

an on-line expert system. Using a network of thermocouples, this the expert system would monitor each parameter and control the precooling process very accurately. Even a brief fluctuation of the water temperature during the process can be taken into account with an on-line expert system.

It is expected that most of these improvements will be available soon and this expert system will prove to be a very effective tool to design a hydrocooler, optimize precooling processes, control product quality, conserve energy, and investigate new applications.

### Conclusion

In this work, an expert system for hydrocoolers was developed. Provided with a user friendly interface, this expert system can perform a wide range of tasks such as: hydrocooler design, precooling process optimization, quality control, energy conservation and investigation of new applications. Further improvement will make the software

more versatile and applicable to a wider variety of commodities.

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## EFFECT OF PH AND STORAGE ON SOLUTIONS OF IMAZALIL

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**Abstract.** Solutions of imazalil at 250 ppm and pH 5, 6, 7, 8, 9 and 10 were held for 19 days then used to treat fruit inoculated with *Penicillium digitatum* Sacc. These treatments were duplicated with freshly made solutions at the same pHs. Valencia oranges were inoculated by puncturing at 4 places around the equator then immersing for 30 seconds in a suspension of approximately 1 million spores per ml. Replicates of 10 fruit per sample (40 inoculations) by three samples per treatment were used. After 10 days fruit treated with aged imazalil preparations averaged 10.1% decay, fruit treated with fresh imazalil preparations averaged 4.3% decay and the untreated checks averaged 92.5% decay. Sporulation of decayed fruit was evaluated using a 0 to 5 scale with 0 = no sporulation and 5 = all mycelia covered with loose spores. Fruit treated with fresh imazalil solutions averaged a sporulation score of 0.8 while fruit treated with aged imazalil solutions averaged a sporulation score of 2.5.

The fungicide imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole) has been used infrequently in Florida. Due to its high cost and lack of acceptance in many important export market countries (Lichty, 1980), it has been used as a supplemental fungicide on citrus only when requested by a particular buyer or when there is concern over the possibility of resistance to other fungicides. For this reason packers will often hold prepared solutions of imazalil for use only when it may be required. Since these prepared solutions may be either as a water solution or in a shipping wax (Hall, 1981; Hayward and

McCornack, 1986), the pH value of the prepared solution may vary widely (Hall, 1980). Some fungicides used in citrus packing are either degraded at some pH (Hall, 1980), or have its efficacy dependant on the pH of the application medium (Hayward and McCornack, 1986). No such effect has been reported with imazalil, but the possibility of formation of metabolites has been recognized (Leemput et al., 1985; Wynants, 1982).

None of the literature reviewed indicated either the pH or the age of the solutions tested. Without other indications, it is likely that workers used either deionized water or potable water available to them. In such case the pH was most likely to be nearly neutral. There is also no indication that the preparations used were other than freshly prepared.

At the recommendation of the manufacturer of imazalil, at least one supplier is marketing an acidifying material to adjust the pH of prepared water solutions to pH 6. Since prepared imazalil solutions may be held at the packinghouse for extended periods at various pH, the possibility of reduced efficacy was investigated. Normal packinghouse preparations of benomyl have been observed to have pH in the range of pH 6 to 10 which is mostly due to the water, wax or other chemicals used in preparing the fungicide suspension (Hall, 1981).

In order to accentuate the possible effect of either pH or storage time on imazalil solutions, a concentration of 250 ppm was chosen. This level is well below the 500 to 1000 ppm needed for acceptable decay control (Brown et al., 1983; Dawson, 1981; Laville et al., 1977; McCornack and Brown, 1977; McCornack et al., 1977; Wardowski and Brown, 1991).

