Commercial and Experimental Developments in California for the Control of Postharvest Citrus Diseases

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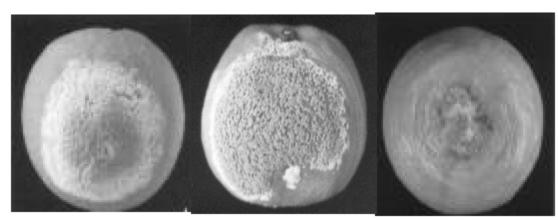
- ***New fungicides***
- ***Maximizing TBZ effectiveness***
- ***Hot water brush***
- ***Ozonated storage rooms***

This work would not be possible without:

Financial support of the CRB

The hard work and ideas of Dave Sorenson, Julie Doctor, Bob Fassel, Charlene Jewell, John Maze, Walter Stutzman, Dennis Margosan, Monir Mansour, Franka Mlikota Gabler, Jonah Aeiyabei, Lluis Palou, Pilar Plaza, and many others.

Green mold	Blue mold	Sour rot
Penicillium digitatum	*P. italicum*	*Geotrichum candidum*



New Postharvest Fungicides

Compound	Class	Diseases	Trade name
PH066	anilino-pyrimidine	GM BM	none
Fludioxinil	phenylpyrrole	GM BM	Switch
Azoxystrobin	methoxyacrylate	GM BM	Abound

New Postharvest Fungicides

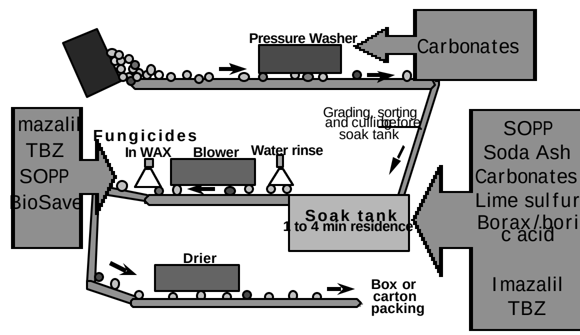
All are classified or requested to be classified as USEPA "reduced risk"

Registration for all expected by 2004 to 2005

Mode of action differs from the currently registered citrus postharvest fungicides (TBZ, IMZ, SOPP)

Maximizing Thiabendazole Effectiveness

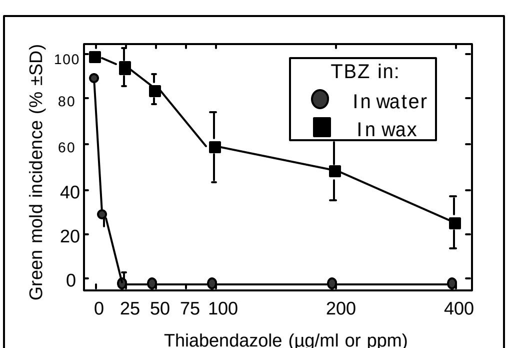
Sites of packline fruit treatments



Fungicide dissolved in	Resulting effectiveness
WARM/HOT WATER	**HIGHEST**
AMBIENT TEMPERATURE WATER	↑
LIGHT WAX	↓
HEAVY WAX	**LOWEST**

Fungicide applied to fruit by	Resulting effectiveness
Immersed in or pressure washed with solution	**HIGHEST**
Float in or drenched with solution	↕
Brief overhead spray	**LOWEST**

TBZ effectiveness in water and wax



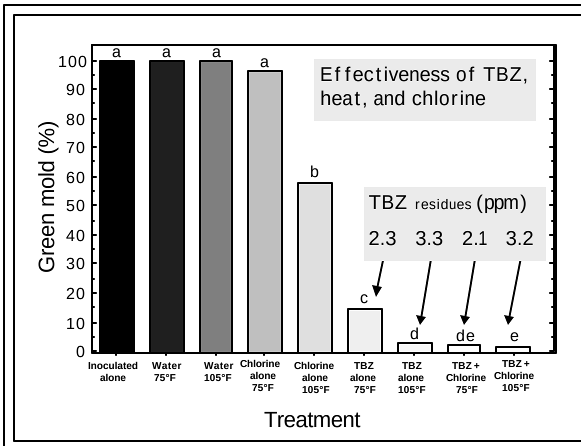


Table 1. Influence of solution temperature on thiabendazole (TBZ) residues (ppm) in lemons. They were immersed in TBZ for 1 min.

