Journal of Food Protection, Vol. 69, No. 7, 2006, Pages 1646–1652

Residues of the Quinone Outside Inhibitor Fungicide Trifloxystrobin after Postharvest Dip Treatments To Control *Penicillium* spp. on Citrus Fruit

MARIO SCHIRRA,[1]* SALVATORE D'AQUINO,[1] AMEDEO PALMA,[1] ALBERTO ANGIONI,[2] PAOLO CABRAS,[2] AND QUIRICO MIGHELI[3]

[1]*Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari, Sezione di Sassari, Via dei Mille 48, 07100 Sassari, Italy;* [2]*Dipartimento di Tossicologia, Università di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy; and* [3]*Department of Plant Protection and Center for Biotechnology Development and Biodiversity Research, University of Sassari, Via E. De Nicola 9, 07100 Sassari, Italy*

MS 05-507: Received 30 September 2005/Accepted 28 February 2006

ABSTRACT

The effectiveness of postharvest dip treatment with trifloxystrobin (TFX) or imazalil (IMZ) was compared for controlling green and blue mold (caused by *Penicillium digitatum* and *Penicillium italicum,* respectively) of citrus fruit. Residues retained by fruit were determined as a function of treatment time, dip temperature, and storage conditions. Trials on 'Avana apireno' mandarin oranges artificially inoculated with *P. digitatum* or *P. italicum* revealed that treatments with 200 to 600 mg/liter active ingredient TFX at 20°C were less effective than 100 mg/liter TFX at 50°C for controlling *P. digitatum* but equally effective for controlling *P. italicum.* IMZ treatments with 200 mg/liter IMZ at 20°C or 25 mg/liter IMZ at 50°C resulted in more than 98% reduction of *P. digitatum* and ca. 93% reduction of *P. italicum* compared with untreated fruit. Total suppression of pathogens was achieved when higher IMZ doses were applied. Studies on artificially wounded lemons, oranges, clementines, and mandarins revealed that treatment with 100 mg/liter TFX at 50°C effectively controlled decay development (mainly due to *P. digitatum*) after 7 days of storage at 20°C. These results were confirmed on nonwounded oranges of cv. Tarocco and on grapefruits of cvs. Marsh Seedless and Star Ruby during 3 weeks of simulated quarantine at 1°C, storage (5 weeks at 8°C for oranges and 8 weeks at 11°C for grapefruits), and an additional 1 week of simulated marketing conditions at 20°C. IMZ at 50°C was highly effective for controlling decay during storage and the simulated marketing period. TFX treatment at 50°C was as effective as IMZ for controlling decay in most samples. After treatment with 100 mg/liter TFX at 20°C, fungicide residues in 'Tarocco' oranges doubled from 0.15 mg/kg to 0.30 mg/kg when dip time increased from 0.5 to 3 min, whereas when treatments were performed at 50°C TFX residues were not related to dipping time. Residues of TFX were significantly correlated with dip temperature. A 3-min dip treatment at 50°C resulted in a deposition of TFX that was approximately twofold higher than that obtained when treatments were carried out at 20°C.

Green and blue mold caused by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer, respectively, are the most common postharvest diseases of citrus fruit worldwide *(25).* On a commercial scale, postharvest protection of citrus fruit is mainly obtained with sodium *o*-phenylphenate, thiabendazole, and imazalil (IMZ), which are the only fungicides registered in the European Union for postharvest treatments of citrus fruit. To manage fungicide resistance in citrus packinghouses, sanitation procedures using sodium carbonate and sodium hypochlorite, treatments with biocontrol agents (yeast and bacteria), improvement of the natural wound-healing processes in citrus peel, and the alternating use of various fungicides with diverse mechanisms of action are strongly recommended *(10).*

Smilanick et al. *(23)* found that when sodium bicarbonate was applied in combination with IMZ, the control of green mold dramatically increased, even when fruits were inoculated with an IMZ-resistant isolate of *P. digitatum.* In recent years, novel classes of active ingredients with a mode of action different from those of other approved pesticides in a crop group have become available and are used currently to cope with problems caused by pathogenic strains resistant to the older fungicides *(13).* Among these new compounds, trifloxystrobin (TFX) is a relatively new synthetic active ingredient belonging to the class of QoI (quinone outside inhibitor) fungicides of the strobilurin group, which act as respiration inhibitors by binding to the Qo center of cytochrome *b (27).* TFX has a very favorable toxicological profile, dissipates rapidly from soils and groundwater, and is harmless to nontarget organisms under field conditions at field application rates *(4).* The versatility and broad spectrum of TFX action against field diseases have been proven for a wide range of horticultural crops *(16).* When applied as a foliar spray, TFX also was effective for controlling various postharvest diseases in avocado (*Persea americana* Mill cv. Hass) fruit *(26).* However, resistance management with these QoIs presents several problems related to the high risk for development of resistance demonstrated by these fungicides soon after their introduction into the market *(1, 3, 9).*

This work was conducted to evaluate the effectiveness

* Author for correspondence. Tel: 39078333224; Fax: 39078333959; E-mail: mario.schirra@ispa.cnr.it.

of TFX and the conventional fungicide IMZ, in combination with hot water treatments, for controlling postharvest *Penicillium* decay of citrus fruit when reduced concentrations of active ingredient (a.i.) were applied before storage. Fungicide residues in fruit were recorded as a function of treatment temperature, treatment duration, and storage conditions.

