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## Sensitivity of *Penicillium digitatum* and *P. italicum* to Imazalil and Thiabendazole in Morocco

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**Abstract:** Green and blue molds (caused by *Penicillium digitatum* and *P. italicum*, respectively) are the main postharvest diseases of citrus fruits in Morocco. Following packing houses reports of reduced efficacy of fungicides used to control these diseases, a survey was conducted during 2005-2006 packing season to characterize, both qualitatively and quantitatively, the resistance of *P. digitatum* and *P. italicum* to imazalil (IMZ) and thiabendazole (TBZ). Isolates of *P. digitatum* and *P. italicum* were obtained from decayed citrus fruits collected from commercial citrus packing houses located in the Souss-Massa-Draa (SMD), South of Morocco and were evaluated *in-vitro* for their sensitivity to IMZ and TBZ. Of the 290 *P. digitatum* isolates, 19% (55/290) were resistant to IMZ and 37% (107/290) were resistant to TBZ tested at discriminatory concentrations of 0.1 or 20  $\mu\text{g mL}^{-1}$ , respectively. In contrast, only 2.5 (5/204) and 21% (44/204) of *P. italicum* isolates collected from packing houses were resistant to IMZ and TBZ, respectively. No resistance to TBZ and IMZ were detected in *Penicillium* sp., isolates collected from a citrus orchard which has no known history of fungicide use. The proportion of collected isolates that were resistant to both fungicides was 1.5% for *P. italicum* and 10% for *P. digitatum*. The mean  $\text{EC}_{50}$  values for *in vitro* inhibition of mycelial growth of the *P. digitatum* resistant-isolates were between 0.81 and 0.98  $\mu\text{g mL}^{-1}$  for IMZ, whereas those of TBZ were between 39.23 and 50.84  $\mu\text{g mL}^{-1}$ . The mean  $\text{EC}_{50}$  values for *P. italicum* resistant-isolates ranged from 0.53 to 0.61  $\mu\text{g mL}^{-1}$  for IMZ and from 52.97 to 59.92  $\mu\text{g mL}^{-1}$  for TBZ, whereas the mean  $\text{EC}_{50}$  values for orchard collected isolates were 0.04  $\mu\text{g mL}^{-1}$  for IMZ and 0.16  $\mu\text{g mL}^{-1}$  for TBZ. The data will provide a baseline for monitoring resistance to IMZ and TBZ in populations of *P. digitatum* and *P. italicum* in the SMD commercial citrus packing houses in the future.

**Key words:** *Penicillium digitatum*, *P. italicum*, imazalil, thiabendazole, fungicide resistance, citrus fruit

### INTRODUCTION

Citrus fruit cultivation is very important in Morocco, being the first exporting agricultural sector and playing a major role in the national economic development. The largest volume of citrus fruit for fresh consumption and export in the Morocco is grown and shipped from packing houses in Souss-Massa-Draa (SMD), Valley. Postharvest decay is one of the major factors affecting fresh citrus fruit quality and marketing value and it is essential to control decay in order to maintain the quality and prolong the shelf life of citrus fruit, particularly in a market where transport from producer to consumer may take several weeks.

The most important diseases that cause commercially significant losses, in Morocco (Elkhamass *et al.*, 1994)

and worldwide (Eckert and Eaks, 1989; Holmes and Eckert, 1999; Zhu *et al.*, 2006), are green mold, caused by *Penicillium digitatum* (Pers.: Fr.) Sacc. and blue mold, caused by *P. italicum* Wehmer. These *Penicillium* species are ubiquitous and produce copious amounts of asexual conidia that are efficiently dispersed via air currents (Holmes and Eckert, 1995). Their inoculums are found in citrus growing-areas and in fruit handling environment. These fungi only infect fruit through injuries created by harvesting and subsequent handling (Brown and Miller, 1999).

