or 200 μg/ml did not significantly reduce the incidence of green mold.

Propylene glycol and glycerine, components of the DF-100 formulation, did not control green mold. DF-100, at a concentration of 5000 μg/ml, provided inconsistent control of green mold. In one test (Table 5, Test 2) after 21 days of storage, it did provide better control of green mold than thiabendazole in the presence of fungal resistance. However, in other tests (Table 4, 5) it was no better than thiabendazole even in the presence of resistance. The use of higher concentrations of DF-100 may prove more effective. However, an improved formulation would be required to prevent excessive foaming of the material which occurred when it was applied at 5000 μg/ml with a non-recovery spray over horsehair brushes.

Summary

The industry presently has fungicides that are quite effective for the control of the major postharvest decays, stem-end rot and green mold. Benomyl and thiabendazole are quite effective against the 2 stem-end rot pathogens. Green mold is also effectively controlled except in instances where the fungus has become resistant to the fungicides. Fortunately, imazalil, sodium o-phenylphenate, diphenyl, or potassium sorbate can be used to control benzimidazole resistant strains of the green mold organism. Of these 4 fungicides, imazalil is by far the most effective alternative to benomyl and thiabendazole where resistance is a problem, but its use is prohibited on fruit exported to Japan because the material is not approved for use on citrus fruit imported by that country. Sour rot, the other major decay of Florida citrus, is not effectively controlled by the approved postharvest fungicides. Sodium o-phenylphenate has some activity, but it has proven inadequate under high inoculum pressure. Registration of guazatine would provide the industry with an effective material for controlling sour rot. Use of the other fungicides evaluated in these tests for decay control is limited because of ineffectiveness or prevented at this time because of lack of registration.

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A QUANTITATIVE DETECTION METHOD FOR *PENICILLIUM DIGITATUM* AND *P. ITALICUM* IN CITRUS PACKINGHOUSES

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Abstract. **Resistance of *Penicillium digitatum* Sacc. and *P. italicum* Wehmer to the benzimidazole fungicides, thiabendazole (TBZ) and benomyl has led to economic losses in the California and Florida fresh citrus industry. The standard air sampling technique of exposing benzimidazole-amended and nonamended potato dextrose agar petri plates for between 1 to 3 minutes is useful in determining spore presence and the level of resistance in lemon packinghouses. Because orange and grapefruit packinghouses generally have fewer *Penicillium* spores present, the standard assay method which is qualitative may not accurately indicate a potential decay problem. A *quantitative* air sampling method was developed that has a high collection efficiency and high sampling volume. Sporeload can now be recorded as number of blue-greens per standard cubic foot of air.**

In 1980, the contributing editor of Florida Grower & Rancher stated, "getting produce to the customer is as important as producing it, since that's the operation that brings returns to the grower" (3). Decayed citrus fruit results in market losses and many of these decays occur after harvest. Prevention and/or the reduction of postharvest

decay and subsequent market losses are what growers, packers and shippers concern themselves with on a daily basis. Providing a postharvest environment that maintains the product in good condition is a major component in a pest management system.

The most prevalent post harvest decay organisms for citrus are the fungi *Pencillium digitatum* (green mold) and *P. italicum* (blue mold) (10). These molds exist in the air and are transported by air currents to healthy fruit (6,2). It is estimated that one spore infecting a wound-bearing fruit can produce as many as 100 million spores in 10 days under optimum environmental conditions (2). Hall & Bice (6) reported decay in a packinghouse is proportional to the concentration of spores in the air and on equipment surfaces. To further compound the problem, these *Penicillium* species develop fungicide resistance (5,8,9,11) and these, too, proliferate in a packinghouse environment.

Murdock (12) determined that these airborne spores could be assayed using microbiological methods in a lemon packinghouse operation. The technique was to simply expose petri dishes filled with potato dextrose agar for a given exposure time and count the spores that had "settled out" on the petri dish. This technique was refined by others (4,1,2) to determine not only spore load but resistant spore population. The agar was refined to enhance the growth of blue/green molds and the media was amended with the appropriate fungicides to determine resistance (7).

The technique has become an important tool to the packinghouse manager in his decay control strategy. He uses the data to assist in evaluating packinghouse sanitation programs and to evaluate appropriate fungicide treatment based on the resistivity assay.

The settling plate technique, however, is a qualitative indicator of airborne contamination load. Many factors affect its performance, exposure time; air currents; placements of petri dishes; nearby packinghouse personnel; and media depth. A need was therefore established to determine quantitatively airborne contamination and thereby establish a uniform assessment of the packinghouse environment.

In 1964, Ft. Detrick laboratories (13) developed a slit air sampler to analyze contamination levels of the ambient air in their biological warfare laboratories. This sampler was later adapted for the aerospace industry, hospitals, laboratories, pharmaceutical industries and food processing manufacturers.

American Machinery Corporation, as a service company to the citrus industry that provides spore assays to the packinghouse manager, selected an air sampler that provided a quick, simple and *portable* method for assessing the microbiological environment quantitatively. The instrument was the SAS sampler (Surface Air Systems). This instrument utilizes a Rodac plate that can also be used for determining surface colonies per cm^2. Surface and air contamination can then be correlated. This report documents the results obtained in 14 Florida packinghouses during the 1984-85 season with the SAS sampler.

