

## Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate

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### ABSTRACT

Previous studies comparing cultivars of different maturity groups in different soils demonstrated that early maturity group cultivars were more sensitive to glyphosate injury than those of other maturity groups. In this work, we evaluated the effect of increasing rates of glyphosate on water absorption and photosynthetic parameters in early maturity group cultivar BRS 242 GR soybean. Plants were grown in a complete nutrient solution and subjected to a range of glyphosate rates either as a single or sequential leaf application. Net photosynthesis, transpiration rate, stomatal conductance, sub-stomatal CO<sub>2</sub>, carboxylation efficiency, fluorescence, maximal fluorescence and chlorophyll content were monitored right before and at different stages after herbicide application; water absorption was measured daily. All photosynthetic parameters were affected by glyphosate. Total water absorbed and biomass production by plants were also decreased as glyphosate rates increased, with the affect being more intense with a single full rate than half the rate applied in two sequential applications. Water use efficiency (WUE) was significantly reduced with increasing rates of glyphosate.

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### 1. Introduction

The expanding global land area for crop production combined with climate change factors of increasing atmospheric CO<sub>2</sub> [1] and surface air temperature [2] are raising important concerns regarding water availability for crops. Knowledge of water requirements by crops and their water use efficiency (WUE) are important for assessing effects of climate change on crop water balance and water resources. It is anticipated that predicted changes in the global climate such as increased CO<sub>2</sub> and temperature, may increase transpiration by plants to impact the input of water required for crop production [3].

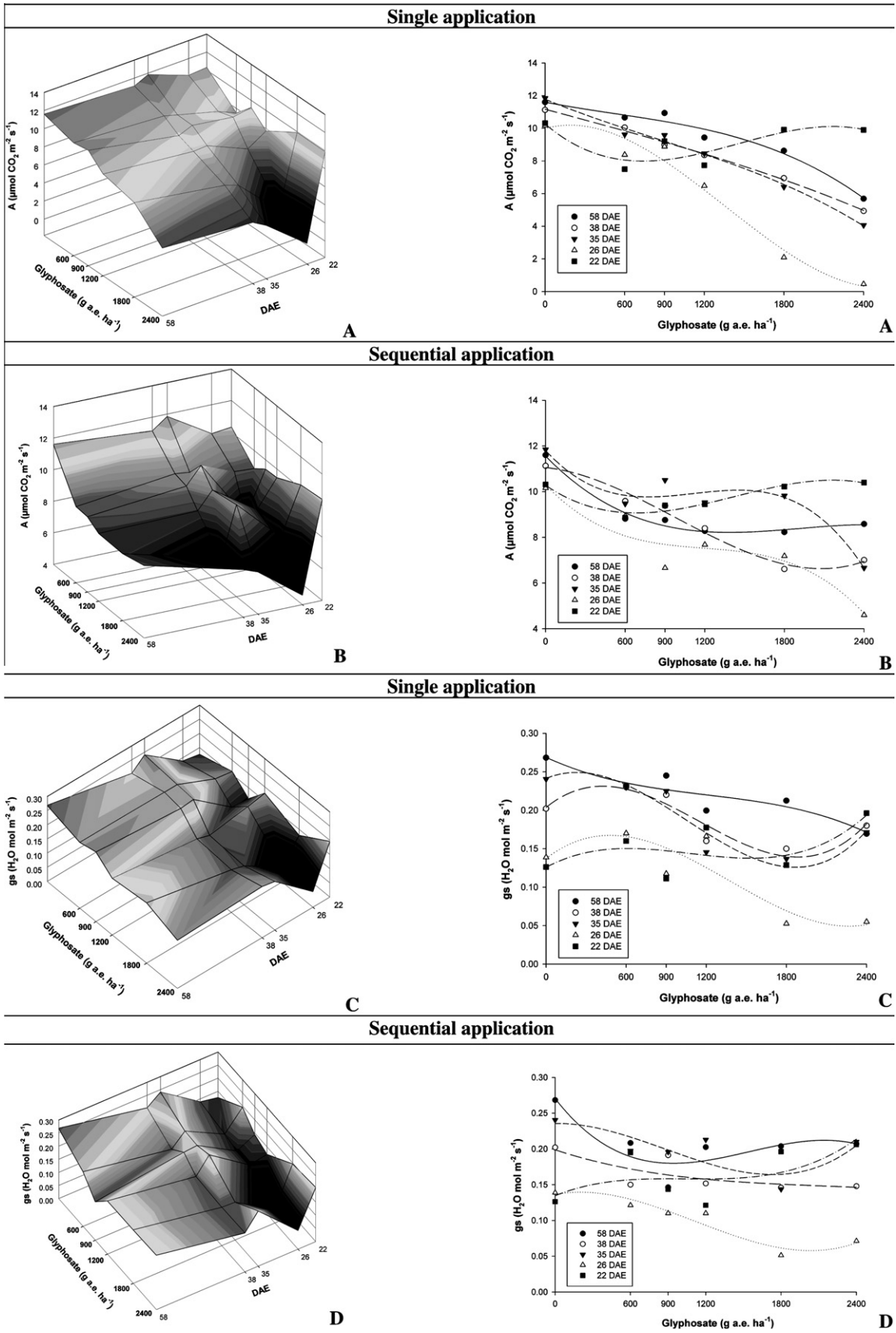
Many farmers have noticed that some transgenic soybeans are sensitive to water stress and others have reported visual plant injuries in glyphosate-resistant (GR) soybean varieties after glyphosate application [4,5]. The nutritional status of GR soybeans also is strongly affected by glyphosate [6]. Glyphosate is a wide-spectrum, foliar-applied herbicide that is translocated throughout the plant to actively growing tissues where it inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway.

This biochemical route is responsible for the biosynthesis of aromatic amino acids, plant defense compounds, and numerous phenolic compounds [7–9].

Despite the widespread adoption of GR technology and the importance of glyphosate in weed control in worldwide cropping systems, few data have been available to understand effects of glyphosate in GR soybean physiology, especially those related to water absorption and photosynthesis as the basic processes for biomass production. A deeper understanding of such effects may lead to a better use of this technology. An initial experiment was conducted at the State University of Maringá during the 2007 summer crop season with cultivars of different maturity groups grown in different soils to evaluate glyphosate injury. Zobiolo et al. [6] demonstrated that such effects were pronounced in the early maturity group (cv. BRS 242 GR), with significant decreases in photosynthetic parameters, shoot mineral concentration and biomass dry weight [6]. In this present work, we evaluated the effect of increasing rates of glyphosate on water absorption and photosynthetic parameters in the previously studied cultivar (cv.) BRS 242, an early maturity group GR soybean. The objective of this research was to evaluate photosynthesis, water absorption and water use efficiency in an early maturity group cultivar of a GR soybean treated with glyphosate at various application rates.

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**Fig. 1.** Photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), sub-stomatal  $\text{CO}_2$  ( $\text{Ci}$ ) and carboxylation efficiency ( $\text{A}/\text{Ci}$ ) in GR soybean as affected by increasing rates of glyphosate applied as a single treatment or sequential, half-rate applications ( $n = 8$ ,  $P < 0.01$ ).