MATERIALS AND METHODS

Fruit samples. Commercial mature lemons (*Citrus limon* (L.) Burm cv. Di Massa), grapefruits (*Citrus paradisi* Macf. cv. Marsh Seedless), clementine and mandarin oranges (*Citrus reticulata* Blanco cvs. Monreal and Avana apireno, respectively), and blood oranges (*Citrus sinensis* (L.) Osbek cv. Tarocco) grown using standard horticultural practices were hand-harvested from an experimental orchard located in central western Sardinia (Italy). 'Avana apireno' mandarins were harvested in February, lemons and clementines were harvested in April, and oranges and grapefruits were picked in May. At harvest, the fruits were placed in plastic trays and delivered to the laboratory immediately. Several groups of fruits of medium size and that were free from rind defects were selected and returned to each box and left overnight.

Fungal cultures and pathogen inoculation. Monosporic isolates of *P. digitatum* (Saccardo: Fries; isolates PD-8, PD-5, and PD-a) and *P. italicum* Wehmer (isolates PI-4, PI-7, and PI-b) were obtained from infected 'Tarocco' oranges harvested in an orchard located in central-western Sardinia (Italy) and were cultured on potato dextrose agar (PDA; Merck & Co., Whitehouse Station, N.Y.) amended with streptomycin sulfate and oxytetracycline hydrochloride (100 + 100 μg/ml) to prevent growth of bacterial contaminants. A conidial suspension (10^8 conidia per ml) 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, filtered through two layers of sterile cheesecloth, and counted with a hemacytometer.

Assays with wounded and artificially inoculated fruit. The efficacies of TFX and IMZ against *P. digitatum* and *P. italicum* were compared in trials carried out on 'Avana apireno' mandarins because of their high susceptibility to *Penicillium* decay. Fruit samples were subdivided into three groups, and each fruit was wounded with two slits (2 by 2 mm) equatorially at four equidistant points. Fruits of the first and second group were artificially inoculated with *P. digitatum* and *P. italicum,* respectively, and fruits of the third group were not inoculated (controls). Inoculation was performed by dipping fruits for 2 min into a 50-liter conidial suspension of each pathogen (final concentration of 10^4 conidia per ml) contained in 72-liter high-density-polyethylene tank. Inoculated and noninoculated fruits were incubated overnight at 20°C and subdivided into 15 subgroups corresponding to the following 3-min dip treatments: water containing 0, 200, 400, or 600 mg/liter TFX or IMZ at 20°C; water containing 0, 25, 50, or 100 mg/liter TFX or IMZ at 50°C; and untreated (control) fruits. Each treatment was performed on three replicate groups of 20 fruits. Dip treatments were performed as previously described *(8).*

Following treatments, fruit were left to dry at room temperature. Noninoculated fruits and those artificially inoculated with *P. digitatim* or *P. italicum* were moved into three separate storage rooms (to avoid possible contamination) and kept at 20°C and ca. 85% relative humidity. The percentage of infected wounds was determined after 7 days.

Trials on simulated natural wounds that favor the development of green and blue mold wound pathogens *(11)* were also performed on 'Di Massa' lemons, 'Tarocco' oranges, and 'Monreal' clementines. Fruits were wounded with four slits (3 by 3 mm) equatorially at four equidistant points and were kept at 20°C for 24 h before treatments. To assess the minimal concentration of fungicide needed to control decay, lemons were subdivided into eight treatment groups corresponding to the following 3-min dip treatments: water at 20 or 50°C (control); 25, 50, or 100 mg/liter a.i. TFX at 50°C; and 25, 50, or 100 mg/liter a.i. IMZ at 50°C. Each treatment was performed on three replicate groups of 40 fruit. There were only four treatment groups for wounded oranges and clementines; treatments with TFX and IMZ at doses of 25 and 50 mg/liter a.i. were not included.

Following treatments, fruit were air dried and stored at 20°C and ca. 85% relative humidity, and the percentage of infected wounds was recorded after 1 week.

Storage trials with sound fruit. 'Tarocco' oranges were grouped into five treatment groups (five fruit boxes per treatment), corresponding to the following 3-min dip treatments: water at 20°C (control), 100 mg/liter TFX at 20°C, water at 50°C, 100 mg/liter TFX at 50°C, and 100 mg/liter IMZ at 50°C. Fungicide concentrations refer to the a.i. There were only four treatment groups for grapefruits 'Marsh Seedless' and 'Star Ruby'; treatment with 100 mg/liter TFX at 20°C was not included.

