Ultraviolet C Irradiation at 0.5 kJ·m^{-2} Reduces Decay without Causing Damage or Affecting Postharvest Quality of Star Ruby Grapefruit (*C. paradisi* Macf.)

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Star Ruby grapefruit [*Citrus paradisi* (Macf.)] were harvested in November, February, and May, treated with ultraviolet C (UV-C) light at 0.5, 1.5, or 3.0 kJ·m^{-2}, and then stored at 7 °C and 90−95% relative humidity (RH) for 4 weeks with 1 additional week at 20 °C and ~80% RH. Untreated fruits were used as control. UV-C irradiation at 0.5 kJ·m^{-2} effectively reduced decay development as compared to nontreated fruit without causing damage. Irradiation at dosages >0.5 kJ·m^{-2} did not further improve decay control and caused rind browning and necrotic peel, the extent of damage depending on treatment dosage and harvest date. The percentage of damaged fruit after irradiation at the higher UV-C dosages was significantly higher in fruit harvested in November; differences between fruits harvested in February and May were negligible. After UV-C irradiation, the phytoalexins scoparone and scopoletin accumulated in flavedo tissue, their amounts depending on harvest date and UV-C dosage. Both phytoalexins showed similar accumulation patterns, although the concentrations of scoparone were much lower than those of scopoletin. Phytoalexin levels increased in most samples as the treatment dosage increased. No detectable levels of scoparone and scopoletin could be found in nonirradiated fruit. The influence of UV-C treatments on soluble solids concentration and titratable acidity of juice was negligible.

Keywords: *Citrus; storage; decay; phytoalexins; 6,7-dimethoxycoumarin; 7-hydroxy-6-methoxycoumarin*

INTRODUCTION

There has been increasing interest over the past few years in "nonconventional" technologies for postharvest decay control of citrus fruit. To reduce the strong dependence on synthetic fungicides, research efforts are currently focused on enhancing host resistance to pathogens through biological or physical inducers (Wilson et al., 1994; Schirra and Ben-Yehoshua, 1999). Among the latter, ultraviolet C (UV-C) irradiation has been shown to elicit in the flavedo tissue such compounds as the phytoalexins scoparone (6,7-dimethoxycoumarin) and scopoletin (7-hydroxy-6-methoxycoumarin) (Kim et al., 1991; Ben-Yehoshua et al., 1992; Rodov et al., 1994), which have been related to enhanced resistance to pathogens (Afek and Sztejnberg, 1994). Kim et al. (1991) have shown that scoparone accumulated in lemon fruit [*Citrus limon* (L.) Burm] after inoculation with *Penicillium digitatum* and that changes in scoparone concentration in flavedo tissue were correlated with the changes in antifungal activity. Later studies on oranges (*Citrus sinensis* Osbeck) (D'hallewin et al., 1999) have shown that fruit response to UV-C irradiation in terms of treatment damage, phytoalexin accumulation, and decay control was dependent on cultivar, treatment dose, and harvest date. Investigations on Marsh seedless grapefruit (*Citrus paradisi* Macf.) have shown that the UV dosage required to develop maximum resistance increases as the season progresses (Droby et al., 1993). Much work is still needed to optimize the experimental conditions of UV-C irradiation dose and treatment duration before application on a commercial scale. The greatest difficulty stems from the constraints of having to operate in conditions that are known to depend on genetic and environmental factors and that may be harmful to the fruit.

The present study deals with how the accumulation of phytoalexins interacts with UV-C irradiaton to induce resistance to mold decay in Star Ruby grapefruit as a function of fruit harvest date and irradiation dosage and demonstrates that UV-C irradiation at the dose of 0.5 kJ·m^{-2} of Star Ruby grapefruit considerably reduced decay without any phytotoxicity in the commercial harvesting season.