One of the major advantages of imazalil is its ability to suppress sporulation in decayed fruit (McCornack and Brown, 1977). By suppressing sporulation, the problems

of resistance in *Penicillium* molds is greatly reduced and the cycle of decay in the packinghouse is kept under control (Hall and Bice, 1977; Wild, 1974). Failure of a fungicide to control sporulation in the packinghouse environment can lead to the selection of resistant strains which will lead to a breakdown of decay control (Dawson, 1981; Eckert, 1988). While it was once thought that the development of resistance to imazalil was improbable or would be only a minor problem, it has now (Kaplan and Dave, 1979; Laville et al., 1977) been demonstrated that imazalil resistance has become a major problem in some areas (Dave and Walia, 1989; Laville, 1981). Any factor that will either reduce the ability of imazalil to control either decay or sporulation would be of serious concern to the citrus packing industry.

Materials and Methods

Commercially available imazalil (Fungaflor 500EC, EPA Reg. No. 43813-6) was diluted with previously prepared Clark and Lubbs buffer solutions (Gabb and Latchem, 1968). Solutions were prepared at 250 ppm imazalil and pH 5, 6, 7, 8, 9 and 10. Deionized water was used in all preparations. Upon the addition of imazalil all solutions developed a cloudiness which seemed to be nearly the same in all preparations except pH 8, which was slightly heavier. Solutions were held for 19 days before treating fruit. At the time the fruit was treated, fresh duplicate solutions were prepared for a total of 12 treatment solutions. The fresh solutions were used within 2 hours of preparation. To insure uniformity solutions were prepared in bulk and divided into three portions just before use. In each of 3 trials, 130 Valencia oranges were inoculated with *P. digitatum* spores by puncturing each fruit 4 times on a conical point approximately 3 mm high by 3 mm diameter at the base. Punctures were spaced around the fruit with a minimum of 2 inches between measurements. The fruit were then immersed for 15 sec in a suspension of spores harvested from agar plates. A sample of the suspension was challenged for growth on potato dextrose agar modified with either 10 ppm thiabendazole or with 0.1 ppm imazalil. The suspensions used in these trials were sensitive to both thiabendazole and imazalil. The fruit were incubated for 20 hours at 70°F and 85% relative humidity and then treated with each of the previously prepared solutions and fresh solutions. At the time of preparation of each solution, the pH was measured with a standardized pH meter, and in the case of the aged solutions, the pH was measured again just before use. These measurements are reported in Table 1.

Table 1. pH of 250 ppm imazalil treatment solutions.

Treatment Nominal pH	pH as Prepared <sup>z</sup>	At Treatment <sup>y</sup>	
	April 27 <sup>x</sup>	April 27 <sup>x</sup>	May 16 <sup>x</sup>
Checks	—	—	—
5	5.07	5.02	5.12
6	6.00	6.09	5.97
7	7.02	7.10	7.23
8	8.01	8.00	7.96
9	8.98	9.04	8.97
10	10.01	10.01	9.98

<sup>z</sup>Solution prepared April 27, 1991. pH at time of preparation.  
<sup>y</sup>pH at time of treating fruit. Both freshly prepared and aged solutions.  
<sup>x</sup>Preparation date of solution. All solutions used May 16.

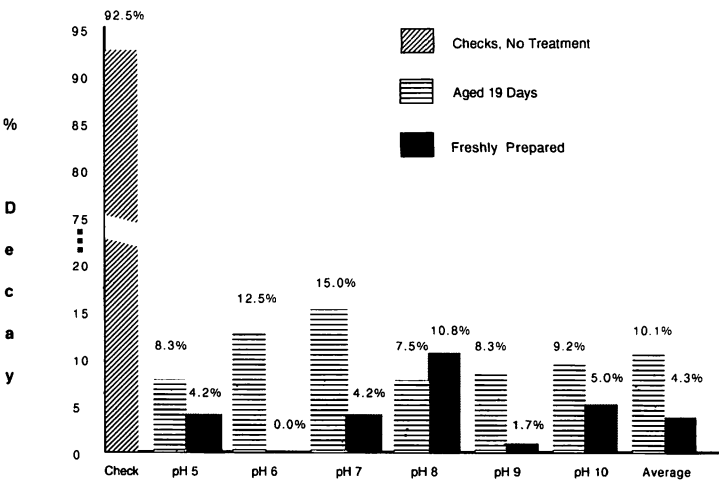


Fig. 1. Decay of fruit treated with fresh and aged 250 ppm imazalil solutions.

