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Residue Uptake and Storage Responses of Tarocco Blood Oranges after Preharvest Thiabendazole Spray and Postharvest Heat Treatment

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Tarocco blood oranges (*Citrus sinensis* Linn. Obsek) were subjected to a single preharvest spray with thiabendazole (TBZ) at a concentration of 1% active ingredient (ai) in water and harvested 2 weeks after fungicide application or heated at 37 °C for 48 h under saturated humidity after harvest. The two treatments were also combined before cold quarantine (3 weeks at 2 °C), subsequent storage (3 weeks at 8 °C), and simulated marketing period (SMP) (1 week at 20 °C). Fruit not treated with TBZ and unheated were used as controls. The residue levels of TBZ (active ingredient, whole fruit basis) after spray were approximately 6.3 and 5.4 mg·kg^{-1} before fruit storage respectively, a level close to the tolerance limit set by the European Community. TBZ showed a high persistence during quarantine, storage, and SMP. TBZ spray significantly reduced the incidence and severity of chilling injury (CI) and decay during the postquarantine period and SMP. Heat treatment (HT) produced beneficial effects in controlling CI, especially during SMP, when applied in combination with TBZ. However, HT remarkably promoted the development of secondary fungal infections such as *Phytophthora* rots and adversely affected fruit flavor and taste. The occurrence of off-flavor and off-taste was found to be perceptible after heating.

KEYWORDS: Thiabendazole; residues; citrus fruit; cold quarantine; chilling injury; decay

INTRODUCTION

The quarantine security regulations of certain citrus-importing countries require that fruit must be certified free from fruit fly. Among postharvest disinfestation procedures, cold quarantine treatments are accepted by the regulatory agencies of most importing countries and are commercially applied to disinfest a wide range of fruit species, including citrus (*1*). Chilling injury (CI) is a major problem when certain citrus fruit cultivars are disinfested by low-temperature treatments. Symptoms of CI may not be apparent during cold treatment but may be fully expressed afterward, when fruit are returned to warm conditions, predisposing them to decay (*2*). Fruit subjected to film packaging or wax coating usually develop less CI (*3*). Heat treatments (hot water treatments or curing) reduce postharvest decay and alleviate CI (*4*). Similarly, certain fungicides such as thiabendazole (TBZ), which suppress postharvest decay, also mitigate the expression of CI (*5*, *6*), indicating that the effectiveness of TBZ is especially valuable when used in combination with hot water (*7*−*10*). However, we found no papers in the literature on residue uptake and chilling response to postquarantine treatment of citrus fruit subjected to preharvest TBZ spray and postharvest heat treatment (HT).

The results of the present study provided evidence for the potential of TBZ spray in controlling CI in Tarocco oranges during postquarantine, and we also demonstrated that the feasibility of this approach on a commercial scale is problematic, because considerably higher levels in preharvest treatment of fungicide are required to achieve equal fungicide residues and effectiveness with respect to conventional postharvest treatments.

MATERIALS AND METHODS

Plant Material, Preharvest Treatments. The investigation was carried out on Tarocco blood oranges (*Citrus sinensis* Linn. Obsek) grown in an experimental grove located in southern Sardinia (Italy) receiving standard horticultural practices. The experiment was set up in a randomized block design with four replicated plots each containing three trees. Preharvest treatments consisted of a single spray of TBZ mixture at a concentration of 1% active ingredient (ai) in water. This concentration was set according to three preliminary separate trials at our laboratories, which indicated that 24 h following spray, the mean concentration of TBZ uptake was 5.47 mg·kg^{-1}, on whole fruit bases

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(author's unpublished data), a residue level close to the tolerance limit established by the European Community (EC) for citrus fruit (6.0 mg·kg^{-1}). The TBZ mixture was prepared with commercially available Tecto 20 S (19.7% ai, TBZ, Merck Sharp & Dohme S.P.A). Each tree was wetted to the point of drip using a low-volume sprayer (~15 L of fungicide per tree). Unsprayed fruits were used as controls. Fruits were harvested 2 weeks after fungicide application, at the first week of February, when susceptibility to CI was still high (*11*). There was no rainfall registered from TBZ treatment to harvest.