TBZ (mg/L)	TBZ residues (ppm) 75°F (24°C)	105°F (41°C)
0	0.0	0.0
100	0.5	1.9
250	1.2	3.2
500	2.2	3.8
1000	3.6	5.5

Mild heating increases TBZ residues

Table 2. Influence of thiabendazole (TBZ) temperature and rinsing & waxing on subsequent residues. The fruit were immersed in aqueous 200 ppm TBZ solutions for 1 minute and: 1) dried in air, or 2) rinsed and waxed.

	80°F (27°C)	105°F (41°C)
Lemons		
1. Not rinsed	0.43 ±0.03	0.49 ±0.11
2. Rinsed & waxed	0.07 ±0.03	0.15 ±0.01
Oranges		
1. Not rinsed	0.44 ±0.08	0.51 ±0.07
2. Rinsed & waxed	0.07 ±0.01	0.09 ±0.03

TBZ residues are easily washed away

Table 3. Green among lemons and oranges treated for one minute in the following 80°F (27°C) solutions. The oranges were _not rinsed_ and stored at 20°C for one wk, the lemons were _rinsed & waxed_ and stored at 50°F for 3 wk.

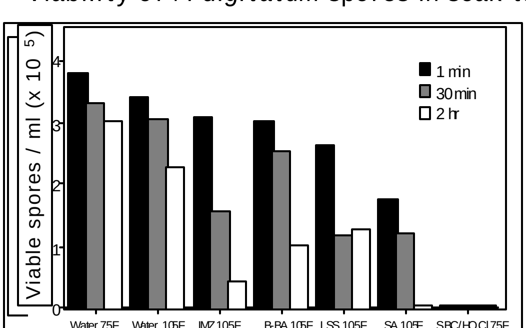
Treatment	Oranges *(not rinsed)* M6R	D201	
Cool control	83.9 a	95.6 ab	[redacted]
SBC 3%	12.2 b	46.1 c	
TBZ 200 ppm	0.0 c	89.4 b	
SBC-TBZ	0.0 c	36.7 c	
TBZ residue(ppm)	*0.44*		

TBZ works well even if most residue removed

Heated aqueous thiabendazole to control green mold

- **Aqueous, warm fungicides work better than those in wax. Cabras and Schirra showed this in Italy with lemons and TBZ.**
- **TBZ is not inactivated by chlorine, while imazalil is. An advantage because TBZ tanks are sanitized by chlorine, while imazalil must be periodically heated or filtered. When in use, fungicide-resistant spores can accumulate in imazalil tanks, but not in TBZ- Cl_2 tanks.**
- **Sodium bicarbonate ($NaHCO_3$ "SBC") controls green mold well, even isolates resistant to fungicides, and sour rot partially. TBZ and SBC can be mixed together.**

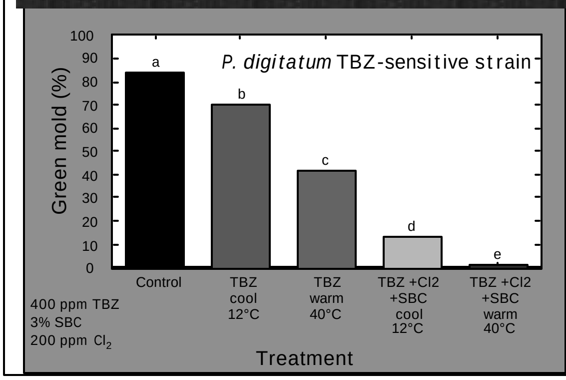
Viability of *P. digitatum* spores in soak tan



