technical Abstract: Spores of fungi that cause postharvest decay of fresh fruit die rapidly in ozonated water. We determined the impact of sporocidal or higher O_3 doses on fruit shelf-life & quality. Fruit were placed in stainless steel baskets immersed in 1.5 to 10 ppm O_3, dried in air, and examined after storage. O_3 concentration did not change during tests. Green mold and sour rot on citrus fruit, caused by *Penicillium digitatum* and *Geotrichum citri aurantii*, respectively, were not reduced by 20 min immersion in 10 ppm O_3. These fungi infect through wounds; their spores were placed in shallow wounds (1 mm wide x 2 mm deep) 24 hr before treatment. On five peach varieties, the average natural incidence of brown rot, caused by *Monilinia fructicola*, was reduced from 10.9 to 5.4% by 1 min immersion in 1.5 ppm O_3. A treatment of 15 min with 5 ppm O_3 further reduced decay to 1.7%, but consistent control of brown rot was associated only with this severe treatment and it caused shallow pits on the fruit. Brown rot caused by spores placed in wounds before treatment was rot controlled. Immersion for 1 or 5 min in 5 ppm O_3 reduced natural aerobic bacteria populations by 1.1 and 1.6 log 10 units, respectively, and yeast and filamentous fungal populations by 0.7 and 1.3 log 10 units, respectively. Spores of *Botrytis cinerea*, cause of gray mold, were sprayed on table grape clusters, the clusters were dried, then immersed for 1 to 6 min in 10 ppm O_3. In two tests, immersion for 1 min in O_3 reduced gray mold from 35% among untreated grapes to about 10%, while in two other tests, the incidence was only reduced from 35 to 26%. Minor injury to the rachis of Crimson Seedless grapes occurred at high O_3 rates. In conclusion, immersion in ozonated water did not control postharvest decay of citrus fruit, injured peaches and nectarines at doses that reliably controlled decay.

DR. CRISOSTO'S OZONE PUBLICATIONS

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FUTURE DATES

2005 Variety Display and Research Update Seminars at the Kearney Agricultural Center.

Mark your calendars for these dates:

- Friday, June 3
- Friday, July 1
- Friday, August 12

8:00 – 9:00 a.m.: Variety display by stone fruit nurseries, breeders and the USDA;
9:00 – 10:00 a.m.: Research Update Topics (For example: Nutrient deficiencies, Dwarfing & semi-dwarfing rootstocks, Keeping trees short, IPM updates, Irrigation management and water stress).

For more information call: Scott Johnson (559) 646-6547; Kevin Day (559) 685-3309, Ext. 211; Harry Andris (559) 456-7557; Brent Holtz (559) 675-7879, Ext. 209; or Bob Beede (559) 582-3211, Ext. 2737.