

QUALITY CONTROL ASSESSMENT METHODOLOGY
RELATED TO CITRUS DECAY CONTROL

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Control of decay is the major requirement for good citrus keeping quality. Fungi that flourish in the high humidity and rainfall environment of Florida can cause extensive decay of citrus fruit during transit, storage and distribution. The proper use and effective application of disinfectants and fungicides are of utmost importance for reducing populations of decay fungi in packing and storage environments and preventing and eradicating infections of the fruit. Knowledge of the existence of any fungicide resistant strains of Penicillium digitatum (green mold) within packing and storage facilities is also necessary to prevent proliferation of decay caused by this fungus.

The purpose of this presentation is to discuss methodology utilized in decay control practices, including those used to assay and combat fungicide resistance.

Decay Control Chemicals

1. Disinfectants - These are compounds used to kill fungal propagules present in packing facilities on floors, walls, and equipment. These materials are added to water in "wet dumps" used to handle fruit, and, in some cases, in water sprays applied to the fruit. Disinfectants may kill fungal propagules on surfaces, but they do not eradicate fruit infections like most fungicides do.

a. Chlorine - This material has been used extensively by the industry as a surface disinfectant in storage rooms, on equipment, and on fruit. In recent years, fruit applications of chlorine have been used to kill canker bacteria possibly present on the fruit surface. Chlorine has the advantage of being inexpensive, but it is rapidly inactivated by organic matter, is pH dependent and corrosive to steel, and it possesses a disagreeable odor.

b. Quaternary Ammonium - Quats, as they are commonly known, are used widely on floors, walls and equipment. These materials cannot be applied to the fruit. Quats are by nature wetting agents, and thus have built-in detergency properties. They are effective penetrants of porous surfaces, non corrosive, stable in the presence of organic matter, but more expensive than chlorine.

2. Fungicides - These are chemicals that can be applied directly to the fruit to prevent and eradicate fungal infections. Some of the materials are slightly systemic and move a slight distance into the rind after application, thus protecting fruit from infections that occur through post-treatment injuries. Fungicides are commonly applied in water or in water wax formulations.

a. Benomyl (Benlate) - This fungicide controls stem-end rots (SER) caused by Diplodia and Phomopsis, anthracnose, and green and blue mold. Because of regulatory changes,

benomyl can no longer be applied postharvest, but it can still be applied preharvest to control postharvest decay.

b. Thiabendazole (TBZ) - Chemistry and fungicidal activity of this fungicide is similar to that of benomyl, except it is not quite as effective on anthracnose. Thiabendazole is applied only postharvest.

c. Sodium O-phenylphenate (SOPP) - This material is best suited for application during washing to clean the fruit. Fruit is rinsed after treatment and most of the fungicide is removed except that which accumulates in rind injuries. Use of this material during washing helps keep the brushes sanitary to reduce contamination of healthy fruit. SOPP is moderately effective against sour rot, but less effective than benomyl or TBZ against green and blue molds and SER.

d. Imazalil - Imazalil is as effective as benomyl or TBZ against green and blue molds, and will control strains resistant to those fungicides. Imazalil inhibits sporulation of green mold and hence prevents soilage. Imazalil is less effective than TBZ or benomyl for the control of SER, particularly in degreened fruit.

e. Biphenyl (diphenyl) - Biphenyl is a volatile vapor-phase fungistat that is effective only when sufficient amounts of it are present in the atmosphere surrounding the fruit. Biphenyl is impregnated into paper pads or sheets and it volatilizes from the pads that are inserted into the carton during packing. Sporulation of Penicillium is reduced by

biphenyl; therefore it is an effective treatment for soilage control. The manufacture of biphenyl has been discontinued, but the material can be used until the present supply is utilized, probably within the next two seasons.

