Seasonal Susceptibility of Tarocco Oranges to Chilling Injury As Affected by Hot Water and Thiabendazole Postharvest Dip Treatments

Mario Schirra,*,† Guy D'hallewin,† Paolo Cabras,‡ Alberto Angioni,‡ and Vincenzo Luigi Garau‡

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

Susceptibility of Tarocco (blood) oranges (*Citrus sinensis* Linn. Obsek) to chilling injury (CI) was investigated with fruit harvested from December through April. The fruit was subjected to a 3 min dip in water with and without thiabendazole (TBZ) at 200 ppm (50 °C) or 1200 ppm (19 °C, room temperature) and then stored at 3 °C and 90−95% relative humidity (RH) for 6 weeks followed by 1 additional week at 20 °C and approximately 80% RH to simulate a marketing period (SMP). CI development was significantly affected by the age of the fruit, decreasing later in the season. Water dips at 50 °C reduced CI. However, dip efficacy was significantly influenced by picking date and storage duration. Treatments with 1200 ppm TBZ at 19 °C also ameliorated CI but had no better effect than water at 50 °C. Treatments with 200 ppm TBZ at 50 °C significantly suppressed CI development on fruits harvested throughout the season. TBZ accumulation on fruits following 200 ppm dips at 50 °C was not significantly dependent on fruit age, while 1200 ppm TBZ at room temperature resulted in significantly higher TBZ uptake in fruits harvested late (April) rather than early (December), with intermediate concentrations of fungicide in fruits harvested from January through March. Residue concentrations in fruits after treatment with 200 ppm TBZ at 50 °C were not significantly different from those found following 1200 ppm TBZ dipping at 19 °C throughout the season. We concluded that postharvest treatments with 1200 ppm TBZ at room temperature or 200 ppm TBZ at 50 °C produced similar TBZ uptake in Tarocco oranges, but TBZ treatments at 50 °C water gave the most effective reduction of CI symptoms.

Keywords: *Citrus; chilling injury; harvest date; heat treatments; storage; thiabendazole*

INTRODUCTION

Long-term storage of citrus fruit requires relatively low temperatures, ranging from 6 to 10 °C, depending upon species, cultivar, and storage duration. High relative humidity, adequate ventilation, and air purification of refrigerated rooms are also recommended (Grierson and Ben-Yehoshua, 1986). Some citrus cultivars, however, are susceptible to chilling injury (CI) when stored below 10 °C. Blood (pigmented) oranges are known to be much more susceptible to CI than nonpigmented oranges (Pratella et al., 1969). Studies with Tarocco (blood) oranges have shown that susceptibility to CI is greatly dependent on the harvest date and that the severity of damage may be alleviated by prestorage 53 °C water dips (Schirra et al., 1997). Postharvest treatments with thiabendazole (TBZ) reduced CI in Tarocco oranges during cold storage and a subsequent simulated marketing period (SMP); the efficacy of TBZ's fungicidal and CI-reducing effects being enhanced when it was used in combination with hot water (Schirra and Mulas, 1995). There have been no reports of seasonal responses of pigmented oranges to chilling storage preceded by TBZ treatments.

This study was therefore designed to investigate the effects of TBZ on chilling injury in cold stored Tarocco oranges harvested at various maturity stages. Relatively low doses (200 ppm) of fungicide at 50 °C were employed in comparison with the standard 1200 ppm treatment at room temperature (19 °C).

MATERIALS AND METHODS

Fruit. The investigation was conducted with Tarocco oranges (*Citrus sinensis* Linn. Obsek) grown in an experimental grove (Southern Sardinia, 39° 55′ N) receiving standard horticultural care. Fruit was harvested at monthly intervals, from the first week of December (when the fruit acquired the full orange color but was not yet commercially mature) to April (overmature fruit). Each harvest involved a random sampling from 15 trees subdivided into 3 replicates of 5 trees each. Twenty fruits were picked from the exterior of the canopy of each tree, placed in plastic boxes, delivered to the laboratory immediately after harvest, and left overnight.

Treatments and Storage. Blemish-free oranges were selected and grouped into 4 treatment lots (each with 4 replications containing 50 fruits each), corresponding to the following 3 min dip treatments: (a) water at 19 °C, (b) water at 50 °C, (c) 1200 ppm TBZ at 19 °C, and (d) 200 ppm TBZ at 50 °C. Dip treatments were applied using an apparatus described previously (Schirra and D'hallewin, 1997). Following treatment, the fruits were dried at room temperature for ca. 5 h. The fruits of 3 boxes of each treatment were used for evaluation of CI, rot incidence, and treatment damage, and the fruits of the remaining box were used for TBZ analysis.

