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1 **PHYSIOLOGICAL RESPONSES AND TOXIN PRODUCTION OF *Microcystis*
2 *aeruginosa* IN SHORT TERM EXPOSURE TO SOLAR UV RADIATION**

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12

13 **ABSTRACT**

14 The aim of this study was to evaluate the effects of short- term (hours) exposure to solar
15 UV radiation (UVR, 280-400nm) on the physiology of *Microcystis aeruginosa*. Three solar
16 radiation treatments were implemented: (i) PAR (PAR, 400-700nm), (ii) TUVA
17 (PAR+UVAR, 315-700nm) and (iii) TUVR (PAR+UVAR+UVBR, 280-700nm). Differential
18 responses of antioxidant enzymes and reactive oxygen species (ROS) production to UVR
19 were observed. Antioxidant enzymes were more active with high UVR doses. However,
20 different responses were observed depending on the exposure to UVAR or UVBR and the
21 dose level. No effects were observed on biomass, ROS production or increased activity of
22 superoxide dismutase (SOD) and catalase (CAT) compared to control when UVR+PAR
23 doses were lower than 9875 kJ m^{-2}. For intermediate doses, UVR+PAR doses between
24 9875 and 10275 kJ m^{-2}, oxidative stress increased while resistance was imparted through
25 SOD and CAT in cells exposed to UVAR. Despite increased antioxidant activity, biomass
26 decrease and photosynthesis inhibition were observed but no effects were observed with
27 added exposure to UVBR. At the highest doses (UVR+PAR higher than 10275 kJ m^{-2}), the
28 solar UVR caused decreased photosynthesis and biomass with only activation of CAT by
29 UVBR and SOD and CAT by UVAR. In addition, for such doses, significant decreases of
30 microcystins (MCs, measured as MC-LR equivalents) were observed as consequence of
31 UVAR.
32 This study facilitates our understanding of the SOD and CAT protection according to
33 UVAR and UVBR doses and cellular damage and reinforces the importance of UVR as an

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environmental stressor. In addition, our results support the hypothesized antioxidant function of MCs.

Keywords: UVR, ROS, SOD, CAT, MC-LR equivalents

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INTRODUCTION

Cyanobacteria are a group of phototrophic organisms that have great ecological and economical importance. They existed on Earth for around 2500-3500 million years, when the weather conditions were extreme, mainly due to high levels of ultraviolet radiation (UVR, 280-400 nm)[1]. During the past decades, springtime stratospheric ozone depletion over the Antarctic and the Southern Ocean has caused enhanced levels of ultraviolet B radiation (UVBR, 280-315 nm) to reach the Earth's surface[2]. Although seasonal ozone depletion continues to occur over the Antarctic, the severity of the depletion is lessening and expectation is that the seasonal depletion will cease by 2050[3].

Some species of *Microcystis* can regulate their position in the water column, due to gas vesicles, while searching favorable depths for their development[4,5]. On shallower depths, cyanobacteria may be exposed to increased solar UVR doses due to less light attenuation in the water column as consequence of low turbidity[6]. UVR can induce significant damage on a variety of cell targets, including DNA[7], proteins[1] and Photosystem II (PS II)[6]. UVAR (315-400 nm), like UVBR, has the potential for cell damage, which is caused by both direct effects and indirect effects via the production of reactive oxygen species (ROS)[8]. UVAR mainly has indirect effects via energy transfer from UVAR stimulated chromophores to the DNA target, or via the photosensitized production of ROS[9,10]. In addition, UVAR induces direct damage to PS II via the same mechanism as UVBR does[11]. Growth and biomass accumulation will result from the complex interactions between direct and indirect harmful effects of UVR, and a series of counteracting repair mechanisms[12]. UVBR are more effective per energy unit[13], however, UVAR is responsible for most of the UVR damage just because its natural levels are much higher[14,15]. In addition, the generalization of UVR effects on cyanobacteria is complex, considering that the responses are specie-specific[16].

The responses of cyanobacteria to UVR effects could include the generation of ROS. In all aerobically living organisms, respiration is thought to be a source of ROS produced inside

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68 the cells. In addition to ROS produced by the respiratory machinery, photosynthetic
69 organisms are challenged by ROS generated by the photosynthetic electron transport
70 chain. Light is essential for photosynthesis, but, at the same time, it can also be a source
71 of major stress. The fact that cyanobacteria constantly produce oxygen under illumination
72 makes it crucial for them to prevent electron escape from normal electron transfer
73 pathways to oxygen, in order to avoid oxidative stress as much as possible. The chemistry
74 of oxygen species is well documented[17]. ROS, including singlet oxygen ($^1O_2$), superoxide
75 anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), are powerful oxidizing
76 agents. Singlet oxygen ($^1O_2$) is produced by energy input to oxygen; it is highly reactive, it
77 has a short half-life in cells[18] and it reacts with target molecules (proteins, pigments, and
78 lipids) in the immediate surroundings. The three oxygen reduction-intermediates (O_2^-,
79 H_2O_2, and OH^-) have different intrinsic features, and therefore possess different reactivity,
80 toxicity levels and targets. Both O_2^- and OH^- have one unpaired electron each that renders
81 them highly reactive with biomolecules. Quian*et al.*[19] showed, using the oxidation of 2´,7´-
82 dichlorofluorescein diacetate (DCFH-DA, general index of oxidative stress), that higher
83 ROS levels destroy the pigment synthesis and the membrane integrity, producing the
84 death of *Microcystis aeruginosa*.
85 On the other hand, cyanobacteria are the oldest autotrophic inhabitants of the planet, and,
86 at some point, they may have been exposed to high UVR levels[20,1]. Therefore, they must
87 have developed effective mechanisms to counteract the detrimental effects of these highly
88 energetic wavelengths. While some of these defenses are enzymatic [catalase (CAT),
89 superoxide dismutase (SOD) and peroxidases], others are non-enzymatic (glutathione, α-
90 tocopherol, β-carotene)[12,17]. When the balance between oxidant levels and antioxidant
91 production is lost, the organisms have to face an oxidative stress that generates a variety
92 of damages[21].
93 Cyanobacteria blooms are recognized as major health risks considering that some
94 cyanobacteria strains produce a wide range of toxins, including neurotoxins, and
95 hepatotoxins, such as microcystins (MCs). Cellular MCs production has been indirectly
96 linked to environmental factors influencing cyanobacterial growth rates[22], which can
97 account for a 3–4 fold variation in total MCs concentrations[23]. In addition, the action
98 mechanisms and the ecophysiological toxin role remain unclear[24,25]. For such reason, the
99 understanding of the environmental factors associated with MCs production is a priority to
100 predict toxic events in nature[26]. Little information is found in the literature regarding the
101 physiological effects and MCs production by exposure of *M. aeruginosa* to increase of

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102 solar UVR doses.Recent experimental data indicate that variations in MCs concentrations
103 were modified under stress conditions[27,28].
104
105 The objective of the present study was determine the activation of different *in vivo*
106 enzymatic antioxidants (CAT and SOD) as a function of solar UVR intensity and quality as
107 well as the consequent ROS increment with higher UVR doses in short term (hours)
108 exposure. We hypothesize that on increasing UVR doses the enzymatic antioxidants
109 protection will not be the same for UVA or UVB irradiance according with the differences in
110 the prevalence of different ROS generated. In addition, we related this differential
111 protection with toxin synthesis, evaluating their effects on growth rate and photosynthesis.
112
113 **MATERIALS AND METHODS**
114 **Experimental set-up**
115 The experiments were performed using *M. aeruginosa* (strain CAAT 2005-3) wild-type
116 strain, isolated from a water body located in the Province of Buenos Aires, Argentina[29].
117 The unialgal cultures were grown in liquid BG-11[30] at 26 ºC. For experiments, we used
118 cells from cultures in the exponential growth phase. In order to avoid cell damage as
119 consequences of changes in irradiance from inside incubator to solar exposure, the *M.*
120 *aeruginosa* culture were pre-adapted to PAR irradiance in an outdoor water bath with
121 running water for temperature control (26ºC ±1) in containers cover with UV cut-off filter
122 than expose the cells to only PAR irradiance (see the treatment “3”), for 1 day previous to
123 the experimental day. After this period, cells were exposed simultaneously to three
124 irradiance treatments:
125 (1) cultures that received full radiation (UVBR, UVAR and PAR)—uncovered quartz tubes
126 (TUVR treatment);
127 (2) cultures that received UVAR and PAR—tubes covered with UV cut-off filter foil
128 (Montagefolie N◦ 10155099, Folex, Germany: 50% transmission at 320 nm) (TUVA
129 treatment); and
130 (3) cultures that received only PAR—containers covered with Ultraphan film (UV Opak,
131 Digefra, Munich, Germany—50% transmission at 395 nm) (PAR treatment).
132 The spectra of the materials used in our experiments are published in Hernando
133 andFerreyra[31].
134