Methodology Affecting Efficacy of Decay Control Treatments

1. Formulations - Decay control chemical formulations should be fresh and pure for maximum activity. All of the fungicides are quite stable in their formulated state, but they should never be stored under extremely high temperatures. The materials should be utilized, if not during the season of purchase, at least during the following season. Liquid chlorine is formulated at a strength near 12%, but will deteriorate with time to a concentration near 9%. Diphenyl pads should be stored at cool temperatures and remain packed in air tight cartons until the day of use.

Wettable powder fungicides such as TBZ should be prepared by stirring a small quantity of water with the material to form a smooth paste. Add additional water, mix, and transfer the mixture to a holding tank where it should be continuously agitated mechanically or with aeration to prevent the fungicide from settling out. Agitation is especially important in drenchers where some of the fungicide may adhere to soil particles that settle rapidly. Emulsifiable concentrate formulations are oily liquids that are not easily removed from containers. Warm water, in excess of 100°F, can be used to more thoroughly rinse these containers.

2. Treatment Conditions

a. Concentration and pH - The concentration, pH and purity of the fungicide should be maintained at the correct levels. Since fungicides applied in non-recovery applications cannot be contaminated before use, this system is the most popular method in the industry of applying postharvest fungicides. With SOPP, a non-recovery foam application during washing has become more popular than a recirculating drench or spray treatment. Because of contamination and lack of easy pH control, most packinghouses have abandoned the recirculating system.

SOPP hydrolyzes in water to the undissociated phenol (o-phenylphenol) and the dissociated anion (o-phenylphenate). The undissociated o-phenylphenol is the active fungicide in the formulation, but it is phytotoxic above certain concentrations. These concentrations lie in the range of 200-400 ppm, depending upon the treating temperature and period of contact. Higher than ambient temperatures and longer than recommended exposure periods increase phytotoxicity. The amount of o-phenylphenol in the SOPP solution is dependent upon pH. Phytotoxic levels of o-phenylphenol can even exist in 0.5% solutions of SOPP if the pH is allowed to drop (Table 1). For this reason, remember that if formulations are diluted to less than 2%, additional buffer must be added to the solution to maintain the pH above 11.5. The principal causes of fruit damage in commercial practice are a drop in

Table 1. Relationship between pH and concentration of O-phenylphenol in solution in sodium O-phenylphenate formulations at 0.5, 1.0, and 2.0% (Eckert, Kolbezin and Kramer 1969)

O-phenylphenol in solution (ppm)			
SOPP (%) ^z			
pH	0.5	1.0	2.0
12.0	45	70	140
11.8	55	110	205
11.6	85	170	320
11.4	140	260	515
11.2	200	390	800
11.0	300	620	- Potential
10.8	450	- ^y	- Injury
10.6	650	-	-

^z SOPP = sodium O-phenylphenate.

^y Not determined.

pH of the treatment solution caused by dilution of the solution with additional water, accumulation of acidic substances such as rotten fruit in recirculating drenches, extended periods of fruit exposure before rinsing, and the use of harsh, usually new, washer brushes with SOPP on very early, tender skinned, degreened fruit.

Any water in the packinghouse that is continuously applied to fruit, such as that in drenchers and soak or dump tanks, should be treated with chlorine to sanitize it. If not, the water becomes contaminated with decay organisms, such as those causing brown and sour rot, and green and blue mold. Since chlorine is rapidly inactivated by organic matter, it must be continually metered into the tank during use to maintain a level of 25-50 ppm of free available chlorine. Activity of the chlorine is dependent upon the presence of hypochlorous acid (HOCl), which is controlled by pH (Figure 1). Hypochlorous acid is more biocidal than the hypochlorite ion ($-\text{OCl}$), and it predominates in solutions at pH 7.5 or less. However, at pH 6.5 and less, chlorine is more volatile and the higher levels in the air are irritating to workers. At low pH, it is also more corrosive to the equipment. Levels of 25-50 ppm chlorine can be monitored with a chlorine kit that measures free available chlorine over a range of 10-200 ppm, and pH can be measured with a pocket pH meter. The meter should be adjusted with calibration solution before use.

