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Short-term UV-B exposure induces metabolic and anatomical changes in peel of harvested lemons contributing in fruit protection against green mold

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ABSTRACT

UV-B radiation (UVBR) is a small fraction of the solar spectrum from 280 to 315 nm. UVBR produces photomorphogenic acclimation responses in plants, modulating their cellular structure and physiology. Here, changes in the peel of harvested lemons after short time exposure to UVBR were analyzed and its potential effects against fungal infection were studied. In the flavedo, UVBR treatment induced variations in the respiratory profiles and increased the phenolic compound contents. Final products of the flavonoid pathway (flavones, flavonols and anthocyanins) increased more markedly than their precursors (flavanones and dihydroflavonols). The increased accumulation of soluble phenolics in the flavedo of treated lemons is associated with the high antioxidant activity found in the flavedo of these samples. Supporting the biochemical determinations, anatomical observations showed abundant intravacuolar deposits of phenolic compounds and an increase in the cell wall thickness in UVBR-treated samples. Metabolic and anatomical modifications associated to UVBR improved natural defenses against *Penicillium digitatum*, the causal agent of green mold disease. Our results suggest that mature postharvest lemons exposed to the artificial radiation showed phenotypic plasticity, allowing an acclimation response to UVBR which confers fruit resistance to pathogens. Thus, combination of UVBR with other treatments could represent an important improvement to control postharvest diseases on citrus.

1. Introduction

Plants use the sunlight as an energy source for photosynthesis and also as an environmental signal that regulates several developmental processes such as the photoperiodicity and photomorphogenesis [40]. UV-B radiation (UVBR) is a small fraction of the solar spectrum (280–315 nm) which can elicit a wide range of responses in plants such as changes in gene expression, physiology, secondary metabolite accumulation and morphology [5,36]. Morphological and anatomical responses include leaf thickening, petiole and stem shortening, increased axillary branching, altered root:shoot ratio, stomatal density fluctuations and chloroplast structure changes [24,34]. UVBR is a positive modulator in plant defense, increasing plant resistance to herbivores and pathogens, due to changes in phenylpropanoid-derivatives, including flavonoids and other soluble phenolic compounds [4]. The stimulation by UVBR of phenylalanine ammonia lyase (PAL), a branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid pathway) metabolisms, has been reported for various plant species [41]. In the epidermal plant tissues, the content of both soluble (flavonoids and UV-B absorbing compounds) and insoluble (lignin) phenolic compounds are increased by UVBR, constituting natural sunscreens [17,18,21,32].

Hilal et al. [18] have demonstrated that in lemon trees both fruit position in the canopy as well as the seasonal period affect the synthesis of UV-B absorbing compounds in the fruit peel. A 3 min dose of artificial UVBR on semi sun lemons harvested during the winter season increased the concentration of UV-B absorbing compounds in flavedo cells. Interdonato et al. [21] showed that the 3 min UVBR dose induced changes in both carbohydrate content and distribution pattern in albedo and flavedo of postharvest lemons.

Green mold, caused by *Penicillium digitatum* Sacc., is the most common postharvest disease of citrus fruit worldwide [11]. It affects an average of 5–8% of the total handled fruit resulting in a significant economic loss to both growers and packers [12]. Alternative treatments to control this disease are needed in order to replace the conventional fungicides, which have led to the occurrence of resistant strains. Several attempts with promising results were performed using both organic

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and inorganic chemicals [8,9,38]. On the other hand, natural resistance in citrus fruit can be elicited by physical treatments, such as heat [20], low temperature [33], UV-C radiation [39], and mechanical injury [6].

The hypothesis of this work was that UVBR induces changes on postharvest lemon peel, representing an acclimation response that enhances fruit resistance against *P. digitatum*. Thus, metabolic and anatomical changes that can be produced by supplemental UVBR and their potential effects against fungal infection were studied.

2. Materials and Methods

2.1. Plant Material

Mature lemons (*Citrus limon* (L.) Burm. F., cv. Eureka) were obtained from a commercial orchard located in the Tucumán province, Argentina (S 26° 50′ and W 65° 12′). Semi sun lemon fruit (color index ≈ -0.6, determined by a scale based on [19]) of uniform size and appearance were hand collected during the austral winter season (June to August) from the north facing half of the canopy [21]. In addition, for green mold incidence determinations, randomly collected mature lemons provided by San Miguel SA Citrus packing house (Tucumán, Argentina) were used. Those lemons were also collected during commercial harvest season and were free from postharvest treatments or coating. All fruit were washed with distilled water and stored at 25 °C after sampling.

2.2. UV-B Treatments

The lemons were exposed to UVBR for 3 min, as described by Interdonato et al. [21]). The lemons were rotated after applying radiation on one side of the fruit, in order to irradiate the other side. UVBR was provided by five UV fluorescent lamps (UVB-313 Q-Panel, Cleveland) placed 50 cm above the fruit. Biologically effective UV-B radiation at lemon peel level was 22,000 J m^{-2} d^{-1}. UVBR-treated (T) and untreated control (C) lemons were stored in a controlled chamber at 25 °C and 65–75% relative humidity in darkness until flavedo sampling or fruit evaluation.