Following treatment, fruit were left to dry at room temperature for approximately 5 h. Each treatment group was then divided into three subgroups. Three replicate fruit boxes from the first subgroup were used for visual assessment, which included evaluation of chilling injury (CI), decay, treatment damage, and external fruit quality *(21).* The fruits from the second subgroup were individually weighed to determine the transpiration rate based on loss of fruit mass, and the fruits of the remaining group were used for analysis of TFX and IMZ residues. No residue analyses were carried out on 'Marsh Seedless' and 'Star Ruby' grapefruits.

Fruits were moved to a ventilated room and kept under cold quarantine conditions at 1°C for 3 weeks. These conditions match those used to comply with quarantine regulations to prevent the spread of fruit flies within flesh fruit *(2).* After quarantine, 'Marsh Seedless' and 'Star Ruby' grapefruits were stored for 8 weeks at 11°C, and oranges were stored for 5 weeks at 8°C. Fruits were then held at 20°C for another week as a simulated marketing period (SMP). Relative humidity during quarantine, storage, and the SMP was set at 90%.

Effect of dip time and temperature on fungicide residues. Oranges were subjected to the following dipping treatments: (i) 0.5, 1.5, or 3.0 min in 100 mg/liter TFX in water at 20 or 50°C and (ii) 3.0 min in 100 mg/liter TFX in water at 20, 30, 40, or 50°C. After treatment, fruits were left to dry for approximately 5 h, and then peel samples were taken and kept frozen at −20°C until analysis. All analyses were performed on four replicate groups of five fruits per treatment.

Chemicals. Acetone and hexane were gas chromatography grade (Merck, Milan, Italy). The a.i. standards for TFX (99%, Bayer AG, Leverkusen, Germany) and IMZ (97%, Eherenstofer, Amburg, Germany) were stock solutions of the a.i. at 500 mg/kg prepared in acetone; standard working solutions were prepared by dilution with extract from the untreated matrix.

Extraction procedure. Five fruits per replicate were weighed, the peel was removed and weighed, and its percentage with respect to the whole fruit was calculated. The peel was then minced with a mincing knife, homogenized, and stored in a freezer at −20°C until analysis. For IMZ extraction, a 2.5 g of peel sample was weighed into a 40-ml screw-cap tube, and 20 ml of an acetone-hexane mixture (1/1, vol/vol) and 6 g of NaCl were added. For TFX extraction, 5 g of homogenized peel sample was weighed into a 40-ml screw-cap flask, and 10 ml of an acetone-hexane (1/1, vol/vol) and 6 g of NaCl were added. The mixtures were then agitated in a rotary shaker for 20 min, the phases were allowed to separate, and the organic layer was injected into a gas chromatograph apparatus for analysis without a clean-up step.

Apparatus. A TQ Trace Gas Chromatographer coupled with a nitrogen-phosphorus detector and an AS200 auto sampler (Termo Quest, Milan, Italy) were used, with the injector and detector temperatures set at 200 and 300°C, respectively. Samples (2 μl) were injected in splitless mode (30 s), and the oven temperature was programmed for 110°C for 1 min and raised to 310°C at 20°C/min. The column was a fused silica capillary DB 5 MS (inside diameter, 30 m by 0.25 mm; 0.25 μm; J & W Scientific, Folsom, Calif.). Helium was the carrier gas and N_2 was the makeup gas at 1.1 and 15 ml/min, respectively. Nitrogen-phosphorus detector conditions were source current of 2.8 and polarization voltage of 3.5. Oxygen and H_2 flows were 60 and 2.3 ml/min, respectively.

Recovery assays. Peel samples from untreated fruits were fortified with appropriate volumes of standard solutions to reach concentrations of 0.1, 0.5, and 1 mg/kg. The samples were allowed to settle for 30 min before extraction and then processed according to the above procedure. Average recovery of TFX from four replicates ranged between 80 and 86%, with a maximum coefficient of variation of 14%; recovery of IMZ ranged between 93 and 104%, with a maximum coefficient of variation of 25%.

Statistical analysis. Analysis of variance (ANOVA) was performed with the MSTAT-C microcomputer statistical program (1991, Michigan State University, East Lansing) according to a single-factor randomized complete block design. Percentages were subjected to arcsine–square-root or square-root transformation before the ANOVA, depending on the range of variation in the data *(12).* Mean comparisons of the effects of treatments were calculated, where applicable, with Tukey's test and were considered significant at $P \leq 0.05$. To determine the pattern of a.i. accumulation in fruit, plots of residue uptake versus 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 AND DISCUSSION

Effect of treatment time, treatment temperature, and storage conditions on TFX residues in fruit. The residue of TFX in 'Tarocco' oranges treated for 0.5 min with 100 mg/liter TFX a.i. at 20°C was 0.15 mg/kg. When the dipping time was 1.5 and 3.0 min, the residues increased by 27 and 100%, respectively (Fig. 1). By contrast, when TFX was applied at 50°C, residues in fruit did not change with different dipping times, reaching the maximum levels after 0.5 min. For TFX, dipping at 50°C for 0.5, 1.5, and 3.0 min produced residues approximately four-, three-, and twofold higher than those from same treatment times at 20°C. Studies on 'Marsh Seedless' grapefruits have revealed that although thiabendazole residues were not affected by length of treatment, the IMZ concentrations in fruit were significantly dependent on treatment time. When dip treatments with 1,200 mg/liter IMZ at 20°C and 200 mg/liter IMZ at 50°C were increased from 0.5 to 3.0 min, residues increased to 100 and 108%, respectively *(8).* A similar relationship between treatment time and IMZ residue was reported for oranges and lemons *(5, 24).* Differences in uptake of fungicides as a function of treatment time can be ascribed to their diverse physicochemical characteristics and different formulations.