Materials and Methods

Packinghouse Locations. During the 1984-85 season, 14 Florida citrus packinghouses were sampled for blue and green molds. At each packinghouse the following 4 stations were sampled: the dump, the degreening room, the wash, and the grading table. At each station, duplicates of RODAC and fall-out plates, containing nonfungicidal (control), TBZ-amended, and benomyl-amended media, were sampled totaling (6) six plates per station.

Media preparation. Control medium was prepared by autoclaving potato dextrose agar, cooling to 120° F, and adding 75% lactic acid at a rate of 50 drops/liter of agar. Botran 75W (2 ppm) was added to the agar to control the expansion of the blue and green colonies and reduce *Rhizopus* contamination (1). The following amended media were prepared from the control. TBZ (20 ppm) was added to 10 ml of isopropyl alcohol per 1000 ml of agar, dissolved, and added directly to the agar. Benomyl (5 ppm) was added to 10 ml of 30% ethyl alcohol-50% acetone solution per 1000 ml of agar, dissolved, and added directly to the agar. The control and amended media were poured aseptically in quantities of 12 ml for 100 x 15 mm petri dishes, and 4 ml for RODAC plates.

Assay methods. For quantitative spore analysis, the SAS air sampler was selected (Spiral System Instruments, Inc., Bethesda, Maryland 20014). It is a portable unit operating from a rechargeable battery with an automatic timer control. To operate, the sampler is set to one. This equates to 20 seconds of sampling time which equals 60 liters of air. The unit cover is cleaned with 70% isopropyl alcohol and removed. A RODAC plate is inserted aseptically. The cover is replaced, the sampler is positioned and the start button is activated. During this time, the sampler maintains sufficient air velocity to impinge existing air flora on the agar surface. When the sampler stops, the cover is removed and the RODAC plate is removed then incubated. All samples are incubated at 25° C for 48-72 hours. Counts are determined by the following calculations:

$$\text{No. of blue/green molds per SCF of air} = \frac{\text{No. of colonies on plate} \times 1000}{60 \times 35.3}$$

Plates are averaged and reported as number of blue/green molds per SCF. For qualitative spore analysis, the fall-out plate method was selected. A 100 by 15 mm petri dish is exposed to air in a designated area in the packinghouse for one minute. The plate is then incubated at 25° C for 48-72 hours. The counts are recorded as the average number of blue/green mold isolates per packinghouse location.

Results and Discussion

Qualitative spore assays in the 14 Florida packinghouses were done in tandem with the quantitative tests. Each house was sampled during the 1984-85 season and a total of 336 plates per assay method were utilized. Table 1 reveals the data obtained for the settling plate technique. The results are recorded as the average number of blue/green mold isolates per fall out plate per minute of exposure.

For Florida, the control counts for fall-out plates (Table 1) were relatively low and as expected, the dump showed the highest levels. However, resistivity at the various sampling sites (Table 2) varied between a high of 93.6% to a low of 18.5%. Also, the spores at the dump indicated extremely high resistivity to both TBZ and benomyl, in excess of 90%. Counts varied from plate to plate and house to house.

TABLE 1: Average number of blue/green molds isolates per fall out plate in Florida packinghouses during 1984-85 season.

Packinghouse Location	Average Number blue/green isolates per amended plate		
	Control (no fungicide)	TBZ, 20ppm	Benomyl, 5ppm
Dump	6.3	5.9	5.8
Degreen	2.8	0.9	1.5
Wash	2.7	0.8	0.5
Grade	3.1	1.6	1.6

TABLE 2: Comparisons of fungicide resistivity by the two sampling techniques in Florida packinghouses.

Location	Sampling Technique	% Resistivity to	
		TBZ	Benomyl
Dump	SAS air samples fall-out plates	62.2 93.6	59.2 92.1
Degreen	SAS fall-out	43.9 68.1	45.5 62.1
Wash	SAS fall-out	68.1 29.6	62.5 18.5
Grade	SAS fall-out	44.7 51.6	50. 51.6

TABLE 3: Average number of blue/green molds per standard cubic foot of air via SAS air sampler in Florida packinghouses during 1984-85 season.

Packinghouse Location	Average Number blue/green isolates per amended plate		
	Control	TBZ	Benomyl
Dump	33.1	20.6	19.6
Degreen	12.3	5.4	5.6
Wash	7.2	4.9	4.5
Grade	11.4	5.1	5.7

In the quantitative measurements, the SAS air sampler captured the molds present in 60 liters of air and impinged the viable molds on the agar surface. Sixty liters is equivalent to approximately 2 SCF of air.

Counts were recorded as the average number of blue/green molds per SCF. The results from the 14 houses are recorded in Table 3. The assay represents the known environmental pollution. As expected, spore load was highest at the dump, and the holding bins showed the second highest concentration. Resistivity was relatively uniform (Table 2), the percent ranging from 43.9 to 62.2% at each site location. Resistivity was shown in comparable amounts to both benomyl and TBZ, confirming that if resistance occurs, it is to both benzimidazole fungicides. Counts were higher than on settling plates and an accurate assessment can be made of resistant spore population.

The SAS sampler provides an easy to operate and readily available portable environmental quality control system. Since measurement is quantitative rather than random, a better assessment of spore population can be determined. The instrument allows for high sampling volume and high collection effiency. This will provide to the packinghouse manager a better treatment response to potential decay pathogens. The instrument can also be used to evaluate the effectiveness of a sanitation program once put in place. In addition, it provides to the industry a uniform method.

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