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Residue Levels and Effectiveness of Pyrimethanil vs Imazalil When Using Heated Postharvest Dip Treatments for Control of *Penicillium* Decay on Citrus Fruit

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The influence of fungicide concentration and treatment temperature on residue levels of pyrimethanil (PYR) in comparison with the commonly used fungicide imazalil (IMZ) was investigated in orange fruits following postharvest dip treatments. The dissipation rate of PYR residues was recorded as a function of storage conditions. The fungicide efficacy against green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, was evaluated on different citrus varieties following the fungicide application at 20 or 50 °C. Residue levels of PYR in Salustiana oranges were significantly correlated with the fungicide dosage, but residue concentrations were notably higher (ca. 13−19-fold) after treatment at 50 °C as compared to treatments at 20 °C. After treatment at temperatures ranging from 20 to 60 °C, PYR and IMZ residues in Salustiana oranges were significantly correlated with dip temperatures. Dissipation rates of PYR during storage were negligible in both Salustiana and Tarocco oranges. Results obtained on wounded, noninoculated Miho satsumas revealed that when treatments were performed at 50 °C, PYR or IMZ concentrations needed to achieve the complete control of decay were 8- and 16-fold less than by treatment at 20 °C. When fruits were inoculated with either *P. digitatum* or *P. italicum*, the application of 400 mg L^{-1} PYR at 20 °C or 100 mg L^{-1} PYR at 50 °C similarly reduced green and blue mold development. These results were corroborated by storage trials on Marsh grapefruits and Tarocco oranges. The lowest concentration of PYR required to achieve almost total protection of the fruit against decay accounted for 100 mg L^{-1} at 50 °C and 400 mg L^{-1} at 20 °C, respectively. Treatments did not affect fruit external appearance, flavor, and taste. It is concluded that postharvest PYR treatment represents an effective option to control green and blue mold in citrus fruit and that integration of fungicide applications and hot water dips may reduce the possibility of selecting fungicide-resistant populations of the pathogen, as a consequence of increased effectiveness of the treatment.

KEYWORDS: Citrus fruit; disease control; hot water dip; fungicide treatments; pyrimethanil; residues; fungicide resistance

INTRODUCTION

Penicillium decay, caused by green (*Penicillium digitatum* Sacc.) or blue (*Penicillium italicum* Wehmer) mold, is the major cause of postharvest decay of citrus fruits worldwide (*1*). Chemical control of these pathogens on a commercial scale is mainly based on postharvest treatments with sodium *o*-phenylphenate, thiabendazole (TBZ), and imazalil (IMZ) (*2*), which are the only fungicides registered for use on citrus fruits in the European community. The extensive use of these chemicals favored the build-up of resistant isolates of *Penicillium* spp. (*3*, *4*), compromising their efficacy. Recent studies (*5*) have shown that TBZ performance against *P. digitatum* increased notably when warm fungicide mixtures (41 °C, 350 mg L^{-1} active ingredient, a.i.) were applied in combination with 3% (wt vol^{-1}) sodium bicarbonate ($NaHCO_3$) and that the combination of TBZ and $NaHCO_3$ was effective even with fruits inoculated with a TBZ-resistant isolate. A similar synergy was observed with IMZ and $NaHCO_3$ (*6*).

Over the past decade, to overcome the problems due to pathogenic strains resistant to "older" fungicides, a wide range of products with novel biochemical modes of action have been

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generated (*7*). Among them, the anilinopyrimidine pyrimethanil (PYR) is a relatively new synthetic active ingredient, which was developed for controlling *Botrytis cinerea* Pers. in viticulture (*7*, *8*). This product is being also used to control a wide range of fungal diseases on fruit, vegetable and ornamental crops (*9*). PYR inhibits mycelial elongation and the secretion of enzymes, such as cutinases, pectinases, and laccases, involved in plant cell wall degradation (*8*, *10*, *11*). In addition to this fungitoxic effect, PYR acts as a stimulator of plant nutrition in grapevines (*Vitis vinifera* L.), thus enhancing the physiological plant status and leading to a better defense capacity toward fungal infection (*12*).

The residue levels and degradation pattern of PYR were investigated on various horticultural crops following field treatments (*13*−*16*). The aim of this study was to determine (i) the influence of fungicide concentration and treatment temperature on residue levels of PYR in comparison with IMZ in oranges following postharvest dip treatments, (ii) the dissipation rate of PYR residues as a function of storage conditions, and (iii) the minimum concentration of PYR and IMZ capable to ensure effective control of *Penicillium* decay on different species of *Citrus* fruit after postharvest dip treatments at 20 or 50 °C.