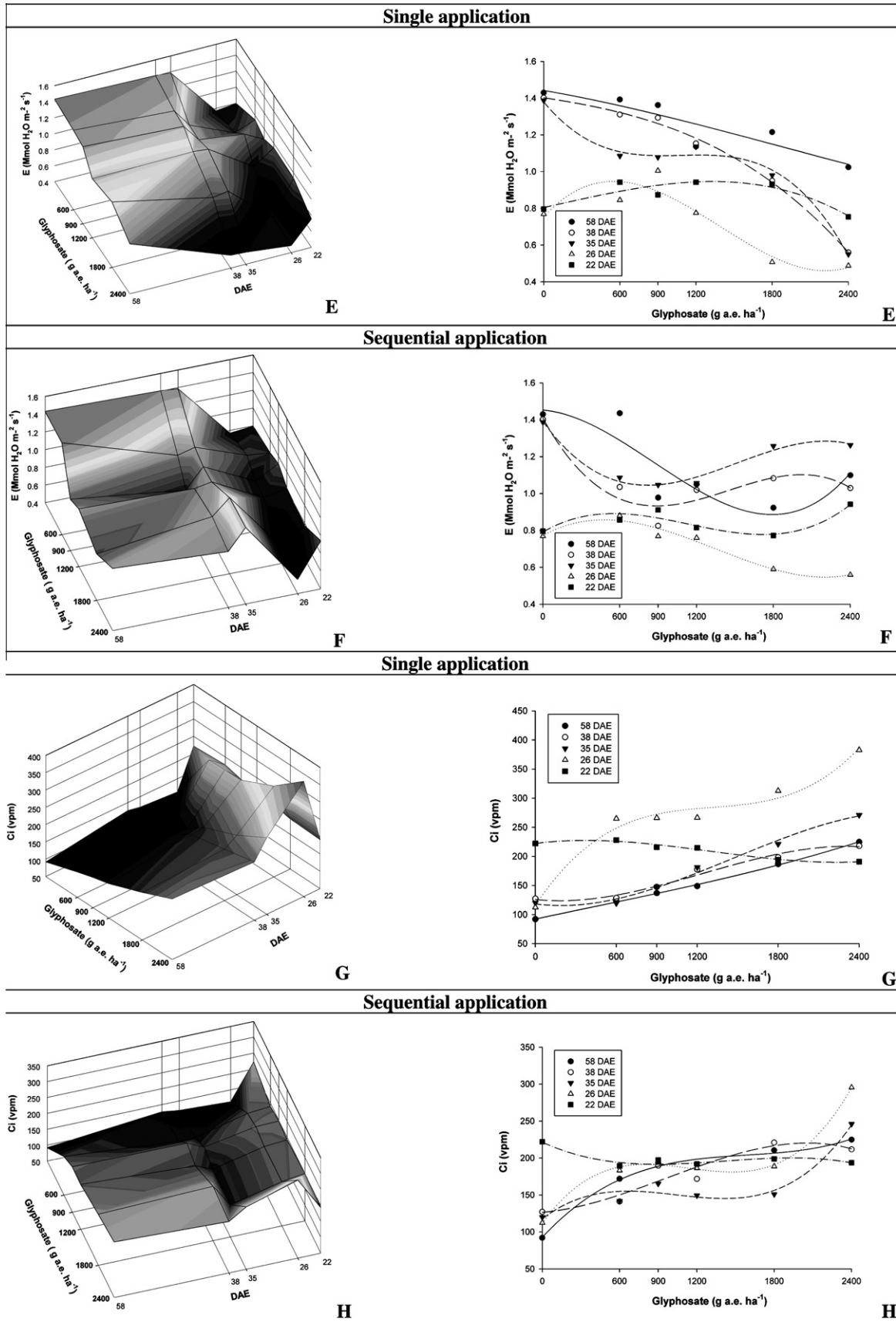


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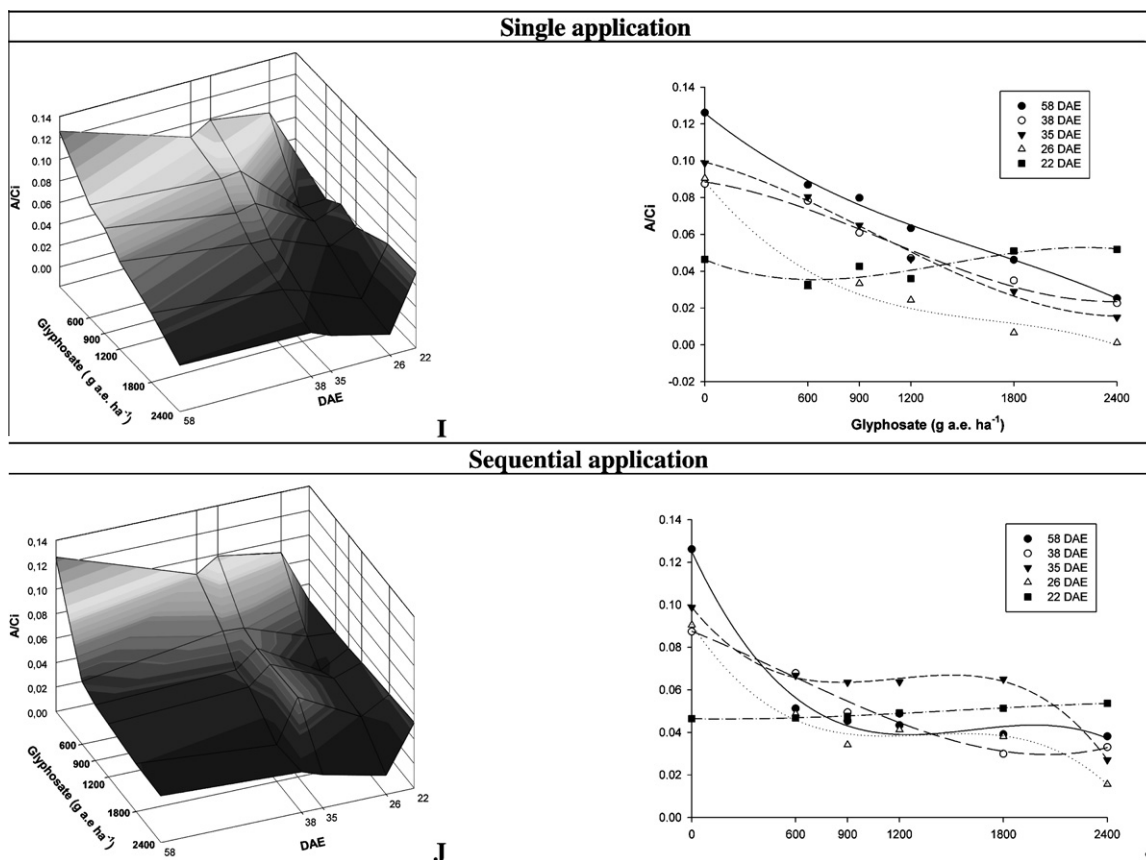


Fig. 1 (continued)

## 2. Material and methods

The experiment was carried out using the cv. BRS 242 GR in a greenhouse equipped with an evaporative cooling system (26–30 °C:20–22 °C day/night) under natural daylight conditions at the State University of Maringá, between July 22th and September 20th, 2008 (location: 23°25'S, 51°57'W) to evaluate the effects of glyphosate at different rates on water absorption and photosynthesis as a possible explanation for the decreased shoot mineral concentrations of cv. BRS 242 GR observed in the previous field study.

Seeds were sterilized for 2 min in 2% NaClO and placed in paper rolls (Germitest) for germination. Seedlings with 5 cm root lengths were transplanted into pots containing nutrient solution. Experimental units were cylindrical polyethylene pots (3.7 dm<sup>3</sup>) under constant aeration. For the first 10 days, the plants were grown in a complete nutrient solution [10] at 1/6 of the usual concentration; in the next 2 weeks, the solution was supplied at 1/3 strength and thereafter it was at full-strength. Nutrient solutions were exchanged every 10 days and pot volume was replenished daily with distilled and deionized water. Before water replacement, the total volume of water absorbed by plants in each pot was recorded. The pH of the solutions was maintained at  $5.8 \pm 0.2$  with additions of NaOH and HCl.

The pots were placed outside the greenhouse for application of the commercially formulated isopropylamine salt of glyphosate 480 g a.e. L-1 (Roundup Ready®, Monsanto Company) using a CO<sub>2</sub> pressurized sprayer equipped with SF110.02 nozzles calibrated to deliver a spray volume of 190 L ha<sup>-1</sup> at a pressure of 2 kgf cm<sup>-2</sup>. Environmental conditions during the applications included air temperature between 25 and 29 °C, relative humidity between

80% and 89%, wind speed between 5 and 10 km h<sup>-1</sup>, and open sky with no clouds. After herbicide applications, the pots were returned to greenhouse. The sprayed solution did not cause run-off from leaves.

The experiment was conducted as a randomized block design, in a factorial arrangement  $(5 \times 2) + 1$ , with eight replicates. Five glyphosate rates (600, 900, 1200, 1800 and 2400 g a.e. ha<sup>-1</sup>) were combined with two application regimes (single and sequential); a control treatment consisted of no herbicide application. Single applications (full rate) were performed at the V4 growth stage (24 days after emergence, DAE), and sequential applications at 50% of the full treatment were applied at V4 and V7 (36 DAE) growth stages of the GR soybeans (cv. BRS 242 GR).

