The sensitivity of sunflower (*Helianthus annuus* L.) plants to UV-B radiation is altered by nitrogen status

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ABSTRACT: Interaction effects between nitrogen and UV-B radiation were studied in sunflower (*Helianthus annuus* L. variety IAC-Iarama) plants grown in a greenhouse under natural photoperiod conditions. Plants were irradiated with 0.8W m⁻² (control) or 8.0W m⁻² (+UV-B) of UV-B radiation for 7h per day. The plants were grown in pots containing vermiculite and watered with 70% of full strength nitrogen-free Long Ashton solution, containing either low (42.3ppm) or high (282ppm) nitrogen as ammonium nitrate. High nitrogen increased dry matter of stem, leaves and shoot, photosynthetic pigments and photosynthesis (A) without any alteration in stomatal conductance (gs) nor transpiration (E) while it reduced the intercellular CO₂ (C) concentration, and malondialdehyde (MDA) content. High UV-B radiation had negative effects on dry matter production, A, gs and E with the effects more marked under high nitrogen, whereas it increased C under high nitrogen. Activity of PG-POD was reduced by high UV-B radiation under low nitrogen but it was not changed under high nitrogen. The UV-B radiation increased the MDA content independently of nitrogen level. Results indicate that the effects of UV-B radiation on sunflower plants are dependent of nitrogen level.

Key words: antioxidants, growth, malondialdehyde, peroxidation, photosynthesis.

RESUMO: Os efeitos da interação entre nitrogênio e a radiação UV-B foram estudados em plantas de girassol (*Helianthus annuus* L. variedade IAC-Iarama), cultivadas em casa de vegetação sob condições fotoperiódicas naturais. As plantas foram irradiadas com 0.8W m⁻² (controle) ou 8.0W m⁻² (+UV-B) durante 7h por dia. As plantas foram cultivadas em vasos contendo vermiculita e regadas com solução nutritiva de Long Ashton 70%, contendo baixa (42.3ppm) ou alta (282ppm) dose de nitrogênio na forma de nitrato de amônio. A alta dose de nitrogênio aumentou a matéria seca das folhas e do caule e parte aérea, pigmentos fotosintéticos e a fotossíntese (A), sem alterar a condutância estomática (gs) ou transpiração (E), enquanto reduziu a concentração intercelular de CO₂ (C) e o conteúdo de malondialdeído (MDA). A radiação UV-B reduziu a produção de matéria seca, A, gs e E, sendo os efeitos mais acentuados sob alta dose de nitrogênio, enquanto aumentou C, sob alta dose de nitrogênio. A atividade da PG-POD foi reduzida sob alta radiação UV-B e baixo nitrogênio, mas não foi alterada sob alta dose de nitrogênio. A radiação UV-B aumentou o conteúdo de MDA independente do nitrogênio. Os resultados indicam que os efeitos da radiação UV-B em plantas de girassol são dependentes da disponibilidade de nitrogênio sendo que a alta dose de nitrogênio torna seus processos fisiológicos mais sensíveis à radiação UV-B.

Palavras-chave: antioxidantes, crescimento, malondialdeído, peroxidação, fotossíntese.

INTRODUCTION

Although, a recovery of the stratospheric ozone layer is expected due to the Montreal Protocol, this recovery is very slow due to the long lifetime of CFCs and the fact that previously undetected ozone-depleting substances were reported in the atmosphere which have been increasing in recent years (LAUBE et al., 2014). Exposure to high UV-B radiation has negative effects on plant growth as a consequence of reduced photosynthesis and DNA damage (FROHNMEYER & STAIGER, 2003). Under stressful conditions, such as high UV-B radiation, an increase in reactive oxygen species (ROS) has been observed (YAO & LIU, 2007), thus resulting in an oxidative stress as a consequence of alteration in the balance between oxidant and antioxidant. In order to minimize and protect the cell against the harmful effects of ROS, plants posses an efficient ROS-scavenging system consisting of enzymatic and non-

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enzymatic compounds that work together to maintain ROS homeostasis (YOU & CHAN, 2015).

Sensitivity of plant growth to nitrogen fertilization is of great importance in agriculture since nitrogen deprivation reduces the leaf production, individual leaf area and total leaf area (VOS & BIEMOND, 1992), resulting in a reduced area for light interception for photosynthesis. Nitrogen is a component of photosynthetic apparatus which is located in the chloroplast and an essential element for amino acids and protein synthesis. In addition, all enzymatic and some non-enzymatic antioxidants contain nitrogen, indicating that plants grown under lower nitrogen availability possess a lower antioxidant capacity (LIN et al., 2011). In nature, plants grow under a combination of environmental stresses which can be lethal or not to them depending on the interaction between the stressors. Due to the importance of nitrogen for plant growth and the harmful effect of UV-B, the objective of this study was to analyze the influence of nitrogen supply and enhanced UV-B radiation on growth, gas exchange characteristics, lipid peroxidation and enzymatic antioxidants of sunflower plants grown in a greenhouse. We hypothesized that the response of sunflower plants to UV-B radiation would change with the level of nitrogen supply.

