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Residue Levels and Storage Decay Control in Cv. Star Ruby Grapefruit after Dip Treatments with Azoxystrobin

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Cv. Star Ruby grapefruit (*Citrus paradisi* Macf.) were subjected to a 3-min dip in water at room temperature (20 °C) or at 50 °C with or without 25, 50, or 100 mg/L azoxystrobin (AZX). Then, the fruits were subjected to cold quarantine at 2 °C and 90−95% relative humidity (RH) for 3 weeks and then stored for 5 weeks at 8 °C and ∼85% RH and for another 2 weeks at 20 °C and 80% RH to simulate a 2-week marketing period (SMP). No AZX residues were detected in the albedo and pulp following treatments at 20 or 50 °C, the total amount of residues being recovered from the flavedo tissue. There was a relationship between the AZX uptake in fruit and the amount of fungicide employed at 20 or 50 °C. When AZX was applied to the fruit at 25 mg/L at 20 °C, the residue level averaged 0.11 mg/kg (active ingredient, whole fruit basis). This residue concentration increased by 50 and 75% when the application rate increased from 25 to 50 or 100 mg/L, respectively. A similar pattern of accumulation was detected in fruit subjected to treatments at 50 °C. However, treatments at 50 °C produced residue levels higher than the treatments at 20 °C, with increases ranging from 63 to 84%, for the same concentration. Storage conditions did not affect the amount of AZX residues in the fruit. Treatment at 50 or 100 mg/L at 20 °C reduced the incidence of moderate to severe chilling injury (CI). Water dips at 50 °C reduced the incidence and severity of CI to a very low extent, with no additional advantages when hot water was used in combination with AZX. Treatments with 50 or 100 mg/L of AZX at 20 °C produced beneficial effects in decay control similar to those of 25 mg/L AZX at 50 °C or hot water alone. Better results were achieved with 50 or 100 AZX at 50 °C, providing complete control of decay during cold storage and with negligible decay after SMP. It was concluded that when AZX was applied at 50 °C, low doses of fungicide and minimal residue levels in fruit were required to control the postharvest decay of grapefruit. This treatment does not impair fruit quality and offers very interesting prospects for large scale application, due to the reduced potential toxicity of AZX to nontarget organisms and to the environment.

KEYWORDS: Chilling injury; decay; hot water dip; strobilurins; storage

INTRODUCTION

Most postharvest losses of citrus fruit are due to decay from green (*Penicillium digitatum* Sacc.) and blue (*Penicillium italicum* Wehmer) molds. Thiabendazole (TBZ) and imazalil (IMZ) are usually applied on a commercial scale to control these fungi (*1*). However, prolonged use of these fungicides favors the proliferation of resistant isolates of *Penicillium* spp. (*2*, *3*), thereby compromising their efficacy. To overcome this problem, it has been suggested to alternate the use of fungicides with different mechanisms of action (e.g., TBZ and IMZ) with the application of nonselective fungicides (*4*) and/or nonconventional technologies, such as physical or biological agents (*5*, *6*). In recent years there has been growing interest in novel, broad spectrum fungicides currently designated "reduced risk" pesticides to humans, nontarget organisms, and environmental resources, with a diverse mechanism of action compared to other currently registered pesticides in a crop group. Among them, azoxystrobin (AZX), a synthetic analogue of the fungal metabolites of the strobilurins and oudemansins (*7*), has a very broad spectrum of activity and is effective against fungal pathogens belonging to the groups of *Oomycetes*, *Ascomycetes*, *Deuteromycetes*, and *Basidiomycetes* (*8*). Oren et al. (*9*) reported that preharvest AZX applications effectively control *Alternaria*

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alternata in citrus fruit. Additionally, AZX was found to be effective as a preventive spray against citrus scab (*Elsinoe faucettii*) in potted seedlings of rough lemon (*Citrus jambhiri* Lush) and against melanose caused by *Diaporthe citri* in grapefruit (*Citrus paradisi* Macf.) seedlings (*10*). However, no publications have been found concerning the levels and the effectiveness of postharvest AZX treatments in decay control in citrus fruit as a function of the fungicide dose. This study was therefore designed to assess the minimal level of AZX capable of achieving a significant control of decay in cv. Star Ruby grapefruit when fungicide mixtures are applied at 20 or 50 °C.