Photosynthetic parameters were recorded at phenological stages V3 (22 DAE – before glyphosate application), V4 (26 DAE – after single application and after the first sequential application), V7 (35 DAE – before the second sequential application), V8 (38 DAE – after the second sequential application) and R1 (58 DAE) and also immediately before and after application of glyphosate. Net photosynthesis (A), transpiration rate (E), stomatal conductance (gs) and sub-stomatal CO<sub>2</sub> concentration (Ci) were evaluated using an infrared gas analyzer (IRGA: ADC model LCpro+, Analytical Development Co. Ltd., Hoddesdon, UK). Carboxylation efficiency was calculated as A/Ci. Evaluations were always carried out between 7:00 and 11:00 am, choosing the last fully expanded trifoliolate (diagnostic leaf) of plants in each pot. The records were taken by automatic time-logging equipment with two measurements of 3 min for each diagnostic leaf.

A portable chlorophyll fluorometer (OS-30 – Opti-Sciences, Inc., Tyngsboro, MA) was used in pulse modulation to determine chlorophyll fluorescence in the same diagnostic leaf under steady state

**Table 1**

Regression statistics and correlations for photosynthetic parameters in GR soybean treated with different rates of glyphosate applied as a single treatment or sequential, half-rate applications.

DAE	Estimation of model parameters adjusted				R <sup>2</sup>
	y <sub>0</sub>	a	b	c	
<b>Fig. 1A</b>					
58	11.60	-0.0016	7.12E7	-4.45E10	0.98 <sup>†</sup>
38	11.16	-0.0022	3.75E8	-8.09E11	0.99 <sup>†</sup>
35	11.77	-0.0034	8.54E7	-3.34E10	0.99 <sup>†</sup>
26	9.98	0.0022	-6.33E6	1.55E9	0.98 <sup>†</sup>
22	10.26	-0.0070	6.31E6	-1.44E9	0.87 <sup>†</sup>
<b>Fig. 1B</b>					
58	11.55	-0.0060	3.36E6	-5.58E10	0.99 <sup>†</sup>
38	11.05	-0.0006	-2.49E6	8.48E10	0.98 <sup>†</sup>
35	11.80	0.0064	6.36E6	-1.90E9	0.97 <sup>†</sup>
26	10.30	-0.0061	4.72E6	-1.31E9	0.95 <sup>†</sup>
22	10.27	-0.0040	3.80E6	-8.90E10	0.96 <sup>†</sup>
<b>Fig. 1C</b>					
58	0.27	-8.02E5	4.94E8	-1.37E11	0.93 <sup>†</sup>
38	0.20	0.0001	-2.04E7	5.92E11	0.93 <sup>†</sup>
35	0.24	7.12E5	-1.74E7	5.59E11	0.94 <sup>†</sup>
26	0.13	0.0001	-1.70E7	4.14E11	0.91 <sup>†</sup>
22	0.12	9.21E5	-1.05E7	3.28E11	0.82 <sup>†</sup>
<b>Fig. 1D</b>					
58	0.27	-0.0002	1.71E7	-3.72E11	0.89 <sup>†</sup>
38	0.20	-5.74E5	2.37E8	-3.73E12	0.79 <sup>†</sup>
35	0.23	3.04E6	-7.47E8	2.83E11	0.81 <sup>†</sup>
26	0.13	3.24E5	-8.87E8	2.64E11	0.96 <sup>†</sup>
22	0.13	7.09E5	-6.99E8	2.25E11	0.80 <sup>†</sup>
<b>Fig. 1E</b>					
58	1.44	-0.0001	-3.44E8	6.09E12	0.92 <sup>†</sup>
38	1.40	0.0001	-2.89E8	-3.04E11	0.99 <sup>†</sup>
35	1.38	-0.0008	7.96E7	-2.46E10	0.99 <sup>†</sup>
26	0.75	0.0008	-8.69E7	2.09E10	0.95 <sup>†</sup>
22	0.80	0.0002	9.91E9	-3.40E11	0.92 <sup>†</sup>
<b>Fig. 1F</b>					
58	1.45	-5.95E5	-4.63E7	1.78E10	0.90 <sup>†</sup>
38	1.41	-0.0013	1.02E6	-2.38E10	0.95 <sup>†</sup>
35	1.39	-0.0010	8.13E7	-1.79E10	0.99 <sup>†</sup>
26	0.77	0.0004	-4.88E7	1.21E10	0.98 <sup>†</sup>
22	0.79	0.0004	-4.81E7	1.43E10	0.93 <sup>†</sup>
<b>Fig. 1G</b>					
58	92.32	0.0497	-2.77E6	2.14E9	0.99 <sup>†</sup>
38	125.89	-0.0241	7.28E5	-1.96E8	0.99 <sup>†</sup>
35	118.27	-0.0301	8.57E5	-1.95E8	0.99 <sup>†</sup>
26	115.96	0.3494	-0.003	6.42E8	0.99 <sup>†</sup>
22	222.30	0.0293	-4.57E5	1.17E8	0.98 <sup>†</sup>
<b>Fig. 1H</b>					
58	92.63	0.1889	-0.0001	2.36E8	0.99 <sup>†</sup>
38	126.66	0.0141	5.26E5	-1.81E8	0.95 <sup>†</sup>
35	117.67	0.1343	-0.0001	4.79E8	0.98 <sup>†</sup>
26	111.51	0.2432	-0.0002	6.84E8	0.99 <sup>†</sup>
22	221.42	-0.0793	6.50E5	-1.53E8	0.96 <sup>†</sup>
<b>Fig. 1I'</b>					
58	0.12	-7.53E5	2.72E8	-5.55E12	0.99 <sup>†</sup>
38	0.09	-1.35E5	-2.33E8	7.36E12	0.99 <sup>†</sup>
35	0.09	-2.44E5	-2.26E8	7.60E12	0.99 <sup>†</sup>
26	0.09	-0.0001	6.09E8	-1.22E11	0.98 <sup>†</sup>
22	0.04	-3.76E5	3.80E8	-8.90E12	0.90 <sup>†</sup>
<b>Fig. 1J</b>					
58	0.12	-0.0002	1.13E7	-2.34E11	0.99 <sup>†</sup>
38	0.08	-3.25E5	-9.50E9	5.66E12	0.99 <sup>†</sup>
35	0.09	-0.0001	9.31E8	-2.63E11	0.99 <sup>†</sup>
26	0.09	-0.0001	9.39E8	-2.32E11	0.99 <sup>†</sup>
22	0.04	-1.18E6	3.71E9	-8.18E13	0.99 <sup>†</sup>

<sup>†</sup> (n = 8, P < 0.01).

conditions ( $F_0$ ), maximal fluorescence under steady state conditions ( $F_m$ ) and the ratio of variable fluorescence to maximal fluorescence ( $F_v/F_m$ ) using the following equation:  $F_v/F_m = (F_m - F_0)/F_m$  Genty et al. [11].

Chlorophyll content was measured before and after application of glyphosate with a SPAD meter (SPAD-502, Minolta, Ramsey, NJ). The meter measures absorption at 650 and 940 nm wavelengths to estimate chlorophyll level [12–14]. SPAD readings were taken of the terminal leaflet of the diagnostic leaf. The SPAD sensor was placed randomly on leaf mesophyll tissue only to avoid the veins. Two leaves were chosen per plant in the pot and measurements were immediately taken per leaf and averaged to provide a single SPAD unit from which chlorophyll content was calculated using the equation of Arnon [15] and expressed as milligrams of chlorophyll per cm<sup>-2</sup> of leaf tissue by the equation of Markwell et al. [16].