Chlorine content will fluctuate in systems where the chlorine is metered in at a constant rate. Under very high fruit treatment rates, chlorine levels will be near 0. At low usage, chlorine may be in excess of 200 ppm. At low chlorine levels, effectiveness will be reduced, and at high levels, some chlorine may be wasted and it will be more volatile and corrosive. Commercial systems are available where the

chlorine is metered accurately upon demand by measuring the oxidizing power of the solution, but such systems are more expensive and have to be evaluated for reliability.

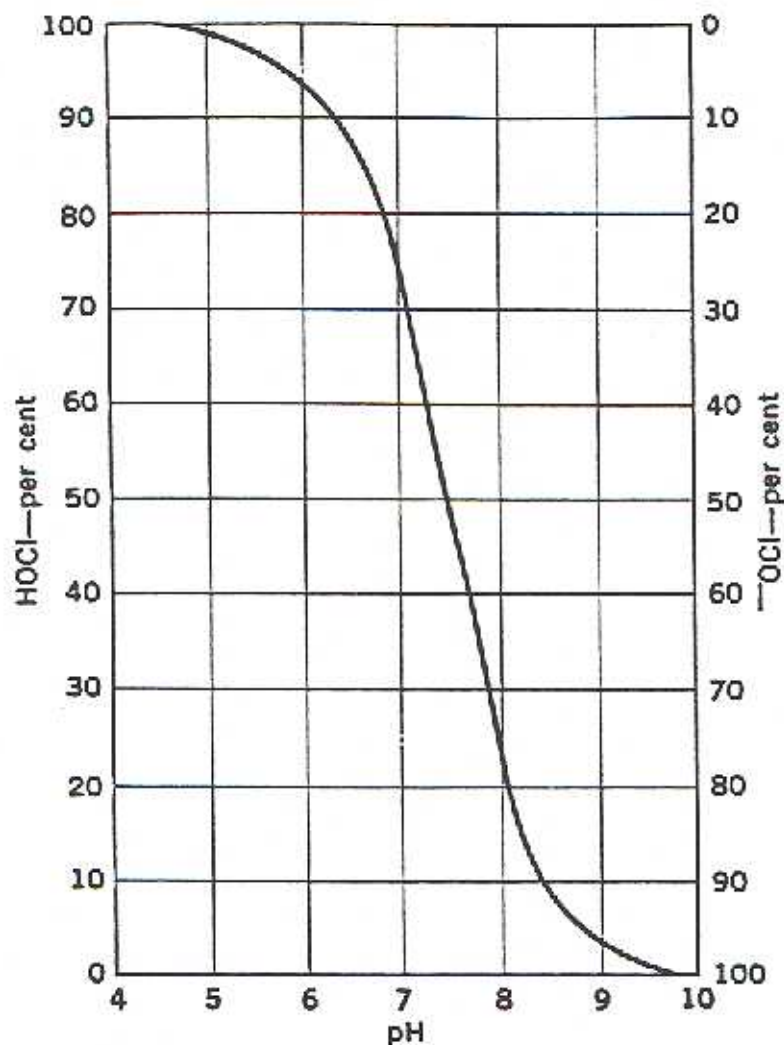


Figure 1. Relationships among hypochlorous acid (HOCl), hypochlorite ion (OCl⁻) and pH (Baker 1959).

Pallet bin drenching is usually the only treatment in Florida packinghouses where the fungicide suspension is recycled and reused. All other applications are usually non-recovery. Thiabendazole is the most commonly used material in drenchers now that benomyl cannot be used for postharvest application. Concentrations of TBZ should be maintained near 1000 ppm for optimum effectiveness. To retain this level, fresh TBZ added back to the tank should be at a concentration of near 1500 ppm. This concentration will vary to some extent among drenchers depending upon how well the TBZ is kept in suspension. The concentration of TBZ in the drencher should be checked weekly. Two relatively simple procedures are available to check TBZ concentrations, but one of these requires a UV spectrophotometer. In the other, an amine-copper reagent in chloroform is reacted with sodium hydroxide and TBZ. After the water-solvent emulsion separates, the solvent portion is filtered and the clear green chloroform-TBZ solution is compared with a set of previously prepared permanent colored standards.