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135 In order to determine the UVBR effects it was calculated as the difference between TUVR
136 and TUVA values for each parameter (Biomass, ROS, CAT and SOD).
137 *M. aeruginosa* culture was exposed to natural sunlight at Buenos Aires (34º 35´S; 58º 22´
138 W) during spring and summer 2014/2015, in an outdoor water bath with running water for
139 temperature control. In order to expose the cells to maximum solar radiation doses, two of
140 the experiments were performed on sunny days, between March 16 and 19, 2015, at the
141 Universidad Nacional de Chilecito (29º 9´S; 67º 28´W, La Rioja, Argentina). Three
142 replicate samples were used for each of the treatments and controls.
143 Intensities and UVR doses to which the unicellular *M. aeruginosa* cells were exposed are
144 common in temperate latitudes. The UVBR doses ranged from 41 to 75 kJ m^{-2}. The overall
145 UVR + PAR doses during the incubation experiments ranged from 9700 to 11200 kJ m^{-2}.
146 Incident solar radiation was monitored continuously during the experiment using a
147 radiometer (model BIC 250, Biospherical Instruments, Inc.), which records irradiances at
148 three wavelengths in the ultraviolet region (305, 320 and 380 nm, approx. 10 nm
149 bandwidth). This radiometer was calibrated against the reference instrument (RGUV)[32] and
150 inter-compared with the GUV 511 sited at INGEBI (Buenos Aires during the experiments).
151 Data were recorded every minute at a site located close to the experimental setup. The
152 equation from Orce and Helbling[33] as used for calculating UVBR doses expressed in kJ m^{-}
153 ^{2}. UVAR and PAR irradiance were monitored continuously using a spectroradiometer
154 (model ILT 950, International Light Technologies, Inc, USA). On it, data (provided in µW
155 cm^{-2} s^{-1}) were recorded every minute at a site next to the BIC 250 radiometer. The
156 calibration was done by International light technologies some weeks before to start the
157 experiments and intercalibrated with GUV 511 from INGEBI during the experiments.
158
159 **Sampling and samples analyses**
160 The experiments started at 9 h. The aliquots of culture samples at time 0 and after the
161 incubation time (8-9h) were taken and the following determinations were made:
162 Chlorophyll *a* (Chl*a*) analyses, cells counts, ROS detection, CAT activity, MCs and
163 photosynthesis measurements.
164 At initial time and after the incubation period of solar radiation exposure, aliquot samples
165 (3 ml) for cells counts were taken, kept in dark bottles and fixed with formalin previously
166 neutralized with sodium borate (final concentration 0.4% w/v). In addition, aliquot samples
167 (15 ml) for 2-7- dichlorodihydrofluorescein diacetate (DCF-DA) oxidation rate analysis,
168 used for *in vivo* ROS detection, as well as Chl*a* (15 ml), MCs (40 ml), SOD activity (15 ml)

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169 and CAT activity (15 ml), were filtered using a GF/F fiber glass filter. Those destined to *in*
170 *vivo* measurements (DCF-DA) were evaluated immediately and those destined to measure
171 MCs (40 ml), Chl*a*, MCs, CAT and SOD activity (see above) were kept at -20ºC until
172 analysis.
173
174 **Chl*a* analyses and cell counts**.
175 Pre-filtered Chl*a* samples were extracted using 4 ml absolute methanol. Absorbance
176 readings of the extracts (24 h later) were used to calculate Chl*a* concentration, after
177 correction of phaeopigments[34] and calibration with standard Chl*a* with a PG
178 spectrophotometer (model P11)[35]. For enumeration of cyanobacteria, cells were analysed
179 with a phase contrast Olympus inverted microscope, according to the procedures
180 described by Villafañe and Reid[36] using a Sedgwick-Rafter counting chamber. In order to
181 separate the colonies into single cells, samples were previously sonicated (approximately
182 10 W; 30 sec) using an ultrasonic homogenizer (US50; Nissei Co., Tokyo, Japan).
183
184 **DCFH-DA oxidation rate**
185 The membrane-permeable non-fluorescent DCFH-DA oxidation has been used for
186 detecting several ROS in biological media[37]. DCFH-DA was initially thought to be useful
187 as a specific indicator of H_2O_2. However, it has already been demonstrated that H_2DCF is
188 oxidized by other ROS, including superoxide anion radical, hydroxyl radical, peroxyl,
189 alkoxyl, hydroperoxyl and peroxynitrite, which are products of normal metabolism[38].
190 DCFH-DA is a fluorogenic probe which passes through cell walls and membranes and is
191 cleaved by cellular esterases. During incubation, DCFH-DA is hydrolysed, by means of
192 intracellular hydrolytic deacetylation, to H_2DCF, which is trapped inside the cell due to its
193 polarity. This substance is then rapidly oxidized to the highly fluorescent compound DCF
194 that allows the evaluation of cellular toxicity.
195 *M. aeruginosa* cells obtained from filtered samples (14 ml filtered on GF/F filters) were
196 incubated *in vivo* in the dark for 30 min in 2 ml of 40 mM Tris-HCl buffer (pH 7.0), in the
197 presence of 5 μM DCFH-DA at 27°C[37]. Fluorescence in the supernatant (without cells) was
198 monitored in a microplate reader (Beckman counter DTX 880, Multimode Detectors) with
199 excitation (λ_{ex}) at 498 nm and emission (λ_{em}) at 525 nm. In all cases, parallel blank controls
200 were included.
201
202 **CAT and SOD activity**

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203 For the CAT activity, cells harvested in GF/F filters were suspended in 5 ml of ice-cold 0.1
204 M potassium phosphate buffer pH 7.0, sonicated in an ice-water bath and clarified by
205 centrifugation 10000 g for 10 min at 4ºC. CAT activity was evaluated as the decomposition
206 rate of hydrogen peroxide (H_2O_2) at 240 nm at 25 ºC[39]. One unit of CAT was defined as
207 the amount of enzyme catalyzing the elimination of 1 mM H_2O_2 per minute.
208 For the SOD activity, cells extracts were obtained using the same process as that used for
209 the CAT assay. The SOD activity was measured using a SOD assay kit (Cayman
210 Chemicals). One unit is defined as the amount of enzyme needed to exhibit 50% of
211 dismutation of the superoxide radical.
212

213 **Photosynthesis measurements**

214 Subsamples of the cultured cyanobacteria (100 ml) were placed in independent (not used
215 for ROS, Chl*a*, MCs, SOD or CAT analysis) experimental quartz tubes (with teflon-lined
216 screw caps), and 0.04 ml of 50 μCi ^{14}C-bicarbonate were added to each tube following
217 SteemanNielsen[40]. Tubes were then placed horizontally on black anodized aluminum
218 frames and exposed to solar radiation in a water bath (25-26ºC, described in experimental
219 set-up).
220 After the incubation period, the samples were filtered through Whatman GF/F glass fiber
221 filters (25 mm), the filters placed in scintillation vials, exposed to HCl fumes for 3-4 h, and
222 dried overnight in a vented hood. Readings of ^{14}C incorporated by cyanobacteria were
223 carried out by liquid scintillation counting using a Packard Liquid Scintillation Analyzer
224 Model 1600 TR (Canberra, Australia). The scintillation cocktail used was OptiPhase
225 "Hisafe" 3, LKB Wallac. The rates of carbon fixation were expressed as Assimilation
226 Numbers (mg C mg Chl-a^{-1} h^{-1}).
227

228 **MCs HPLC analysis**

229 Cells samples (15 ml) were broken by 3 frozen–unfrozen cycles followed by 30 min
230 ultrasonication (Omni ruptor 400), then were centrifuged for 15 min at 5000 r/min to
231 eliminate cell debris. The supernatant was passed through conditioned (10 ml 100%
232 methanol, 50 mL 100% distilled water) Sep-Pak C18 cartridges (Waters). The MCs were
233 eluted with 80% methanol. Quantitative chromatographic analysis of MCs was performed
234 by HPLC with a photodiode array detector (LC- 20A, SPD-M20A, Shimadzu Scientific
235 Instruments, Columbia, MD, USA) and C_{18} column (Thermo ODS-Hypersil, 150 × 4.60mm,
236 5μm). The column was equilibrated with a mixture composed by 65% of A solution [water

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237 with 0.05% (v/v) trifluoroacetic acid] and 35% of B solution [acetonitrile with 0.05% (v/v)
238 trifluoroacetic acid]. The mobile phase consisted of a discontinuous gradient of A and B
239 solutions. The flow rate was 1.0 ml/min. MCs were identified on the basis of their UV
240 spectra and retention time. Standard of MC-LR was purchased from Sigma (St Louis, MO,
241 USA).
242
243 **Effect of UVR on different physiological parameters of *M. aeruginosa***
244 The relative photosynthesis variation (RPV) and the relative variation in biomass
245 (RBiomassV) due to UVR were calculated as follows:
246
247 RPV, $RBiomassV_{UVAR}$ (%)= (PAR-TUVA)*100/PAR
248 RPV, $RBiomassV_{UVBR}$ (%)= (TUVA-TUVR)*100/PAR
249
250 The variation in ROS concentration (RROSV) as well as variation on SOD and CAT
251 activity (RSODV and RCATV, respectively) were calculated as follows:
252
253 RROSV, RSODV, $RCATV_{UVAR}$ (%)= (TUVA-PAR)*100/PAR
254 RROSV, RSODV, $RCATV_{UVBR}$ (%)= (TUVR-TUVA)*100/PAR
255
256 where PAR, TUVA and TUVB denote the measurements of the respective parameter
257 under each of the irradiance treatment.
258
259 **Statistical analyses**
260 One–way ANOVA analyses and then a Tukey test were performed (Statistica, version 9) to
261 determine the significance of the differences observed between treatments for each
262 parameter values during experiments at different solar irradiance. Normality was verified
263 using a Kolmogorov-Smirnov test[41].
264
265 **RESULTS**
266 ***M. aeruginosa* biomass and abundance**
267 The initial number of the cells was, on average, of 8 x 10^5 cell ml^{-1}. The effect of solar
268 radiation on biomass of *M. aeruginosa* was evaluated at the end of the experiment.
269 The effect of UVBR in RPV and RBiomassV was estimated from the difference between
270 UVAR+PAR and UVR+PAR. For experimental UVBR doses lower than 65kJ m^{-2}