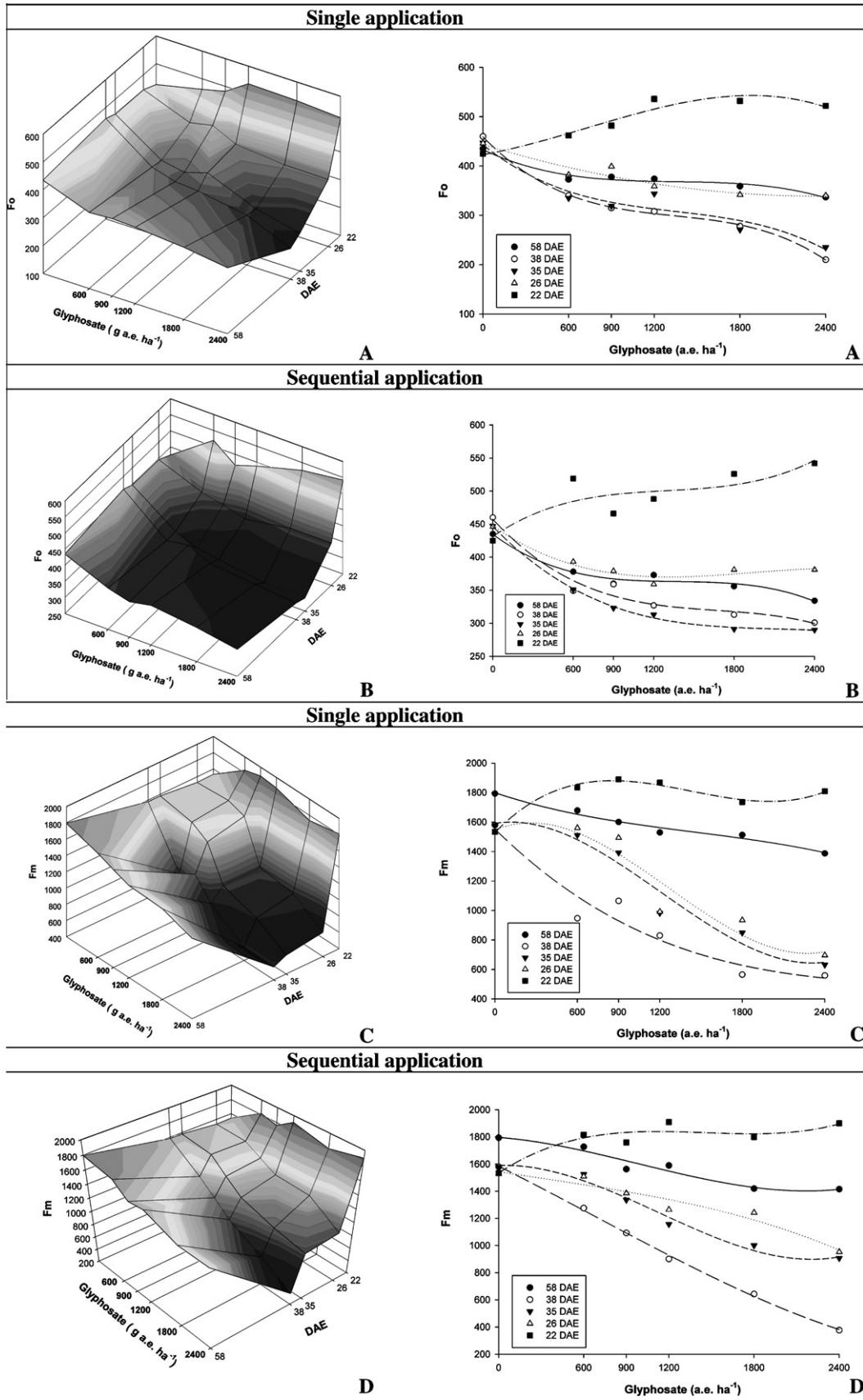
When the plants reached the R1 growth stage, accumulated water absorption was recorded to calculate the water use efficiency by the relationship of dry matter produced to water consumption. All photosynthetic parameters and plant height were measured. After these assessments, shoots were clipped and all harvested materials including roots were packed in paper bags and dried in an air circulation oven at 65–70 °C to a constant weight, in order to determine the dry biomass. Data errors were passed through the test of Shapiro and Wilk [17], in order to evaluate data normality. Data were subjected to analysis of variance, and when *F* values were significant (*P* < 0.01), regression analysis were conducted. For the graphics, curves plotted for photosynthetic parameters and total water absorption at different DAE were made using regression analysis by the adjustment of the equation of the collected data using the polynomial cubic model:  $\hat{y} = y_0 + ax + bx^2 + cx^3$ , calculated by the no linear statistical model through the SAS statistical program [18] and SigmaPlot 10.0 statistical package [19]. Regression analysis was used to select the equation expressing the highest significance to a maximum of the second grade using SigmaPlot 10.0 [19] for variables analyzed at R1 growth stage.

### 3. Results and discussion

#### 3.1. Photosynthetic parameters

Photosynthetic parameters (*A*, *C<sub>i</sub>*, *E*, *g<sub>s</sub>*) were decreased as glyphosate rate increased. Photosynthetic rates (*A*) before glyphosate (22 DAE) were between 10 and 11 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (Fig. 1A and B; Table 1). These values are very similar to the values of *A* (11–12 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) reported by Procópio et al. [20] at 39 DAE for *Glycine max* and *Phaseolus vulgaris*. This range of value are considered optimal for this vegetative phase [21]. However, both single and sequential applications of glyphosate (24 DAE) decreased *A* values at rates above 1200 g a.e. ha<sup>-1</sup> (Fig. 1A and B; Table 1). Two days after glyphosate application (26 DAE), *A* was between 6 and 0 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for doses of 1200 and 2400 g a.e. ha<sup>-1</sup> with the single application (Fig. 1A; Table 1), and between 7 and 4 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for the same doses applied sequentially (Fig. 1B; Table 1). Values for *A* in plants that did not receive glyphosate were similar to those found before glyphosate was applied. At 35 DAE, *A* for single applications was still lower than for the corresponding sequential application. After the second sequential glyphosate application (36 DAE), *A* decreased further; however, it remained higher than the single application (Fig. 1A and B; Table 1). At the R1 growth stage (58 DAE), the value for *A* for a single glyphosate application was lower than for sequential applications and ranged between 5 and 11 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for the single application and 8–11 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for sequential application, respectively (Fig. 1A and B; Table 1).

Similar to *A*, *g<sub>s</sub>* and *E* were also reduced by glyphosate (Fig. 1C–F; Table 1). Previous studies demonstrated that photosynthetic parameters (*A*, *E*, *g<sub>s</sub>*) were severely affected by glyphosate in different maturity group cultivars of GR soybeans growing in different



**Fig. 2.** Fluorescence ( $F_0$ ), maximal fluorescence ( $F_m$ ) and the ratio of variable fluorescence to maximal fluorescence ( $F_v/F_m$ ) under the steady state condition in GR soybean as affected by increasing rates of glyphosate applied either singly or in split applications ( $n = 8, P < 0.01$ ).