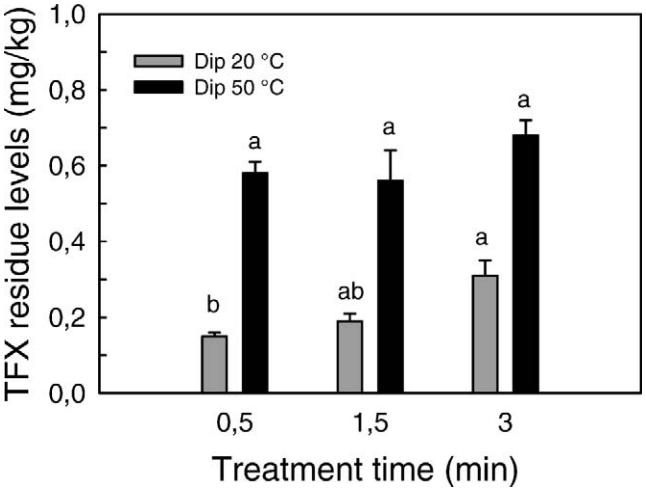

FIGURE 1. *Influence of treatment time on residues (milligrams per kilogram on a whole-fruit basis) of trifloxystrobin (TFX) in 'Tarocco' oranges following treatment with 100 mg/liter TFX-based commercially available fungicide mixture at 20 or 50°C. The fungicide concentrations refer to the active ingredient (a.i.). Vertical bars indicate the standard deviation* (n = 4). *Within each treatment time, different letters indicate significant differences according to Tukey's test,* $P \leq 0.05$.

An increase in the temperature of the dip mixture from 32.2 to 43.3°C increased IMZ residues by 1.5 to 2 times *(24).* Similar results were obtained with thiabendazole and IMZ *(8).* Following treatment with 100 mg/liter TFX at 20 to 50°C, the residues of fungicide were significantly correlated ($R^2 = 0.9769$, $P < 0.05$) with dip temperature, according to a sigmoidal trend (Fig. 2). IMZ treatment at 50°C produced a residue approximately eightfold higher than treatment with TFX (Table 1). TFX has a general pattern of movement (translaminar activity) unique to QoI (strobilurin) fungicides *(14, 16).* The residues remain stable over a long period, indicating that continuous penetration from the surface deposits replaces compound lost through metabolism. The present study revealed that TFX residues remained fairly constant over both the storage period and the SMP. Conversely, IMZ dissipated, with mean concentrations of ca. 25 and 47% their initial value after the storage period and SMP, respectively (Table 1).

Effect of treatment on decay development in wounded noninoculated fruit. Trials with wounded lemons revealed that TFX efficacy against decay increased with increasing fungicide concentrations (Fig. 3). At 25 mg/liter, TFX was significantly less effective than IMZ, whereas when higher concentrations of TFX were used, no signifi-

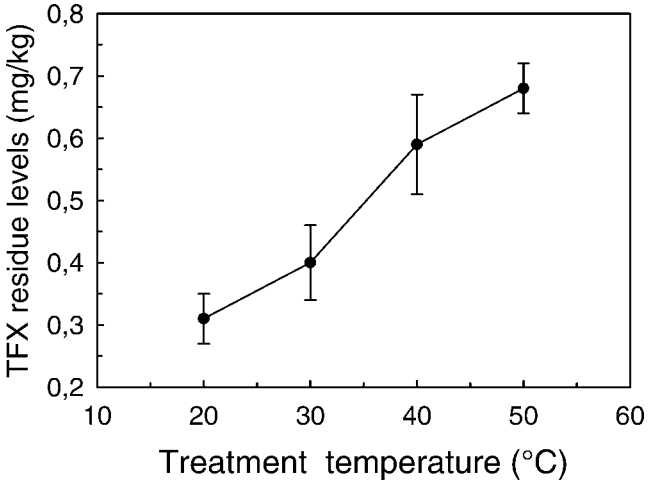

FIGURE 2. *Influence of treatment temperature on trifloxystrobin (TFX) residues (milligrams per kilogram on a whole-fruit basis) in 'Tarocco' oranges following a 3-min dip treatment with a 100-mg/liter TFX-based commercially available fungicide mixture. The fungicide concentrations refer to the active ingredient (a.i.). Vertical bars indicate the standard deviation (*n = 4*).*

