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## Chemical stress reduces the lateral shoot growth in vernalized garlic

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### ABSTRACT

Brazil's low garlic productivity is attributed, mainly to the lateral shoot growth, a physiological anomaly characterized by early lateral shoot growth of cloves in noble group garlic cultivars. Considering the aforementioned information, the aim was to evaluate the occurrence of lateral shoot growth and productivity characteristics in garlic plants, cv. Chonan, subject to herbicide chemical stress. In order to do so, an experiment was conducted in outlining randomized blocks in a 3x5 factorial scheme, constituted by three herbicides (paraquat 500 g ha<sup>-1</sup>; ammonium glufosinate 400 g ha<sup>-1</sup> and glyphosate 720 g ha<sup>-1</sup>) and five subdoses (0, 5, 10, 15 and 20% of the minimum recommended dose of each herbicide). The electron transport rates (ETR) were evaluated after herbicide application, as well as the culture's morphological and productive characteristics. The chemical stress caused by herbicides paraquat, ammonium glufosinate and glyphosate in underdoses of 25, 40 and 36 i.a. ha<sup>-1</sup>, respectively, was efficient in reducing the electron transport rate and the lateral shoot growth of the Chonan garlic cultivar, and also technically viable in increasing the productivity of commercial bulbs.

**Keywords:** *Allium sativum*, herbicide, electron transport rate.

### RESUMO

#### Estresse químico reduz pseudoperfilhamento no alho vernalizado

A baixa produtividade de alho no Brasil é atribuída, principalmente, ao pseudoperfilhamento, anomalia fisiológica caracterizada pela brotação antecipada dos bulbilhos em cultivares de alho do grupo nobre. Considerando as informações supracitadas, objetivou-se avaliar a ocorrência de pseudoperfilhamento e características de produtividade em plantas de alho, cultivar Chonan, submetidas ao estresse químico de herbicidas. Para isso foi conduzido um experimento em delineamento de blocos casualizados com esquema fatorial 3x5, constituído por três herbicidas (paraquat 500 g ha<sup>-1</sup>; amônio glufosinato 400 g ha<sup>-1</sup> e glyphosate 720 g ha<sup>-1</sup>) e cinco subdoses (0, 5, 10, 15 e 20%, da dose mínima recomendada de cada herbicida). Foram avaliadas a taxa de transporte de elétrons (ETR) após a aplicação dos herbicidas, assim como as características morfológicas e produtivas da cultura. O estresse químico dos herbicidas paraquat, amônio glufosinato e glyphosate nas subdoses de 25, 40 e 36 i.a. ha<sup>-1</sup>, respectivamente, foi eficiente na redução da taxa de transporte de elétrons e do pseudoperfilhamento da cultivar de alho Chonan, assim como tecnicamente viável no aumento da produtividade de bulbos comerciais.

**Palavras-chave:** *Allium sativum*, herbicida, taxa de transporte de elétrons.

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Garlic (*Allium sativum*) is cultivated in most regions of Brazil, both by large and small producers. In Brazil's South, concentrated production is highlighted in the state of Santa Catarina, where cultivation happens with a greater frequency in small family agriculture properties, with low productivity of commercial standard bulbs.

Lateral shoot growth is one of the factors which causes a decrease in Brazil's garlic productivity. This genetic-physiologically caused anomaly is characterized by the early lateral shoot growth of cloves, which not only

reduces the culture productivity, but also depreciates the product, compromising its commercial value (Souza & Casali, 1986). In this sense, several factors have been related to the lateral shoot growth in garlic, such as photoperiod (Wu *et al.*, 2015, 2016), temperature (Wu *et al.*, 2015, 2016), the cultivar (Resende *et al.*, 2011, 2013; Wu *et al.*, 2016), excessive nitrogen (Marouelli *et al.*, 2002; Trani *et al.*, 2008), irrigation (Marouelli *et al.*, 2002), as well as the regulatory action of gibberelins hormones (Vieira *et al.*, 2014; Wu *et al.*, 2016) and jasmonic acid (Wu *et al.*, 2016).