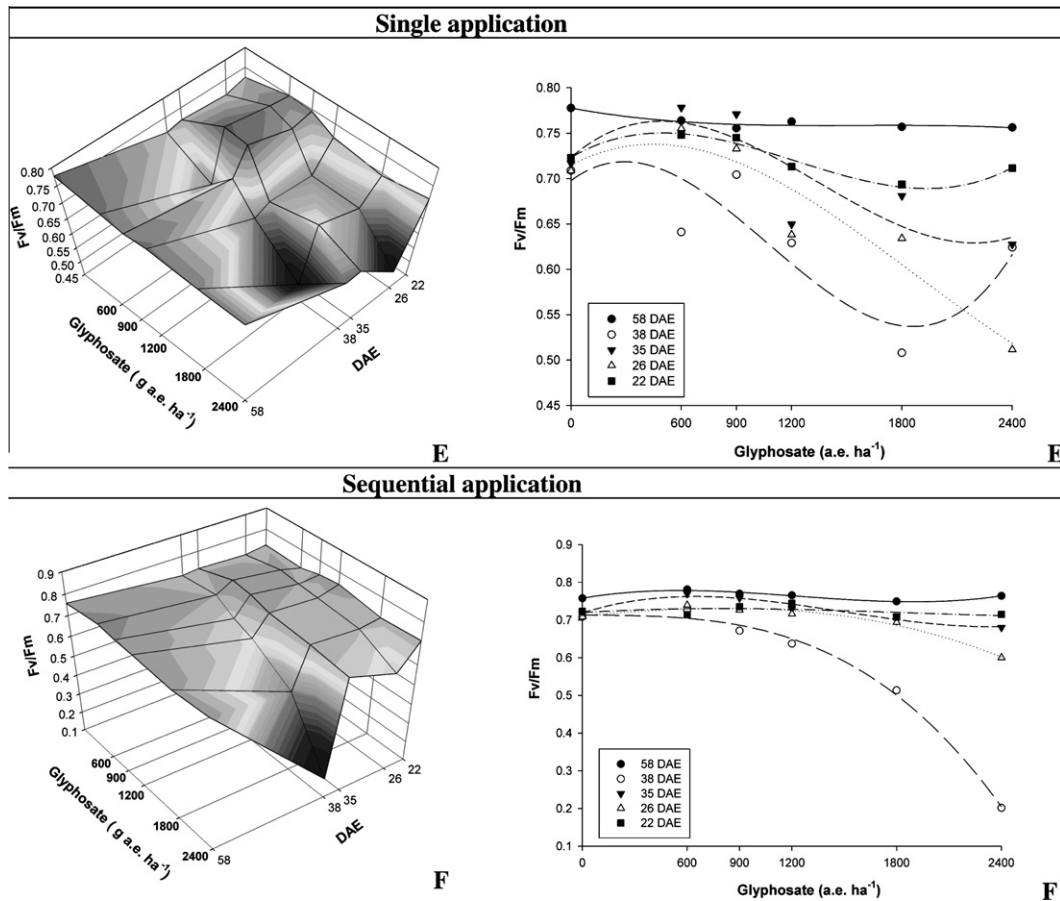


Fig. 2 (continued)

soils; however, there were no differences between the non-treated GR soybeans and their respective near-isogenic non-GR parental lines [6]. Stomatal closure is an important factor contributing to depressed  $\text{CO}_2$  assimilation [22] and stomata also respond to  $\text{CO}_2$  as stomatal conductance decreases as  $\text{CO}_2$  concentration increases [23,24]. Magalhães Filho et al. [25] also reported that a partial stomatal closure leads to decreased stomatal conductance ( $g_s$ ) and consequently increases sub-stomatal  $\text{CO}_2$  ( $C_i$ ). As stomatal conductance declined with increasing rates of glyphosate, a sharp decrease was observed for transpiration rate (E), photosynthesis rate (A),  $\text{CO}_2$  assimilation and carboxylation efficiency (A/ $C_i$ ). This led to a considerable increase in  $C_i$  after glyphosate application (Fig. 1G and H; Table 1) which was most pronounced with the single application at 250–400 vpm at 26 DAE (Fig. 1G; Table 1). The increase in  $C_i$  after the first glyphosate application (26 DAE) was lower for sequential applications than after the single application (Fig. 1G and H; Table 1) although the second glyphosate application further increased  $C_i$  (Fig. 1H; Table 1). At the R1 growth stage,  $C_i$  was lower for plants receiving a sequential relative to those receiving a single application of glyphosate (Fig. 1G and H; Table 1).

With decreased A and increased  $C_i$ , the carboxylation efficiency (A/ $C_i$ ) was extremely affected by both application regimes (Fig. 1I and J; Table 1), and was proportional to glyphosate rate applied. At the R1 stage, plants not treated with glyphosate had an almost 6× higher carboxylation efficiency than glyphosate treated plants (Fig. 1I and J; Table 1) as reflected in increased dry biomass production because diffusion of  $\text{CO}_2$  to the chloroplast is essential for photosynthesis. The cuticle that covers the leaf is nearly impermeable to  $\text{CO}_2$  so that the main port of entry of  $\text{CO}_2$  into the leaf is the sto-

matil pore from which  $\text{CO}_2$  diffuses through the pore into the substomatal cavity and into the intercellular air spaces among mesophyll cells [26].

### 3.2. Fluorescence

Chlorophyll fluorescence is a measure of photosynthetic efficiency and plant productivity [27]. It can be used as a tool to study several aspects of photosynthesis because it reflects changes in thylakoid membrane organization and function. Changes in fluorescence are associated with plants treated with herbicides that inhibit amino acid synthesis [28] or the respiratory pathway [29].

Light energy used to drive photosynthesis can be dissipated as heat or re-emitted as light at a longer wavelength, the latter process is known as fluorescence [30,27]. Before glyphosate application (22 DAE), measurements with a chlorophyll fluorometer under steady state conditions showed that the arbitrary units of fluorescence ( $F_o$ ) were between 430 and 530 (Fig. 2A and B; Table 2); however, after a single glyphosate application (24 DAE),  $F_o$  decreased proportionately with all rates of glyphosate (Fig. 2A and B; Table 2).  $F_o$  decreased until 38 DAE, after which  $F_o$  apparently recovered as the R1 growth stage was reached (58 DAE). For a single application, apparent glyphosate injury was more pronounced than with sequential applications of a comparably rate because  $F_o$  units were between 300 and 200 for the 1200 and 2400 g a.e.  $\text{ha}^{-1}$  for a single application at 38 DAE (Fig. 2A; Table 2), respectively, and between 350 and 300 for the 1200 and 2400 g a.e.  $\text{ha}^{-1}$  applied sequentially, (Fig. 2B; Table 2).  $F_o$  also decreased after the first sequential application (24 DAE) from 430 and 530 to 360 and 390 arbitrary units (Fig. 2B; Table 2). After

**Table 2**

Regression statistics and correlations for fluorescence ( $F_o$ ), maximal fluorescence ( $F_m$ ) and the ratio of variable fluorescence to maximal fluorescence ( $F_v/F_m$ ) in GR soybean treated with different rates of glyphosate applied as a single treatment or sequential, half-rate applications.

DAE	Estimation of model parameters adjusted				$R^2$
	$y_0$	$a$	$b$	$c$	
<b>Fig. 2A</b>					
58	433.28	-0.1361	9.77E5	-2.41E8	0.98*
38	459.46	-0.2982	0.0002	-4.93E8	0.99*
35	443.00	-0.2322	0.0001	-3.61E8	0.97*
26	443.74	-0.0887	1.85E5	7.83E11	0.96*
22	423.13	0.0515	4.71E5	-2.16E8	0.97*
<b>Fig. 2B</b>					
58	434.57	-0.1498	0.0001	-2.54E8	0.99*
38	457.49	-0.2202	0.0001	-2.46E8	0.98*
35	446.50	-0.2096	9.69E5	-1.53E8	0.99*
26	447.36	-0.1418	8.16E5	-1.40E8	0.98*
22	431.40	0.1382	-9.80E5	2.52E8	0.88*
<b>Fig. 2C</b>					
58	1800.01	-0.3131	0.0001	-3.05E8	0.99*
38	1553.62	-0.9219	0.0003	-3.20E8	0.97*
35	1592.22	0.1440	-0.0007	1.81E7	0.98*
26	1546.74	0.3851	-0.0008	2.16E7	0.96*
22	1529.21	0.9614	-0.0008	1.88E7	0.99*
<b>Fig. 2D</b>					
58	1796.64	-0.0880	-0.0002	6.00E8	0.97*
38	1584.39	-0.5217	-4.99E5	2.43E8	0.99*
35	1589.78	0.0395	-0.0005	1.37E7	0.99*
26	1544.35	-0.1661	2.28E5	-2.24E8	0.97*
22	1533.90	0.6932	-0.0005	1.16E7	0.95*
<b>Fig. 2E</b>					
58	0.77	-3.77E5	2.42E8	-5.12E12	0.96*
38	0.69	0.0002	-3.02E7	9.36E11	0.88*
35	0.72	0.0002	-2.19E7	5.44E11	0.86*
26	0.71	0.0001	-1.39E7	2.48E11	0.96*
22	0.72	0.0001	-1.50E7	4.11E11	0.97*
<b>Fig. 2F</b>					
58	0.76	8.05E5	-9.28E8	2.51E11	0.98*
38	0.71	-5.08E6	6.87E9	-3.87E11	0.99*
35	0.72	0.0001	-1.47E7	3.29E11	0.97*
26	0.71	4.51E5	-1.78E8	-8.25E12	0.98*
22	0.72	3.03E5	-2.46E8	4.41E12	0.82*

\* ( $n = 8$ ,  $P < 0.01$ ).

the second application (36 DAE),  $F_o$  remained at 310–390 arbitrary units, suggesting that plants exposed twice to glyphosate did not recover from herbicide injury until reaching the R1 stage. Maximal fluorescence ( $F_m$ ) showed the same tendency as  $F_o$ . Before glyphosate was applied (22 DAE), there was no difference between the plants (Fig. 2C; Table 2) regardless of subsequent application regime ( $1550 < F_m < 1850$ ). Nevertheless, the single glyphosate application (24 DAE), decreased  $F_m$  proportional to the applied rate of glyphosate (Fig. 2C; Table 2). A similar response to sequential applications occurred, in which  $F_m$  decreased from 1800 and 1890 to 1500 and 950 for the lowest and highest rates, respectively.