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271 (UVR+PAR doses of 9776 kJm^{-2}) there were no differences in biomass (cell ml^{-1}) between
272 treatments ($p > 0.05$). For UVBR doses between 65.7and 67.9kJ m^{-2} (UVR+PAR doses
273 between 9875 and 10275 kJ m^{-2}), there were significant differences between treatments (p
274 < 0.05) (**Figure 1 A**).
275 The effect of the UVBR on the cells count was evaluated using an RBiomassV index and
276 determining the difference between TUVR and TUVA as was defined in *Materials and*
277 *Methods*. There were no differences in cells number between TUVR and TUVA for UVB
278 doses lower than 65 kJ m^{-2} (**Figure 1 B**). For UVB doses between 65 and 67.9 kJ m^{-2},
279 there were no UVBR inhibition (**Figure 1 B**) because not significant differences were found
280 ($p > 0.05$) between TUVA and TUVR treatment (**Figure 1 A**). The UVAR inhibition for such
281 UVB doses interval was in average 65%. Such results clearly show a decrease in biomass
282 by exposure to UVAR and no effects for UVBR. For UVBR doses higher than 70.8 kJ m^{-2}
283 (UVR+PAR doses of 10674 kJ m^{-2}) significant differences were observed between the
284 three radiation treatments ($p < 0.01$) (**Figure 1 A**). The percentage of relative UVA
285 inhibition was similar in average to those calculated for intermediate UVR doses, however,
286 the UVBR inhibition was in average of 20% (**Figure 1 B**). Such results show that the
287 UVBR as well as UVAR are diminishing the *M. aeruginosa* biomass and that it was not
288 possible to determine a dose-dependent effect within that range of irradiances. However, it
289 was observed a significant decreased in biomass inhibition for both UVBR and UVAR in
290 exposure to highest doses (**Figure 1 B**).
291
292 **ROS**
293 For experimental UVBR doses lower than 65 kJ m^{-2} (UVR+PAR doses of 9776 kJm^{-2}),
294 there were no differences in cellular ROS concentration between treatments ($p > 0.05$).
295 For UVBR doses between 65.7 and 67.9 kJ m^{-2} (UVR+PAR doses between 9875 and
296 10275 kJ m^{-2}), there were significant differences between treatments ($p < 0.01$), being
297 ROS concentrations significantly higher in UVA treatments compared with PAR (**Figure 2**
298 **A**). Such increment of ROS concentrations as consequence of exposure to UVAR, reach a
299 maximum of 390% in average (RROSV) at UVR+PAR doses of 9975 kJ m^{-2}. When the
300 UVBR effect was null or negative, the ROS concentration was higher in the TUVA
301 compared to the TUVR treatment (**Figure 2 B**). For UVBR doses higher than 70.8 kJ m^{-2}
302 (UVR+PAR doses of 10674 kJ m^{-2}), significant differences were observed between the
303 three radiation treatments ($p < 0.01$) (**Figure 2 A**). However, the UVAR produced a higher
304 RROSV (290%) compared with UVBR (200%). It was observed a lower ROS increment of

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305 both UVAR and UVBR at maximum exposure doses, compared with lower doses. Not
306 differences were found between both treatments (around 110%) (**Figure 2 B**).
307
308 **SOD activity**
309 For experimental UVBR doses lower than 65 kJ m^{-2} (UVR+PAR doses of 9776 kJm^{-2}),
310 there were no differences in SOD activity between treatments ($p > 0.05$). For UVBR doses
311 between 65.7 and 67.9 kJ m^{-2} (UVR+PAR doses between 9875 and 10275 kJ m^{-2}), there
312 were significant differences between treatments ($p < 0.05$) (**Figure 3 A**). The SOD activity
313 was significantly higher in cells exposed to UVAR compared with PAR ($p < 0.01$) reaching
314 a maximum increment (RSODV) of 620% in average at UVR+PAR doses of 10075 kJ m^{-2}.
315 In cells exposed to UVBR there was a negative increment, which means that the SOD
316 activity was lower in TUVR (probably due to a consumption in presence of UVBR)
317 compared with TUVA treatment (**Figure 3 B**). For UVBR doses higher than 70.8 kJ m^{-2}
318 (UVR+PAR doses of 10674 kJ m^{-2}), significant differences were observed only for TUVA
319 treatment compared to TUVR or PAR ($p < 0.01$) (**Figure 3 A**). The SOD activity was higher
320 in cell exposed to UVAR reaching a maximum of 300% (RSODV) for UVR+PAR doses of
321 10674 kJ m^{-2} (**Figure 3 B**).
322
323 **CAT activity**
324 For experimental UVBR doses lower than 65 kJ m^{-2} (UVR+PAR doses of 9776 kJm^{-2}),
325 there were no differences in CAT activity between treatments ($p > 0.05$). For UVBR doses
326 between 65.7 and 67.9 kJ m^{-2} (UVR+PAR doses between 9875 and 10275 kJ m^{-2}), there
327 were significant differences between treatments ($p < 0.05$) (**Figure 4 A**). The CAT activity
328 was only increased in cells exposed to UVAR reaching a maximum of 70 % in average
329 (RCATV) at UVR+PAR doses of 10275 kJ m^{-2} (**Figure 4 B**). Such results clearly show an
330 increased CAT activity by exposure to UVAR and no effects of UVBR. For UVBR doses
331 higher than 70.8 kJ m^{-2} (UVR+PAR doses of 10674 kJ m^{-2}), significant differences were
332 observed between the three radiation treatments (**Figure 4 A**). Cells exposed to UVBR
333 showed the maximum CAT activity reaching in average an increment of 110 % (RCATV)
334 for UVBR doses of 70.8 and 72.2 kJ m^{-2} and decreasing for the higher experimental
335 doses. In cells exposed to UVAR, however, the trend was to an increased activity with
336 higher irradiance, with a maximum at UVR+PAR doses of 11173 kJ m^{-2} an average of 80%
337 of increment in CAT activity (**Figure 4 B**).
338

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339 **Photosynthesis**

340 At 65.7and 67.9 kJ m^{-2}UVBR doses, no significant differences between TUVR and TUVA
341 treatments were found for the assimilation number; however, in both treatments, they were
342 significantly lower compared to PAR (**Figure 5 A**). Consequently, UVBR photosynthesis
343 inhibition was determined (**Figure 5 B**). At higher UVR doses, the assimilation number
344 was significantly lower in both UVR treatments compared to PAR, but it is lower in UVB
345 than in UVA treatments (**Figure 5 A**). The RPV was increased, being higher at 72.2 and
346 74.4 kJ m^{-2} UVBR doses with 25% inhibition of the photosynthetic rate in average (**Figure**
347 **5 B**). The photosynthesis inhibition produced by UVAR was higher compared to that
348 produced by UVBR corresponding to a 50% in average from 66.4 to 74.4 kJ m^{-2} of UVBR
349 doses (**Figure 5 B**).

350

351 **MCs concentration**

352 The most abundant MC was [Leu[1]] MC-LR. It was expressed as toxin per cell (quota $Q_{[Leu1]\ MC\text{-}LR}$)
353 with levels between 0.1 and 80 fg.cell^{-1} (expressed as MC-LR equivalent) after the
354 incubation period in different experiments analyzed. With UVBR doses between 41 and 73
355 kJ m^{-2}, significant differences between treatments were not found. However, a decreased
356 quota trend for cells exposed to TUVA treatments was observed. $Q_{[Leu1]\ MC\text{-}LR}$ decreased
357 significantly ($p < 0.05$) in cells exposed to TUVR and TUVA treatment compared to control
358 (**Figure 6**) at UVBR doses of 74.4 kJ m^{-2}.

359

360 **DISCUSSION**

361 The response of organisms including cyanobacteria to stress, is the production of ROS[8,42],
362 being the photosynthetic process an important source of ROS for photosynthetic
363 organisms[43]. We determine a UVBR threshold doses of 67.9 kJ m^{-2} (UVR+PAR doses of
364 10275 kJ m^{-2}), below which no significant increment in ROS nor significant biomass
365 decreased were observed in *M. aeruginosa*. No cell damage in TUVB treatment for low
366 and moderate UVBR doses may be attributable to the highly efficient repair of DNA lesions
367 in cyanobacteria in the presence of UVAR and PAR[44]. UVBR doses of 108 kJ m^{-2} induced
368 a significant increase of ROS as compared with lower doses with maximum concentration
369 at 647 kJ m^{-2} in *Anabaena* sp.[8]. An important clarification to be considered is that the
370 relatively low cell concentration used in our experiments avoids a considerable self-
371 shading of the cells exposed to UVR.

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372 For UVBR doses higher than 67.9 kJ m^{-2} (UVR+PAR doses of 10275 kJ m^{-2}), the ROS
373 content increased significantly in cells exposed to UVBR and UVAR, producing a
374 significant biomass decrease. The main reason for the quick dissolution of cells exposed
375 to high UVR doses is most likely a result of acute physiological stress and chronic
376 depression of key physiological processes that resulted in rapid cellular necrosis[45]. A
377 similar observation was reported by Singh et al.[42], who applied high UVBR irradiance and
378 found significantly decreased biomass accumulation in *Phormidium foveolarum* and
379 *Nostoc muscorum* by inhibiting the process of photosynthesis caused by UV-induced
380 formation of ROS[18,9]. ROS may act as a signal and/or secondary messenger enabling
381 cyanobacteria to regulate the expression of a number of genes, resulting in a protection
382 from environmental stresses, especially UV irradiance[9,46]. A decrease in cell number of *M.*
383 *aeruginosa* was reported after the exposure to 11 kJ m^{-2} of UVBR[45], however in such
384 experiments, the cells were not exposure to UVAR nor PAR during the incubation with
385 UVBR avoiding the possible repairing processes by UVAR and PAR[44,45,46]. In addition,
386 growth inhibition was shown as a consequence of UVBR exposure in *M. aeruginosa*, using
387 long incubation periods (10 days) and cumulative doses of 146 and 210 kJ m^{-2}, without
388 effects using cumulative doses of 75 kJ m^{-2} [47].
389 Living organisms have developed several defenses to protect themselves against ROS
390 damage[18]. While some of these defenses are enzymatic (CAT, SOD and peroxidases)[50],
391 others are non-enzymatic (glutathione, vitamin A, C, E, carotenoids, etc.), and further they
392 may repair DNA damage[51]. When the balance between oxidant levels and antioxidant
393 production is lost, the organisms have to face an oxidative stress that generates a variety
394 of damages.
395 H_2O_2, $O_2^{\cdot -}$ and lipid hydroperoxides are considered to be suitable signaling species for
396 initiating functional modulation avoiding disruption of cellular integrity, which allows
397 organisms to adapt to the stress conditions[52]. H_2O_2 is the most stable ROS and, to avoid
398 damage caused by them in cyanobacteria, these have evolved various enzymes that are
399 able to detoxify this compound. CAT is one of the most-studied enzymes that exclusively
400 dismutate H_2O_2[53]. In our experiments, CAT activity increased for cells exposed to UVAR,
401 with UVR+PAR doses between 9776 and 10275 kJ m^{-2}, as well as for exposure to UVBR
402 and UVAR with UVBR doses higher than 67.9 kJ m^{-2} (UVR+PAR doses of 10275 kJ m^{-2}).
403 In fact, the maximum increment in CAT activity for cells exposed to UVAR was coincident
404 with a low inhibition of biomass and a significant decreased in ROS concentration for
405 UVAR and UVBR in maximum experimental UVR doses. One of the possible reasons for

