

# Physiological and productive traits of soybean with metsulfuron-methyl application at early desiccation

**Abstract** – The objective of this work was to evaluate the effects of metsulfuron-methyl rates, applied at different times, on the physiological and productive traits of soybean (*Glycine max*) in two crop years, in field conditions, using cucumber (*Cucumis sativus*) as a bioindicator plant, in greenhouse conditions. The experiments were conducted in a randomized complete block, in a 4x5 factorial arrangement, with four replicates. Factor A was the herbicide application time (45, 30, 15, and 0 days before soybean sowing, DBS), and factor B was the metsulfuron-methyl rate (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup> a.i.). The following variables were evaluated: phytotoxicity, gas exchange, chlorophyll *a* fluorescence, and yield components. The highest phytotoxicity in soybean plants is observed after the use of the highest rate of metsulfuron-methyl and with sowing at 0 DBS. The electron transport rate drastically reduces with an increasing metsulfuron-methyl rate and sowing proximity, whereas non-photochemical quenching, acting as a defense mechanism, increases in soybean plants exposed to a herbicide carryover of up to 5.4 g ha<sup>-1</sup>. Metsulfuron-methyl carryover reduces soybean 1,000-grain weight and productivity as a function of application rate and sowing period. For the cucumber plants, negative effects on physiological traits were also verified. An interval of more than 45 days is recommended for sowing soybean after metsulfuron-methyl application for desiccation.

**Index terms:** *Cucumis sativus*, *Glycine max*, herbicide, phytotoxicity.

## Características fisiológicas e produtivas de soja com aplicação de metsulfurom-metílico na dessecação precoce

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos de doses de metsulfurom-metílico, aplicadas em diferentes épocas, sobre as características fisiológicas e produtivas da soja (*Glycine max*), em dois anos agrícolas, em condições de campo, tendo-se utilizado o pepino (*Cucumis sativus*) como planta bioindicadora, em casa de vegetação. Os experimentos foram conduzidos em blocos ao acaso, em arranjo fatorial 4x5, com quatro repetições. O fator A foi o tempo de aplicação do herbicida (45, 30, 15 e 0 dias antes da semeadura da soja, DAS), e o fator B foi a dose de metsulfurom-metílico (0, 3,6, 5,4, 7,2 e 9,0 g ha<sup>-1</sup> i.a.). Foram avaliadas as seguintes variáveis: fitotoxicidade, trocas gasosas, fluorescência da clorofila *a* e componentes de rendimento. A maior fitotoxicidade em plantas de soja é observada após o uso da maior dose de metsulfurom-metílico e com semeadura em 0 DAS. A taxa de transporte de elétrons reduz drasticamente com o aumento da dose de metsulfurom-metílico e da proximidade da semeadura, enquanto a extinção

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Received  
March 23, 2021

Accepted  
July 05, 2021

### How to cite

SILVA, J.D.G. da; GALON, L.; PAWELKIEWICZ, R.; FRANCESCETTI, M.B.; PORTES, J.C.; ROSSETTO, E.R. de O.; PERIN, G.F.; MÜLLER, C. Physiological and productive traits of soybean with metsulfuron-methyl application at early desiccation. *Pesquisa Agropecuária Brasileira*, v.56, e02496, 2021. DOI: <https://doi.org/10.1590/S1678-3921.pab2021.v56.02496>.

não fotoquímica, como mecanismo de defesa, aumenta em plantas de soja expostas ao transporte de herbicida de até 5,4 g ha<sup>-1</sup>. Os resíduos de metsulfurom-metilico reduzem o peso de mil grãos e a produtividade de soja em função da dose de aplicação e do período de semeadura. Para as plantas de pepino, também foram verificados efeitos negativos nas características fisiológicas. Um intervalo de mais de 45 dias é recomendado para a semeadura da soja após a aplicação de metsulfurom-metilico para dessecação.

**Termos para indexação:** *Cucumis sativus*, *Glycine max*, herbicida, fitotoxicidade.

## Introduction

The no-tillage system has been widely used in recent years due to advantages over the conventional system, such as reduced erosion, better structured soil, increased water retention capacity, greater accumulation of organic matter, carbon sequestration in the soil, and greater biodiversity (Perotti et al., 2020). In addition, the viability of this system is causally related to the use of herbicides for weed management and crop desiccation, with glyphosate being the main product used (Benbrook, 2016).

The effectiveness in weed control and widespread use of glyphosate has allowed it to replace other herbicides in agricultural systems, with the possibility of a reduced number of applications, which has favored crop conservation and decreased the demand for alternative herbicides with a greater toxicity (Gilbert, 2013).

However, the dependence on glyphosate as the main method for weed management has caused resistance problems, resulting from inadequate management practices and an increase in the number of applications (Duke, 2012). This is interesting considering that one of the main challenges in chemical weed management has been the evolution of resistant weeds. Weed resistance is responsible for productivity losses, reduced commodity prices, lower land value, and increased management costs (Norsworthy et al., 2012). Furthermore, the use of transgenic crops resistant to herbicides has expanded, while the development of new herbicide molecules, particularly with new mechanisms of action, has decreased in the agrochemical industry (Duke, 2012).

In the no-tillage system, the most suitable method for controlling weeds is management during the off-season and pre-sowing period (Placido et al., 2015).

Early desiccation results in a greater yield in the sowing operation, a lower infestation of crops established in succession, and a higher efficiency of the herbicides applied after emergence (Lamego et al., 2013; Matos et al., 2016).

In this context, the adoption of practices for weed control allows slowing down the evolution of resistance to herbicides. Among the proposed alternatives, stand out herbicide rotation with different mechanisms of action and a residual effect (Santos et al., 2016), as well as soil cover, crop rotation, and the use of quality seed, among others. Herbicides with a longer activity period in the soil provide weed control for a longer time, suppressing new flows and reducing the number of necessary applications (Oliveira et al., 2018). However, herbicide residues in the soil can cause phytotoxicity and affect the growth and development of a crop grown in succession, a process known as carryover (Melo et al., 2016). This can occur with herbicides used for weed control in winter crops and in the management of desiccation, such as metsulfuron-methyl, which can cause damage to subsequent crops if the interval between their application and sowing is not respected, mainly for sensitive species (Rodrigues & Almeida, 2018).

The herbicide metsulfuron-methyl is one of the most used from the group of sulfonylureas, due to its low toxicity to mammals, good selectivity for cultures, and recommendation for application at low rates (Yang et al., 2018). Like other sulfonylurea herbicides, metsulfuron-methyl acts by inhibiting the enzyme acetolactate synthase (ALS), which is responsible for catalyzing two parallel reactions in the biosynthetic pathway of the branched-chain amino acids valine, leucine, and isoleucine that are essential for the growth of plants (Ntoanidou et al., 2016). Producers have used this herbicide for early desiccation to reduce the density of species that are difficult to control, such as grove (*Conyza* spp.), and most of them sow soybean [*Glycine max* (L.) Merr.] after the use of metsulfuron-methyl. Although the interval recommended between the application of metsulfuron-methyl and sowing of soybean is of at least 60 days (Rodrigues & Almeida, 2018), it may differ according to soil characteristics, climate, management, and tolerance of the soybean cultivars. It should be noted that the possibility of using the herbicide in a period closer to soybean sowing would be important to reduce the incidence

of weeds that develop in the off-season and infest the subsequent crop during its emergence.

The objective of this work was to evaluate the effects of metsulfuron-methyl rates, applied at different times, on the physiological and productive traits of soybean in two crop years, in field conditions, using cucumber as a bioindicator plant, in greenhouse conditions.

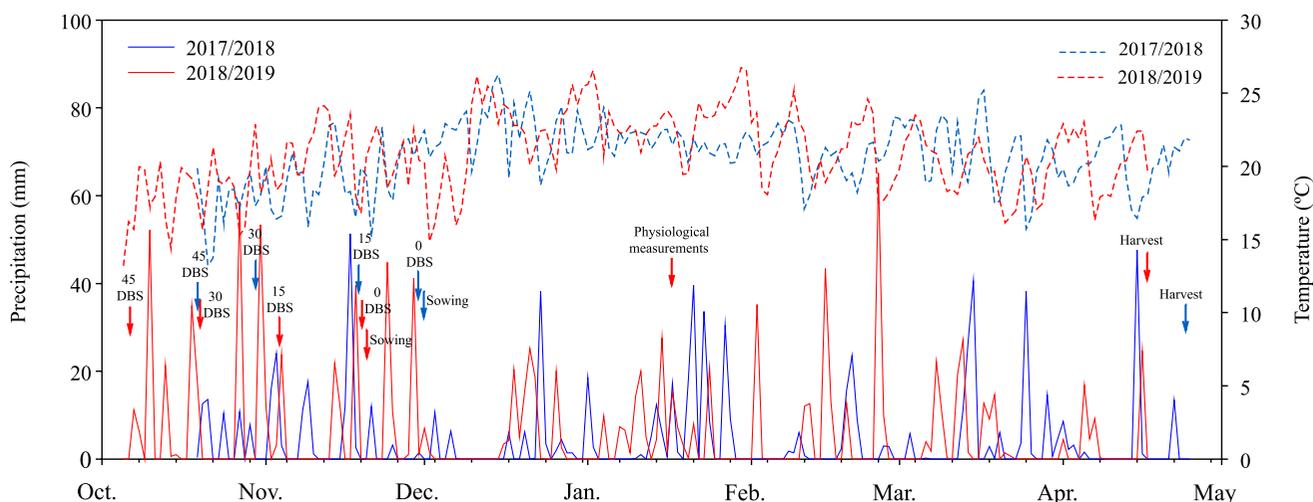
## Materials and Methods

Two experiments were carried out in the experimental area of Universidade Federal da Fronteira Sul, located in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil (27°43'47"S, 52°17'37"W, at 670 m altitude), in the 2017/2018 and 2018/2019 crop years. The climate of the region is of the Cfa type, humid subtropical, according to the Köppen-Geiger classification (Peel et al., 2007), with well-distributed rains throughout the year and hot summers and cold winters. The soil was classified as a Latossolo Vermelho húmico aluminoférrico (Santos et al., 2018b), i.e., a Humic Aluminoferric Ferralsol, with the following physicochemical characteristics: pH 4.7 in water, 3.04% organic matter, 8.5 mg dm<sup>-3</sup> P, 106 mg dm<sup>-3</sup> K, 1.0 cmol<sub>c</sub> dm<sup>-3</sup> Al<sup>3+</sup>, 5.1 cmol<sub>c</sub> dm<sup>-3</sup> Ca<sup>2+</sup>, 3.4 cmol<sub>c</sub> dm<sup>-3</sup> Mg<sup>2+</sup>, effective cation exchange capacity (CEC) of 9.9 cmol<sub>c</sub> dm<sup>-3</sup>, CEC<sub>pH7</sub> of 18.6 cmol<sub>c</sub> dm<sup>-3</sup>,