The effective PS2 quantum yield represents the plant's capacity to convert photon energy into chemical energy once steady state electron transport has been achieved [11]. Thus, considering that glyphosate affected  $F_o$  and  $F_m$ , the ratio of variable fluorescence to maximal fluorescence ( $F_v/F_m$ ) was also affected by glyphosate, although there was a different behavior observed between the application schemes. With a single glyphosate application,  $F_v/F_m$  decreased at all glyphosate rates (1200, 1800 and 2400 g a.e. ha<sup>-1</sup>)

24 DAE and continued to decrease through 38 DAE. Plants then appeared to initiate a recovery; however, the decline continued until the R1 stage (Fig. 2E; Table 2).  $F_v/F_m$  was also affected by sequential glyphosate applications; however, glyphosate injuries were lower than observed with the higher rates of the single applications (Fig. 2F; Table 2). Horton et al. [31] concluded that decreases in  $F_v/F_m$  are associated with increased excitation energy quenching in the PSII antennae and are generally considered indicative of "down regulation" of electron transport, which is reflected in lower photosynthesis rates.

### 3.3. Chlorophyll content

Chlorophyll content generally increases as plants grow (Fig. 3A and B; Table 3), but is affected by glyphosate. Although chlorophyll is not affected immediately after glyphosate application, a reduction in chlorophyll is observed within 2 days after glyphosate application (26 DAE), with the greatest decline observed 38 DAE. Effects were greatest with rates  $\geq 1200$  g a.e. ha<sup>-1</sup>. This decrease could be due to direct damage of the chloroplast [32–34] in the presence of glyphosate. Zobiolo et al. [6] also noticed that plants from all maturity groups exposed to a single or sequential application of glyphosate frequently had chlorophyll concentrations lower than plants that were not exposed to this herbicide.

### 3.4. Water absorption

Changes in water absorption were observed during soybean development (Fig. 4A and B; Table 4). Glyphosate treated plants absorbed approximately the same volume of water until 40 DAE after which the final volume of water absorbed by plants decreased proportional to glyphosate rate. The difference between plants that were not sprayed with those which received the highest glyphosate rate was about 6.3 L per plant. This difference for sequential applications was about 5.0 L per plant. The influence of glyphosate on total water absorbed was about 0.3 L per plant lower with the single application compared with the sequential applications until the R1 stage (Fig. 5). It is known that plants respond to water stress and regulate transpiration by decreasing stomatal conductance. Although this decrease reduces photosynthetic potential, possible dehydration of cells and tissues is avoided [35].

The plasma membrane is the key structure which can be disrupted by external factors (e.g., freezing and thawing or chemical agents) thus the water transport is abruptly diminished [36]. Glyphosate must pass through the plasma membrane and enter the symplast to cause phytotoxicity [37]; thus, plasmalemma disruption by glyphosate leading to decreased water absorption may not explain the data in Fig. 4.

Plants can sense water availability near the root and respond by chemically signaling the shoot [38,39]. Such signals include abscisic acid (ABA), which is produced under drought stress by roots and transported through xylem via transpiration to the leaves where it decreases stomatal conductance and consequently reduces leaf expansion [38–41]. Because this research was conducted in nutrient solution which eliminated drought stress, other factors might decrease water absorption, including interference of glyphosate with photosynthetic parameters (Figs. 4 and 5).

Since aquaporins are integral membrane proteins that form water-selective channels across the membrane [26], aquaporin activity in root or leaf membranes may affect changes in conductance and transpiration [42,43]. The molecular mechanisms leading to aquaporin opening or closing are not well understood, although a number of essential amino acid residues have been identified and several structural motifs have been predicted that may be related to aquaporin activity [44]. A possible mechanism suggested for pH-dependent gating involves low pH (cytosol acido-



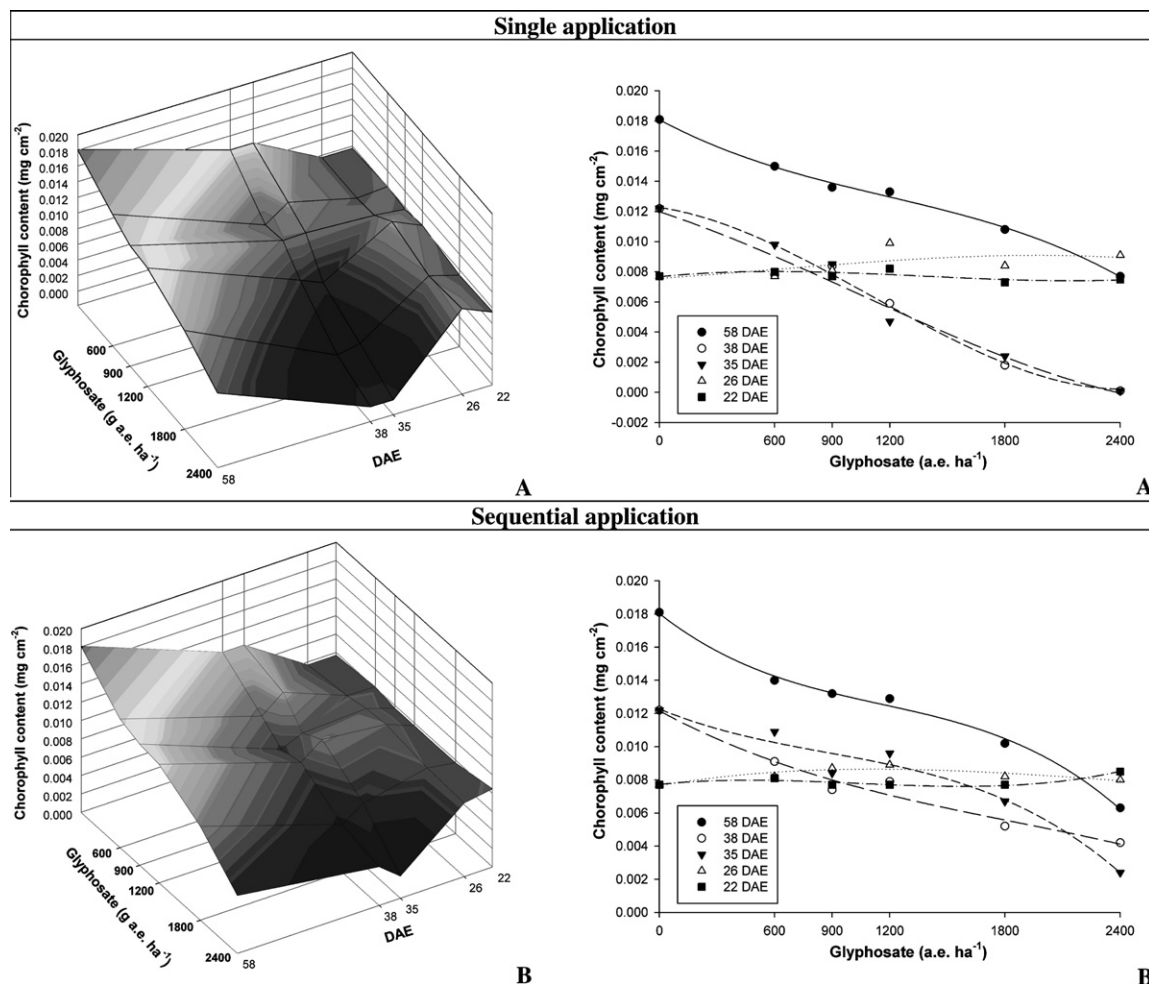


Fig. 3. Chlorophyll content ( $\text{mg cm}^{-2}$ ) in GR soybean as affected by increasing rates of glyphosate applied either singly (A) or as a split application (B) ( $n = 8$ ,  $P < 0.01$ ).

Table 3

Regression statistics and correlations for chlorophyll content ( $\text{mg cm}^{-2}$ ) in GR soybean treated with different rates of glyphosate applied as a single treatment or sequential, half-rate applications.