MATERIALS AND METHODS

Plant Material. Red-fleshed grapefruits (*Citrus paradisi* Macf. cv. Star Ruby) were harvested in the third week of April (late-season) from a single lot of an experimental orchard located in central western Sardinia (Italy), receiving standard horticultural care. Harvest involved a random sampling from 10 trees. The fruits were picked from the outside of the canopy of each tree, placed in plastic boxes, delivered to the laboratory immediately after harvest, graded, sized, returned to the boxes (40 fruits individually numbered per box), and grouped into eight treatment lots (each with nine fruit boxes), corresponding to the following 3-min dip treatments: (I) water at 20 °C (control fruit); (II) 25 mg/L AZX at 20 °C; (III) 50 mg/L AZX at 20 °C; (IV) 100 mg/L AZX at 20 °C; (V) water at 50 °C; (VI) 25 mg/L AZX at 50 °C; (VII) 50 mg/L AZX at 50 °C; and (VIII) 100 mg/L AZX at 50 °C.

The AZX mixtures in water were prepared with commercially available Quadris [22.9% active ingredient (ai), ZENECA Agrochemicals Grangemouth, Scotland, U.K.]. Dip treatments were performed using an apparatus described by Schirra et al. (*11*). Following treatment, the fruits were left to dry at room temperature for ~5 h. Each treatment group was then divided into three subgroups. The fruit in the first subgroup included four replicate fruit boxes used for assessment, which included chilling injury, decay, treatment damage, and external fruit quality. Four replicate fruit boxes from the second subgroup were used for AZX analysis. The fruits from the remaining boxes were individually weighed to determine the transpiration rate from fruit mass loss. Finally, the fruits were moved to a storage room and kept at cold quarantine conditions at 2 °C and ~85% relative humidity (RH) for 3 weeks. The fruits were then stored for 5 weeks at 8 °C and ~85% RH and for another 2 weeks at 20 °C and 80% RH to simulate a 2-week marketing period (SMP).

Fruit Weight Loss, Visual Assessment, Flavor, and Taste. Fruit weight loss and visual assessments were determined after quarantine, storage, and SMP. CI (peel pitting and brown staining) was scored as slight when light-brown CI covered <5% of the rind surface, the damage was not objectionable, and it would not deter the consumer from buying the fruits; a moderate score was given when darker brown spots and depressions covered up to 25% of the rind surface (some consumers might reject these fruit unless they could be purchased at a reduced price); and a severe score indicated that injury covered >25% of the rind surface, and the fruit would therefore be rejected. The percentage of fruit in each rating was then calculated. Decay incidence was assessed as total rots caused by blue mold (*P. italicum* Wehmer), green mold (*P. digitatum* Sacc.), and brown rot (*Phytophtora citrophthora*) or as miscellaneous rots of other fungi. Overall visual quality, fruit flavor, and taste were rated subjectively into one of five categories [5 (excellent), 4 (good), 3 (fair), 2 (poor), and 1 (very poor)] by an informal panel familiar with this cultivar.

Analysis of AZX. *Chemicals.* Ethyl acetate and hexane were pesticide grade solvents (Merck, Milan, Italy). The analytical standard of azoxystrobin was kindly supplied by Zeneca (Milan, Italy). Stock standard solutions of azoxystrobin (500 mg/kg) were prepared in acetone. Working standard solutions were prepared by dilution with extract from untreated matrix.

Apparatus. A TQ trace gas chromatograph, coupled with an NPD 80 detector and an AS 2000 autosampler (Termo Quest, Milan, Italy), was used. The column was a fused silica capillary CP Sil 5 CB (15 m × 0.25 mm; film thickness = 0.25 μm; Chrompack, Middelburg, The Netherlands). The injector and detector were at 200 and 280 °C, respectively. The 1 μL sample was injected in the splitless (60 s) mode. The oven was programmed as follows: 100 °C, raised to 150 °C (8 °C/min), raised to 300 °C (20 °C/min), and held for 3 min.

