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Effect of glyphosate-based herbicides on the photosynthetic responses of the macrophyte *Egeria densa* Planch. from topical lotic ecosystems

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ABSTRACT

Effect of glyphosate-based herbicides on the photosynthetic responses of the macrophyte *Egeria densa* Planch. from topical lotic ecosystems

The effects of pesticide pollution on eco-physiological responses in primary producers are understudied. To partly overcome this gap, this study evaluated the effects of a glyphosate-based herbicide, Roundup® (three nominal concentrations: 0.28 mg/L [Treatment T0.28]; 3.5 mg/L [T3.5]; and 6 mg/L [T6]), and its main degradation product, aminomethylphosphonic acid (AMPA) (0.03 mg/L [T0.03]), on the photosynthetic responses of an aquatic macrophyte species, *Egeria densa* Planch. The net photosynthetic rate (NPR) and dark respiration rate (DRR) were evaluated by the dissolved oxygen evolution method, while some PSII performance parameters were measured by a Chl-*a* fluorescence-based method. Additionally, the chlorophyll *a* content was measured. The experimental data showed that *E. densa* had significant losses in photosynthetic efficiency under Roundup® and AMPA exposure, with reductions in the NPR (reduction of -61 % for T3.5; –90 % for T6; and –161 % for T0.03, compared to control) and chlorophyll *a* content (–82 % for T0.28, –79 % for T3.5, –82 % for T6, and –28 % for T0.03) and increases in the DRR (+150 % for T3.5, +130 % for T6, and +271 % for T0.03). The results from the Chl- *a* fluorescence measurements registered statistically significant differences between the Roundup® treatments and the control group for the effective quantum yield (+56 % for T0.28, +43 % for T3.5, and +56 % for T6) and for non-photochemical extinction (+67% for T0.28, +73 % for T3.5, and +59 % for T6). Taken together, the findings of the experiments with *E. densa* indicate that the negative effects from the glyphosate-based herbicide Roundup® on primary producers might be common and widespread in tropical and subtropical aquatic environments in Brazil.

Key words: AMPA, Chl-*a* fluorescence, freshwater ecosystem, net photosynthetic rate, primary production, Roundup®

RESUMO

Efeito de herbicidas à base de glifosato sobre as respostas fotossintéticas da macrófita* Egeria densa *Planch. de ecossistemas lóticos tropicais

Os efeitos da poluição por pesticidas sobre as respostas eco-fisiológicas em produtores primários são pouco estudados. Para superar parcialmente essa lacuna, o presente estudo avaliou os efeitos de um herbicida à base de glifosato, Roundup® (três concentrações nominais: 0.28 mg/L [Tratamento T0.28]; 3.5 mg/L [T3.5]; e 6 mg/L [T6]) e seu principal produto de degradação, o ácido aminometilfosfônico, AMPA (0.03 mg/L [T0.03]), sobre os parâmetros fotossintéticos de uma espécie de macrófita aquática, Egeria densa *Planch. As taxas de fotossíntese líquida (TFL) e de respiração no escuro (TRE) foram avaliadas pelo método da evolução do oxigênio dissolvido, enquanto alguns parâmetros fotossintéticos foram mensurados por um método baseado na fluorescência Chl-*a*. Adicionalmente, o teor de clorofila* a *foi mensurado. Dados experimentais mostraram que* E. densa *registrou perdas significativas de eficiência fotossintética quando exposta ao Roundup® e ao AMPA, com reduções na TFL (redução de -61 % para T3.5; -90 % para T6 e -161 % para T0.03, comparados com o controle) e no teor de clorofila* a *(-82 % para T0.28, -79 % para T3.5, -82 % para T6 e -28 % para T0.03) e aumentos na TRE (+150 % para T3.5, +130 %*

*para T6 e +271 % pra T0.03). Os resultados das medidas de fluorescência de Chl-*a*, registraram diferenças estatisticamente significativas entre os tratamentos com Roundup® e o grupo controle para rendimento quântico efetivo (+56 % para T0.28, +43 % para T3.5 e +56 % para T6) e para extinção não-fotoquímica (+67 % para T0.28, +73 % para T3.5 e +59 % para T6). Tomados em conjunto, os resultados dos experimentos com* E. densa *indicam que os efeitos negativos do herbicida à base de glifosato, Roundup®, sobre produtores primária pode ser comum e generalizado nos ecossistemas aquáticos tropicais e subtropicais brasileiros.*