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MATERIALS AND METHODS

Plant Material. The investigation was conducted on Star Ruby grapefruit grown in an experimental grove in southwestern Sardinia (Italy) under standard horticultural management practices. In November (when fruits were not yet commercially mature), February (mid-season), and May (late-

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season) 1995, the fruits were harvested from the exterior of the canopy and immediately delivered to the laboratory. All harvests were carried out within the first week of each respective month, and each involved a random sampling from 15 trees. Defect-free fruits were selected, washed with water, allowed to dry overnight, placed in boxes, and then removed for UV-C treatment before being returned to boxes for storage.

Treatments. UV-C irradiation was carried out in a small (60 × 120 × 70 cm) ventilated irradiation chamber with four lamps (G15T8 type, Tana Industries, Kibbutz Netiv Ha Lamed- He, Israel), each with a nominal power output of 3.6 W; the peak wavelength emitted by each lamp was 254 nm. The grapefruits were placed individually on opposite rotating rods, ~25 cm from the irradiation source, and turned continuously during UV-C treatment to provide uniform irradiation over the whole fruit surface (Stevens et al., 1998). Temperature inside the treatment chamber remained within 20 ± 2 °C, and the UV-C dosages were 0.5, 1.5, and 3.0 kJ·m^{-2} (treatment duration of 26, 75, and 151 s, respectively). The UV-C fluency was measured with a UVx radiometer (UV Products Inc., San Gabriel, CA) as reported by D'hallewin et al. (1993); nontreated fruits served as controls.

All experiments were repeated four times (replicates) using 25 fruits per replicate. After treatment, fruits were kept in the dark at 21 °C for ~1 h before storage in a ventilated room at 7 °C and 90−95% relative humidity (RH) for 4 weeks with one additional week at 20 °C and ≈80% RH as simulated a marketing period (SMP) (Schirra, 1992). Following UV-C treatment and during storage fruit were kept in the dark to minimize any possible photoreactivation processes (Stevens et al., 1998).

Peel Color. Peel color measurements were taken 1 h following UV-C irradiation and after the SMP on five fruits per replicate, individually numbered. Peel color was measured using a colorimeter (Macbeth series 1500, Newburgh, NY). The measurements were taken at four equally spaced sites around the fruit equator, and an average score was calculated. CIELAB L^* = lightness, a^* = bluish green/red purple hue component, and b^* = yellow/blue hue component were measured. Chroma, C^*, and hue angle $h°$ (0° = red-purple, 90° = yellow, 180° = blue green, and 270° = blue) values were calculated according to the requirements described by McGuire (1992). That is, when $a^* > 0$ and $b^* > 0$, $h° = \text{arctangent}(b^*/a^*)$, whereas when $a^* < 0$ and $b^* < 0$, $h° = 180 + \text{arctangent}(b^*/a^*)$. The instrument's illuminant, calibration plate, and illuminant/viewing geometry were *C*, orange color (CR-A47O plate), and *d*/0, respectively (McGuire, 1992).

Visual Assessments, Flavor, and Taste. Treatment damage (peel necrosis and/or browning to the rind) was expressed as a percentage of the total number of fruits in the sample. Decay was visually assessed as rots caused by blue mold (*Penicillium italicum* Wehmer), green mold (*P. digitatum* Sacc.), or brown rot (*Phytophtora citrophthora*), or as miscellaneous rots of unidentified fungi, and total percentage of decay was calculated. Overall visual quality was rated subjectively into one of five categories [5 (excellent), 4 (good), 3 (fair), 2 (poor),and 1 (very poor)] by an informal panel of five people familiar with this cultivar. Visual rating was carried out under daylight conditions, in a separate room. Flavor and taste were subjectively rated as 3 (good), 2 (fair), or 1 (poor) on fruits without infections or physiological defects.

Juice Analyses. Before storage and after SMP, three replicates of five healthy fruits were randomly selected for juice analysis. The juice was extracted from individual fruits with a small laboratory hand reamer (Type MPZ2 AG, Braun, Frankfurt, Germany), and juice content was expressed as percentage of fruit weight. Percentage of soluble solids concentration (SSC) was determined with a digital Abbe refractometer (Reichert model A1171, Wien, Austria). Titratable acidity (TA) was determined by titratating an aliquot of juice to pH 8.2 with 0.1 mol·L^{-1} NaOH and expressing the result as percentage of anhydrous citric acid. Maturity index was evaluated as the SSC/TA ratio (Soule and Grierson, 1986).