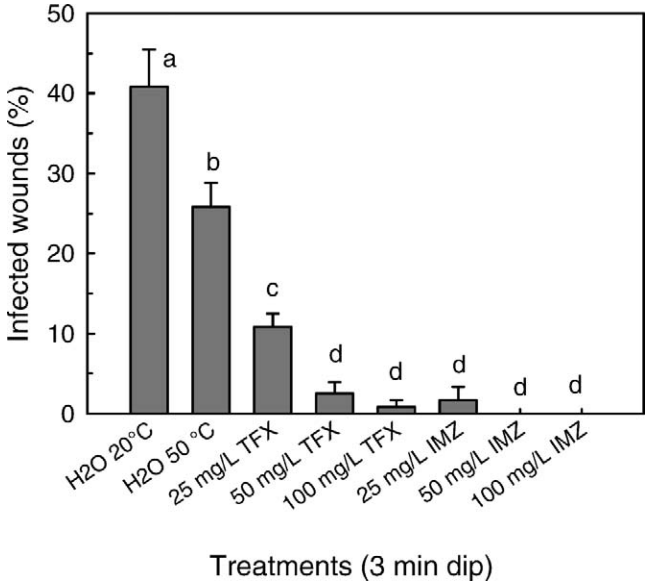

FIGURE 3. *Influence of a 3-min dip treatment in water at 20 (control) or 50°C or in trifloxystrobin (TFX) or imazalil (IMZ) commercially available fungicide mixtures containing 25, 50, or 100 mg/liter active ingredient (a.i.) at 50°C on the percentage of infected wounds in 'Di Massa' lemons after 7 days of storage at 20°C. Each value is the mean (±standard deviation) of three replicates (40 wounded fruits per replicate). Different letters indicate significant differences according to Tukey's test,* $P < 0.05$.

cant differences were found between the TFX and IMZ results. The minimal concentrations that provided almost complete control of *Penicillium* infection were 50 mg/liter TFX or 25 mg/liter IMZ at 50°C. In wounded untreated 'Tarocco' oranges, the incidence of decay was approximately 76% after 7 days of storage at 20°C (Table 2). This percentage was significantly reduced by fungicide treatments especially those with IMZ, which was more effective than TFX. The influence of hot water dipping on control of decay in wounded oranges was not significant. In 'Monreal' clementines (Table 2) and 'Avana apireno' mandarins (Table 3), hot water dipping effectively reduced decay development. In 'Monreal' clementines, both TFX and IMZ were more effective than hot water, with no significant differences between them. In 'Avana apireno' mandarins, treatments with 200 and 400 mg/liter TFX at 20°C or with 25 and 50 mg/liter at 50°C significantly reduced the incidence of infected wounds (from ca. 80 to 89%). All other treatments suppressed the development of pathogens completely. The infections were mainly caused by *P. digitatum* and to a much less extent by *P. italicum* (data not shown).

Effect of treatment on decay development in wounded artificially inoculated fruit. The percentage of lesions in 'Avana apireno' mandarins that were infected by *P. digitatum* and *P. italicum* was significantly affected by hot water treatment, reducing the incidence of infection by 33 and 54%, respectively, compared with untreated fruit (Table 3).

Treatments with 200, 400, or 600 mg/liter TFX at 20°C were as effective as 25 and 50 mg/liter TFX at 50°C but were less effective than 100 mg/liter TFX at 50°C for controlling *P. digitatum.* Treatments with 200 mg/liter IMZ provided almost complete control of green mold development, and no visible green mold infections were observed in fruit treated with 400 or 600 mg/liter IMZ at 20°C or with 25, 50, or 100 mg/liter IMZ at 50°C.

P. italicum development was not significantly affected by 25 mg/liter TFX at 50°C but was significantly reduced

TABLE 1. *Residues of trifloxystrobin (TFX) and imazalil (IMZ) in 'Tarocco' oranges following a 3-min dip treatment and then 3 weeks at 1°C (quarantine), storage for 5 weeks at 8°C (quarantine + storage), and an additional 1-week simulated marketing period (SMP) at 20°C*

Treatment[a]	Fungicide residue (mg/kg active ingredient) after[b]:			
	Treatment	Quarantine	Quarantine + storage	SMP
TFX, 20°C	0.21 ± 0.03	0.20 ± 0.02	0.24 ± 0.04	0.26 ± 0.03
TFX, 50°C	0.52 ± 0.13	0.44 ± 0.08	0.52 ± 0.09	0.45 ± 0.06
IMZ, 50°C	4.16 ± 0.58	4.18 ± 1.16	3.12 ± 0.50	2.21 ± 0.45

[a] Treatments were 3-min dips followed by air drying. Fungicides were applied at 100 mg/liter active ingredient.
[b] Each value is the mean (±standard deviation on a whole-fruit basis) of four replicates (five fruits per replicate).

TABLE 2. *Influence of postharvest treatments on the percentage of infected wounds in artificially wounded noninoculated 'Tarocco' oranges and 'Monreal' clementines after 7 days of storage at 20°C*

Treatment[a]	% infected wounds[b]	
	'Tarocco' oranges	'Monreal' clementines
Water, 20°C	75.6 A	15.0 A
Water, 50°C	66.7 A	8.3 B
TFX, 50°C	31.1 B	0.8 C
IMZ, 50°C	11.1 C	2.5 C

[a] Treatments were 3-min dips followed by air drying. Fungicides were applied at 100 mg/liter active ingredient.