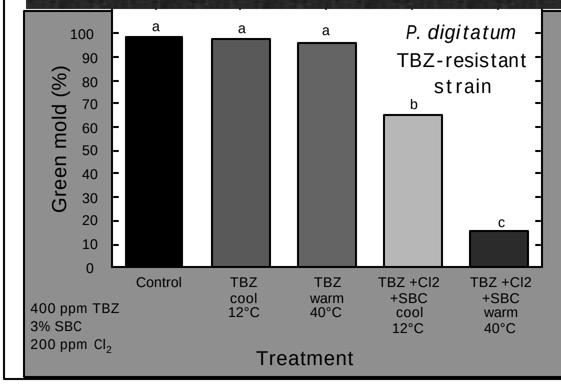
Spores died almost immediately in chlorinated $NaHCO_3$

Large scale TBZ tests at University of California Lindcove Packline

TBZ, chlorine, and sodium bicarbonate (SBC) effectiveness in combinations with and without heat



TBZ, chlorine, and sodium bicarbonate (SBC) effectiveness in combinations with and without heat - Lindcove December 2001



Maximizing TBZ effectiveness

Immersion in a warm mix of TBZ, sodium bicarbonate, and chlorine was superior to any of them alone.

Their effectiveness was additive.

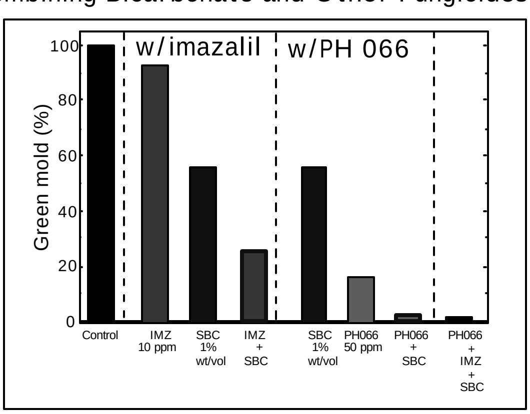
TBZ residues were increased by heat.

The combination controlled a TBZ-resistant isolate of *P. digitatum*.

Impact on TBZ effectiveness

Measure	Magnitude
Applied in water vs wax	+++
Immersed vs sprayed	++
Heat	+
Sodium bicarbonate	+++
Chlorine	+

Combining Bicarbonate and Other Fungicides



Pros & Cons of Sodium Bicarbonate

Pros

- Inexpensive
- Controls fungicide resistant isolates
- Accepted by organic growers and others
- Compatible with other fungicides & chlorine

Cons

- Effectiveness only fair, no SER control
- Salty solution hard to dispose of some places
- pH high and rises as its used
- Scale on equipment from calcium carbonate
- Some risk of fruit injury, more if not rinsed