Postharvest Treatment and Storage Conditions. Untreated and TBZ-treated fruits were placed in plastic trays, delivered to the laboratory within 3 h after harvest, graded, sized, returned to the trays, and respectively divided into two groups. Fruits of the first group were moved to a ventilated storage room kept at 2 °C and 90−95% relative humidity (RH) for 3 weeks (simulated cold quarantine conditions). The remainding fruits were transferred to a heated room at 37 °C and 95−98% RH. Air temperature and humidity of the heated chambers were recorded simultaneously using a portable drum instrument (TIG-1TH Thermohygrograph, LSI, Milan, Italy). After 48 h, the fruit was moved to the cold storage area containing the first group.

Each treatment group included three subgroups. Fruits of the first subgroup (four replicate fruit trays) were used for assessment. The second subgroup (four replicate fruit trays) was used for analysis of TBZ, whereas fruits of the remaining subgroup (40 fruits) were individually weighed for the determination of transpiration rate as fruit mass loss. Following quarantine, fruits were stored for 3 additional weeks at 8 °C and ~85% RH (optimal storage conditions for Tarocco oranges). At the end of cold storage, fruit was maintained at 20 °C and 80% RH for 1 week to simulate marketing period (SMP).

Visual Assessments, Fruit Weight Loss, and Organoleptic Acceptance. Visual assessments, fruit weight loss, and organoleptic characteristics (fruit flavor and taste) were carried out at the end of quarantine, storage, and SMP. These included CI, rot incidence, treatment damage, and external fruit quality. The amount of peel surface affected by CI (peel pitting and brown staining) was scored as none (score 0, no CI), slight (score 1, few scattered pittings), moderate (score 2, covering up to 25% of the rind surface), and severe (when injury covered >25% of the rind surface). Then, the percentage of fruit in each rating was calculated. Decay incidence was recorded as total rots caused by green mold (*Pennicillium digitatu*m Sacc.), blue mold (*Penicillium italicum* Wehmer), brown rot (*Phytophthora citrophthora*), and sour rot (*Geotrichum candidum*) or as miscellaneous rots of other fungi. Overall visual quality was rated subjectively as one of five categories: 5 (excellent), 4 (good), 3 (fair), 2 (poor), and 1 (very poor). Fruit flavor and taste were scored as 3 (good), 2 (fair), and 1 (poor). An informal panel of three people, familiar with blood oranges, assessed visual quality and organoleptic characteristics (*11*).

Thiabendazole Analysis. *Chemicals.* Thiabendazole was analytical standard (Ehrenstorfer), and analytical grade triphenyl phosphate (99%, Janssen, Geel, Belgium) was used as the internal standard (i.s.). Ethyl acetate and methanol were of HPLC grade, whereas hexane was of pesticide grade (Carlo Erba, Milan, Italy). Anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba). A stock standard solution of thiabendazole (~500 mg/kg) was prepared in methanol. Working standard solutions, containing 0.3 mg/kg i.s., were obtained by dilution with the extract from untreated flavedo without interfering peaks. The extraction solution was an ethyl acetate/hexane (50:50, v/v) mixture containing the i.s. at 0.3 mg/kg (*9*).

Apparatus and Chromatography. An HRGC trace gas chromatograph (ThermoQuest, Milan, Italy) was employed. It was fitted with an NPD-80 nitrogen−phosphorus detector, an AS 2000 autosampler (Carlo Erba), a split−splitless injector, and a chromcard chemstation for trace. A Durabond fused silica capillary column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) with DB-5 (5% phenylmethylpolysiloxane) stationary phase (film thickness = 0.25 μm) was employed. The injector and detector were operated at 250 and 280 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), oven temperature was set at 110 °C for 1 min, raised to 280 °C (20 °C/min), and held for 6 min. Helium was the carrier and makeup gas at 120 and 130 kPa, respectively. The calibration curve was constructed drawn with the i.s. method, by measuring peak heights versus concentrations. Good linearity was achieved in the 0−10 mg/kg range, with a correlation coefficient of 0.9992.