Control of green and blue molds is mainly based on the use of fungicides treatments principally thiabendazole (TBZ) and imazalil (IMZ) sprayed, alternately or simultaneously, on fruit during waxing operation in the packing house. In most commercial packing houses in



SMD, pre-storage chemical control with benomyl (BEN) or thiophanate-methyl (THM) is commonly applied, as postharvest drench, to reduce the incidence of green and blue molds in citrus fruit that are stored in cold before processing. Unlike IMZ, the postharvest fungicides, BEN, THM and TBZ belong to the chemical class of benzimidazole and have a similar mode of action. The imidazole fungicide IMZ is effective for controlling TBZ-resistant isolates of *P. digitatum* and *P. italicum* (Wild, 1994; Smilanik *et al.*, 2005).

Several reports (Staub, 1991; Hewitt, 1998) indicated that when populations of fungal pathogens are repeatedly exposed to site-specific fungicides, resistant strains could be readily selected. The result of this selection has been documented in cases in which TBZ and IMZ were used extensively in controlling *P. digitatum* and *P. italicum* in commercial packing houses (Bus, 1992; Wild, 1994; Holmes and Eckert, 1999; Besri *et al.*, 1983; Kinay *et al.*, 2007).

However, although study has been done in several other countries (Eckert *et al.*, 1994; Holmes and Eckert, 1995; Brown and Miller, 1999), little is known about the fungicide sensitivity of *P. digitatum* and *P. italicum* populations in the SMD citrus packing houses. Thus, the research reported herein was conducted to determine, under packing house conditions, the proportion and the level of resistance of *P. digitatum* and *P. italicum* to imazalil and thiabendazole in the SMD Valley, South of Morocco.

## MATERIALS AND METHODS

**Fungicides:** The commercial citrus postharvest fungicides TBZ (50% a.i., Tecto 500 SC, RODA, Morocco) and IMZ (80% a.i., Magnate 80 EC, SAOAS, Morocco) were used. Fungicide solutions were first prepared by dissolving each commercially-formulated fungicide in acetone. An aqueous stock solution of each fungicide was then prepared. To obtain desired concentrations, samples from stock solutions of fungicides were added aseptically to molten (50°C) sterile Potato Dextrose Agar (PDA) medium at pH 5.9±0.2. Control plates contained PDA and acetone and the final concentration of acetone in the medium did not exceed 1% (v/v). All concentrations were expressed as microgram of active ingredient (a.i.) per milliliter of growth medium.

**Sampling locations and fungal isolations:** The isolates of *P. digitatum* (290) and *P. italicum* (204) used in this study were recovered from infected citrus fruit collected in 4 packing houses located in SMD Valley, South of Morocco, during the 2005-2006 packing season. These packing houses are referred to as packing houses I, II, III

and IV, respectively, throughout the remainder of the article. Isolates of *Penicillium* sp., were evaluated for fungicide sensitivity in comparison with reference isolates recovered from a citrus orchard where TBZ and IMZ are not used. At least five infected citrus fruits, with symptoms of green and blue molds, were collected monthly (from November 2005 to May 2006) from each packing house. Fruits were placed in separate plastic bags and transported to the laboratory.

Using the procedure described by Bus *et al.* (1991), conidia were collected from rotted citrus fruits, previously surface-disinfected with 90% ethanol, using a sterile cotton swabs that were stored in sterile test tubes. The conidia were transferred to Petri plates containing potato dextrose agar acidified with 0.5 mL of lactic acid (80%) per liter (pH 5.3±0.2). After a four days incubation period at 25°C, plates were examined under a stereomicroscope to determine colonies identities. Isolates were confirmed to belong to *P. digitatum* and *P. italicum* species, according to Domsch *et al.* (1980) identification key. Pure subcultures of *P. digitatum* and *P. italicum* were stored on PDA slants at 5°C in the dark, until needed.

