Sensitivity of yellow passion fruit to ultraviolet-B radiation

Inês Cechin[1], Valéria de Jesus Rocha[1] and Terezinha de Fátima Fumis[1]

[1]Universidade do Estado de São Paulo, Faculdade de Ciências, Departamento de Ciências Biológicas, Avenida Luiz Edmundo Carrijo Coube, nº 14-01, Vargem Limpa, CEP 17033-360 Bauru, SP, Brazil. E-mail: icechin@fc.unesp.br, valery_roch@yahoo.com.br, tffumis@fc.unesp.br

Abstract – The objective of this work was to evaluate the effects of UV-B radiation on the vegetative growth and on the gas exchange characteristics of passion fruit plants (*Passiflora edulis*) grown in greenhouse. The average unweighted UV-B radiation near the apex of the plants was 8 W m^{-2} for the UV-B treatment (high UV-B), and 0.8 W m^{-2} for the control plants (low UV-B). Plants were irradiated with UV-B for 7 hours per day, centered on solar noon, during 16 days. High UV-B radiation resulted in lower shoot dry matter accumulation per plant. The content of UV-B absorbing compounds and anthocyanins was increased in the plants exposed to high UV-B radiation, when compared with the control. UV-B radiation did not affect stomatal conductance or transpiration rate, but reduced photosynthesis and instantaneous water-use efficiency, and increased intercellular CO_2 concentration. The accumulation of UV-B-absorbing compounds and anthocyanins did not effectively shield plants from supplementary UV-B radiation, since the growth and photosynthetic processes were significantly reduced.

Index terms: *Passiflora edulis*, anthocyanins, growth, phenolic compounds, photosynthesis.

Sensitividade do maracujazeiro-amarelo à radiação ultravioleta-B

Resumo – O objetivo deste trabalho foi avaliar o efeito da radiação UV-B no crescimento vegetativo e nas características das trocas gasosas de plantas de maracujazeiro-amarelo (*Passiflora edulis*), cultivadas em casa de vegetação. A radiação média não ponderada de UV-B próximo ao topo das plantas foi de 8 W m^{-2}, para plantas tratadas com UV-B (alta UV-B), e de 0,8 W m^{-2} para plantas controle (baixa UV-B). As plantas foram irradiadas com UV-B por 7 horas por dia, centralizadas ao redor do meio-dia, durante 16 dias. Alta radiação de UV-B reduziu o acúmulo de matéria seca da parte aérea da planta. O conteúdo de compostos que absorvem UV-B e de antocianinas foi aumentado nas plantas expostas à alta radiação UV-B, quando comparado às plantas controle. A radiação UV-B não alterou a condutância estomática ou a taxa de transpiração, mas reduziu a fotossíntese e a eficiência instantânea do uso da água, e aumentou a concentração intercelular de CO_2. O acúmulo de compostos que absorvem UV-B e antocianinas não protegeram de forma efetiva as plantas contra o aumento da radiação suplementar UV-B, visto que o crescimento e os processos fotossintéticos foram significativamente reduzidos.

Termos para indexação: *Passiflora edulis*, antocianinas, crescimento, compostos fenólicos, fotossíntese.

Introduction

Ultraviolet radiation is arbitrarily divided into three bands of different wavelengths. The atmospheric ozone absorbs 100% of UV-C (100–280 nm) and 90% or more of UV-B (280–315 nm), whereas UV-A (315–400 nm) passes through it with little change. Changes in the ozone layer will, therefore, result in an increase in solar UV-B radiation reaching the Earth, but not in UV-A.

Significant declines in ozone concentration were observed since satellite records began in 1979, with a more severe loss recorded in 2011 in the Arctic and Antarctic stratosphere (Blunden & Arndt, 2012), which was mainly caused by chemical destruction of ozone. According to data of Brazil's Instituto Nacional de Pesquisas Espaciais, there was a reduction in the ozone layer over the country at lower latitudes (Sahai et al., 2000). Portmann et al. (2012) highlighted the role of nitrous oxide anthropogenic emissions as an O_3-destroying compound.

Plants are vulnerable to increased UV-B radiation because many cellular components can absorb it directly (Jordan, 1996). Increased UV-B radiation has many effects on plant morphology, physiology and development, and its impacts on growth and development are seen in many plant species (Frohnmeyer & Staiger, 2003). Reduction in biomass

accumulation due to UV-B exposure has been verified in several species, either under field (Gao et al., 2003) or greenhouse conditions (Nogués et al., 1998). Many plants exposed to this radiation exhibit decreased rates of photosynthesis (Correia et al., 2005; Cechin et al., 2008).