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406 the increased CAT activity in cells exposed to UVAR for intermediate UVR doses
407 (UVR+PAR doses between 9875 and 10275 kJ m^{-2}) is the activity of SOD.
408 Disproportionation of O_2^- to H_2O_2 and oxygen is catalyzed by SOD, and the dismutation of
409 H_2O_2 to oxygen and water is catalyzed by CAT. SOD activity was elevated only for the
410 exposure to UVAR when cells were exposed to UVR+PAR doses higher than 9776 kJ m^{-2}
411 (UVBR doses higher than 65 kJ m^{-2}). Low SOD activity or consumption of it was observed
412 for the cells exposure to intermediate UVBR doses but no significant differences were
413 observed at high UVBR doses. Consequently, this could mean that there was no O_2^{-}
414 $^{\cdot}$production for the exposure to UVBR at high UVBR doses. Another possible explanation
415 for the lack of increased SOD activity in UVBR exposure could be the $O_2^{\cdot-}$ reduction to OH^-
416 via Fenton reaction which could cause highly damaging effects[12]. Such reaction would be
417 produced considering the high Fe concentration from BG11 culture medium. Nomura et
418 al.[55] showed that CAT and peroxidase in *Synechococcus* sp. PCC 7002 mutants and the
419 wild-type, were relatively unaffected compared to the increase in SOD activity. The
420 significantly higher CAT activity in cells exposed to UVBR for doses higher than 67.9 kJ m^-
421 ^{2} shows a high H_2O_2 concentration which could be produced not only by the activity of
422 SOD, but also by an indirect UVBR effect with increased ROS, particularly H_2O_2 content.
423 For intermediate UVBR doses it was observed a SOD consumption, in coincidence with a
424 decrease of ROS, which may preserve cell damage. For higher UVR doses, UVAR exhibit
425 similar effects of decreased biomass, as UVBR did, as indicated by the induction of ROS.
426 Exposure of cyanobacteria to UVAR has been found to cause oxidative stress by
427 producing ROS via photosensitized reactions[9], damaging photosynthetic apparatus,
428 decreasing survival and inhibiting growth[9,55]. In our work, only PAR did not induce
429 oxidative stress or photoinhibition compared to the initial exposure time (data not showed).
430 This is because the PAR dose used in our study was not in excess irradiance.
431 Differential type of response of plant to UVBR was demonstrated, which is dependent on
432 its irradiance[56,57]. High UVBR irradiance produces ROS and may cause damage to DNA,
433 proteins and lipids, while low irradiance may produce a protective response against other
434 stresses[56]. However, it was reported that low UVBR irradiance can serve as a signal to
435 regulate plant growth and development[58], without damage effects. The lack of UVBR
436 effects for low and moderate doses (between 65.7 and 67.9 kJ m^{-2}) could be explained
437 considering a high enzyme activity determined or an increased *de novo* synthesis of
438 molecular antioxidants such as ascorbic acid and carotenoids (not measured in our study).

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439 There were no significant decrease of biomass or significant increase of ROS cellular
440 content in cells exposed to UVAR at experimental doses lower than 9776 kJ m^{-2}
441 (UVR+PAR). For UVR doses between 9776 and 10275 kJ m^{-2} (UVR+PAR), the UVAR
442 produced a significant decrease in biomass and a higher ROS content compared to PAR
443 treatment.This can be due to the fact that there is a larger amount of UVAR reaching the
444 Earth because it is not influenced by the depletion of stratospheric ozone[59].
445 UVBR is a highly variable environmental signal, and fluctuations in irradiance will probably
446 modulate the level of ROS and photoprotective signaling molecules, as well as the
447 induction of UV-stress proteins[9]. In order to accurately perceive the light environment and
448 prevent damage caused by toxic light exposure, cyanobacteria possess photoreceptors,
449 such as phytochromes, UVAR/blue photosensors and still undefined photoreception
450 systems of mediation responses to UVBR[60]. The differential antioxidant responses and
451 ROS production to UVR observed in our study, could be the result of photoreception
452 systems and other enzymatic or non-enzymatic antioxidants not measured by us.
453 Another consequence of the loss of balance between oxidant levels and antioxidant
454 production is the photosynthesis inhibition. The photosynthesis inhibitory effect was absent
455 for UVBR exposures to low and moderate doses (between 65.7 and 67.2 kJ m^{-2}), despite
456 the fact that, on higher doses, damage had been observed. It appears that the adaptive
457 response of the photosynthetic function occurred and the repair system for photosynthetic
458 damage is efficient when using UVBR doses lower than 65.7 kJ m^{-2}. PS II is very sensitive
459 to changes in the environment and may decline under unfavorable environmental
460 conditions[61]. The extent of the repair of PS II is determined by the rate of synthesis of the
461 D1 protein *de novo*[62]. Suppression of the *de novo* synthesis of the D1 protein by $^1O_2$ and
462 H_2O_2 was demonstrated in *Anabaena* sp[63] and in *Synechocystis*[64]. While UVBR acts
463 directly on DNA and proteins and thus damages them, UVAR is thought to cause long-
464 term photosensitized oxidation[65]. The light-saturated photosynthetic rates of *M. aeruginosa*
465 854, exposed to 15 kJ m^{-2} of UVBR doses, were significantly lower than unexposed
466 controls. This higher sensitivity, compared with that observed in our experiments, may be
467 due to the fact that *M. aeruginosa* 854 has been exposed only to UVB comparing with our
468 exposure to the complete solar spectrum. UVB-induced damage can be repaired by
469 photoreactivation by UVA[42,47] as well as by low and middle regions of PAR[48]. Recently
470 was reported photosynthesis inhibition and increased oxidative stress by exposure of *M.*
471 *aeruginosa* to UVBR long incubation periods (10 days) and accumulative doses of 210 kJ
472 m^{-2} [49]. UVAR inhibition of primary production can be observed in the experiments for all

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473 UVBR doses, reaching an average of around 50%. It was significantly higher than the
474 inhibition produced by UVBR starting at 67.9 kJ m^{-2} doses (UVR+PAR doses of 10275 kJ
475 m^{-2}), of an average 15%. Even though UVBR are more effective per energy unit[13], and,
476 hence, potentially more damaging than those at longer wavelengths, many studies
477 conducted in different locations have showed that UVAR is responsible for most of the
478 photosynthetic inhibition just because its natural levels are much higher[14,15].
479 Another adaptive way to avoid the damage for an increased ROS concentration, is the
480 presence of MCs. There is scientific evidence that under oxidative stress, MC producers,
481 compared with no producers, have a comparative advantage as MC acts as a protein-
482 modulating metabolite and protectant, increasing the fitness of their host[66]. Under our
483 experimental conditions, the short term exposure of *M. aeruginosa* to UVAR produced a
484 significant [Leu[1]] MC-LR decrease ($p < 0.05$) when the UVR+PAR doses reached 11173
485 kJ m^{-2} (UVBR doses of 74.4 kJ m^{-2}). In addition, for such doses there were observed a
486 lower ROS concentration as well as a decreased biomass inhibition. For lower doses, a
487 tendency of decreased MC quota was observed when cells were exposed to UVAR. In
488 other studiers, it was demonstrated that that several environmental factors have influenced
489 the biosynthesis of cyanotoxins for several defined isolates. Kaebernick et al.[67] proposed
490 that the MCs synthetase gene cluster is regulated by light quality, either directly or via
491 another regulatory factor, and that transcription requires different thresholds of light
492 intensity for initiation and upregulation. Dziallas and Grossart[27] and Hernando et al.[21]
493 showed that MCs weaken the detrimental effect of H_2O_2 on *M. aeruginosa* and proposed a
494 function of cyanobacterial toxins as radical scavengers relevant for cyanobacterial growth.
495 Yang and Kong[49] showed a decreased mycD transcription after the exposure of *M.*
496 *aeruginosa* to high UVBR doses (210 kJ m^{-2}) and long period of incubation, whereas lower
497 irradiance stimulated the expression (75 kJ m^{-2} accumulative doses after 10 days of
498 exposure). As more evidence supporting the role of MC related to oxidative stress, several
499 studies showed an increased sensitivity of MC-deficient mutants under high light and
500 oxidative stress conditions[67,68,69]. In addition, Briand et al.[70] observed a decrease in MC cell
501 quota in the late exponential growth phase, probably due to an increase in MC binding to
502 proteins in senescent cultures that are accumulating ROS.
503 Finally, an interesting property of several cyanobacteria is their capacity to overcome UVR
504 damage using UV-absorbing/screening compounds as a third line of defense (not
505 measured in our study). Results from Sommaruga et al.[71] suggest that the bloom-forming
506 *Microsystis* utilizes direct UV sunscreens compounds such as MAAs. However, Jiang and