MATERIALS AND METHODS

Fruit. Oranges [*Citrus sinensis* (L.) Osbek] cvs. Salustiana, Tarocco, and Sanguinello, grapefruits [*Citrus paradisi* (Marcf.)] cv. Marsh seedless, and satsumas (*Citrus unshiu* Marc.) cv. Miho were hand-harvested when commercially mature from the experimental orchard of the C.N.R. Istituto di Scienze delle Produzioni Alimentari, located in central-western Sardinia (Italy), receiving standard horticultural practices. Fruits were placed in plastic trays and delivered to the laboratory immediately after harvest. Then, fruits of medium size and free from rind defects were selected, returned to each box, and left overnight at 20 °C.

Influence of Treatment Temperature and Fungicide Concentration on Residue Levels of PYR and IMZ. *Fungicides.* The commercial formulations of IMZ (DECCOZIL 50, 44.66%. Janssen Pharmaceutica N.V., Turmhoutseweg 30, Beerse, Belgium) and PYR (SCALA, 37.4%, Hoechst Schering AgrEvo GmbH, Wolfenbüttel, Germany) were used in this study. All concentrations were expressed as active ingredients (a.i.).

Fruit. Oranges cv. Salustiana were subjected to the following 3 min dip treatments: (i) treatment with a PYR or IMZ water mixture containing 25, 50, 100, 200, or 400 mg L^{-1} a.i. at 20 or 50 °C; or (ii) treatment with a PYR or IMZ water mixture containing 150 mg L^{-1} a.i. at 20, 30, 40, 50, or 60 °C. After treatment, fruits were left to dry at room temperature for approximately 5 h, and then, peel samples were taken and kept frozen at −18 °C until analysis. All treatments were performed on four replicates of six fruits per treatment.

Chemicals. Acetone and hexane were of gas chromatography (GC) grade (Merck, Milan, Italy). Sodium chloride was analytical grade (Carlo Erba, Milan, Italy). The a.i. standards, PYR (at 95%) and IMZ (at 97%), were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solutions of the a.i. (500 mg kg^{-1}) were prepared in acetone. Working standard solutions of a.i. were prepared by diluting the stock solutions with extract from untreated matrix.

Extraction Procedure and Residue Analysis. Fruit samples were weighed, and their peels were removed. The peel was weighed, and its percentage with respect to the whole fruit weight was calculated. It was then minced with a mincing knife and homogenized. The samples were stored in a freezer at −20 °C until analysis. For PYR extraction, 5 g of homogenized sample was weighed in a 40 mL screw-capped flask to which 10 mL of acetone/hexane (1/1) and 6 g of NaCl were added. For IMZ extraction, a 2.5 g aliquot of peel sample was weighed in a 40 mL screw-capped tube; 20 mL of a acetone/hexane mixture (1/1, v/v) and 6 g of NaCl were added. Then, the mixtures were agitated in a rotatory shaker for 20 min. Subsequently, the phases were allowed to separate, and the organic layer was injected in GC for the analysis without any cleanup step. Residue analysis was performed by a TQ Trace Gas Chromatographer, coupled with a NPD 80 detector, a split/splitless injector, and a AS200 autosampler (Termo Quest, Milan, Italy) as described in a previous report (*16*).

Fungal Cultures and Pathogen Inoculation. Monosporic isolates of *P. digitatum* (coded PD-8, PD-5, and PD-a) and *P. italicum* (coded PI-4, PI-7, and PI-b) were obtained from rotten oranges cv. Tarocco harvested in an orchard located in central-western Sardinia (Italy) and cultured on potato dextrose agar (PDA; Merck & Co., Whitehouse Station, NY) amended with streptomycin sulfate and oxytetracycline hydrochloride (100 + 100 μg mL^{-1}) to prevent growth of bacterial contaminants. A conidial suspension (1×10^8 conidia mL^{-1}) of *P. italicum* or *P. digitatum* was prepared as follows: Fungal isolates were grown in Petri plates (90 mm diameter) containing 15 mL of PDA under constant fluorescent light; after 2 weeks of incubation at 25 °C, spores were collected by scraping the colony surface with a sterile scalpel, resuspended in sterile Ringer's solution (NaCl, 8.6 g L^{-1}; KCl, 0.3 g L^{-1}; and $CaCl_2$, 0.48 g L^{-1}), filtered through two layers of sterile cheesecloth, and counted with a hemocytometer.