Scoparone and Scopoletin Analysis. The experiment was repeated in 1996−1997 and 1997−1998 with the same

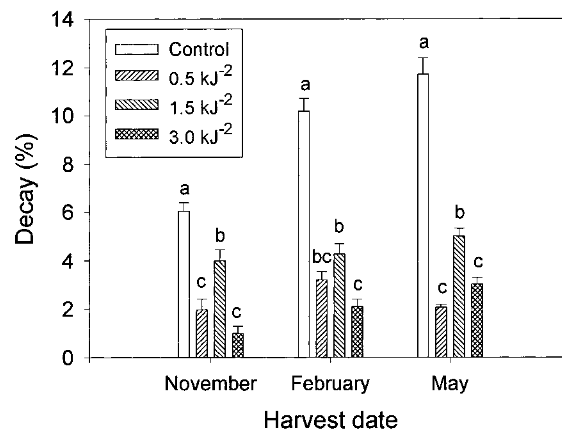

Figure 1. Influence of UV-C$_{254nm}$ postharvest irradiation on decay percentage (mean values of nine replicate fruit samples: 3 replicates × 3 years) in Star Ruby grapefruit harvested at various maturity stages and stored for 4 weeks at 7 °C plus 1 week at 20 °C. Within each harvest date, different letters denote significant differences by Tukey's test, $P \leq 0.01$. Vertical bars represent SE.

experimental design. In addition, scoparone and scopoletin development in flavedo tissue was assessed in 1997−1998. Extraction of phytoalexins was performed 6 days after UV-C treatment, as described by Kim et al. (1991). Quantitative analysis was achieved by high-performance liquid chromatography (HPLC) using a pump (Varian 9012, Walnut Creek, CA) equipped with a manual injection system (10 μL loop) and a reversed-phase column Erbasil-S C_{18} (Carlo Erba, Milan, Italy), 120 × 10 mm i.d., 0.5 μm particle size. The column was placed in an oven (Merck L 7350, Darmstadt, Germany) at 50 °C. In all analyses, the mobile phase consisted of combinations of methanol and 0.05 mol·L^{-1} ammonium acetate buffer (pH 4.25). Step-gradient elution was used: starting with an 80:20 (v/v) methanol/buffer ratio, changing to 60:40 at 5 min and to 40:60 at 10 min, and reaching a 20:80 ratio at the stopping time of 18 min. An additional 4-min postrun time was used to return to the 80:20 starting ratio. The flow rate at time 0 was 0.6 mL·min^{-1} with a step increase of 0.1 mL·min^{-1} after each 5 min. Quantitative measurement was performed with a fluorescence detector (Varian 9070) at an elicitation wavelength (λ_{ex}) of 350 nm and an emission wavelength (λ_{em}) of 430 nm. Retention times for scopoletin and scoparone were 8.9 and 10.9 min, respectively, based on standards: scopoletin (Sigma Chemical Co., St. Louis, MO, product. S-2500) and scoparone (Sigma product D-4912). The amounts of the eluted compounds were determined by Varian Work Station software (Varian).

Data Analysis. Analysis of variance (ANOVA) was performed by MSTAT-C software (Michigan State University, East Lansing, MI, 1988). Damage and decay percentages were transformed to arcsine values before statistical analysis. Mean separations were calculated by Tukey's Studentized range test at $P \leq 0.05$ or 0.01 where appropriate.

RESULTS

Decay percentage in nontreated fruit increased as the season progressed, accounting for approximately 6 and 10%, respectively, in November and February and 12% in May (Figure 1). UV-C irradiation at 0.5 kJ·m^{-2} significantly reduced decay development in comparison to nontreated fruit. Treatment at 1.5 and 3.0 kJ·m^{-2} induced no further improvement in decay control with respect to the lowest dosage. *Penicillium* species, especially *P. digitatum*, accounted for >85% of total decay (data not shown).