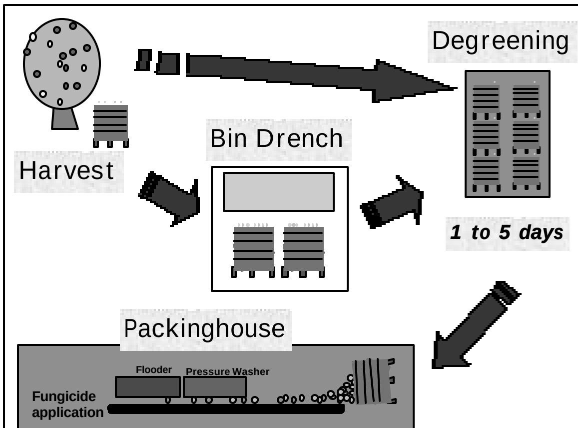
Commercial Tests

Sunkist Growers

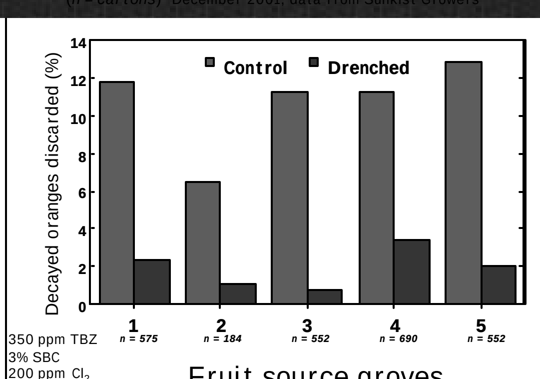
The unusual conditions of the 2001 navel season

- Many navel oranges spilt while green and on trees in groves - usually this is rare.
- The split fruit often developed green mold.
- High spore densities in tree canopies occurred.
- Decay rates after harvest, particularly after degreening, were very high.

Early split navel oranges were common at Lindcove Fall 2001



ommercial drencher with TBZ, chlorine, and sodium bicarbonate
(*n = cartons*) December 2001, data from Sunkist Growers



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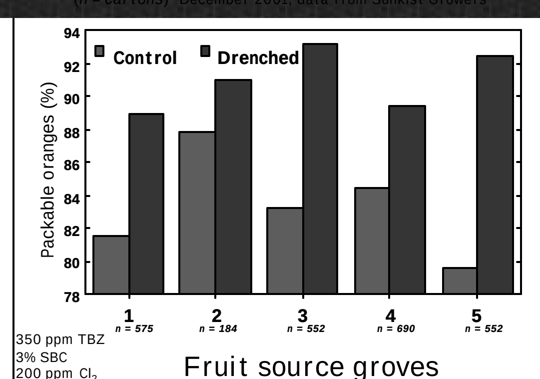


Commercial Tests

TBZ/SBC/Cl_2 drenching reduced decay of oranges during degreening by 82%

Packable fruit per grove increased from 83.3% to 91.0%

"Hot Water Brush"

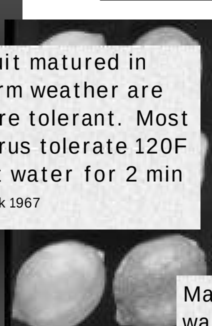
Prior literature

- Hot water combined with sodium carbonate/bicarbonate or borax/boric acid controls brown rot and green/blue molds. These enter common use. **Smith 1907 Barger 1925 Winston 1935 Fawcett 1936**
- Hot water (53C for 5 min) recommended for green mold control on orange, less effective on grapefruit. Rates that control it close to those that injure fruit. Brown rot controlled at lower temp. **Smoot and Melvin 1963, 1965 Palou 2001**
- California tank recommendations: 118-120F for 2 to 4 minutes, in Florida: 128F for 5 min. **Official ASHRAE citrus guidelines 1966 Eckert 1967**
- Insecticidal hot water treatments (46C, >40 m) often injure citrus fruit. **Miller et al 1988 McGuire 1991 Williams et al 1994 Shellie et al 1998**

Prior literature

- Synergistic combinations of hot water and fungicides (imazalil, sopp, sorbate, and thiabendazole) and growth regulators (GA and 2,4-D). **1987 Wild Barkai Golan 1991 Rodov et al 1996, Schirra et al 1995, 1997 Ben-Yehoshua 1998**
- One to four minutes hot water treatment reduces subsequent chilling injury, wt loss, & softening of many citrus fruits. **MacDonald et al 1991 Rodov et al 1995 Schirra and Mulas 1995 McLauchlan et al 1997 Porat et al 1999 Rodov et al 1996**
- Very hot-water immersion (80C for 1 min or 70C for 2 min) reduces both fruit-surface and initial juice microbial loads without altering quality of fresh juice. **Pao and Davis 1999**
- Brief (20 s) very hot water (56C) reduces chilling injury and decay, and induces resistance to subsequent infections. **Porat et al 2000 Rodov et al 2000 Pavoncello et al 2001**