**Fungicide sensitivity tests:** The isolates of *P. digitatum* and *P. italicum* were *in vitro*-tested for sensitivity to IMZ and TBZ at a discriminatory concentration of 0.1 or 20 µg mL<sup>-1</sup>, respectively; a concentration completely inhibitory for the sensitive isolates but not for the resistant isolates. Hyphal plugs (5 mm diameter) from the periphery of actively growing colony were inverted and placed in the centers of 3 replicate Petri plates containing PDA medium supplemented with fungicide. After a ten days incubation period at 25°C, the isolates were considered either resistant if any growth was observed on fungicide-amended medium or sensitive if no growth has occurred.

**Pathogenicity tests:** Isolates under study, which included both sensitive isolates obtained from citrus fruit collected in an orchard and resistant isolates recovered from citrus fruit collected in packing houses, were inoculated into citrus fruit to ensure the pathogenicity of *P. digitatum* and *P. italicum* isolates. Orange fruit were washed, surface-disinfected with 90% ethanol and then inoculated by the injection of 10 µL of a spore suspension (10<sup>5</sup> spores mL<sup>-1</sup>) of each *Penicillium* sp., isolate about 1 cm below the rind surface. Three oranges constituted a single replicate and each treatment was replicated two times. After inoculation, all fruit were stored for one week at 20°C and 95% RH, after which they were examined for decay development and sporulation.



**Measurement of fungicide sensitivity:** For each packing house *P. digitatum* and *P. italicum* resistant-isolates were used to determine the effective concentration for inhibition of 50% of mycelium radial growth (EC<sub>50</sub>). These isolates were arbitrary chosen and represent the 4 sampled packing houses.

A 5 mm diameter disk was taken from the margin of a seven day-old culture of each isolate of *P. digitatum* and *P. italicum* and placed at the center of PDA plate. Fungicide concentrations in amended PDA medium were 0, 0.1, 0.2, 0.5 and 1.0 µg of TBZ per mL; 0, 0.015, 0.025, 0.035, 0.045 and 0.05 µg of IMZ per mL, for the sensitive isolates collected from a citrus orchard. For the TBZ- and IMZ-resistant isolates the following concentrations were used: 0, 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0 µg of IMZ per mL; 0, 10, 20, 40, 50 and 70 µg of TBZ per mL. All isolates were collected from commercial packing houses. Three Petri plates were used for each fungicide concentration. Colony diameters were measured after seven days at 25°C. The experiment was performed twice. Percent growth inhibition at each fungicide concentration was calculated according to the following formula:

$$\text{Growth inhibition (\%)} = \frac{(\text{Unamended} - \text{Fungicide amended})}{\text{Unamended}} \times 100$$

**Statistical analysis:** The EC<sub>50</sub> values were calculated for each isolate and each fungicide by regression analysis of the percent inhibition of fungal growth versus the log of the fungicide concentration (µg mL<sup>-1</sup>) (Mondal *et al.*, 2005). The data were subjected to statistical Analysis of Variance (ANOVA) using the STATISTICA software, version 6, StatSoft, 2001, France. Mean separation was performed following the method of Newman and Keuls test at p = 0.05.

## RESULTS

**Fungicide sensitivity:** In this study, 290 isolates of *P. digitatum* were collected from 4 commercial citrus packing houses located in SMD Valley. All isolates were tested *in vitro* for resistance to either TBZ or IMZ at discriminatory concentrations of 20 or 0.1 µg per mL, respectively. Present results show that 3.79, 40, 4.41 and 12.9% of *P. digitatum* isolates collected from packing houses I, II, III and IV, respectively, were resistant to IMZ (Table 1). Whereas, all *P. digitatum* isolates collected from a citrus orchard were sensitive to IMZ. The percentage of TBZ-resistant isolates of *P. digitatum* collected from the four packing houses ranged from 9 to 75%. In contrast, all 25 isolates of *P. digitatum* recovered from infected fruit collected in a citrus orchard

Table 1: Evaluation of the sensitivity to imazalil and thiabendazole of *Penicillium digitatum* and *P. italicum* isolates from 4 locations in the Souss-Massa-Drâa Valley, Morocco