9.7 cmol<sub>c</sub> dm<sup>-3</sup> H+Al, base saturation of 48%, and 640 g kg<sup>-1</sup> clay. The environmental conditions observed during the experimental period in the two crop years are shown in Figure 1.

The used experimental design was randomized complete blocks, arranged in a 4x5 factorial arrangement, with four replicates. In factor A, the application time of metsulfuron-methyl was tested at 45, 30, 15, and 0 days before sowing of soybean (DBS), and, in factor B, the following herbicide rates were tested: 0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup> a.i. Each experimental unit occupied an area of 15 m<sup>2</sup> (5.0x3.0 m). In the winter period, the area was cultivated with black oat (*Avena strigosa* Schreb.) as a cover crop, in order to simulate the formation of straw in the no-tillage system. Prior to the installation of the experiments, the area was desiccated, providing 7,720.35, 5,431.5, 4,417.75, and 3,483.7 kg ha<sup>-1</sup> dry matter in 2017/2018 and 7,226.0, 5,154.8, 4,273.2, and 4,221.2 kg ha<sup>-1</sup> in 2018/2019 for herbicide applications at 45, 30, 15, and 0 DBS, respectively.

The metsulfuron-methyl herbicide (FMC Química do Brasil Ltda., Campinas, SP, Brazil) was applied using a backpack sprayer pressurized with CO<sub>2</sub>, equipped with the DG 110.02 four-spray boom (Névoa Comercial, Campinas, SP, Brazil), spaced at 50 cm, and calibrated to provide a spray volume of 150 L ha<sup>-1</sup>.



**Figure 1.** Average temperature and precipitation during the experimental periods, in two consecutive soybean (*Glycine max*) crop years (2017/2018 and 2018/2019), in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil. DBS, days before soybean sowing.

The soybean cultivar used in the 2017/2018 and 2018/2019 crop years was NA 5909 RG, which was sown under a no-tillage system. For sowing, a seeder/fertilizer machine with six lines and 50 cm spacing between lines was used, and a density of 280 thousand plants per hectare was adopted. Fertilization was carried out according to the soil analysis, following the technical recommendations for soybean cultivation (Manual..., 2016), using 350 kg ha<sup>-1</sup> of the N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O (05-20-20) fertilizer at sowing in both experiments. When necessary, the crop was managed according to these same recommendations.

In parallel, the effect of metsulfuron-methyl was evaluated on cucumber plants (*Cucumis sativus* L.) of the Pioneiro cultivar, under greenhouse conditions. The greenhouse had an automatic irrigation system, temperature of 25±5°C, and relative humidity of 65±5%, being covered with a 150 µm transparent plastic. The plants were placed apart to avoid shading. The experimental design was randomized complete blocks, with four replicates. The soil used was obtained from the experimental field, showing the previously indicated characteristics. In factor A, the application time of metsulfuron-methyl (45, 30, 15, and 0 DBS) was tested, and, in factor B, the application rate (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup> a.i.). Three cucumber seed were sown in a 0.5 dm<sup>3</sup> pot, constituting an experimental unit. The plants were grown for 21 days.

Phytotoxicity was assessed at 7, 14, 21, 28, and 35 days after emergence (DAE) for field experiments and at 7 and 14 DAE for the greenhouse experiment, using a percentage scale, where zero (0%) represented no damage and one hundred (100%) indicated all dead plants (Velini et al., 1995).

Gas exchange was determined using the LCA PRO infrared gas analyzer (Analytical Development Co. Ltd, Hoddesdon, UK), evaluated on the last completely expanded leaf at the vegetative development stage (V8). Photosynthetic rate (µmol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>), transpiration (mol m<sup>-2</sup> s<sup>-1</sup>), and the ratio between internal and external concentration of CO<sub>2</sub> (C<sub>i</sub>/C<sub>a</sub>, µmol mol<sup>-1</sup>) were obtained between 8:00 a.m. and 11:00 a.m. under environment photosynthetically active radiation (~ 1,000 µmol m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> concentration (C<sub>a</sub>, ~400 µmol mol<sup>-1</sup>), and temperature (~26.5°C).

Chlorophyll *a* fluorescence variables were determined using the OS5p portable modulated

fluorometer (Opti-Sciences, Inc., Hudson, NH, USA). Leaves were initially adapted to the dark for 30 min to obtain initial fluorescence (F<sub>0</sub>), maximum fluorescence (F<sub>m</sub>), and the potential quantum yield of photosystem II (PSII) (F<sub>v</sub>/F<sub>m</sub> = (F<sub>m</sub> - F<sub>0</sub>)/F<sub>m</sub>). Leaves were exposed to illumination and a pulse of saturated actinic light to obtain: steady state fluorescence (F); light-adapted maximum fluorescence (F<sub>m</sub>' ); the effective quantum yield of PSII (Y<sub>II</sub> = (F<sub>m</sub>' - F)/F<sub>m</sub>' ); nonphotochemical quenching (NPQ = (F<sub>m</sub> - F<sub>m</sub>' )/F<sub>m</sub>' ); and the electron transport rate (ETR = Y<sub>II</sub> × PAR × 0.84 × 0.5), where PAR was the flow of photon (µmol m<sup>-2</sup> s<sup>-1</sup>) incidence on the leaves, 0.5 is the value corresponding to the fraction of excitation energy distributed to PSII, and 0.84 is the value corresponding to the fraction of incident light that is absorbed by leaves.

Plant material was collected from the greenhouse at 21 DAE. Cucumber shoots were collected, packed in paper bags, and dried in an oven with forced-air circulation at 65±5°C until the material reached a constant weight to obtain shoot dry matter.

The number of pods per plant and of grains per plant were evaluated in five plants collected randomly, in each experimental unit in the field. The trait 1,000-grain weight (g) was determined by extrapolation after counting samples of 100 grains for each replicate. Yield was evaluated from the grain harvest at 18% humidity in a useful area of 3.0 m<sup>2</sup> for each experimental area. Grain moisture was corrected to 13%, and yield data was extrapolated to kg ha<sup>-1</sup>.

The data obtained from the field experiments were subjected to the factorial analysis of variance by the F-test, at 5% probability, and adjusted to response surfaces, where the herbicide rate was represented on the X axis, the soybean sowing interval after herbicide application was represented on the Y axis, and the analyzed variable was represented on the Z axis. For the greenhouse experiment, when significant differences were detected by the analysis of variance (p≤0.05), means were compared by Duncan's test, at 5% probability. All analyses were performed using the easyanova package of the R, version 3.6.1, software (R Core Team, 2013), and graphs were plotted using the SigmaPlot, version 10.0, software (Systat Software Inc., San Jose, CA, USA).

## Results and Discussion

The occurrence of phytotoxicity was modeled according to the soybean sowing interval after the application of metsulfuron-methyl at different rates, and a response surface was evaluated over time in DAE, in the two crop years (Figure 2 and Table 1). Metsulfuron-methyl is active at very low concentrations and is characterized as slow acting, with a carryover potential that depends on the class and rate of the used herbicide, sensitivity of the crop in succession, soil characteristics, and environmental conditions before and after herbicide application (Ismail & Azlizan, 2002; Santos et al., 2016; Gonçalves et al., 2018; Fraga et al., 2019).

A significant increase in phytotoxicity was observed throughout crop development, with more severe symptoms occurring at a shorter interval between herbicide application and soybean sowing. However, soybean plants in 2017/2018 (Figure 2 A, C, and E and Table 1) were more responsive to herbicide application than in 2018/2019, the following crop year (Figure 2 B, D, and F and Table 1). Metsulfuron-methyl application at the highest rate caused a phytotoxicity of 40% at 7 DAE (Figure 2 A and Table 1), reaching 80% at 35 DAE (Figure 2 E and Table 1) in 2017/2018.

In both crop years, phytotoxicity increased more with the time than with the rate of application. Phytotoxicity was significantly reduced after 15 DBS, whose influence was greater than that of the herbicide rate itself. Leaf area was damaged by up to 83 and 77% in the 2017/2018 and 2018/2019 crop years, respectively, when metsulfuron-methyl was applied at 0 DBS (Table 1). Notably, when the proximity of sowing occurred together with a reduced presence of mulch, the highest rates of phytotoxicity caused by the herbicide were verified. However, this feature should be carefully evaluated. Although straw represents a barrier for the herbicide to reach the soil, it has been recommended to adopt herbicides with a greater solubility and a low vapor pressure and octanol-water partition coefficient (Matos et al., 2016), such as metsulfuron-methyl.

