Factors Affecting Imazalil and Thiabendazole Uptake and Persistence in Citrus Fruits Following Dip Treatments

Paolo Cabras,† Mario Schirra,*,‡ Filippo M. Pirisi,† Vincenzo L. Garau,† and Alberto Angioni†

Dipartimento di Tossicologia, Università di Cagliari, viale Diaz 182, 09126 Cagliari, Italy, and CNR Istituto per la Fisiologia della Maturazione e della Conservazione del Frutto delle Specie Arboree Mediterranee, Località Palloni, 09170 Oristano, Italy

The effect of concentration, temperature, and length of treatment with imazalil (IMZ) and thiabendazole (TBZ) was studied with application to citrus fruit. The amount of residues retained by fruit after "home" washing was also monitored. IMZ uptake in citrus fruit was related to treatment duration, whereas TBZ residues was not. Residues of IMZ or TBZ fungicides were significantly correlated with dip temperature ($r = 0.943$ for IMZ; $r = 0.911$ for TBZ). Treatment at 50 °C produced a deposition ∼8 and ∼2.5 times higher than when treatments were carried out at 20 °C in IMZ and TBZ, respectively. No significant differences in terms of IMZ deposition were detected after treatments carried out alone or in combination. Uptake of the two fungicides was associated with their physicochemical characteristics as well as different formulation types. IMZ residues showed a great persistence during storage when applied separately, and >83% of active ingredient was present after 9 weeks of storage. IMZ residues increased with dip length, doubling when dip time increased from 0.5 to 3 min. In contrast, TBZ residues did not change with the different dip times. Following postharvest dip treatments of citrus fruit at 20 or 50 °C, home washing removed ∼50% of the IMZ and ∼90% of the TBZ.

Keywords: *Citrus; postharvest; heat treatments; imazalil; thiabendazole; residues*

INTRODUCTION

Imazalil (IMZ) and thiabendazole (TBZ) are systemic fungicides employed to control a wide range of fungal diseases on fruit, vegetables, and ornamentals (Tomlin, 1994). Both fungicides are most widely used in packinghouse treatments to control postharvest decay in citrus fruit (Eckert and Eaks, 1988). IMZ is especially effective against green mold (*Penicillium digitatum*), including benzimidazole-resistant strains, and against sporulation, but is less effective than TBZ for stem-end rot control and is ineffective against sour rot and brown rot (Wardowski and Brown, 1991). Therefore, it is an industrial practice to use them in combination. However, after imazalil-resistant isolates of *P. digitatum* were noticed (Holmes and Eckert, 1992; Bus et al., 1991) the combined use of TBZ and IMZ was considered an inappropriate strategy as it can lead to double resistance (Eckert et al., 1994). At ambient temperature (20 °C) recommended doses of these fungicides are 1000 mg/L as water mixture or 2000 mg/L in water-based wax (Wardowski and Brown, 1991).

It was demonstrated that it is possible to drastically reduce the fungicide doses usually employed in conventional treatments at room temperature without jeopardizing fruit quality and fungicide effectiveness by combining IMZ and TBZ with hot water, as greater deposits of fungicide are achieved in fruit when fungicide is applied in hot water (Schirra et al., 1996, 1997, 1998a,b; Smilanick et al., 1997). The concentration and persistence of the fungicide following application to citrus fruit have been found to depend on various pre- and postharvest factors. These include the dosage and treatment mode (spray or dip), method of application (aqueous or wax-based mixtures), species, cultivar, and storage conditions (Dezman et al., 1986; Papadopoulou-Mourkidou, 1991).

This paper deals with the residue uptake and persistence in citrus fruit following IMZ and TBZ treatments, in relation to concentration, dip temperature, and treatment duration. The amount of residues retained by fruit after "home" washing was also monitored.

