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RESISTANCE OF DIFFERENT STRAINS OF *PENICILLIUM DIGITATUM* TO IMAZALIL TREATMENT IN CALIFORNIA CITRUS PACKINGHOUSES

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Abstract. **Imazalil has been used to control postharvest disease of citrus in California since 1981. It gives good protection against fungal diseases caused by such organisms as *Penicillium digitatum* and *Penicillium italicum*. In the past two years several papers have been published that report the occurrence of resistant strains of *Penicillium digitatum* and *Penicillium italicum* to imazalil. Several experiments were conducted to determine resistance and competitiveness of these strains. Results indicated that there are some strains which are resistant to very low levels of imazalil. However, the less sensitive strains are not able to fully compete with the sensitive strains and as a consequence such tolerant strains are of low practical significance to the citrus industry in general. The history of imazalil residues on citrus has shown that the residues are adequate to control decay. Stricter sanitation procedures and alternation of fungicides or combination uses of fungicides seem to give the desired control of citrus decay organisms.**

The use of the fungicide imazalil on citrus to control postharvest diseases was discussed by Kaplan and Dave' (2). Eckert reported the concern about repeated application of imazalil and build up of resistance by some strains of *Penicillium* to this fungicide (1). A survey for imazalil resistant *Penicillium* spores in 1987 found green sporulation on lemons treated with 2000 ppm of imazalil. The decay on treated fruits was typical of green mold caused by *P. digitatum* Sacc. The initial isolations from the collected fruit were successful in recovering colonies of *P. digitatum.* In many cases *Geotrichum candidum* Lk. ex pers., the sour rot organism, was also recovered. However, when the *P. digitatum* colonies initially isolated from the decayed fruit were plated again on imazalil amended media, many of the fungi grew and sporulated. Furthermore, many of the isolated fungi suspected of being pathogens caused decay on untreated oranges. However, these pathogens could not be reisolated onto imazalil amended media. This indicated that these pathogenic organisms are very weakly resistant to imazalil, and suggests that growth on untreated fruit caused a reversion of the organisms to the wild type (Dave', B.A. unpublished).

In any case a study was undertaken by our group as well as a plant pathologist in Beerse, Belgium to determine this "weakness theory of non survival of so called tolerant strains" (3).

Materials and Methods

Strain collection. Five different strains of *P. digitatum* were used for these experiments. Strains one and two were collected from decayed fruit treated with 2000 ppm of imazalil in California packinghouses (CAL1 and CAL2). Strains three and four were provided by Janssen Pharmaceutica (JAN3 and JAN4). The fifth strain was collected from an exposed PDA plate at a California packinghouse (SAS).

Strain plating. A spore dilution was made from each of the above strains in distilled water. A 0.1 ml aliquot of each suspension was spread evenly on an imazalil amended plate. The plates were left at room temperature for 5 days after which the number of colonies were counted. The plating was replicated three times.

Fruit inoculation. To determine competition of sensitive and resistant strains, two suspensions of sensitive and resistant strains were made with an equal number of spores. Two oranges were inoculated with sensitive strains, and two with resistant strains of *P. digitatum.* A 50:50 spore suspension of resistant and sensitive strains was made and two oranges were inoculated with the suspension. Green mold which developed from these oranges was plated on PDA plates. After 5 days the spores from this plate were diluted and plated on to imazalil amended media plates. The plates were left at room temperature and the growth (–) or inhibition (+4) of survivors was determined after five days.

Results and Discussion

Four strains of *P. digitatum* with different tolerance levels to imazalil and a sensitive strain were used for this experiment. Table 1 presents the initial screening of the strains. Four strains were resistant to 20 ppm of TBZ and showed some resistance to 10 ppm of SOPP. Each strain was resistant to a different degree at 0.5 ppm of imazalil. Table 2 shows the average number of colonies in amended imazalil plates and the ED 50 value for the different strains. As figure 1 indicates the maximum ED 50 for the strains was 0.44 ppm. The ED 50 for strain CAL2 was 0.39 ppm of imazalil; howerer, the ED 50 for the same strain of CAL2 was about 80 ppm of TBZ.

When the spores from the sensitive strains and resistant strains were mixed in equal amount and injected under the orange's skin the newly developed spores were sensitive to low levels of imazalil. Table 3 indicates the growth or inhibition of these cultures on imazalil amended plates.

Table 1. Initial screening of the cultures.

	Growth (–) or inhibition (+4) of different strains of *P. digitatum*in presence of different fungicides			
Strain	Check	TBZ (20 ppm)	IMZ (0.5 ppm)	SOPP (10 ppm)
SAS	–	+4	+4	+4
CAL1	–	–	–	+2
CAL2	–	–	–	+2
JAN3	–	+2	+2	+3
JAN4	–	–	+1	+2

Table 2. Average number of colonies on different imazalil amended plates.

