EFFECT OF INCREASING DOSES OF GLYPHOSATE ON WATER USE EFFICIENCY AND PHOTOSYNTHESIS IN GLYPHOSATE-RESISTANT SOYBEANS

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Abstract The increase cultivated area of GR soybeans across different regions of the world have raised questions not yet answered as related secondary effects of glyphosate on physiology of GR soybeans. In this work, we attempted to measure the effect of increasing glyphosate doses on water absorption and photosynthetic parameters of soybean, cv. BRS 242 GR. Plants were grown in completed nutrient solution (Hoagland & Arnon, 1950) in a greenhouse equipped with an evaporative cooling system (25–35 : 20–22□C day/night) under natural daylight. The commercially formulated isopropylamine salts of glyphosate was sprayed on foliar doses of 450, 675, 900, 1350 and 1800 g. e.a ha^{-1} either in single application at four leaf stage (24 DAE) and in sequential application (24 and 36 DAE) (50%-50%) at four and five leaf stages, respectively. Using an infrared gas analyzer (IRGA), fluorometer (pulse-modulated) and chlorophylometer, before and after herbicide application net photosynthesis (A), transpiration rate (E), stomatal conductance (gs), sub-stomatal CO_2 (Ci), carboxilation efficiency (A/Ci), fluorescence (Fs), maximal fluorescence (Fms); yield of quantum efficiency (Y=Fm-Fs/Fms) and chlorophyll contents were monitored. In addition, water absorption was measured daily and biomass yield and water use efficiency (WUE) were estimated by harvesting plants at R1 stage. All measures of photosynthetic parameters (A, E, gs, Ci) and Fs, Fms and Y were affected by increasing glyphosate doses. Chlorophyll contents were reduced right after glyphosate use, however with decreased in chlorophyll content and carboxilation efficiency, (A/Ci) the values suggest that glyphosate might have interfering, in some way, in the synthesis of chlorophyll and/or in carboxilative metabolism of photosynthesis (Calvin cycle), which contributed to dry biomass reductions. Total amount of water absorbed and biomass production by plants were also decreased as glyphosate doses increased, with a more intense effect of single application, as compared to sequential. WUE was also significantly reduced with increasing glyphosate doses. Under single and sequential application, soybean plants need 13 to 20% and 8 to 14% more water to produce the same amount of biomass, respectively.

Keywords GR soybean; glyphosate; water; photosynthesis

Introduction

Increasing cropped area in the world, especially transgenic crops, and with the effects of climate change, increasing atmospheric CO_2 and surface air temperature (Houghton et al., 2001), probably an important concern in the near future will be the water requirement of crops. Knowledge of water requirements by crops and their water use efficiency (WUE) is important for assessing effects of climate changes on crop water balance and water resources. It is expected that the predicted main changes in the global climate, like the increase in CO_2 and temperature, may lead to increases in transpiration rate by plants, which could impact dramatically the input of water required for crop production (Allen et al., 2003).

Many farmers have noticed that some GR soybeans are more sensitive to water stress; others have reported visual injuries in some GR soybean varieties after use of glyphosate (Zablotowicz & Reddy, 2007). 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 that is, responsible for the biosynthesis of aromatic amino acids, plant defense mechanisms, and phenolic compounds (Singh et al. 1991; Hernandez et al. 1999).

The enormous adoption of GR technology and the importance of glyphosate in weed control in the world, not much has been done so far to understand eventual effects of glyphosate in GR soybean physiology, especially those related to water absorption and photosynthesis, which are the basic processes for biomass production. A deeper understanding of such questions may lead to a better use of this technology. The objective of this research was to evaluate water absorption and photosynthesis in GR soybeans under increasing doses of glyphosate.