DAE	Estimation of model parameters adjusted				$R^2$
	$y_0$	$a$	$b$	$c$	
<i>Fig. 3A</i>					
58	0.0181	-6.84E6	3.24E9	-9.18E13	0.99*
38	0.0120	-4.17E6	-1.56E9	5.07E13	0.98*
35	0.0123	-1.89E6	-4.63E9	1.38E12	0.99*
26	0.0076	8.57E7	3.12E10	-1.79E13	0.80*
22	0.0077	1.27E6	-1.36E9	3.32E13	0.85*
<i>Fig. 3B</i>					
58	0.0180	-9.11E6	5.68E9	-1.64E12	0.99*
38	0.0122	-6.20E6	2.09E9	-3.75E13	0.98*
35	0.0123	-4.76E6	3.00E9	-1.14E12	0.98*
26	0.0076	1.99E6	-1.16E9	1.59E13	0.92*
22	0.0077	1.19E6	-1.66E9	5.42E13	0.96*

\* ( $n = 8$ ,  $P < 0.01$ ).

sis) in which aquaporin activity is reduced [45,46,43]. In humans, other compounds can inhibit aquaporin water permeability, such as mercurial compounds ( $\text{HgCl}_2$ ) [47] or tetraethylammonium [48]. More research is needed to understand the relationship between glyphosate, water absorption and aquaporin.

### 3.5. Biomass production and plant height

Any chemical that alters leaf metabolism will affect the level of intermediates and/or activity of enzymes of the Calvin cycle [28]. Decreased  $\text{CO}_2$  assimilation may decrease biomass and carbohydrate accumulation [25]. As glyphosate rates increased, both root and shoot were affected, probably by additive effects on photosynthesis and water absorption. Plants receiving the sequential application were less affected than those receiving the single application of glyphosate (Figs. 6–8).

Shibles and Weber [49] reported that total biomass production by soybean fundamentally depends on energy supplied by photosynthesis. Photosynthetic organisms use solar energy to synthesize carbon compounds and, with adequate leaf area, carbon production is optimized with this input of energy [26]. Thus, the reduction in all photosynthetic parameters and the decrease in water absorption in GR soybeans observed at the R1 stage (Figs. 1–4; Tables 1–4), long after herbicide application, suggests that either glyphosate or its metabolites may have long term effects on physiology of the plant. Several previous reports suggest that glyphosate molecules can remain in plants until complete physiological maturity [50,51,4,5].

Similarly, plant height was decreased by increasing glyphosate rates with a single application affecting plant height more than sequential applications of the same rate (Fig. 8).

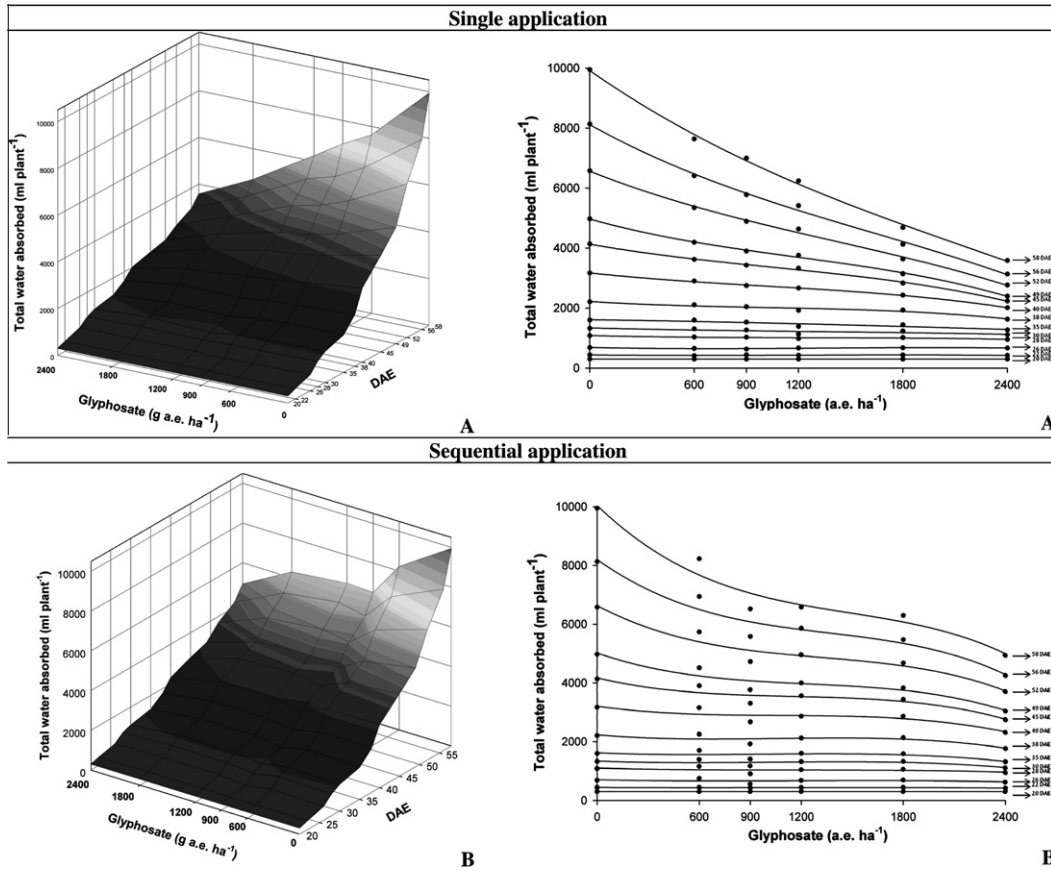


Fig. 4. The affect of glyphosate applied singly or sequentially to GR soybeans on total water absorption at different growth stages ( $n = 8, P < 0.01$ ).

Table 4

Regression statistics and correlations for total water absorption at different growth stages in GR soybean treated with different rates of glyphosate applied as a single treatment or sequential, half-rate applications.

DAE	Estimation of model parameters adjusted				$R^2$
	$y_0$	$a$	$b$	$c$	
<i>Fig. 4A</i>					
58	9919	-4.0458	0.0009	-1.34E7	0.99 <sup>*</sup>
56	8117	-3.2284	0.0009	-1.90E7	0.99 <sup>*</sup>
52	6566	-2.3128	0.0007	-1.66E7	0.99 <sup>*</sup>
49	4971	-1.6345	0.0007	-2.02E7	0.99 <sup>*</sup>
45	4134	-1.0350	0.0004	-1.38E7	0.99 <sup>*</sup>
40	3174	-0.6240	0.0003	-9.26E8	0.99 <sup>*</sup>
38	2221	-0.3712	0.0003	-8.29E8	0.98 <sup>*</sup>
35	1614	-0.0827	-2.81E5	2.57E9	0.93 <sup>*</sup>
30	1340	-0.1447	5.70E5	-1.41E8	0.89 <sup>*</sup>
28	1086	-0.1475	0.0001	-2.72E8	0.94 <sup>*</sup>
26	688	-0.1312	0.0001	-3.18E8	0.89 <sup>*</sup>
22	439	-0.0530	5.84E5	-1.62E8	0.80 <sup>*</sup>
20	301	-2.04E17	1.19E20	2.21E25	0.99 <sup>*</sup>
<i>Fig. 4B</i>					
58	10048	-5.5940	0.0032	-7.13E7	0.98 <sup>*</sup>
56	8206	-4.1330	0.0025	-6.19E7	0.97 <sup>*</sup>
52	6624	-3.0358	0.0019	-4.86E7	0.97 <sup>*</sup>
49	5021	-1.9991	0.0014	-3.75E7	0.96 <sup>*</sup>
45	4178	-1.3877	0.0011	-3.14E7	0.94 <sup>*</sup>
40	3209	-0.8089	0.0007	-2.20E7	0.90 <sup>*</sup>
38	2237	-0.5102	0.0006	-1.74E7	0.88 <sup>*</sup>
35	1624	-0.2465	0.0003	-1.07E7	0.81 <sup>*</sup>
30	1346	-0.2422	0.0003	-9.73E8	0.80 <sup>*</sup>
28	1101	-0.1651	0.0001	-3.84E7	0.82 <sup>*</sup>
26	701	-0.1294	0.0001	-3.55E8	0.80 <sup>*</sup>
22	442	-0.0337	3.85E5	-1.18E8	0.83 <sup>*</sup>
20	302	-2.04E17	1.19E20	2.21E25	0.99 <sup>*</sup>

<sup>\*</sup> ( $n = 8, P < 0.01$ ).