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

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

‡ Università di Cagliari.

S0021-8561(97)00776-0 CCC: $15.00
Published on Web 02/27/1998

After treatment, the fruits were stored at 3 °C and 90−95% relative humidity (RH) for 6 weeks, with a complete air change every hour. These conditions favor development of CI. At the end of storage, the fruits were maintained at 20 °C and about 80% RH for 1 week to simulate a 1 week marketing period.

Visual Assessment. At weekly intervals during cold storage and after SMP, the fruits were examined for treatment damage, CI, and rot incidence. CI was rated as 0 (no damage), 1 (slight, <10% peel pitting), 2 (moderate, 10−30% peel pitting), and 3 (severe, >30% peel pitting). To obtain a weighted average for the chilling index, the number of fruits with each CI rating was multiplied by the rating number and the sum of these products was divided by the total number of fruits in the sample to give an average CI for the sample. Decay was caused by *Penicillium italicum* Wehmer, *Penicillium digitatum* Sacc., *Alternaria citri* Ell. & Pierce, *Botrytis cinerea* Pers. ex Fr., or miscellaneous rots of unidentified fungi, and the total decay percentage was calculated.

Thiabendazole Analysis. *Chemicals.* Thiabendazole was an analytical standard purchased from Ehrenstorfer (Augsburg, Germany). Triphenyl phosphate (99%) was used as the internal standard (i.s.) and was analytical grade (Janssen, Geel, Belgium). Ethyl acetate and methanol were HPLC grade, while 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 (ca. 500 mg/kg) was prepared in methanol. Working standard solutions, containing the i.s. at 0.3 mg/kg, were obtained by dilution with the extract from untreated flavedo without interfering peaks. Extraction solution: ethyl acetate/hexane (50/50 (v/v)) mixture containing the i.s. at 0.3 mg/kg.

Apparatus and Chromatography. An HRGC Mega 5160 gas chromatograph (Carlo Erba) was employed. It was fitted with an NPD-40 nitrogen phosphorus detector, an AS 550 autosampler (Carlo Erba), and a split−splitless injector. It was connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with DB 5 (5% phenylmethypolysiloxane) liquid phase (film thickness 0.25 μm). The injector and detector were operated at 250 and 280 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 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 graph was constructed with the i.s. method by measuring peak heights vs concentrations. Good linearity was achieved in the range 0−10 mg/kg, with a correlation coefficient of 0.9992.

Sample Preparation. Three replicates of three fruits each were used for thiabendazole analysis. Fruits were weighed, and the flavedo was removed with a vegetable peeler. The flavedo was weighed, and its percentage with respect to the whole fruit was calculated. It was then triturated with a mincing knife and homogenized. The samples were stored in a refrigerator at −20 °C until analysis.

Extraction Procedure. A 2.5 g aliquot of homogenized sample (1 g for samples at highest concentrations) was weighed in a 30 mL screw-capped tube; 2 g of sodium chloride and 10 mL of ethyl acetate/hexane (50/50 (v/v)) mixture containing the i.s. at 0.3 mg/kg were added, and the tube was shaken in a rotary shaker (GFL, Germany) 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 flavedo samples were fortified with 1, 10, and 25 mg/kg of thiabendazole and processed according to the procedure described above. Recoveries from four replicates showed values ranging from 87 to 106%,

Data Analysis. Analysis of variance (ANOVA) was performed by MSTAT-C software (1991) according to a randomized complete block design. Mean comparisons were performed by Duncan's multiple range test ($P = 0.05$).