2.3. Flavedo Sampling

Flavedo tissue slides (~2 mm thickness) were cut from postharvest lemons (50 fruit per treatment). Fig. 1 indicates sampling time outline in respect to harvest. The peel samples were immediately used for fresh tissue determinations (water content, oxygen consumption), fixed (microscopy) or stored at −20 °C (chemical determinations).

2.4. Water Content Determination

To determine the flavedo water status fresh and dry weights were recorded. The water content was calculated by using the following equation: *Water content* (%) = (*Fresh weight* − *Dry weight*) / *Fresh weight* × 100.

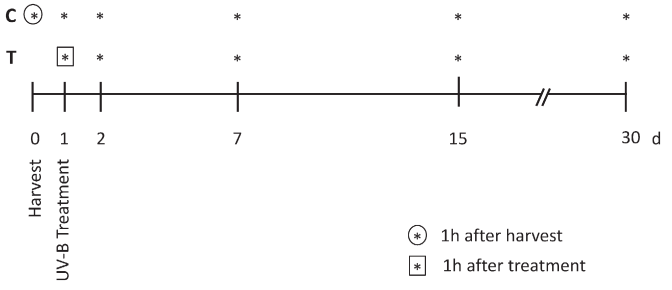

Fig. 1. Lemon peel sampling outline. Flavedo samples were taken on indicated days (asterisks) from control (C) and 3 min UVBR-treated (T) lemons. First samples were taken either 1 h after harvest (circle) or 1 h after UVBR application (square), respectively.

2.5. Oxygen Consumption Determination

To evaluate oxygen consumption of lemon flavedo, the tissue samples (50 mg fresh weight) were split into ~1 mm^2 pieces and incorporated into a 2 mL thermostated cell containing 5 mmol L^{-1} TRIS–HCl buffer, pH 7.2, under continuous stirring. The cell was coupled to an oxygen detector (Oxygraph-2k, Oroboros) and the oxygen consumption was recorded by using specific software. 1 mmol L^{-1} potassium cyanide (KCN) was used to inhibit the cytochrome oxidase pathway (COX) and 3 mmol L^{-1} salicylhydroxamic acid (SHAM) to inhibit the alternative oxidase pathway (AOX) [16]. The total oxygen consumption corresponds to COX plus AOX plus residual respirations.

2.6. ROS Determination

Reactive oxygen species (ROS) were determined using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) probe [10,14]. Briefly, 1 mg flavedo tissue homogenized with 2 mL^{-1}distilled water was centrifuged at 6000 rpm for 20 min. The supernatant was added with 1 mmol L^{-1} H_2DCFDA (final concentration). Resulting 2′,7′-dichlorofluorescein (DCF) was measured at 525 nm (excitation at 488 nm) using an ISS-PCI spectrofluorometer (Champaign, IL, USA). Accumulated DCF is proportional to the concentration of H_2O_2 in flavedo cells. Data were normalized to flavedo fresh weight.

2.7. Flavedo Antioxidant Activity

Antioxidant capacity was determined in a methanol extract of frozen flavedo by using the chromogenic 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonate cation radical ($ABTS^+$) [28]. Antioxidant capacity was determined as ($ABTS^+$) consumption (solution discoloration) by reading the decrease of absorbance at 734 nm in UV–visible spectrophotometer (Spectromax plus 250, Molecular Device, CA, USA).

2.8. Soluble Phenolic Compound Determination

Soluble phenolics were extracted from 500 mg of frozen flavedo with 2 mL of acidified methanol (methanol:water:HCl, 79:20:1) at 40 °C for 12 h in darkness [18]. The total phenolic content was determined according to Singleton et al. [37] and was expressed as μg phenol equivalent mg fresh $weight^{-1}$. Different flavonoids were determined by specific colorimetric techniques. Flavanone and dihydroflavonol were measured as described by Popova et al. [31] and expressed as μg naringenin equivalent mg fresh $weight^{-1}$. Flavone and flavonol were determined according to Woisky and Salatino [44] and were expressed as μg quercetin equivalent mg fresh $weight^{-1}$. Anthocyanin, according to Abdel-Aal and Hucl [1] were expressed as mg cyanidin-3-glucoside g fresh $weight^{-1}$.

2.9. Anatomical Studies

Transversal sections of flavedo were taken from the equatorial zone of lemons on days 2 and 7 according to time outline, and fixed in FAA (formalin:acetic acid:50% ethanol, 5:5:90) at room temperature. Free hand sections were stained with $FeCl_3$:MeOH (1:99) to visualize phenolic compound deposits [13]. Flavedo samples were also fixed in Karnovsky's solution (8% formaldehyde, 16% glutaraldehyde and 100 mmol L^{-1} sodium phosphate buffer, pH 7.4) [23]. After incubation at 4 °C overnight, the samples were included in Spurr resin, cut in ultrathin sections, and stained with toluidine blue [15]. Microscopical observations were performed using an optical microscope (Carl Zeiss, Axiostar plus). Microphotographs were taken with a coupled digital

camera (Canon A620, Power Shot 7.1 MP). Anatomical studies were performed using 5 fruits for each treatment.

