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Effects of low UV-B doses on the accumulation of UV-B absorbing compounds and total phenolics and carbohydrate metabolism in the peel of harvested lemons

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ABSTRACT

This paper examined the peel (albedo and flavedo) of postharvest lemon fruits after UV-B exposure in order to analyze relationships between soluble carbohydrate metabolism and secondary metabolite accumulation. Lemons (*Citrus limon*, cv. Limoneira 8A) were harvested in winter months (June to August), treated with 0.43 W m^{-2} (22 kJ m^{-2} d^{-1} UV-BBE) of UV-B radiation during 0 (control), 0.5, 1.0, 2.0, 3.0, and 5.0 min, and then stored at 25 °C for 24 h. Peel samples from irradiated areas were obtained with a razor blade and frozen in liquid nitrogen until use for measurements. Data obtained showed that 2 and 3 min of UV-B exposure effectively increased the level of UV-B absorbing compounds and total phenolics in flavedo without causing visual alterations of the peel colour as compared with non-irradiated lemons. By contrast, there were no significant changes in albedo secondary metabolite accumulation. The amount of secondary metabolites was depending upon UV-B time–dose. Exposure over 3.0 min did not further improve the accumulation of UV-B absorbing and phenolic compounds. Soluble sugars (sucrose, glucose and fructose) also accumulated in the lemon peel after UV-B exposure, but the distribution patterns were different. After 3 min time–dose, sucrose and hexoses increased in flavedo, whereas in albedo only increased the sucrose and glucose. This effect was related to UVB-induced changes in the activity of sucrose-hydrolyzing and sucrose-synthesizing enzymes: soluble and cell-bound invertase, sucrose synthase (SS) and sucrose phosphate synthase (SPS). Data indicate that lemon peel retains the capacity to modify the enzyme activity of sucrose metabolism in response to UV-B exposure. Our results also suggest that the exposure of postharvest lemons to low supplemental UV-B doses produces changes in the carbon allocation of peel tissues including synthesis, but probably not only limited to them, of UV-B absorbing and phenolic compounds.

1. Introduction

Environmental factors can exert disadvantageous influences on the plant's life leading to disruption of metabolic processes at molecular, cellular, whole organism or even ecosystem level. Disadvantageous influences are referred as “stress” and unfavourable environmental factors as “stress factors” or simply “stressors” (Bijlsma and Loeschcke, 2005). The focus in stress physiology has been driven to know as plants respond and survive to short-term and long-term (chronic) suboptimal growth conditions (Gaspar et al., 2002). Many environmental factors such as ultraviolet radiation, low temperature, microorganisms and insects, nutrient availability, and heavy metals, have a great impact on the synthesis of secondary metabolites (Bijlsma and Loeschcke, 2005). In fact, plant secondary metabolites are important determinants of plant stress responses (Jansen et al., 2008). Ultraviolet wavelengths greater than 280 nm (UV-B radiation) are a ubiquitous component of the solar radiation, but their levels considerably vary in the biosphere spatially and temporally. During the later part of the 20th century the average of UV-B irradiances in the biosphere have increased primarily due to the chlorine- and bromine-containing compounds released into the atmosphere by anthropogenic activities (Butler et al., 1999). UV-B effects include complex changes in DNA repair capacity, photosynthetic activity, growth, plant morphology, gene expression, pest- and pathogen-resistance, and secondary metabolism (Frohnmeyer and Staiger, 2003). Plant tissues respond to UV-B by inducing cellular protective processes that include changes in phenylpropanoid metabolism with increased synthesis of UV-B absorbing compounds, mostly flavonoids and other related phenolics (Tegelberg et al., 2001). Although UV-absorbing compounds primarily protect the DNA molecule (Stapleton, 1992), they also play an important role in the plant antioxidant defence and against

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pathogens and/or herbivores attack (Ortuño et al., 2006; Hagen et al., 2007).

Allocation and availability of the photoassimilate are important parameters in determining plant responses to UV-B radiation (Gwynn-Jones, 2001). The provision of resources may be crucial for protection and repair mechanisms to UV-B exposure through the synthesis of UV-B absorbing compounds (Logemann et al., 2000). UV-B radiation is linked to CO_2 assimilation by the rate to which the carbon skeleton is diverted from the primary metabolism to the secondary one (Kasige and Takashi, 2009). Therefore, factors that affect the carbohydrate metabolism will also affect the production of UV-B absorbing compounds and other phenolics, partly as result of the supply of substrates (carbohydrates) and/or intermediates (*e.g.* cinnamic acids), and partly from the action of rate-limiting enzymes (Gwynn-Jones, 2001). In this context, the UV-B radiation may act as a regulator of both CO_2 assimilation and carbohydrate metabolism. Then, UVB-induced relationships between carbohydrates and secondary metabolites are expected that may be occurring in plants.