Assays with Wounded and Artificially Inoculated Fruit. The efficacy of PYR against *P. digitatum* and *P. italicum* was compared with that of IMZ in trials conducted on Miho satsumas and Salustiana oranges. Fruit samples of each cultivar were subdivided into three groups, and each fruit was wounded by two slits (2 mm × 2 mm) equatorially at the two opposite sides. Then, fruits of the first and second group were artificially inoculated with *P. digitatum* and *P. italicum*, respectively, while fruits of the third group were not inoculated. Fruits were inoculated by dipping fruits for 2 min into a 72 L high-density polyethylene tank that contained 50 L of conidial suspension of each pathogen (final concentration, 1×10^4 conidia mL^{-1}).

Inoculated and noninoculated fruits were kept overnight at 20 °C. Then, fruits were subdivided into 15 subgroups corresponding to the following 3 min dip treatments: water at 20 °C; PYR at 200, 400, or 600 mg L^{-1} at 20 °C; IMZ at 200, 400, or 600 mg L^{-1}at 20 °C; water at 50 °C; PYR at 25, 50, or 100 mg L^{-1} at 50 °C; IMZ at 25, 50, or 100 mg L^{-1} at 50 °C; and untreated (control) fruits. Dip treatments were performed as described previously (*17*).

Following treatments, fruits were left to dry at room temperature and then transferred to a storage room and kept at 20 °C and ca. 90% relative humidity (RH). Each treatment was applied to three replicates of 20 fruits each. The percentages of infected wounds on noninoculated fruits and their causative agents of green and blue mold decay in wounds artificially inoculated with *P. digitatum* or *P. italicum* were recorded after 5 days in Miho satsumas and after 7 and 12 days in Salustiana oranges.

Trials on simulated natural wounds were also performed on Tarocco, Sanguinello, and Valencia Late oranges, to assess the minimal concentration of fungicide capable of controlling decay. Oranges were grouped into 10 treatment groups corresponding to 3 min dipping treatments in water at 20 or 50 °C and PYR at 50, 100, 200, or 400 mg L^{-1} at 20 or 50 °C.

Following treatments, fruits were left to dry at room temperature and then transferred to a storage room and kept at 20 °C and ca. 90% RH. After 1 week, the percentage of infected wounds was determined. Each treatment was applied to three replicates of 40 fruits each.

Storage Trials with Sound Fruit. Grapefruits cv. Marsh Seedless were placed into eight treatment groups (three fruit boxes per treatment), corresponding to the following 3 min dip treatments: water at 20 or 50 °C and PYR at 100, 200, or 400 mg L^{-1} at 20 or 50 °C. After treatment, fruits were stored at 10 °C for 11 weeks plus 2 weeks at 20 °C (simulated marketing period, SMP). The RH during storage and SMP were set at 90 and 75%, respectively.

Oranges cv. Tarocco were grouped into four treatment groups (five fruit boxes per treatment), corresponding to the following 3 min dip treatments: (i) water at 20 °C (control); (ii) PYR at 400 mg L^{-1} at 20 °C; (iii) water at 50 °C; and (iv) PYR at 100 mg L^{-1} at 50 °C. Following treatment, oranges were kept under cold quarantine conditions at 1 °C for 3 weeks (*18*). After quarantine, fruits were stored for 6 weeks at 8 °C and then at 20 °C for 2 additional weeks SMP. The RH during quarantine and storage was 90% while during SMP it was 75%. Each treatment group included two subgroups. Three replicate fruit boxes