Although no treatment damage occurred at 0.5 kJ·m^{-2} in fruit harvested in February and May, 1.0% of the fruits harvested in November exhibited damage (Figure

Table 1. Quantity of Scopoletin and Scoparone in Flavedo Tissue 6 Days after Irradiation in Star Ruby Grapefruit in Relation to Harvest Date

harvest date	scopoletin[a] (μg·g^{-1} of fresh wt)			scoparone[a] (μg·g^{-1} of fresh wt)		
	UV-C$_{254nm}$ doses (kJ·m^{-2})					
	0.5	1.5	3.0	0.5	1.5	3.0
Nov	91.1 C(A)	107.1 B(C)	275.1 A(B)	25.7 C(B)	50.2 B(C)	73.7 A(B)
Feb	100.0 C(A)	380.9 B(B)	550.3 A(A)	32.5 C(AB)	80.5 B(A)	94.8 A(A)
May	72.7 C(B)	410.8 B(A)	536.5 A(A)	38.2 B(A)	75.7 A(B)	73.7 A(B)

[a] In each row or column grouping, means separation by Tukey's test, $P \leq 0.01$, are indicated: upper case letters without parentheses relate to comparisons of the effects of UV-C doses within each harvest date; upper case letters in parentheses relate to comparisons of the influence of different harvest dates within each treatment dose.

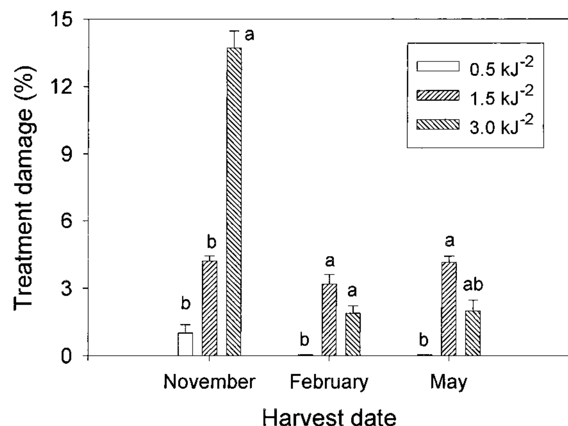

Figure 2. Influence of UV-C$_{254nm}$ postharvest irradiation on treatment damage percentage (mean values of nine replicate fruit samples: 3 replicates × 3 years) in Star Ruby grapefruit harvested at various maturity stages and stored for 4 weeks at 7 °C plus 1 week at 20 °C. Within each harvest date, different letters denote significant differences by Tukey's test, $P \leq 0.01$. Vertical bars represent SE.

2). Treatment damage appeared as rind browning and tissue necrosis. Upon UV-C treatments at 1.5 kJ·m^{-2} damage to fruit ranged from 3.2 to 4.2%. The percentage of damaged fruit after irradiation at 3.0 kJ·m^{-2} was high (13.7%) in fruits harvested in November and relatively low (1.9–2.0%) in those harvested later.

After the SMP, the external appearance, flavor, and taste of fruit, when free of treatment damage and decay, were judged to be good, differences due to treatments being negligible (data not shown).

Levels of scoparone and scopoletin in flavedo tissue of nontreated fruit were undetectable. Following UV-C, scopoletin accumulation increased as the irradiation dose increased (Table 1). Concentrations of scopoletin in fruit irradiated at 3.0 kJ·m^{-2} UV-C light were ~3–7-fold higher than those of fruit treated with 0.5 kJ·m^{-2}. The response to UV-C stimuli was also affected by fruit age. The rate of scopoletin accumulation was significantly lower in fruit harvested in November with respect to fruit harvested late in the season. The concentration of scoparone was very low in comparison to that of scopoletin. Differences in scoparone occurrence in relationship to UV-C doses and harvest date displayed a pattern similar to that in scopoletin. There were no treatment-dependent differences in SSC, TA, and maturity index at any harvest date within each picking date. Therefore, all data (Table 2) are reported as means of 48 separate analyses (4 replicate samples × 4 treatments × 3 years).