Extraction Procedure. From each treatment group, three fruits per replication were weighed; their flavedo, albedo, and pulp were obtained and weighed, and their percentage was calculated with respect to the whole fruit. Each sample was then triturated with a mincing knife, homogenized, and stored in a freezer at −20 °C until analysis. A 2.5 g aliquot of flavedo was weighed in a 40 mL screw-capped tube; 20 mL of hexane/ethyl acetate mixture (1:1, v/v) and 2 g of NaCl were added, and the mixture was agitated in a rotatory shaker (Stuart Scientific) for 20 min. A 2.5 or 10 g aliquot was used for albedo and pulp analysis, respectively. A 1 mL aliquot of the organic solution was evaporated under a gentle nitrogen stream, and the solid was dissolved with 200 μL of acetone. The solution was then injected in GC for analysis.

Recovery Assays. Flavedo, albedo, and pulp samples of untreated grapefruits were fortified with appropriate volumes of standard solutions to reach concentrations of 0.05, 0.1, and 0.5 mg/kg of ai. The samples were allowed to settle for 30 min prior to extraction and were then processed according to the above procedure. Average recovery from four replicates showed values ranging from 89 to 100% with a maximum coefficient of variation (CV) of 6%.

Statistical Analysis. Analysis of variance (ANOVA) was performed, and mean comparisons were determined by Tukey's test at $P \leq 0.05$, where appropriate.

Table 1. Azoxystrobin Levels (on a Whole Fruit Basis) in Star Ruby Grapefruit Following a 3-min Dip Treatment, after Cold Quarantine for 3 Weeks at 2 °C, Subsequent Storage for 5 Weeks at 8 °C (Quarantine + Storage), and an Additional 2 Weeks of Simulated Marketing Conditions (SMP) at 20 °C[a]

		azoxystrobin residues (mg/kg of active ingredient ± SD)			
treatment temp (°C)	fungicide concn (mg/L)	treatment	quarantine	quarantine + storage	SMP
20	25	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.02
	50	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.01	0.16 ± 0.01
	100	0.19 ± 0.02	0.21 ± 0.02	0.22 ± 0.00	0.25 ± 0.02
50	25	0.18 ± 0.07	0.18 ± 0.03	0.16 ± 0.05	0.15 ± 0.04
	50	0.28 ± 0.03	0.25 ± 0.02	0.24 ± 0.03	0.22 ± 0.04
	100	0.35 ± 0.06	0.30 ± 0.05	0.32 ± 0.04	0.31 ± 0.03

[a] Treatments are 3-min dips followed by air-drying of dipped fruit.

RESULTS

The total amount of AZX was retained by the flavedo as no residue was detected in the albedo or the pulp following treatments at 20 or 50 °C (data not shown). After treatments, there was a linear (but not proportional) relationship ($r = 0.945$) between the residue level in the fruits and the amount of fungicide employed at 20 °C. Following treatment with 25 mg/L AZX at 20 °C, the residue uptake was 0.11 mg/kg (ai, on a whole fruit basis) (**Table 1**). A double fungicide concentration (50 mg/L AZX) produced 0.16 mg/kg (ai), with an ~50% increase compared to the treatment with 25 mg/L AZX, whereas a 4-fold concentration (100 mg/L AZX) doubled the residue uptake. The pattern of ai accumulation in fruit subjected to treatments at 50 °C showed a relationship ($r = 0.958$) between fungicide dose and residue concentration, similar to that with the treatment at 20 °C. However, treatments at 50 °C produced residue levels higher than the respective treatments at 20 °C, with increases of residue level at 50 °C compared to 20 °C

