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Postinfection Activity, Residue Levels, and Persistence of Azoxystrobin, Fludioxonil, and Pyrimethanil Applied Alone or in Combination with Heat and Imazalil for Green Mold Control on Inoculated Oranges

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The postinfection activity of azoxystrobin (AZX), fludioxonil (FLU), and pyrimethanil (PYR), applied alone or in combination with imazalil (IMZ), in controlling postharvest green mold in 'Salustiana' oranges inoculated with *Penicillium digitatum* was studied. Fruits were immersed for 30 or 60 s in (i) water or water mixtures at 20 °C containing AZX, FLU, or PYR at 600 mg/L; and (ii) IMZ at 600 mg/L, alone or in combination with AZX, FLU, or PYR at 600 mg/L. Similar treatments were performed at 50 °C using the active ingredients at half rates with respect to the treatments at 20 °C. Fungicide residues in fruits were analyzed following treatments and after 14 days of simulated shelf life at 17 °C. AZX or FLU mixtures at 20 °C for 30−60 s similarly but moderately reduced green mold decay with respect to control fruit; differences due to dip time were not significant. Superior control of decay was achieved by PYR and, especially, IMZ, applied alone or in combination with AZX, FLU, or PYR. The activity of PYR at 20 °C was significantly dependent on treatment time, whereas that of IMZ and combined treatments at 20 °C was not. The effectiveness of FLU or PYR mixtures at 50 °C in controlling decay was similar and superior to that of AZX. The action of single- or double-fungicide application was not dependent on dip time in most samples. IMZ or combined mixtures at 50 °C were consistently more effective with respect to single-fungicide treatments with AZX, FLU, or PYR. The application of heated fungicide mixtures resulted in significantly higher residue accumulation in most fruit samples compared to treatments performed at 20 °C. The degradation rate of fungicides was generally low and dependent on treatment conditions such as time, temperature, and the presence or not of other fungicides.

KEYWORDS: *Citrus*; *Penicillium* decay; hot water dip; reduced risk fungicides; residues

INTRODUCTION

Green and blue molds, respectively caused by *Penicillium digitatum* Sacc. and *Pennicillium italicum* Wehmer, are major postharvest diseases of citrus fruit worldwide (*1*). Postharvest management of these pathogens is mainly based on spore-load reduction through sanitation with chlorine or sodium orthophenylphenate (SOPP) and by control of spore germination or fungal growth with thiabendazole (TBZ) or imazalil (IMZ) (*1*,*2*). However, the intensive use of SOPP, TBZ, and IMZ, separately or in combination, in citrus packinghouses in various countries favored the development of single-, double-, and triple-resistant isolates of *Penicillium* spp. and, hence, reduced the effectiveness of these active ingredients (*3*−*5*). Therefore, alternatives to these fungicides are required where resistant isolates have appeared to reduce the risk of selecting and spreading new resistant populations of the pathogens.

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In recent years various effective and relatively novel compounds have been developed to control a wide range of fungal diseases (*6*). Of these, azoxystrobin (AZX), fludioxonil (FLU), and pyrimethanil (PYR) are compounds belonging to different chemical classes and thus have different modes of action against fungal pathogens (*6*). These compounds are classified as reduced-risk fungicides by the U.S. Environmental Protection Agency (*7*) and have recently been registered there also for postharvest treatment of various horticultural crops, including citrus fruit (*8*). Studies have been performed to evaluate the potential of these compounds in controlling postharvest decay of citrus fruit (*8*−*11*). The comparative efficacies of AZX, FLU, and PYR were also evaluated when applied alone or in combination to lemon fruit that were wound-inoculated with *P. digitatum*-sensitive or -resistant to IMZ/TBZ (*8*). The addition of food preservatives such as sodium bicarbonate (*12*−*15*) or potassium sorbate (*16*) was shown to improve their performance. In addition, the residues and effectiveness of AZX (*17*), FLU (*18*), and

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PYR (*11*, *19*) were enhanced when they were used in combination with hot water. In the present study we investigated the residue level, persistence, and postinfection activity of AZX, FLU, and PYR, applied alone or in combination with IMZ, in controlling postharvest green mold in 'Salustiana' oranges inoculated with *P. digitatum* and subjected to standard treatments for 30 or 60 s at ambient temperature or using heated mixtures at 50 °C.

MATERIALS AND METHODS

Fruit. Commercially mature 'Salustiana' oranges [*Citrus sinensis* (L.) Osbek] were hand-harvested in an experimental orchard of the Institute of Sciences of Food Production of the National Research Council in central-western Sardinia, Italy, managed under standard horticultural practices. Fruits were placed in plastic trays and delivered to the laboratory immediately after harvest. Medium size fruits, free from rind defects, were selected, returned to each box, and left overnight at 20 °C.