b. Degreening - Degreening favors development of SER and anthracnose, caused by Diplodia and Colletotrichum, respectively. Recommended methods of degreening utilizing ethylene at about 5 ppm and relative humidities of 90-96% should be followed to minimize the effect degreening has on decay. Ethylene concentrations that exceed the recommended range will not enhance the degreening rate, and they will

significantly increase disease. Anthracnose on inoculated Robinson tangerines was increased from 16.7 to 85.9% by degreening with 50 rather than 10 ppm ethylene (Table 2). The incidence of *Diplodia* SER in Valencia oranges was increased significantly by degreening with 50 ppm ethylene, even when fruit were treated with TBZ for decay control (Figure 2). However, if the proper high relative humidity levels are maintained during degreening, the incidence of green mold can be reduced by the degreening treatment. High moisture levels promote healing of injuries in the flavedo, thereby protecting the rind from infection (Table 3). High temperatures also play a role by retarding the growth of Penicillium and

Table 2. Influence of ethylene concentration on the incidence of anthracnose in Robinson tangerines inoculated with Colletotrichum gloeosporioides (Brown and Barmore 1977)

Ethylene concentrations ² (ppm)	Fruit with anthracnose (%)
0	0.0
10	16.7
20	56.7
30	65.5
40	86.7
50	85.9

² Fruit were exposed to ethylene for 2 days, and stored for 1 week at 78°F at near 100% relative humidity.

allowing the healing process to proceed before the fungus has an opportunity to invade the injury. At low relative humidity, injured tissue desiccates and dies without forming a barrier to the fungus.