MATERIALS AND METHODS

Seeds of sunflower (Helianthus annuus L. variety IAC-Iarama) were sown in 4L pots filled with vermiculite. Seedlings were thinned to one per pot after emergency and were grown in a greenhouse made of polycarbonate situated at the city of Bauru (22°21’28”S, 49°01’37”W), São Paulo, Brazil under natural light:dark length ratio. The mean maximum and minimum temperatures were 34 and 22°C, respectively. The plants were supplied with 300mL of 70% of full strength nitrogen-free Long Ashton solution (HEWITT, 1966), containing either low (42.3ppm) or high (282ppm) nitrogen as ammonium nitrate three times a week, and with tap water on the other days. UV-B radiation was supplied by 40W fluorescent sunlamps (UVB-313, Q-Panel Co., Cleveland, USA) held in mobile adjustable frames over the plants as described by CECHIN et al. (2008). The UV-B radiation near the top of the plants was 0.8W m⁻² for control plants or 8.0W m⁻² for treated plants. Plants were irradiated with UV-B for 7h per day, centered around solar noon. The UV-B radiation and nitrogen supply treatments were initiated five days after sowing. Maximum photosynthetic active radiation (PAR) intensity at noon time under a clear sky of a summer day was about 2,100µmol m⁻² s⁻¹. In the greenhouse, solar UV-B radiation was virtually absent, and PAR intensity was nearly 70% of that measured outside under clear sky conditions due to polycarbonate transmission characteristics. Plants were subjected to one of the following treatments: (a) low nitrogen supply and low UV-B (low N+low UV-B), (b) low nitrogen supply and high UV-B (low N+high UV-B), (c) high nitrogen supply and low UV-B (high N+low U-VB), and (d) high nitrogen supply and high UV-B (high N+high UV-B).

Measurements of photosynthesis (A), stomatal conductance (gₖ), transpiration (E) and intercellular CO₂ concentration (C) were made after 21 days of treatment on the youngest fully expanded leaves by using a portable infra-red gas analyser (LCpro, ADC, Hoddesdon, UK). Measurements were taken between 8 and 10am inside the greenhouse under ambient temperature, partial pressure of carbon dioxide and water vapour pressure of air. Photosynthetic photon flux density of 1,200µmol m⁻² s⁻¹ was supplied by a light unit mounted on the top of leaf chamber.

After photosynthetic measurements were taken, photosynthetic pigments were extracted on three leaf discs collected from each plant in 80% aqueous acetone and the content was calculated according to the equations proposed by LICHTENTHALER (1987). Soluble protein was determined according to LOWRY et al. (1951) and its content used to express the activity of pirogalol peroxidase (PG-POD). Extraction of peroxidase (POD; EC 1.11.1.7) was performed according to EKLER et al. (1993) and its activity was determined according to TEISEIRE & GUY (2000) by using pyrogallol as substrate. Change in absorbance due to the formation of purpurogallin was measured in a spectrophotometer at the wavelength of 430nm. The activity of PG-POD was expressed as µmol purpurogallin min⁻¹ mg⁻¹ protein. Level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, according to HEATH & PACKER (1968). The MDA content was calculated by its extinction coefficient of 155mM⁻¹ cm⁻¹ and expressed as nmol MDA per g fresh weight.

After 22 days of treatment, 5 plants of each group were selected randomly for biomass measurements. The plants were divided into leaves and stem before being oven dried at 70°C for at least 48 hours. All data were submitted to two-way analysis of variance procedure and the differences...
between the means of treatments were compared by using Least Significant Difference (LSD) procedure at 5% level. All data were processed using SPSS 9.0 software for Windows.

RESULTS AND DISCUSSION

In nature, plants are subjected to several stressors which may interact with each other, thus resulting in adverse effects on the plants, effects that may differ if the factors were acting alone. The data presented in the meta-analysis by LI et al. (2010) show that both woody and herbaceous plants present reductions in biomass under UV-B radiation. UV-B radiation reduces dry matter by affecting cell production and shortened growth zone (FINA et al., 2017), thus resulting in reductions in leaf expansion and final leaf size. Sunflower plants grown under elevated UV-B radiation showed a reduction in dry matter accumulation in the above ground parts, with a negative effect on both stem and leaves (Table 1). Shoot dry matter under high nitrogen was 2.4 times higher than in low nitrogen due to a higher investment in dry matter into leaves than into stem demonstrating that leaf expansion rate is very sensitive to nitrogen supply. Supplemental UV-B radiation under high nitrogen decreased the biomass of plants with no effect when nitrogen stress was imposed, suggesting secondary protection against UV-B damage under nitrogen deficiency (HUNT & MCNEIL, 1998).