Fungicides. The following commercial formulations were used in this study: (a) IMZ (Deccozil 50, 44.66%, active ingredient (ai), Decco Italia, Piano Tavola Belpasso, Catania, Italy); (b) AZX (Ortiva, 23.2 ai, Syngenta Crop Protection S.p.A., Milan, Italy); (c) FLU (Scholar, 20.4% ai, Syngenta Crop Protection S.p.A.); (d) PYR (Scala, 37.4% ai, BASF Italia, Milan, Italy).

Fungal Strains and Growth Conditions. Conidia of a *P. digitatum* isolate, obtained from decayed fruit harvested in the Institute's experimental orchard, where occasionally only copper fungicides were used, were cultured in Petri plates (90 mm diameter) containing 15 mL of potato dextrose agar (PDA; Merck & Co., Whitehouse Station, NY), stored at 4 °C, and periodically renewed using the same substrate. Inoculum for the experiment was obtained by inoculating Petri plates of potato dextrose agar with conidia of *P. digitatum* from plates held at 4 °C. After 1 week of incubation at 20 °C, conidia were collected by scraping the colony surface with a sterile scalpel, suspended in Ringer's solution (NaCl, 8.6 g/L; KCl, 0.3 g/L; and $CaCl_2$, 0.48 g/L), filtered through two layers of sterile cheesecloth, counted with a hemacytometer, and adjusted at 10^6 conidia/mL by adding sterile distilled water. Conidia of this isolate of *P. digitatum* were not able to germinate in PDA amended with either TBZ (15 μg/mL) or IMZ (0.1 μg/mL).

Assays with Wounded Artificially Inoculated Fruit. To determine the influence of treatments on decay, control fruits were segregated in 32 groups corresponding to the following dip treatments for 30 or 60 s: (a) water alone at 20 or 50 °C (controls); (b) IMZ, AZX, FLU, or PYR at 600 mg/L and 20 °C; (c) AZX, FLU, or PYR at 600 mg/L in combination with 600 mg/L IMZ at 20 °C; (d) IMZ, AZX, FLU, or PYR at 300 mg/L and 50 °C; (e) AZX, FLU, or PYR at 300 mg/L in combination with 300 mg/L IMZ at 50 °C. Dip treatments were performed by using a device described previously (*15*).

Then, oranges were disinfected by immersion for 30 s in 1% household bleach, rinsed with water, and left to dry at ambient temperature; each fruit was wounded by two slits equatorially, at the two opposite sides with a 2 mm wide and 2 mm long stainless steel rod and inoculated by dipping fruits for 1 min in a 72 L, high-density polyethylene tank containing 50 L of conidial suspension of *P. digitatum* (final concentration 1×10^6 conidia/mL). After inoculation, fruits were incubated at 20 °C and 90% relative humidity (RH) for approximately 20 h before treatments to simulate infection occurring at harvest either in the field or during transit.

Following treatments fruits were left to dry at room temperature and subsequently stored at 20 °C at ca. 90% RH. Fruits were evaluated for incidence of decay caused by green mold (percentage of fruit with decay lesions of any size compared to the total, within each replicate) after 7 days. Four replicates were used for each treatment. Each replicate contained 40 fruits.

Influence of Treatments and Storage Conditions on Residues and Persistence of IMZ, AZX, FLU, or PYR. Treatment groups for residue analysis were 28, which was 4 less than those used for the assays with inoculated fruit, not including the treatments with water alone.

Following treatments fruits were left to dry at ambient temperature, moved to a ventilated room, and kept at 17 °C and 90% RH (simulated shelf-life conditions). Analyses of fungicides were performed at time 0 (immediately after treatment) and after 14 days. All analyses were performed on four replicates per treatment.

Chemicals. Acetone and hexane were of GC grade (Merck, Milan, Italy). Sodium chloride was of analytical grade (Carlo Erba, Milan, Italy). The analytical standards of IMZ (at 97%), FLU (at 95%), AZX (at 99%), and PYR (at 97%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock standard solutions of the ai (500 mg/L) were prepared in acetone. Working standard solutions of ai were prepared by diluting the stock solution with the extract from untreated fruit.

Extraction Procedure and Residue Analysis. Six oranges per replication were weighed and their peels removed. The peels were weighed and frozen at −18 °C until residue analysis. Five grams of minced and homogenized peel was weighed in a 40 mL screw-cap flask, and 10 mL of acetone/hexane mixture (1:1, v/v) and 4 g of NaCl were added. For recovery assay samples of untreated orange peel were fortified with appropriate amounts of standard stock solutions to reach concentrations from 0.1 to 10 mg/kg. The recovery ranged from 97.8 to 103.6%, with a maximum 7% coefficient of variation (CV). Good linearity was achieved in the 0.1−10.0 mg/kg range, with correlation coefficients between 0.9996 and 0.9993.