Table 2. Influence of 3-min Dip Treatments with Azoxystrobin and Storage Conditions on the Incidence of Chilling Injury (CI) and Decay in Star Ruby Grapefruits Submitted to Cold Quarantine for 3 Weeks at 2 °C, Subsequent Storage for 5 Weeks at 8 °C (Quarantine + Storage), and an Additional 2 Weeks of Simulated Marketing Conditions (SMP) at 20 °C[a,b]

variable	treatment temp (°C)	fungicide concn 0 mg/L	25 mg/L	50 mg/L	100 mg/L
		Quarantine + Storage			
slight CI	20	27.4 a(A)	22.6 a(A)	17.9 a(A)	28.6 a(A)
	50	1.2 a(B)	0.0 a(B)	0.0 a(B)	1.2 a(B)
moderate to severe CI	20	34.5 a(A)	31.2 a(A)	25.9 b(A)	23.8 b(A)
	50	1.2 a(B)	0.0 a(B)	1.2 a(B)	1.2 a(B)
decay (%)	20	8.3 a(A)	11.9 a(A)	2.4 b(A)	3.6 b(A)
	50	1.2 a(B)	3.6 a(B)	0.0 a(A)	0.0 a(A)
		SMP			
slight CI	20	25.0 a(A)	19.0 a(A)	17.9 a(A)	25.0 a(A)
	50	1.2 a(B)	0.0 a(B)	0.0 a(B)	1.2 a(B)
moderate to severe CI	20	38.8 a(A)	35.4 a(A)	23.8 b(A)	27.4 b(A)
	50	2.4 a(B)	0.0 a(B)	2.4 a(B)	1.2 a(B)
decay (%)	20	16.4 a(A)	15.5 a(A)	4.8 b(A)	6.0 b(A)
	50	5.9 a(B)	4.7 a(B)	0.0 a(A)	1.2 a(A)

[a] Treatments are 3-min dips followed by air-drying of dipped fruit. [b] In each row or column grouping, means separation was carried out by Tukey's range test at $P = 0.05$ (lower case letters) or $P = 0.01$ (upper case letters). Letters without parentheses refer to comparisons of the effect of fungicide concentration within each dip temperature. Letters in parentheses refer to comparisons of the influence of dip temperature within each fungicide concentration.

ranging from 63 to 84%. After quarantine, storage, and SMP, residue levels of AZX were very similar to their initial levels.

No fruit with visible symptoms of CI could be detected during quarantine (data not shown). Afterward, AZX treatments at 20 °C did not significantly affect the incidence of fruit with slight CI compared to control fruit (**Table 2**). Treatments at 50 or 100 mg/L at 20 °C gave some measure of control on the incidence of moderate to severe CI. Best results were achieved by hot water dips at 50 °C, which reduced the incidence and severity of CI to very low values. AZX applied at 50 °C did not produce more beneficial effects than hot water dips alone.

There was no decay in untreated fruit after quarantine (data not shown). By the end of cold storage and after SMP, the decay incidences (mainly *Penicillium* spp.) were 8.3 and 16.4%, respectively (**Table 2**). Treatment with 25 mg/L AZX at 20 °C did not affect decay development. By contrast, with 50 or 100 mg/L AZX at 20 °C a significant control of decay was obtained both during storage and SMP. Treatment with 25 mg/L at 50 °C was found to be as effective as hot water alone. By contrast, higher doses of AZX (50 and 100 mg/L) at 50 °C gave better protection against decay.

Fruit weight loss was not significantly affected by treatment. Therefore, the data are presented as means (±SE, 99% confidence) of 32 replicate samples (four replications × eight treatments) (**Figure 1**). After SMP, fruit weight loss averaged 6.5 ± 0.39.

No phytotoxic effects (peel necrosis, browning on the rind) due to AZX treatments at 20 °C or to 25 mg/L AZX at 50 °C could be observed after storage and SMP, whereas small reddish spots were occasionally observed (<2%) in fruits treated with 50 or 100 mg/L AZX at 50 °C (data not shown). The flavor and taste of fruits with no physiological or pathological disorders were scored as good even after SMP, differences among treatments being negligible (data not shown).

