

**Thiabendazole Uptake and Storage Performance of Cactus Pear
 [*Opuntia ficus-indica* (L.) Mill. Cv Gialla] Fruit Following
 Postharvest Treatments with Reduced Doses of Fungicide
 at 52 °C**

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The storage response of cactus pears [*Opuntia ficus-indica* Miller (L.) cv. Gialla] was investigated over 6 weeks at 6 °C, plus an additional week of simulated marketing period (SMP) at 20 °C, after a 3-min dip treatment with thiabendazole (TBZ) at 1000 mg/L at 20 °C or 150 mg/L TBZ at 52 °C. Untreated fruits were used as control. Following TBZ treatments at 20 and 52 °C, total residues were recovered from the peel of cactus pear, as the concentration of residues in the pulp was negligible. Treatments with 1000 mg/L TBZ at 20 °C resulted in a 2.82 mg/kg residue uptake (active ingredient, whole-fruit basis), whereas treatment at 150 mg/L TBZ left 1.09 mg/kg. TBZ showed great persistence over both storage and SMP: on average, in the fruits treated at 20 and 52 °C, over 72 and 68%, respectively, of TBZ was still present after SMP. Postharvest treatments with 1000 mg/L TBZ at room temperature did not affect the expression of slight-to-moderate chilling injury (CI), but reduced severe CI by approximately 50% and decay development by 63.4% in comparison to those of untreated fruit after SMP. The effectiveness of TBZ was much higher with the treatment at 150 mg/L TBZ at 52 °C, providing 91% control of severe CI and approximately 89% suppression of decay; no treatment damage occurred during storage and SMP. External appearance was better in fruit treated with 150 mg/L TBZ at 52 °C. Respiration rate, titratable acidity, soluble solids concentration, and acetaldehyde in the flesh were not significantly influenced by treatments. Ethylene production rate and ethanol levels in the flesh were significantly higher in the TBZ-treated fruit as opposed to those in the untreated control fruit.

KEYWORDS: *Opuntia ficus-indica*; hot dip treatment; thiabendazole; residue analysis; chilling injury; rots; storage

INTRODUCTION

Cactus (prickly) pear [*Opuntia ficus-indica* (L.) Mill.] represents a very important food source to humans and animals in arid and semi-arid lands (1). The cactus pears can be eaten as fresh fruit or after processing in many other ways (2). The young cladodes have been a traditional vegetable in the Mexican diet for centuries and a speciality vegetable in the U.S. (3). In Mexico the cladodes are also used to treat diabetes mellitus, and

experimental evidence suggests they may have additional human health benefits (2).

In Italy cactus pear is grown primarily as a fresh-market fruit. Its domestic marketing season starts in mid-August with fruit of the spring flower flush, and lasts until November or December with fruit of a second crop, the result of a special management practice known as *scozzolatura* involving the removal of the flowers and cladodes that appear at the end of the natural bloom in spring. This causes a flush of new floral and vegetative buds that lead to a new crop about two months later in the season (4). Like many tropical and subtropical crops, cactus-pear fruits are highly perishable once harvested: when kept below 10 °C they are susceptible to chilling injury (CI), and they deteriorate rapidly under shelf life conditions as a result of dehydrating

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and decay favored by mechanical injury to the peel and stem-end during harvest (5). Storage conditions at 5–8 °C with 90–95% relative humidity (RH) and adequate ventilation of refrigerated rooms have proved to be the best compromise in controlling CI and decay for approximately 30 days (6). Postharvest treatment with thiabendazole (TBZ) has been found to produce some benefit to fruit quality with respect to that of untreated fruit, but it has not been effective enough in controlling decay (7). Postharvest treatments with heated fungicides mixtures are known to be much more effective than treatments with unheated chemicals having equally active ingredient concentrations (8). These effects enable producer to minimize the dose of fungicide needed to control postharvest decay. This is the result of the combined effect between heat and increased activity of heated fungicide due to the improved penetration and coverage of active ingredient (a.i.) on the fruit. The potential of treatments with heated fungicides in controlling postharvest decay have been proven on various horticultural crops (8). However, no specific studies have been dealt with on cactus pear fruit.

The present study investigated the storage performance of cactus pears and residue levels of TBZ at reduced doses (150 mg/L) of fungicide applied at 52 °C in comparison to the that of standard treatment at 20 °C.