Our results showed that the negative effect of UV-B radiation on the above ground biomass occurs under both low and high nitrogen. The presence of interaction between the two factors showed that the negative effect was more marked under high nitrogen implicating in an alteration in the sensitivity of plants under these conditions thus contrary to the results observed by YAO & LIU (2009). Our findings are in agreement with SINGH et al. (2012) who suggested that increased sensitivity towards UV-B radiation may result where fertilisers are applied in excess of recommended levels.

Although, some studies have shown that O3 depletion and the concurrent rise in UV-B radiation is not a direct threat to photosynthetic productivity of crops, KATARIA et al. (2014) pointed out in their review that UV-B radiation can affect photosynthesis in various aspects such as damage of photosystem II, loss in integrity of the thylakoid membranes and reduced Rubisco activity and content besides stomatal conductance. The inconsistent findings may be explained by the fact that plant species have different sensitivity to UV-B radiation in addition to the adverse growth conditions among the different studies such as the ratio of PAR to UV-B radiation during the growth period. The UV-B radiation damage to plants can be reduced by combining UV-B irradiation with visible light (OTA et al., 2017). In

Table 1 - Interactive effects of nitrogen and UV-B radiation on shoot, stem and leaves dry matter (g), stomatal conductance (g, mol m⁻² s⁻¹), photosynthesis (A, μmol m⁻² s⁻¹), transpiration (E, mmol m⁻² s⁻¹), intercellular CO₂ concentration (C, μmol mol⁻¹), total chlorophyll and carotenoid content (mg g⁻¹ FW) activity of peroxidase (PG-POD, μmol min⁻¹ mg⁻¹ protein) and malondialdehyde content (MDA, mmol g⁻¹ fresh weight) of sunflower plants.

<table>
<thead>
<tr>
<th>Low N</th>
<th>High N</th>
<th>Low N</th>
<th>High N</th>
<th>UV</th>
<th>N</th>
<th>U x N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>0.42±0.02aA</td>
<td>0.80±0.02aB</td>
<td>0.25±0.02bA</td>
<td>0.43±0.02bB</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.61±0.02aA</td>
<td>1.71±0.01aB</td>
<td>0.44±0.04bA</td>
<td>0.98±0.06bB</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Shoot</td>
<td>1.03±0.03aA</td>
<td>2.51±0.03aB</td>
<td>0.69±0.06aA</td>
<td>1.41±0.09bA</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>g,</td>
<td>0.70±0.01aA</td>
<td>0.69±0.01aA</td>
<td>0.48±0.02bA</td>
<td>0.54±0.02bB</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>21.9±0.4aA</td>
<td>26.8±0.4aB</td>
<td>17.9±0.5aA</td>
<td>20.5±0.5bA</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>E</td>
<td>5.36±0.05aA</td>
<td>5.10±0.06aB</td>
<td>4.26±0.07aB</td>
<td>4.52±0.04bB</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>255.7±3.3aA</td>
<td>223.3±1.9aB</td>
<td>251.3±1.4aA</td>
<td>238.8±2.6bB</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>Chl a+b</td>
<td>1.13±0.04</td>
<td>1.53±0.03</td>
<td>1.17±0.06</td>
<td>1.37±0.07</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>Car</td>
<td>0.35±0.02</td>
<td>0.43±0.01</td>
<td>0.33±0.01</td>
<td>0.39±0.02</td>
<td>NS</td>
<td>***</td>
</tr>
<tr>
<td>PG-POD</td>
<td>0.49±0.07aA</td>
<td>0.36±0.04aA</td>
<td>0.33±0.02bA</td>
<td>0.47±0.05aA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MDA</td>
<td>1.59±0.08</td>
<td>0.93±0.08</td>
<td>2.62±0.16</td>
<td>1.92±0.29</td>
<td>***</td>
<td>NS</td>
</tr>
</tbody>
</table>