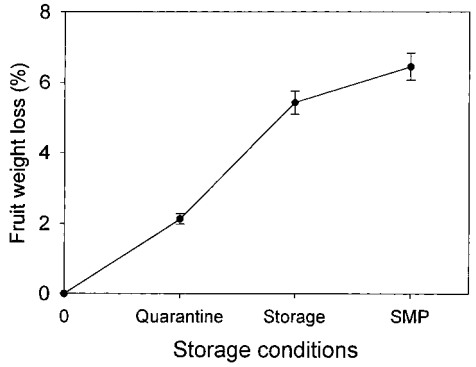

Figure 1. Fruit weight loss in Star Ruby grapefruit after cold quarantine for 3 weeks at 2 °C, subsequent storage for 5 weeks at 8 °C (storage), and another 2 weeks of simulated marketing conditions (SMP) at 20 °C. Each datum point is a mean (±SE, 95% confidence) of 32 replicate samples (four replications × eight treatments).

DISCUSSION

It is recognized that the effectiveness of postharvest treatments with conventional fungicides are greatly enhanced compared to treatments at room temperature when used as warm mixtures to citrus fruit (*11*−*15*). At a higher temperature there is a synergistic effect of heat, enhanced active ingredient uptake, better encapsulation, and diffusion of ai in the cuticular wax (*16*). Accordingly, the present research has demonstrated that treatments at 50 °C produced higher residue levels than the respective treatments at 20 °C, with increases ranging from 63 to 84% for the same concentrations applied at 20 or 50 °C.

In the present study at 20 °C AZX at concentrations of 50 or 100 mg/L decreased decay, whereas 25 mg/L was ineffective. The 50 °C dip was equally effective against chilling injury whether or not AZX was present. Treatment with 25 mg/L AZX at 50 °C was as effective as hot water alone in controlling decay. However, when the application rate of AZX increased from 25 to 50 or 100 mg/L, treatment efficacy further increased, providing complete control of decay during cold storage with negligible decay after SMP.

It has been reported that preharvest treatments with AZX at a concentration of 0.075% effectively controlled *Alternaria* rot in cv. Minneola mandarin hybrid fruits (*9*). Various citrus fruit species may be affected by this fungus in the orchard or during storage, but fungicidal treatments that are effective against *Penicillium* and stem-end-rot are not adequate against *Alternaria* fungus (*17*). In this study treatments with 50 or 100 mg/L AZX at 50 °C were highly effective in controlling *Penicillium* decays in cv. Star Ruby grapefruit during storage and SMP. The lowest threshold of AZX residue concentration in fruit capable of achieving an effective control decay was ∼0.28 mg/kg and was produced by 50 mg/L AZX at 50 °C. Such residue levels are far below those left by treatments with 50 mg/L IMZ at 50 °C (2.0 mg/kg) (*11*), that is, ∼7-fold higher than after AZX treatment at 50 °C. Moreover, the residue of IMZ decreased during storage, especially when the fruits were removed from cold storage to SMP conditions, and this reduced its effectiveness against *Penicillium* spp. (*11*). IMZ activity decreased following the degradation rate of the ai, reaching 1.27 and 0.83 mg/kg, respectively, by the end of storage and subsequent shelf life. Cabras et al. (*18*) reported that, when AZX is applied on grapes as a preharvest spray, the residues in fruit disappeared with a pseudo-first-order kinetics ($r = 0.98$) and a half-life of 15.2 days. The results reported herein revealed that AZX residues in fruit remained unchanged during both cold storage and shelf life.

CONCLUSIONS

This is the first study that provides an indication of the effectiveness, residue uptake, and persistence of AZX when applied at room temperature or at 50 °C in the management of postharvest decay in grapefruit. The major findings are the following: (a) a very low dose (50 mg/L) of AZX at 50 °C was required to suppress postharvest decay completely; (b) the residue level in the fruit was minimal, <0.3 mg/kg/whole fruit, which is far below the tolerance level (2 mg/kg) fixed in the United States for bananas and in Italy for tomatoes and grapes. Finally, because the mode of action of AZX against pathogens is different from that of TBZ and IMZ (*19*), the alternate use of these fungicides (TBZ, IMZ, and AZX) should overcome the problem of TBZ- and IMZ-resistant biotypes of *Penicillium* spp.

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