MATERIALS AND METHODS

Plant Material. Cactus pear fruit cv. Gialla were obtained from a commercial orchard located in Sicily (37° 22' Lat N) receiving standard horticultural practices. All trees began to flower during the first week of June; at this stage, the spring flush of flowers and cladodes was removed from 25 trees to promote a second flush of flowers and cladodes (by the end of June) that opened during the third week of July. Cladode autumn crop load was adjusted to six fruit to maximize fruit growth potential. Forty-five fruiting cladodes were left on each tree. Fruit were harvested when they had reached their commercial maturity stage, as indicated by the peel color breakage (4).

Treatments and Storage Conditions. Fruits were delivered to the laboratory on the day of harvest, sorted for uniform size and freedom from defects, placed in plastic boxes (50 fruit individually numbered per box), and divided into 3 treatment groups, corresponding to the following 3-min dip treatments: (a) water dip treatments with 1000 mg/L TBZ at 20 °C, (b) 150 mg/L TBZ at 52 °C, and (c) untreated (control) fruit. TBZ mixtures in water were prepared from commercially available Mertect (41.8% active ingredient, Janssen Pharmaceutica N. V., Belgium). Dip treatments were performed in a bath fitted with heating elements and an electronic recirculation pump. The bath contained 200 L of water or fungicide mixture for the treatments; one box of fruit was dipped at a time. 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 fruit were left to dry at room temperature for 4–5 h. Each treatment group was then divided into two subgroups (four replicate fruit boxes each) respectively used for visual assessment and chemical analysis. Finally, fruit were moved to a storage room and kept at 6 °C for 6 weeks and ca. 85% relative humidity (RH). Then, fruit were held one additional week at 17 °C and 80% RH to simulate a one-week marketing period (SMP).

Visual Assessment and Organoleptic Acceptance. Visual assessments included chilling injury (CI), decay, and external fruit quality. Inspections were carried out after 3 or 6 weeks of storage and after SMP. CI (peel pitting and brown spots) was scored as slight, moderate, and severe. Then the percentage of fruit in each rating was calculated. Decay incidence was assessed as total percentage of rotten fruit caused by *Penicillium spp.*, *Fusarium spp.*, *Alternaria spp.*, or as miscellaneous rots of other fungi or bacteria. Overall visual quality was rated subjectively into one of five categories: 5 (excellent), 4 (good), 3 (fair), 2 (poor), and 1 (very poor) and an average value was calculated.

Organoleptic acceptance (fruit flavor and taste) was scored as 3 (good), 2 (fair), and 1 (poor).

Physiological Response and Internal Quality Characteristics. Physiological (respiration and ethylene production rates) and internal quality attributes as evaluated by titratable acidity (as % citric acid), soluble solids concentration (SSC), and acetaldehyde and ethanol in the flesh were determined at harvest, by the end of storage, and after SMP. Respiration and ethylene production rates by freshly harvested fruit were determined after they were held at room temperature (20 ± 1 °C) for 24 h. All analyses were performed according to Schirra et al. (9).

Thiabendazole Analysis. Chemicals. Thiabendazole was analytical standard (Ehrenstorfer, Germany), and analytical grade triphenyl phosphate (99%, Janssen, Geel, Belgium) was used as the internal standard (i.s.). Ethyl acetate and methanol were HPLC grade, and hexane was 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 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.

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 column (30 m \times 0.25 mm i.d.) (J&W Scientific, Folsom, CA) with DB 5 (5% phenylmethylpolysiloxane) liquid phase (film thickness 0.25 μ m) was employed. The injector and detector were operated at 250 and 280 °C, respectively. The sample (2 μ L) was injected in the splitless mode (60 s), and the oven temperature was set at 110 °C for 1 min, then 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 drawn with the i.s. method by measuring peak heights vs concentrations. Good linearity was achieved in the 0–10 mg/kg range, with a correlation coefficient of 0.9992.

Sample Preparation. Three fruits per replication were weighed and their peel was removed and weighed, and its percentage of the whole fruit was calculated. Peel and flesh were then homogenized. Samples were stored in a freezer at -20 °C until analysis.

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. 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, then injected for gas chromatographic analysis.

Recovery Assays. Untreated samples (peel and flesh) 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 and mean comparisons were determined by Tukey's test at $P \leq 0.05$, where appropriate.