Materials and Methods

Growth conditions and cultivation practices

The experiment was conducted in greenhouse equipped with an evaporative cooling system (25–35 : 20–22^oC day/night) under natural daylight conditions at the State University of Maringá, Paraná Stata, Brazil, between July 22th and September 20th, 2008 (location: 23° 25' S, 51° 57' W). Seeds were sterilized for 2 min in 2% NaClO and placed to germinate in paper roll (germitest). Seedlings with 5 cm of root length, were transplanted into pots containing nutritive solution. Experimental units were polyethylene pots with 3.7 dm^3, kept constantly aerated. For the first 10 days, the plants were kept in complete nutritive Hoagland & Arnon (1950) solution, diluted to 1/6 of the usual concentration; in the next two weeks they were grown in solution diluted to 1/3 and after that they were grown under total usual concentration. Solutions were exchanged every 10 days and its volume completed daily with distillate and deionized water. During water replacement, the total volume of water absorbed per pot was recorded. The pH of the solutions was maintained at 5.8 ± 0.2 with additions of NaOH and HCl.

Glyphosate application

The pots were placed outside the greenhouse to spray the commercially formulated isopropylamine salts of glyphosate (360 g a.e. L^{-1}) treatments, using a backpack sprayer with SF110.02 nozzles, under constant pressure of CO_2 (2 kgf cm^{-2}) and sprayed at 190 L ha^{-1}. Environmental conditions during the applications were air temperature between 25 and 29 °C, relative air moisture between 80 and 89%, wind speed between 5 and 10 km h^{-1} and open sky without cloudiness. After herbicide applications, the pots were returned to greenhouse. The sprayed solution did not cause run-off from leaves.

Measurement of response variables

In different phenological stages (V3 – 22 DAE; V4 – 26 DAE; V7 – 35 DAE; V8 – 38 DAE and R1 – 58 DAE) and also immediately before and after application of glyphosate, the photosynthetic parameters: net photosynthesis (A), transpiration rate (E); stomatal conductance (gs) and sub-stomatal CO_2 concentration (Ci) were evaluated using an infrared gas analyzer (IRGA), and and the chlorophyll index (CI) was measured using a Minolta SPAD-502.

At R1 stage, accumulated water absorption was recorded and all photosynthetic parameters were measured. After these assessments, shoot and root system were clipped and all harvested materials were packed in paper bags and dried in air circulation oven at 65 – 70 ^oC until constant weight, in order to determine the dry biomass.

Data analysis

The experiment was accomplished in a randomized blocks design, in a factorial scheme (5x2) + 1, with four replicates. Five glyphosate doses (450, 675, 900, 1350 and 1800 g a.e. ha^{-1}) were combined with two application modalities (single and sequential), with an additional treatment with no herbicide application. Single applications were performed at V4 stage (24 DAE, days after emergence), and sequential applications were applied at V4 (24 DAE - 50% of dose) and V7 stage (36 DAE - 50% of dose) of GR soybeans (cv. BRS 242 GR). All data were subjected evaluated using regression analysis, it was chosen the equation from highest significant degree, to a maximum the second grade using the statistical package SigmaPlot 10.0.

Results and Discussion

Photosynthetic parameters

Before glyphosate applications (22 DAE) the photosynthetic rate (A) was between 10 – 11 µmol CO_2 m^{-2} s^{-1} (Figures 1A and 1B). considered optimal for this vegetative phase (Liu et al., 2005). However, after glyphosate applications (24 DAE), A decreased with doses above 675 g a.e. ha^{-1} both for single and sequential applications (Figure 1A and 1B). When these plants reached R1 stage (58 DAE), A in single application was lower than in sequential application, ranging between 5 – 11 µmol CO_2 m^{-2} s^{-1} for the single application and 8 – 11 µmol CO_2 m^{-2} s^{-1} for sequential application, respectively (Figure 1A and 1B). Similar behavior was found for gs and E, which were also reduced by glyphosate applications (Figures 1C, 1D, 1E and 1F). Ci was considerably increased after glyphosate application (Figures 1G and 1H), however this increase was more pronounced for single application. As stomatal conductance declined with increasing doses of glyphosate, a deep decrease is observed for transpiration rate (E), photosynthesis rate (A), CO2 assimilation and carboxilation efficiency (A/Ci) (Figure 1I and 1J).