In vernalized noble group garlic cultivars, the lateral shoot growth has been the cause of greatest concern, given they are more sensitive to this anomaly. According to Burba (1983), the vernalization of cloves stimulates the accumulation of cytokinins and gibberelins, resulting in a change of hormonal balance and premature lateral shoot growth. Therefore, one of the alternatives to reduce the lateral shoot growth, used by Brazilian garlic growers, consists of suspending irrigation during the bulbification period, causing a hydric deficit. The plants' response to

the hydric deficit is closing its stomata, consequently reducing the diffusion of CO<sub>2</sub> to the leaf mesophyll, which causes a decrease in photosynthesis (Souza *et al.*, 2001).

The stress in garlic plants induces the production of abscisic acid, which acts as a substrate to gibberellins produced during vernalization, in addition to helping the formation of larger bulbs due to the translocation of solutes (Taiz & Zeiger, 2013).

In Brazil's South, although it possesses the adequate temperature and photoperiod for garlic cultures, rains are frequent during the year and it makes the suspension of irrigation infeasible for promoting hydric stress in bulbification. Thus, the excessive humidity increases in this period, resulting in the lateral shoot growth and compromised productivity in commercial garlic bulbs.

In this context, new technology is needed for supplanting in a field level the necessary hydric stress for good culture yield in environments without a defined dry season. In order to achieve this, the application of herbicides acting in specific metabolic routes, diminishing or inhibiting the production of precursor compounds of important molecules for metabolism, such as hormones, chlorophylls, among others (Salmazo, 2009), may, as a means of chemical stress, contribute to the reduction of the lateral shoot growth in garlic. However, there's scarce literature on information about herbicide use aiming to promote chemical stress in vernalized garlic cultivars, in order to minimize the effects of the lateral shoot growth, without affecting the plant significantly.

Therefore, the aim of this research is to promote stress in a garlic culture, through application of herbicide underdoses in the pre-differentiation of cloves, and evaluating its effects on the occurrence of the lateral shoot growth and the commercial yield of bulbs.

## MATERIAL AND METHODS

The experiment was conducted in field on the Olericulture Sector and

the Vegetable Physiology/Horticulture Laboratory of the Agronomy Department, belonging to the Universidade Estadual do Centro-Oeste, in Guarapuava-PR (25°23'00"S, 51°29'38"W, 1.024 m altitude). The local climate is classified as humid subtropical mesotermic (Cfb), with mild summers, winters with frost occurrence and frequent rain during the year (without a defined dry season).

The meteorological data occurred during the experiment conduction are found in Figure 1, and were obtained in the Instituto Agrônômico do Paraná, located 150 m from the experimental area.

The experimental outlining used was randomized blocks, with fifteen treatments and four repetitions. The treatments were represented by factorial 3x5, where three herbicides were used (paraquat 500 g ha<sup>-1</sup>; ammonium glyphosate 400 g ha<sup>-1</sup> and glyphosate 720 g ha<sup>-1</sup>) and five underdoses (0, 5, 10, 15 and 20% of the minimum recommended dose of each herbicide).

The planting of the cloves was made in May 25, 2016, using patches 1 m width and 0.2 m height, 0.3 m space between lines and 0.10 m between plants, the final population being 333,000 plants ha<sup>-1</sup>. The experimental units were composed of 3.6 m<sup>2</sup> divisions, with three simple lines 4 m long garlic plants, totalizing 120 plants. The area considered to be useful was 30 plants in the central lines.

The noble group cultivar Chonan was used, which presents high susceptibility to the lateral shoot growth. The used bulbs were class 5 (transversal diameter between 42 and 47 mm) and cloves with an average mass between 2.7-3.3 g/clove. The cloves were stored in a cold chamber at 4°C and 75% moisture, during 20 days for the vernalization process.

The plantation's fertilization was performed in accordance to the soil's chemical analysis results, with the application of 100 kg ha<sup>-1</sup> of simple superphosphate. (20% P<sub>2</sub>O<sub>5</sub> and 12% S) and 500 kg ha<sup>-1</sup> of 04-14-08 formulation (NPK). In the fertilization, performed 50 days after planting, 50 kg of N ha<sup>-1</sup> were used (46% N).