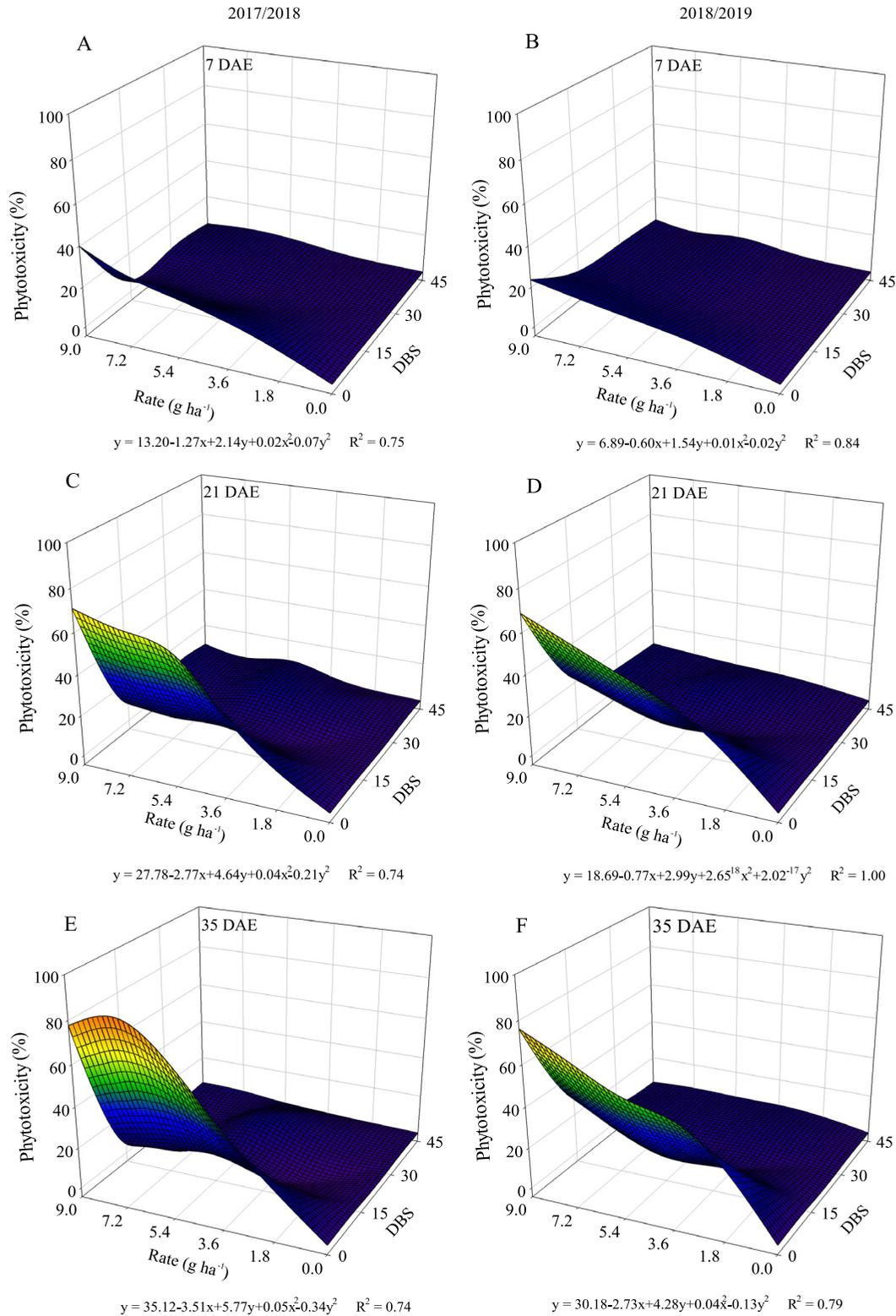
At 15 DBS, it was possible to observe a phytotoxicity of up to 30% in 2018/2019, which gradually decreased with the increase in the interval between herbicide application and soybean sowing. In the same year, the lowest rate of the herbicide, together with the longest interval, resulted in 6.5% leaf damage (Figure 2 F and Table 1). After the application of the herbicide at

soybean pre-sowing, there was an accumulation of rain above 200 and 350 mm in 2017/2018 and 2018/2019, respectively (Figure 1), which may have contributed to the differences noted in the second crop year.

Conversely, the absence of precipitation during sowing and of good soil moisture favored herbicide uptake at 0 DBS, during the two crop years, which caused a high phytotoxicity and a high plant mortality. This result was further aggravated by a slight water deficit in the following two weeks after the emergence of the plants, which affected their metabolism, reduced their growth rate, and accentuated the damage caused to them during the second crop year.

Similarly, other authors have reported phytotoxicity due to the residual effects of ALS-inhibiting herbicides, both in corn (*Zea mays* L.), after 90 days of imazethapyr application (Santos et al., 2018a), and in soybean, after 445 days of imazapyr + imazapic application (Agostinetto et al., 2018). When studying the effects of the pre-emergent application of sulfentrazone on soybean, Aisenberg et al. (2016) found a phytotoxicity above 60% for the NA 5909 RG cultivar, which also affected its productivity. The authors emphasized that phytotoxicity is an important characteristic to be evaluated, even in selective cultures. In many cases, phytotoxicity (Figure 2 and Table 1) can affect yield components and, consequently, final crop yield, as was verified at the 0 DBS interval in both crop years.

Physiological data were modeled by response surfaces (Figure 3). Lower photosynthetic rates were observed at 0 and 45 DBS at all application rates (Figure 3 A and Table 2). Transpiration showed lower values in soybean plants grown when exposed to the highest rates of metsulfuron-methyl at 0 and 15 DBS (Figure 3 B and Table 2). In addition, the  $C_i/C_a$  ratio was lower in soybean plants when the herbicide was applied at 15 and 30 DBS, regardless of the applied rate (Table 2). The lowest values of stomatal conductance were observed at the rate of 7.2 g ha<sup>-1</sup> at 0 DBS and at all rates at the other application intervals – from 15 to 45 DBS. Despite having no mode of action acting directly on photosystems, ALS-inhibiting herbicides have been described as having a secondary or indirect effect on plant physiological processes. A reduction in photosynthetic rates and in the quantum yield of PSII has been reported previously in different plants, such as wheat (*Triticum aestivum* L.) exposed to metsulfuron-methyl (Agostinetto et al., 2016) and sugarcane



**Figure 2.** Phytotoxicity at 7 (A and B), 21 (C and D), and 35 (E and F) days after emergence (DAE) of the NA 5909 R soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>), in two consecutive crop years – 2017/2018 (A, C, and E) and 2018/2019 (B, D, and F) – in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil. DBS, days before soybean sowing.

(*Saccharum* spp.) exposed to trifloxysulfuron-sodium (Galon et al., 2010). The impairment of photosynthesis due to herbicide toxicity has been related to a lower CO<sub>2</sub> fixation, plant development (Su et al., 2018), and crop productivity (Gonçalves et al., 2018).

Associated with gas exchange traits, chlorophyll *a* fluorescence is a quick and noninvasive tool for

determining photosynthetic effects after herbicide exposure. The F<sub>v</sub>/F<sub>m</sub> ratio had a slight reduction at 0 DBS at the highest rate, and photochemical quenching (qP) and the photochemical extinction coefficient (qL) were reduced by about 30% with the increase in metsulfuron-methyl rates, regardless of the application interval (Table 3). Changes in q<sub>P</sub> and q<sub>L</sub> indicate that

**Table 1.** Phytotoxicity at 7, 14, 21, 28, and 35 days after emergence (DAE) of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>), in two consecutive crop years (2017/2018 and 2018/2019), in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil<sup>(1)</sup>.