Chlorophyll content

Chlorophyll contents tend to increase as plants grow (Figure 2A and 2B), but contents are affected by glyphosate use. Although contents are not affected immediately after glyphosate application, expressive reduction of chlorophyll contents are observed starting at 26 DAE (2 days after application) for both modalities, reaching the lowest content at 38 DAE. Effects were more expressed for rates ≥ 900 g a.e. ha-1. This decrease could be due to direct damage of chloroplast (Campbell et al., 1976) by glyphosate presence.

Water absorption

Changes in water absorption were observed during soybean development (Figures 3A and 3B). Until 40 DAE, plants under different glyphosate treatments absorbed approximately the same volume of water, but by the time important differences were found. The final volume of water absorbed by plants was decreased proportionally to glyphosate doses applied. For single applications of glyphosate, the difference between plants that were not sprayed to glyphosate and those which received the highest dose was about 6.3 L per plant and for sequential application about 5.0 L per plant. The influence of glyphosate in total water absorption affected more the single application than sequential application until R1 stage, which comparing the application modalities, consumption by plants in sequential application was about 0.3 L per plant higher than for single application (Figure 4).

Biomass production

As glyphosate doses increased, both root and shoot were affected, probably by additive effects from previous effects in all photosynthetic parameters and water absorption. Plants under sequential application were less affected than those under single application (Figures 5 and 6). Reductions in all photosynthetic parameters and decrease in water absorption in GR soybeans observed at R1 stage (Figures 1, 2 and 3), long after herbicide application, suggests that either glyphosate or its metabolites may have long term effects on the plant's physiology. Many papers have reported that glyphosate molecules can remain in plants until complete physiological maturity (Arregui et al., 2003; Duke et al., 2003; Zablotowicz & Reddy, 2007).

Water use efficiency

WUE agronomic can be determinated through the relationship between dry matter produced and water consumption by crop (Kramer & Boyer, 1995). WUE was severely reduced as glyphosate rates increased (Figure 10). In fact, the amount of water that GR soybean without glyphosate needs to produce 1 gram of dry biomass is 204% and 152% less than the need of plants exposed to 1800 g a.e. ha-1 of single and sequential glyphosate applications (Figure 7).

Using the currently recommended doses (450 to 900 g a.e. ha-1) under single application in GR soybeans, plants needed 13% to 20% more water to produce the same dry biomass than without application. In sequential application this effect is lower, however by using recommended doses GR soybean needs from 8% to 14% more water to produce the same dry biomass as compared to treatment without glyphosate (Figure 7).

Therefore, evidences that glyphosate use lead to negative effects in photosynthetic indexes and chlorophyll contents are in accordance to visual symptoms described as the “yellow flashing” by other authors (Krausz & Young, 2001; Reddy & Zablotowicz, 2003). Effects of glyphosate or its metabolites on WUE lead to the conclusion that GR soybean plants submitted to glyphosate use are less efficient in converting water into biomass, as compared to GR plants that do not receive glyphosate.

Conclusions

Photosynthesis and water use efficiency were strongly affected by glyphosate in GR soybean. With increasing glyphosate doses such effects of decrease in photosynthetic parameters and water absorption are pronounced.