Apparatus. A TQ Trace gas chromatograph, coupled with an NPD 80 detector, a split/splitless injector, and an AS200 autosampler (TermoQuest, Milan, Italy), was used. The column was a fused silica capillary DB 5 MS (30 m × 0.25 mm i.d.; film thickness = 0.10 μm, J&W Scientific, Folsom, CA). The injector and detector temperatures were set at 220 and 300 °C, respectively. The sample (2 μL) was injected in splitless mode (30 s). The oven temperature was programmed as follows: 90 °C (1 min), raised to 310 °C (at 15 °C/min). Helium was the carrier gas and N_2 the make up gas at 1.1 and 15 mL/min, respectively. NPD conditions were as follows: source current, 2.8; polarization voltage, 3.5. Oxygen and H_2 flows were at 60 and 2.3 mL/min, respectively.

Statistical Analysis. Statistical analysis was performed by Statgraphics software (Statgraphics Centurion, version XV, Herndon, VA). Analysis of variance (one-way ANOVA) was carried out using a unifactorial complete randomized block design. Percentages were transformed in arcsin $\sqrt{x}$ before ANOVA. Mean comparisons of the effects of treatments were calculated by Duncan's multiple-range test at $P \leq 0.05$.

RESULTS

Influence of Treatments on Decay Development in Inoculated Fruit. Treatments with AZX or FLU at 20 °C for 30 s significantly ($P < 0.05$) but moderately (ca. 12−13% control) reduced the incidence of decay with respect to fruit treated with water at 20 °C, where 100% of the fruit were visibly affected by green mold (**Figure 1**). Superior control of decay was achieved by PYR (ca. 38% control) and especially by IMZ, which resulted in total or almost total control of decay when applied alone or in combination with AZX, FLU, or PYR. The effectiveness of AZX and FLU mixtures at 20 °C did not improve when treatment time was extended from 30 to 60 s, whereas the performance of PYR at 20 °C increased significantly with increased dip time.

Water dips at 50 °C were ineffective in controlling green mold development (100% decay incidence) (**Figure 2**). FLU applied at 50 °C was as effective as PYR and more effective than AZX. Best control of decay was provided by IMZ, which reduced the incidence of green mold to very low levels (ca. 2%). The action of single and combined treatments at 50 °C was not significantly affected by dip time. However, IMZ mixtures were consistently more effective than single-fungicide treatments with AZX, FLU, or PYR when applied alone or in combination with AZX, FLU, or PYR at 50 °C.

Influence of Treatments and Storage Conditions on IMZ Residues. Following treatment with IMZ at 600 mg/L and 20 °C, alone or in combination with AZX or FLU for 30 or 60 s, residue levels of IMZ were similar (**Table 1**). Accordingly, IMZ residues were not affected by dip time even when IMZ was applied alone at 50 °C. By contrast, following treatment with IMZ in combination with PYR at 20 °C or with AZX, FLU, and PYR at 50 °C, residues of IMZ were notably dependent on dip time. Coapplication

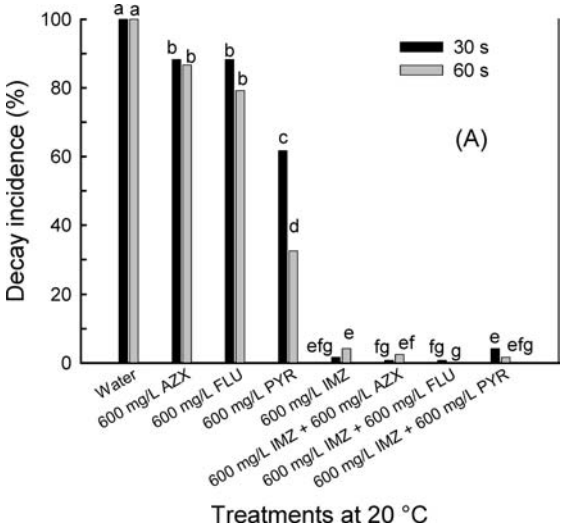

Figure 1. Incidence of postharvest green mold on 'Salustiana' oranges, inoculated with conidia of *P. digitatum* (10^6 conidia/mL) of an imazalil sensitive isolate 20 h prior to dip treatments at 20 °C for 30 or 60 s with azoxystrobin (AZX), fludioxonil (FLU), pyrimethanil (PYR), or imazalil (IMZ) at 600 mg/L or with IMZ at 600 mg/L in combination with AZX, FLU, or PYR at 600 mg/L. Unlike letters denote significant differences by Duncan's multiple-range test, $P \leq 0.05$.