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507 Qiu[72] were not able to detect the presence of UV-absorbing compounds in *M. aeruginosa*
508 strain 384.
509

510 **CONCLUSION**

511 The cosmopolitan distribution of cyanobacteria shows that they can cope with a wide
512 spectrum of environmental stresses such as heat, cold, desiccation, salinity, nitrogen
513 starvation, photo-oxidation and osmotic stress. During present investigation we have
514 successfully attempted our proposed hypothesis. We found that the antioxidant enzymes
515 were more active with high UVR doses in short term exposure. However, different
516 responses were activated depending on the exposure to UVAR or UVBR and the doses
517 level. No effects were observed on biomass, ROS production or increased activity of SOD
518 and CAT compared to control when UVR+PAR doses were lower than 9875 kJ m^{-2}. For
519 intermediate doses oxidative stress and resistance was imparted through SOD and CAT in
520 cells exposed to UVAR. Despite such responses it was observed biomass decrease and
521 photosynthesis inhibition but no effects were observed with the exposure to UVBR. In
522 highest doses, the solar UVR caused decreased photosynthesis and biomass with the only
523 activation of CAT for UVBR and SOD and CAT for UVAR. In addition, significant
524 decreases of [Leu^1] MC-LR were observed as consequence of UVAR. This was in
525 agreement with the hypothesis of MC as ROS scavenger. The metabolic characteristics of
526 *M. aeruginosa* in solar UVR exposure and the differential enzymatic antioxidant and MC
527 responses, have proven useful in assessing the underlying biochemical mechanisms of
528 UVR damage and acclimation. It´s an evidence of some internal cellular mechanisms in
529 UVR mediated signaling pathways in the context of the UVR perception mechanism. UVR
530 signaling is an important but poorly understood aspect of light responsiveness in
531 cyanobacteria, on the molecular and biological levels. Therefore, further study is
532 necessary to improve our understanding of cellular signaling processes associated with
533 UV-induced cell death and survival strategies in cyanobacteria.
534 Our results open new perspectives on the influence of UV on aquatic ecosystems, and on
535 its impact on population dynamics and photosynthesis.
536
537

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538 **Figure captions**

539 **Figure 1**:

540 A. *M. aeruginosa* biomass (cells μl^{-1}) after the incubation period as a function of solar
541 doses when cells were exposed to: TUVR (UVBR+UVAR+PAR); TUVA
542 (UVAR+PAR) and PAR. Each point represents the mean ± sd. Significant (Tukey
543 test) differences between treatments are marked with * for $p<0.05$.

544 B. Relative biomass variation (RBiomassV) in percentage (%) calculated according to
545 experimental biomass (cell μL^{-1}) as a function of incubation solar doses. RBiomassV
546 indicates the increase in biomass calculated as a percentage of control PAR
547 treatment considered as 100%. The bars show the statistically significant increase
548 in biomass induced by UVAR and/or UVBR denoted in A.

549

550 **Figure 2:**

551 A. ROS (UA $cell^{-1}$) after the incubation period as a function of solar doses when cells
552 were exposed to: TUVR (UVBR+UVAR+PAR); TUVA (UVAR+PAR) and PAR.
553 Each point represent the mean ± sd. Significant (Tukey test) differences between
554 treatments are marked with * for $p <0.05$.

555 B. Relative ROS (RROSV) variation in percentage (%), calculated according to
556 experimental ROS concentration as a function of incubation solar doses. RROSV
557 indicates the increase in ROS calculated as a percentage of control PAR treatment
558 considered as 100%. The bars show the statistically significant increase in ROS
559 induced by UVAR and/or UVBR denoted in A.

560

561 **Figure 3**:

562 A. SOD activity (UA $cells^{-1}$) after the incubation period as a function of solar doses
563 when cells were exposed to: TUVR (UVBR+UVAR+PAR); TUVA (UVAR+PAR) and
564 PAR. Each point represent the mean ± sd. Significant (Tukey test) differences
565 between treatments are marked with * for $p <0.05$.

566 B. Relative SOD variation (RSODV) in percentage (%) calculated according to
567 experimental SOD activity as a function of incubation solar doses. RSODV
568 indicates the increase in SOD calculated as a percentage of control PAR treatment
569 considered as 100%. The bars show the statistically significant increase in SOD
570 induced by UVAR and/or UVBR denoted in A.

571

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572 **Figure 4:**

573 A. CAT activity (UA $cell^{-1}$) after the incubation period as a function of solar doses
574 when cells were exposed to: TUVR (UVBR+UVAR+PAR); TUVA (UVAR+PAR)
575 and PAR. Each point represent the mean ± sd. Significant (Tukey test)
576 differences between treatments are marked with * for $p < 0.05$.

577 B. Relative CAT variation (RCATV) in percentage (%) calculated according to
578 experimental CAT activity as a function of incubation solar doses. RCATV
579 indicates the increase in CAT calculated as a percentage of control PAR
580 treatment considered as 100%. The bars show the statistically significant
581 increase in CAT induced by UVAR and/or UVBR denoted in A.

582

583 **Figure 5**:

584 A. Assimilation numbers (mg C mg Chla^{-1} h^{-1}) after incubation period as a function
585 of solar doses when cells were exposed to: TUVR (UVBR+UVAR+PAR); TUVA
586 (UVAR+PAR) and PAR. Each point represent the mean ± sd. Significant
587 (Tukey test) differences between treatments are marked with * for $p < 0.05$ and
588 ** for $p < 0.01$.

589 B. The bars show the statistically significant relative photosynthesis inhibition (%)
590 induced by UVAR and/or UVBR as a function of incubation doses denoted in
591 A.

592

593 **Figure 6:**

594 Effect of solar experimental doses after the incubation period on Quota [Leu^1]MC-LR (fg
595 $cell^{-1}$) in each experimental treatment. Each bar represents the mean ± sd. The same
596 level of horizontal bars for each UVBR doses show not significantly differences at p
597 <0.05 level by Tukey test.

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605

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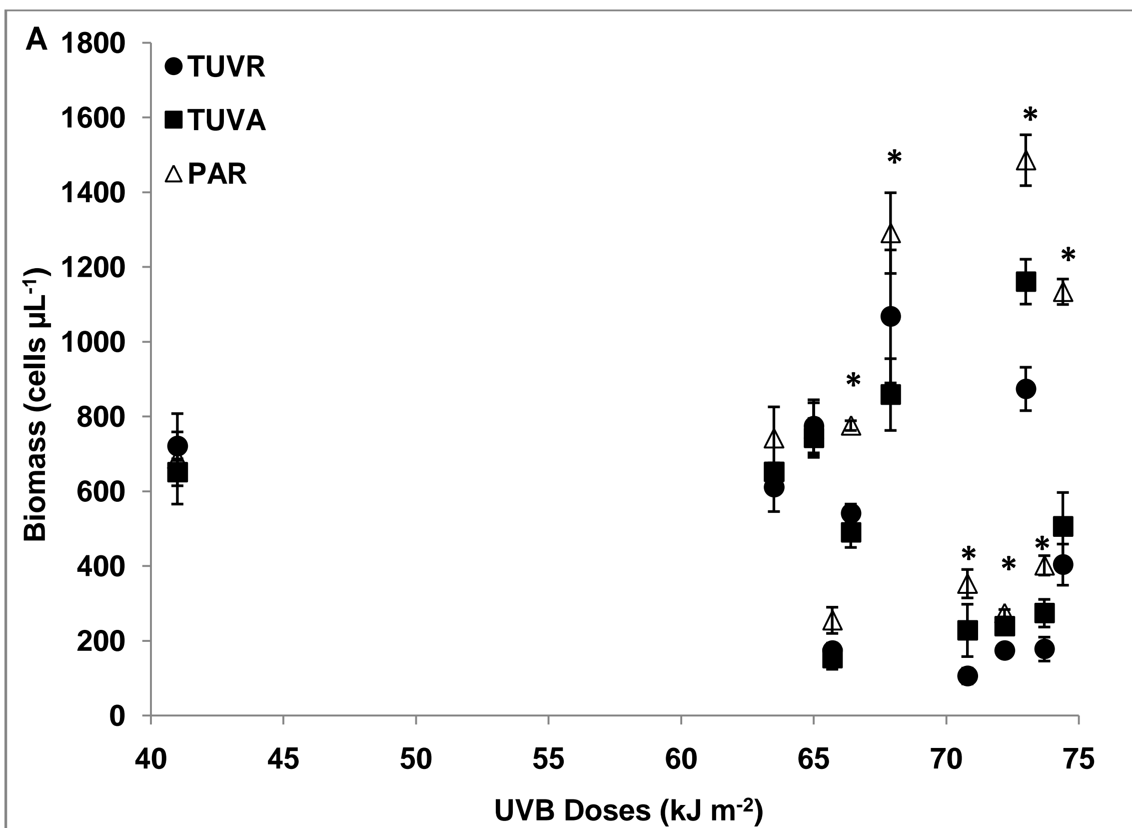