Acknowledgement

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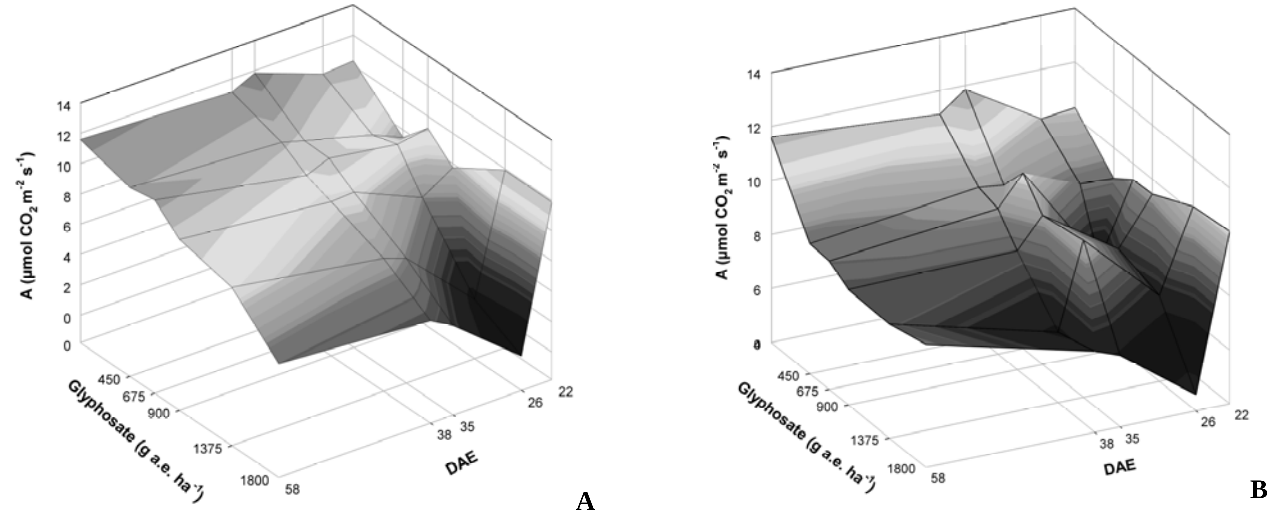
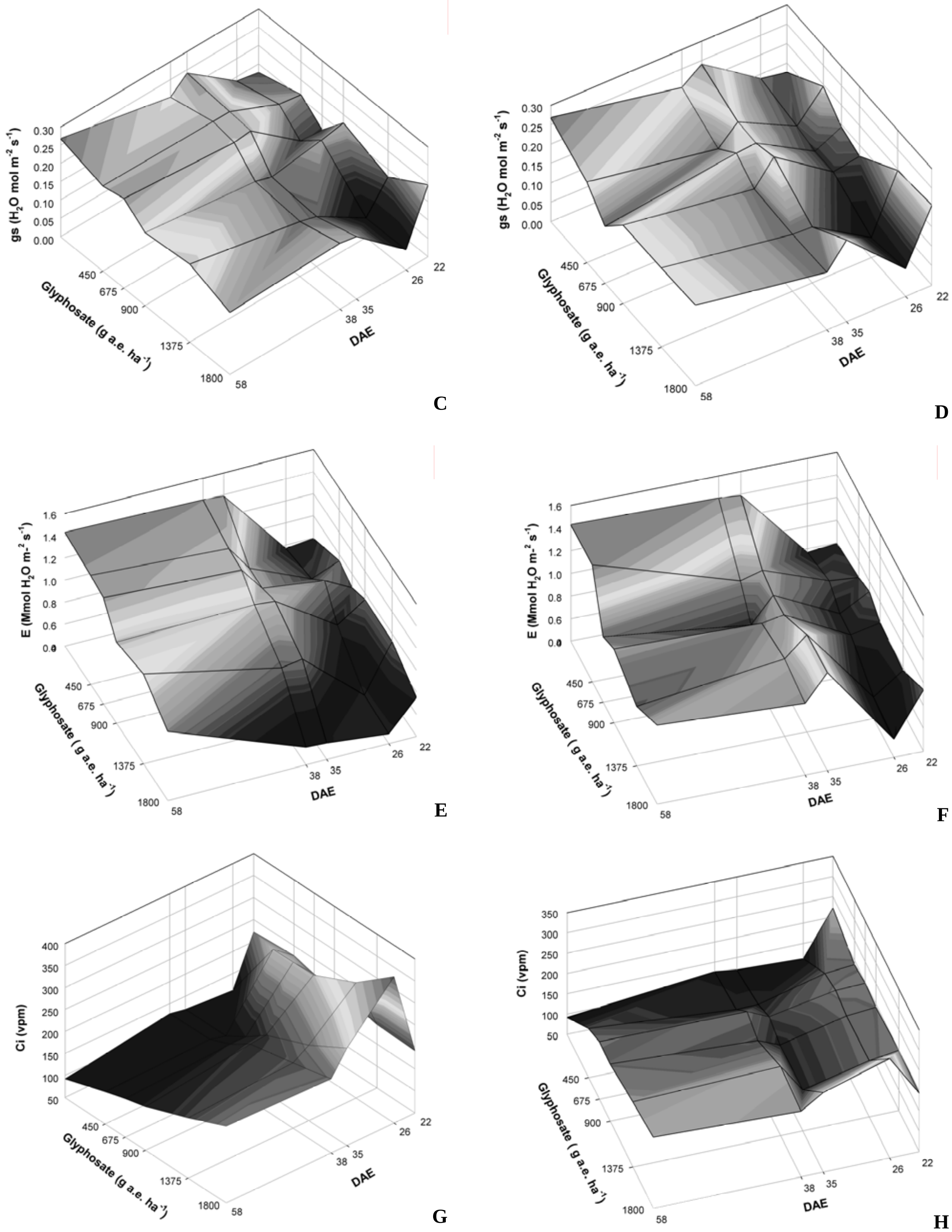
Single application	**Sequential application**