Weeds were manually handled

whenever necessary, so that the culture would remain unaffected by interference. Pests and diseases were controlled daily monitoring the experimental area with application of fungicides and insecticides registered in accordance to the culture's needs, in accordance to technical recommendations (Pavan & Sakate, 2014). Approximately 100 days after planting started the differentiation phase, characterized by the occurrence of cloves in early formation at the stems' base. The chemical stress was induced 105 days after planting with application of herbicides, with the purpose of reducing the lateral shoot growth on the plants.

For the application of the herbicides, a CO<sub>2</sub> pressurized coastal pulverizer equipped with a model AD 110.015 two-tipped bar, with 0.5 m space between them and 0.5 m from the plants, in a 3.6 km h<sup>-1</sup> movement speed was used. This application constituted a 150 L ha<sup>-1</sup> rate. In the moment of this application, performed between 10 h 00 min and 11 h 00 min, climate conditions were monitored with a portable digital thermo-anemometer. The temperature, relative air moisture and wind speed were 25°C, 65% and 2 km h<sup>-1</sup>, respectively.

At 7 and 14 days after the herbicide application (DAA) the electron transport rate was evaluated (ETR = μmol m<sup>-2</sup> s<sup>-1</sup>) in garlic plants, using a portable fluorometer, model Y(II) Meter, from Opti-Science®.

Plants were harvested 180 days after planting, when they showed signs of ripening characterized by yellowing and drying 2/3 of the aerial part. In this period evaluations were made of the plants' height (PH, cm), number of leaves (NL) and pseudo stem diameter (PD, mm). Plants were harvested manually, being subjected to a pre-curing process, remaining exposed for three days to the sun in such a way that the leaves of one plant would cover the bulbs of the other, shielding it from solar radiation. Afterwards the curing was performed, in which the plants remained for a 35-day period in dry and aerated area.

After the curing process, bulbs were cleaned and evaluations made of total bulb productivity (TBP in t ha<sup>-1</sup>), bulbs

with the lateral shoot growth LSG (%) and commercial bulb productivity (CBP, in t ha<sup>-1</sup>).

After verifying the normality and homogeneity of variances through the Shapiro-Wilk and Bartlett tests, respectively, the data were subjected to the Sisvar<sup>®</sup> statistic software and the variance analysis performed. Averages were compared by the Scott-Knott grouping test ( $p \leq 0.05$ ). In addition, all characteristics were subjected to regression analysis, with the polynomial

square model.

## RESULTS AND DISCUSSION

The application of paraquat, ammonium glufosinate and glyphosate at 5% of the commercial dose, respectively represented by 25, 20 and 36 g ha<sup>-1</sup>, reducing the electron transport rate (ETR) in the Chonan garlic cultivar plants, indicating that chemical stress was in general more

expressive at 7 days if compared to 14 DAA (Figure 2). In addition, the garlic cultivar Chonan presented a very similar behavior in relation to ETR reduction, when subjected to the three herbicide underdoses in the beginning of the cloves' differentiation, although possessing different mechanisms and behaviors (Roman *et al.*, 2007).

It is important to highlight that herbicide underdoses cause chemical stress in the cloves' differentiation period, inducing the formation of

**Table 1.** Plant height (PH), number of leaves (NL), pseudo stem diameter (PD), total bulb productivity (TBP), lateral shoot growth (LSG), commercial bulb productivity (CBP) and regression equation. Guarapuava, UNICENTRO, 2018.