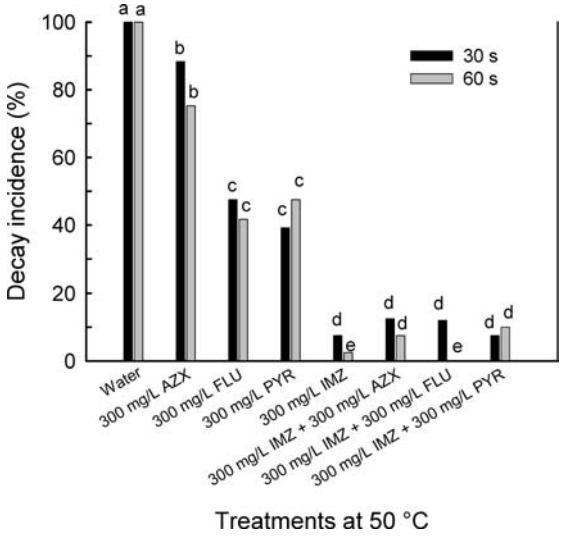

Figure 2. Incidence of postharvest green mold on 'Salustiana' oranges, inoculated with conidia of *P. digitatum* (10^6 conidia/mL) of an imazalil sensitive isolate 20 h prior to dip treatments at 50 °C for 30 or 60 s with IMZ, AZX, FLU, or PYR at 300 mg/L or with IMZ at 300 mg/L in combination with AZX, FLU, or PYR at 300 mg/L. The incidence of fruit decay was evaluated after 7 days of incubation at 20 °C. Unlike letters denote significant differences by Duncan's multiple-range test, $P \leq 0.05$.

of AZX, FLU, or PYR for 30−60 s at 20 °C consistently reduced IMZ residues with respect to fruit treated with IMZ alone; the influence of the other active ingredients at 50 °C was not clear, being dependent on type of fungicide and treatment duration. At equal treatment time, treatments with IMZ at 300 mg/L and 50 °C, alone or in combination with AZX, FLU, or PYR, produced higher residues (0.7−4.0-fold higher) than treatment with IMZ at 600 mg/L and 20 °C in all samples except those treated with IMZ−AZX or IMZ−PYR for 30 s, in which similar residues were recorded. IMZ residues showed marked persistence during 14 days of shelf life, decreasing slowly in most samples.

Table 1. Influence of Postharvest Treatments with Imazalil (IMZ), Alone or in Combination with Azoxystrobin (AZX), Fludioxonil (FLU), or Pyrimethanil (PYR), on IMZ Residues in 'Salustiana' Oranges after Treatment (Time 0) and after 14 Days of Simulated Shelf Life at 17 °C

		IMZ residues[b] (mg/kg, on a whole fruit basis)	
treatment[a]	dip time (s)	time 0	shelf life
600 mg/L IMZ 20 °C	30	3.00 de	1.38 ghi
	60	3.54 cd	1.65 fgh
600 mg/L IMZ + 600 mg/L AZX 20 °C	30	1.91 g−l	1.18 ghi
	60	2.53 efg	1.27 ghi
600 mg/L IMZ + 600 mg/L FLU 20 °C	30	1.10 l	0.95 i
	60	1.31 il	1.04 i
600 mg/L IMZ + 600 mg/L PYR 20 °C	30	2.22 g−l	0.86 i
	60	2.73 de	0.92 i
300 mg/L IMZ 50 °C	30	5.75 ab	2.10 de
	60	6.06 a	2.71 c
300 mg/L IMZ + 300 mg/L AZX 50 °C	30	2.34 fgh	0.94 i
	60	5.50 ab	2.45 cd
300 mg/L IMZ + 300 mg/L FLU 50 °C	30	2.54 efg	2.59 cd
	60	6.58 a	3.37 b
300 mg/L IMZ + 300 mg/L PYR 50 °C	30	2.23 g−l	1.78 ef
	60	4.60 bc	4.41 a

[a] Concentrations refer to active ingredient. [b] Means in columns followed by unlike letters differ significantly by Duncan's multiple-range test, $P \leq 0.05$.

Table 2. Influence of Postharvest Treatments with Azoxystrobin (AZX), Alone or in Combination with Imazalil (IMZ), on AZX Residues in 'Salustiana' Oranges after Treatment (Time 0) and after 14 Days of Simulated Shelf Life at 17 °C

		AZX residues[b] (mg/kg, on a whole fruit basis)	
treatment[a]	dip time (s)	time 0	shelf life
600 mg/L AZX 20 °C	30	0.45 bc	0.41 bcd
	60	0.48 bc	0.50 b
600 mg/L AZX + 600 mg/L IMZ 20 °C	30	0.41 bc	0.37 d
	60	0.40 c	0.40 bcd
300 mg/L AZX 50 °C	30	0.53 abc	0.47 bc
	60	0.57 ab	0.46 bcd
300 mg/L AZX + 300 mg/L IMZ 50 °C	30	0.47 bc	0.39 cd
	60	0.66 a	0.60 a

[a] Concentrations refer to active ingredient. [b] Means in columns followed by unlike letters differ significantly by Duncan's multiple-range test, $P \leq 0.05$.