Palavras chave: *AMPA, ecossistema de águas continentais, fluorescência da Chl-*a*, produção primária, Roundup®, taxa fotossintética líquida*

INTRODUCTION

In recent decades, the increase in agricultural productivity per area of global crops to ensure there is enough food for the population has become a major global concern (Galli et al., 2005; Kleijn et al., 2009). Therefore, herbicides have been used to eliminate unwanted plants, called weeds, which compete for resources with plants of agronomic interest. In this context, glyphosate-based herbicides have been one of the most widely used herbicides in the world in an attempt to eliminate (completely, if possible) the frequency of weed occurrence in commercial agricultural areas (Galli et al., 2005; Kleijn et al., 2009; Gill et al., 2018). In fact, the increase in the use of commercial glyphosate herbicide formulations occurred with the increase in the planted area of genetically modified (GM) crops, mainly soybeans, worldwide. There are about 185.1 million hectares of GM crops in 26 countries, led by the USA and Brazil, of which ca. 50 % is used for the production of GM soybeans (Fernandes et al., 2019).

Glyphosate is a non-selective herbicide absorbed by plants through leaves that competitively inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) in the synthesis of aromatic amino acids (Forlani et al., 2008; Oliveira et al., 2016). In practice, the exposure of plants to glyphosate leads to the inhibition of the action of EPSPs in the metabolic pathway of aromatic amino acids, consequently causing a decrease in the production of certain amino acids indispensable for plant metabolism, such as phenylalanine, tyrosine, and tryptophan (Sáenz & Di Marzio, 2009). In addition, glyphosate can affect the plant by reducing the concentrations of chlorophyll *a*, phytoalexins (important in protecting the plant against herbivory, for example), and lignin (one of the major structural components of plant biomass) (Yamada & Castro, 2007; Gomes et al., 2017).

It has been widely documented that, in general, continental aquatic environments receive large amounts of herbicides, including glyphosate-based herbicides, which are carried by rainfall from plantations in their vicinity (Aparicio et al., 2013). Several studies have shown significant concentrations of glyphosate in surface waters worldwide, including the USA (1.24 mg/L, Newton et al., 1994; 0.07 mg/L, Battaglin et al., 2014), Canada (2.8 mg/L, Legris & Couture, 1989; 1.1 and 1.5 mg/L, Feng et al., 1990; 1.54 mg/L, Couture et al., 1995), Argentina (0.7 mg/L, Peruzzo et al., 2008), and, obviously, Brazil (0.1 mg/L, Silva et al., 2003). In one of the most serious cases, a study carried out in the USA detected a glyphosate concentration of 5.2 mg/L in runoff waters (Edwards et al., 1980).

Upon reaching these environments, part of these herbicides is broken down via microbial action, generating aminomethylphosphonic acid (AMPA) molecules, which constitute the major glyphosate degradation product (Bonnet et al., 2007). Several studies have suggested that AMPA may also be a potentially toxic substance to plants. For example, Gomes et al. (2016) demonstrated that AMPA has phytotoxic effects since it decreases the photosynthetic rate and quantum yield of photosystem II (PSII), and it reduces chlorophyll *a* biosynthesis. The occurrence of AMPA has also been reported in continental aquatic ecosystems. For example, Villeneuve et al. (2011) reported a variation of 0.00004 to 0.066 mg/L in Canada, 0.00002 to 0.041 mg/L in the USA, and 0.0021 to 0.0481 mg/L in France. Other studies have also shown a wide variation in the concentration of AMPA in surface waters: concentrations between 0.00007 and 0.43 mg/L were reported by Coupe et al. (2012); concentrations between 0.002 and 0.028 mg/L were found by Battaglin et al. (2014); and concentrations between 0.0021 and 0.0026 mg/L were registered by Poiger et al. (2017).

Although sometimes considered a model of invasive exotic species and a nuisance for reservoirs, especially those related to hydroelectricity generation (Bini et al., 1999; Uieda & Marçal, 2020), *Egeria densa* is a widely distributed freshwater plant that is native to Brazil, and it is an important primary producer in some lotic habitats (Branco et al., 2017). In addition, *E. densa* has been identified as having an important role as a habitat for other organisms, such as algae, zooplankton, and juvenile fishes (Pelicice & Agostinho, 2005).