Risk of fruit injury



Fruit matured in warm weather are more tolerant. Most citrus tolerate 120F hot water for 2 min
Houck 1967

"Wilted" fruit more tolerant to hot water Fawcett 1936 Klotz 1973

Injured lemons release oils, d-limonene in particular, which may cause phytotoxicity.
Klotz and DeWolfe 1961 Obenland et al 1995

Many facilities don't heat water above 105F so as to avoid liability for fruit injuries

How do heat treatments work?

Brief hot-water causes a transient inhibitory effect on pathogens, arresting their growth for 24-48h.

Similarly, curing occurs at temperatures above that tolerated by the pathogen.

During this lag period, resistance to infection increases in the peel and stops pathogens.

Nafussi et al 2001 J. Agric. Food Chem. 49(1):107-113

"Hot-water brush"

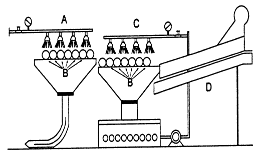


Fig. 1. Scheme of the commercial-scale automatic rinsing and disinfecting machine: (A) tap water (20–23°C); (B) brushes; (C) hot water (55°C); (D) drying tunnel (40°C).

Developed and made at Kibbutz Lotan, Israel

Accomplishes cleaning and some decay control with a combination of rotating brushes and (very) hot water. other versions similar, some use Biox (eugenol) or fungicides in the water

We made a hot-water drench system using a modified pressure washer

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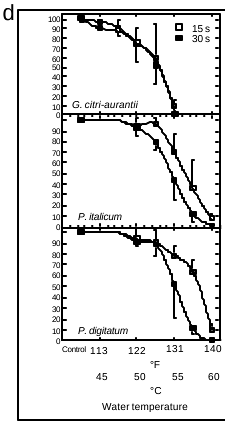
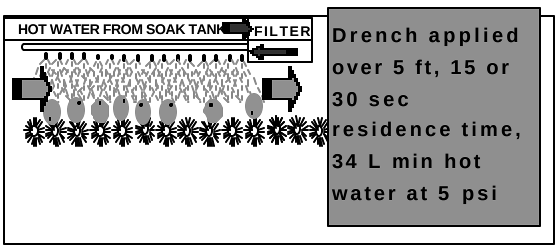
Spores of green and blue mold died similarly; those of sour rot died at cooler temperatures

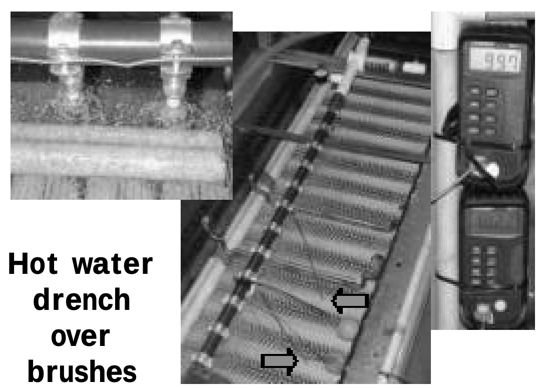
Table 2. Water temperatures that killed 99% (LD_{99}) of the spores of *P. digitatum*, *P. italicum* or *G citri-aurantii* in 30s.