UV-C irradiation at 0.5 kJ·m^{-2} did not affect peel color as evaluated by the lightness coefficient (L^*), hue angle ($h°$), and chroma (C^*) (Table 3). At 1.5 and 3.0 kJ·m^{-2} irradiation affected the color of only the November-picked fruits.

Table 2. SSC, TA, and Maturity Index (SSC/TA) in Star Ruby Grapefruit Harvested at Various Picking Dates and Stored for 4 Weeks at 7 °C plus 1 Week of SMP at 20 °C[a]

quality attribute	harvest date	time of measurement	
		harvest[b]	SMP[c]
SSC (%)	Nov	8.3 C	8.7 C
	Jan	8.6 B	9.4 B
	Mar	9.1 A	9.9 A
TA (%)	Nov	2.1 A	1.8 A
	Jan	1.9 B	1.7 B
	Mar	1.6 C	1.2 C
SSC/TA	Nov	3.9 C	4.8 C
	Jan	4.5 B	5.5 B
	Mar	5.7 A	8.3 A

[a] Mean values of 48 separate analyses. Mean separation within columns by Tukey's test is indicated. $P \leq 0.01$. [b] One hour following treatment. [c] After 4 weeks of storage at 7 °C plus 1 week of SMP at 20 °C.

DISCUSSION

The major finding of the present study is that UV irradiation at 0.5 kJ·m^{-2} considerably reduced decay, from a range of 6–12 to 2–3%. This desirable result confirms previous work on other citrus fruit species such as Avana mandarins (*Citrus reticulata* Blanco) (D'hallewin et al., 1993), Ovale kumquat [*Fortunella margarita* (Lour) Swingle] (Rodov et al., 1992), and Valencia late oranges (D'hallewin et al., 1999). In the present study, Star Ruby grapefruit appeared to be fairly resistant to UV-C damage as relatively low percentages of treatment damage occurred in mature fruit harvested in February and May after UV-C irradiation at 1.5 and 3.0 kJ·m^{-2}, whereas adverse effects in fruit treated with 0.5 kJ·m^{-2} were observed only on fruit harvested in November, which was not yet mature with respect to international maturity indices (Soule and Grierson, 1986). UV-C irradiation did not affect fruit flavor and taste even at the highest UV-C dose. On the other hand, a general decline in the sensory preference for juice and pulp flavor has been reported in Marsh grapefruit subjected to γ-irradiation at 0.6 kGy but not at 0.3 kGy (Miller and McDonald, 1996). However, the γ-irradiation is much more penetrating than UV-C and is accordingly expected to cause more damage.

Experimental evidence indicates that the effect of UV in reducing the citrus *P. digitatum*, the most important postharvest rot of citrus fruit, is an induced resistance phenomenon rather than a germicidal effect (Chalutz and Droby, 1992). Following UV-C stimuli, scoparone displayed a pattern of accumulation in flavedo tissue of Star Ruby grapefruit similar to that of scopoletin, as

Table 3. Effect of UV-C_{254nm} Postharvest Irradiation on Peel Color Measurements of Star Ruby Grapefruit in Relationship to Treatment Dose and Harvest Date

harvest date	UV-C dose (kJ·m^{-2})	lightness (L^*)		hue ($h°$)		chroma (C^*)	
		harvest[a]	SMP[b]	harvest[a]	SMP[b]	harvest[a]	SMP[b]
Nov	0.0	51.6 ns[c]	52.5 C	112.7 ns	111.5 A	52.8 ns	51.8 D
	0.5	51.2	52.3 C	112.9	112.4 A	53.0	53.2 C
	1.5	52.1	54.1B	112.5	109.0 B	52.8	54.7 B
	3.0	51.9	66.5A	112.7	92.5 B	52.7	61.3 A
Feb	0.0	68.5 ns	70.2 ns	75.7 ns	72.9 ns	60.9 ns	60.7 ns
	0.5	68.1	70.2	75.4	73.4	61.1	61.3
	1.5	69.0	70.6	75.7	73.5	60.7	59.0
	3.0	68.2	70.5	76.0	72.0	61.2	58.8
May	0.0	77.8 ns	76.5 ns	52.3 ns	53.4 ns	89.3 ns	88.6 ns
	0.5	76.8	75.4	52.1	52.8	89.2	88.8
	1.5	77.9	76.3	52.4	53.1	88.7	87.6
	3.0	78.0	75.1	52.6	53.8	89.1	86.8