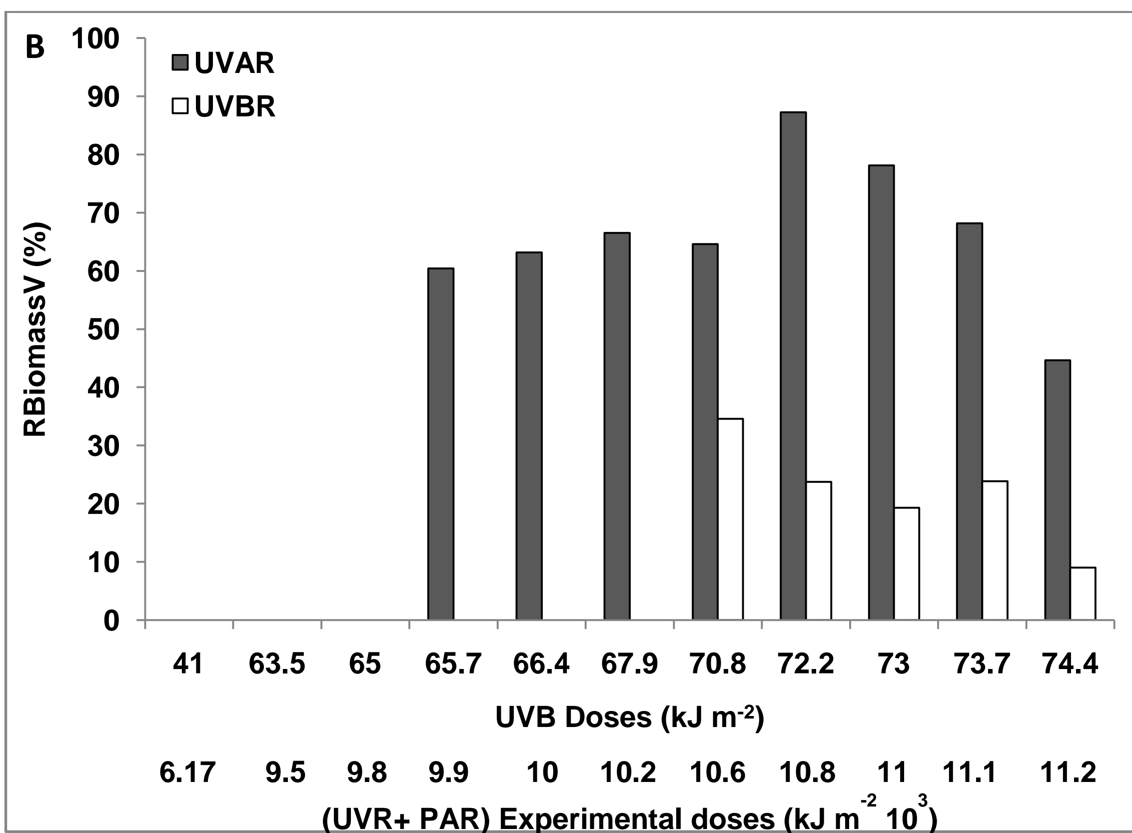

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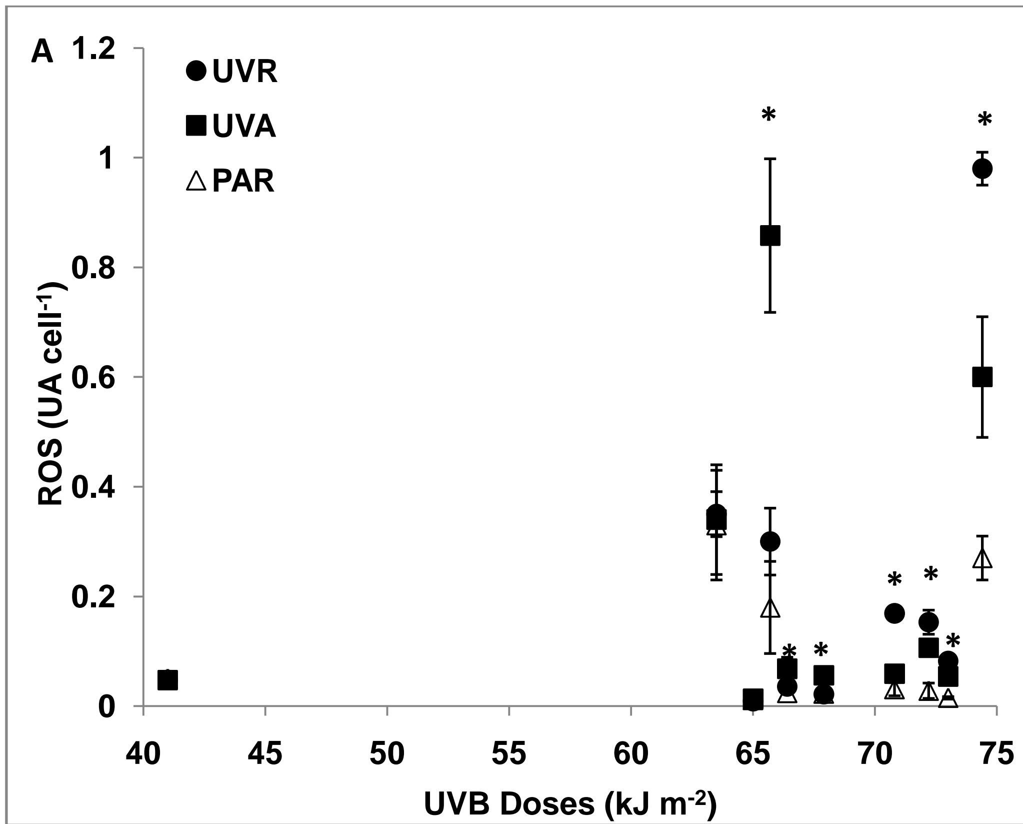

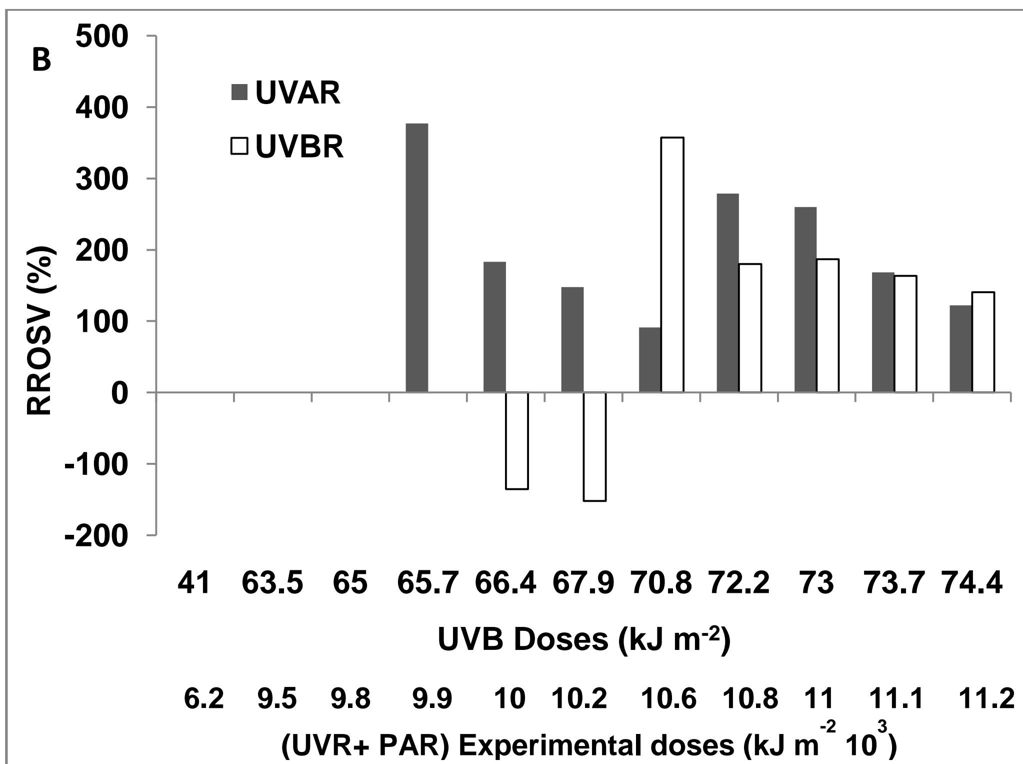

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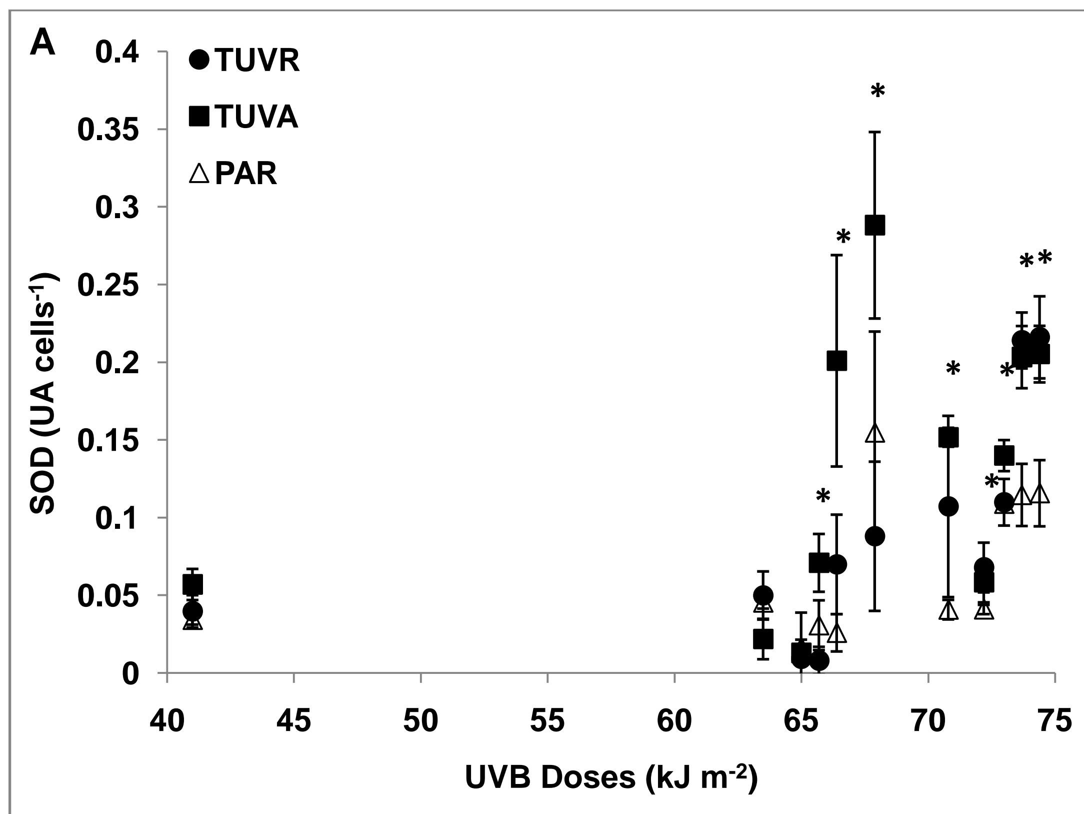