MATERIALS AND METHODS

Plant Material and Treatments. Mature oranges (*Citrus sinensis* Linn. Obsek) cv. Salustiana and grapefruits (*Citrus paradisi* Macf.) cv. Star Ruby and Marsh seedless were obtained from a single lot of an experimental orchard located in central western Sardinia (39° 55′ N latitude). Fruits were delivered to the laboratory immediately after harvest. They were sorted for uniform size and freedom from defects, placed in plastic boxes, and used for the following experiments:

Experiment I (Effect of Dip Time on Fungicide Uptake). Marsh seedless grapefruits were placed in plastic boxes (30 fruits per box) and dipped for 0.5, 1.5, or 3.0 min in a water mixture of (a) 1200 mg/L IMZ at 20 °C, (b) 200 mg/L IMZ at 50 °C, (c) 1200 mg/L TBZ at 20 °C, and (d) 200 mg/L TBZ at 50 °C. Analyses of TBZ and IMZ were carried out 4−5 h after treatment and after 3 days of simulated marketing conditions (SMP) at 17 °C and ∼90% relative humidity.

Experiment II (Influence of Dip Temperature on Fungicide Deposition). Salustiana oranges were placed in plastic boxes (30 fruits per box) and dipped for 3.0 min in mixture of (a) 150 mg/L IMZ at 20, 30, 40, and 50 °C; (b) 150 mg/L TBZ at 20, 30, 40, and 50 °C; and (c) 150 mg/L IMZ plus 150 mg/L

* Corresponding author (telephone +39.783.33224; fax +39.783.33959; e-mail M.Schirra@imfpp.ss.cnr.it).

† Università di Cagliari.

‡ CNR Istituto per la Fisiologia della Maturazione e della Conservazione del Frutto delle Specie Arboree Mediterranee.

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Table 1. Influence of Dip Length on Residue Levels of IMZ and TBZ in Marsh Seedless Grapefruits

dip treatment	days after dip treatment	dip length 0.5 min	1.5 min	3.0 min
IMZ Residue[a] (mg/kg)				
1200 mg/L IMZ (20 °C)	0[b]	2.79b	4.11ab	5.59a
	3	3.05b	4.28ab	6.85a
200 mg/L IMZ (50 °C)	0	1.87b	2.54b	3.90a
	3	1.86b	2.43ab	3.99a
TBZ Residue[a] (mg/kg)				
1200 mg/L TBZ (20 °C)	0	3.08a	3.54a	2.82a
	3	3.48a	3.36a	3.36a
200 mg/L TBZ (50 °C)	0	1.16b	1.48ab	1.92a
	3	1.46a	1.52a	1.40a

[a] Different letters denote significant differences within each row by Duncan's multiple range test, $P = 0.05$. [b] 4−5 h after treatment.

Table 2. Influence of Dip Temperature and Storage Duration at 17 °C on Residue Levels of IMZ and TBZ in Salustiana Oranges Following Fungicide Application Separately (I) or in Combination (II)

treatment	dip temp (°C)	storage duration 0 weeks	3 weeks	6 weeks	9 weeks
IMZ Residue[a] (mg/kg)					
I	20	0.72Aa	0.81Aa	0.85Aa	0.64Aa
II	20	0.70Aa	0.45Ba	0.33Ba	0.46Ba
I	30	1.36Aa	1.59Aa	1.69Aa	1.13Aa
II	30	1.31Aa	0.85Bb	0.89Bb	0.70Bb
I	40	3.42Aa	3.07Aba	2.87Ba	2.85Ba
II	40	2.20Aa	1.79Ba	1.46BCb	1.29Cb
I	50	5.87Aa	6.22Aa	5.01Aa	5.25Aa
II	50	5.58Aa	5.19Aa	4.82Aa	3.06Bb
TBZ Residue[a] (mg/kg)					
I	20	0.62Aa	0.45Ba	0.39Ca	0.33Da
II	20	0.42Ab	0.31BCb	0.24Cb	0.34Ba
I	30	0.79Aa	0.62ABa	0.65Aba	0.49Ba
II	30	0.71Aa	0.62Aba	0.44BCb	0.41Ca
I	40	0.69Aa	0.68Aa	0.56Aa	0.51Aa
II	40	0.79Aa	0.57Ba	0.48Bb	0.46Ba
I	50	1.55Aa	1.00Ba	0.68Ba	0.51Ba
II	50	1.32Ab	0.83Ba	0.73Ba	0.65Ba

[a] In each row or column group different letters denote significant differences by Duncan's multiple range test, $P = 0.05$. Capital letters for comparison of the influence of storage duration within each treatment; lower case letters for comparison of the effect of treatment within each dip temperature.