Thus, considering the potential ecological risk of glyphosate-based herbicides in continental aquatic environments (Maria et al., 2020), the present study aimed to evaluate the phytotoxic effects of Roundup® and AMPA on the aquatic macrophyte *E. densa* under controlled conditions. For 7 days, samples of *E. densa* were exposed to three nominal concentrations of Roundup®, 0.28 mg/L, 3.5 mg/L, and 6 mg/L, and to a nominal concentration of 0.03 mg/L of AMPA in a semi-static test. These concentrations are common in the environment. The commercial formulation of the glyphosate-based herbicide chosen in the ecotoxicological tests was Roundup® Original because it is one of the most used worldwide. The response variables considered were the chlorophyll *a* content and photosynthetic parameters, which were measured and calculated by the dissolved oxygen evolution and by Chl-*a* fluorescence-based methods.

Based on the literature and considering that macrophytes have the same metabolic pathways, in which glyphosate acts as an inhibitor, it was expected that the photosynthetic response of *E. densa* would be significantly impaired by Roundup® and AMPA exposure. Additionally, the magnitude of the negative effects on the photosynthetic response of *E. densa* was expected to be proportional to the herbicide doses used in the experimental treatments.

MATERIALS AND METHODS

Collection and preparation of the *Egeria densa* samples

Specimens of *E. densa* were used to perform the experimental analyses during this study. All specimens used in the experimental analyses were collected in small streams (1st to 3rd order) located in southeastern Brazil, particularly in the Rio do Cervo basin in the central-west region of São Paulo State. After collection, the specimens were placed in bottles containing water from the stream and taken *in vivo* to the laboratory, where they were initially cleaned and weighed. During the cleaning process, sediments and epiphytic organisms associated with the surface of the stems and roots were removed using a stereoscopic microscope, hard bristle brush, and distilled water jets. After cleaning, plants were incubated in sterilized 110 mL Erlenmeyer flasks containing 100 mL of sterilized Bold's basal medium for a 3-day acclimation period in a B.O.D. incubator (Nova Ética brand, model 411/FDP355) under the same temperature (20 ± 0.5 °C), irradiance (140 ± 15 µmol m^{-2} s^{-1}), and photoperiod (12/12 h) that were used in the experimental analyses (Branco et al., 2017). The irradiance to which the plants were subjected was continuously measured with a quantometer, Li-Cor model LI-189 (Lincoln, Nebraska, USA), coupled to a spherical quantum sensor, LI-193 SA. The Erlenmeyer flasks and Bold's basal medium used throughout the experimental procedures were sterilized by autoclaving at 121 °C for 20 min using a vertical autoclave (AV-30, Phoenix Luferco, Araraquara, SP, Brazil).

Determination of the nominal concentrations and procedures for preparing experimental concentrations of Roundup® and AMPA

Three nominal concentrations were used in the Roundup® treatments: 0.28 mg/L of the active ingredient (treatment T0.28), 3.5 mg/L of the active ingredient (treatment T3.5), and 6 mg/L of the active ingredient (treatment T6). These nominal experimental concentrations were selected because they represent, respectively, the maximum concentration allowed by Brazilian law (CONAMA Resolution No. 357/05) for waters used for irrigation and animal consumption, the concentration recommended by the manufacturer for the control of aquatic and terrestrial pests (Giesy et al., 2000; Vera et al., 2012), and one of the highest concentrations ever found in natural aquatic environments (Edwards et al., 1980). For AMPA, we used a treatment concentration of 0.03 mg/L of the active ingredient (treatment T0.03), which is a median value reported for lotic environments (Villeneuve et al., 2011; Coupe et al., 2012).

The nominal experimental concentrations of Roundup® were calculated based on acid equivalent (a.e.) values (360 g/L) so that the preparation of each concentration took into account the following dilutions (according to Oliveira et al., 2016): First, to obtain an initial concentration of 0.28 mg/L, 0.77 µL of Roundup® was added to 1 L of sterilized Bold's basal medium. Then, to obtain an initial concentration of 3.5 mg/L, 9.7 µL of Roundup® was added to 1 L of sterilized Bold's basal medium. Finally, to obtain an initial concentration of 6 mg/L, 16.6 µL of Roundup® was added to 1 L of sterilized Bold's basal medium. Considering the nominal experimental concentration of AMPA, to obtain a concentration of 0.03 mg/L, 1 mg of AMPA (≥ 99 % purity, CAS # 1066-51-9) was initially added to 1 L of sterilized distilled water, and then a 30 mL aliquot of this 1 % aqueous AMPA solution was added to 1 L of sterilized Bold's basal medium (Oliveira et al., 2016). In addition, for each treatment tested, a control group containing sterilized Bold's basal medium without herbicide (0 mg/L) was simultaneously evaluated.