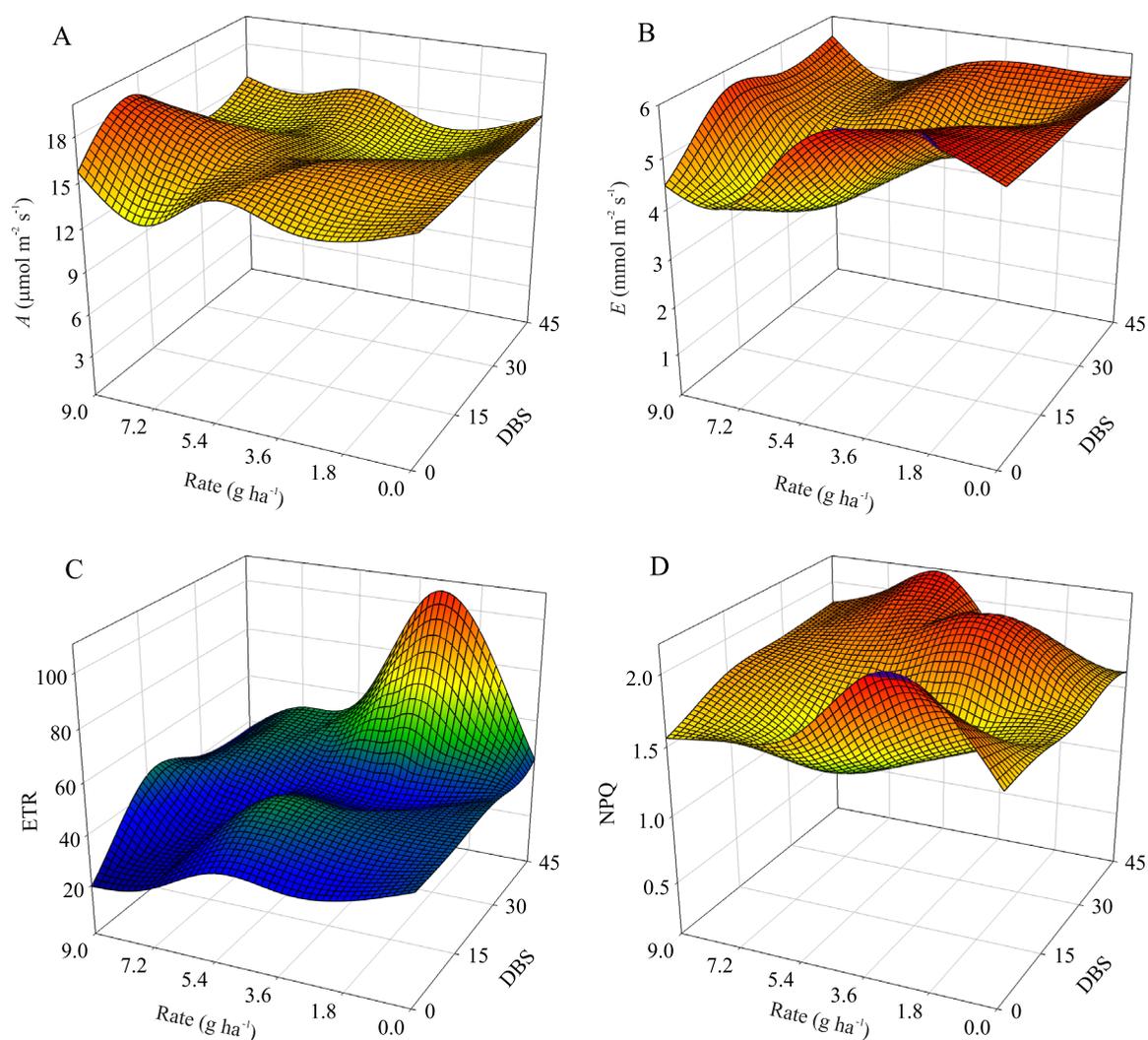
Factor		Phytotoxicity (%) – 2017/2018 crop year					Phytotoxicity (%) – 2018/2019 crop year				
Time (DBS) <sup>(2)</sup>	Rate (g ha <sup>-1</sup> a.i.)	7 DAE	14 DAE	21 DAE	28 DAE	35 DAE	7 DAE	14 DAE	21 DAE	28 DAE	35 DAE
45	0	0.0±0.0aA <sup>2</sup>	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aD	0.0±0.0aB	0.0±0.0aB	0.0±0.0aB	0.0±0.0aA
30	0	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aD	0.0±0.0aC	0.0±0.0aC	0.0±0.0aC	0.0±0.0aA
15	0	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aA	0.0±0.0aE	0.0±0.0aC	0.0±0.0aC	0.0±0.0aD	0.0±0.0aD
0	0	0.0±0.0aD	0.0±0.0aD	0.0±0.0aC	0.0±0.0aD	0.0±0.0aC	0.0±0.0aE	0.0±0.0aD	0.0±0.0aE	0.0±0.0aE	0.0±0.0aD
45	3.6	2.0±1.2bA	3.3±2.0bA	4.0±2.3bA	0.0±0.0bA	1.5±0.9bA	2.8±0.3bC	6.5±0.9bA	5.3±1.0bAB	4.5±2.1bAB	6.0±3.0bA
30	3.6	2.8±1.9bA	1.7±1.2bA	7.0±1.2bA	5.8±2.2bA	4.5±1.7bA	3.0±0.0bC	5.8±0.8bB	5.8±0.8bAB	4.0±0.6bBC	5.8±0.8bAB
15	3.6	5.3±1.0bA	5.0±0.0bA	3.8±2.4bA	6.5±2.2bA	2.5±1.4bA	3.8±0.5bD	7.8±1.0bB	8.0±0.0bB	7.0±1.2bC	8.0±0.0bC
0	3.6	18.3±4.3aC	18.8±5.2aC	34.5±5.9aB	42.5±7.8aC	42.5±10.9aB	12.0±0.0aD	25.5±1.7aC	38.8±3.8aD	41.3±1.3aD	47.5±1.4aC
45	5.4	4.8±1.2bA	1.3±1.3bA	8.8±1.3bA	3.3±2.0bA	2.8±1.0bA	5.5±0.9bAB	7.3±0.8bA	6.3±1.3bAB	6.0±1.2bA	6.3±1.3bA
30	5.4	4.3±1.7bA	3.0±3.0bA	9.5±3.6bA	3.8±2.4bA	10.0±10.0bA	5.3±0.9bB	6.0±1.2bB	9.5±1.0bA	5.8±0.8bBC	5.3±1.0bAB
15	5.4	5.3±2.3bA	3.8±1.3bA	7.5±3.2bA	9.5±5.5bA	8.8±5.9bA	6.0±0.7bC	9.5±0.5bB	8.0±1.8bB	10.0±0.8bC	7.5±1.7bC
0	5.4	24.3±8.7aBC	38.8±10.9aB	58.8±12.1aA	65.0±13.7aB	68.5±15.0aA	15.8±0.8aC	28.8±3.1aC	48.8±3.8aC	50.0±4.6aC	53.8±3.8aC
45	7.2	5.8±2.1bA	7.5±3.2bA	5.8±4.3bA	10.7±5.2bA	5.0±3.5bA	3.8±0.5cBC	10.0±0.8bA	7.8±1.0bA	7.3±1.5cA	6.8±1.3cA
30	7.2	5.3±2.5bA	3.8±2.4bA	5.0±3.0bA	5.8±2.2bA	1.3±1.3bA	5.5±0.9cB	9.0±2.1bAB	8.3±1.2bA	7.8±1.0cB	7.3±0.8cA
15	7.2	6.5±2.0bA	7.5±3.2bA	6.8±2.7bA	10.0±5.0bA	7.8±5.8bA	8.5±0.5bB	10.5±1.0bAB	14.0±4.0bB	17.8±3.1bB	15.8±4.0bB
0	7.2	30.0±11.0aAB	37.5±4.8aB	65.0±7.9aA	72.5±10.3aAB	84.8±3.9aA	19.5±0.5aB	36.3±1.3aB	58.8±2.4aB	63.8±4.3aB	65.0±4.1aB
45	9.0	4.5±0.5bA	10.0±5.0bA	9.3±1.5bA	8.5±2.9bA	5.8±3.3bA	6.8±0.8cA	7.8±1.0cA	9.5±1.0cA	7.5±1.4dA	6.0±1.2cA
30	9.0	6.0±1.2bA	2.0±2.0bA	9.5±1.0bA	4.3±2.8bA	2.0±2.0bA	8.5±0.5cA	13.0±1.7bA	11.0±0.6cA	13.5±1.5cB	8.8±1.3cA
15	9.0	5.8±2.1bA	4.5±1.7bA	8.8±1.5bA	10.0±3.5bA	4.5±2.1bA	10.8±1.5bA	14.3±1.4bA	21.8±6.9bA	30.0±3.5bA	27.8±5.7bA
0	9.0	40.0±8.4aA	51.3±12.1aA	71.0±14.7aA	83.0±7.7aA	77.8±14.3aA	23.8±1.3aA	43.8±3.8aA	68.8±1.3aA	76.8±2.7aA	76.3±4.7aA
45		3.4±0.7b	4.4±1.4b	5.6±1.2b	4.5±1.5b	3.0±1.0b	3.8±0.6c	6.3±0.8c	5.8±0.8c	5.1±0.9c	5.0±0.9c
30		3.7±0.8b	2.1±0.9b	6.2±1.2b	3.9±1.0b	3.6±2.0b	4.5±0.7c	6.8±1.1bc	6.9±0.9c	6.2±1.1c	5.4±0.8c
15		4.6±0.9b	4.2±0.9b	5.4±1.2b	7.2±1.8b	4.7±1.7b	5.8±0.9b	8.4±1.1b	10.4±2.2b	13.0±2.5b	11.8±2.5b
0		22.5±4.3a	29.3±5.2a	45.9±7.1a	52.6±7.7a	54.7±8.2a	14.2±1.9a	26.9±3.5a	43.0±5.5a	46.4±6.1a	48.5±6.2a
0		0.0±0.0C	0.0±0.0C	0.0±0.0C	0.0±0.0C	0.0±0.0C	0.0±0.0E	0.0±0.0D	0.0±0.0E	0.0±0.0E	0.0±0.0D
3.6		7.1±2.0B	7.2±2.2B	12.3±3.7B	13.7±4.7B	12.8±5.1B	5.4±1.0D	11.4±2.2C	14.4±3.7D	14.2±4.1D	16.8±4.6C
5.4		9.6±3.0AB	11.7±4.8AB	21.1±6.3B	20.4±7.5AB	22.5±8.1A	8.1±1.2C	12.9±2.5C	18.1±4.7C	17.9±4.9C	18.2±5.4C
7.2		11.9±3.8AB	14.1±3.9A	20.6±7.0B	24.8±7.7A	24.7±9.2A	9.3±1.6B	16.4±3.0B	22.2±5.6B	24.1±6.1B	23.7±6.4B
9.0		14.1±4.3A	16.9±6.0A	24.6±7.7A	26.4±8.7A	22.5±8.9A	12.4±1.8A	19.7±3.8A	27.8±6.4A	31.9±7.1A	29.7±7.5A
Variation source											
Application time (T)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Rate (R)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T x R		0.0044	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Block		0.03	0.0512	0.1043	0.0256	0.03	0.0863	0.2867	0.0782	0.0413	0.0226
CV (%)		89.43	85.04	64.08	58.13	71.44	18.52	24.71	27.57	22.50	26.65

<sup>(1)</sup>Means followed by equal letters, lowercase letters compare application times and uppercase letters compare the different rates applied, do not differ by Duncan's test, at 5% probability. <sup>(2)</sup>Days before soybean sowing. CV, coefficient of variation.

a lower photochemical dissipation may be associated with a closed proportion of PSII reaction centers, that is, with an increase in the proportion of the reduced state of quinone A and a decrease in the excitation energy available for photochemical processes (Genty et al., 1989). In addition, the reaction centers are constituted by chlorophylls, which have already been reported as being reduced in soybean plants exposed

to the carryover of imazapyr + imazapic, both ALS-inhibiting herbicides (Fraga et al., 2019).