The inoculated fruit of each trial was then randomized into 13 lots of 10 fruit each and each lot was treated with the prepared imazalil solutions. The treated fruit were held at ambient temperature for 11 days at ambient conditions, then examined for decay at each inoculation point. The percent decay for each trial replicate and the average of all replicates at each pH, fresh vs. aged, was calculated and are presented in Figure 1. Those fruit that decayed were subjectively evaluated for sporulation on a scale of 0 to 5, 0 being no visible spores and 5 being the entire fruit covered with loose spores. These results are summarized in Table 2. Spores from imazalil treated, decayed fruit, were tested for imazalil resistant by challenging them on agar with 0.1 ppm imazalil. Growth was observed in all samples tested, indicating some resistance to the fungicide.

Results and Discussion

During the aging period the pH of the prepared solutions remained stable (Table 1). No changes in color or odor were noted; however, the solution at pH 8 seemed to be cloudier than the others. No measurements were made at the time of fruit treatment as all treatments were cloudy to some extent. The average decay of all pH levels was 10.1% decay for solutions held 19 days at various pH and 4.3% for freshly prepared solutions, which is significant at the 95% level of confidence. The fresh solutions at pH 6 and pH 7 were

Table 2. Sporulation of decayed fruit.

Treatment Nominal pH	Sporulation Index of Decayed Fruit <sup>z</sup>	
	Aged Solution	Fresh Solution
Checks	—	5
5	4	2
6	3	0 <sup>y</sup>
7	3	1
8	1	1.5
9	2	0
10	2	1.5

<sup>z</sup>Sporulation Index: 0 = No visible spores, 1 = Trace of tight spores, 2 = ¼ surface covered with loose spores, 3 = ½ surface covered with loose spores, 4 = ¾ surface covered with loose spores, 5 = Entire fruit covered with loose spores.  
<sup>y</sup>No decayed fruit.

significantly better (99% level of confidence) than the aged solutions. At pH 8, the aged solution had less decay than the fresh solution but the difference was not significant. This may be related to the cloudiness observed and is a subject for further investigation.

The level of imazalil used in these trials (250 ppm) was selected in order to allow some decay and accentuate any differences in the treatments. This was achieved in that the decay in all samples was higher than has reported for imazalil (Harding, 1976; Kaplan and Dave, 1979; McCornack and Brown, 1977) when used at levels of 500 to 1000 ppm. The current recommendations for the use of imazalil in water is 1000 ppm (Wardowski and Brown, 1991). The total decay for fruit treated with fresh solutions ranged from 0% to 10.8% with the median at 4.2% and a mean value of 4.3%. This is similar to the values in a previous report using imazalil at 250 ppm (Laville et al., 1977).

Sporulation in decayed fruit was affected to a greater extent than decay control both by pH and by storage. In freshly prepared solutions some loss of sporulation control was observed at pH 5, while at other pH values, suppression of sporulation by imazalil was retained (Table 2). In the aged solutions, sporulation was definitely greater at all pH levels except pH 8, which was about the same for both fresh and aged solutions. In the aged solutions, there was a marked loss of sporulation control at pH 5, 6, and 7, while the treatments at pH 8, 9 and 10 were somewhat better. When tested for resistance to imazalil, all samples checked grew well and sporulated, indicating that resistance had been selected by the treatment. Such selection of resistance in a single generation has been noted with benomyl (Wardowski and Brown, 1991), and it is not surprising that it should be found under these conditions.

The whole of these results indicate that the current practice of holding prepared solutions of imazalil for extended times in citrus packinghouses is inadvisable. Low pH solutions should be avoided as well. Reduced efficacy of fungicide and the possibility of contributing to the population of resistant mold spores in the packinghouse is a major concern. Packers should prepare the imazalil solution just before it is to be used to pack fruit and should use it promptly.

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