Experimental procedures and photosynthetic performance measurements to evaluate the effect of Roundup® and AMPA on the photosynthesis of *E. densa*

The procedures to expose *E. densa* samples to Roundup® and AMPA were carried out based on a semi-static test model with the analysis of the photosynthetic responses performed on the seventh day (T7) after exposure to herbicides and the renewal of the experimental media on the third and fifth days to prevent the loss of nutrients and active ingredient (Nešković et al., 1996; Oliveira et al., 2016).

After exposure of the samples, the effects of Roundup® and AMPA on the photosynthetic performance of *E. densa* were assessed by applying analytical methods based on the dissolved oxygen evolution and Chl-*a* fluorescence measurements.

Experiments using dissolved oxygen evolution were performed to calculate the net photosynthetic rate (NPR) and dark respiration rate (DRR), and they followed the procedures described by Necchi Júnior (2004a, 2004b), Oliveira et al. (2016), and Branco et al. (2017), while experiments involving Chl-*a* fluorescence measurements were used to calculate specific photosystem II (PSII) performance parameters (Vilas Boas et al., 2018).

Considering the NPR, the measurements were performed in T7 in quintuplicate (n = 5) for each experimental group, including treatments with different concentrations and the control groups. Thus, after the acclimation period, the specimens were transferred to 110 mL borosilicate glass bottles with 98.5 % transparency (clear bottles) containing Bold's basal medium, with the concentrations corresponding to each experimental treatment, and the specimens were then incubated for a 1 h period in B.O.D. incubators under the same conditions used in the acclimation period, namely, a temperature of 20 ± 0.5 °C, an irradiance of 140 ± 15 µmol m^{-2} s^{-1}, and a photoperiod of 12/12 h. Frontal illumination was provided by three cool white fluorescent lamps (Osram 15 W), and during the incubation time, the flasks were repositioned inside the incubator every 6 min to ensure that all replicas received the same average irradiance, regardless of their position in the incubator. Calculations of the NPR were performed from the dissolved oxygen concentrations quantified at the beginning (initial concentration or [I]) and at the end (final concentration or [F]) of the incubation period, which were measured using an oximeter equipped with a self-agitating probe (Yellow Springs Instruments, YSI, model 5100, Ohio, USA).

Considering the DRR, the same procedures described above for the NPR were repeated (in triplicate, n = 3), but in this case, the *E. densa* samples were incubated in amber bottles covered with aluminum foil (dark bottles) under the complete absence of illumination. Calculations of the NPR and DRR were based on Littler & Arnold (1985), and the following formulas were used: $NPR = ([F] - [I])\ V\ Ti^{-1}\ DW^{-1}$ and $DRR = ([I] - [F])\ V\ Ti^{-1}\ DW^{-1}$, where *NPR* is the net photosynthetic rate, *DRR* is the dark respiration rate, *[F]* is the final dissolved oxygen concentration after the incubation period, *[I]* is the initial dissolved oxygen concentration before the incubation period, *V* is the volume of the bottle, *Ti* is the incubation time, and *DW* is the dry weight of the sample.

The evaluation of PSII performance based on the Chl-*a* fluorescence measurement using a pulse-amplitude-modulated underwater fluorometer (i.e., Diving-PAM, Walz, Effeltrich, Germany) was performed on T7 under the same conditions used in the acclimation period. After a 30 min period of dark acclimation (Lichtenthaler et al., 1981; Yannicari et al., 2012), samples of each treatment and control group were analyzed (in quintuplicate, n = 5) using the "Induction Curve" function (Schreiber et al., 1995), applying an actinic light of 285 µmol photons $m^{-2}\ s^{-1}$ and a saturation pulse of 15 s with a duration of 0.8 s and intensity of 2000 µmol photons $m^{-2}\ s^{-1}$. The following PSII performance parameters were calculated: effective quantum yield of PSII (YII) (Genty et al., 1989; Schreiber et al., 1995; Necchi Júnior, 2005), quantum yield of non-regulated energy dissipation (YNO) (Demming-Adams et al., 1996), and quantum yield of regulated energy dissipation (YNPQ) (Demming-Adams et al., 1996).