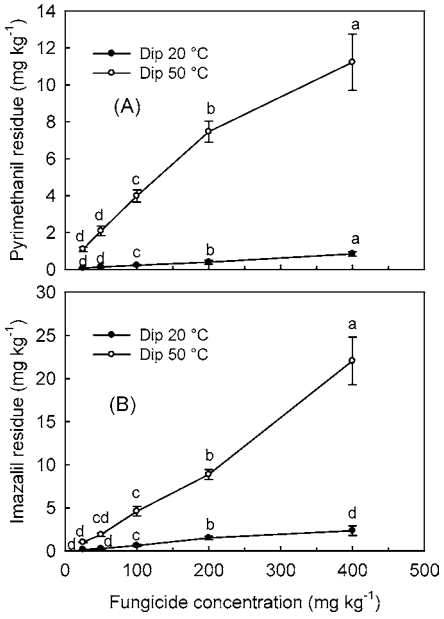

Figure 1. Influence of PYR (**a**) and IMZ (**b**) concentration on residue levels (mg kg^{-1} on a whole fruit basis) in Salustiana oranges following treatment with PYR- or IMZ-containing commercially available fungicide mixtures at 20 or 50 °C. Values are the means of four replicates. Vertical bars indicate standard deviation. The treatment was a 3 min dip followed by air drying of the fruit. Within each treatment temperature, different letters indicate statistical differences as per Tukey's test, $P \leq 0.05$.

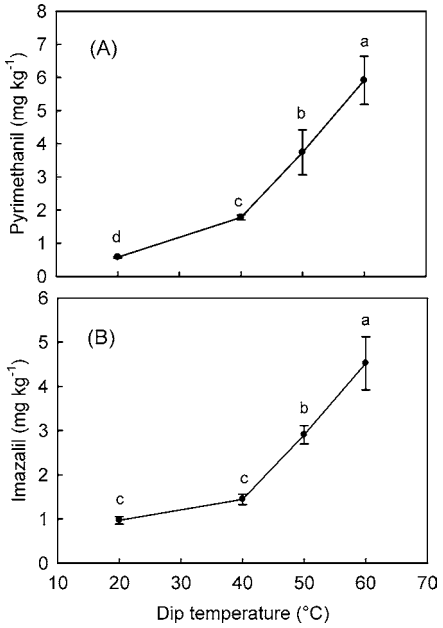

Figure 2. Influence of dip temperature on PYR (**a**) and IMZ (**b**) on residue levels (mg kg^{-1} on a whole fruit basis) in Salustiana oranges following treatment with 150 mg L^{-1} PYR- or IMZ-containing commercially available fungicide mixtures. Values are the means of four replicates. Vertical bars indicate standard deviation. The treatment was a 3 min dip followed by air drying of the fruit. Different letters indicate statistical differences as per Tukey's test, $P \leq 0.05$.

from the first subgroup were used for visual assessment (treatment damage and decay) and organoleptic characteristics, while fruits of the remaining group were used for PYR residue analysis.

Salustiana oranges were grouped into four groups (two fruit boxes per treatment) used for treatment with 100 or 400 mg L^{-1} PYR at 20 or 50 °C, respectively. Following treatment, fruits were transferred to a ventilated room and stored at 17 °C and 75% RH for 3 weeks. Analysis of PYR residues was performed after treatment and at the end of storage.

Statistical Analysis. Statistical analysis was performed by Statgraphics software (Manugistics, version 5 Professional, 2000, Herndon, VA) statistical program. Analysis of variance (ANOVA) was carried out according to a single factor, complete randomized block design with three or four replicates for each treatment, respectively, for visual assessment and residue analysis. Percentages were subjected to the ANOVA or transformed in $arcsin\sqrt{x}$ or $\sqrt{x}$ before the ANOVA, depending on the range of variation of data (*19*). Mean comparisons of the effects of treatments were calculated, where applicable, by Tukey's test, $P \leq 0.05$. Actual values are shown. To determine the pattern of PYR or IMZ accumulation in fruit, plots of residue levels vs dip temperature or fungicide concentration were computed for each data set, and the maximum squares of correlation coefficients were used to determine the equation of the best-fit curve.

RESULTS

Influence of Fungicide Concentration on Residue Level. There was a significant relationship between PYR residue levels in Salustiana oranges and the amount of fungicide employed after treatment with 25−400 mg L^{-1} at 20 or 50 °C, according to a multiplicative relationship either at 20 ($P < 0.001$; $R^2 = 0.9435$; $y = 0.0042x^{0.868}$) or 50 °C ($P < 0.001$; $R^2 = 0.9803$; $y = 0.0729x^{0.856}$) (**Figure 1a**). Treatments at 50 °C produced residue levels notably higher than those at 20 °C, from ca. 13- to 19-fold (**Figure 1a**). The pattern of IMZ accumulation after treatments was similar to that of PYR, when applied at the same temperature. That is, at 20 °C, $P < 0.001$, $R^2 = 0.9529$, and $y = 0.00568x^{1.01704}$ while at 50 °C, $P < 0.001$, $R^2 = 0.9902$, and $y = 0.0259x^{1.11508}$). The increase in residues raising the temperature from 20 to 50 °C was lower (ca. 4−9-fold) than in treatment with PYR (**Figure 1b**).