Herbicides	Herbicide dose (%)					Mean	Regression equation	R <sup>2</sup>
	0	5	10	15	20			
<b>PH (cm)</b>								
Paraquat	84.05 aA	77.77 bB	73.87 bC	73.02 bC	71.77 bC	76.10 b	$0.0375x^2 - 1.3353x + 83.831$	0.98
Ammonium glufosinate	84.05 aA	82.65 aA	82.52 aA	81.17 aA	79.55 aA	81.99 a	$-0.0047x^2 - 0.1147x + 83.847$	0.96
Glyphosate	84.05 aA	82.75 aA	81.25 aA	80.80 aA	79.15 aA	81.60 a	$0.0010x^2 - 0.2550x + 84.000$	0.98
Mean	84.05 A	81.05 B	79.21 C	78.33 C	76.82 C			
<b>NL</b>								
Paraquat	7.95 aA	7.25 aB	6.92 aB	6.85 aB	6.82 aB	7.16 b	$0.0046x^2 - 0.1446x + 7.9186$	0.98
Ammonium glufosinate	7.95 aA	7.30 aB	7.22 aB	7.02 aB	7.05 aB	7.31 b	$0.0035x^2 - 0.1125x + 7.9011$	0.95
Glyphosate	7.95 aA	7.87 aA	7.60 aA	7.55 aA	7.07 aA	7.61 a	$-0.0017x^2 - 0.0085x + 7.9411$	0.95
Mean	7.95 A	7.47 B	7.25 B	7.14 B	6.98 B			
<b>PD (mm)</b>								
Paraquat	16.62 aA	14.06 aB	13.96 aB	13.63 aB	13.69 aB	14.39 a	$0.0143x^2 - 0.4121x + 16.366$	0.90
Ammonium glufosinate	16.62 aA	14.61 aB	14.54 aB	14.19 aB	13.84 aB	14.76 a	$0.0087x^2 - 0.2933x + 16.390$	0.90
Glyphosate	16.62 aA	14.91 aA	14.75 aA	14.13 aA	13.55 aA	14.79 a	$0.0051x^2 - 0.2413x + 16.433$	0.94
Mean	16.62 A	14.53 B	14.42 B	13.98 B	13.69 B			
<b>TBP (t ha<sup>-1</sup>)</b>								
Paraquat	16.61 aA	13.91 aB	13.52 aB	13.07 aB	12.63 aB	13.95 b	$-0.0009x^2 - 0.1969x + 16.814$	0.94
Ammonium glufosinate	16.61 aA	15.52 aA	16.05 aA	14.24 aB	13.88 aB	14.71 a	$0.0127x^2 - 0.4309x + 16.334$	0.93
Glyphosate	16.61 aA	16.39 aA	14.23 aB	13.77 aB	12.54 aB	15.26 a	$-0.0025x^2 - 0.0845x + 16.482$	0.84
Mean	16.61 A	15.27 B	14.60 C	13.70 C	13.01 C			
<b>LSG (%)</b>								
Paraquat	63.35 aA	25.12 bB	42.03 aA	47.77 aA	51.76 aA	46.00 b	$0.2093x^2 - 4.1975x + 56.579$	0.48
Ammonium glufosinate	63.35 aA	39.06 aB	33.16 aB	44.36 aB	45.08 aB	45.00 b	$0.1918x^2 - 4.4602x + 60.839$	0.81
Glyphosate	63.35 aA	43.55 aA	51.75 aA	53.27 aA	62.06 aA	54.80 a	$0.1443x^2 - 2.7429x + 60.582$	0.70
Mean	63.35 A	35.91 D	42.31 C	48.47 C	52.96 B			
<b>CBP (t ha<sup>-1</sup>)</b>								
Paraquat	6.01 aB	10.23 aA	7.57 bB	6.83 aB	6.12 bB	7.35 b	$-0.0227x^2 + 0.3901x + 6.8537$	0.46
Ammonium glufosinate	6.01 aB	9.48 aA	10.75 aA	7.82 aB	7.66 aB	8.34 a	$-0.0327x^2 + 0.6877x + 6.3789$	0.72
Glyphosate	6.01 aB	9.17 aA	6.86 bB	6.43 aB	4.60 bB	6.61 b	$-0.0231x^2 + 0.3517x + 6.5689$	0.70
Mean	6.01 C	9.62 A	8.39 B	7.03 C	6.12 C			