RESULTS

Residues of TBZ were detected in peel and, to a much lesser extent, in pulp; no relationship was found between peel and flesh residue concentrations (Table 1). After treatments at 20 °C or 52 °C, the TBZ uptake, calculated on a whole-fruit basis, averaged 2.82 and 1.09 mg/kg, respectively. Thus, when treatment was applied at 20 °C, the fungicide mixture contained TBZ residues at 6.6-fold more than that used in treatment at 52 °C, but residue concentration was only 2.5-fold more than it was after treatment at 52 °C. TBZ showed great persistence over both storage and SMP: on average, in fruit treated at 20

Table 1. Thiabendazole Residues in the Peel, Flesh, and Whole Fruit (calculated) in Cactus Pear (*Opuntia ficus-indica* cv Giolla) Fruit Following Treatment (0 wk), by the End of Cold Storage (6 weeks at 6 °C), and after One Subsequent Week of Simulated Marketing Conditions at 17 °C (6 + 1)

fruit sample	postharvest treatments ^a					
	1000 mg/L TBZ, 20 °C			150 mg/L TBZ 52 °C		
	storage duration (weeks)					
	0	6	6+1	0	6	6+1
thiabendazole residues (mg/kg active ingredient) ^b						
peel	6.12 ± 0.60	5.03 ± 0.70	4.42 ± 0.65	2.41 ± 0.31	1.82 ± 0.40	1.64 ± 0.17
flesh	0.10 ± 0.06	0.15 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	0.01 ± 0.01	0.05 ± 0.01
whole fruit	2.82 ± 0.29	2.57 ± 0.37	2.11 ± 0.38	1.09 ± 0.14	0.87 ± 0.18	0.83 ± 0.07

^a Treatments were 3-min dips followed by air-drying of dipped fruit. ^b Mean values (±SD) of 5 replicates of 3 fruit samples each.

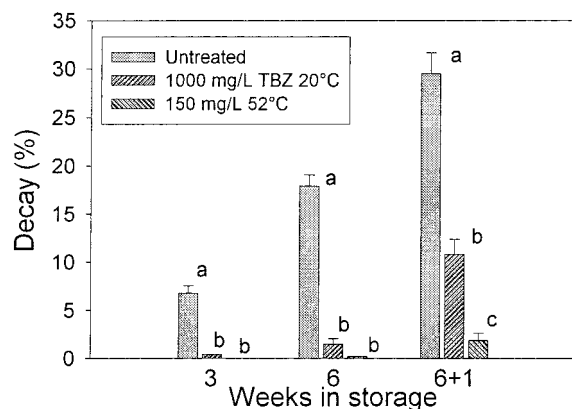


Figure 1. Influence of 3-min dip treatments with thiabendazole (TBZ) at 20 °C and at 52 °C on percentage of fruit showing decay in cactus pears (*Opuntia ficus-indica* cv Giolla) after 3 and 6 weeks of storage at 6 °C (designated 3 and 6), and after 6 weeks of storage at 6 °C plus 1 week at 17 °C (6+1).

or 52 °C over 72% and 68% of it was still present after SMP.

After 3 and 6 weeks storage and after SMP the incidence of decay in untreated fruit averaged approximately 7, 18, and 30%, respectively (**Figure 1**). Treatment with 1000 mg/L TBZ at 20 °C resulted in almost complete suppression of decay during storage and a 3-fold reduction after SMP. However, treatment efficacy was considerably improved with 150 ppm TBZ at 52 °C with no decay during storage and less than 2% rot after SMP. *Penicillium italicum* was the predominant fungi, although other pathogens such as bacteria were found occasionally (data not shown).

There were no visible symptoms of CI after 3 weeks' cold storage (data not shown), and even after 6 weeks' storage the percentage of fruit with slight to moderate CI was negligible. By contrast, untreated fruit with severe CI averaged 6.8% (**Table 2**) and, upon removal from cold storage, CI increased rapidly, accounting for 27.1% slight-to-moderate CI and 21.2% severe CI in untreated control. Treatment with 1000 mg/L TBZ at 20 °C did not affect the incidence of slight-to-moderate CI, but it remarkably reduced the incidence of severe CI during both storage and SMP. Better results were achieved by treatment with 150 mg/L TBZ at 52 °C, which notably alleviated the incidence and severity of CI after SMP.