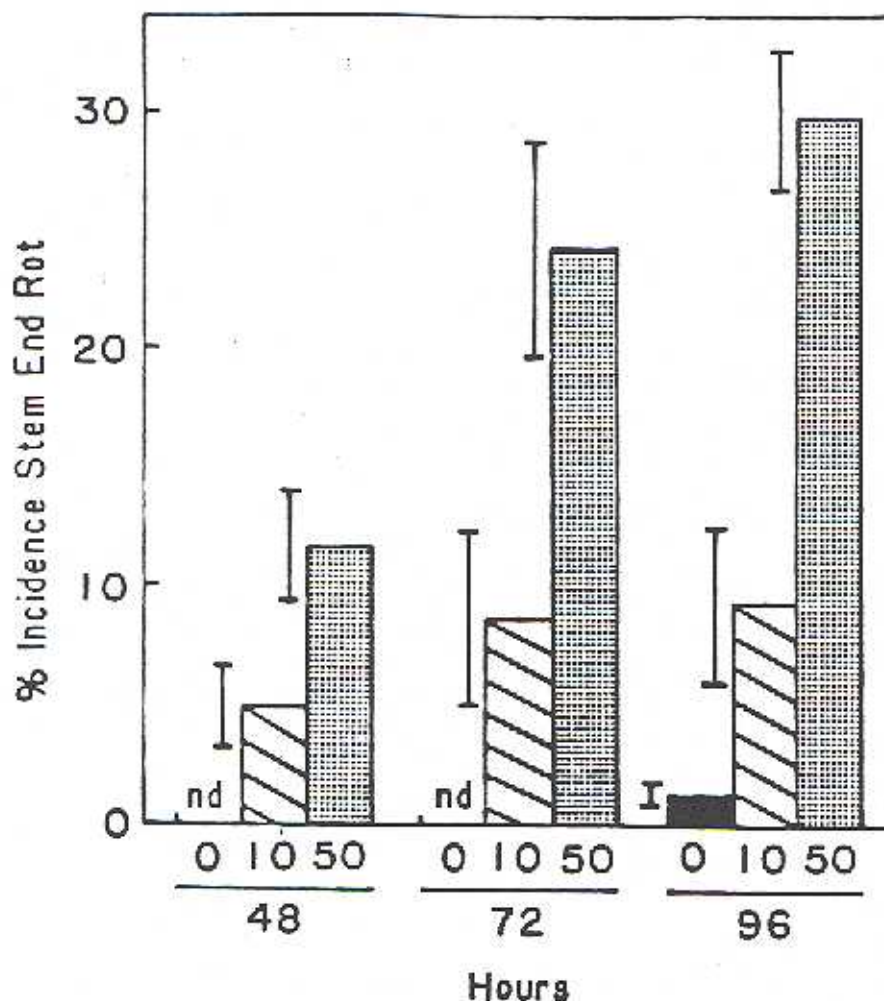


Figure 2. Incidence of stem-end rot caused by *Dipolida natalensis* in Valencia oranges treated with thiabendazole (1000 ppm) after exposure to ethylene (0, 10, or 50 ppm) for 48, 72 or 96 hours and stored for 4 weeks at 85°F and 94-96% relative humidity. Bars represent standard errors of the means of 3 replications (Brown 1986).

c. Application - Time of application of decay control treatments is very critical. Delays in treatment allow fungal infections at injuries and at the stem-end to penetrate beyond the reach of fungicides that may be applied later. The relationship between infection and the time of treatment with SOPP is shown in Figure 3 where efficacy of the treatment dropped drastically after 24 hours. Efficacy of the treatment was less a few hours after inoculation because ungerminated spores are less sensitive to the fungicide than germinated ones. Delays between harvest and treatment should not exceed 24 hours unless the ambient temperature is so cold (40-50°F) that growth of the fungi is retarded. When fruit is degreened, treatments following degreening become progressively less effective the later they are applied after degreening (Figure 2). Efficacy of all postharvest fungicides against SER is best with treatments applied before rather than after degreening (Table 4). Thus, a good fungicide application before degreening, such as a preharvest spray or postharvest drench, is much more effective than later applications.

Applications on the packingline are most effective when the fungicides are applied in water. However, some packers prefer to apply the fungicides in the water wax. The concentration in the wax should be double that of the water application to attain comparable efficacy. Even then, movement of imazalil in the rind is hindered when it is applied in resin solution water wax. In either water or wax, the fungicide should not

Table 3. Influence of temperature and relative humidity on development of *Penicillium digitatum* in oranges during degreening and subsequent storage (Brown 1973).

Degreening temperature ^z (°F)	Fruit infected with green mold (%) after degreening at:	
	90-96 RH (%)	55-75 RH (%)
81	89	71
86	63	93
91	21	97

^z Fruit were degreened at each temperature at each range of relative humidity for 3 days and then stored at 77°F and 100% relative humidity for 4 days.

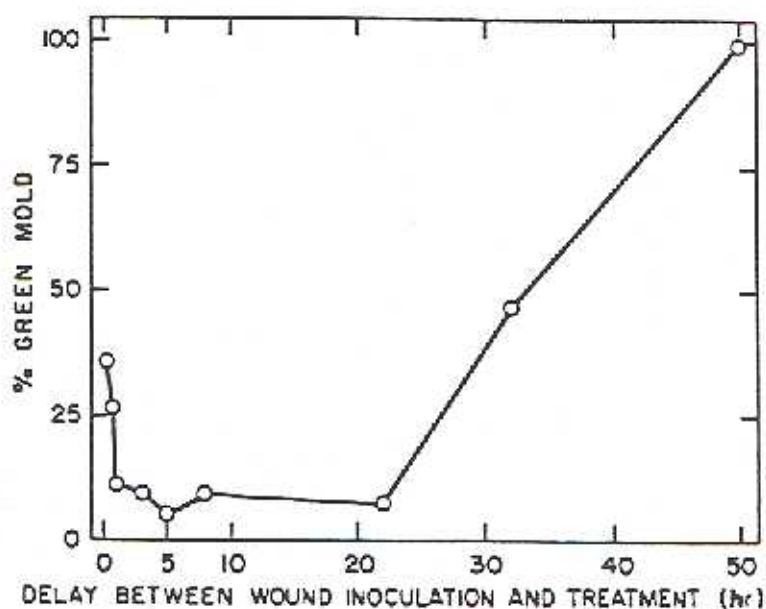


Figure 3. Effect on time between wound inoculation and treatment upon the effectiveness of sodium *o*-phenylphenate (SOPP) in controlling green mold on oranges. The oranges were submerged in a 2.0% SOPP solution pH 12 for 2 minutes and then rinsed with water (Seberry 1969).