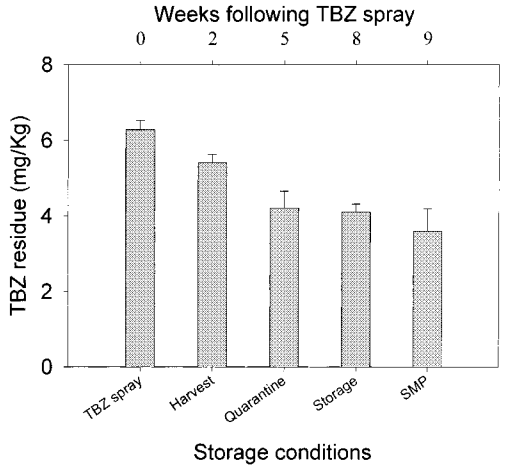

Figure 1. Residue levels (milligrams per kilogram active ingredient, whole fruit basis) following 1% (active ingredient) thiabendazole (TBZ) preharvest treatment, at harvest, after quarantine (3 weeks at 2 °C), storage (3 additional weeks at 8 °C), and simulated marketing period (1 week at 20 °C). Following TBZ spray and at harvest data are mean of four replicate analysis. After quarantine, storage, and SMP data are means of eight replicate fruit samples (four replicates × two treatments, TBZ and TBZ + HT). Vertical bars denote SE.

Sample Preparation. Analysis of TBZ was performed following treatment and after quarantine, storage, and SMP. Five fruits per replication were weighed, and their peels were removed and weighed; the percentage 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. Each analysis was replicated four times.

Extraction Procedure. A 2.5-g aliquot of homogenate was weighed in a 30-mL screw-capped tube; 2 g of sodium chloride and 10 mL of an ethyl acetate/hexane (50:50, v/v) mixture containing the i.s. (0.3 mg/kg) were added, and the tube was then shaken in a rotary shaker (GFL) for 20 min. The phases were allowed to separate, and the organic layer was poured into another flask containing 1 g of anhydrous sodium sulfate and then injected for gas chromatographic analysis.

Recovery Assays. Untreated samples were fortified with thiabendazole at 1, 10, and 25 mg/kg for peel and at 0.01, 0.05, and 0.20 mg/kg for flesh and processed according to the procedure described above. Recoveries from four replicates showed values ranging from 87 to 106%.

Statistical Analysis. Analysis of variance (ANOVA) was performed by MSTAT-C software (1991). Mean comparisons were performed by Tukey's test, $P \leq 0.05$, where appropriate.

RESULTS

The residue level of TBZ (whole fruit basis) after treatment was ~6 mg·kg^{-1} and 5.4 mg·kg^{-1} 2 weeks later (**Figure 1**). From a regulatory standpoint, before storage, the amount of TBZ residue in fruit was close the tolerance limit set by the EC. However, it should be pointed out that residue levels of fungicide applied to the tree may vary with environmental and other field conditions. There were no significant differences in residue level between fruits treated with TBZ separately or in combination with HT. Therefore, after quarantine, storage, and SMP, data on TBZ residues are presented as an overall average of eight replicates (four replicates × two treatments, TBZ and TBZ + HT). The degradation rate of TBZ was very low during refrigeration as 67% of it was still present after SMP.

No visible symptoms of CI were detected after 3 weeks of cold quarantine treatment (**Table 1**). However, after poststorage