The attenuation of UV-B radiation that reaches the leaf surface can occur by the production and deposition of UV-absorbing compounds in leaf epidermal cells or hairs (Manetas, 2003). Increase in their concentration has been reported in plants under UV-B radiation (Boeger & Poulson, 2006). Anthocyanins, for example, are phenolic compounds found in the vacuoles of parenchyma cells that protect against the excess of visible and ultraviolet radiation (Solovchenko & Merzlyak, 2008).

Passion fruit, originated in the American tropics, was introduced and grown in most tropical and subtropical parts of the world. Yellow passion fruit (*Passiflora edulis* f. *flavicarpa* O. Deg.) is the most cultivated species, and its fruits may be eaten in natura or destined for juice production. However, the impact of UV-B radiation on the growth and physiology of passion fruit has not been studied so far.

The objective of this work was to evaluate the effects of UV-B radiation on the vegetative growth and on the gas exchange characteristics of passion fruit plants (*Passiflora edulis*) grown in greenhouse.

Materials and Methods

Approximately 60-day-old plants of yellow passion fruit, grown in plastic tubes filled with Plantmax substrate, were transferred to a greenhouse located in Bauru, SP, Brazil (22°21'28"S and 49°01'37"W, at a 630 m altitude) in October 2008, with maximum and minimum temperatures around 31 and 20°C, respectively. Plants were cultivated under natural photoperiod and supplied with tap water, when necessary.

In the greenhouse, solar UV-B radiation was virtually absent, and the photosynthetic active radiation (PAR) intensity was nearly 70% of that measured with the infrared gas analyzer sensor outside, under clear sky conditions, due to the transmission characteristics of the polycarbonate cover and to the shading by UV-B lamps and greenhouse structural elements. UV-B radiation was supplied by 40 W fluorescent sunlamps UVB-313, (Q-Panel Co., Cleveland, OH, USA) held in mobile adjustable frames over the plants. The system consisted of two raisable aluminum frames over two benches. Each frame had eight pairs of lamps at a distance of 0.5 m from each other. The two frames were separated by a polyester curtain to avoid lateral UV-B radiation from reaching the control plants. The distance between the lamps and the top of the growing plants was kept constant at 10 cm, by adjusting the frame above the plants.

The experimental design included two levels of UV-B radiation: low-UV-B (control) and high (UV-B treatment) with 20 plants in each treatment. In the high UV-B treatment, the lamps over the bench were wrapped with a 0.10 mm thick cellulose acetate filter (Malaga Produtos Metalizados Ltda., Osasco, SP, Brazil) to cut off radiation below 280 nm. In the control treatment, the plants were kept under lamps wrapped with a 0.125 mm thick polyester filter (Du Pont Co., Wilmington, Delaware, USA), which absorbs radiation below 320 nm. These filters were replaced every four and eight days, respectively. The average unweighted UV-B radiation near the top of the plants was 8 W m^{-2}, for the high UV-B treatment, and 0.8 W m^{-2} for the control. A voltage meter (Marconi Equipamentos para Laboratórios Ltda., Piracicaba, SP, Brazil) connected to a broad band UV-B sensor with preamplifier 280–350 nm, with a peak wavelength at 313 nm, (Delta-T Devices Limited, Cambridge, UK) was used to measure the total irradiance in the UV-B band reaching the top of the plants. The plants were irradiated with UV-B for 7 hours per day, centered around solar noon, during 16 days. The highest UV-B level used in this experiment was about 30% higher than the one obtained under clear sky conditions at solar noon, outside the greenhouse. This level is classified as low level and represents ozone depletion between 9–20%.

A portable infrared gas analyzer LCpro, (ADC Bioscientific Ltd., Hoddesdon, UK) was used to measure photosynthesis (A), stomatal conductance to water vapor (g_s), transpiration (E), and intercellular CO_2 concentration (C_i). Measurements were made on the youngest fully expanded leaves developed under UV-B radiation, 16 days after the beginning of the UV-B treatment. Photosynthetic active radiation of 1,000 μmol m^{-2} s^{-1} was supplied by a light unit, mounted on top of the leaf chamber. The leaves were kept under this PAR until steady-state. Instantaneous

water-use efficiency was calculated by dividing net CO_2 assimilation by transpiration (A/E).