Influence of Treatments and Storage Conditions on AZX Residues. After treatment with AZX at 20 or 50 °C, AZX fruit residues did not change significantly when treatment time was increased from 30 to 60 s (**Table 2**). After treatments with IMZ−AZX at 50 °C, AZX residues were significantly dependent on treatment time but were not with IMZ−AZX at 20 °C.

Whereas treatment with AZX, alone at 20 or 50 °C, or with IMZ−AZX at 20 °C did not reveal significant differences in AZX

Table 3. Influence of Postharvest Treatments with Fludioxonil (FLU), Alone or in Combination with Imazalil (IMZ), on FLU Residues in 'Salustiana' Oranges after Treatment (Time 0) and after 14 Days of Simulated Shelf Life at 17 °C

		FLU residues[b] (mg/kg, on a whole fruit basis)	
treatment[a]	dip time (s)	time 0	shelf life
600 mg/L FLU 20 °C	30	0.86 e	0.81 fg
	60	1.50 cd	1.36 bcd
600 mg/L FLU + 600 mg/L IMZ 20 °C	30	0.75 e	0.68 g
	60	0.79 e	0.77 fg
300 mg/L FLU 50 °C	30	2.24 b	1.29 cde
	60	3.19 a	1.91 a
300 mg/L FLU + 300 mg/L IMZ 50 °C	30	2.06 bc	0.87 efg
	60	3.71 a	1.03 efg

[a] Concentrations refer to active ingredient. [b] Means in columns followed by unlike letters differ significantly by Duncan's multiple-range test, $P \leq 0.05$.

Table 4. Influence of Postharvest Treatments Pyrimethanil (PYR), Alone or in Combination with Imazalil (IMZ), on PYR Residues in 'Salustiana' Oranges after Treatment (Time 0) and after 14 Days of Simulated Shelf Life at 17 °C

		PYR residues[b] (mg/kg, on a whole fruit basis)	
treatment[a]	dip time (s)	time 0	shelf life
600 mg/L PYR 20 °C	30	2.40 def	1.27 d
	60	2.84 cd	1.34 d
600 mg/L PYR + 600 mg/L IMZ 20 °C	30	1.43 f	1.41 d
	60	1.73 ef	1.60 cd
300 mg/L PYR 50 °C	30	3.55 c	2.88 c
	60	5.79 b	5.82 b
300 mg/L PYR + 300 mg/L IMZ 50 °C	30	5.94 b	4.89 b
	60	11.04 a	9.24 a

[a] Concentrations refer to active ingredient. [b] Means in columns followed by unlike letters differ significantly by Duncan's multiple-range test, $P \leq 0.05$.

residues as a function of dip time, IMZ−AZX at 50 °C for 60 s produced significantly more residues than treatment for 30 s. AZX residues were generally unaffected by dip temperature except for IMZ−AZX at 50 °C for 60 s, which produced significantly more residues (0.65-fold higher) than the respective treatment at 20 °C. After 14 days of storage, AZX residues decreased slowly, with reductions from 0 to approximately 19%, depending on treatment type.

Influence of Treatments and Storage Conditions on FLU Residues. When FLU was applied alone at 20 or 50 °C, or in combination with IMZ at 50 °C, residues increased significantly as treatment time increased from 30 to 60 s (**Table 3**). Conversely, differences in FLU residues as a function of treatment time were not significant after IMZ−FLU at 20 °C. FLU residues were unaffected by the presence of IMZ in most samples. At equal dip times, FLU residues after treatments at 50 °C were ca. 1.1−3.7-fold higher than those of treatments at 20 °C. The degradation rate of FLU residues was notably affected by treatment type, ranging from ca. 3 to 72% when compared to initial values.

Influence of Treatments and Storage Conditions on PYR Residues. Whereas PYR residues were similar after treatment with PYR or IMZ−PYR at 20 °C for 30−60 s, significantly higher residue values were found when these treatments were performed at 50 °C (**Table 4**). The coapplication of IMZ significantly increased PYR residues with respect to fruit treated with PYR alone. When PYR and IMZ−PYR mixtures were used at 50 °C, PYR residues were respectively higher (0.5-fold higher) and remarkably higher (5.4-fold higher) compared to the same treatments at 20 °C. After the shelf life period, residues of PYR in the fruit were still 47−100% of the initial values.