Influence of Dip Temperature on Fungicide Deposition. PYR residues in Salustiana oranges after treatments at temperatures ranging from 20 to 60 °C were significantly correlated ($P < 0.0001$; $R^2 = 0.9949$) with dip temperatures. The pattern of residue accumulation described a double reciprocal regression equation [$y = 1/(-0.652355 + 47.7186/x)$] (**Figure 2a**). In particular, treatments at 40, 50, and 60 °C produced a deposition of PYR, which was approximately 3-, 4-, and 10-fold higher, respectively, than deposition at 20 °C. Treatment with IMZ described a reciprocal-y curve [$y = 1/(1.49 - 0.021726x)$] with a highly significant correlation ($P < 0.0001$; $R^2 = 0.9544$) between treatment temperature and residue deposition (**Figure 2b**). IMZ treatments at 40, 50, and 60 °C produced approximately 1.5-, 3-, and 5-fold, respectively, more residues than treatment at 20 °C.

Influence of Fungicide Treatment and Storage Conditions on Residue Dissipation Rate. PYR residues in Salustiana oranges after storage were very similar to those found immediately after treatment (**Table 1**). Similarly, PYR residues in Tarocco oranges after SMP were similar to their initial levels (**Table 2**).

Effects of Treatments on Decay Development in Wounded Noninoculated Fruit. Trials on Miho satsumas confirmed their high susceptibility to decay (**Table 3**). Indeed, after 5 days of storage at 20 °C, all wounded, noninoculated fruit, untreated or dipped in water at 20 °C, were infected (mainly by *P. digitatum*). Hot water treatment reduced decay incidence by 57%, as compared to untreated control fruit, while treatment with 200 mg L^{-1} PYR or IMZ at 20 °C provided 92 and 97.5% decay control, respectively. Complete suppression of decay in wounded, noninoculated fruit was achieved with PYR or IMZ at 400 mg L^{-1} and 20 °C, whereas remarkably lower concentra-

Table 1. PYR Residues (on a Whole Fruit Basis) in Salustiana Oranges Immediately Following Dip Treatments (Time 0) and after 3 Weeks of Storage at 17 °C

	postharvest conditions fungicide residues (mg kg^{-1})[b]	
treatments[a]	time 0	3 weeks at 17 °C
400 mg L^{-1} at 20 °C	0.84 ± 0.12	0.61 ± 0.03
100 mg L^{-1} at 50 °C	3.98 ± 0.03	3.32 ± 0.16

[a] The treatment was a 3 min dip followed by air drying of the dipped fruit. [b] Each value is a mean of four replicates (± standard deviation).

Table 2. PYR Residues (on a Whole Fruit Basis) in Tarocco Oranges Immediately Following Dip Treatments (Time 0) and after Cold Quarantine for 3 Weeks at 1 °C Plus Standard Storage for 6 Weeks at 8 °C and an Additional 2 Weeks of SMP at 20 °C

	postharvest conditions fungicide residues (mg kg^{-1})[b]	
treatments[a]	time 0	SMP
400 mg L^{-1} at 20 °C	0.64 ± 0.11	0.44 ± 0.04
100 mg L^{-1} at 50 °C	3.29 ± 0.14	3.28 ± 0.20

[a] The treatment was a 3 min dip followed by air drying of the dipped fruit. [b] Each value is a mean of four replicates (± standard deviation).

Table 3. Influence of Postharvest Treatments on the Percentages of Natural Incidence of Decay in Noninoculated Wounds and of Green and Blue Mold Decay in Wounds Artificially Inoculated with *P. digitatum* or *P. italicum* in Miho Satsumas after 5 Days of Incubation at 20 °C