2.10. Atmospheric Monitoring of Penicillium spp. in the Incubation Chamber

Atmospheric conidial concentration in the incubation chamber was periodically monitored following the gravimetric method described by Palou et al. [30]. Microbial samples were taken using PDA open Petri dishes exposed to the chamber atmosphere during 1 min. Colony-forming units (CFU) for *Penicillium* spp. per 3 Petri dish area (174 cm^2) were determined after 7 d of incubation at 25 °C. Natural contamination was classified based on the following scale: 1–2 CFU: clean; 3–10 CFU: polluted; >10 CFU: strongly polluted.

2.11. Green Mold Incidence Determination

Disease development was evaluated on T and C lemons as follows. Two days after harvest, the lemons were wounded with a sterilized stainless steel rod tip and transferred to an incubation chamber with air-polluted degree between 3 and 10 CFU of *P. digitatum*. The fruits were incubated at 25 °C and 95% relative humidity for 5 d. 150 lemons were used per treatment. Green mold incidence was expressed as percentage of decayed fruit per total lemons evaluated.

2.12. Effect of UVBR on P. digitatum Growth

To evaluate the effect of UVBR on *P. digitatum* growth, conidia were challenged with the radiation. *P. digitatum* conidia were isolated from the naturally infected lemons and grown on potato dextrose agar (PDA) dishes, pH 5.5, at 25 °C for 7 d. A protocol adapted by Cerioni et al. [8] was followed to prepare 10^6 conidia mL^{-1} suspensions. Briefly, 5 μL of conidial suspension were spotted on PDA medium in crystal clear polystyrene (UV-B transparent) Petri dishes. Conidial cells were grown for 24 h at 25 °C and were exposed to increasing time-dose of UVBR between 3 and 240 min placed at 50 cm under the lamps. Colony diameter was measured after 7 d of incubation at 25 °C.

2.13. Statistical Analysis

Data is the result of three different trials and were subjected to analysis of variance (ANOVA) followed by Tukey's test. Differences at $P < 0.05$ were considered significant.

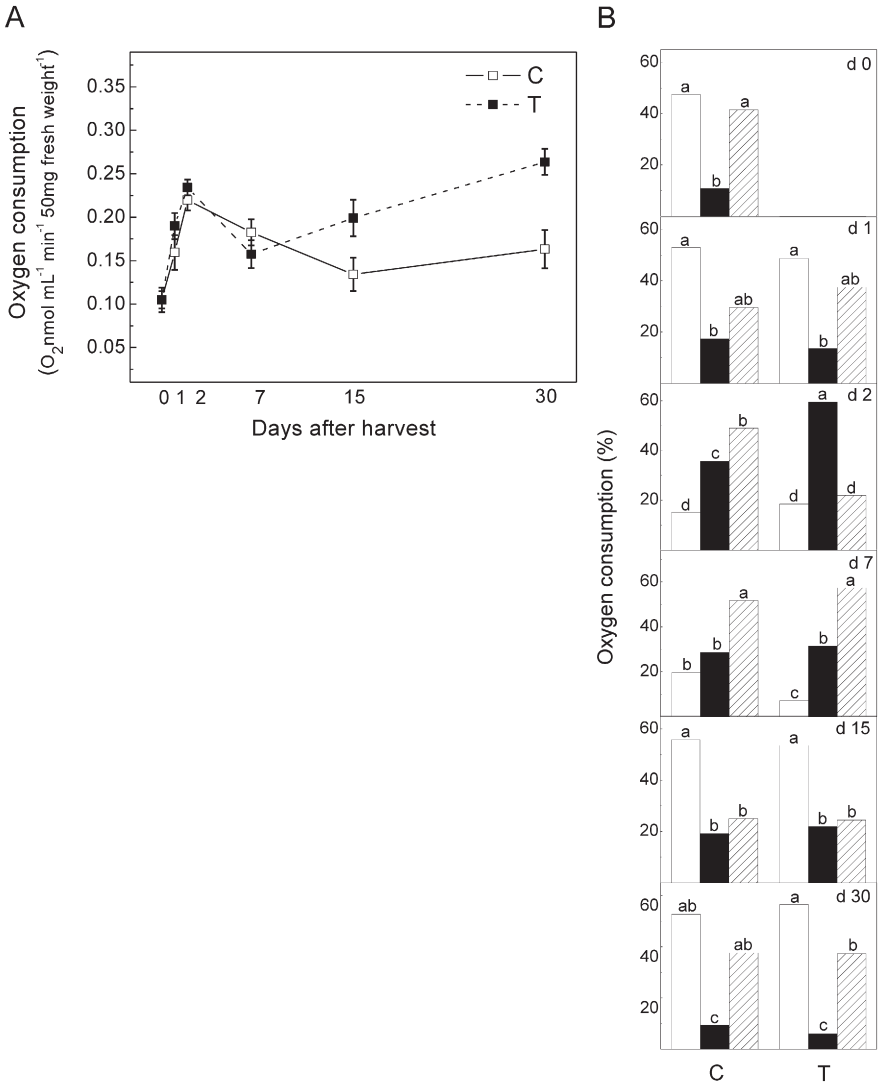

Fig. 2. Flavedo oxygen consumption of Control (C) and UVBR-treated (T) flavedo samples was measured on the indicated day after harvest. A) Total respiration; B) Cytochrome respiration (white bar), alternative respiration (black bar) and residual respiration (striped bar). Data are means of 3 independent experiments by triplicate. Different letters indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