DISCUSSION

Previous studies have shown that when FLU is applied on artificially wounded, but not inoculated, fruit so as to facilitate the spread of natural wound infections and on sound fruit in simulated storage trials, it has proved to be very effective and almost completely controlled postharvest decay on 'Tarocco' oranges when applied at 400 mg/L and 20 °C or at 100 mg/L and 50 °C (*18*). Our present study revealed that FLU application at 600 mg/kg and 20 °C or at 300 mg/kg and 50 °C to 'Salustiana' oranges inoculated with *P. digitatum* had little effect in controlling green mold. These results are consistent with those of Smilanick et al. (*16*), who found that FLU at 300 mg/L (50% of the lowest recommended dose) did not effectively control green mold on 'Valencia' oranges artificially inoculated with *P. digitatum* before treatments; the influence of heat and the addition of potassium sorbate on its performance was small. On the other hand, Zhang (*9*) reported that although aqueous FLU mixtures, applied using a simulated commercial packingline at 250 mg/L, proved to be ineffective in reducing green mold on naturally infected 'Valencia' oranges, FLU effectiveness at concentrations of 500−1200 mg/L improved, becoming similar to that of IMZ or TBZ at 1000 mg/L, and was even effective in controlling TBZ-resistant *P. digitatum* isolates on oranges. However, trial conditions used by Schirra et al. (*18*) were quite different from those of the present study and from those of Zhang (*9*) and Smilanick et al. (*16*).

The application of aqueous mixtures of PYR at 300 mg/L on oranges and at 500 mg/L on lemons effectively controlled green mold and produced 1−2 mg/kg residues (*11*). The addition of sodium bicarbonate (*11*) or potassium sorbate (*16*) improved PYR control of green mold, whereas the influence of heat was irregular and small, although PYR residues in fruit were greatly increased by heat (*11*). On the other hand, D'Aquino et al. (*19*) proved the beneficial effects of heat in controlling green and blue mold when applied in combination with PYR, the reason presumably being the relatively long treatment time (3 min), whereas the treatment times (30−60 s) employed by Smilanick et al. (*11*) and those in the current study were not long enough to achieve the beneficial effects of heat, such as inhibition of pathogens, activation of host defense responses (*20*), enhanced diffusion and penetration of the ai into cuticular wax (*15*), and increased permeation of the ai into rind wounds (*21*).

Kanetis et al. (*10*) investigated the comparative efficacy of AZX, FLU, and PYR for management of citrus green mold decay and stated that when the fungicides were applied as aqueous mixtures, PYR exhibited the highest level of green mold control caused by both IMZ/TBZ-sensitive and -resistant isolates of *P. digitatum*. PYR was very effective, even when applied 21 h after inoculation when the pathogen developed into fruit tissue, revealing a local systemic-like activity. Conversely, whereas the efficacy of FLU and AZX was very high at the early stages of infection, it decreased as postinoculation time increased from 9 to 21 h, demonstrating their protective, nonsystemic action.

Accordingly, our results show that PYR was more effective than AZX and FLU in treatments performed at ambient temperature, but its efficacy was similar to that of FLU when applied at half rates and 50 °C; AZX was less effective. Differences in fungicide performance at 20 and 50 °C were ascribed to differences in fungicide mobility in fruit tissue. IMZ alone and in combined mixtures at 20 or 50 °C was consistently more effective than single-fungicide treatments with AZX, FLU, or PYR, resulting in total or almost total control of decay. The relatively low decay control of FLU and AZX when applied separately may be related to various factors, including the interval (20 h) between fruit inoculation and chemical treatment, fruit wound size (2 × 2 mm) adopted in the test, and the systemic properties of fungicides. With a deep injury and long period of time between inoculation and fungicide application, pathogen could penetrate into the fruit tissues, and the chemical may not reach the active infection sites if the fungicide formulations have poor systemic activity. Strategies to optimize the efficacy of these new fungicides for managing citrus green mold and minimizing the buildup of resistant isolates of the pathogen are reported by Kanetis et al. (*14*).

The rate of uptake and diffusion of organic chemicals into fruit cuticle is known to be affected by various factors, including cuticular wax composition (*22*, *23*), cuticle permeability (*24*, *25*), ai diffusion through the plant cuticle (*26*), and temperature (*27*, *28*). Our study reveals that while residues of FLU, PYR, and IMZ increased remarkably when dip temperature was raised from 20 to 50 °C, lower residue accumulation was recorded for AZX. These results are consistent with those reported previously indicating that residues of IMZ (*19*, *29*), AZX (*17*), FLU (*18*), and PYR (*11*, *19*) in citrus fruit were dependent on fungicide dosage and treatment temperature, concentrations being higher after treatment at 50 °C compared to treatments at 20 °C; treatments with FLU, PYR, or IMZ at 50 °C, for example, produced 2.6−4-, 13−19-, and 4−9-fold higher residues of FLU, PYR, and IMZ than the respective treatments at 20 °C. The increase in residues of AZX as temperature is raised from 20 to 50 °C was lower than in treatment with FLU, PYR, or IMZ (ca. 0.6−0.8-fold).