			inoculated wounds	
treatments[a]	fungicide concn (mg L^{-1})	noninoculated wounds (natural infections %)[b]	*P. digitatum* (%)	*P. italicum* (%)
untreated		100 d	100 e	100 h
water at 20 °C		100 d	100 e	100 h
PYR at 20 °C	200	8.0 b	27.0 c	20.0 de
	400	0.0 a	17.0 abc	5.0 a
	600	0.0 a	16.0 abc	8.0 a
IMZ at 20 °C	200	2.5 a	15.0 abc	21.7 e
	400	0.0 a	15.0 abc	8.8 abc
	600	0.0 a	17.5 abc	8.3 ab
water at 50 °C		43.0 c	59.0 d	89.0 g
PYR at 50 °C	25	1.0 a	24.0 bc	19.2 cde
	50	0.0 a	13.0 ab	18.8 b–e
	100	0.0 a	8.0 a	9.0 a–d
IMZ at 50 °C	25	0.0 a	16.0 abc	35.8 f
	50	0.0 a	17.0 abc	27.0 ef
	100	0.0 a	8.0 a	20.4 e

[a] Natural infections in noninoculated fruit were mainly due to *P. digitatum*. [b] The treatment was a 3 min dip followed by air drying of the fruit. Values in each column followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$. Each value is a mean of three replicates (20 fruits per replicate and two wounds per fruit).

tions of PYR (50 mg L^{-1}) and IMZ (25 mg L^{-1}) were still effective when applied at 50 °C, that is, 8- and 16-fold less than treatments at 20 °C with PYR and IMZ, respectively. In Salustiana oranges, hot water treatment reduced the percentage of natural infections over 90% and approximately 19% after 7 and 12 days, respectively, in comparison with untreated control fruits (**Table 4**).

In wounded, noninoculated Tarocco, Sanguinello, and Valencia oranges, hot water treatment significantly reduced the percentage of decay. Treatments with PYR were significantly more effective than hot water. The complete suppression of decay was achieved with 200 mg L^{-1} PYR applied at 20 °C or with 50 mg L^{-1} PYR applied at 50 °C (**Table 5**).

Table 4. Influence of Postharvest Treatments on the Percentages of Infected Wounds after 7 and 12 Days of Storage at 20 °C in Salustiana Oranges Noninoculated or Artificially Inoculated with *P. digitatum* or *P. italicum*[a]

		storage duration (days)					
		noninoculated wounds (natural infections %)		inoculated wounds			
				P. digitatum (%)		*P. italicum* (%)	
treatments[a]	fungicide concn (mg L^{-1})	7	12	7	12	7	12
untreated		34.2 b	84.2 ab	99.2 a	100 a	68.3 a	96.7 a
water at 20 °C		53.3 a	94.2 a	98.3 a	100 a	54.2 a	95.8 a
PYR at 20 °C	200	0.0 d	0.0 c	0.0 c	8.3 b	0.0 c	0.0 c
	400	0.0 d	0.0 c	0.0 c	3.3 bc	0.0 c	0.0 c
	600	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
IMZ at 20 °C	200	0.0 d	0.0 c	0.0 c	1.7 cd	0.0 c	0.0 c
	400	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
	600	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
water at 50 °C		3.3 c	68.3 b	76.7 b	100 a	12.5 b	49.2 b
PYR at 50 °C	25	0.0 d	0.8 c	0.0 c	0.0 d	0.0 c	0.0 c
	50	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
	100	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
IMZ at 50 °C	25	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
	50	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c
	100	0.0 d	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c

[a] The treatment was a 3 min dip followed by air drying of the fruit. Values in each column followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$. Each value is a mean of three replicates (20 fruits per replicate and two wounds per fruit).

Table 5. Influence of Postharvest Treatments on the Percentages of Infected Wounds in Artificially Wounded Tarocco, Sanguinello, and Valencia Late Oranges after 12 Days at 20 °C[a]

		% infected wounds[b] PYR concentrations				
orange cultivars	dip temp (°C)	0	50	100	200	400
Tarocco	20	89.2 (a)d	5.0 (a)c	0.8 (a)b	0.0 (a)a	0.0 (a)a
	50	26.7 (b)b	0.0 (b)a	0.0 (a)a	0.0 (a)a	0.0 (a)a
Sanguinello	20	76.7 (a)a	14.7 (a)b	1.3 (a)a	0.0 (a)a	0.0 (a)a
	50	22.7 (b)b	0.0 (b)a	0.0 (a)a	0.0 (a)a	0.0 (a)a
Valencia	20	60.1 (a)d	4.2 (a)b	2.5 (a)ab	1.7 (a)ab	0.0 (a)a
Late	50	15.8 (b)b	0.0 (b)a	0.0 (a)a	0.0 (a)a	0.0 (a)a

[a] The treatment was a 3 min dip followed by air drying of the fruit. [b] Each value is a mean of three replicates (40 fruits per replicate). In each row or column grouping, means separation by Tukey's test, $P \leq 0.05$. Letters without parentheses relate to comparisons of the effects of PYR concentration within each dip temperature. Letters in parentheses relate to comparisons of the influence of dip temperature within each PYR concentration.

