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


New Postharvest Fungicides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Diseases</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH066</td>
<td>anilino-pyrimidine</td>
<td>GM BM</td>
<td>none</td>
</tr>
<tr>
<td>Fludioxonil</td>
<td>phenylpyrrole</td>
<td>GM BM</td>
<td>Switch</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>methoxyacrylate</td>
<td>GM BM</td>
<td>Abound</td>
</tr>
</tbody>
</table>

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)

Green mold  | Blue mold  | Sour rot
Penicillium digitatum | P. italicum | Geotrichum candidum
Maximizing Thiabendazole Effectiveness

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

TBZ effectiveness in water and wax

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

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

<table>
<thead>
<tr>
<th>TBZ (mg/L)</th>
<th>75°F (24°C)</th>
<th>105°F (41°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>250</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td>500</td>
<td>2.2</td>
<td>3.8</td>
</tr>
<tr>
<td>1000</td>
<td>3.6</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>80°F (27°C)</th>
<th>105°F (41°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Not rinsed</td>
<td>0.43 ±0.03</td>
<td>0.49 ±0.11</td>
</tr>
<tr>
<td>2. Rinsed &amp; waxed</td>
<td>0.07 ±0.03</td>
<td>0.15 ±0.01</td>
</tr>
<tr>
<td>Oranges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Not rinsed</td>
<td>0.44 ±0.08</td>
<td>0.51 ±0.07</td>
</tr>
<tr>
<td>2. Rinsed &amp; waxed</td>
<td>0.07 ±0.01</td>
<td>0.09 ±0.03</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oranges (not rinsed)</th>
<th>Lemons (rinsed/waxed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool control</td>
<td>83.9 a 95.6 ab</td>
<td>89.4 b 99.1 a</td>
</tr>
<tr>
<td>SBC 3%</td>
<td>12.2 b 46.1 c</td>
<td>8.0 b 20.4 cd</td>
</tr>
<tr>
<td>TBZ 200 ppm</td>
<td>0.0 c</td>
<td>0.0 c 85.6 b</td>
</tr>
<tr>
<td>SBC-TBZ</td>
<td>0.0 c 36.7 c</td>
<td>0.0 c 15.1 d</td>
</tr>
</tbody>
</table>

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- Cl₂ tanks.
- Sodium bicarbonate (NaHCO₃ “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 tanks

Spores died almost immediately in chlorinated NaHCO₃

Large scale TBZ tests at University of California Lindcove Packline
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

<table>
<thead>
<tr>
<th>Measure</th>
<th>Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied in water vs wax</td>
<td>+++</td>
</tr>
<tr>
<td>Immersed vs sprayed</td>
<td>++</td>
</tr>
<tr>
<td>Heat</td>
<td>+</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>+++</td>
</tr>
<tr>
<td>Chlorine</td>
<td>+</td>
</tr>
</tbody>
</table>

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 it's used
- Scale on equipment from calcium carbonate
- Some risk of fruit injury, more if not rinsed
The unusual conditions of the 2001 navel season

- Many navel oranges split 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

### Graphs and Data

#### Graph 1
- Title: Commercial drencher with TBZ, chlorine, and sodium bicarbonate
- Description: Data from Sunkist Growers
- X-axis: Fruit source groves
- Y-axis: Decayed oranges discarded (%)
- Data points:
  - Control: 0, 1, 2, 3, 4, 5
  - Drenched: 1, 2, 3, 4, 5
- Details:
  - 350 ppm TBZ
  - 3% SBC
  - 200 ppm Cl₂

#### Graph 2
- Title: Commercial drencher with TBZ, chlorine, and sodium bicarbonate
- Description: Data from Sunkist Growers
- X-axis: Fruit source groves
- Y-axis: Packable oranges (%)
- Data points:
  - Control: 1, 2, 3, 4, 5
  - Drenched: 1, 2, 3, 4, 5
- Details:
  - 350 ppm TBZ
  - 3% SBC
  - 200 ppm Cl₂
Commercial Tests
TBZ/SBC/Cl₂ 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 Burger 1925 Winston 1935 Fawcett 1996
• Hot water (53°C 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. Smart and Melvin 1963, 1965 Palou 2001
• California tank recommendations: 118-120°F for 2 to 4 minutes, in Florida: 128°F for 5 min. Official ASHRAE citrus guidelines 1966 Eckert 1967

Prior literature

• Very hot-water immersion (80°C for 1 min or 70°C 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 (56°C) reduces chilling injury and decay, and induces resistance to subsequent infections. Porat et al 2000 Rodov et al 2000 Pavanello et al 2001

Risk of fruit injury

“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 105°F 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.

"Hot-water brush"

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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Table 2. Water temperatures that killed 99% (LD99) of the spores of P. digitatum, P. italicum or G. citri-aurantii in 30s.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Water temp °F (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. digitatum</td>
<td>139.6 (59.8)</td>
</tr>
<tr>
<td>P. italicum</td>
<td>140.2 (60.1)</td>
</tr>
<tr>
<td>G. citri-aurantii</td>
<td>133.3 (56.3)</td>
</tr>
</tbody>
</table>

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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.

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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.

![Graph showing reduction in yeasts and molds with hot water treatment.]

- **Lemons**
- **Oranges**

**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 were 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.

**Why do it?**

To reduce sporulation from diseased fruit in storage.
Ozone was present at 300 ppb at night only in a commercial citrus storage room.

- 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 Colorimeter) among lemons, nor significant differences in soluble solids or titrable acidity among Valencia oranges.

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 Colorimeter) among lemons, nor significant differences in soluble solids or titrable acidity among Valencia oranges.

300 ppb O₃ nightly did not stop infections, but inhibited the production of spores.

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

Packinghouse test
March 2000 in Fresno 30 days, 4.5°C, 300 ppb O₃ 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.