Phenolic compounds and anthocyanins were extracted according to Musil et al. (2003) with some modifications. Samples of dried leaves were ground to a fine powder. A total of 18 mg of each sample was extracted in a 6 mL mixture of MeOH:H_2O:HCl (79:20:1 v/v/v), in the dark, for 48 hours. After that, the extraction media was filtered and the absorbance was measured at 300, 530, and 657 nm. The absorbance of the extract due to UV-B absorbing compounds or anthocyanins was assumed to be directly proportional to the molar concentration of these metabolites in the extracts (Murray & Hackett, 1991). The concentration of total phenolic compounds was quantified by dividing their absorbance at 300 nm wave length by dry matter (Ab_{300} g^{-1}), and the concentration of anthocyanins by the equation (Ab_{530} - 1/3Ab_{657}) / dry matter (Lindoo & Caldwell, 1978).

After 16 days of UV-B radiation treatment, nine plants of each treatment were selected randomly for shoot dry matter determination. The plants were divided into stems and leaves before being oven dried at 65°C for 48 hours.

Statistical tests were done using SPSS/PC 9.0 for Windows. Statistical analysis was performed using Student's t test, at 5% probability.

Results and Discussion

Under unfavorable environmental conditions, plants accumulate pigments – such as mycosporine-like amino acids, phenolic compounds, alkaloids, and carotenoids – that can attenuate the radiation, both in the UV and in the visible parts of the spectrum (Solovchenko & Merzlyak, 2008). UV-B absorbing compounds have been recognized as a general response to this radiation. They can protect the plants from the radiation harm by reducing the transmittance of UV photons through the leaf tissue. The protective role of UV-B-absorbing compounds has been reported by Li et al. (1993) in flavonoid-deficient *Arabidopsis* mutants. In the present study, the concentration of UV-B absorbing compounds at 300 nm, which is characteristic of flavonoids, increased 60%, when compared to the control plants, in response to UV-B radiation (Figure 1A). These results are in agreement with the findings in the recent meta-analysis by Li et al. (2010), which showed that, in herbaceous plants, UV-B-absorbing compounds increased by 37% under high UV-B treatments. Moreover, there is a high correlation between total leaf phenolic concentration and UV-B absorbing capacity in plants (Levizou & Manetas, 2002). Therefore, the increase in total leaf phenolic concentration observed here and in other studies can render protection to plants against elevated UV-B levels.

The main spectral feature of in vivo anthocyanin absorption is a peak around 550 nm, which occurs in small amounts in almost every leaf. For this reason, anthocyanins absorb UV less efficiently when compared to others flavonoids. Anthocyanins synthesized in the leaf have also been associated with UV-B radiation and can act as an antioxidant compound, helping to reduce photooxidative damage (Steyn et al., 2002). Passion fruit plants grown under high UV-B radiation had a 32% increase in anthocyanin concentration in comparison to the control plants (Figure 1B). This result differs from those of other research, which showed that UVB radiation did not increase anthocyanin concentration (Ibañez et al., 2008). In the present study, the increase in UV-B absorbing compounds and anthocyanins indicates that passion fruit plants may activate a defense mechanism against the radiation harmful effect. In this sense, the screening pigments would attenuate the incident radiation by removing, to a considerable extent, the harmful UV-B and the excessively absorbed visible quanta.

UV-B radiation (280–320 nm) of solar light inevitably has detrimental effects on earthly organisms because many cell components absorb in the UV range (Jordan, 1996). Li et al. (2010) observed that supplemental UV-B radiation had significant negative effects on herbaceous photosynthetic rates of a wide

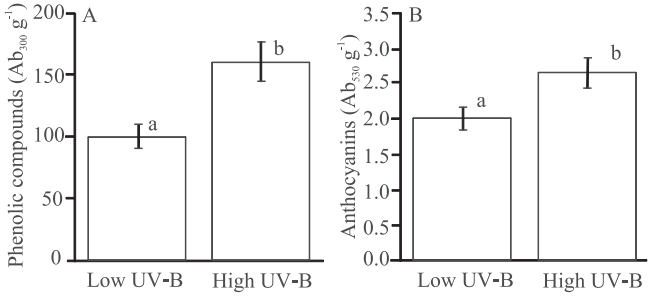

Figure 1. Accumulation of phenolic compounds (A) and anthocyanins (B) in yellow passion fruit grown under low or high UV-B radiation for 16 days. Bars represent the standard error (n = 4 to 5).

range of species, from woody to herbaceous ones. The negative effects of UV-B on photosynthetic processes have been verified under both field (Correia et al., 2005) and greenhouse conditions (Cechin et al., 2008).