Table 1. Influence of Preharvest Thiabendazole Spray (TBZ, 1% Active Ingredient), Postharvest Heat Treatment (HT, 48 h at 37 °C), Combined Treatments (TBZ + HT), and Storage Conditions on the Incidence of Chilling Injury, *Penicillium* Species and *Phytophthora* Infections, and Total Decay in Tarocco Oranges[a]

	chilling injury (%)		rot (%)		total
treatment	slight	moderate + severe	*Penicillium* spp.	*P. citrophthora*	decay (%)
3 Weeks at 1 °C + 3 Weeks at 8 °C					
control	10.0a	20.0a	10.8a	0.8b	11.6a
TBZ	0.0b	2.5b	0.0b	0.8b	0.8b
HT	4.2ab	6.7b	2.5b	6.7a	9.2a
TBZ + HT	0.0b	3.3b	0.0b	0.8b	0.8b
3 Weeks at 1 °C + 3 Weeks at 8 °C + 1 Week at 20 °C					
control	12.0a	35.0a	20.8a	2.5b	23.3a
TBZ	0.8b	8.3bc	0.0b	0.8b	0.8c
HT	4.2b	11.7b	5.8b	15.0a	20.8ab
TBZ + HT	1.7b	4.2c	0.0b	15.8a	15.8b

[a] Mean separation within each column group by Tukey's test, $P \leq 0.05$.

at 8 °C for 3 weeks, 10% of untreated fruit had slight CI, whereas 20% of fruit exhibited moderate to severe injury. No further increases in slight CI were detected after SMP. By contrast, fruit with moderate to severe CI increased remarkably, accounting for 35% after SMP. TBZ spray and HT significantly reduced CI during storage and SMP. After SMP, TBZ + HT was significantly more effective than HT alone in reducing the extent and severity of CI.

There was very little decay (<1%) in untreated fruit after quarantine (data not shown). Afterward, decay incidence increased rapidly, accounting for approximately 12 and 23% after storage and subsequent SMP, respectively. The main agents of decay in untreated fruit were green and blue molds. Brown rot also developed on fruit but at very low extent. TBZ resulted in almost complete control of decay, giving a total suppression of *Penicillium* spp. HT significantly reduced *Penicillium* decays but remarkably promoted the development of brown rot. Similar results were obtained with the combined treatment due to the lack of efficacy of TBZ against brown rot.

No treatment-dependent damage (peel necrosis or browning to the rind) could be observed after storage and SMP. However, HT produced adverse effects on organoleptic acceptance. The appearance of off-favor and bad taste has been perceived on fruit removal to the heating room and became more pronounced as storage duration proceeded. After storage and SMP, fruit flavor and taste were scored as fair and poor, respectively. By contrast, unheated fruits were scored as good, even after SMP (data not shown). The influence of treatments on fruit weight loss was negligible (**Figure 2**).

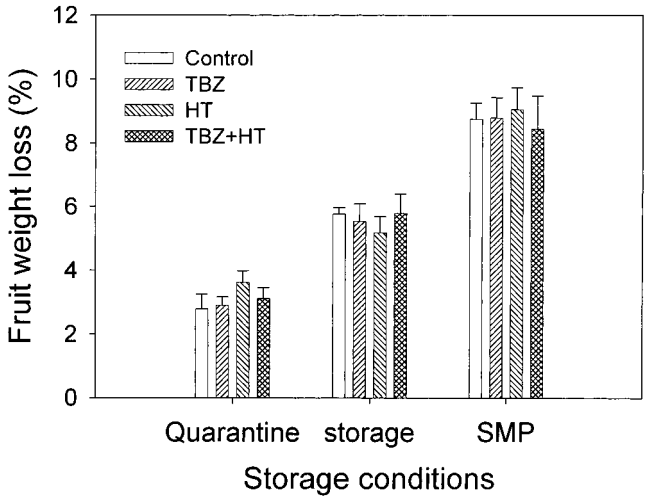

Figure 2. Fruit weight loss (percent) in Tarocco oranges after quarantine (3 weeks at 2 °C), storage (3 additional weeks at 8 °C), and simulated marketing period (1 week at 20 °C). Each data point is the mean (±SE, 99% confidence) of four treatment fruit samples.