After the analysis of the photosynthetic parameters, the samples of *E. densa* were used to measure the chlorophyll *a* content, applying the methodology proposed by Wetzel & Likens (2000). Finally, the samples were kept in a drying oven with forced air circulation (Tecnal, model TE-394/2) at a constant temperature (70 °C) until obtaining constant mass. After drying, the samples were weighed with the aid of an analytical balance (Shimadzu, model AUW220D) to obtain the dry weights. Dry weights were then used to express the chlorophyll *a* content in relation to the biomass measurement of the *E. densa* samples.

Data analysis

Regarding the data analysis, all data was checked for normality and homogeneity using the Kolmogorov–Smirnov test and Levene's test, respectively. One-way ANOVA was performed to identify significant differences in NPR, DRR, YII, YNO, YNPQ, and chlorophyll *a* content among the control group and treatments. When significant differences were found ($p < 0.05$), the Tukey test was performed to identify the contrasting groups. Significant differences ($p < 0.05$) between the photosynthetic parameters of the control group and treatment with AMPA were tested by applying the Student's *t*-test. All tests were performed using the statistical software Statistica 7.1.30.0 (StatSoft, Inc. 2005).

RESULTS

One-way ANOVA showed that there were statistically significant differences in the values of the net photosynthetic rate (NPR) of *E. densa* among the experimental treatments using the herbicide Roundup® ($F = 16.35$). In turn, the Tukey test performed *a posteriori* revealed that, in fact, there were significant reductions in the NPR for treatments T3.5 (a reduction of 61 % compared to the control) and T6 (–90 %) when compared with the control group (Fig. 1). Likewise, there were statistically significant differences in the DRR among the Roundup® treatments ($F = 42.39$), with higher values being observed for treatments T3.5 (an increase of 150 % compared to the control) and T6 (+130 %) (Fig. 1).

One-way ANOVA revealed that there were differences in the content of chlorophyll *a* among the control group and the Roundup® treatments ($F = 4.68$), with significantly lower values observed in treatments T0.28 (–82 %), T3.5 (–79 %), and T6 (–82 %) compared to the control group (Fig. 1).

Statistically significant differences between Roundup® treatments and the control group were observed for YII ($F = 8.66$) and YNPQ ($F = 10.18$). In this context, significant increases