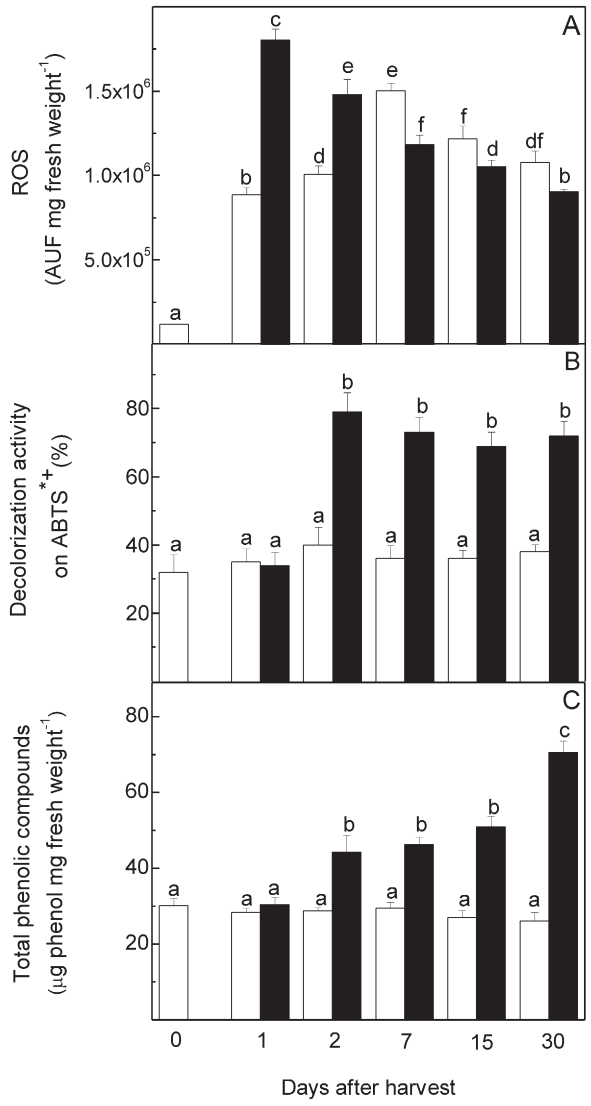

Fig. 3. Reactive oxygen species (A), antioxidant activity (B), and total phenolic compound (C). Lemon flavedo samples were taken following the outline in Fig. 1. Data are means of 3 independent experiments by triplicate. Different letters indicate significant differences according to Tukey's test ($P < 0.05$). White bars: control lemons; black bars: UVBR-treated lemons. AUF: arbitrary units of fluorescence.

3. Results

3.1. Respiratory Activity in Flavedo of Postharvest Lemons

Profiles of total respiration of flavedo cells from both C and T lemons were similar up to day 7 (Fig. 2A). Thereafter, the total respiration of C lemons remained low, while T lemon respiration increased until day 30. The relative contribution of different respiratory activities to total respiration is shown in Fig. 2B. On days 0 and 1, the COX activity represented 50–55% of the total respiration, AOX activity corresponded to 10–15%, and residual activity accounted for 30–40%. COX activity decreased on day 2 in both samples, and continues to decrease in T lemons on day 7. From day 15 on, the COX activity increased in both lemons until reach the initial proportion (similar to day 1). AOX activity increased significantly on day 2 in both samples, being markedly higher in T lemons, and it decreased until reaching less than the 10% of initial activity at 30 d. Noteworthy, at day 30 relative proportions of different respiratory activities recovered the profile of day 0.

Both C and T lemons underwent similar water loss (dehydration) of approximately 10% toward day 30 (data not shown).

3.2. Reactive Oxygen Species and Antioxidant Activity in Flavedo of Postharvest Lemons

In Fig. 3A, ROS level in C flavedo increased after harvest, reaching on day 1 values 6-fold higher than data recorded in flavedo at harvest, with a maximum on day 7. From this point a progressive decrease until the end of the experimental period occurred. UVBR triggered ROS production on flavedo almost immediately (1 h after treatment) doubling its level in respect to the C sample and progressively decreased.

The antioxidant activity of T flavedo increased significantly on day 2 (i.e. 24 h after UV-B treatment, see Fig. 1), maintaining high levels until day 30 (Fig. 3B). In contrast, control flavedo did not show significant changes throughout the experiment.

3.3. Phenolics in Flavedo of Postharvest Lemons

Fig. 3C shows the total soluble phenolics in the flavedo of C and T lemons. Soluble phenolics of T lemons showed a sustained increase from day 2, reaching values of around 2–3-fold higher than those of