Treatment Effect on Decay Development in Wounded Artificially Inoculated Fruit. The number of infections of green and blue mold in Miho satsumas artificially inoculated with *P. digitatum* and *P. italicum* was significantly reduced by hot water treatment (**Table 3**). Infections caused by *P. digitatum* were reduced by about 40% by hot water treatment, while those caused by *P. italicum* were reduced by only 10%. Treatments with 200 mg L^{-1} PYR and IMZ were significantly more effective than hot water and similarly reduced decay caused by *P. digitatum* and *P. italicum*. Treatments with 400 mg L^{-1} PYR or IMZ at 20 °C were significantly more effective than the respective treatments with 200 mg L^{-1}, while treatment with 600 mg L^{-1} was as effective as treatment with 400 mg L^{-1} of both fungicides. In Salustiana oranges, the effect of hot water against *P. digitatum* and *P. italicum* was significant during 7

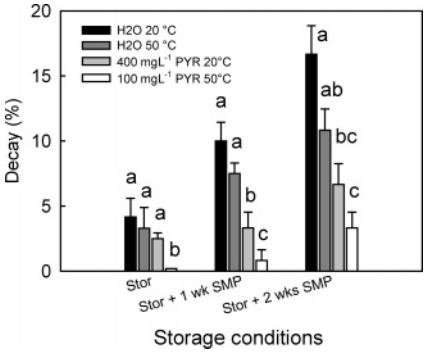

Figure 3. Influence of 3 min dip treatments with PYR at 20 or 50 °C on the decay incidence in Tarocco oranges after cold quarantine for 3 weeks at 1 °C plus standard storage for 6 weeks at 8 °C (storage) and subsequent simulated marketing period (SMP) at 20 °C for 1 or 2 weeks. Values are the means of four replicates (40 fruits per replicate). Vertical bars indicate standard deviation. Within each storage period, different letters indicate statistical differences as per Tukey's test, $P \leq 0.05$.

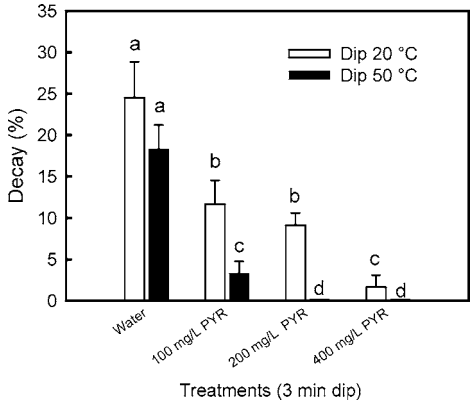

Figure 4. Influence of 3 min dip treatments with PYR at 20 or 50 °C on the decay incidence in Marsh Seedless grapefruits after 11 weeks of storage at 10 °C plus 2 weeks of simulated SL at 20 °C. Values are the means of three replicates (40 fruits per replicate). Vertical bars indicate standard deviation. The fungicide concentrations refer to active ingredients. Treatments are 3 min dips followed by air drying of the dipped fruit. Different letters indicate statistical differences as per Tukey's test, $P \leq 0.05$.

and 12 days of storage, respectively (**Table 4**). Treatments with 200, 400, and 600 mg L^{-1} PYR at 20 °C provided ca. 92, 97, and 100% control of green mold after 12 days of storage. IMZ treatment at 20 °C was significantly more effective than the respective treatment with PYR, while when higher doses of active ingredient were used, differences between PYR and IMZ were not significant. All treatments at 50 °C completely suppressed green mold decay development. Blue mold was not detected over 12 days of incubation in the inoculated wounds when fruits were subjected to fungicide treatment at 20 or 50 °C

Fruit Storage Response to Dip Treatments. The decay incidence among Tarocco oranges after cold quarantine for 3 weeks at 1 °C plus standard storage for 6 weeks at 8 °C (quarantine + storage) was approximately 4% in fruits subjected to treatment in water at 20 °C with no significant differences between hot water dipping and 400 mg L^{-1} PYR treatment at 20 °C (**Figure 3**). By contrast, 100 mg L^{-1} PYR at 50 °C nearly eliminated green mold. After the subsequent first and second weeks of SMP, the decay incidence (mainly incited by *P. digitatum*) increased by 2.5- and 4-fold, respectively.