Table 1. Influence of Harvest Date and Postharvest Dip Treatments on Chilling Injury Index in Tarocco Oranges after 3 and 6 Weeks of Storage at 3 °C and after an Additional Week at 20 °C

	picking date[b]				
treatment[a]	December	January	February	March	April
3 Weeks at 3 °C					
water 19 °C	1.67^{aB}	1.90^{aB}	2.59^{aA}	1.24^{aB}	0.16^{aC}
water 50 °C	0.38^{cB}	0.36^{bB}	2.02^{aA}	0.26^{cB}	0.03^{aB}
1200 TBZ 19 °C	0.94^{bB}	0.95^{bB}	2.19^{aA}	0.64^{bBC}	0.03^{aC}
200 TBZ 50 °C	0.03^{dC}	0.16^{bB}	1.12^{bA}	0.06^{cBC}	0.00^{aC}
6 Weeks at 3 °C					
water 19 °C	2.67^{aA}	2.67^{aA}	2.82^{aA}	1.87^{aB}	1.07^{aC}
water 50 °C	2.30^{bA}	1.60^{bB}	2.55^{aA}	0.62^{bC}	0.57^{bcC}
1200 TBZ 19 °C	2.64^{abA}	2.47^{aA}	2.63^{aA}	0.99^{bB}	0.90^{abB}
200 TBZ 50 °C	1.08^{cB}	0.94^{cBC}	1.99^{bA}	0.64^{bCD}	0.36^{cD}
6 Weeks at 3 °C + 1 Week at 20 °C					
water 19 °C	2.77^{aA}	2.72^{aA}	2.83^{aA}	2.17^{aB}	1.45^{aC}
water 50 °C	2.63^{aAB}	2.23^{bB}	2.74^{abA}	1.97^{aC}	0.83^{bD}
1200 TBZ 19 °C	2.89^{aA}	2.75^{aA}	2.84^{aA}	2.14^{aB}	1.06^{abC}
200 TBZ 50 °C	1.63^{bC}	2.12^{bAB}	2.38^{bA}	1.44^{bBC}	0.63^{bD}

[a] Treatments are 3 min dips followed by air-drying of the dipped fruits. [b] In each row or column group, means followed by a common letter are not significantly different by Duncan's multiple range test, $P \leq 0.05$. Lower case letters relate to comparisons of the effects of different treatments, within each harvest date. Capital letters relate to comparisons of the influence of different harvest dates, within each treatment.

RESULTS AND DISCUSSION

Decay was minimal (<2.0%) after SMP in the fruits harvested from December through February and dipped in water at 19 or 50 °C (data not shown). The decay percentages in the fruits harvested in March and April were 11.1 and 3.2%, respectively, in oranges dipped in water at 19 °C and 13.0 and 3.4%, respectively, in the fruits dipped in water at 50 °C. The relatively high decay caused by *Penicillium* spp. and *B. cinerea*, in fruits harvested in March may be attributed to prolonged rainy and warm weather before harvest. The decay incidence in the fruits treated with fungicide at 19 or 50 °C was negligible in all samples.

The susceptibility of Tarocco oranges to CI was greatly dependent on the age of the fruit, declining later in the season (Table 1). Visible symptoms of CI in the fruits harvested from December to March and treated with 19 °C water were observed during the second week of storage and 2 weeks later in the fruits picked in April (data not shown). At the end of cold storage and after SMP, the CI index was greatest in these fruits harvested from December to February, significantly lower in the fruits harvested in March, and even lower in oranges picked in April. Dipping the fruits in water at 50 °C reduced CI development in comparison to dipping at 19 °C. However, the efficacy of the 50 °C water dip was significantly dependent on the picking date.

The seasonal susceptibility of Tarocco oranges to CI was first reported by Schirra et al. (1997). They also demonstrated that when the fruits were harvested in mid-season (January−February), postharvest dips in water at 53 °C reduced CI and decay during subsequent cold storage and SMP. However, when oranges were harvested earlier or later in the season, the treatment could be harmful to the fruit. In contrast, the results of the present research showed that when dipping is carried out at 50 °C, no heat damage occurred to the peel.

Treatment with 1200 ppm TBZ compared to water at room temperature alleviated CI but did not produce any

Table 2. Thiabendazole Residues in Tarocco Oranges (μg/g, Whole Fruit Basis) As Influenced by Treatment, Time in Storage, and Picking Date

		picking date[c]				
treatment[a]	time in storage[b] (weeks)	December	January	February	March	April
1200 TBZ, 19 °C	0	3.40^{aC}	4.03^{aBC}	4.79^{aB}	4.62^{aBC}	6.50^{aA}
200 TBZ, 50 °C	0	3.48^{aB}	4.36^{aB}	4.29^{aB}	5.32^{aB}	4.98^{aB}
1200 TBZ, 19 °C	6 + 1	2.34^{bB}	3.08^{aB}	3.13^{bB}	2.33^{bB}	4.76^{aA}
200 TBZ, 50 °C	6 + 1	3.12^{abB}	3.86^{aAB}	4.73^{aA}	4.77^{aA}	3.63^{aB}