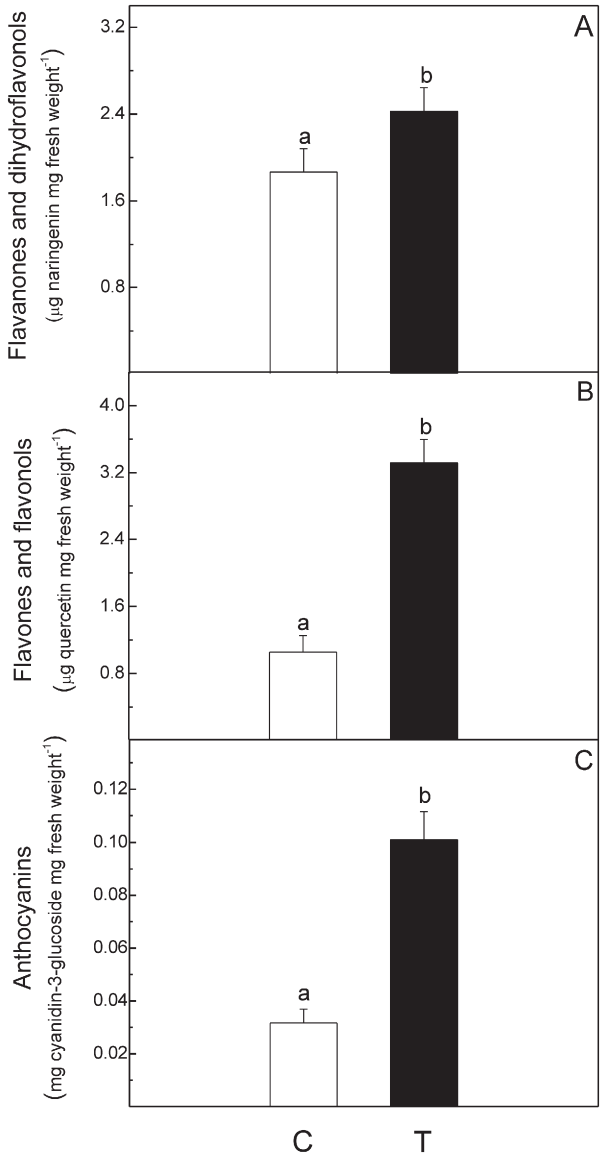

Fig. 4. Flavonoid types in lemon flavedo. A) Flavanones and dihydroflavonols, B) Flavones and flavonols; C) Anthocyanins. C (control) and T (UVBR-treated). Samples were taken 2 d after harvest. Different letters indicate significant differences according to Tukey's test ($P < 0.05$).

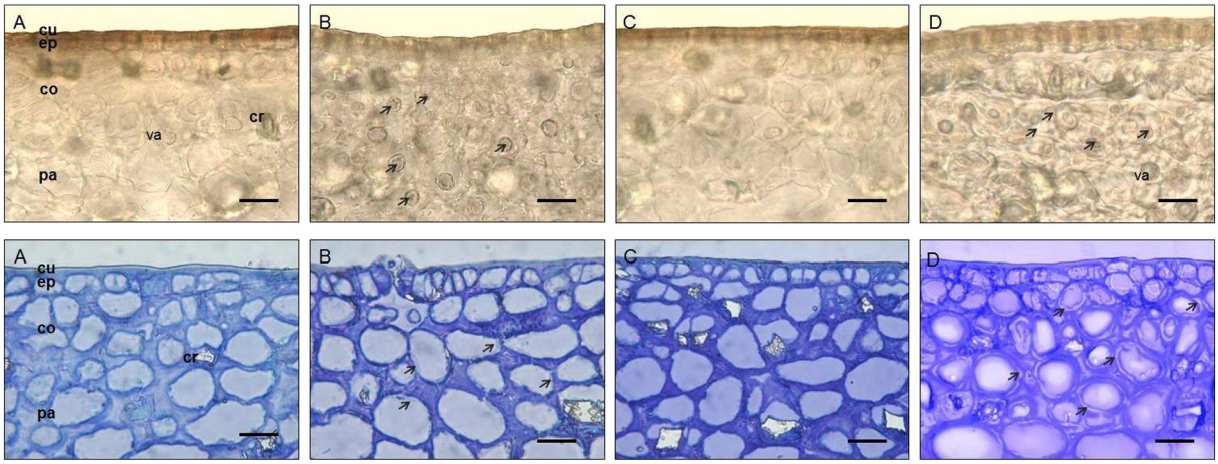

Fig. 5. Stained cross-sections of both control and UVBR- treated lemon flavedo. Upper panel: $FeCl_3$ staining, arrows indicate phenolic deposits (bars = 20 μm); lower panel: toluidine blue staining, arrows indicate thickened cell walls (bars = 12 μm). Control on day 2 (A) and day 7 (C), UVBR-treated on day 2 (B) and day 7 (D). ep: epidermis, co: collenchyma, cr: crystal, cu: cuticle, pa: parenchyma, va: vacuole.

control on day 30. On the contrary, no significant changes of soluble phenolics were observed in C lemons. Flavonoids were evaluated on day 2 in flavedo (Fig. 4). Flavanones–dihydroflavonols, flavonols and anthocyanins were increased in 29, 214 and 218%, respectively, in T lemons in respect to C lemons.

3.4. Anatomical Changes in Flavedo of Postharvest Lemons

Microphotographs of both C and T flavedo samples taken on days 2 and 7 are shown in Fig. 5. Phenolics were visualized in T flavedo as gray colored spots inside the vacuoles of both collenchyma and parenchyma cells (arrows in Fig. 5B and D, upper panel). UVBR radiation produced cell wall thickening (epidermis, collenchyma, and parenchyma), which was more pronounced toward day 7 (arrows in Fig. 5B and D, lower panel).