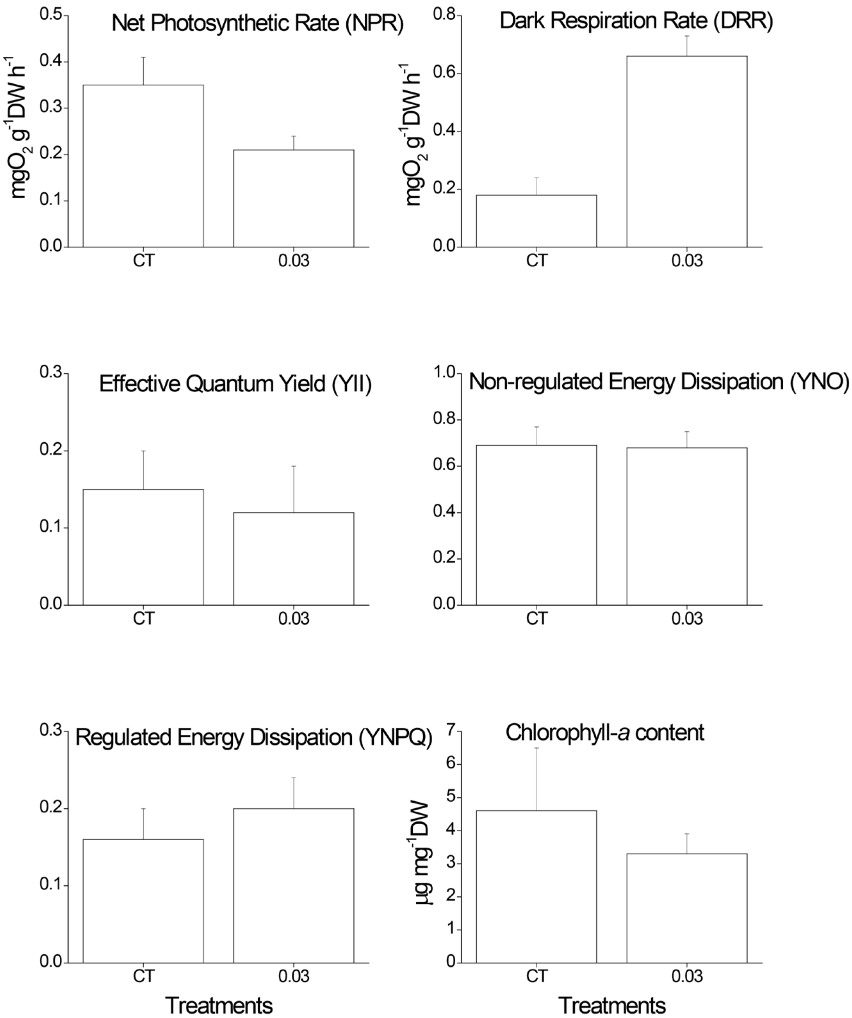

Figure 1. Values (mean and standard deviation) of the net photosynthetic rate (NPR; n = 5), dark respiration rate (DRR; n = 3), chlorophyll *a* content (Chl-*a* content; n = 8), effective quantum yield (YII; n = 8), non-regulated energy dissipation (YNO; n = 8), and regulated energy dissipation (YNPQ; n = 8) for *Egeria densa* for the control group (CT) and each Roundup® treatment (nominal concentrations of 0.28 mg/L, 3.5 mg/L, and 6 mg/L). *Valores (média e desvio padrão) das taxas fotossintéticas líquidas (NPR; n = 5), taxas de respiração no escuro (RRD; n = 3), teor de clorofila* a *(Chl-*a *content; n = 8), rendimento quântico efetivo (YII; n = 8), dissipação não-regulada de energia (YNO; n = 8) e dissipação regulada de energia (YNPQ; n = 8) de* Egeria densa *para o controle grupo (CT) e cada tratamento com Roundup® (concentrações nominais: 0.28 mg/L; 3.5 mg/L; e 6 mg/L).*

in the values of YII were registered for all concentrations (T0.28 = +56 %, T3.5 = +43 %, and T6 = +56 %) and there were significant increases in the YNPQ values (T0.28 = +67 %, T3.5 = +73 %, and T6 = +59 %) (Fig. 1).

The experimental results showed statistically significant differences in the values of both NPR and DRR of *E. densa* among samples from the control group and those exposed to AMPA (*T*-value = 19.36). There was a strong reduction (–161 %) in the NPR values of the plants exposed to AMPA, relative to the control group, whereas the DRR values of these plants increased (+271 %) (Fig. 2).

There were significant differences in the chlorophyll *a* content between the control group and the AMPA treatment (*T*-value = 3.21), with lower values (–28 % compared to the control group) reported for plants exposed to AMPA (Fig. 2).

Despite the reductions in the nominal values of the PSII performance parameters YII (–20 %) and YNO (–2 %) of the samples of *E. densa* exposed to AMPA relative to those of the control group and the increase in the nominal values of YNPQ (+25 %), no statistically significant differences were found for the values of these parameters between treatment with AMPA and the control group (Fig. 2).

DISCUSSION

The results of the experiments exposing *E. densa* to different concentrations of Roundup® and its main degradation product, AMPA, suggests, as a rule, the occurrence of significant negative effects on the measured photosynthetic parameters.