[a] Treatments are 3 min dips followed by air-drying of the dipped fruit. [b] 0 = following treatment; 6 + 1 = 5 weeks at 3 °C + 1 week at 20 °C. [c] In each row or column group, means followed by a common letter are not significantly different by Duncan's multiple range test, $P \leq 0.05$. Lower case letters relate to comparisons of the effects of different treatments, within each harvest date. Capital letters relate to comparisons of the influence of different harvest dates, within each treatment.

additional advantages with respect to water dipping at 50 °C. In contrast, the incidence of CI in the fruits treated with 200 ppm TBZ at 50 °C was significantly reduced throughout the season.

The increased efficacy of TBZ when applied in combination with hot water has been demonstrated in various citrus fruit cultivars susceptible to CI (Wild and Hood, 1989; McDonald et al., 1991; Rodov et al., 1994; Schirra and Mulas, 1995), and the enhanced activity of heated chemical has been associated with the higher deposition of active ingredient (a.i.) on fruit (Schirra et al., 1996). However, these investigations were carried out on the fruits from a single harvest, and comparisons between the effects of cold and heated fungicide application were performed by using equal mixture concentrations.

Thanks to the linear relationship we found between the residue levels of TBZ in fruit following treatment at 50 °C and fungicide solution concentration (Schirra, M.; Cabras, C. unpublished results), in this study we estimate that 200 ppm TBZ at 50 °C should produce an a.i. uptake similar to that measured after 1200 ppm dipping at room temperature (19 °C). This appraisal was largely confirmed by the present study (Table 2). Immediately after dipping, fruit residues of TBZ between these two treatments were similar throughout the harvesting season. As far as the influence of harvest date on a.i. uptake is concerned, the fruits harvested in April and treated with 1200 ppm TBZ at room temperature contained significantly more TBZ than the fruits picked earlier. It is known that during growth and ripening the epicuticular wax layer of citrus fruit undergoes a change from a crystalline to an amorphous state, with the appearance of rough and granular surface structures with a network of deep cracks coinciding with fruit maturation and senescence (Freeman et al., 1979; El-Otmani et al., 1989). Therefore, differences in a.i. uptake in relation to the maturity stage, when TBZ is applied at room temperature, may be due to the different physical status of epicuticular wax.

In contrast, TBZ deposition following 200 ppm dipping at 50 °C was not significantly dependent on the maturity stage of the fruits. Studies with "Fortune" mandarins revealed that hot water dips induced a melting and redistribution of the epicuticular wax, resulting in the coverage of surface cracks (Schirra and D'hallewin, 1997). Therefore, we speculate that the deposition of the hot fungicide may not depend on wax morphology but on its enhanced penetration through the epicuticular wax during treatment, rather than through gaps in the wax. Specific studies of this point are in progress at our laboratory.

Fungicide concentrations at the end of SMP in the fruits harvested in December, February, and March and treated with 1200 ppm TBZ at room temperature were significantly lower than their initial levels. Conversely, when the fruits were treated with 200 ppm TBZ at 50 °C, the concentrations of a.i. after SMP were not significantly different from those measured immediately after treatment. The persistence of TBZ when the fruits were dipped at 50 °C may be due to better encapsulation and coverage of the fungicide during melting and solidifying of the wax, giving better protection to the chemical.

Thus far, the mode of action of TBZ in reducing CI is unclear. Schiffmann-Nadel et al. (1972) have suggested that latent fungal infections in the peel begin to develop with loss of fruit resistance during unfavorable storage conditions (e.g., chilling temperature), thereby causing CI. Therefore, the inhibition of latent fungal infection by TBZ treatment may also affect CI development. Studies with grapefruits (Schiffmann-Nadel et al., 1975) showed that TBZ efficacy increased with residue concentration, and the physiological effect was attributed to a lower rate of peel senescence and reduced microbial activity.