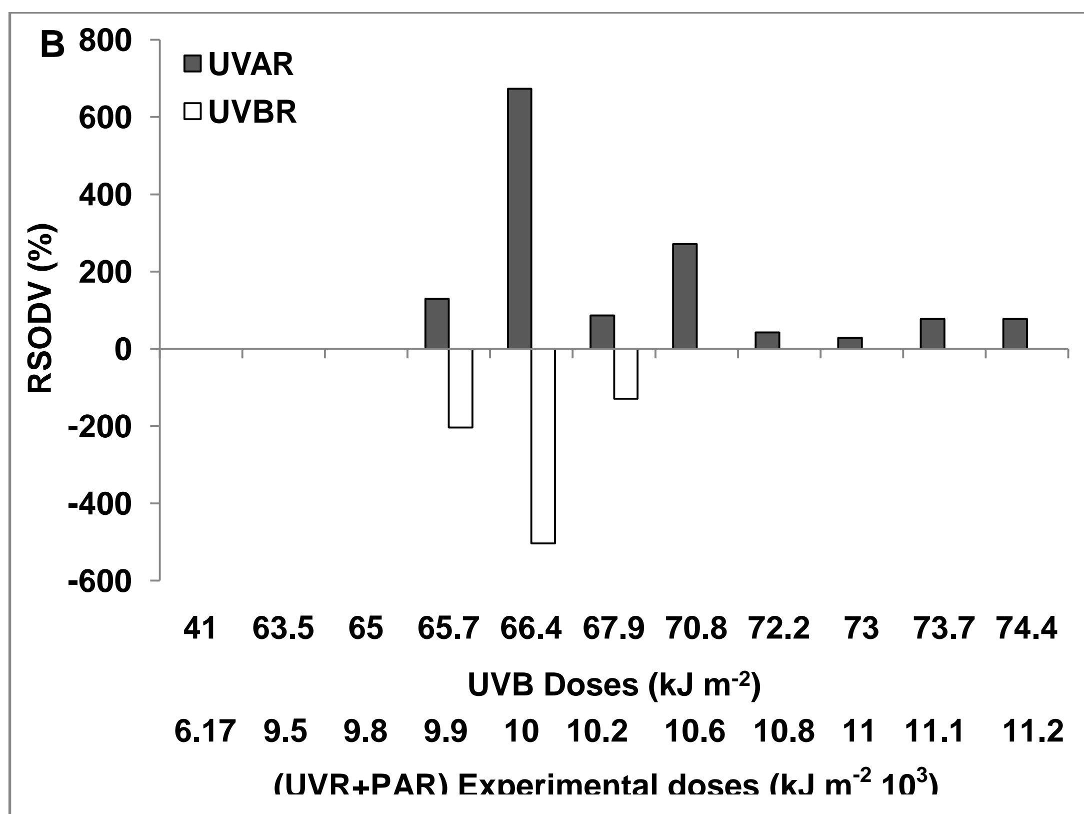

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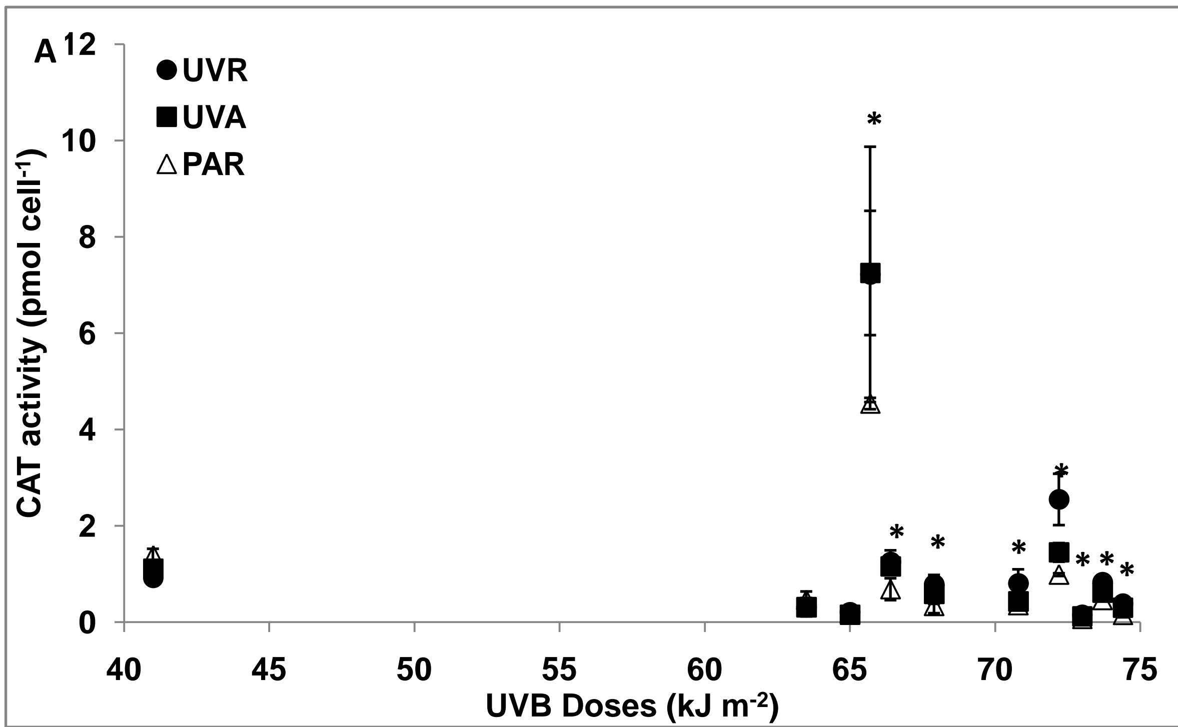