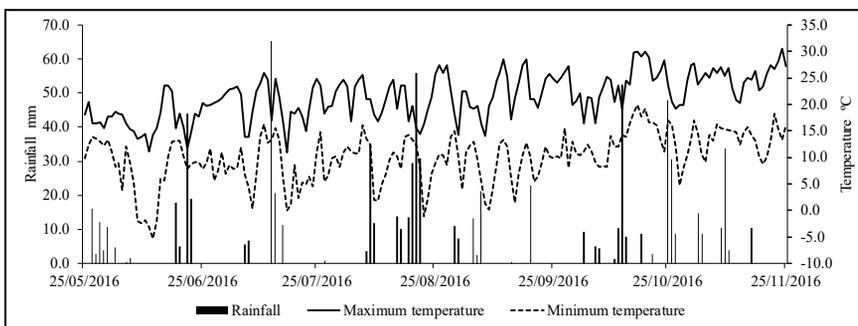
\*Means followed by same lowercase letters in the column and uppercase letters in the row belong to the same group by the Scott-Knott test,  $p \leq 0.05$ .

abscisic acid, responsible for solute translocation, opening and closing of stomata and antagonizing the gibberelins (Taiz & Zeiger, 2013). During the garlic vernalization period the gibberelins synthesis is related to clove differentiation and promotion of the lateral shoot growth in the culture (Vieira *et al.*, 2014).

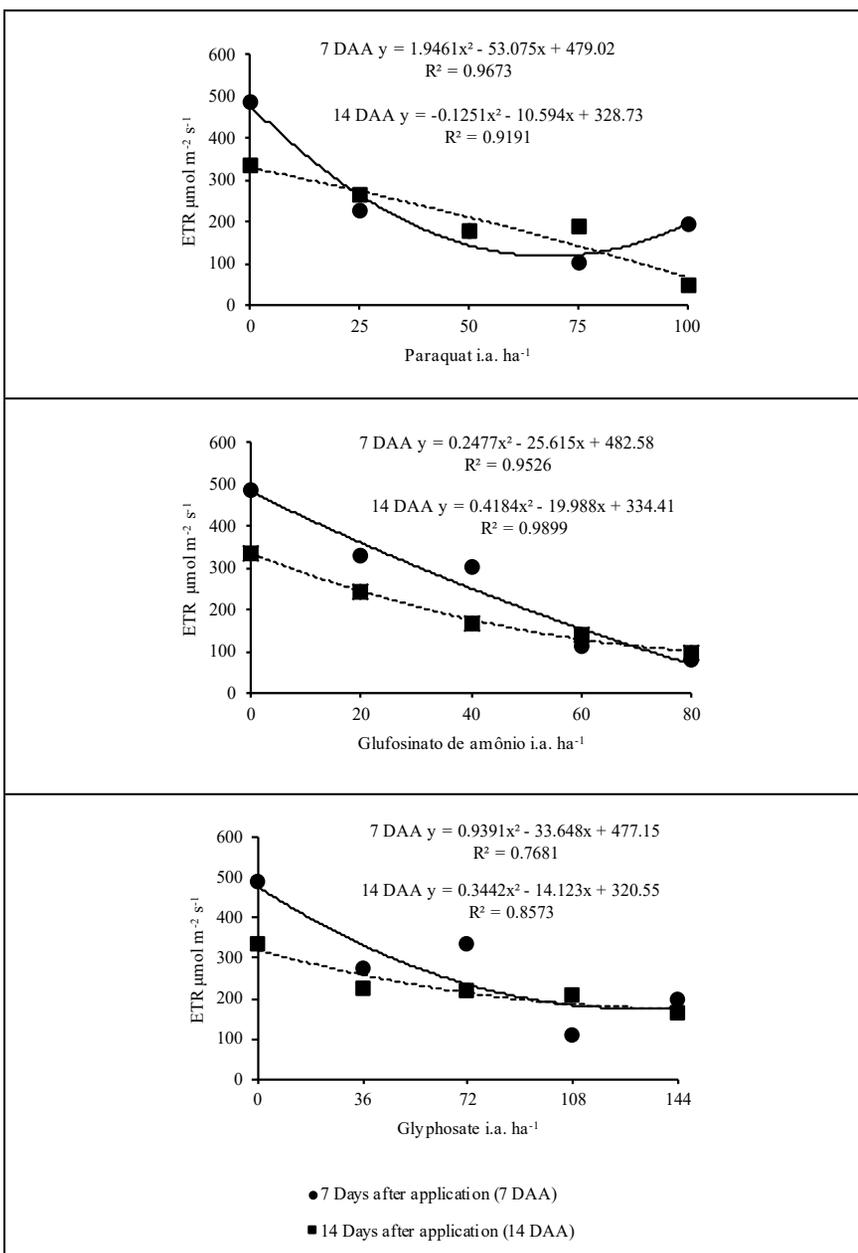
Evaluations of ETR in chemical handling of weeds are frequently used in many cultures to indicate the susceptibility of some herbicides, such as photosynthesis inhibitors, in quantifying the interference degree on plants (Baker 2008; Dayan & Zaccaro, 2012; Girotto *et al.*, 2012; Tropaldi *et al.*, 2015). It should be remarked that, due to what is proposed in this work, the interference of chemical stress in garlic plants should not be so intense to reduce productivity and the bulbs' commercial quality. In this way, fluorescence is used as a non-destructive technique, capable of obtaining abiotic stress results caused by herbicides in a rapid diagnosis after application (Korres *et al.*, 2003).