Sample location	Fungi	Fungicide sensitivity <sup>a</sup> (R <sup>y</sup> /T <sup>z</sup> )	
		Thiabendazole	Imazalil
Packing house I	<i>P. digitatum</i>	11.0/79	3.0/79
	<i>P. italicum</i>	3.0/37	0.0/37
Packing house II	<i>P. digitatum</i>	84/112	45/112
	<i>P. italicum</i>	31/89	3.0/89
Packing house III	<i>P. digitatum</i>	9.0/68	3.0/68
	<i>P. italicum</i>	4.0/28	0.0/28
Packing house IV	<i>P. digitatum</i>	3.0/31	4.0/31
	<i>P. italicum</i>	6.0/50	2.0/50
Orchard	<i>P. digitatum</i>	0.0/25	0.0/25
	<i>P. italicum</i>	0.0/14	0.0/14

<sup>a</sup>Sensitivity of isolates to fungicide was tested on PDA medium amended with TBZ at 20 µg mL<sup>-1</sup> or IMZ at 0.1 µg mL<sup>-1</sup>. Isolates which failed to grow on the amended medium were considered sensitive. <sup>y</sup>No. of isolates resistant to fungicide, <sup>z</sup>Total No. of *Penicillium* isolates per packing house

(nonexposed population) were sensitive to TBZ tested at a discriminatory concentration of 20 µg mL<sup>-1</sup> (Table 1).

A discriminatory concentration of 0.1 µg of IMZ per mL was used for testing *P. italicum* resistance. As shown in Table 1, IMZ-resistant isolates of *P. italicum* were relatively rare. Among 204 isolates collected during 2005-2006 packing season only 5 were resistant to IMZ; resistance was found at two packing houses (II and IV). Whereas, all *P. italicum* isolates collected in a citrus orchard were sensitive to IMZ. Based on a discriminatory concentration of 20 µg of TBZ per mL, resistant isolates of *P. italicum* were recovered from decayed fruit collected in the 4 citrus commercial packing houses (Table 1). However, none of the *P. italicum* isolates, recovered from fruit collected in a citrus orchard, showed growth at 20 µg mL<sup>-1</sup> of TBZ, thus all isolates were considered sensitive to TBZ. Four groups of resistant isolates were identified among all *Penicillium* sp., isolates recovered from packing houses:

- Isolates sensitive to both IMZ and TBZ
- Isolates resistant to IMZ only
- Isolates resistant to TBZ only
- Isolates resistant to both IMZ and TBZ

The *in vivo* pathogenicity test indicated that the IMZ- and TBZ-resistant isolates of *P. digitatum* and *P. italicum* were not significantly different from IMZ- and TBZ-sensitive isolates in their growth and sporulation on untreated fruit (data not shown).

**Level of resistance:** Quantification of the sensitivities of *P. digitatum* and *P. italicum* to IMZ and TBZ was based on *in vitro* inhibition of mycelial growth. As shown in Table 2, the mean EC<sub>50</sub> values of 25 TBZ- and IMZ-sensitive isolates of *P. digitatum* collected from a



Table 2: Sensitivity ( $EC_{50}$ ) of wild-type citrus orchard isolates of *P. digitatum* and *P. italicum* to imazalil and thiabendazole

Fungicide	Fungi	Mean $EC_{50}$ value ( $\mu\text{g mL}^{-1}$ ) <sup>a</sup>
Imazalil	<i>P. digitatum</i>	0.034±0.007
	<i>P. italicum</i>	0.040±0.006
Thiabendazole	<i>P. digitatum</i>	0.140±0.090
	<i>P. italicum</i>	0.160±0.030

<sup>a</sup> $EC_{50}$ : Effective concentration of fungicide in the culture medium needed to inhibit 50% of mycelial growth. Mean values were determined for ten isolates