In passion fruit plants, after 16 days of UV-B radiation stress, photosynthetic performance showed significant alterations (Figure 2). Plants grown under elevated UV-B radiation had slightly, but not significantly, lower transpiration rates and stomatal conductance than the control; however, the calculated C_i value increased by approximately 10% in UV-B stressed plants. Rates of photosynthesis of these plants were reduced by 41% when compared to the control. Instantaneous water-use efficiency in the control plants was 3.46, whereas in UV-B stressed ones it was 2.5, a value 28% lower. This marked photosynthetic reduction may be a consequence of the shock caused by the sudden high UV-B exposure. Cechin et al. (2008) observed lower reduction (24%) in photosynthesis, studying sunflower plants nearly under the same UV-B radiation level. Jansen & van den Noort (2000) found evidence that increased UV-B radiation stimulate stomatal opening or closure. Although stomatal conductance was not significantly affected by UV-B radiation in the present study, the calculated C_i value was higher. Therefore, the rise in C_i in UV-B stressed plants may be a reflection of biochemical or photochemical dysfunction instead of stomatal limitation. Feng et al. (2003) reported that Rubisco content and activity decreased in soybean seedlings supplemented with UV-B radiation. Decreased rates of photosynthesis have also been attributed to damage to the photosynthetic apparatus (Rodrigues et al., 2006).

The increase in C_i observed here could also represent an increase in respiration. However, reports have shown that respiration of sunflower and maize are insensitive to enhanced UV-B radiation (Mark & Tevini, 1996). It is worth noting that the ability of screening pigments to attenuate incident radiation was insufficient to fully protect the photosynthetic process in passion fruit plants under UV-B radiation.

Leaves of passion fruit plants grown under UV-B radiation were smaller and visibly curled towards the abaxial surface (Figure 3), as observed in *Arabidopsis* (Boeger & Poulson, 2006) and *Acer mono* Maxim. plants (Yao & Liu, 2006) grown under UV-B radiation. Leaf curling has been considered as a photoprotective mechanism to minimize the UV-B radiation effect (Boeger & Poulson, 2006). Because the amount of UV-B used in the present study was detrimental to photosynthesis and shoot biomass accumulation, the curling response may be an expression of UV-B damage.

Li et al. (2010) showed that herbaceous and woody plants had a decrease in shoot biomass of 16.6 and 8.4%, respectively, due to UV-B exposure.

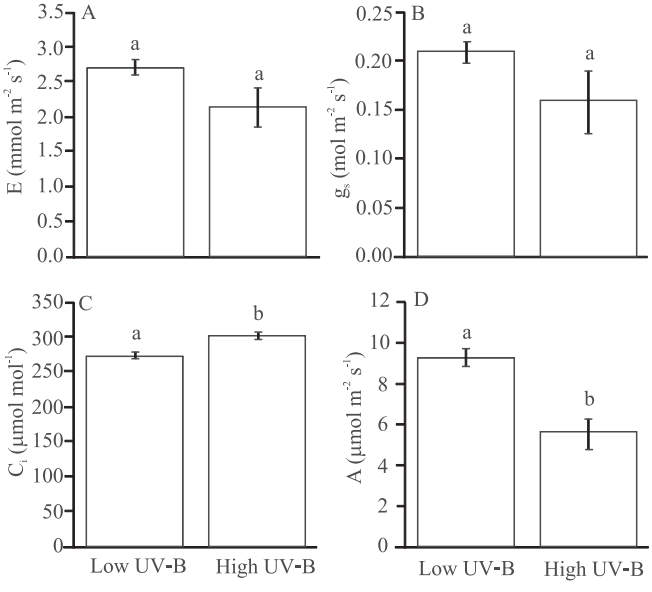

Figure 2. Effects of low or high UV-B radiation on yellow passion fruit: A, transpiration (E); B, stomatal conductance (g_s); C, intercellular CO_2 concentration (C_i); and D, photosynthesis (A). Bars represent the standard error (n = 7).