3.5. Green Mold Incidence on Postharvest Lemons

Methodologies used to assess the effectiveness of postharvest treatments against fungi require ensuring infection of most control samples [11]. In the present work, experimental conditions included artificial wound made by a steel tip and an atmospheric environment contaminated with conidia (see Section 2.11). In these conditions, UVBR treatment was able to control around 60% of green mold incidence in respect to controls (Fig. 6).

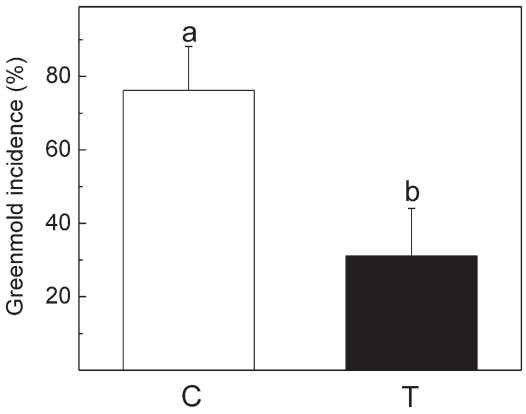

Fig. 6. Green mold incidence on postharvest lemons. Control (white bars) and treated with UVBR (black bars). Data are means of 3 independent experiments by triplicate using n = 50 for each condition tested. Different letters indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

It is worth to mention that a 3 min UV-B dose did not have germicide effect on the phytopathogen (data not shown). It is necessary to have an exposure time of at least 180 min to completely inhibit fungal growth.

4. Discussion

A 3 min non-germicide dose of supplemental UVBR on postharvest lemons induced metabolic and anatomical changes in flavedo tissue and reduced the incidence of green mold development. The applied radiation dose has no effect on peel water status and maintains the fruit cosmetic values, one of the most relevant factors affecting lemon export trade. The 3 min dose has been chosen as it has been shown to induce significant increases of carbohydrate precursors and soluble phenolic compounds in the peel of postharvest lemons [18,21].

In plants, phenylpropanoid-derivatives act as defensive molecules and are involved in cell wall thickening [7,26]. It is well known that solar UV radiation is responsible for the accumulation of phenylpropanoids, mainly in epidermal cells of sun-exposed organs such as leaves, flowers and fruits [7]. Hilal et al. [17] demonstrated that UV-B radiation stimulates an increase of fructose and phenolic compound contents and induces cell wall thickening in quinoa cotyledons. Our present results show that radiation treatment with artificial UVBR induced the phenolic compound accumulation in flavedo of postharvest lemons, indicating that a photo-induced synthesis of phenolics. It should be noted that final products of the flavonoid pathway (flavones, flavonols and anthocyanins) increased more markedly than their precursors (flavanones and dihydroflavonols). Further biochemical studies should be done to establish the mechanisms underlying to sustain the accumulation of those final products. The increased accumulation of soluble phenolics in flavedo of treated lemons can be associated with the high antioxidant activity found in the flavedo of these samples.

In addition, supplemental UVBR enhances the AOX activity of flavedo tissue which is in agreement with Zhao et al. [46]. The AOX respiratory pathway plays both regulatory and protective roles to modulate the effects of oxidative stress in plants exposed either to biotic or abiotic factors [42]. The AOX pathway therefore acts as an "overflow" for the excess of electrons when the COX pathway is saturated or becomes restricted which can occur in the presence of excess sugar, allowing the maintenance of the carbon flow into the tricarboxylic acid (TCA) cycle [3]. Interdonato et al. [21] demonstrated that 3 min dose of supplemental UVBR increased over 400% the concentration of fructose in lemon flavedo with a concomitant increase of soluble phenolics. Thus, continued carbon flux through the TCA cycle supported by AOX might be critical to provide carbon intermediates during periods of extensive biosynthesis [25]. Increased AOX activity produces up-

regulation of genes encoding PAL and chalcone synthase enzymes in stressed plants [35] and the activation of the oxidative pentose phosphate pathway (OPPP) [43], which provides phenylpropanoid precursors such as fructose-derived erythrose-4-P (E4P). In this regard, present results indicate that a sustained increase of phenolic compounds occurs in UVBR-treated flavedo from day 2 (24 h after irradiation), maybe triggered by a mechanism dependent on AOX peak.

Supporting the biochemical determinations, anatomical observations showed abundant intravacuolar deposits of phenolic compounds in UVBR-treated samples. An increase in cell wall thickness was also found after the 3 min dose of supplemental UVBR. A positive correlation between accumulation of soluble phenolics and lignin deposition in cell wall against biotic and abiotic stresses including UVBR was reported [2,17,22,29,32]. Changes in plant cell wall structure and composition under UV light could produce mechanical barriers and protective substances such as secondary compounds including lignin [27,45]. Anatomical changes in lemon peel represent a protective barrier of cells against UV-B radiation that could be useful to prevent the pathogen infection. However, further rigorous studies should be done as a proof of concept in regard to the application of UVBR in lemon disease control.

In conclusion, data suggest that a short-term exposure of mature postharvest lemons to artificial UV-B induces an acclimation response to radiation. This phenotypic plasticity allows increasing of fruit natural defenses by mechanical/chemical modifications of the flavedo tissue which prevent the dissemination of *P. digitatum*. Combination of UVBR with other treatments could represent an important improvement to control postharvest diseases on citrus fruit.