In trials on Marsh grapefruits subjected to 11 weeks of standard storage at 10 °C plus 2 weeks SMP, hot water dipping did not significantly reduce the incidence of rotten fruits (**Figure 4**). Treatments at 20 °C with 200 mg L^{-1} PYR were equally effective ($P > 0.05$) as those with 100 mg L^{-1} PYR at 20 °C but significantly less effective than those with 400 mg L^{-1} PYR at 20 °C and with 100 mg L^{-1} PYR at 50 °C, which resulted in almost complete control of decay. Total suppression of decay was achieved with both 200 and 400 mg L^{-1} PYR at 50 °C. There were no treatment-dependent damages (peel necrosis, browning to the rind) and fruit flavors and tastes were unaffected by treatments (data not shown).

DISCUSSION

Many studies have been conducted in recent years to reduce the strong dependence on fungicides used for controlling postharvest decay of horticultural crops (*2*). Nonetheless, fungicides play a key role in pest management, and at present, they cannot be completely replaced by alternative methods. Novel, low-risk, broad-spectrum fungicides, with diverse mechanisms of action as compared to other approved pesticides in a crop group, are available on the market and may also be used to cope with the problems due to the selection of fungal populations resistant to older fungicides.

The baseline sensitivities of *P. digitatum* populations to novel fungicides belonging to different chemical classes such as azoxystrobin, fludioxonil, and PYR have been established by Kanetis et al. (*20*). Investigations on TBZ-sensitive and -resistant isolates in postinoculation treatments of pear fruit (*Pyrus communis* L.) have shown that the new "reduced-risk" fungicides fenhexamid, fludioxonil, and PYR were highly active against gray mold caused by *B. cinerea*, whereas blue mold caused by *P. expansum* was effectively controlled only by fludioxonil and PYR (*21*). In preinoculation treatments, fenhexamid was very effective against gray mold, whereas fludioxonil and PYR were effective against blue mold. Sholberg et al. (*22*) have shown that pre- or postharvest application of PYR was effective in controlling gray and blue mold on stored apples and may represent a possible alternative to TBZ where TBZ-resistant populations of these pathogens have been selected.

Investigations on pear (*P. communis*) and bitter orange (*Citrus aurantium* L.) have shown that rates of diffusion and penetration of organic chemicals into leaves and fruits increase greatly; increasing temperatures and equilibriums will be obtained faster at higher temperatures (*23*). This may explain why, when treatments are performed with heated fungicides, considerably lower concentrations are required to achieve comparable performances and residues left by standard treatments with fungicide mixtures at room temperature (*24*). Accordingly, the present study showed that following postharvest treatments, PYR and IMZ residues in oranges were closely dependent on fungicide concentration and temperature.

Following treatment with 400 mg L^{-1} PYR at 20 °C, residue levels in oranges were far below (approximately 5-fold) those left by treatments with 100 mg L^{-1} PYR at 50 °C and remained stable during storage. By contrast, PYR residue levels in tomato fruits decreased rapidly, dropping by half within 6 days after spraying of tomato plants grown in a commercial greenhouse and reaching the detection limit after 2 weeks (*15*). Similar results were reported by Garau et al. (*14*). In strawberries, PYR residues declined over the 10 day sampling, with a half-life time of 6 days (*16*). As photodegradation plays an important role in the disappearance of PYR (*14*), the high persistence of PYR recorded in the present study was ascribed to the absence of light and to the relatively low storage temperature.

Postharvest treatments with PYR represent an effective tool to control green and blue mold in citrus fruits, especially when applied at 50 °C. Considering that the mode of action of PYR is different from that presented by the products currently in use for *Penicillium* decay management, i.e., TBZ and IMZ, the use of alternative effective fungicides, such as PYR, should remediate problems related to the appearance of populations of *Penicillium* spp. resistant to TBZ and IMZ (*3*, *4*). Also, integration of fungicide applications and hot water dips may reduce the possibility of selecting fungicide-resistant populations of the pathogen by increasing the effectiveness of the treatment.

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