TBZ at 20, 30, 40, and 50 °C. Analyses of IMZ and TBZ were carried out 4−5 h after treatment and after 3, 6, and 9 weeks at 17 °C.

Experiment III (Effect of Fruit Washing on Fungicide Removal). Star Ruby grapefruits were divided into four treatment groups and dipped for 3.0 min in mixture of (a) 1200 mg/L IMZ at 20 °C, (b) 200 mg/L IMZ at 50 °C, (c) 1200 mg/L TBZ at 20 °C, and (d) 200 mg/L TBZ at 50 °C. Before analysis, fruits of the respective groups were divided into two subgroups. Fruits of the first subgroup were washed with cold (∼16 °C) tap water for 30 s by passing a sponge over the fruit surface, whereas fruits of the remaining group were used as control. Analyses of TBZ and IMZ were carried out 4−5 h after treatment, after 6 weeks of storage at 8 °C, and after a subsequent 1 week at 20 °C.

Treatment Device. Dip treatments were performed in a bath fitted with heating elements and an electronic recirculation pump. Two hundred liters of fungicide mixtures was used for the treatments, dipping one box of fruit at a time. After two dips, the fungicide mixtures were discharged and renewed. Bath temperature was constantly maintained within ±0.5 °C of the programmed temperature by an electronic thermostat (OEM/HT, Carel, France) and probes (PTC 40, Carel, France). Following treatment, the fruits were left to dry at room temperature for ∼5 h.

Chemicals. IMZ and TBZ mixtures in water were prepared with commercially available Fungazil 500 EC (44.66% active ingredient, Janssen Pharmaceutica N.V. Belgium) and Tecto 20 S (22% active ingredient, Merck Sharp and Dhome, Nedherland), respectively.

Analysis of IMZ and TBZ. Three fruits per replication were weighed, and their peels were removed and weighed; the percentage of peel with respect to the whole fruit was calculated. Samples of peel were then triturated with a mincing knife, homogenized, and stored in a freezer at −20 °C until analysis. Extraction procedures, recovery assays, sample preparation, and IMZ and TBZ determinations were performed as described by Schirra et al. (1996, 1998b).

Data Analysis. Each experiment was replicated four times. Analysis of variance (ANOVA) was performed by MSTAT-C software (1991). Mean comparisons were performed by Duncan's multiple range test at $P = 0.05$, when appropriate.

RESULTS AND DISCUSSION

The residue level of IMZ in Marsh grapefruit after treatment with 1200 mg/L at 20 °C for 0.5 min was increased from 2.79 mg/kg to levels 47−100% higher by dipping for 1.5 and 3.0 min, respectively (Table 1). Similarly, dipping with 200 mg/L IMZ at 50 °C for 1.5 and 3 min increased the residues 35.8−108% higher than residues from dipping for 0.5 s. In contrast, TBZ residues did not change with the different dip times after treatment with 1200 mg/L TBZ at 20 °C or with 200 mg/L TBZ at 50 °C. Similar results were obtained by Brown and Dezman (1990) and Smilanick et al. (1997) by increasing the treatment duration with IMZ.