Table 3: Sensitivity ( $EC_{50}$ ) of *P. digitatum* and *P. italicum* resistant-isolates collected from 4 commercial packing houses to imazalil and thiabendazole

Sample location	Fungi	Mean $EC_{50}$ value ( $\mu\text{g mL}^{-1}$ ) <sup>a</sup>	
		Thiabendazole	Imazalil
packinghouse I	<i>P. digitatum</i>	42.83±6.200 <sup>a</sup> (10) <sup>b</sup>	0.81±0.12 a (3)
	<i>P. italicum</i>	55.87±3.000A (3)	-
packinghouse II	<i>P. digitatum</i>	50.84±4.240a (10)	0.98±0.09 a (10)
	<i>P. italicum</i>	59.92±4.270A (10)	0.61±0.08 A (3)
packinghouse III	<i>P. digitatum</i>	45.12±9.350a (9)	0.87±0.13 a (3)
	<i>P. italicum</i>	52.97±3.090A (4)	-
packinghouse IV	<i>P. digitatum</i>	39.23± 8.57a (3)	0.85±0.15 a (4)
	<i>P. italicum</i>	53.85± 2.89A (6)	0.53±0.03 A (2)

<sup>a</sup> $EC_{50}$ : Effective concentration of fungicide in the culture medium needed to inhibit 50% of mycelial growth. <sup>b</sup>No. of isolates used per sampling location are given in parentheses. <sup>c</sup>Values within columns followed by the same letter(s) (a or A) are not significantly different ( $p = 0.05$ ). Values are means of three replicates per isolate followed by the standard deviation of the mean

citrus orchard on fungicide-amended PDA were 0.14 and 0.046  $\mu\text{g mL}^{-1}$ , respectively. The mean  $EC_{50}$  values for mycelial growth reduction for 14 isolates of *P. italicum* obtained from infected fruit collected from an orchard, with no history of fungicides use, were 0.07  $\mu\text{g}$  of imazalil per mL and 0.15  $\mu\text{g}$  of thiabendazole per milliliter. These values are much lower than the values recorded by isolates recovered from fruit collected from commercial citrus packing houses. The citrus orchard populations were therefore considered as being indicative of wild-type populations.

The mean  $EC_{50}$  values of *P. digitatum* resistant-isolates, sampled from the four packing houses, varied from 39.23 to 50.84  $\mu\text{g mL}^{-1}$  of TBZ and from 0.81 to 0.98  $\mu\text{g mL}^{-1}$  of IMZ and were not significantly different ( $p = 0.05$ , Table 3). For *P. italicum*, the mean  $EC_{50}$  values for mycelial growth reduction for resistant-isolates were from 52.97 to 59.92  $\mu\text{g mL}^{-1}$  for TBZ and from 0.53 to 0.61  $\mu\text{g mL}^{-1}$  for IMZ, with no significant difference ( $p = 0.05$ , Table 3). On the basis of these results, it appears that mycelial growth of *P. digitatum* and *P. italicum* was more sensitive to IMZ than to TBZ.

## DISCUSSION

In the present study, the baseline sensitivity to IMZ and TBZ was determined using 25 and 14 wild-types

isolates of *P. digitatum* and *P. italicum*, respectively, collected in a citrus orchard without a previous history of fungicide exposure. Their sensitivity was compared to a representative *P. digitatum* and *P. italicum* populations isolated from decayed citrus fruits (oranges and mandarins) collected from commercial packing houses located in the SMD Valley. The development of fungal resistance to IMZ and TBZ has been well documented (Holloman, 1993; Holmes and Eckert, 1999; Ghosop *et al.*, 2007). The results presented here for SMD citrus packing houses isolates of *P. digitatum* and *P. italicum* also concur with these findings.