gs (H2O mol m-2 s-1)
0.00 0.05 0.10 0.15 0.20 0.25 0.30
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
C
gs (H2O mol m-2 s-1)
0.00 0.05 0.10 0.15 0.20 0.25 0.30
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
D
E (Mmol H2O m-2 s-1)
0.4 0.6 0.8 1.0 1.2 1.4 1.6
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
E
E (Mmol H2O m-2 s-1)
0.4 0.6 0.8 1.0 1.2 1.4 1.6
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
F
Ci (vpm)
50 100 150 200 250 300 350 400
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
G
Ci (vpm)
50 100 150 200 250 300 350
Glyphosate (g a.e. ha-1)
450 675 900 1375 1800
DAE
58 38 35 26 22
H

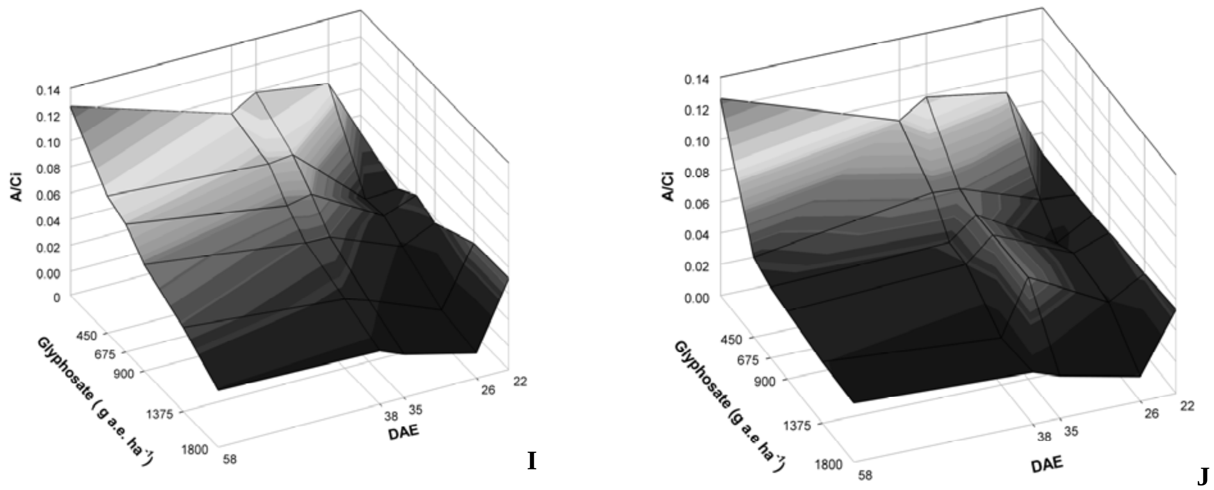


Fig. 1 Photosynthetic rate (A), transpiration rate (E), stomatic conductance (gs), substomatal CO_2 (Ci) and carboxilation efficiency (A/Ci) in GR soybean after application of increasing glyphosate doses in two modalities. Each point represents average of four independent replicates.