Residue levels in fruit are reported to be affected by treatment time and to depend on type of fungicide formulation and temperature. In citrus, for example, IMZ residues were found to be significantly dependent on treatment time (*29*−*31*), but TBZ residues were not (*31*). Our data reveal that when AZX, FLU, and PYR were applied at 50 °C, ai residues were essentially dependent on length of treatment and less affected by treatment time when mixtures were used at 20 °C. The influence of treatment time on IMZ residues was dependent on the presence of other fungicides, increasing significantly with treatment time, when IMZ was applied alone or in combination with PYR; residues were not affected by time when IMZ was applied in combination with AZX or FLU. Inter-relationships of residue deposition in citrus fruit as a function of treatment time have also been reported after application of the novel fungicides trifloxistrobin (*32*) and cyprodinil (*33*).

The U.S. maximum residue limit (MRL) for IMZ, AZX, FLU, and PYR for citrus fruit is 10 mg/kg (*34*). Our results show that post-treatment residues were within this tolerance limit except for those of IMZ−PYR at 50 °C for 60 s, which produced slightly higher PYR residues (10.05) than the U.S. MRL. However, residues decreased to lower values (9.24 mg/kg) after shelf life. All active ingredients showed marked persistence during 14 days of storage, depending on treatment conditions (time, temperature, and presence of other active ingredients), in agreement with previous studies on AZX (*17*), FLU (*18*), IMZ, and PYR (*19*).

LITERATURE CITED

(1) Eckert, J. W.; Eaks, I. L. Postharvest disorders and diseases of citrus fruit. In *The Citrus Industry*; Reuther, W., Calavan, E. C., Carman, G. E., Eds.; University of California Press: Berkeley, CA, 1989; Vol. 5, pp 179−260.

(2) Barkai-Golan, R. *Postharvest Diseases of Fruits and Vegetables. Development and Control*; Elsevier Science: Amsterdam, The Netherlands, 2001.

(3) Bus, V. G.; Bongers, A. J.; Risse, L. A. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. *Plant Dis.* **1991**, *75*, 1098–1100.

(4) Holmes, G. J.; Eckert, J. W. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology* **1999**, *89*, 716–721.

(5) Kinay, P.; Mansour, M. F.; Mlikota Gabler, F.; Margosan, D. A.; Smilanick, J. L. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Prot.* **2007**, *26*, 647–656.

(6) Tomlin, C. *The Pesticide Manual*, 11th ed.; British Crop Protection Council: London, U.K., 1997.

(7) U.S. Environmental Protection Agency (USEPA). General overview: reduced-risk pesticide program. Environmental Protection Agency of Pesticide Programs, Staff Background, **1998**; pp 2−4.

(8) Adaskaveg, J. E.; Kanetis, L.; Soto-Estrada, A.; Förster, H. A new era of postharvest decay control in citrus with the simultaneous introduction of three new 'reduced-risk' fungicides. *Proc. Int. Soc. Citriculture* **2004**, *10*, 999−1004

(9) Zhang, J. The potential of a new fungicide fludioxonil for stem-end rot and green mold control on Florida citrus fruit. *Postharvest Biol. Technol.* **2007**, *46*, 262–270.

(10) Kanetis, L.; Förster, H.; Adaskaveg, J. F. Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. *Plant Dis.* **2007**, *91*, 1502–1511.

(11) Smilanick, J. L.; Mansour, M. F.; Gabler, F. M.; Goodwine, W. R. The effectiveness of pyrimethanil to inhibit germination of *Penicillium digitatum* and to control citrus green mold after harvest. *Postharvest Biol. Technol.* **2006**, *42*, 75–85.

(12) Smilanick, J. L.; Mansour, M. F.; Margosan, D. A.; Gabler, F. M. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Dis.* **2005**, *89*, 640–648.

(13) Smilanick, J. L.; Mansour, M. F.; Sorenson, D. Pre-and postharvest treatments to control green mold of citrus fruit during ethylene degreening. *Plant Dis.* **2006**, *90*, 89–96.

(14) Kanetis, L.; Förster, H.; Adaskaveg, J. F. Optimizing efficacy of new postharvest fungicides and evaluation of sanitizing agents for managing citrus green mold. *Plant Dis.* **2008**, *92*, 261–269.

(15) Schirra, M.; D'Aquino, S.; Palma, A.; Angioni, A.; Cabras, P. Factors affecting the synergy of thiabendazole, sodium bicarbonate, and heat to control postharvest green mold of citrus fruit. *J. Agric. Food Chem.* **2008**, *56*, 10793–10798.