Previous investigations on Valencia oranges and Eureka lemons (Smilanick et al., 1997) have demonstrated that by doubling the IMZ concentration or length of dipping or by increasing the solution temperature by 5.6 °C the residue uptake increased approximately 1.5−2times on the fruit. The present study showed that following treatment with IMZ or TBZ at 150 mg/L concentration, the residue levels of fungicides (Table 2) were significantly correlated with dip temperature ($r = 0.943$ for IMZ; $r = 0.911$ for TBZ). Differences in uptake of fungicides could be associated with their diverse physicochemical characteristics and different formulation types used. Treatment with IMZ at 50 °C produced a deposition of 5.87 mg/kg, which was ∼8-fold higher than deposition from treatment at 20 °C (0.72 mg/kg). Fruits dipped in TBZ at 50 °C had residues of 1.55 mg/kg, which is 2.5-fold higher than concentrations in fruit treated at 20 °C (0.62 mg/kg). No significant differences in initial residues of IMZ were detected between separate or combination treatments. However, after 9 weeks of storage, significantly lower amounts of IMZ were measured in fruit treated in combination versus separately (on average, 42 versus 14%). Therefore, the presence of TBZ reduces IMZ absorption by cuticular wax. The only degraded portion of IMZ is that which has not penetrated into the wax; for this reason, the increase in the fraction of IMZ that does not penetrate into the wax leads to an increase in the amount of IMZ that is degraded. This occurrence is corroborated by the results of experiment III. Considering that commercially IMZ is used together with TBZ, the greater losses in persistence of IMZ in the combined treatment may significantly impact its efficacy. TBZ applied in combination did not significantly affect its deposition in oranges in comparison with treatments carried out separately. After 9 weeks of storage, residues decreased

Table 3. Influence of Fruit Washing before Analysis on Residue Levels of IMZ and TBZ in Star Ruby Grapefruits Following Fruit Dipping in Fungicide Mixtures As Indicated

treatment	washing before analysis	storage duration: 0 weeks	6 weeks	6 + 1 weeks
		IMZ Residue[a] (mg/kg)		
1200 mg/L, 20 °C	no	4.40a	3.35a	2.43a
	yes	2.18b	2.34b	2.66a
200 mg/L, 50 °C	no	5.33a	3.64a	2.74a
	yes	2.87b	3.39a	3.34a
		TBZ Residue[a] (mg/kg)		
1200 mg/L, 20 °C	no	3.77a	4.00a	4.30a
	yes	0.40b	0.30b	0.47b
200 mg/L, 50 °C	no	3.76a	2.50a	2.48a
	yes	0.44b	0.31b	0.35b

[a] Different letters denote significant differences within each treatment group by Duncan's multiple range test, $P = 0.05$.

by an average of ~40% in separated or combination treatments. The concentrations of IMZ from dipping at 50 °C are similar to those measured in lemons treated with comparable IMZ levels (Schirra et al., 1997). However, uptake of TBZ in oranges was much less than that in lemons (Schirra et al., 1997). Both fungicides showed great persistence during storage (Schirra et al., 1996, 1997, 1998a,b) especially IMZ, which declined minimally during storage; >83% of it was still present after 9 weeks of storage.

Previous studies (Brown et al., 1983) demonstrated that IMZ residues in oranges were not easily removed by washing fruit on tumbler brushes as only 3% of the residues were removed from fruit treated with IMZ in the water mixture, whereas 31% of the residues were removed by washing fruit treated with IMZ in the water wax. The present investigation showed that home washing, carried out 4−5 h after dip treatment with mixtures of 1200 mg/L at 20 °C or 200 mg/L at 50 °C, removed ~50% of the IMZ residues and ~90% of the TBZ in both treatments (Table 3). The quantities of residues that cannot be removed by washing are those that have penetrated into the cuticle (Rieder and Schreiber, 1996). Residues of IMZ decreased during storage, whereas residues remaining after washing did not change. Thus, only the IMZ fraction that penetrated into the wax did not undergo any degradation. TBZ residues did not change during storage either in washed or in unwashed fruit. Taking into account the facts that the residues of TBZ and IMZ in citrus fruit are retained mostly by the peel portion (Brown et al., 1983; Tadeo et al., 1988) and that 50% of IMZ and 10% TBZ are absorbed by the peel and not removed by home washing, the tolerance thresholds for the whole fruit when used for processing should be critically re-examined.

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