Single application	Sequential application

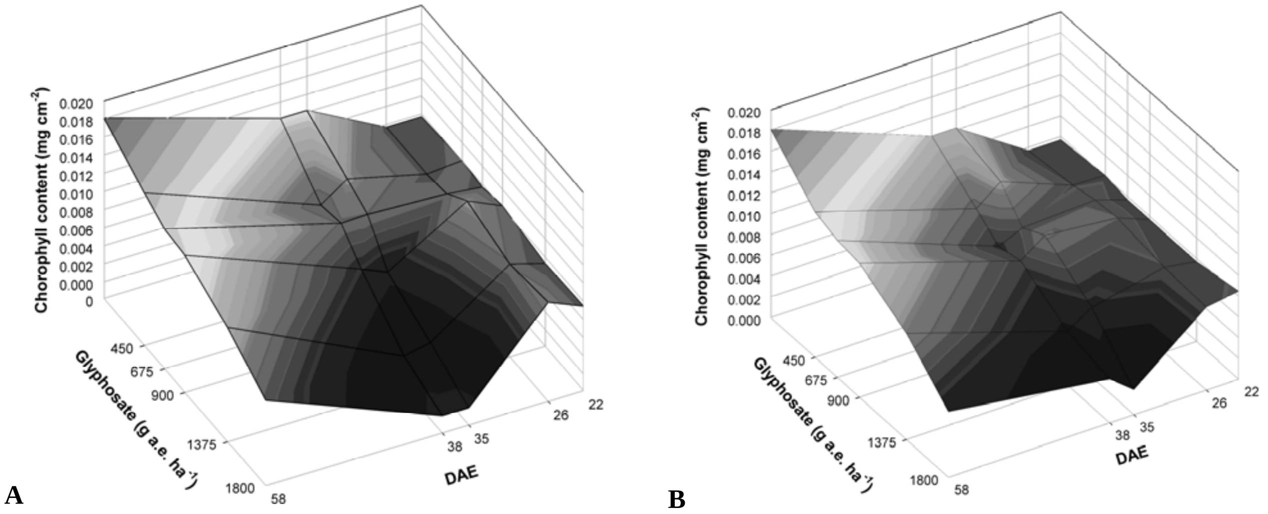


Fig. 2 Chlorophyll content (mg cm^{-2}) in GR soybean under increasing glyphosate doses in two modalities. Each point represents average of four independent replicates.

Single application	Sequential application

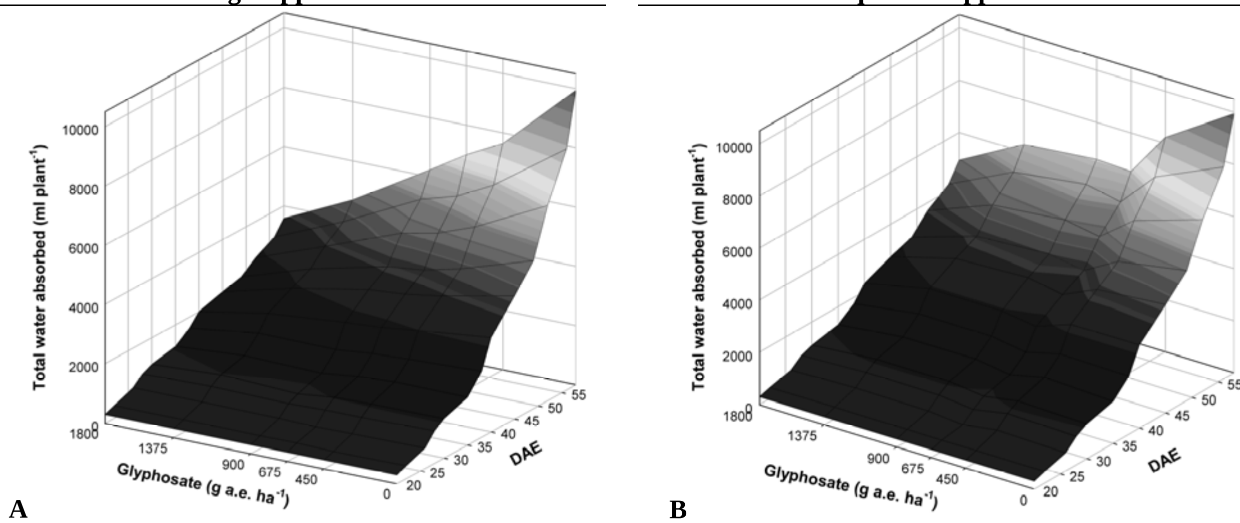


Fig. 3 Total water absorption in different DAE under increasing glyphosate doses Each point represents average of four independent replicates.