[a] One hour following treatment. [b] After 4 weeks of storage at 7 °C plus 1 additional week of SMP at 20 °C. [c] Mean separation within columns for a color measurement by Tukey's test is indicated, $P \leq 0.01$. ns = nonsignificant.

also reported for various orange cultivars (D'hallewin et al., 1999). However, scoparone levels in oranges were found to be much greater than scopoletin, whereas in grapefruit we found the reverse. Therefore, scopoletin may play the role of the major phytoalexin in grapefruit, unlike in other citrus cultivars (Ben-Yehoshua et al., 1992). The effectiveness of UV-C radiation in decay control, even at the lowest dose, and the treatment-dependent accumulation of scopoletin and scoparone support the close relationship between reduction of decay and induction of the phytoalexins, at least up to the levels reported, and the finding that their interaction is capable of controlling pathogens (Kim et al., 1991; Ben-Yehoshua et al., 1992; Rodov et al., 1992; D'hallewin et al., 1999). The effectiveness of scoparone and scopoletin against *P. italicum* was proven to be higher than that against *P. digitatum*, both in vitro and in vivo (Angioni et al., 1998). When applied at a concentration of 50 mg/L, scoparone was more effective than scopoletin in inhibiting *P. italicum* growth, whereas a higher activity of scoparone against *P. digitatum* and similar activities against *P. italicum* were measured at 100 mg/L concentrations.

The results of this study showed that UV-C irradiation was more effective in inducing higher levels of the phytoalexins in fruit harvested in February and May than in November. This may be of particular importance because citrus fruits at the end of the season have usually lost much of their resistance (Ben-Yehoshua et al., 1995). Decay control at the end of the season is difficult, and any improvement at this period is of great importance.

The clear effect of UV irradiation on the accumulation of phytoalexins would not rule out the possibility that, besides the phytoalexins scoparone and scopoletin, other physical and biological occurrences (e.g., acceleration of wound healing or production of lignin and pathogen related-proteins, such as chitinase or β-1,3-glucanase) may have been induced by UV-C irradiation and improved fruit resistance against decay (Wilson et al., 1994). The pronounced increase in the level of phytoalexins induced by higher doses of UV irradiation was not adequate in this study to reduce decay further, probably because of the damage induced at these higher doses.

Only UV-C irradiation at the 3.0 kJ·m^{-2} dose affected color development of Star Ruby grapefruit harvested in November as compared to nontreated fruit. Yet, even at this high treatment rate, UV-C did not affect SSC, TA, and maturity index.

Investigations of Marsh seedless grapefruit have shown (Droby et al., 1993) that inoculated fruit harvested in February required approximately double the UV-C irradiation dose (8 kJ·m^{-2}) to induce maximum resistance against *P. digitatum* in comparison to fruit harvested in November. However, no chemical evidence has been reported in that paper on phytoalexin accumulation in Marsh seedless grapefruit following UV-C treatment. The present study showed that no treatment-dependent differences were found in decay control in Star Ruby grapefruit throughout the harvest season.

In conclusion, the present findings support a recommendation for a future large-scale study with Star Ruby grapefruit to determine whether mature fruit is free of damage. It appears that UV-C irradiation may be a new tool in the decay control arsenal of citrus fruits. Such a tool is urgently needed as the decay control of citrus fruit is dependent on the use of a few fungicides, the registrations of which are often challenged by health authorities.

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