Even at 45 DBS, a reduction in ETR was observed at rates higher than  $3.6 \text{ g ha}^{-1}$  (Figure 3 C and Table 3). The impairment of PSII affects the ETR and the production of adenosine triphosphate and nicotinamide adenine dinucleotide phosphate necessary for Calvin cycle functioning and  $\text{CO}_2$  fixation (Timm et al., 2016). Fraga et al. (2019) also found an increase in lipid



**Figure 3.** Photosynthetic rate ( $A$ ) (A), transpiration ( $E$ ) (B), electron transport rate (ETR) (C), and nonphotochemical quenching (NPQ) (D) of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and  $9.0 \text{ g ha}^{-1}$ ), in the 2018/2019 crop year, in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil. DBS, days before soybean sowing.

peroxidation in soybean leaves, which could cause oxidative stress and the degradation of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme, which fixes atmospheric carbon dioxide within photosynthesis (Das et al., 2016).

Nonphotochemical quenching was greater at 0 DBS than at the lowest application rates. High values were also observed at 45 DBS, regardless of the applied herbicide

rate (Figure 3 D and Table 3). Nonphotochemical quenching acts as a stress suppression mechanism by the thermal dissipation of excess energy absorbed in PSII, through the xanthophyll cycle (Niyogi et al., 1997), aiming to avoid oxidative stress and reductions in photosynthetic rate, which is the primary electron sink, also reducing the potential quantum derived from photochemical reactions. However, the activation

**Table 2.** Photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ), and ratio between internal and external  $\text{CO}_2$  concentration ( $C_i/C_a$ ) of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0  $\text{g ha}^{-1}$ ), in the 2018/2019 crop year, in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil<sup>(1)</sup>.

Factor		$A$	$g_s$	$E$	$C_i/C_a$
Application time (DBS) <sup>(2)</sup>	Rate ( $\text{g ha}^{-1}$ a.i.)	( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	( $\text{mol m}^{-2} \text{s}^{-1}$ )	( $\text{mmol mol}^{-1}$ )	( $\mu\text{mol mol}^{-1}$ )
45	0	15.69±0.62aA <sup>2</sup>	1.37±0.14aA	5.53±0.37aA	0.827±0.022aAB
30	0	15.69±0.62aA	1.37±0.14aA	5.53±0.37aA	0.827±0.022aA
15	0	15.69±0.62aB	1.37±0.14aA	5.53±0.37aA	0.827±0.022aA
0	0	15.69±0.62aA	1.37±0.14aB	5.53±0.37aA	0.827±0.022aA
45	3.6	14.28±1.58aA	1.17±0.05bA	5.51±0.53abA	0.809±0.023abB
30	3.6	13.43±1.16aA	1.45±0.17bA	4.41±0.70bB	0.829±0.025aA
15	3.6	16.05±1.81aAB	1.23±0.08bA	5.10±0.05abAB	0.796±0.029bB
0	3.6	14.51±1.39aA	2.24±0.48aA	6.17±0.43aA	0.816±0.022abA
45	5.4	15.75±1.35aA	1.36±0.16aA	5.20±0.28abA	0.815±0.011aAB
30	5.4	14.55±1.68aA	1.46±0.09aA	4.82±0.15abAB	0.824±0.021aA
15	5.4	15.56±1.58aB	1.40±0.06aA	3.86±0.41bB	0.805±0.028aAB
0	5.4	15.34±1.71aA	1.51±0.20aB	5.58±0.55aA	0.819±0.022aA
45	7.2	14.80±1.27abA	1.30±0.17aA	4.77±0.22aA	0.837±0.020aA
30	7.2	14.09±1.63bA	1.31±0.20aA	4.64±0.29aA	0.824±0.018aA
15	7.2	17.20±0.69aAB	1.30±0.04aA	3.84±0.53aA	0.796±0.027bB
0	7.2	12.95±1.26bA	1.53±0.22aB	4.28±0.64aA	0.832±0.025aA
45	9.0	15.53±1.67bA	1.25±0.08aA	5.57±0.50aA	0.818±0.017abAB
30	9.0	14.46±1.55bA	1.46±0.08aA	5.37±0.25aAB	0.823±0.020abA
15	9.0	18.50±0.78aA	1.29±0.10aA	5.62±0.59aA	0.800±0.029bB
0	9.0	15.72±0.93bA	1.15±0.08aB	4.47±0.93aB	0.834±0.027aA
45		15.21±0.56b	1.29±0.05b	5.32±0.17a	0.821±0.008a
30		14.44±0.58b	1.41±0.06ab	4.95±0.19ab	0.826±0.009a
15		16.60±0.55a	1.32±0.04b	4.79±0.25b	0.805±0.011b
0		14.84±0.54b	1.56±0.13a	5.21±0.29ab	0.826±0.009a
0		15.69±0.32AB	1.37±0.06A	5.53±0.17A	0.827±0.010A
3.6		14.57±0.72B	1.52±0.16A	5.30±0.27AB	0.812±0.011B
5.4		15.30±0.72AB	1.43±0.06A	4.87±0.24BC	0.816±0.010AB
7.2		14.76±0.69AB	1.36±0.08A	4.38±0.22C	0.822±0.011AB
9.0		16.05±0.69A	1.29±0.05A	5.26±0.30AB	0.819±0.011AB
Variation source					
Application time (T)		0.0021	0.0555	0.0994	<0.001
Rate (R)		0.1163	0.3592	<0.001	0.0862
T x R		0.4352	0.0666	0.0125	0.284
Block		<0.001	0.1971	<0.001	<0.001
CV (%)		11.67	23.89	14.24	1.93

<sup>(1)</sup>Means followed by equal letters, lowercase letters compare application times and uppercase letters compare the different rates applied, do not differ by Duncan's test, at 5% probability. <sup>(2)</sup>Days before soybean sowing. CV, coefficient of variation.

of thermal dissipation mechanisms was not sufficient to prevent changes in the electron transport and photosynthetic efficiency of soybean plants, as was also found for cucumber plants exposed to the residual effect of bensulfuron-methyl (Sun et al., 2019).

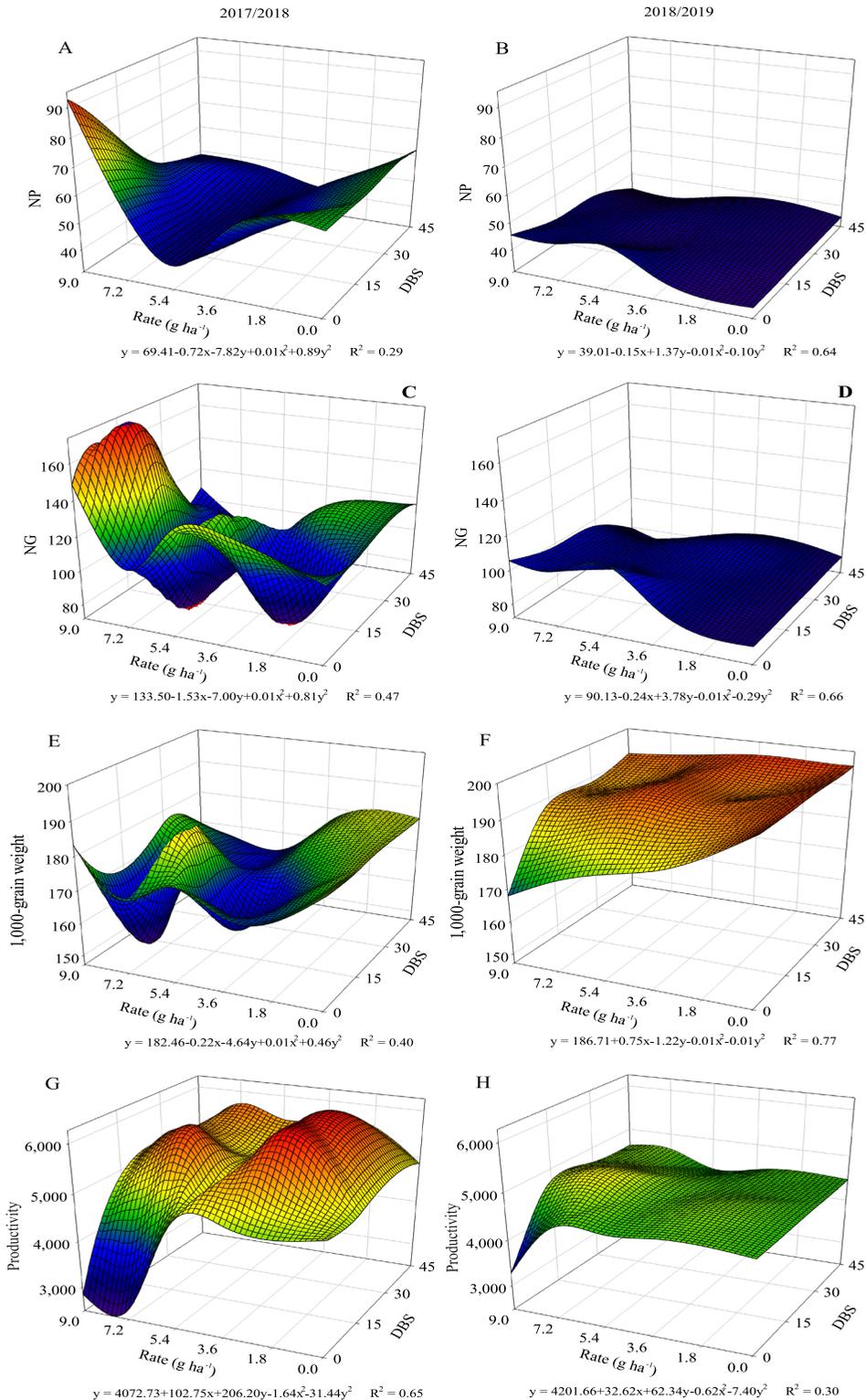
The yield components, in general, were more responsive to herbicide application in 2017/2018. In this crop year, the number of pods and number of