Postharvest treatments with 1200 ppm TBZ at room temperature or 200 ppm TBZ at 50 °C produced similar fungicide residue in Tarocco oranges, but heated fungicide proved to be more useful in reducing CI. This could be due to positive synergistic effects of heat and TBZ, when applied in combination, in reducing antimicrobial activity, as suggested by Schiffmann-Nadel et al. (1975) and/or by enhancing fruit resistance to chilling stress (e.g., cell wall reinforcement, induction of heat shock proteins, membrane stabilization) (Klein and Lurie, 1992; Paull and McDonald, 1994). Such an effect might also explain the beneficial effect of the 50 °C dip relative to the control treatment.

ACKNOWLEDGMENT

We are indebted to Dr. J. M. Labavitch, Pomology Department, University of California, Davis, CA, for critical review of the paper.

LITERATURE CITED

El-Otmani, M.; Arpaia, M. L.; Coggins, Ch. W., Jr.; Pehrson, J. E.; O'Connell, N. W. Developmental changes in "Valencia" orange fruit epicuticular wax in relation to position on the tree. *Sci. Hortic.* **1989**, *41*, 69−81.

Freeman, B.; Albrigo, L. G.; Biggs, R. H. Ultrastructure and chemistry of epicuticular waxes of developing *Citrus* leaves and fruits. *J. Am. Soc. Hortic. Sci.* **1979**, *104*, 801−808.

Grierson, W.; Ben-Yehoshua, S. Storage of Citrus Fruits. In *Fresh Citrus Fruits;* Wardowski, V. F., Nagy, S., Grierson, W., Eds.; Avi Publishing Co. Inc.: Westport, CT, 1986; pp 479−507.

Klein, J. D.; Lurie, S. Heat treatments for improved postharvest quality of horticultural crops. *HortTechnology* **1992**, *2*, 316−320.

McDonald, R. E.; Miller, W. R.; McCollum, T. G.; Brown, G. E. Thiabendazole and imazalil applied at 53C reduce chilling injury and decay of grapefruits. *HortScience* **1991**, *26*, 397−399.

Paull, R. E.; McDonald, R. E. Heat and cold treatments. In *Insect Pests and Fresh Horticultural Products*: *Treatments and Responses.* Paull R. E., Armstrong J. W., Eds.; CAB International: Wallingford, U.K., 1994; pp 191−222.

Pratella, G. C.; Tonini, G.; Cessari, A. Postharvest disease problems of Italian citrus fruit. *Proc. Int. Citrus Symp., 1st* **1969**, *3*, 1317−1323.

Rodov, V.; Ben-Yehoshua, S.; Albagli, R.; Fang, D. Q. Reducing chilling injury and decay of stored citrus fruit by hot water dips. *Postharvest Biol. Technol.* **1994**, *5*, 119−127.

Schiffman-Nadel, M.; Chalutz, E.; Waks, J.; Lattar, F. S. Reduction of pitting of grapefruit by thiabendazole during long-term cold storage. *HortScience* **1972**, *4*, 394−395.

Schiffman-Nadel, M.; Chalutz, E.; Waks, J.; Dagan, M. Reduction of chilling injury in grapefruit by thiabendazole and benomyl during long-term storage. *J. Am. Soc. Hortic. Sci.* **1975**, *100*, 270−272.

Schirra, M.; D'hallewin, G. Storage performance of Fortune mandarins following hot water dips. *Postharvest Biol. Technol.* **1997**, *10*, 229−237.

Schirra, M.; Mulas, M. Improving storability of Tarocco oranges by postharvest hot-dip fungicide treatments. *Postharvest Biol. Technol.* **1995**, *6*, 129−138.

Schirra, M.; Cabras, P.; Angioni, A.; Melis, M. Residue level of imazalil fungicide in lemons following prestorage dip treatment at 20 and 50 °C. *J. Agric. Food Chem.* **1996**, *44*: 2865−2869.

Schirra, M.; Agabbio, M.; D'hallewin, G.; Pala, M.; Ruggiu, R. Response of Tarocco oranges to picking date, postharvest hot water dips and chilling storage temperature. *J. Agric. Food Chem.* **1997**, *45*, 3216−3220.

Wild, B. L.; Hood, J. M. Hot dip treatments reduce chilling injury in long-term storage of "Valencia" oranges. *HortScience* **1989**, *24*, 109−110.

Received for review September 11, 1997. Revised manuscript received January 6, 1998. Accepted January 6, 1998. Research was supported in part by the National Research Council (Italy): "Progetto Speciale INC, Agro Industria − Miglioramento della Qualità dei Prodotti Agroalimentari".

JF970776S