	Imazalil concentration (ppm)					
Strain	0	0.125	0.25	0.50	1.0	ED 50
SAS	98	0	0	0	0	—
CAL1	120	100	98	52	0	0.44
CAL2	100	105	68	29	0	0.39
JAN3	70	38	0	0	0	0.13
JAN4	65	52	15	1	0	0.19

Table 3. Growth (–) or inhibition (+4) on amended imazalil plates.

	Imazalil concentration (ppm)				
Strain	0	0.125	0.25	0.50	1.0
SAS	–	+4	+4	+4	+4
CAL1+SAS	–	+2	+4	+4	+4
CAL2+SAS	–	+1	+4	+4	+4
JAN3+SAS	–	+4	+4	+4	+4
JAN4+SAS	–	+1	+4	+4	+4

This data indicates that the so called resistant strains are not able to fully compete with the wild strains.

However a note based on EUP results (2) printed on all the product labels of imazalil formulation in the market, clearly states that for good sporulation control of penicillum organisms the recommended dosage is 4000 ppm. It is only for economic reasons that the citrus industry elected to use the 2000 ppm level. Data from our monitoring experiences in one particular packing house (CCH) recently using the higher level confirmed that good sporulation control is achieved by 4000 ppm of imazalil. Sublethal dosage may have given rise to the survival of the so called imazalil less sensitive strains in the citrus industry.

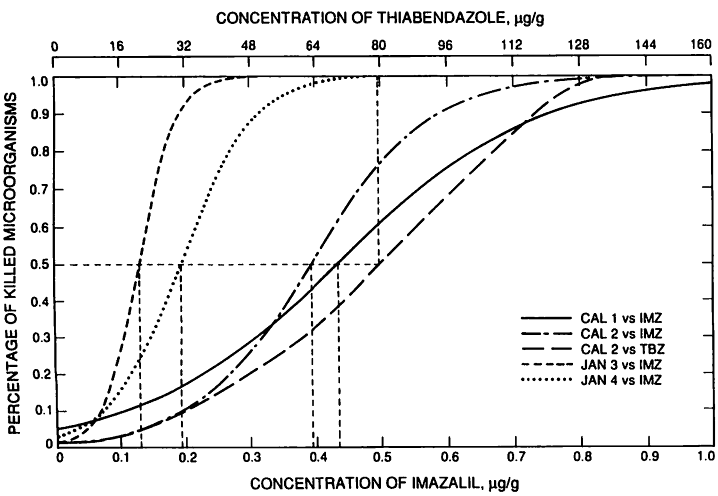

Fig. 1. ED_{50} of several penicillium strains.

There should be some "resistance response strategies" in the industry to combat any problem that might exist or occur in the future. Good sanitation of packinghouses, monitoring of resistant strains, good and uniform application of pesticides and fast fruit movement in the market can be the first steps.

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REDUCTION IN SODIUM ORTHO-PHENYLPHENATE CONCENTRATION ON CITRUS BY CONTROLLING pH

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Abstract. **For five (5) years, orthophenylphenate (OPP) residues were monitored in both East Coast and West Coast Citrus packing houses, utilizing foam solutions of sodium orthophenylphenate (SOPP). It was found that if pH was tightly controlled through the use of hexamine, initial use concentration could be reduced while maintaining adequate residues. The benefits resulted in a cost savings to the packinghouse; a reduction of OPP to sewage; and reduced risk potential of fruit burn to early and late season fruit.**

Sodium orthophenylphenate (SOPP) has been used longer than any of the other currently registered fungicide for post-harvest treatment of citrus. It also has the broadest spectrum or anti-microbial action of any of the organic fungicidal compounds used in the packinghouse (1,2). Efficacy against several post-harvest decay organisms and SOPP's abililty to readily destroy most incipient infections in fruit make it an extremely valuable tool in preventing decay (4). As public concern over food safety and groundwater pollution continue to increase yearly, steps need to be taken in our industry to use valuable treatments like SOPP in an efficient manner that minimizes the risk of excessive residues on food and reduces waste and effluence. This paper reviews five years worth of field data on reduced concentration treatments of SOPP in foam wash applications by use of hexamine as an additive.

SOPP tetrahydrate is soluble in water and is hydrolyzed to form o-phenylphenol (undissociated) and o-phenylphenate (dissociated) (Fig. 1). The concentration of the species is dependent on the solution's pH (5). Ortho-phenylphenol is lethal to post-harvest organisms, but is phytotoxic to citrus fruit in concentrations above 200 ppm depending on contact time, temperature and other variables. While o-