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References

[1] E.S.M. Abdel-Aal, P. Hucl, A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats, Cereal Chem. 76 (1999) 350–354.
[2] M.B. Ali, N. Singh, A.M. Shohael, E.J. Hahn, K.Y. Paek, Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress, Plant Sci. 171 (2006) 147–154.
[3] B. Arnholdt-Schmitt, J.H. Costa, D. Fernandes de Melo, AOX — a functional marker for efficient cell reprogramming under stress? Trends Plant Sci. 11 (2006) 281–287.
[4] C.L. Ballaré, Light regulation of plant defense, Annu. Rev. Plant Biol. 65 (2014) 335–363.
[5] C.L. Ballaré, M.M. Caldwell, S.D. Flint, S.A. Robinson, J.F. Bornman, Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change, Photochem. Photobiol. Sci. 10 (2011) 226-4.
[6] A.R. Ballester, M.T. Lafuente, C.H.R. De Vos, A. Bovy, L. González-Candelas, Citrus phenylpropanoid and defense against pathogens. Part I: Metabolic profiling in elicited fruits, Food Chem. 136 (2013) 178–185.
[7] L. Carrasco-Ríos, Efecto de la radiación Ultravioleta-B en plantas, Idesia 27 (2009) 59–76.
[8] L. Cerioni, L. Rodríguez-Montelongo, J. Ramallo, F.E. Prado, V.A. Rapisarda, S.I. Volentini, Control of lemon green mold by a sequential oxidative treatment and sodium bicarbonate, Postharvest Biol. Technol. 63 (2012) 33–39.
[9] L. Cerioni, M. Sepulveda, Z. Rubio-Ames, S.I. Volentini, L. Rodríguez-Montelongo, J.L. Smilanick, J. Ramallo, V.A. Rapisarda, Control of lemon postharvest diseases by low-toxicity salts combined with hydrogen peroxide and heat, Postharvest Biol. Technol. 83 (2013) 17–21.
[10] J.F. Davidson, B. Whyte, P.H. Bissinger, R.H. Schiestl, Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. 93 (1996) 5116–5121.
[11] J.W. Eckert, G.E. Brown, Evaluation of Postharvest Treatments for Citrus Fruit, in: K.D. Hickey (Ed.), Method for Evaluating Pesticides for Control of Plant Pathogens, APS Press, St. Paul, MN, USA 1986, pp. 92–97.
[12] J.W. Eckert, I.L. Eaks, Postharvest Disorders and Diseases of Citrus Fruit, in: W. Reuter, E.C. Calavan, G.E. Carman (Eds.), The Citrus Industry, vol. 5, University of California Press, Berkeley, CA, USA 1989, pp. 17–260.
[13] P.B. Gahan, Plant Histochemistry and Cytochemistry: An Introduction, Academic Press. U.K, London, 1984.
[14] B. Halliwell, M. Whiteman, Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142 (2004) 231–255.
[15] Y. Heslop-Harrison, J. Heslop-Harrison, The digestive gland of pinguicula: structure and cytochemistry, Ann. Bot. 47 (1981) 293–319.
[16] M. Hilal, A.M. Zenoff, G. Ponessa, H. Moreno, E.M. Massa, Saline stress alters the temporal patterns of xylem differentiation and alternative oxidase expression in developing soybean roots, Plant Physiol. 117 (1998) 695–701.
[17] M. Hilal, M.F. Parrado, M. Rosa, M. Gallardo, E.M. Massa, J.A. González, F.E. Prado, Epidermal lignin deposition in quinoa cotyledons in response to UV-B radiation, Photochem. Photobiol. 79 (2004) 205–210.
[18] M. Hilal, L. Rodríguez-Montelongo, M. Rosa, M. Gallardo, J.A. González, R. Interdonato, V.A. Rapisarda, F.E. Prado, Solar and supplemental UV-B radiation effects in lemon peel UV-B-absorbing compound content — seasonal variations, Photochem. Photobiol. 84 (2008) 1480–1486.
[19] HunterLab, Hunter Lab Color scale, Insight on color, 8, Hunter Associates Laboratories, Reston, VA, USA 1996, pp. 1–4.
[20] S. Infla, D. Boonyakiat, Induction of resistance to *Penicillium digitatum* in tangerine fruit c.v. Sai Num Phung flavedo by hot water treatment. Songklanakarin J, Sci. Technol. 2 (2010) 445–451.
[21] R. Interdonato, M. Rosa, C.B. Nieva, J.A. González, M. Hilal, F.E. Prado, Effects of low UV-B doses on the accumulation of UV-B absorbing compound and total phenolics and carbohydrate metabolism in the peel of harvested lemons, Environ. Exp. Bot. 70 (2011) 204–211.
[22] J. Jozef Kováčik, B. Klejdus, Dynamics of phenolic acids and lignin accumulation in metal-treated *Matricaria chamomilla* roots, Plant Cell Rep. 27 (2008) 605–615.
[23] M.J. Karnovsky, A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy, J. Cell Biol. 27 (1965) 137–138.
[24] E. Kostina, A. Wulff, R. Julkunen-Tiito, Growth, structure, stomatal responses and secondary metabolites of birch seedlings (*Betula pendula*) under elevated UV-B radiation in the field, Trees 15 (2001) 483–491.
[25] H. Lambers, Cyanide-resistant respiration: a non-phosphorylating electron transport pathway acting as an energy overflow, Physiol. Plant. 55 (1982) 478–485.
[26] V. Lattanzio, A. Cardinali, V. Linsalata, Plant Phenolics: a Biochemical and Physiological Perspective, in: V. Cheynier, P. Sarni-Manchado, S. Quideau (Eds.), Recent Advances in Polyphenol Research, vol. 3, John Wiley & Sons, Ltd., Chichester, UK 2012, pp. 1–39.
[27] H. Le Gall, F. Philippe, J.-M. Domon, F. Gillet, J. Pelloux, C. Rayon, Cell Wall metabolism in response to abiotic stress, Plants 4 (2015) 112–166.
[28] N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, Clin. Sci. 84 (1993) 407–412.
[29] J.C.M.S. Moura, C.A.V. Bonine, J. de Oliveira Fernandes Viana, M.C. Dornelas, P. Mazzafera, Abiotic and biotic stresses and changes in the lignin content and composition in plants, J. Integr. Plant Biol. 52 (2010) 360–376.
[30] L. Palou, J. Usall, J. Pons, M.C. Cerdà, I. Viñas, Micoflora en centrales citrícolas de Tarragona, Investig. Agrar. Prod. Prot. Veg. 16 (2001) 447–462.
[31] M. Popova, V. Bankova, D. Butovska, V. Petkov, B. Nikolova-Damyanova, A.G. Sabatini, G.L. Marcazzan, S. Bogdanov, Validated method for the quantification of biologically active constituents of poplar-type propolis, Phytochem. Anal. 15 (2004) 235–240.
[32] F.E. Prado, M. Rosa, C. Prado, G. Podazza, R. Interdonato, J.A. González, M. Hilal, UV-B Radiation, its Effects and Defense Mechanisms in Terrestrial Plants, in: A. Parvaiz, M.N.V. Prasad (Eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change, Springer Science + Business Media, New York-USA 2012, pp. 57–83.
[33] A. Rab, M. Sajid, N.U. Khan, K. Nawab, M. Arif, M.K. Khattak, Influence of storage temperature on fungal prevalence and quality of citrus fruit (CV. Blood Red), Pak. J. Bot. 44 (2012) 831–836.
[34] T.M. Robson, K. Klem, O. Urban, M.A.K. Jansen, Re-interpreting plant morphological responses to UV-B radiation, Plant Cell Environ. (2014), http://dx.doi.org/10.1111/pce.12374.
[35] E. Santos Macedo, D. Sircar, H.G. Cardoso, A. Peixe, B. Arnholdt-Schmitt, Involvement of alternative oxidase (AOX) in adventitious rooting of *Olea europaea* L. microshoots is linked to adaptive phenylpropanoid and lignin metabolism, Plant Cell Rep. 31 (2012) 1581–1590.
[36] M. Schreiner, I. Mewis, S. Huyskens-Keil, M.A.K. Jansen, R. Zrenner, J.B. Winkler, A. Krumbein, UV-B-induced secondary plant metabolites — potential benefits for plant and human health, Crit. Rev. Plant Sci. 31 (2012) 229–240.
[37] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178.
[38] J.L. Smilanick, M.F. Mansour, F. Mlikota Gabler, D. Sorenson, Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides, Postharvest Biol. Technol. 47 (2008) 226–238.
[39] C. Stevens, V.A. Khan, C.L. Wilson, J.Y. Lu, E. Chalutz, S. Droby, The effect of fruit orientation of postharvest commodities following low dose ultraviolet light-C treatment on host induced resistance to decay, Crop. Prot. 24 (2005) 756–759.
[40] L. Taiz, E. Zeiger, I.M. Møller, A. Murphy, in: A.D. Sinauer (Ed.), Plant physiology and development, Sinauer Associates, Inc., Sunderland, USA, 2014.