[b] Each value is the mean of three replicates (40 fruits per replicate). In each column, values followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$.

by hot water and 50 mg/liter TFX at 50°C and especially by 200, 400, or 600 mg/liter TFX at 20°C and by 100 mg/liter IMZ at 50°C. However, better results were achieved with IMZ treatments, resulting in more than 90% control of *P. italicum* development after application of 25 mg/liter IMZ at 50°C and in total control after other IMZ treatments.

Fruit responses to dip treatments during storage. There were no visible CI symptoms after quarantine and after cold storage; CI was still negligible in both oranges and grapefruits (data not shown). After the SMP, 5 and 7.6% of the 'Marsh Seedless' grapefruits had slight and moderate CI, respectively, and 13.8% of these fruits had severe CI. In 'Star Ruby' grapefruits and 'Tarocco' oranges, the incidence of CI was considerably low even after SMP; no fruits were scored as slight CI and less than 4 and 2% of the 'Star Ruby' grapefruits and 'Tarocco' oranges, respectively, had moderate to severe CI (data not shown). The low incidence of CI in control grapefruits and oranges might be related to the age of these fruits, which were harvested late in the season *(21).* Hot water dip at 50°C reduced the incidence of CI to a very low level, with no additional advantages when hot water was used in combination with TFX or IMZ. These results are in agreement with previous findings for 'Star Ruby' grapefruit subjected to postharvest treatments with heated IMZ *(21)* and azoxystrobin *(18).*

The weight loss percentage in 'Tarocco' oranges was significantly reduced by TFX treatment at 20°C with respect to control fruit, but the influence of hot water with or without fungicides was not significant (Table 4). No phytotoxic effects (e.g., peel necrosis or browning on the rind) due to treatments were observed in oranges and grapefruits during storage and the SMP (data not shown).

No decay was observed in either untreated or treated fruit during quarantine (data not shown). By the end of cold storage and after the SMP, the decay incidences (mainly caused by *P. digitatum*) in oranges were 9.2 and 19.2%, respectively (Table 5). Decay development was unaffected by treatment with 100 mg/liter TFX at 20°C, but heated TFX and IMZ at 50°C provided almost complete control of

TABLE 3. *Influence of postharvest treatments on the percentage of infected wounds in 'Avana apireno' mandarins not inoculated or artificially inoculated with* P. digitatum *or* P. italicum *after 7 days of storage at 20°C*

Treatment[a]	% infected wounds[b]		
	Noninoculated wounds (natural infections)	Inoculated wounds	
		P. digitatum	*P. italicum*
Untreated	30.0 D	85.0 E	89.2 FG
Water at 20°C	29.2 D	84.2 E	96.7 G
Water at 50°C	19.2 C	56.7 D	40.8 CD
200 mg/liter TFX at 20°C	4.2 B	37.5 CD	31.7 C
400 mg/liter TFX at 20°C	3.3 B	30.0 C	31.7 C
600 mg/liter TFX at 20°C	0.0 A	25.8 C	25.0 C
200 mg/liter IMZ at 20°C	0.0 A	1.7 AB	0.0 A
400 mg/liter IMZ at 20°C	0.0 A	0.0 A	0.0 A
600 mg/liter IMZ at 20°C	0.0 A	0.0 A	0.0 A
25 mg/liter TFX at 50°C	5.0 B	29.2 C	71.7 EF
50 mg/liter TFX at 50°C	5.8 B	33.3 CD	58.3 DE
100 mg/liter TFX at 50°C	0.0 A	6.7 B	19.2 BC
25 mg/liter IMZ at 50°C	0.0 A	0.0 A	5.8 AB
50 mg/liter IMZ at 50°C	0.0 A	0.0 A	0.0 A
100 mg/liter IMZ at 50°C	0.0 A	0.0 A	0.0 A

[a] Treatments were 3-min dips followed by air drying.

[b] Each value is the mean of three replicates (20 fruits per replicate, two wounds per fruit). In each column, values followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$.

Penicillium infection during cold storage, with very low percentage of decayed fruit after the SMP. In 'Marsh Seedless' grapefruit, treatments with heated fungicides had similar efficacy in for decay control during both the storage period and the SMP, whereas in 'Star Ruby' grapefruits IMZ was more effective than TFX (Table 5). The influence of hot water on decay in 'Marsh Seedless' was nonsignificant. In 'Tarocco' and 'Star Ruby' fruits, hot water dipping significantly reduced decay development with respect to

TABLE 4. *Influence of postharvest treatments on the percent weight loss in 'Tarocco' oranges after 3 weeks of quarantine at 1°C plus 5 weeks of storage at 8°C (quarantine + storage) and an additional 1-week simulated marketing period (SMP) at 20°C*

Treatment[a]	Weight loss (%) after[b]:	
	Quarantine + storage	SMP
Water, 20°C	5.73 A	8.11 A
TFX, 20°C	4.67 B	6.71 B
Water, 50°C	5.28 AB	7.41 AB
TFX, 50°C	5.55 AB	7.66 AB
IMZ, 50°C	5.50 AB	7.74 AB

[a] Treatments were 3-min dips followed by air drying. Fungicides were applied at 100 mg/liter active ingredient.