Fungus	Water temp °F (°C)
P. digitatum	139.6 (59.8)
P. italicum	140.2 (60.1)
G. citriaurantii	133.3 (56.3)



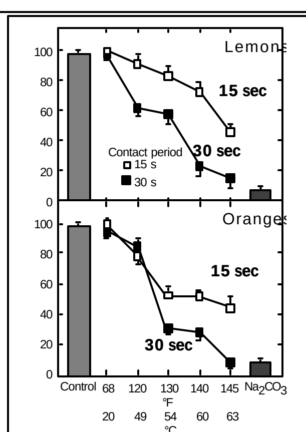
Hot-water brush device at Lindcove



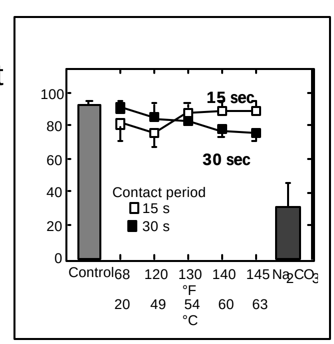


Hot water drench over brushes

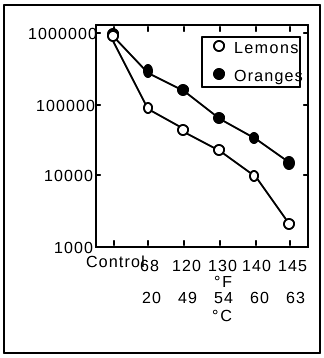
Control of green mold with water at 145°F for 30 sec in a drench was equal in effectiveness to immersion in 3% sodium carbonate at 95°F



Sour was not controlled by hot water, while immersion in 3% sodium carbonate at 95°F reduced it significantly



Naturally occurring yeasts and molds were greatly reduced by hot water treatment



Hot-water drench over brushes

Results similar to Israeli work. Green mold reduced about 85% on lemons and oranges by 30 sec at 145°F and equal to sodium carbonate.

Sour rot was not controlled by hot water, but it was reduced about 75% by sodium carbonate.

We saw no injury to any fruit in these tests. HWB more feasible in summer/fall when the fruit are less susceptible to heat injury.

Because natural microbes are greatly reduced, it could be an element in ISO or HACCP programs.

Why was sour rot not controlled?

This pathogen died at much lower temperatures than the penicillia, which <u>were</u> controlled by hot water.

***Geotrichum* may be more resistant to fruit resistance elements (lignification, citral, scoparone, PR-proteins, etc.) that were induced by heat than the *penicillia*.**

Furthermore, *Geotrichum* can grow at higher temperatures than the penicillia, so the hot water may not have caused a lag in its growth, shown to be important in control of penicillia by hot water.

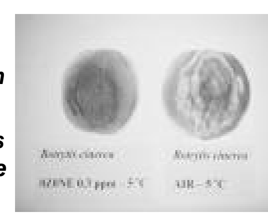
Ozonated citrus storage rooms

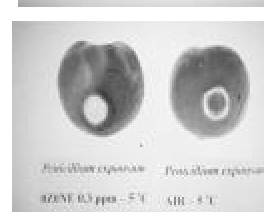
Why do it?

To reduce sporulation from diseased fruit in storage

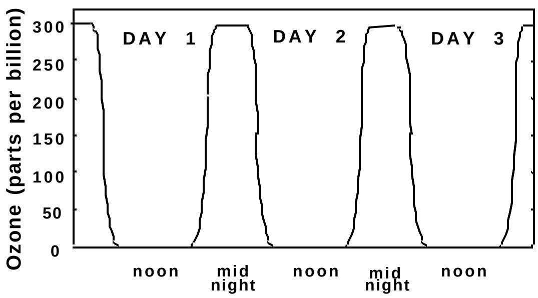
Sporulation of many fungi stopped by O3 at 0.3 ppm

