

RESISTANCE TO POSTHARVEST FUNGICIDES IN FLORIDA'S CITRUS PACKINGHOUSES

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Abstract. Developed resistance to postharvest fungicides is a serious problem in many citrus packing areas. Florida has apparently avoided this problem for many years. During the past 25 years, assays for resistant molds have been conducted in Florida citrus packinghouses. This report is a summary of the results of these assays. While rarely encountered, those cases where resistance was encountered can help understand how the problem may be avoided.

The heavy reliance on two citrus postharvest fungicides commonly used in Florida, thiabendazole and imazalil, will allow for the development of resistance. In some citrus producing areas, such as California, Australia and New Zealand, this has become a serious problem resulting in much expense to the packer. By the late 1980s the problem of resistance was being described as a "crisis" in California (Anonymous, 1989; Wild, 1994).

In California, assays conducted by the method reported herein have often found over 300 spores per minute with up to 100% resistance to either thiabendazole or imazalil, and sometimes both. One difference between our method and the general California method is that they usually use 0.25 ppm imazalil rather than our 0.1 (Sorenson, 2006).

One method of fungicide use is to use alternate fungicides, using one until resistance develops to it, then withdrawing that fungicide and substituting another having a different mode of action (Goodwine, 2006). To aid in the decision which fungicide to use, a classification system has been developed which identifies the fungicides' modes of action (Adaskaveg, 2006; Fishel, 2006). Thiabendazole, a benzimidazole, is in Class #1. Other Class #1 fungicides are Benelate and Topsin-M. Imazalil is categorized as Class #3. There is no significance to these numbers other than the order in which they have been identified and assigned. New fungicides of other classes have been introduced, and others are under development in an effort to provide alternative treatments to control resistant strains (Table 1).

During the period from 1980 through 2005, assays were conducted on an average of 7 times a year in various Florida packinghouses. These assays were conducted either at the request of the packinghouse management or their postharvest fungicide supplier. In total 27 packinghouses were involved.

Materials and Methods

Petri dishes containing Potato Dextrose Agar were exposed in sets of two or three. In all tests, the agar was amended with 50 ppm Novobiocin to suppress bacteria growth. Up

Table 1. New fungicides for postharvest application on citrus.

Fungicide	Common/trade name	Group
Sodium o-phenylphenate	SOPP/Dowicide	— ^z
Thiabendazole	TBZ/Mertect	1
Imazalil	Fungafflor	3
Pyrimethanil ^y	Penbotec/Filabuster	9
Azoxystrobin	Abound	11
Fludioxonil ^x	Graduate	12

^zNot in FRCS list. Considered Group 0 by some.

^yNot currently available for citrus except in combination with imazalil (July 2006)

^xNot currently registered for postharvest application (July 2006).

until the introduction of imazalil, plates were exposed in pairs, one with no fungicide, which was used for the base count, and a second plate amended with thiabendazole at 20 ppm for estimation of resistance. After the introduction of imazalil, a third plate was added amended with 0.1 ppm imazalil. At that time, the thiabendazole plate was reduced to 10 ppm to conform to California practice.

The prepared plates were exposed at various locations in and around the packinghouse as deemed appropriate at the time. The usual locations for exposing the plates were:

1. Any active degreening Room
2. At the Pack Dump
3. At The Dryer Exit (In the Air Stream)—Closed bottom Dryers.
4. The Pack Area.
5. Cooler
6. Other locations included Pregrade, Pack grade, Brush polisher, near cull belts.

Most plates were exposed for 60 s, the exception being cold rooms where the exposure was 120 s. The plates were then incubated for 96 to 120 h at 70 to 75°F until the presence or absence of identifiable colonies had manifested (Fig. 1).

The number of *Penicillium* colonies on the plain agar plate was taken as the base count and reported in spores per minute. Resistance was estimated by comparing the number of colonies growing on the plain agar with the number of colonies growing on the fungicide-amended agar. This was reported as a percentage of the total count.

Results and Discussion

In the 176 assays conducted over this period, no Imazalil resistant Green Mold spores were found in a Florida packinghouse's atmosphere. However, the potential for resistance to Imazalil does exist as spores collected from decaying fruit found in cartons and rot bins have tested positive for imazalil resistance when imazalil was part of the prepack treatment. Resistance to thiabendazole was found, but with the exception noted below, either the percent resistance or the total

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Fig. 1. Agar plate containing several *Penicillium* colonies.

count was very low. This fact can be encouraging to the Florida citrus packer as Green Mold is a wound pathogen and must find the injury before it can start to decay the fruit. Low counts and or low resistance levels mean that there are very long odds against continuing the resistance cycle.

The results of 174 assays are summarized in Table 2. There are two exceptions to these results, but they have been excluded from the table so as to not skew the picture.

The first exception was in a packinghouse where plates exposed at the waxer and pack tables showed over 300 spores per minute with an estimated 100% resistance. It was noted that at the time of the test, cartons of grapefruit with a high amount decayed fruit were being repacked. The decayed fruit had a heavy load of loose spores and were being tossed about 4 feet into a bin less than 20 feet from the area where the plates were exposed.

The second exception was an unusually high count found near a washer in a small packinghouse. Later investigation revealed that broken fruit were being collected in buckets, which were accumulated near an open doorway for occasional disposal. The draft from the doorway was carrying spores into the packinghouse. In this case the packinghouse was not using TBZ or Imazalil and no resistance was found.

In California, resistance to the Benzimidazoles (TBZ, Benomyl, Thiophanate-Methyl) was found in packinghouses between 15 months and two years after their commercial introduction (Houck, 1977; Serotzki and Gisi, 2004). Commercial levels of resistance to imazalil was found in California packinghouses 7 y after its introduction (Tedford, 2005).

Table 2. Results 1980-2005 (174 Assays).

Location	Average spores per minute	Maximum found	Maximum TBZ resistant spores	Average % resistant (when found)
Degreening room	<1	12	8	25%
Dump	10-12	150	34	12%
Dryer Exit	<1	3	1	100%
Pack Area	<1	5	1	100%
Cooler	<1	10	2	50%
Miscellaneous	<1	23	17	100%

The lack of significant fungicide resistance in Florida packinghouses can be attributed to many factors, the major ones are:

1. Degreening conditions used in Florida tend to suppress the growth of Green Mold.
2. Florida packers seldom practice long term prepack storage as is common in California lemon houses.
3. Most Florida packinghouses are empty of fruit during the hot, humid months of June, July and August.

These factors and the lack of resistance problems similar to those that plague other growing areas, does not mean that it cannot become a problem in the future. New fungicides of different classes (Table 1) have been introduced with the specific goal of combating resistance to existing fungicides. However each of these has the potential for developing strains resistant to themselves and even multiple fungicide resistance if not used with good resistance control practices (Adaskaveg et al., 2005).

To prevent resistance from becoming a problem in the packinghouse, the packer must make every effort to control those conditions that could lead to its development (Hall and Bice, 1977; Tedford, 2004). These include:

1. Do not handle decaying fruit where spores may contact sound fruit.
2. Thoroughly sanitize storage boxes and bins before use.
3. Do not use fungicides at lower than recommended doses.
4. Regularly assay for resistance.
5. When using fungicide combinations, use them from the start.
6. If using a fungicide of one group in the grove, use one of a different group alone or in combination for drenching before degreening.
7. Store and handle all fruit under appropriate conditions.

While resistance is not currently a problem in Florida, new practices could cause it to become one. Long term storage of fresh fruit for summer sale or fresh juice use and the introduction of earlier and later maturing varieties could contribute to the development of problematic resistance for Florida's citrus packers.

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