grains were compromised at all application intervals from 0 to 45 DBS and at the metsulfuron-methyl rates from 1.8 to 7.2 g ha<sup>-1</sup> (Figure 4 A and C and Table 4). In 2018/2019, an increase of 35 and 39%, respectively, was observed in number of pods and number of grains between applications at 0 and 15 DBS for rates above 3.6 g ha<sup>-1</sup> (Figure 4 B and D and Table 4). The density of the plants was causally related to soybean yield

**Table 3.** Initial fluorescence (F<sub>0</sub>), potential quantum yield of PSII (F<sub>v</sub>/F<sub>m</sub>), electron transport rate (ETR), quenching photochemical (qP), photochemical extinction coefficient (qL), and nonphotochemical quenching (NPQ) of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>), in the 2018/2019 crop year, in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil<sup>(1)</sup>.

Time (DBS) <sup>(2)</sup>	Rate (g ha <sup>-1</sup> a.i.)	F <sub>0</sub>	F <sub>v</sub> /F <sub>m</sub>	ETR	qP	qL	NPQ
45	0	163.7±5.3aA	0.762±0.026aA	44.2±11.7aBC	0.925±0.131aA	0.623±0.123aA	1.62±0.09aAB
30	0	163.7±5.3aA	0.762±0.026aA	44.2±11.7aA	0.925±0.131aA	0.623±0.123aA	1.62±0.09aA
15	0	163.7±5.3aA	0.762±0.026aA	44.2±11.7aA	0.925±0.131aA	0.623±0.123aA	1.62±0.09aA
0	0	163.7±5.3aA	0.762±0.026aAB	44.2±11.7aA	0.925±0.131aA	0.623±0.123aA	1.62±0.09aAB
45	3.6	145.0±8.6bAC	0.790±0.016aA	99.4±3.3aA	0.898±0.180aA	0.583±0.153aA	1.68±0.24abAB
30	3.6	171.0±9.4aA	0.756±0.027bA	35.6±6.8bA	0.840±0.197aA	0.528±0.148aA	2.08±0.41aA
15	3.6	117.7±5.4cC	0.772±0.028abA	46.8±14.8bA	0.626±0.042aA	0.352±0.036aA	1.62±0.09bA
0	3.6	147.3±8.6bAB	0.778±0.024abA	33.5±11.4bA	0.817±0.097aA	0.527±0.090aA	2.18±0.34aA
45	5.4	137.0±14.8aBC	0.784±0.028aA	56.2±15.7aB	0.758±0.103aA	0.468±0.094aA	2.18±0.07aA
30	5.4	121.0±5.0aB	0.765±0.034aA	39.0±10.3aA	0.674±0.014aA	0.393±0.009aA	1.90±0.30aA
15	5.4	116.7±2.3aC	0.785±0.016aA	47.0±17.5aA	0.720±0.024aA	0.432±0.026aA	1.35±0.07bA
0	5.4	126.5±7.3aBC	0.775±0.019aA	35.2±10.6aA	0.721±0.028aA	0.439±0.024aA	1.73±0.24abAB
45	7.2	161.3±9.9aAB	0.786±0.023aA	50.0±16.3aBC	0.623±0.153aA	0.388±0.123aA	2.00±0.06aA
30	7.2	160.7±12.7aA	0.785±0.018aA	46.1±11.0abA	0.869±0.106aA	0.577±0.107aA	1.82±0.28aA
15	7.2	116.3±1.3bBC	0.795±0.013aA	29.2±6.7abA	0.638±0.028aA	0.359±0.022aA	1.55±0.05aA
0	7.2	111.0±1.5bC	0.783±0.029aA	23.9±1.6bA	0.653±0.047aA	0.375±0.036aA	1.61±0.03aAB
45	9.0	127.7±11.4bC	0.765±0.024aA	26.0±11.9abC	0.636±0.083aA	0.390±0.074aA	1.22±0.45aB
30	9.0	160.0±9.2aA	0.766±0.026aA	39.8±12.7abA	0.731±0.122aA	0.457±0.102aA	1.79±0.22aA
15	9.0	141.3±14.3abAB	0.772±0.016aA	46.9±17.9aA	0.777±0.165aA	0.493±0.140aA	1.76±0.07aA
0	9.0	128.3±10.9bBC	0.742±0.034aB	19.6±0.1bA	0.717±0.016aA	0.432±0.012aA	1.56±0.02aB
45		146.9±5.2a	0.778±0.010a	55.2±7.52a	0.768±0.061a	0.490±0.051a	1.74±0.12ab
30		155.3±5.3a	0.767±0.011a	40.9±4.34bc	0.808±0.055a	0.516±0.047a	1.84±0.12a
15		131.1±5.2b	0.777±0.009a	42.8±5.92b	0.737±0.046a	0.452±0.041a	1.51±0.05b
0		135.4±5.0b	0.768±0.011a	31.3±3.91c	0.767±0.037a	0.479±0.034a	1.74±0.09ab
0		163.7±2.4A	0.762±0.012B	44.2±5.24AB	0.925±0.059A	0.623±0.055A	1.62±0.04A
3.6		145.3±6.1B	0.774±0.011AB	53.8±8.27A	0.795±0.069AB	0.497±0.057B	1.82±0.16A
5.4		125.3±4.4C	0.777±0.012AB	44.4±6.55AB	0.718±0.026B	0.433±0.024B	1.77±0.12A
7.2		137.3±7.1B	0.787±0.010A	37.3±5.46B	0.696±0.051B	0.425±0.044B	1.75±0.08A
9.0		139.3±6.2B	0.761±0.012B	33.1±6.25B	0.715±0.051B	0.443±0.043B	1.58±0.13A
Variation source							
Application time (T)		<0.001	0.2599	<0.001	0.7449	0.7091	0.0514
Rate (R)		<0.001	0.0073	0.0106	0.0122	0.011	0.3375
T x R		<0.001	<0.001	0.0016	0.7621	0.7846	0.0314
Block		0.0419	0.7684	<0.001	0.0021	0.0013	0.0017
CV (%)		11.54	2.85	39.09	26.21	35.95	22.08

<sup>(1)</sup>Means followed by equal letters, lowercase letters compare application times and uppercase letters compare the different rates applied, do not differ by Duncan's test, at 5% probability. <sup>(2)</sup>Days before soybean sowing. CV, coefficient of variation.



**Figure 4.** Number of pods (NP) (A and B), number of grains (NG) (C and D), 1,000-grain weight (g) (E and F), and productivity (kg ha<sup>-1</sup>) (G and H) of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>), in two consecutive crop years – 2017/2018 (A, C, E, and G) and 2018/2019 (B, D, F, and H) – in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil. DBS, days before soybean sowing.

components. As mentioned before, the application of metsulfuron-methyl at 0 DBS caused high plant mortality, changing the density of the plants by area. This reduction in plant population stimulated the phenotypic plasticity of soybean plants, a phenomenon that has been reported to allow plants to change their morphology and regulate their growth and production components according to different densities, emitting more branches and increasing branch growth at low

densities (Ferreira et al., 2016; Balbinot Junior et al., 2018). The authors concluded that number of pods was the main variable associated with phenotypic plasticity, which, in the present study, explains its significant increase in the second crop year, mainly at 0 DBS (Table 4).

Conversely, the 1,000-grain weight showed lower values with the increase of the metsulfuron-methyl rate and proximity to soybean sowing (Figure 4 E and

**Table 4.** Number of pods (NP), number of grains (NG), 1,000-grain weight, and productivity of the NA 5909 RG soybean (*Glycine max*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>), in two consecutive crop years (2017/2018 and 2018/2019), in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil<sup>(1)</sup>.