DISCUSSION

Many papers have been published on TBZ uptake, persistence, and effectiveness in decay control of citrus fruit following postharvest treatments and subsequent standard storage conditions (*9*, *12*−*14*). However, much less is known about the feasibility of preharvest application of TBZ for control of storage decay of citrus fruit (*15*, *16*). We found no papers in the literature about residue deposition on citrus fruit after preharvest TBZ sprays or about the potential of these treatments in controlling CI. Specific studies on residue uptake have been carried out on other benzimidazoles such as benomyl (*17*, *18*). The present study provides, for the first time, an indication of the efficacy of TBZ spray as a possible approach to reducing the expression of CI in Tarocco oranges during a postquarantine period without impairing fruit quality. Postharvest heat treatment gave equivalent control of decay but adversely affected fruit flavor and taste. A worsening of the flavor−taste traits was perceptible after HT and became more pronounced as storage duration proceeded. Thus, HT cannot be recommended as a possible approach to alleviate CI in Tarocco oranges.

Schirra et al. (*9*) have shown that a conventional dip treatment with 1200 mg/L TBZ at room temperature, or with 200 mg/L TBZ at 50 °C, produced similar residue uptakes in Tarocco oranges (from 3.4 to 6.5 mg·kg^{-1}, depending on harvest date), but lower doses of heated fungicide proved to be more effective in alleviating CI. The present investigation showed that to reach such levels of residues in fruit, the concentration of fungicide to be used should be ∼1%, approximately 8- and 14-fold higher than conventional postharvest dip treatments at room temperature or at 50 °C, respectively, indicating that considerably more TBZ must be used.

Green and blue molds are the major agents of postharvest decay of citrus fruit produced in areas with scant summer rainfall (*19*, *20*) and occasionally may cause rots in the orchard, depending on fruit fly population and environmental conditions. As both fungi are wound pathogens (*21*) and incipient infections are difficult to suppress by postharvest treatments (22), preharvest fungicide treatments may have the advantage of being present on fruit before injuries occur during harvesting and postharvest handling (*15*). However, preharvest treatments may not be effective in controlling *Penicillium* rots because most rind injuries occur during harvesting and handling. Thus, when fruits are treated upon arrival at the packinghouse, rind injuries may absorb enough active agent to suppress spores and eradicate most of the occurring infections (*20*). Results reported herein show that TBZ spray completely prevented the development of *Penicillium* decays in Tarocco oranges during quarantine, subsequent storage, and SMP.

Lanza et al. (*23*) have shown that heat treatment at 36 °C for 48 or 72 h considerably reduced the incidence of decay in Tarocco oranges artificially wounded and inoculated with *P. digitatum*. Accordingly, the present study confirms the beneficial effect of HT in controlling the occurrence of natural infections of *Penicillium* spp. but favored the development of "secondary" decays such as brown rot during the postquarantine period, even when HT was preceded by TBZ spray, as the chemical was

inactive against this pathogen. Various studies have been performed to explain the HT's mode of action in controlling postharvest decay (*4*), and several physiological and biochemical treatment-dependent changes have been related to the induction of fruit resistance to CI (*24*). Similarly, the mechanism of action of TBZ against pathogen is recognized (*25*, *26*). However, the physiological effects of this fungicide in alleviating CI have not yet been elucidated.

CONCLUSIONS

Established concentrations of TBZ in conventional postharvest treatments of citrus fruit to ensure best protection against green and blue mold decay range from 1000 to 2000 mg/L as aqueous suspension and up to a 4000 mg/L or even more when TBZ is used as a water-based wax coating of the fruit (*27*). Such levels of TBZ effectively reduced the expression of CI during cold quarantine treatment (*6*) and should provide the best protection against green and blue molds (*27*). The present study indicated that a single spray with TBZ 14 days before harvest controlled decay and alleviated the incidence and severity of CI in Tarocco oranges during the postquarantine period, thereby confirming the physiological performance of TBZ, as widely demonstrated when used in postharvest treatments (*5*, *6*, *9*, *10*). However, a much higher concentration of the fungicide is required for equivalent residue uptake and effectiveness in comparison to postharvest treatments. In addition, application of such high TBZ concentrations in the orchard is extremely risky in relation to the development of resistant biotypes of the green and blue mold pathogens and to the environmental impact due to TBZ's anthelmintic properties.

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