Least Significant difference procedure (LSD) for comparing treatment means was only performed for the variable with significant F-test for the main effects (N and UV-B) and/or their interaction. The means followed by the same small letters (for N at a given UV-B level) and capital letters (for UV-B at a given N level) are not significant different at P = 0.05. Values are means±SE of 4-6 plants. Significance levels are: *, P<0.05; **, P<0.01; *** P<0.001; NS, not significant.
this study, UV-B radiation showed a negative effect on photosynthesis while nitrogen had a positive effect (Table 1), with no visible bleaching or necrosis on the leaves under UV-B radiation. Maximum reduction in photosynthesis by UV-B radiation was recorded in higher-nitrogen grown plants due to the presence of interaction. It is important to note that even the photosynthetic capacity is strongly related to foliar nitrogen concentration, in this study high nitrogen supply was not able to fully counteract the harmful effect of UV-B radiation. According to SINGH et al. (2012), individual nutrient applied at higher than recommended dose made the plants more susceptible to UV-B radiation compared to NPK combination. Our results contrast with those reported by RIQUELME et al. (2007) who observed that the UV-B in common bean was effective in reducing photosynthesis only when it was accompanied by nitrogen restriction. In addition, they reported that under these conditions photosynthesis was correlated with a decrease in $g_s$ rather than with a decrease in Rubisco activity.

The effect of nitrogen on photosynthesis occurs through changes in the stomatal aperture in addition to being part of photosynthetic apparatus (URAIRI et al., 2016). Under the conditions of our experiment, nitrogen did not affect $g_s$ or $E$. However, UV-B radiation reduced $g_s$ and $E$ under low and high nitrogen supply, with the reduction more marked under low than under high nitrogen (Table 1). Under high nitrogen, the greater demand for CO$_2$ resulted in lower $C_i$ compared to low nitrogen. Conversely, enhanced UV-B radiation increased $C_i$ under high nitrogen, with no effect under low nitrogen. Total chlorophyll and carotenoid were increased by high nitrogen independently of UV-B radiation. Since the photosynthetic pigments were not affected by UV-B, the results obtained in this study suggested that the reduction in photosynthesis under low nitrogen was partially correlated to stomatal limitation, while under high nitrogen the photosynthesis is also under control of mesophyll metabolism which reduced the demand for CO$_2$. The N-deprived plants had lower sensitivity to UV-B radiation in terms of photosynthesis which was correlated with lower decreases in Rubisco and PEPCase activity when compared with well N-nourish plants (CORREIA et al., 2005). It is possible that in our study the reduction in the activity and/or pool size of Rubisco may have contributed to an increase in CO$_2$ under high nitrogen.

The ability of plants to cope with an increase in UV-B radiation depends on their capacity to eliminate the ROS produced under stress conditions (CHOUDHARY & AGRAWAL, 2014). Carotenoids can function as a photosynthetic pigment and also as an antioxidant thus protecting the plants from oxidative stress (VIEIRA et al., 2017). There was an increase in total carotenoids in sunflower plants by high nitrogen, which was independent of UV-B radiation level applied (Table 1). RIQUELME et al. (2007) observed that under conditions of high nitrogen there was an increase in carotenoid content with no effect of UV-B radiation thus supporting our findings. Although, the antioxidative defense can be increased by UV-B radiation, it was observed that the defense system of plant is not always effective in detoxify ROS (CHOUDHARY & AGRAWAL, 2016). The activity of PG-POD in some species can be reduced (KALANTAR AHMADI et al., 2015) or increased (MOVUDI et al., 2014) by addition of nitrogen. Although, the effect of nitrogen and UV-B radiation on PG-POD activity was not significant, there was a significant interaction between the two factors which resulted in a reduction in the activity of PG-POD in plants grown under low nitrogen while under high nitrogen it was not affected.

The primary function of the antioxidant system is to control the accumulation of ROS which are responsible for membrane damage. UV-B radiation caused membrane damage measured in terms of MDA (SINGH et al., 2014). In this study, the MDA content was significantly affected by nitrogen and UV-B radiation and their effects were opposite (Table 1). Interaction between the two factors was not significant which means that both factors acted independently. Supplemental nitrogen reduced the MDA content suggesting a lower production of ROS as observed by YAO & LIU (2007). Conversely, MDA content was increased by UV-B radiation under both low and high nitrogen (Table 1). This indicated that addition of nitrogen could not alleviate the damage of UV-B radiation on cell membrane. Although, high UV-B radiation can stimulate the activity of some enzymes due to increasing requirement of scavenging ROS (YAO & LIU, 2007), in our study the activity of PG-POD was not increased but instead it was reduced under low nitrogen and not changed under high nitrogen. Therefore, the increase in MDA content under high UV-B radiation may have resulted from increased ROS production and the lack of enzyme activation.

**CONCLUSION**

The present study clearly illustrated that the sunflower plants performance is negatively affected by an increase in UV-B radiation. Although, the
addition of nitrogen improved the growth of plants, it made the physiological process of plants more sensitive to increase in UV-B radiation indicating that the plant response to UV-B depends on the interaction between different stressor factors which the plants are subjected to.

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