Spore production resumes when removed from ozone

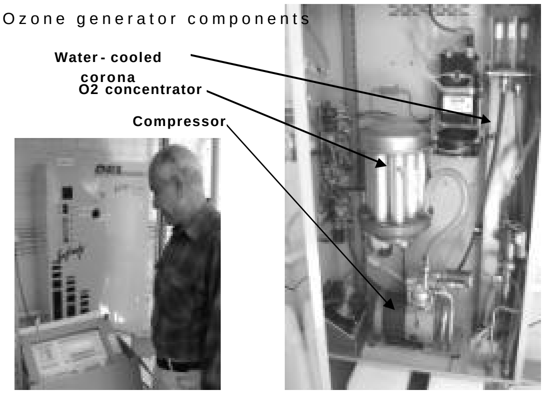




Ozone was present at 300 ppb at night only in a commercial citrus storage room

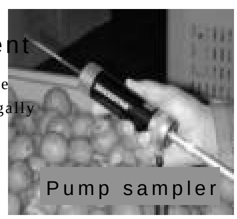


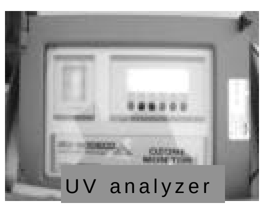
Ozone generator components



Ozone gas measurement

(Workers were not present when ozone was in the room; although they can legally enter 300 ppb ozone for 15 min)

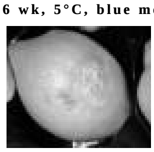


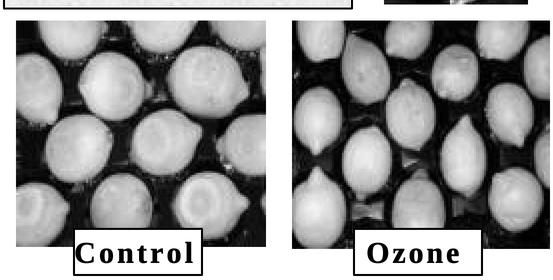


- The test was repeated three times, each time for a 6 to 8 week period.
- No lemons or oranges were injured due to ozone treatment.
- There were no differences in surface color (CIELab values recorded with a Minolta Colormeter) among lemons, nor significant differences in soluble solids or titrable acidity among Valencia oranges.

300 ppb O_3 nightly did not stop infections, but inhibited the production of spores

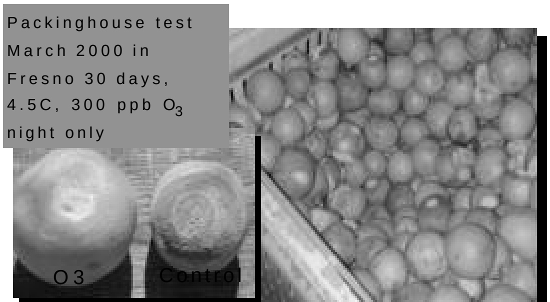
6 wk, 5°C, blue mold

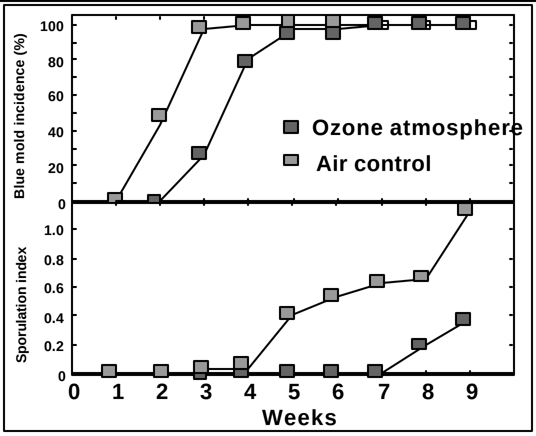




Ozone at low concentrations greatly reduces the sporulation of green and blue mold

Packinghouse test March 2000 in Fresno 30 days, 4.5C, 300 ppb O_3 night only





Control of sporulation is important because: 1) The production of spores that then contaminate healthy fruit is reduced. 2) Most spores that cause decay during storage are fungicide resistant isolates; retarding sporulation of these strains reduces their proliferation.

In other tests, we found ozone penetration into commercial cartons and bags was very poor; adequate penetration occurred in RPCs, lemon storage boxes, and field bins.

To kill spores rapidly with ozone, doses of 200 ppm in one hour or more are needed.