No treatment damage to the peel (browning of the rind) was found during storage or SMP (data not shown). After cold storage and SMP, fruit treated with 150 mg/L TBZ at 52 °C

Table 2. Incidence of Slight to Moderate and Severe Chilling Injury (CI) in Cactus Pear (*Opuntia ficus-indica* cv Giolla) Fruit after 6 Weeks of Storage at 6 °C Plus 1 Week at 17 °C, Preceded by Thiabendazole Dips at 20 or 52 °C as Indicated

treatment ^a	slight to moderate CI (%)	severe CI (%)
	6 weeks at 6 °C ^b	
untreated	0.4a	6.8a
1000 mg/L TBZ 20 °C	0.5a	1.5b
150 mg/L TBZ 52 °C	0.0a	0.0b
6 weeks at 6 °C + 1 week at 17 °C		
untreated	27.1a	21.2a
1000 mg/L TBZ, 20 °C	23.2a	10.7b
150 mg/L TBZ, 52 °C	15.1b	1.9c

^a Treatments were 3-min dips following by air-drying of dipped fruit. ^b Different letters denote significant differences within a column group at $P = 0.05$ by Tukey's test.

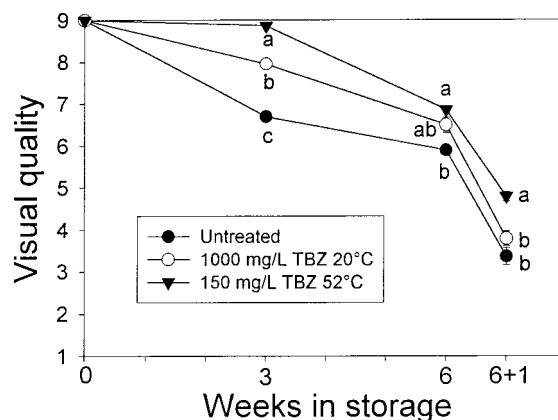


Figure 2. Effect of 3-min dip treatments with thiabendazole (TBZ) at 20 °C and at 52 °C on visual quality in cactus pears (*Opuntia ficus-indica* cv Giolla) after 3 and 6 weeks of storage at 6 °C (designated 3 and 6), and after 6 weeks of storage at 6 °C plus 1 week at 17 °C (6 + 1).

Table 3. Influence of TBZ Dips at 20 and 52 °C on Respiration and Ethylene Production Rate in Cactus Pear (*Opuntia ficus-indica* cv Giolla) Fruit after 6 Weeks at 6 °C Plus 1 Week at 17 °C

treatment ^a	respiration rate (mgCO ₂ kg ⁻¹ h ⁻¹)	ethylene (μgkg ⁻¹ h ⁻¹)
	at harvest ^b	
	43.5	0.33
6 weeks at 6 °C + 1 week at 17 °C ^c		
untreated	36.7 ^{ns}	1.88c
1000 mg/L TBZ, 20 °C	33.4	2.08b
150 mg/L TBZ, 52 °C	36.9	2.74a

^a Treatments were 3-min dips following by air-drying of dipped fruit. ^b Harvest data are only included to provide a comparison to the other treatments. ^c Different letters denote significant differences within a column group at $P = 0.05$ by Tukey's test.

had better overall appearance than untreated control and fruit treated with 1000 mg/L TBZ at 20 °C (**Figure 2**). Treatments had no significant effect on fruit respiration rate, but ethylene production rate increased after SMP (**Table 3**). Titratable acidity, soluble solids concentration, and acetaldehyde in the flesh were not significantly affected by treatments (**Table 4**). TBZ treatments resulted in significantly higher levels of ethanol in the flesh after SMP with respect to that in the untreated fruit. No

Table 4. Effect of TBZ Dips at 20 and 52 °C on Titratable Acidity, Soluble Solids Concentration (SSC), and Acetaldehyde and Ethanol Amount in the Flesh of Cactus Pear (*Opuntia ficus-indica* cv Gialla) Fruit after 6 Weeks at 6 °C Plus 1 Week at 17 °C

treatment ^a	titratable acidity (%)	SSC (%)	acetaldehyde (mg/100 mL)	ethanol (mg/100 mL)
	at harvest ^b			
	0.05	14.2	0.46	0.0
	6 weeks at 6 °C + 1 week at 17 °C ^c			
untreated	0.07 ^{ns}	12.0 ^{ns}	0.38 ^{ns}	1.99 ^b
1000 mg/L TBZ, 20 °C	0.07	13.1	0.42	3.21 ^c
150 mg/L TBZ, 50 °C	0.06	12.2	0.50	5.90 ^a

^a Treatments were 3-min dips following by air-drying of dipped fruit. ^b Harvest data are included only to provide a comparison to the other treatments. ^c Different letters denote significant differences within a column group at $P = 0.05$ by Tukey's test.

off-flavors and off-taste were found in the flesh after SMP (data not shown).