[41] D. Treutter, Significance of flavonoids in plant resistance: a review, Environ. Chem. Lett. 4 (2006) 147–157.
[42] O. Van Aken, E. Giraud, R. Clifton, J. Whelan, Alternative oxidase: a target and regulator ofstress responses, Physiol. Plant. 137 (2009) 354–361.
[43] J.T. van Dongen, K.J. Gupta, S.J. Ramírez-Aguilar, W.L. Araújo, A. Nunes-Nesi, A.R. Fernie, Regulation of respiration in plants: A role for alternative metabolic pathways, J. Plant Physiol. 168 (2011) 1434–1443.
[44] R. Woisky, A. Salatino, Analysis of propolis: some parameters and procedures for chemical quality control, J. Apic. Res. 37 (1998) 99–105.
[45] S. Yamasaki, N. Noguchi, K. Mimaki, Continuous UV-B irradiation induces morphological changes and the accumulation of polyphenolic compounds on the surface of cucumber cotyledons, J. Radiat. Res. 48 (2007) 443–454.
[46] M.G. Zhao, Y.G. Liu, L.X. Zhang, L. Zheng, Y.R. Bi, Effects of enhanced UV-B radiation on the activity and expression of alternative oxidase in red kidney bean leaves, J. Integr. Plant Biol. 49 (2007) 1320–1326.