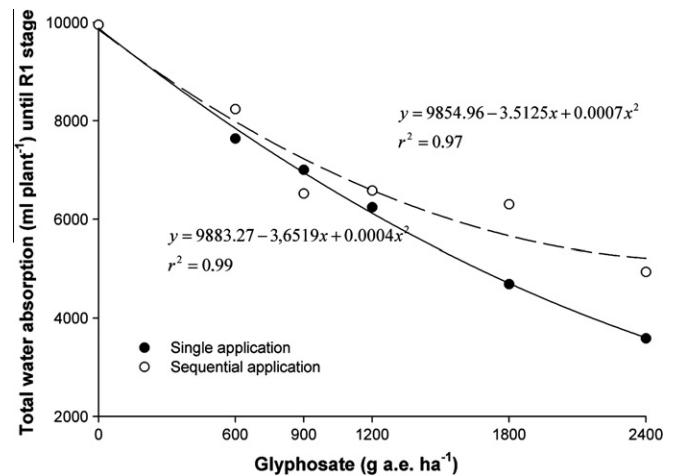


Fig. 5. Total water absorption of GR soybean plants from 0 to 58 DAE after treatment with glyphosate. Data represent the average of eight independent replicates.

### 3.6. Water use efficiency

Agronomic WUE can be determined by the relationship of dry matter produced to water consumption by the crop [52,53,36]. WUE was severely reduced as glyphosate rates increased (Fig. 9). In fact, the volume of water that non-treated GR soybean plants required to produce 1 g of dry biomass is 204% and 152% less than required when the plant is exposed to 2400 g a.e. ha<sup>-1</sup>, either in a single or sequential applications (Fig. 9). Since glyphosate is

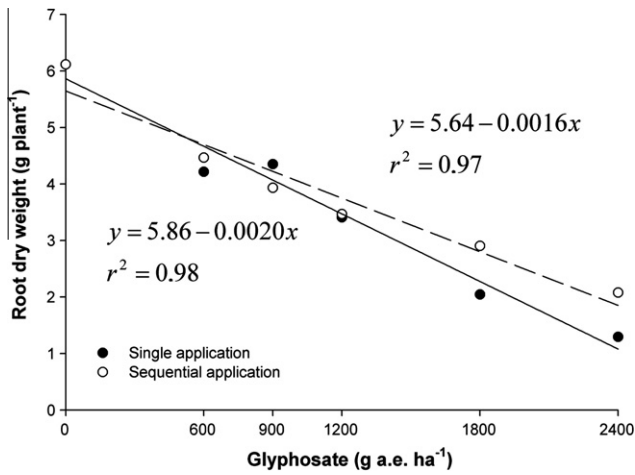


Fig. 6. Root dry biomass of GR soybeans at the R1 growth stage after treatment with glyphosate. Data represent the average of eight independent replicates.

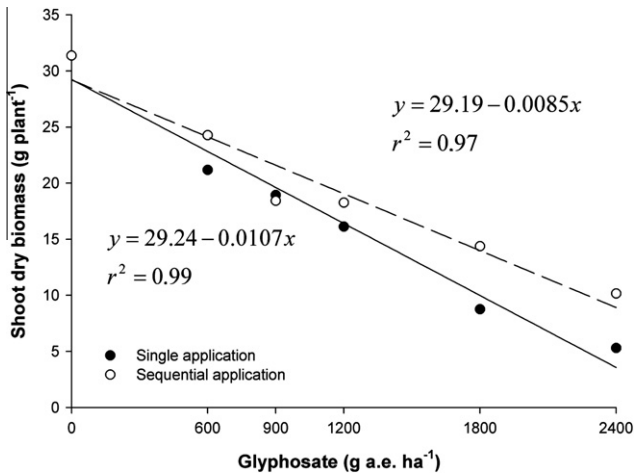


Fig. 7. Shoot dry biomass of GR soybeans at the R1 growth stage after treatment with glyphosate. Data represent the average of eight independent replicates.

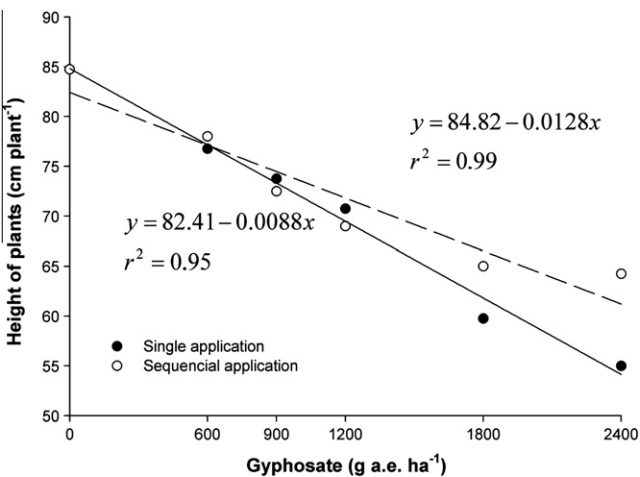


Fig. 8. Height of GR soybean plants at the R1 growth stage after treatment with glyphosate. Data represent the average of eight independent replicates.

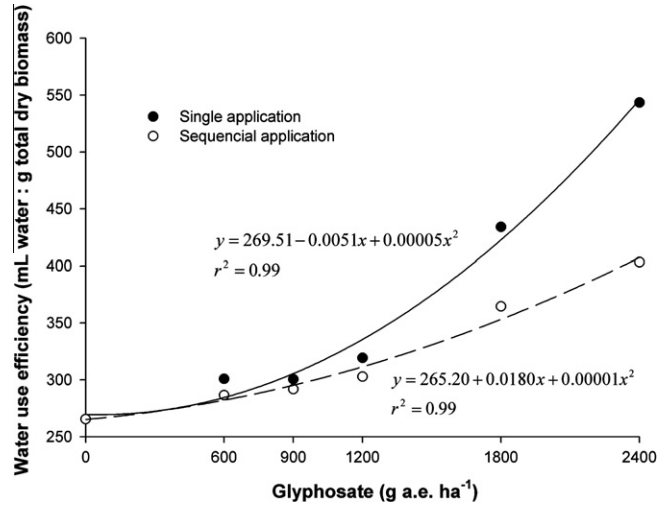


Fig. 9. Water use efficiency (WUE) of GR soybean after treatment with glyphosate. Data represent the average of eight independent replicates.

particles in the field or in greenhouse container studies [58,59]; thus, lower injury might be observed for GR soybean under field conditions.

GR soybean plants receiving a single application of the currently recommended rates of glyphosate (600–1200 g a.e. ha<sup>-1</sup>) needed 13–20% more water to produce the same amount of dry biomass than non-glyphosate treated plants. Although the effect is less pronounced with sequential applications at lower rates, application of glyphosate at recommended rates to GR soybean required from 8% to 14% more water to produce the same dry biomass as plants without glyphosate (Fig. 9).

The negative effects of glyphosate on photosynthetic indexes and chlorophyll content parallel visual symptoms described as “yellow flashing” in previous reports [60,61]. Decreased water absorption caused by glyphosate observed in this study could explain the decreases in shoot mineral concentration and biomass production of cv. BRS 242 GR treated with glyphosate in the study by Zobiolo et al. [6]. Effects of glyphosate or its metabolites on WUE explains why GR soybean plants treated with glyphosate are more sensitive to drought and less efficient in converting water into biomass compared to GR plants that do not receive glyphosate.

#### 4. Conclusions

As glyphosate rates increased, photosynthetic parameters, water absorption and biomass production decreased drastically, consequently photosynthesis and water use efficiency of GR soybean were strongly affected by glyphosate. Regarding potential climate change in the future, GR soybeans with increased transpiration might impact the input of water required for soybean production.

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exuded from plants into the rhizosphere [54–57], and considering that this study was conducted in nutrient solution, soybean plants may have been subjected to a continuous re-absorption of glyphosate or its metabolites. Exuded glyphosate may be sorbed to soil

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