All *P. digitatum* and *P. italicum* isolates collected in a citrus orchard were sensitive *in vitro* to IMZ and TBZ. This result was consistent with earlier study (Holmes and Eckert, 1995, 1999; Kinay *et al.*, 2007) and suggests that naturally occurring resistant isolates of *P. digitatum* and *P. italicum* are rare to absent in citrus orchards, especially in those orchards without a prior history of fungicides usage. Nevertheless, isolates of *P. digitatum* resistant to TBZ, to IMZ, or to both fungicides were detected in all packing houses sampled. Resistance to IMZ was less common than resistance to TBZ in the SMD citrus packing houses. This result was consistent with earlier published data for *P. digitatum* populations from China (Zhu *et al.*, 2006) and from Sao Paulo (Brazil) citrus packing houses (Fischer *et al.*, 2009). The proportion of *P. digitatum* resistant isolates was as high as 19% (55/290) for IMZ and 37% (107/290) for TBZ. Considering the extensive and heavy use of both fungicides in commercial citrus packing houses, it is not surprising that resistance to TBZ and IMZ was so prevalent in *P. digitatum* populations sampled from SMD, Valley. As reported in a similar study (Bus *et al.*, 1991), among 115 *P. digitatum* isolates collected from citrus fruits at the wholesale markets in Rotterdam (Holland) and originating from Morocco, 30% of the isolates showed *in vitro* resistance to TBZ at 10  $\mu\text{g mL}^{-1}$  and only 2% showed resistance to IMZ tested at 0.2  $\mu\text{g mL}^{-1}$ . Unsatisfactory control of green mold has been observed in some commercial citrus packing houses in SMD. However, no relationship has been established between unsatisfactory control of the disease and a decrease in sensitivity to IMZ and TBZ in *P. digitatum* isolates recovered from fruit collected in packing houses. Present results demonstrate that the high proportion of isolates with reduced sensitivity to IMZ and TBZ may account for the unsatisfactory control of green mold in commercial citrus packing houses in the SMD Valley.



In contrast, only 2.5 (5/204) and 21% (44/204) of the *P. italicum* isolates collected in citrus packing houses showed growth on PDA amended with IMZ at  $0.1 \mu\text{g mL}^{-1}$  or TBZ at  $20 \mu\text{g mL}^{-1}$ , respectively. Similar proportion of *P. italicum* resistant isolates to IMZ and TBZ have been observed in Rotterdam from mandarin and orange fruits produced in Morocco (Bus *et al.*, 1991). The low occurrence of resistant *P. italicum* isolates to IMZ may be due to the low frequency of this species among *Penicillium* sp., populations in citrus packing houses (Bus *et al.*, 1991; Holmes and Eckert, 1999).

In this study, among 290 *P. digitatum* isolates tested 29 were resistant to both IMZ and TBZ; whereas only three out of 204 *P. italicum* isolates tested were resistant to both fungicides. This phenomenon of double resistance to IMZ and TBZ has also been reported on *P. digitatum* (Holmes and Eckert, 1999) and *P. italicum* (Bus *et al.*, 1991). The mutations in *CYP51* gene and in  $\beta$ -tubulin gene have been described as the most common mechanisms of resistance to IMZ and TBZ, respectively (Ma and Michailides, 2005; Schmidt *et al.*, 2006; Zhu *et al.*, 2006; Ghosop *et al.*, 2007). The number of resistant isolates collected from the 4 packing houses varied greatly, indicating that many factors influencing the development of resistance were involved, such as sanitation measures, layout of the packing house and fungicide-spray programs used in each packing house.

Present study indicated that the resistance of *P. digitatum* and *P. italicum* to IMZ and TBZ was not linked with any decrease in pathogenicity. This finding is consistent with earlier study, in which increased resistance to these fungicides was not associated with reduced pathogenicity (Gutter *et al.*, 1981; De Waard and van Nistelrooy 1990; Seidel *et al.*, 1990; Holmes and Eckert, 1995).