Fruits (*e.g.* apple, peach, citrus, plum, grape) are rich in peel flavonoids and other phenolic compounds (Velioglu et al., 1998; Cantos et al., 2001; Kataoka et al., 2003; Ortuño et al., 2006; Hagen et al., 2007). Citrus fruits are the most important source of flavonoids and phenolics (Gattuso et al., 2007), particularly, flavanones (*e.g.* naringin, hesperidin) and many polymethoxylated flavones (*e.g.* tangeretin, sinensetin, nobiletin) (Ortuño et al., 2006). These compounds are very rare in other fruits and show a high effectiveness against fungal pathogens (Arcas et al., 2000). Citrus is the most important fruit tree crop in the world, with an annual production of approximately 102 million tonnes (Perez-Perez et al., 2005). Lemon (*Citrus limon*) is the third most important species of citrus after orange and mandarin, with a production totalling more than 4,400,000 tonnes during the 2001/2002 season. Argentina with 1.2 million tonnes is currently the world's largest producer of lemons (FAO, 2003). Lemon is a leading crop in the Northwest of Argentina, where approximately 1.1 million tonnes of the fragrant fruit were harvested in 2008. Tucumán is the nation's top lemon-producing province and Tafí Viejo is nicknamed the Citrus Capital of the World. Worldwide the lemon production is mostly marketed as fresh fruit and frozen juice. Lemon fruit is not a highly perishable product, but 'postharvest losses' frequently occur. They include attack by fungi and other microorganisms, and physical damage during handling and/or transportation (Smilanick et al., 2003). Nevertheless, approximately 90% of "postharvest losses" is produced by the green mold *Penicillium digitatum* (Pers.:Fr.) Sacc (Ismail and Zhang, 2004). This mold is controlled primarily by extensive use of chemical fungicides such as imazalil and thiabendazole (Cabras et al., 1999). The apparition of *P. digitatum* fungicide-resistant strains has led to search alternative strategies to postharvest fungal control. These strategies include use of antagonistic microorganisms, natural compounds, and chemical and physical treatments (Ben-Yehoshua et al., 1992; D'hallewin et al., 2000). Among these latter, the heat, and ionizing (gamma rays) and non-ionizing (UV) radiations seem to be the most important. UV treatments are usually performed using short wavelengths (190–280 nm, UV-C) (Droby et al., 1993). Results have shown that short-time UV-C irradiations induce fungal resistance against both *P. digitatum* (green mold) and *P. italicum* (blue mold) in the peel of harvested citrus (Arcas et al., 2000; D'hallewin et al., 2000). Despite beneficial effects against fungal infections, the UV-C radiation affects the colour of lemon peel being particularly dangerous to skin and eyes (Baadsgaard, 1991; Ben-Yehoshua et al., 1992). Then, alternative UV treatments are desirable in order to achieve safer results. In that context, the UV-B radiation could be a suitable option for a beneficial treatment of postharvest lemons (Wargent et al., 2006). The aim of this work was to study the effect of short-time UV-B doses on the accumulation of UV-B-absorbing compounds, total phenolics, soluble carbohydrates and sucrose-related enzymes in the peel (flavedo and albedo) of postharvest mature lemons, in order to establish relationships between UVB-induced secondary metabolites synthesis and soluble carbohydrates.

2. Materials and methods

2.1. Plant material and UV-B treatment

Mature fruits of *C. limon* (cv. Limoneira 8A) were kindly provided by CITRUSVIL SA (San Pablo-Tucumán, Argentina, 26°50′S, 65°12′W). Lemons of uniform size and appearance, from the superficial-inner portion of the canopy, were harvested during the austral winter season (June, July and August). Superficial-inner fruits were at least 70–150 cm from the tree periphery and 130–180 cm above the ground. Tree rows were oriented in a north-south direction, with in-row and between-row spacing of 5.5 and 6.5 m, respectively. Lemons were randomly picked from the north canopy side of healthy trees. Forty trees were chosen to avoid an unwanted thinning effect due to repeated sampling and to get always fruits with similar size and ripeness stage. Photosynthetic active radiation (PAR) and UV-B radiation within the tree canopy were measured 100 cm inside the canopy and 170 cm above the ground on the north tree side with a quantum sensor (LI-190SA) coupled to a Data-Logger (LI-1000) (Li-Cor, Lincoln) and a silicon photoelectric cell coupled to a Photometer/Radiometer (PMA2100, Version 1.17; Solar Light Company, Inc.). Instantaneous measurements were done in 2008 on June 17 for cloudy condition and on July 13 for sunny condition, respectively. Measurements were recorded at 9:30, 13:00, and 17:30 h and were done near a fruit with the sensor and photoelectric cell facing up. Reference radiation data were measured outside the tree canopy immediately before inside measurements. For each timing hour, measurements were done on four different trees and were completed in ≤15 min.

Picked lemons were immediately transported to the laboratory, washed with distilled water and stored at 25 °C for 24 h before supplemental UV-B irradiation. Thirty lemons were surface irradiated on a well-delimited peel area with supplemental UV-B radiation using UV-fluorescent lamps. A rack of six fluorescent UV-B lamps (Q-Panel 313, Cleveland, USA) provided the supplemental UV-B radiation. To assure uniform irradiation conditions, the lamp rack was mounted 60 cm above lemons. UV emission from UV-B fluorescent lamps in the range 280–400 nm was measured with a fiber optic spectroradiometer (BLACK-Comet, StellarNet, Inc., FL, USA), recently factory calibrated. The supplemental biologically effective UV-B daily dose (UV-BBE) weighted using the generalized plant action spectrum normalized at 300 nm (Caldwell, 1971) was 0.43 W m^{-2} (22 kJ m^{-2} d^{-1}) (Nogués et al., 1998). Lemons were irradiated during 0 (control), 0.5, 1.0, 2.0, 3.0, and 5.0 min time–dose. After UV-B treatment, the irradiated and non-irradiated (control) lemons were stored in a controlled chamber at 25 °C and 65% relative humidity in darkness for 24 h to minimize any possible photoreactivation process (Stevens et al., 2005). After that, the flavedo (~2 mm thickness) and albedo (~3 mm thickness) tissues were obtained with a razor blade. Samples were cut in small pieces, frozen in liquid nitrogen, and then were milled to fine powder with a coffee mill. Powdered samples were stored at −20 °C until use for chemical and enzymatic measurements. Possible UV-B injury in the peel of lemons was surveyed visually every day after the UV-B treatment began for at least 20 d.

2.2. Total phenolics and UV-B absorbing compounds

Total phenolics were extracted from 0.2 g FW of powdered tissue with 3 ml of 96% ethanol and placed in a cold room (6 °C) in