Table 4. Efficacy of fungicides applied before or after degreening for control of stem-end rot caused by *Diplodia natalensis* (Brown 1986)

Fungicide	Rate (ppm)	Percentage decay control ²	
		Before	After
Benomyl	600	100	91
Thiabendazole	1000	91	59
		97	52
Imazalil	1000	84	63
		78	44
Guazatine	1000	86	41

² Decay control in experiments where fungicides were applied as drench treatments to unwashed fruit before degreening for 72 hours or as nonrecovery sprays to washed fruit after degreening before waxing.

be applied to excessively wet fruit because the water will dilute the strength of the fungicide and wax. Polisher-drier brushes tend to remove some of the surface residues left on the fruit from aqueous applications. This may interfere with sporulation control activity of imazalil. To circumvent this, an aqueous application of 1000 ppm can be followed with another application of 1000 ppm in the wax. This will provide maximum control of infection by utilizing the systemic action of the aqueous treatment, and the sporulation control provided by the surface residues that remain from the wax application. Thorough coverage of the fruit with fungicide must be achieved

during application. This can only occur if all applicator nozzles or sprays are functioning, and if the fruit has an opportunity to rotate and contact fungicide-saturated brushes evenly on all surfaces. This will not occur if the dump rate exceeds that designed for the equipment, where fruit are so crowded that they may never contact the brushes.

Enough fungicide residue (0.2 - 0.3 ppm) must be deposited on the fruit for decay control. However, the amount of residue is usually not as important as the timing of the fungicide application. Drenches of fungicide before degreening that leave residues of 0.2 ppm are often much more effective as decay control treatments than water wax fungicide applications made after degreening that leave residues of 1.2 ppm. Residues from postdegreening treatments may never reach the invading hyphae of the fungus. Higher residues are important, however, for effective control of sporulation of P. digitatum on infected fruit (Table 5). Residues of imazalil from aqueous applications had to exceed 1 ppm before sporulation on diseased fruit was effectively prevented. Residues from aqueous applications of imazalil provide better sporulation control of Penicillium on infected fruit than comparable residues deposited in water wax.

With diphenyl, some caution has to be observed to prevent residues from exceeding legal tolerances (U.S., 110 ppm; European EEC, 70 ppm). Oranges and tangerines absorb at least twice as much diphenyl per unit of surface area than lemons

Table 5. Relationship between residues of imazalil from aqueous applications and control of sporulation of Penicillium digitatum

Residues (ppm) ^z	Sporulation index ^y
0.00	4.9
0.89	3.7
1.00	2.9
1.17	1.6
1.42	0.6
1.45	0.8
1.93	0.2
2.33	0.2
2.81	0.0

^z Residues of imazalil on a whole fruit basis from aqueous nonrecovery applications for 15 seconds.

^y Sporulation index: 5 = fruit surface covered entirely with green sporulating mycelium; 0 = fruit surface without mycelium and/or with white nonsporulating mycelium.

or grapefruit, and early green-colored fruit absorb more diphenyl than more mature, well-colored fruit. With these varieties and in these instances, one rather than two pads per carton may be adequate. Diphenyl tends to be less effective for control of sporulation of Penicillium on varieties that absorb more material. Since more is absorbed by the fruit, less diphenyl remains in the atmosphere and suppression of sporulation is less effective.

d. Storage - Low temperature storage maintains fruit quality and retards development and spread of fungal infections. Development of sour rot and *Diplodia* SER is essentially halted below 40°F. All decay fungi will resume development once fruit are transferred back to higher temperatures. Keep in mind that grapefruit and lemons are susceptible to chilling injury and should not be stored below 50-55°F for long periods of time. To better identify problems that may occur after fruit are shipped, representative samples should be taken after packing and placed in storage at 70-80°F for observation. These can be observed weekly for 2 weeks with domestic shipments, and at least 4 weeks with export fruit. Disorders observed upon arrival at the destination can often be identified from those observed in the test samples held at the packing source.