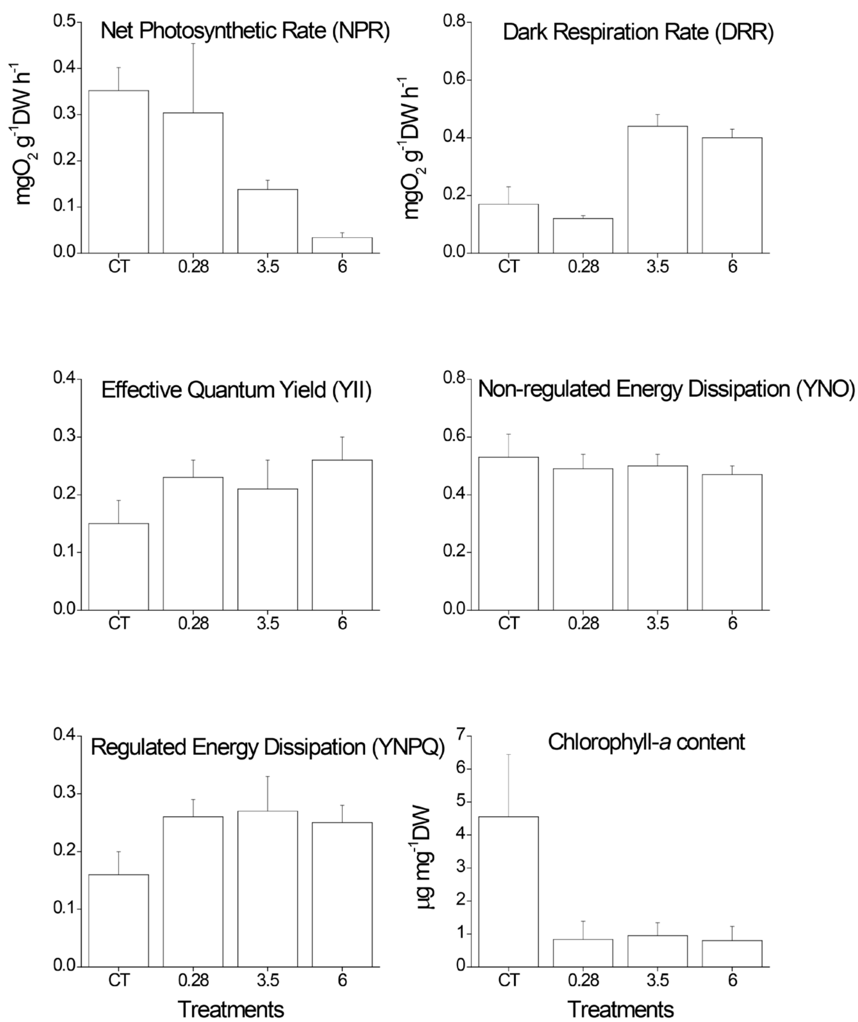

Figure 2. Values (mean and standard deviation) of NPR (n = 5), DRR (n = 3), Chl-*a* content (n = 8), YII (n = 8), YNO (n = 8), and YNPQ (n = 8) for *E. densa* for the CT and the AMPA treatment (nominal concentration of 0.03 mg/L). *Valores (média e desvio padrão) das taxas fotossintéticas líquidas (NPR; n = 5), taxas de respiração no escuro (RRD; n = 3), teor de clorofila a (Chl-*a *content; n = 8), rendimento quântico efetivo (YII; n = 8), da dissipação não-regulada de energia (YNO; n = 8) e da dissipação regulada de energia (YNPQ; n = 8) de* Egeria densa *para o controle grupo (CT) e o tratamento com AMPA (concentração nominal: 0.03 mg/L).*

Under exposure to Roundup®, specimens of *E. densa* showed a significant reduction in NPR and DRR, particularly for treatments with the highest concentrations of the herbicide (3.5 mg/L and 6 mg/L). In this sense, NPR was significantly lower in treatments T3.5 and T6, while DRR was significantly higher for these same treatments. These results indicate that, in fact, the photosynthetic response of *E. densa* is negatively affected when exposed to concentrations of the herbicide Roundup® of above 3.5 mg/L. The loss of photosynthetic efficiency under exposure to Roundup® has been observed for some other primary producers of stream ecosystems, among which is the species of charophyte *Nitella microcarpa* var. *wrightii* (Charophyceae, Chlorophyta) (Oliveira et al., 2016). As observed in the present study for *E. densa*, it appears that the NPR of *N. microcarpa* var. *wrightii* decreased as a function of increased DRR under exposure to the herbicide. The significant increase in DRR values, which, in turn, negatively impacts the NPR, suggests that *E. densa* experiences relevant physiological stress and increases in metabolic rate when exposed to Roundup® (Kramer et al., 2004; Klughammer & Schreiber, 2008; Vilas Boas et al., 2018). The indication that exposure to Roundup® produces significant physiological stress in *E. densa*, which was observed from the data from the dissolved oxygen evolution technique and chlorophyll *a* content, apparently conflicts somewhat with data from the Chl-*a* fluorescence measurements. In this context, significant increases in parameter YII were observed, apparently suggesting that there was an increase in photosynthetic efficiency. However, although the increase in YII may suggest a positive effect on the photosynthetic response, when this increase is associated with the increase in YNPQ values, it is suggested that the plant is trying to adjust its photosynthetic metabolism to acclimate to the imposed environmental conditions. In fact, by increasing the energy dissipation by the antenna complex (a fact revealed precisely by the YNPQ values), the plant shows an attempt to prevent excess energy from damaging the reaction center of PSII (Demmig-Adams et al., 1996; Mantoan et al., 2016). Thus, by combining the data obtained by the dissolved oxygen evolution and Chl-*a* fluorescence-based techniques, it appears that when exposed to Roundup®, *E. densa* tried to adjust its photosynthetic apparatus in the face of environmental stress; however, despite this attempt, the specimens still showed an effective reduction in NPR. This reduction in NPR may be related to the effect of glyphosate in another stage of photosynthesis, such as the Calvin cycle. Some studies have shown that disrupting the metabolic pathway of aromatic amino acids can affect the Calvin cycle (Geiger et al., 1986; Shieh et al., 1991; Gomes et al., 2014). In addition, glyphosate can inhibit Rubisco gene expression, also affecting CO_2 assimilation and degrading chlorophyll, without demonstrating any inhibitory effect on Chl-*a* fluorescence-based parameters (Soares et al., 2020).