Factor	Time (DBS) <sup>(2)</sup>	Rate (g ha <sup>-1</sup> a.i.)	2017/2018 crop year				2018/2019 crop year			
			NP	NG	1,000-grain weight (g)	Productivity (kg ha <sup>-1</sup> )	NP	NG	1,000-grain weight (g)	Productivity (kg ha <sup>-1</sup> )
45	0	0	62.1±3.4aA	115.9±16.8aA	180.2±6.0aA	4857.2±158.4aB	35.4±3.3aA	81.9±9.0aA	195.78±1.0aA	4468.0±81.2aA
30	0	0	62.1±3.4aA	115.9±16.8aA	180.2±6.0aA	4857.2±158.4aB	35.4±3.3aA	81.9±9.0aA	195.78±1.0aA	4468.0±81.2aA
15	0	0	62.1±3.4aA	115.9±16.8aAB	180.2±6.0aAB	4857.2±158.4aB	35.4±3.3aA	81.9±9.0aA	195.78±1.0aA	4468.0±81.2aA
0	0	0	62.1±3.4aB	115.9±16.8aA	180.2±6.0aA	4857.2±158.4aA	35.4±3.3aC	81.9±9.0aC	195.78±1.0aA	4468.0±81.2aA
45	3.6	3.6	42.1±3.9aB	94.1±2.7abA	175.1±3.6aA	5662.3±291.5aA	37.9±1.5aA	88.0±3.6aA	196.0±3.4aA	4428.7±120.0aA
30	3.6	3.6	47.8±2.2aAB	109.5±3.3abA	171.1±6.1aA	5920.6±313.3aA	40.4±1.5aA	96.1±3.3aA	191.4±4.4aA	4525.2±60.6aA
15	3.6	3.6	42.6±2.0aB	72.3±9.2bB	182.3±6.9aA	5698.1±145.1aA	39.0±2.5aA	92.5±5.0aA	191.3±6.9aA	4640.2±70.5aAB
0	3.6	3.6	54.7±11.0aB	134.7±25.0aA	169.3±4.2aA	4825.5±229.7bA	40.2±2.2aBC	95.0±3.8aBC	181.8±3.8bB	4527.1±104.4aA
45	5.4	5.4	45.6±1.1aB	80.4±4.0bA	164.3±5.1aA	5474.5±51.9aAB	35.3±1.5bA	82.7±1.3bA	194.7±4.2aA	4310.4±127.6aA
30	5.4	5.4	41.6±3.9aB	80.3±3.8bA	165.1±4.0aA	5099.3±67.2abB	39.9±4.8bA	94.0±12.0bA	193.6±4.9aA	4606.4±89.7aA
15	5.4	5.4	45.3±6.1aB	86.9±9.1bAB	159.9±1.5aC	5868.1±24.2aA	37.93±1.4bA	94.8±8.1bA	193.0±1.6aA	4602.2±34.7aAB
0	5.4	5.4	40.3±5.8aC	131.8±39.9aA	175.7±6.8aA	4700.9±420.4bA	47.9±1.9aA	113.4±3.6aA	178.7±1.2bB	4455.3±289.6aA
45	7.2	7.2	48.1±4.0abAB	87.9±8.9aA	165.6±4.0aA	5730.1±204.2aA	32.3±0.6bA	73.9±2.2bA	193.1±1.1aA	4644.9±77.7abA
30	7.2	7.2	53.1±2.8abAB	107.9±0.6aA	169.6±5.3aA	5498.9±168.2aAB	38.8±1.1abA	90.7±2.1aA	188.9±1.2aA	4721.4±120.3abA
15	7.2	7.2	40.7±4.2bB	110.1±4.6aAB	166.6±2.0aBC	5118.0±62.5aAB	41.4±2.9aA	93.9±5.9aA	189.9±2.6aA	4970.6±84.4aA
0	7.2	7.2	57.5±7.7aB	105.6±12.8aA	169.2±7.0aA	2678.2±305.0bB	45.2±1.4aAB	104.1±4.7aAB	174.5±4.4bBC	4430.6±277.1aA
45	9.0	9.0	48.5±5.3bAB	107.4±4.6abA	169.1±5.9abA	4988.0±223.9aAB	33.6±3.6bA	79.3±8.4bA	192.1±4.4aA	4620.3±165.8aA
30	9.0	9.0	45.4±1.7bB	94.3±5.5bA	171.7±7.2abA	5515.1±76.9aAB	40.5±1.6abA	95.9±3.0abA	188.5±3.0aA	4674.7±92.0aA
15	9.0	9.0	52.7±5.8bAB	127.2±3.0abA	159.8±6.1bC	5671.6±253.0aA	40.9±2.3abA	98.2±5.1aA	188.3±4.6aA	4743.6±65.0aAB
0	9.0	9.0	92.7±3.0aA	148.7±9.2aA	183.3±9.3aA	2832.2±673.5bB	45.4±1.4aAB	105.7±3.0aAB	168.0±4.0bC	3274.3±344.1bB
45			49.3±2.1b	97.1±4.7b	170.8±2.5a	5342.4±116.7a	34.9±1.1c	81.2±2.6c	194.5±1.2a	4494.5±54.8a
30			50.0±2.0b	101.6±4.4b	171.5±2.6a	5378.2±110.6a	39.0±1.2b	91.7±3.1b	191.6±1.5a	4599.1±41.9a
15			48.7±2.6b	102.5±6.0b	169.8±3.0a	5442.6±107.2a	38.9±1.1b	92.2±3.0b	191.7±1.7a	4684.9±47.4a
0			61.4±4.8a	127.3±9.9a	175.5±3.0a	3978.8±279.5b	42.8±1.3a	100.0±3.2a	179.7±2.5b	4231.1±146.5b
0			62.1±1.5A	115.9±7.5AB	180.2±2.7A	4857.2±70.8B	35.4±1.5B	81.9±4.0B	196.0±0.4A	4468.0±36.3B
3.6			46.8±3.0B	102.6±8.4AB	174.5±2.7AB	5526.6±156.3A	39.4±0.9A	92.9±1.9A	190.1±2.5B	4530.3±45.5AB
5.4			43.2±2.2B	94.9±10.8B	166.2±2.6C	5285.7±147.5A	40.3±1.7A	96.2±4.4A	190.0±2.3B	4493.6±80.4AB
7.2			49.8±2.8B	102.9±4.3AB	167.7±2.25BC	4756.3±327.9B	39.4±1.4A	90.6±3.3A	186.6±2.2BC	4691.9±87.8A
9.0			59.8±5.3A	119.4±6.0A	171.0±3.9BC	4751.7±338.9B	40.1±1.5A	94.8±3.5A	184.2±3.0C	4328.2±181.0B
Variation source										
Application time (T)			<0.001	0.006	0.3364	<0.001	<0.001	<0.001	<0.001	<0.001
Rate (R)			<0.001	0.0996	0.0029	<0.001	0.0443	0.0095	<0.001	0.0186
T x R			<0.001	0.2582	0.2418	<0.001	0.2447	0.36	0.0102	<0.001
Block			0.6977	0.6617	0.0038	0.8991	0.2589	0.0131	<0.001	0.2345
CV (%)			18.45	26.62	6.10	10.27	12.69	12.90	2.71	6.46

<sup>(1)</sup>Means followed by equal letters, lowercase letters compare application times and uppercase letters compare the different rates applied, do not differ by Duncan's test, at 5% probability. <sup>(2)</sup>Days before soybean sowing. CV, coefficient of variation.

F and Table 4). In 2018/2019, the lowest 1,000-grain weight was observed with the application of rates above  $3.6 \text{ g ha}^{-1}$ , at 0 and 15 DBS (Figure 4 F and Table 4). In both crop years, productivity was reduced mainly due to the highest rates of metsulfuron-methyl and the shorter interval between its application and soybean sowing, which was attributed to the residual effects of the herbicide in the soil. In 2017/2018, there was a reduction of up to 47% in productivity from 15 DBS onwards (Figure 4 G and Table 4), whereas, in the following crop year, there was a 42% reduction when the highest rate was applied at 0 DBS (Figure 4 H and Table 4). These yield components were also reported to be reduced in soybean plants after exposure to carryover of atrazine, metribuzin, flumioxazin, and a mixture of diuron, hexazinone, and sulfometuron-methyl (Gonçalves et al., 2018). The 1,000-grain weight is sensitive to various stress conditions, which directly affect crop yield (Souza et al., 2015; Comin et al., 2018), and may be greater with a lower number of pods per plant, which reduces competition for photoassimilates (Cruz et al., 2016).

As observed for the phytotoxicity data, there was a reduction in productivity in the shortest interval between herbicide application and soybean sowing (Figure 4 and Table 4). A decrease in yield was also found in soybean plants exposed to the residual effects of carotenoid synthesis- and photosystem-inhibitor herbicides after 42 days of application (Soltani et al., 2011). According to these authors, different herbicides caused injuries from 35% (isoxaflutole and atrazine) to 90% (dimethenamid and dicamba/atrazine) on soybean plants grown afterwards, with a consequent reduction in biomass and crop yield. Therefore, to determine the necessary withdrawal period before sowing soybean, it is necessary to verify the interaction of the herbicide with the soil, whose particles may degrade or absorb it. These processes can last only a few days or even months or years in the case of highly persistent compounds (Filizola et al., 2002). This shows the importance of studies on the residual effects of different herbicides, in order to allow the selection of products less harmful to subsequent crops.

Since metsulfuron-methyl is applied at very low rates, its detection in the soil is difficult. This shows the need of using methods to determine the presence of its compounds in the soil (Marinho et al., 2019), in order to avoid sowing sensitive crops in the same area.

Among the methods used to identify soils contaminated by herbicides, bioindicator plants stand out as being quick and inexpensive, with cucumber being one of the most sensitive species to metsulfuron-methyl (Ismail & Azlizan, 2002).

A moderate increase of ~5% was observed in the phytotoxicity rates of cucumber plants grown after the application of the metsulfuron-methyl herbicide (Table 5). Moreover, there was an interaction between the rate and application interval of metsulfuron-methyl for initial fluorescence and nonphotochemical quenching. The herbicide promoted an increase in initial fluorescence and nonphotochemical quenching of up to 38 and 265% at 15 and 0 DBS, respectively. Initial fluorescence, like nonphotochemical quenching, is activated in adverse situations, specifically by a mechanism that involves the dissipation of excess energy via fluorescence in the antenna complex (Baker & Rosenqvist, 2004). In cucumber plants, the increase in initial fluorescence and nonphotochemical quenching was probably sufficient to mitigate the negative effects of metsulfuron-methyl on the photosynthetic process, since the potential quantum yield of PSII ( $F_v/F_m$ ) and ETR were not altered.

