New Methods to Control Postharvest Decay of Citrus

Joseph L. Smilanick
USDA-ARS, Parlier

The purpose of this project is to develop new methods to control postharvest decay of citrus for use in California packinghouses. Practical experiments with the newly approved postharvest fungicide pyrimethanil (PYR) were completed in 2005. PYR has recently been approved for use in California to control green mold, caused by *Penicillium digitatum*, though acceptance of its residues in some overseas markets is still in progress.

The EC_{50} (the concentration of PYR that inhibits germination of 50% of a population of *P. digitatum* spores) was 0.2 to 0.4 μg/ml and was similar from pH 4 to 7. Green mold on citrus fruit was reduced more than 90% by PYR at 500 μg/ml or higher applied by immersing for 30 seconds or drenching the fruit, while its application in wax over rotating brushes at 1,000 or 2,000 μg/ml reduced green mold about 65%. Control of sporulation by PYR in aqueous solutions was better than the same concentration applied in wax, but it was inferior to imazalil (IMZ). IMZ and thiabendazole (TBZ)-resistant *P. digitatum* isolates were controlled by PYR.

The addition of sodium bicarbonate improved PYR performance, as we reported previously for TBZ (Plant Disease 90:89-96) and IMZ (Plant Disease 89:640-648). PYR was not compatible with chlorine. An increase in the temperature of the PYR solution slightly but significantly improved its effectiveness to control green mold, although its residues on fruit were greatly increased by heat; they approximately doubled for each 10°F increase in solution temperature above 86°F. PYR was very effective when applied up to 24 hours after inoculation but much less effective when it was applied before inoculation. PYR effectively controls green mold and is a useful tool to control isolates of *P. digitatum* resistant to other fungicides.

We completed the evaluation of two approaches to minimize postharvest green mold during degreening: (1) fungicide applications to trees before harvest; and (2) drenching fruit after harvest just before degreening (Plant Disease 90:89-96). Preharvest applications of thiophanate methyl (TM) controlled postharvest green mold consistently. In five tests, green mold among "degreened" oranges was 16% when TM was applied one week before harvest, while among fruit not treated the incidence was 89.5%. Thiabendazole (TBZ) applied to harvested fruit in bins before degreening was also very effective. TBZ effectiveness was enhanced by mild heating (106°F), adding sodium bicarbonate, and immersing fruit, rather than drenching them, with the solution. With these measures, an isolate of *P. digitatum* with a high level of TBZ resistance was controlled.

In semi-commercial tests with naturally inoculated fruit, TBZ and sodium bicarbonate treatment reduced green mold incidence from 11% among untreated oranges to 2%. TBZ residues in lemons at 106°F were about twice those treated at 75°F. Neither TM before harvest nor TBZ and sodium bicarbonate applied after harvest influenced green color removal during degreening of oranges. Sodium bicarbonate very slightly reduced the rate of lemon color change. Because TM and TBZ share the same mode of action, efforts to monitor the occurrence of resistant isolates is very important, and TM should only be used in disease-conducive years.

Treatment	Artificially inoculated fruit (%) [y] 2004	2005	Natural infections (%; 2005 only) [x]
Control	98.9 a[z]	84.5 a	3.2 a
Azoxystrobin	79.0 b	29.9 b	2.1 ab
Topsin M WSB	19.2 c	12.9 c	0.6 b
P	*0.0001*	*0.0001*	*0.0905*

[y] Percentage of fruit infected with green mold. Each value is the mean of four replicates of 100 fruit each. They were inoculated by immersing a metal probe with a 1 mm by 2 mm tip into a solution containing one million spores of *P. digitatum* and making a single wound in each fruit immediately after harvest and before degreening.
[x] Percentage of fruit infected with green mold. Each value is the mean of 12 replicates of 100 fruit each.
[z] Values followed by unlike letters differ significantly.

In the fall of 2005, we repeated in Field 12 at Lindcove a portion of an earlier experiment where thiophanate methyl (Topsin M WSB) and azoxystrobin (Abound) were applied before harvest to control postharvest green mold during degreening. Results in both years with Topsin M were similar to each other and those of prior years (Table 1). Results with naturally infected fruit followed a similar pattern, but infected fruit were few and statistical confidence (*P*) in these results is lower than among those with inoculated fruit.

Abound was more effective in the 2005 tests than those in 2004. A reason a difference in Abound effectiveness occurred may be related to rainfall. In 2004, rain fell immediately after the fungicide applications, while in 2005 no rain fell between the application of the fungicides and harvest. It is likely that Topsin M activity is more resistant to rainfall than that of Abound. Abound, although inferior in effectiveness to Topsin M, may merit use in drier years and if there are concerns about fungicide resistance in packinghouses.

Thiabendazole (TBZ) and imazalil (IMZ) have been in widespread commercial for many years in packinghouses to control citrus green mold, caused by *Penicillium digitatum.* Resistance to both has developed in populations of the pathogen. We collected isolates from infected lemons and oranges from many diverse locations in California, with the goal to determine the mechanisms of resistance to both fungicides.

We have reached some conclusions about the mechanism of TBZ resistance (Plant Disease 90: accepted Oct 6, 2005), while work with IMZ is in progress. TBZ binds to beta-tubulin, which inhibits its assembly into microtubules needed for pathogen growth. Among 35 isolates from groves all were sensitive to TBZ, while among 74 isolates collected within ten packinghouses 20 were resistant to TBZ. RAPD analysis was applied so that isolates selected for further characterization differed from each other.

All twenty TBZ-resistant isolates characterized displayed a point mutation in the beta-tubulin gene sequence relating to amino acid codon position 200. Thymine was replaced by adenine (TTC → TAC), which changed the phenylalanine (F) to tyrosine (Y). Presumably, this mutation retards attachment of TBZ to tubulin. In contrast, all forty-nine TBZ-sensitive isolates that were sequenced and had no mutations at this or any other codon positions and their amino acid sequences were identical. All of the isolates of *P. digitatum* resistant to TBZ collected from a geographically diverse sample of California packinghouses employed the same mechanism to acquire resistance to this fungicide. The diversity of RAPD fingerprints suggests many isolates may have independently developed TBZ resistance. Our results provide a basis to develop practical means to detect and manage fungicide resistance in packinghouses.

Figure 1. Green mold has developed among these stored navel oranges treated with thiabendazole and imazalil before storage, indicating the pathogen, Penicillium digitatum*, has developed resistance to these fungicides. Contact Citrus Research Board for table.*