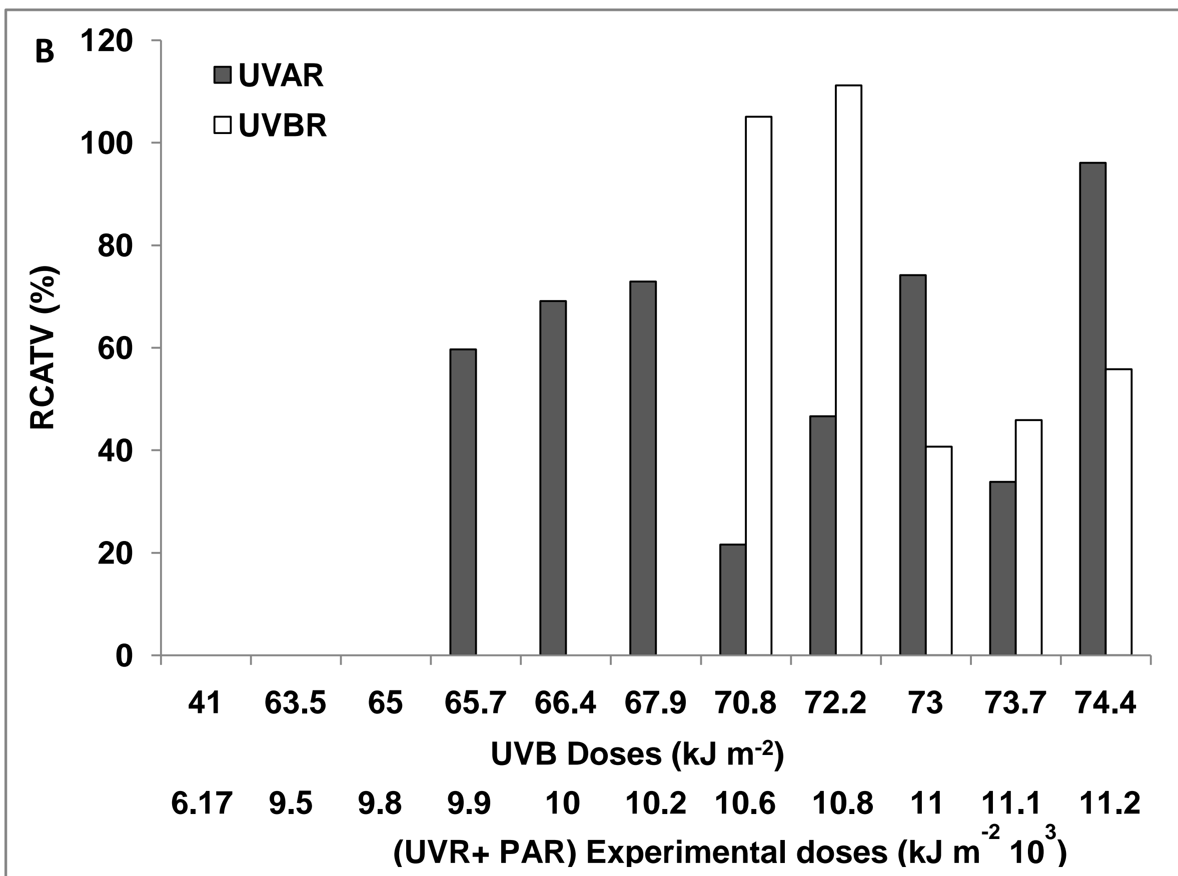

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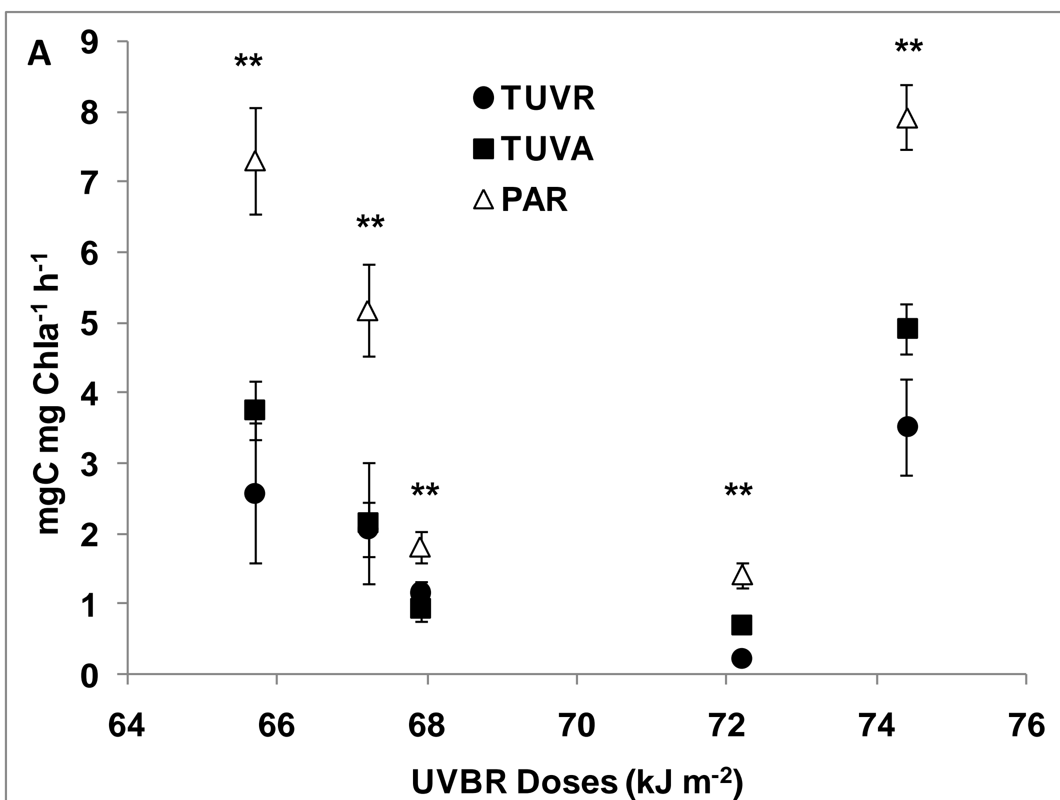

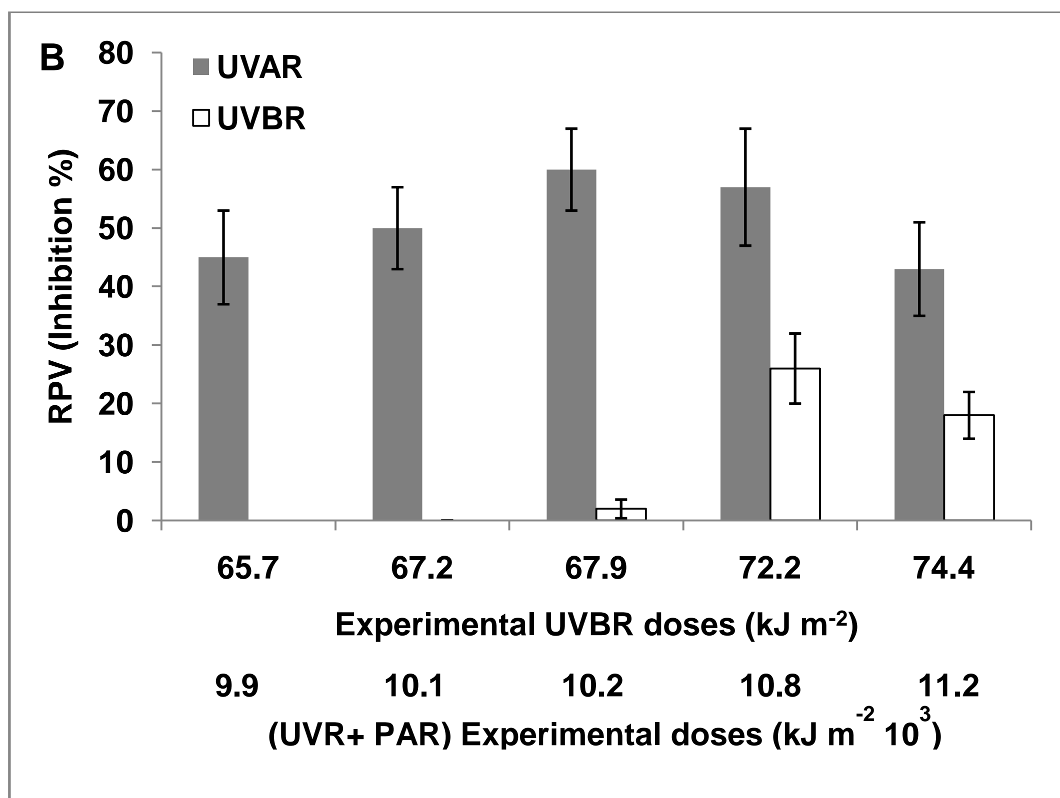

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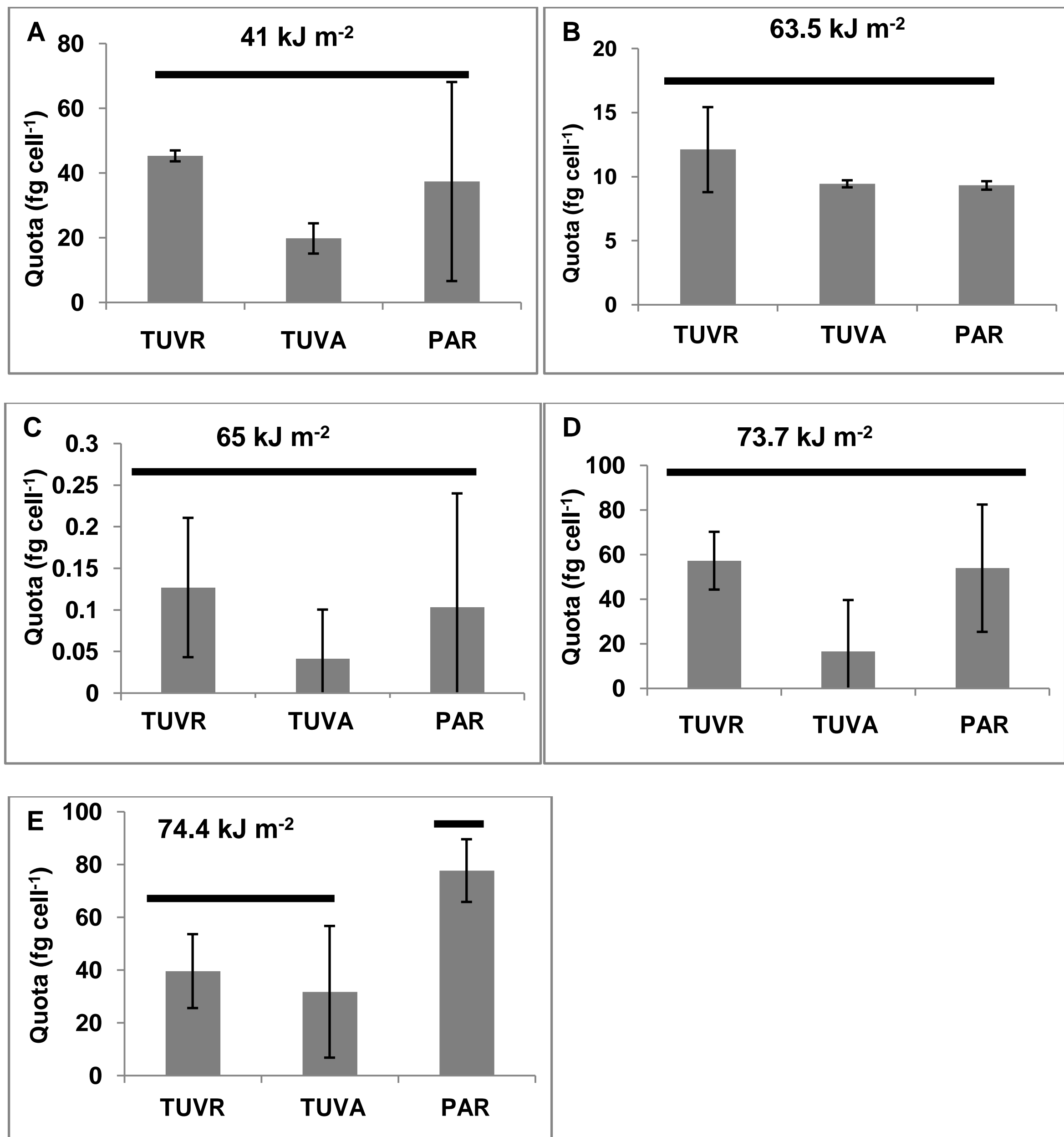

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UVR+PAR Doses	Effects	Cells Biomass	ROS	CAT activity	SOD activity	MC consumption	Photosynthesis
< 9776 kJ m^{-2}	UVBR	=	=	=	=	=	=
	UVAR	=	=	=	=	=	=
9875 to 10275 kJ m^{-2}	UVBR	=	=	=	=	=	=
	UVAR	--	+++	+++	+++	=	---
> 10275 kJ m^{-2}	UVBR	-	++++	++++	=	=	-
	UVAR	----	++++	+++	+++	++	---

We found a UVBR threshold and different responses were activated depending on the exposure to UVAR or UVBR and doses. =: no changes; -: decrease; +: increase. The amount of signals represent the intensity of the effect.

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