However, unlike instantaneous physiological measurements, plant dry matter represents the accumulated effect of the metsulfuron-methyl residue in the soil. The shoot dry matter of cucumber plants showed a linear reduction of up to 18% with an increasing herbicide rate. A similar response was observed for the application intervals, with a greater reduction in shoot dry matter closer to cucumber sowing (Table 5). Similarly, residual bensulfuron-methyl affected the morphological traits of cucumber plants (Sun et al., 2019).

For the NA 5909 RG soybean cultivar, even though there were no major variations in productivity in the longest interval, the data set considering both experiments in the field does not allow a recommendation for safe sowing in a period equal to or less than 45 days after the application of metsulfuron-methyl. It has been suggested that the soybean crop should be sown at least 60 days after metsulfuron-methyl application since uncontrollable factors, such as environmental conditions, contribute to the process of herbicide persistence and degradation.

**Table 5.** Phytotoxicity at 7 and 14 days after emergence (DAE), initial fluorescence ( $F_0$ ), potential quantum yield of PSII ( $F_v/F_m$ ), electron transport rate (ETR), and nonphotochemical quenching (NPQ) of the Pioneiro cucumber (*Cucumis sativus*) cultivar sown 45, 30, 15, and 0 days after the application of metsulfuron-methyl at different rates (0, 3.6, 5.4, 7.2, and 9.0 g ha<sup>-1</sup>) in the municipality of Erechim, in the state of Rio Grande do Sul, Brazil<sup>(1)</sup>.

Factor		Phytotoxicity (%)		Chlorophylla <i>A</i> fluorescence				
Time (DBS) <sup>(2)</sup>	Rate (g ha <sup>-1</sup> a.i.)	7 DAE	14 DAE	$F_0$	$F_v/F_m$	ETR	NPQ	SDM <sup>(3)</sup> (g)
45	0	0.00±0.00AB	0.00±0.00AC	187.00±3.89AB	0.655±0.032AA	61.91±11.13AA	0.447±0.057AB	0.530±0.022AA
30	0	0.00±0.00AB	0.00±0.00AB	187.00±3.89AB	0.655±0.032AA	61.91±11.13AA	0.447±0.057AB	0.530±0.022AA
15	0	0.00±0.00AC	0.00±0.00AB	187.00±3.89AB	0.655±0.032AAB	61.91±11.13AA	0.447±0.057AB	0.530±0.022AA
0	0	0.00±0.00AB	0.00±0.00AB	187.00±3.89AA	0.655±0.032AAB	61.91±11.13AA	0.447±0.057AB	0.530±0.022AA
45	3.6	2.25±0.25AA	2.33±0.24AAB	232.50±32.34AA	0.662±0.027AA	63.92±6.60AA	1.166±0.081AA	0.433±0.016AAB
30	3.6	3.00±1.22AA	2.00±0.71AAB	206.50±8.17AAB	0.665±0.037AA	66.10±6.73AA	0.478±0.084BB	0.518±0.029AA
15	3.6	2.00±0.71AB	3.75±1.75AA	209.25±9.67AB	0.649±0.040AAB	69.43±7.20AA	0.499±0.034BB	0.498±0.031AAB
0	3.6	2.75±0.48AA	2.75±0.75AA	188.25±5.23AA	0.688±0.021AAB	65.66±4.67AA	0.659±0.024BB	0.526±0.032AA
45	5.4	1.75±0.63BAB	1.33±0.47BBC	238.25±32.82AA	0.665±0.031ABA	60.72±5.63AA	1.122±0.114AA	0.498±0.040AAB
30	5.4	3.00±0.71ABA	3.25±0.63ABA	203.00±12.36AAB	0.702±0.031AA	63.95±4.91AA	0.753±0.025AAB	0.462±0.030AAB
15	5.4	4.75±1.11AA	4.25±1.44AA	195.00±2.48AB	0.631±0.011BB	53.55±7.09AA	0.746±0.075AB	0.364±0.051BC
0	5.4	2.00±0.00BA	2.00±0.71ABAB	209.67±11.55AA	0.680±0.021ABAB	58.40±4.50AA	0.884±0.223AB	0.512±0.038AA
45	7.2	3.50±0.50AA	2.75±0.75AAB	224.25±24.16ABAB	0.635±0.055AA	61.30±9.20AA	1.018±0.142AA	0.475±0.027AAB
30	7.2	4.00±0.58AA	2.50±0.29AA	244.75±18.32AA	0.677±0.022AA	67.13±8.75AA	1.085±0.070AA	0.372±0.013BB
15	7.2	3.00±0.71AAB	3.75±1.44AA	258.50±8.03AA	0.695±0.019AA	72.31±6.03AA	1.198±0.197AA	0.423±0.034ABBC
0	7.2	2.75±1.60AA	2.00±0.71AAB	190.00±6.72BA	0.653±0.017AB	51.94±6.28AA	0.561±0.048BB	0.459±0.029ABA
45	9.0	2.50±0.29AA	4.00±0.58AA	200.75±13.17ABAB	0.668±0.038AA	53.53±3.88AA	0.944±0.328BA	0.399±0.039AB
30	9.0	3.00±0.71AA	3.50±0.50AA	227.50±21.66AAB	0.662±0.025AA	65.32±3.62AA	0.897±0.310BAB	0.418±0.028AB
15	9.0	4.25±0.48AA	3.50±0.65AA	180.00±0.41BB	0.708±0.015AA	61.61±8.76AA	0.768±0.185BB	0.413±0.012ABC
0	9.0	2.33±0.24AA	2.25±0.25AAB	217.33±12.23ABA	0.716±0.034AA	64.94±4.47AA	1.632±0.220AA	0.497±0.065AA
45		2.00±0.31 A	2.08±0.36AB	216.55±10.56A	0.657±0.015A	60.27±3.18A	0.939±0.091A	0.467±0.016AB
30		2.60±0.43 A	2.25±0.35AB	213.75±7.36A	0.672±0.012A	64.88±3.02A	0.732±0.082B	0.460±0.017B
15		2.80±0.48 A	3.05±0.60A	205.95±6.85A	0.668±0.012A	63.76±3.61A	0.732±0.080B	0.446±0.019B
0		1.97±0.38 A	1.80±0.31B	198.45±4.36A	0.678±0.011A	60.60±2.83A	0.837±0.110AB	0.505±0.017A
0		0.00±0.00 B	0.00±0.00B	187.00±1.74C	0.655±0.014B	61.91±4.98A	0.447±0.026C	0.530±0.010A
3.6		2.50±0.35 A	2.71±0.49A	209.13±8.84AB	0.666±0.015AB	66.28±2.90A	0.700±0.077B	0.494±0.016AB
5.4		2.88±0.45 A	2.71±0.49A	211.48±9.28AB	0.669±0.013AB	59.16±2.70A	0.876±0.071AB	0.459±0.023BC
7.2		3.31±0.44 A	2.75±0.43A	229.38±9.79A	0.665±0.016AB	63.17±3.96A	0.965±0.085A	0.432±0.016C
9.0		3.02±0.28 A	3.31±0.28A	206.40±7.82BC	0.689±0.014A	61.35±2.78A	1.060±0.148A	0.432±0.021C
Variation source								
Application time (T)		0.1363	0.0672	0.2062	0.3856	0.6909	0.093	0.0369
Rate (R)		<0.001	<0.001	0.0041	0.2135	0.7297	<0.001	<0.001
T x R		0.3031	0.6058	0.0444	0.1961	0.9171	<0.001	0.0929
Block		0.8446	0.2577	0.1012	<0.001	0.0972	0.2395	0.3357
CV (%)		58.14	65.81	13.95	5.96	23.58	36.66	13.80

<sup>(1)</sup>Means followed by equal letters, lowercase letters compare application times and uppercase letter compare the different rates applied, do not differ by Duncan's test, at 5% probability. <sup>(2)</sup>Days before soybean sowing. <sup>(3)</sup>SDM, shoot dry matter. CV, coefficient of variation.

## Conclusions

1. The use of metsulfuron-methyl has negative effects on the NA 5909 RG soybean (*Glycine max*) cultivar, mainly when applied closer to soybean sowing (0 day before sowing) and at highest rate (9.0 g ha<sup>-1</sup> a.i.).

2. Metsulfuron-methyl drastically reduces the photosynthetic electron transport rate when applied at rates greater than 3.6 g ha<sup>-1</sup> a.i. and in periods less than 45 days from soybean sowing, whereas nonphotochemical quenching increases in soybean plants exposed to a herbicide carryover of up to 5.4 g ha<sup>-1</sup> a.i.

3. Metsulfuron-methyl carryover reduces soybean 1,000-grain weight and productivity as a function of the application rate (higher than 5.4 g h<sup>-1</sup> a.i.) and sowing period (intervals shorter than 15 days after herbicide application).

4. Cucumber (*Cucumis sativus*), used as a bioindicator plant, confirms the negative effects of metsulfuron-methyl on physiological traits, regardless of the rate applied until 45 days before sowing.

5. The use of metsulfuron-methyl is not indicated safely in a period of less than 45 days before soybean sowing, regardless of the applied rate.

### Acknowledgments

To Universidade Federal da Fronteira Sul (UFFS) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for financial support (process numbers PES-2018-0884 and 406221/2016-2, respectively); and to Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Fapergs), to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for fellowships (process numbers Fapergs/Capes 05/2017, Capes/PNPD 88887.352933/2019-00, and CNPq/PQ-ID 306927/2019-5).

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