Fungal Resistance to Fungicides

1. Type of Resistance - Citrus decay fungi can develop resistance to postharvest fungicides. *P. digitatum* and *P. italicum* (green and blue mold) have frequently caused commercial losses because of their ability to resist SOPP, diphenyl, TBZ, benomyl, and recently, imazalil. The other decay fungi, as yet, have not developed resistance. Because of their ability to produce large numbers of airborne spores, the mold fungi can rapidly produce large numbers of resistant spores in packinghouse and storage environments. Potentially, a single spore of *P. digitatum* can produce about 100 million

spores on an infected fruit in 7 days under optimum environmental conditions. In a natural population of P. digitatum, resistant TBZ or benomyl spores are produced at a rate of 1 to 10 per 100 million spores. Once TBZ or benomyl is used continuously, the resistant spores infect and multiply rapidly and eventually replace the sensitive types. Since TBZ and benomyl are chemically similar, strains of Penicillium resistant to one are usually resistant to the other.

Shift of the natural population to resistant under the selection pressure of the fungicide is reached either through a disruptive or a directional selection. In disruptive selection, the fungicide is essentially inactive against resistant strains and these proliferate suddenly causing a quick and sudden loss of disease control. This situation has happened with TBZ and benomyl. With a directional selection situation, the loss of fungicide activity is much more gradual beginning with a few less sensitive strains which increase in numbers slowly over time. Such directional selection has occurred with imazalil in California lemon houses.

2. Factors that Favor Development of Resistance - Repeated, continuous use of the same fungicide increases the possibilities of developing a resistance problem. The selection pressure exerted by a postharvest fungicide treatment on citrus fruit is considerably greater than that produced by the same fungicide sprayed in the field. Postharvest fungicides are applied in a manner that usually

results in complete coverage, and the fungicide deposit deteriorates only slightly during the storage life of the fruit. The treated fruit are stored under conditions that permit growth and sporulation of fungicide-resistant strains of the pathogen. The resistant spores are dispersed readily by air currents in the packinghouse to other fungicide-treated fruit, continuing the process of selection for fungicide resistance.

Pre-harvest sprays with the same fungicide used in the packinghouse will also enhance the selection process for resistance. Pre-harvest sprays of benomyl used in Florida for postharvest decay control may select for strains resistant to the TBZ application in the packinghouse.

Storage of treated fruit for extended periods of time give the resistant strains time to infect, sporulate, and reinfect. Thus, resistance became a major problem in California lemon packing, and was also first noted in Florida with lemons. In both situations, treated fruit are stored for several weeks or even months. Holding fruit in cold storage for summer sale is another example where resistance may be manifested. In most Florida packing operations, treated fruit are shipped before infected fruit sporulate in the packinghouse. However, with export fruit sporulation may occur in the storage facilities at the docks, or in transit aboard ship.

3. Assessing Resistance - Resistance to fungicides is evaluated by collecting mold spores from the atmosphere or

from surfaces of walls, floors, and equipment in the packinghouse. These are then dispersed on a culture medium, usually potato dextrose agar-yeast extract-peptone, that is amended with the fungicide of interest. Amendments to the media, such as dichloran (3 ppm) or Q-phenylazole (100 ppm) and PCNB (500 ppm), have been used to suppress contaminating fungi and to cause colonies of Penicillium to be compact and nondiffusive for easier counting. Concentrations of fungicides used in the resistance assays have varied and attempts have been made to standardize these levels so comparisons among surveys can be made. Recommended levels in ppm of fungicides for use in the culture media are TBZ 10; benomyl 2; Q-phenylphenate 15-20; imazalil 0.05-0.1. Spores are also cultured at the same time on nonamended media so a measure of the total population can be obtained to determine the percentage of resistance.