(16) Smilanick, J. L.; Mansour, M. F.; Mlikota Gabler, F.; Sorenson, D. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Biol. Technol.* **2008**, *47*, 226–238.

(17) Schirra, M.; Cabras, P.; Angioni, A.; Brandolini, V. Residue levels and storage decay control in 'Star Ruby' grapefruit after dip treatments with azoxystrobin. *J. Agric. Food Chem.* **2002**, *50*, 1461–1465.

(18) Schirra, M.; D'Aquino, S.; Palma, A.; Marceddu, S.; Angioni, A.; Cabras, P.; Scherm, B.; Migheli, Q. Residue level, persistence, and storage performance of citrus fruit treated with fludioxonil. *J. Agric. Food Chem.* **2005**, *53*, 6718–6724.

(19) D'Aquino, S.; Schirra, M.; Palma, A.; Angioni, A.; Cabras, P.; Migheli, Q. Residue levels and effectiveness of pyrimethanil versus imazalil when using heated postharvest dip treatments for control of *Penicillium* decay on citrus fruit. *J. Agric. Food Chem.* **2006**, *54*, 4721–4726.

(20) Schirra, M.; D'hallewin, G.; Ben-Yehoshua, S.; Fallik, E. Host pathogen interaction modulated by heat treatment. *Postharvest Biol. Technol*. **2000**, *21*, 71–85.
(21) Dore, A.; Molinu, M. G.; Venditti, T.; D'hallewin, G. Immersion of lemons into imazalil mixtures heated at 50 °C alters the cuticle and promotes permeation of imazalil into rind wounds. *J. Agric. Food Chem*. **2009**, *57*, 623–631.
(22) Heredia, A. Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. *Biochim. Biophys. Acta* **2003**, *1620*, 1–7.
(23) Hendrik, B.; Koch, K.; Cerman, Z.; Neinhuis, C. Evans Review no. 3: structure−function relationships of the plant cuticle and cuticular waxes − a smart material? *Funct. Plant Biol*. **2006**, *33*, 893–910.
(24) Schönherr, J.; Riederer, M. Foliar penetration and accumulation of organic chemicals in plant cuticles. *Rev. Environ. Contam. Toxicol*. **1989**, *108*, 1–70.
(25) Baur, P.; Marzouk, H.; Schönherr, J.; Bauer, H. Mobilities of organic compounds in plant cuticles as affected by structure and molar volumes of chemicals and plant species. *Planta* **1996**, *199*, 404–412.
(26) Jenks, M. A.; Ashworth, E. N. Plant epicuticular waxes. Function, production and genetics. *Hortic. Rev*. **1999**, *23*, 1–68.
(27) Baur, P.; Schönherr, J. Temperature dependence of the diffusion of organic compounds across plant cuticles. *Chemosphere* **1995**, *30*, 1331–1340.
(28) Schönherr, J.; Baur, P. Effects of temperature, surfactants and other adjuvants on rates of uptake of organic compounds. In *Plant Cuticles − an Integrated Functional Approach*; Kerstiens, G., Ed.; BIOS Scientific Publishers: Oxford, U.K., 1996; pp 135−155.
(29) Smilanick, J. L.; Michael, I. F.; Mansour, M. F.; Mackey, B. E.; Margosan, D. A.; Florers, D.; Weist, C. F. Improved control of green mold of citrus fruit with imazalil with warm water compared with its use in wax. *Plant Dis*. **1997**, *81*, 1299–1304.
(30) Brown, G. E.; Dezman, D. J. Uptake of imazalil by citrus fruit after postharvest application and the effect of residue distribution on sporulation of *Penicillium digitatum*. *Plant Dis*. **1990**, *74*, 927–930.
(31) Cabras, P.; Schirra, M.; Garau, V. L.; Angioni, A. Factors affecting imazalil and thiabendazole uptake and persistence in oranges following dip treatments. *J. Agric. Food Chem*. **1999**, *47*, 3352–3354.
(32) Schirra, M.; D'Aquino, S.; Palma, A.; Angioni, A.; Cabras, P.; Migheli, Q. Residues of the quinone outside inhibitor fungicide trifloxystrobin after postharvest dip treatments to control *Penicillium* spp. on citrus fruit. *J. Food Prot*. **2006**, *69*, 1646–1652.
(33) Schirra, M.; D'Aquino, S.; Cabras, P.; Angioni, A. Influence of postharvest application rates of cyprodinil, treatment time and temperature on residue levels and efficacy in controlling green mould on 'Valencia' oranges. *Food Addit. Contam*. **2009**, *26A*, 1033−1037.
(34) USDA Foreign Agricultural Services MRL Data Base, **2005**; www.mrldatabase.com (Dec 10, 2009).

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