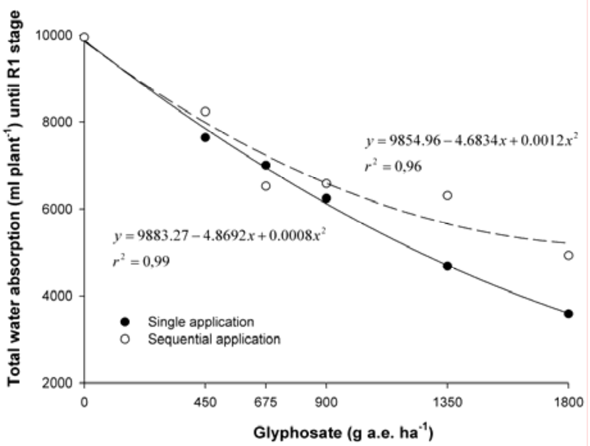


Fig. 4 Total water absorption of GR soybean plants measured from 0 to 58 DAE under different treatments with glyphosate. Data represents average of four independent replicates.

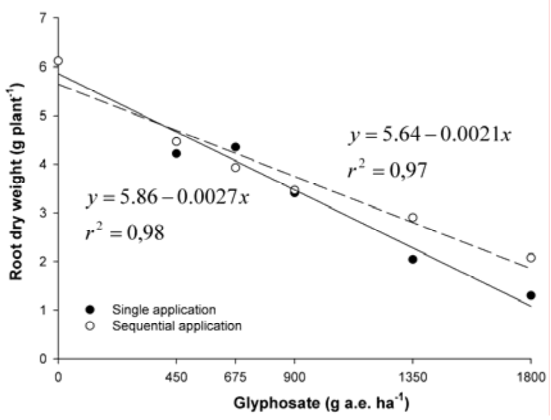


Fig. 5 Root dry biomass at R1 stage under different treatments with glyphosate. Data represents average of four independent replicates.

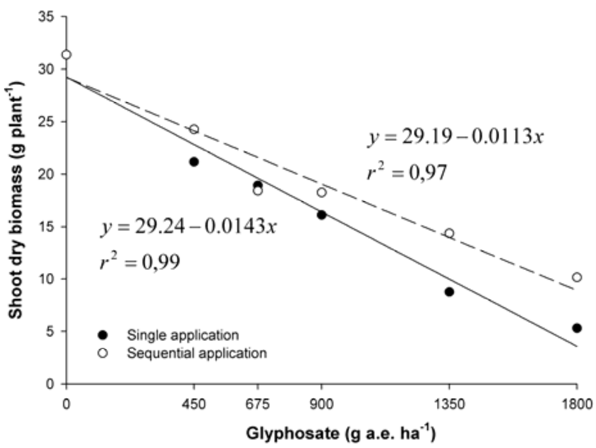


Fig. 6 Shoot dry biomass at R1 stage under different treatments with glyphosate. Data represents average of four independent replicates.

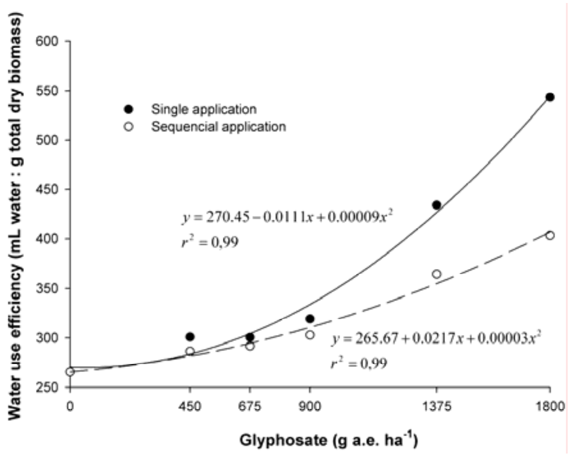


Fig. 7 Water use efficiency (WUE) in GR soybean under different treatments with glyphosate. Data represents average of four independent replicates.