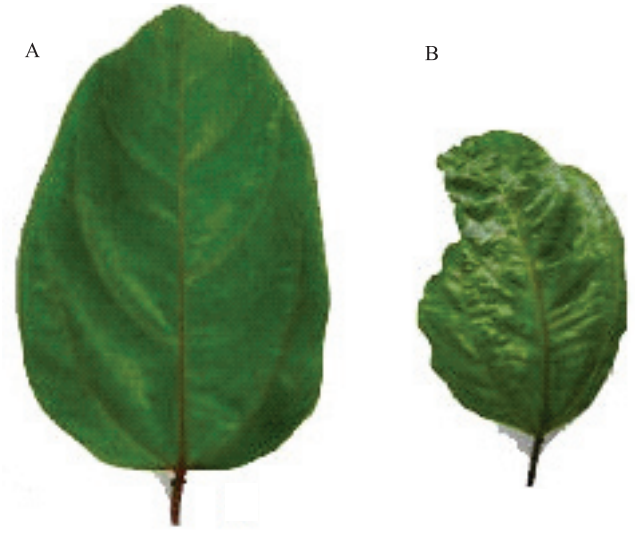

Figure 3. Leaves of yellow passion fruit grown under low (A) or high (B) UV-B radiation for 16 days.

Contradictory results reported in the literature can be explained by the fact that plant responses to UV-B radiation depend on a number of parameters, including the developmental stage of tissue, the plant species, and growth conditions. Moreover, plant responses to UV-B radiation are dependent on the UV-B level of exposure and the ratio of PAR to UV-B under experimental conditions (Pradhan et al., 2006). In passion fruit plants, the reduction in the CO_2 assimilation rates due to UV-B exposure were accompanied by a significant inhibition in shoot dry matter accumulation, from 2.47 g in control to 1.77 g in the stressed plants (Figure 4). By the end of the experiment, the stem and leaf dry matter of individual plants was significantly reduced by 20 and 36%, respectively, compared to the control plants. Therefore, the difference in shoot dry matter production was a consequence of the effect of the UV-B radiation on both leaf and stem growth.

Dry matter production was less sensitive to UV-B radiation than photosynthesis, indicating that the effects of UV-B on biomass may result from reductions in leaf area rather than reductions in CO_2 assimilation per unit area. The reduction in growth has been attributed to non-photosynthetic mechanisms, such as leaf expansion, as a consequence of the effect of UV-B radiation on the rate and duration of both cell division and elongation (Hopkins et al., 2002). Growth retardation may also be related to lower ability to photorepair cyclobutane pyrimidine dimer by higher amounts of anthocyanins (Hada et al., 2003), known to filter out the blue/UV-A light required for photolyase function (Britt, 1995) and the visible light for photosynthesis (Hughes et al., 2005).

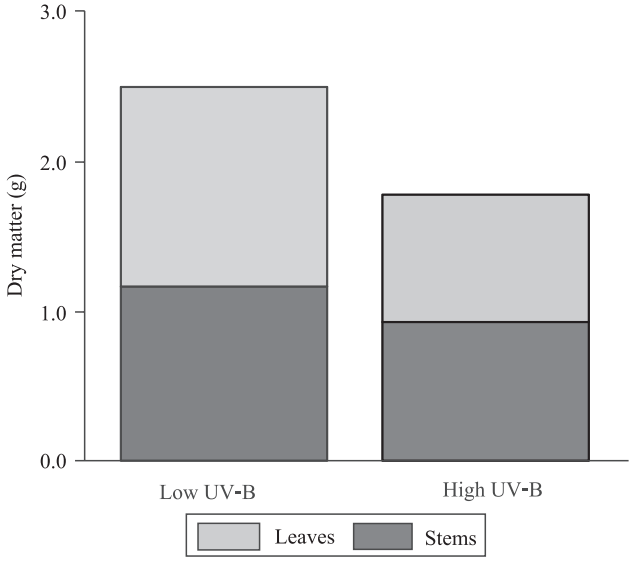

Figure 4. Shoot dry matter of yellow passion fruit grown under low or high UV-B radiation for 16 days. Values are mean of nine plants.

Conclusions

1. UV-B radiation induces an increase in UV-B-absorbing compounds and anthocyanins, in passion fruit plants.

2. Higher accumulation of these compounds do not effectively shield plants from supplementary UV-B radiation, since the growth and photosynthetic processes were significantly reduced.

3. Passion fruit production may be challenged by future increase in UV-B radiation.

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