Several methods have been utilized to collect spores. The most common procedure is to remove lids from petri dishes containing the media, usually for a minute, and to allow the spores to settle on the agar surface. Exposure times are reduced to 5-15 seconds if spore concentrations are high, such as at dump sites. Dishes are returned to the lab, incubated at 75-80°F for several days, and colonies that are formed by each germinating spore are counted. Another version of this assay method is to expose filter papers in sterile uncovered petri dishes for several days at various locations in the

packinghouse. The dishes are covered, returned to the lab, and spores on the papers are removed by washing with sterile water and measured aliquots of the water are spread over the surface of agar plates. These are dried, covered, and handled in a manner similar to the previous procedure. In these two methods, the assays are used to sample airborne spores that happen to settle from the atmosphere on the exposed test surfaces. These techniques provide an indication of the airborne contamination level. In another but more quantitative method, measured quantities of air are drawn across the assay media with an air sampler unit. The air flora are impinged directly on the agar surface, and the number of spores can be determined per cubic foot of air. When the resistant portion of a relatively high Penicillium population in the packinghouse approaches 10%, performance of the fungicide treatment is usually significantly reduced.

4. Combatting Resistance - Good sanitary practices are required if the Penicillium population is to be kept at a minimum in the packinghouse, storage, and transit facilities. An attempt should always be made to prevent infected fruit from sporulating in the packinghouse. Irrespective of the proportion of resistant individuals in the population, if the total population is minimal then the occurrence of resistance will be a minor problem. To maintain low inoculum levels, all infected fruit should be discarded daily and the equipment and premises cleaned with water and detergent. This can be

followed with a spray of an appropriate disinfectant. Cartons of unshipped fruit with excessive green mold should never be repacked in the packinghouse, but should be handled at a separate location where spores cannot be released back into the packinghouse atmosphere. Repacking may well have been necessary because resistant strains of P. digitatum had caused the decay. A significant reduction in decay has been noted when the dump and primary grade areas, where decayed fruit are first encountered, are spatially separated from the rest of the packingline and loading facilities. Exhaust fans at a totally enclosed dump can be used to remove spores from the packinghouse, and the packingline can be designed so prevailing wind currents do not spread spores from the degreening and dump areas to the rest of the line. A sanitizing spray of chlorine can be applied to spore-contaminated fruit immediately after dumping to reduce the inoculum load.

With the loss of benomyl and diphenyl, only three fungicides are now available for postharvest application. Fortunately, TBZ, SOPP and imazalil are chemically unrelated and can be applied in a mixture or in an appropriate sequence to delay the build-up of Penicillium resistance. Resistance would only occur if two genes for resistance to unrelated fungicides occurred in a single cell, and this is a rare event in a natural population of spores. Use of unrelated fungicides is especially valuable when fruit are treated

before and after storage. For example, when a TBZ drench is applied to fruit before degreening the drenched fruit can subsequently be treated with SOPP and imazalil. However, since imazalil is less effective than TBZ against SER and is not approved for fruit exported to Japan, much of the drenched fruit in Florida houses is treated both times with TBZ. Where this occurs, it is particularly important to practice good sanitation and to monitor the mold population for resistance.

If upon assaying for resistance, a significant resistant population is detected, all efforts should be made to thoroughly sanitize the house to minimize the spore load. Stringent sanitation should then be practiced, and if possible, use of a fungicide of a different chemistry should be employed. Benzimidazole resistant strains of *P. digitatum* (green mold) do not compete well with sensitive strains, and once use of TBZ is discontinued, resistant populations decline. This is not true with blue mold (*P. italicum*), where resistant strains have been shown to persist even after discontinuing the use of TBZ. Fortunately, blue mold spore populations are low in Florida packinghouses.

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