The mean  $\text{EC}_{50}$  values of *P. digitatum* sensitive isolates were  $0.036 \mu\text{g mL}^{-1}$  for IMZ and  $0.14 \mu\text{g mL}^{-1}$  for TBZ and were of the same order as those reported by Holmes and Eckert (1999) and Kinay *et al.* (2007) for nonexposed population. The 14 *P. italicum* isolates that were tested from citrus orchard were sensitive to IMZ and TBZ with mean  $\text{EC}_{50}$  values of 0.04 and  $0.16 \mu\text{g mL}^{-1}$ , respectively. Establishing the level of sensitivity of *P. digitatum* and *P. italicum* is critical for monitoring the evolution of resistance to TBZ and IMZ in populations of these important pathogens in the SMD citrus packing houses. This information is of great importance in planning efficient measures for the postharvest control of green and blue molds of citrus fruit and for

implementing effective anti-resistance strategies in the SMD commercial citrus packing houses.

In resistant *P. digitatum* isolates collected from packing houses, the mean  $\text{EC}_{50}$  values were from 39.23 to  $50.84 \mu\text{g mL}^{-1}$  for TBZ and from 0.81 to  $0.98 \mu\text{g mL}^{-1}$  for IMZ. Despite inhibitory differences, the  $\text{EC}_{50}$  values for TBZ and IMZ were consistent with other reports. For example, as reported by Holmes and Eckert (1991), the  $\text{EC}_{50}$  value for TBZ was greater than  $47 \mu\text{g mL}^{-1}$ , whereas  $\text{EC}_{50}$  values for IMZ varied from 0.87 to  $0.92 \mu\text{g mL}^{-1}$ , in isolates of *P. digitatum* collected in California citrus packing houses. In the case of *P. italicum* resistant isolates, the mean  $\text{EC}_{50}$  values for TBZ ( $52.97$ - $59.92 \mu\text{g mL}^{-1}$ ) were higher than reported by Bus in 1992 ( $22.6 \mu\text{g mL}^{-1}$ ). In contrast, the mean  $\text{EC}_{50}$  values ( $0.53$ - $0.61 \mu\text{g mL}^{-1}$ ) for *P. italicum* IMZ-resistant isolates used in this study were slightly higher compared to the mean  $\text{EC}_{50}$  value ( $0.52 \mu\text{g mL}^{-1}$ ) for 4 IMZ-resistant isolates collected in California citrus packing houses (Holmes and Eckert, 1999). According to Holmes and Eckert (1999), variability in the  $\text{EC}_{50}$  values reported by various researchers can be explained by differences in experimental procedures (e.g., inoculum age, medium pH) and the calculation of the  $\text{EC}_{50}$  value. Present observation that the levels of resistance to IMZ and TBZ did not differ significantly within populations of *P. digitatum* and *P. italicum* recovered from the 4 packing houses may be explained by the fact that these populations are subjected to a constant selection pressure, due to repeated application of the same fungicide. Imazalil and thiabendazole are applied in the SMD citrus packing houses at relatively high dosage rates ( $2,000 \mu\text{g mL}^{-1}$  for IMZ and  $4,800 \mu\text{g mL}^{-1}$  for TBZ) in a wax formulation that covers the surface of fruit.

The favorable environmental conditions in packing house, the abundant sporulation of the *Penicillium* sp., and the fact that all SMD packing houses use common fungicide-spray programs are factors contributing to the development of fungicide resistance (Dekker, 1986). Nevertheless, there has been no report of complete failure of green and blue molds control in any of the commercial citrus packing houses from which these resistant *P. digitatum* and *P. italicum* isolates were recovered. However, the sole reliance on TBZ and IMZ for more than 26 years and since, there is currently no effective chemical or biological alternatives available for the control of green and blue molds, could put the citrus industry in the SMD in a precarious position. Continuous monitoring programs for fungicide sensitivity determination are required in order to indicate the need to change the applied spray programs and thus, prevent



failures of control. To our knowledge, this report constitutes the first mention of resistance in populations of *P. digitatum* and *P. italicum* in SMD citrus packing houses to IMZ.

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