Considering the data obtained by the dissolved oxygen evolution technique, the experimental results of the exposure of *E. densa* to AMPA showed complete correspondence with those reported for exposure to Roundup®, with a significant reduction in NPR associated with a significant increase in DRR. Considering the chlorophyll *a* content, a significant reduction in this pigment concentration was also observed. Although the mechanisms are not yet fully understood, AMPA is also able to interfere with chlorophyll *a* biosynthesis, perhaps through inhibition of δ-aminolevulinic acid (ALA) production, which is an intermediate of chlorophyll biosynthesis (Gomes et al., 2014). However, when considering the parameters obtained by the Chl-*a* fluorescence measurements, the data on the exposure of this macrophyte to AMPA, unlike what was observed for Roundup®, did not register statistically significant differences when compared to data from the control group, suggesting that AMPA also affects CO_2 assimilation, without any photochemical damage detected by Chl-*a* fluorescence-based parameters.

A few other experimental studies have also shown the occurrence of negative impacts of exposure to glyphosate on some aspect of the photosynthesis of aquatic macrophytes (e.g., Kielak et al., 2011; Santos et al., 2020; Zong et al., 2018). Santos et al. (2020), for example, revealed that glyphosate solutions (both from a commercial formulation, Roundup®, and from an analytical standard), depending on the concentration, significantly affected the photosynthesis of *Salvin-*

ia biloba, an aquatic macrophyte, decreasing the contents of chlorophyll *a* and *b* and causing disturbances in PSII. In addition, the results suggested that exposure to glyphosate caused oxidative stress and an increased concentration of shikimic acid in these plants. In this same direction, Kielak et al. (2011) showed that the phytotoxic effects in *Lemna minor* caused by exposure to the herbicide Roundup Ultra 360 SL (containing the isopropylamine salt of glyphosate as the active ingredient) have been associated with decreases in the contents of chlorophyll *a*, *b*, and *a*+*b* and reduced biomass growth.

The results of the present study become even more relevant when we consider that *E. densa* is one of the best-distributed macrophyte species in tropical and subtropical continental aquatic ecosystems in South America, occurring from southeastern Brazil to the coastal areas of Argentina and Uruguay (Cook & Urmi-Konig, 1984; Yarrow et al., 2009). In these environments, *E. densa* is an important primary producer (Branco et al., 2017). This relevant functional role in continental aquatic environments shows that the results of the present study may have identified a potential environmental risk generated by the indiscriminate and uncontrolled use of the herbicide Roundup® in agricultural areas connected to lotic and lentic ecosystems. In this context, impacts on the primary production of *E. densa* can have a cascade effect, reaching consumers who depend on this producer and, ultimately, the aquatic ecosystem as a whole, with potential unpredictable impacts throughout the trophic web.

Therefore, considering the results obtained through the exposure experiments of *E. densa* to the nominal concentrations of the herbicide Roundup® and to its main degradation product, AMPA, it is possible to making the following conclusions: i) The data obtained by the dissolved oxygen evolution, chlorophyll *a* content, and Chl-*a* fluorescence-based techniques revealed that both Roundup® and AMPA negatively affected the photosynthetic responses, leading to a loss of photosynthetic efficiency of these plants. ii) The most relevant negative effects on photosynthesis were observed for the Roundup® concentrations of 3.5 mg/L and 6 mg/L, indicating that the degree of severity of the impacts produced by the herbicide on these plants, as a rule, is accentuated in the highest concentrations; although, it is not expressed in a directly proportional way. iii) From an ecological perspective, the present study reveals the potential ecological risk for continental aquatic ecosystems in South America since this species of macrophyte is an important primary producer in these environments.

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