[b] Each value is the mean of three replicates (40 fruits per replicate). In each column, values followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$.

TABLE 5. *Influence of storage conditions on decay percentage in 'Tarocco' oranges and 'Marsh Seedless' and 'Star Ruby' grapefruits following a 3-min dip treatment and then 3 weeks at 1°C (quarantine) plus storage (5 weeks at 8°C for oranges or 8 weeks at 11°C for grapefruits; quarantine + storage) and an additional 1-week simulated marketing period (SMP) at 20°C*

Treatment[a]	% decayed fruit after[b]: Quarantine + storage	SMP
'Tarocco' oranges		
Water, 20°C	9.2 A	19.2 A
TFX, 20°C	7.5 A	15.0 A
Water, 50°C	2.5 B	7.4 B
TFX, 50°C	0.8 B	1.7 C
IMZ, 50°C	0.0 B	0.8 C
'Marsh Seedless' grapefruits		
Water, 20°C	36.3 A	43.2 A
Water, 50°C	31.6 A	35.5 A
TFX, 50°C	5.0 B	7.5 B
IMZ, 50°C	6.3 B	9.0 B
'Star Ruby' grapefruits		
Water, 20°C	30.0 A	39.0 A
Water, 50°C	21.0 B	24.0 B
TFX, 50°C	7.0 C	10.0 C
IMZ, 50°C	1.0 D	2.0 D

[a] Treatments were 3-min dips followed by air drying. Fungicides were applied at 100 mg/liter active ingredient.

[b] Each value is the mean of three replicates (40 fruits per replicate). In each column, values followed by the same letters do not differ significantly according to Tukey's test, $P \leq 0.05$.

control fruit but was less effective after the SMP than was treatment with IMZ or TFX at 50°C. The better performance of TFX at 50°C was ascribed to the synergistic effect of heat, enhanced a.i., better penetration into the wound sites that are exploited by the fungus *(6, 20),* and increased diffusion of the a.i. across the plant cuticle *(17, 22).*

Several studies in recent years have dealt with the use of heated fungicides for postharvest control of decay in horticultural crops. This approach is aimed at improving the performance of the active ingredient for controlling decay by reducing the concentration of chemicals usually employed in conventional postharvest treatments at ambient temperature *(20).* Investigations carried out with citrus fruit revealed the potential of postharvest treatments with conventional fungicides as a function of the fungicide dose applied at room temperature or in combination with hot water *(20).* More recently, these studies have included novel formulations designated as reduced-risk pesticides, such as azoxystrobin *(18)* and fludioxonil *(19).* We adopted the fungicide IMZ as a standard reference because of its high effectiveness and wide application in control of *Penicillium* decay on a commercial scale. Nevertheless, alternative control strategies must be developed to avoid or delay any decrease in the efficacy of this fungicide as a consequence of the selection of resistant pathogenic populations. The results of the present study suggests that postharvest application of TFX may be an effective option for controlling green and blue molds in citrus fruit, which have developed resistance to the fungicides currently in use *(7, 15).* However, because resistance to TFX has been reported for many crops and diseases in different countries around the world *(1, 3, 9),* exclusive application of TFX should be avoided in a program of sustainable management of *Penicillium* decay of oranges.

ACKNOWLEDGMENTS

This research was supported by MiPAFm, Progetto ''Ricerche e sperimentazioni nel settore dell'Agrumicoltura Italiana'' paper 129, and by European Union PIC Interreg III A–Italy–France (CITRUS).