DISCUSSION

Much effort has been made in recent years to control postharvest decay of horticultural crops by using heat treatment in combination with fungicides (8). This combined treatment is done for the following reasons (a) to improve the effectiveness of the active ingredients, (b) to reduce the amounts of chemicals required with respect to amounts needed for conventional treatments performed at room temperature, and (c) to achieve positive synergistic effects of combined chemical and hot water treatments which have been corroborated on various horticultural crops (8). Bergher et al. (10) have shown that a 2-min dipping of cactus pear fruit in Benomyl (250 mg/L a.i.) or Betran (1050 mg/L a.i.) mixtures heated to 48–50 °C effectively reduced decay due to *Cladosporium spp.* and *Alternaria spp.* over 2 months storage at 0 °C and 80–85% RH. By contrast, treatments at room temperature with Benlate (0.2% w/v) plus Ridomil (0.1% w/v) fungicides applied in combination did not effectively reduce decay development with respect to untreated fruit in cactus pear fruit (*Opuntia amyclaea* T.) (11).

The present study indicates that a 1000 mg/L TBZ postharvest treatment at 20 °C significantly reduced the percentage of decay and alleviated the expression of CI. TBZ treatment with reduced doses of active ingredient (150 mg/L) at 52 °C was also significantly more effective than the conventional treatment at room temperature, thus confirming the positive synergistic effects between hot water and TBZ in controlling CI and decay as previously found with other fruit species (12, 13).

The greater efficacy of heated fungicide may be related to enhanced penetration of the active ingredient in the microlesions caused by glochids during fruit handling. Moreover, the partial melting of the epicuticular wax layer in the wounded areas (14), with concomitant occlusion of the possible micro-gates for wound pathogens, may also have contributed to protecting fruit from decay.

Studies on citrus fruit have shown that antioxidant enzyme systems in flavedo tissue play a role in the expression of CI (15), and that heat treatment improves the efficiency of antioxidant enzyme systems and induces cold tolerance (16, 17). The present study indicates that TBZ, in addition to its antifungal activity, also alleviates CI, especially when the fungicide mixture was applied at 52 °C. However, the physiological effect of TBZ in alleviating CI and how this effect improves when TBZ is applied in combination with hot water remain to be elucidated.

Thus far, very low residue concentrations were recovered from the flesh of cactus pear fruit. Accordingly, previous studies on citrus fruit have shown that TBZ is retained by the fruit surface, as traces of TBZ residues have been detected in fruit flesh (18). Königler and Wallnöfer (19) showed that hand-peeled oranges and tangerines left contaminating TBZ residues of 14% and 7%, respectively, on hands and 5% and 12%, respectively, on the edible portion of the fruits. Cabras et al. (20) reported that only a minimal part of the active ingredient can penetrate the cuticle of citrus fruit.

Therefore, the lack of relationship between pulp and flesh residue levels found in this study indicates that TBZ is retained by the peel surface without, or with very limited, penetration of the flesh. Thus, the TBZ residues recovered from the flesh may be attributed to contamination during peeling when preparing samples for peel and flesh analyses.

TBZ treatments caused higher levels of ethanol in the flesh after SMP with respect to that of untreated fruit. However, such increases did not adversely affect the flavor and taste of the flesh.

CONCLUSIONS

Overall, our data show that it is possible to extend the marketing period of cv. Gialla cactus pears by as much as 7 weeks with minimal losses of fruit by using cold storage preceded by a postharvest dip treatment with relatively low doses (150 mg/L) of TBZ at 52 °C. This means that there is about a 6.6-fold saving on fungicide with respect to that used for treatments performed at room temperature (1000 mg/L).

There is scope for further studies on a large scale to endorse the effectiveness of postharvest treatments at reduced doses of heated TBZ in decay control of cactus pear fruit during storage, transport, and shelf life. Positive results of such further treatments with TBZ could pave the way for the application of this approach as an alternative to conventional fungicide treatments.

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