REFERENCES

1. Amand, O, F. Calay, L. Coquillart, T. Legat, B. Bodson, J. M. Moreau, and H. Maraite. 2003. First detection of resistance to QoI fungicides in *Mycosphaerella graminicola* on winter wheat in Belgium. *Commun. Agric. Appl. Biol. Sci.* 68(4B):519–531.
2. Armstrong, J. W. 1994. Heat and cold treatment, p. 103–119. *In* R. E. Paull and J. W. Armstrong (ed.), Insect pests and fresh horticultural products: treatments and responses. CAB International, Wallingford, UK.
3. Avila-Adame, C., O. Olaya, and W. Koller. 2003. Characterization of *Colletotrichum graminicola* isolates resistant to strobilurin-related QoI fungicides. *Plant Dis.* 87:1426–1432.
4. Barlett, D. W., J. M. Clough, J. R. Godwin, A. A. Hall, M. Hamer, and B. Parr-Dobrzanski. 2002. The strobilurin fungicides. *Pest Manag. Sci.* 58:649–662.
5. Brown, G. E., and D. J. Dezman. 1990. Uptake of imazalil by citrus fruit after postharvest application and the effect of residue distribution on sporulation of *Penicillium digitatum. Plant Dis.* 74:927–930.
6. Brown, G. E., S. Nagy, and M. Marauljia. 1983. Residues from postharvest nonrecovery spray applications of imazalil to oranges and effects on green mould caused by *Penicillium digitatum. Plant Dis.* 67:954–957.
7. Bus, V. G., A. J. Bongers, and L. A. Risse. 1991. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. *Plant Dis.* 75:1098–1100.
8. Cabras, P., M. Schirra, F. M. Pirisi, V. L. Garau, and A. Angioni. 1999. Factors affecting imazalil and thiabendazole uptake and persistence in citrus fruits following dip treatments. *J. Agric. Food Chem.* 47:3352–3354.
9. Chin, K. M., D. Chavaillaz, M. Kaesbohrer, T. Staub, and F. G. Felsenstein. 2001. Characterizing resistance risk of *Erysiphe graminis* f.sp *tritici* to strobilurins. *Crop Prot.* 20:87–96.
10. Eckert, J. W. 1995. Postharvest disease control: experience with citrus fruit. *Tree Fruit Postharvest J.* 6(2):9–12.
11. Eckert, J. W., and I. L. Eaks. 1988. Postharvest disorders and diseases of citrus fruit, p. 179–260. *In* W. Reuther, E. C. Calavan, and G. E. Carman (ed.), The citrus industry, vol. 5. University of California Press, Berkeley.
12. Gomez, K. A., and A. A. Gomez. 1984. Statistical procedures for agricultural research. Wiley and Sons, New York.
13. Gullino, M. L., P. Leroux, and C. M. Smith. 2000. Use and challenges of novel compounds for plant disease control. *Crop Prot.* 19: 1–11.
14. Hermann, D., W. Fisher, G. Knauf-Baider, A. Steinemann, P. Margot, U. Gisi, and D. Laird. 1998. Behaviour of the new strobilurin fungicide trifloxystrobin on and in plants. *Phytopathology* 88:S37. (Abstract.)
15. Holmes, G. H., and J. W. Eckert. 1992. Reduced sensitivity of *Penicillium digitatum* to imazalil, thiabendazole and *o*-phenylphenol. *Phytopathology* 82:1069. (Abstract.)
16. Margot, P., F. Huggenberger, J. Amrein, and B. Weiss. 1998. CGA279202: a new broad-spectrum strobilurin fungicide, p. 375–

382. *In* Proceedings of the Brighton Crop Protection Conference on Pest Diseases. BCPC Publications, Brighton, UK
17. Riederer, M., and L. Schreiber. 1995. Waxes—the transport barriers of plant cuticles, p. 131–156. *In* R. J. Hamilton (ed.), Waxes: chemistry, molecular biology and functions, vol. 6. The Oily Press, Dundee, UK.
18. Schirra, M., P. Cabras, A. Angioni, and V. Brandolini. 2002. Residue levels and storage decay control in 'Star Ruby' grapefruit after dip treatments with azoxystrobin. *J. Agric. Food Chem.* 50:1461–1465.
19. Schirra, M., S. D'Aquino, A. Palma, S. Marceddu, A. Angioni, P. Cabras, B. Scherm, and Q. Migheli. 2005. Residue level, persistence and storage performance of citrus fruit treated with fludioxonil. *J. Agric. Food Chem.* 53:6718–6724.
20. Schirra, M., G. D'hallewin, S. Ben-Yehoshua, and E. Fallik. 2000. Host pathogen interaction modulated by heat treatment. *Postharvest Biol. Technol.* 21:71–85.
21. Schirra, M., G. D'hallewin, P. Cabras, A. Angioni, S. Ben-Yehoshua, and S. Lurie. 2000. Chilling injury and residue uptake in cold stored Star Ruby grapefruits following thiabendazole and imazalil dip treatments at 20 and 50°C. *Postharvest Biol. Technol.* 20:91–98.
22. Schönherr, J., and P. Baurr. 1996. Effects of temperature, surfactants and other adjuvants on rates of uptake of organic compounds, p. 135–155. *In* G. Kerstiens (ed.), Plant cuticles—an integrated functional approach. BIOS Scientific Publications, Ltd., Oxford.
23. Smilanick, J. L., M. E. Mansour, D. A. Margosan, and F. Mlikota Gabler. 2005. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold of citrus fruit. *Plant Dis.* 89:640–648.
24. Smilanick, J. L., I. F. Michael, M. F. Mansour, B. E. Mackey, D. A. Margosan, D. Florers, and C. F. Weist. 1997. Improved control of green mould of citrus fruit with imazalil with warm water compared with its use in wax. *Plant Dis.* 81:1299–1304.
25. Smoot, J. J., L. G. Houck, and H. B. Johnson. 1983. Market diseases in citrus and other subtropical fruits, p. 9–12. *In* USDA-ARS agricultural handbook, vol. 398. U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C.
26. Willingham, S. L., K. G. Pegg, L. M. Coates, A. W. Cooke, J. R. Dean, P. W. B. Langdon, and D. R. Beasley. 2001. Field management of avocado postharvest diseases. *Acta Hort.* 553:435–438.
27. Wood, P. M., and D. W. Hollomon. 2003. A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Q(o) site of complex III. *Pest Manag. Sci.* 59:499–511.