In the data analysis, no significant interactions were found between herbicide x underdose for the evaluated characteristics in the garlic culture. However, considering the variation factors in an isolated manner, there was no significant difference of the herbicide doses for pseudostem diameter (PD), lateral shoot growth (LSG) and total bulb productivity (TBP). Considering the underdose factor, no significant differences were observed only for PH with ammonium glufosinate, as well as for PH, NL, PD, and LSG with glyphosate (Table 1).

Generally, the herbicide underdoses affected negatively the production components of Chonan vernalized garlic cultivar and promoted chemical stress with decrease in lateral shoot growth (Table 1). This factor can be verified in witness without herbicide application (dose 0%), in which a higher PH, NL, PD, TBP and LSG, and a smaller commercial bulb productivity (CBP) was observed. In this context, the main highlight among herbicides occurred for paraquat in a 5% underdose causing a PH, NL, PD, TBP and LSG decrease, and a 58.74% increase in CBP.



**Figure 1.** Daily weather data of maximum and minimum temperatures (°C) and of rainfall (mm) occurred during the period of conduction of the experiment. Guarapuava, UNICENTRO, 2018.



**Figure 2.** Electron transport rate (ETR) in the Chonan garlic cultivar at 7 and 14 days after application (DAA) of the paraquat, ammonium glufosinate and glyphosate herbicides. Guarapuava, UNICENTRO, 2018.

This herbicide presents contact action with fast leaf absorption and limited translocation, as well as an electron flux blocking mechanism in the photosystem I (PSI) transport chain, which can, in few hours, cause phytotoxic effects on plants directly exposed to sunlight (Roman *et al.*, 2007; Oliveira Junior, 2011; Larotte *et al.*, 2015).

Concerning PH, no differences among ammonium glufosinate and glyphosate occurred when compared to the witness (no application). Contrarily, paraquat has negatively affected this characteristic, from an application of 5% of the commercial dose (25 g ha<sup>-1</sup>) (Table 1). For NL, no significant difference occurred among the herbicides, and only difference for paraquat and ammonium glufosinate doses, concerning the witness without application (Table 1). From 5% of paraquat and ammonium glufosinate, chemical stress was evidenced by the reduction of the garlic plant's NL.

The fact of the PH and NL variables not having been influenced by glyphosate might be attributed by the characteristics of the studied species and dose, as well as of the herbicide's mechanism of action, since it may take from two to four weeks to cause phytotoxic effects on the plants (Rodrigues & Almeida, 2011). Glyphosate acts inhibiting the action of enzyme enol piruvil shikimate phosphate synthase (EPSPs), paralyzing the synthesis of amino acids phenylalanine, tyrosine and tryptophan, and secondary compounds such as alkaloids, coumarins and flavonoids, in addition to inhibiting the production of chlorophylls and stimulating the production of ethylene and ascetic indol acid (AIA) (Kruse *et al.*, 2000; Galli & Montezuma, 2005; Roman *et al.*, 2007).

Similar results were observed for the pseudostem diameter (PD), in NL (Table 1). Resende *et al.* (2013) found in Guarapuava-PR that noble group garlic cultivars presented a larger pseudostem diameter, total bulb production and percentage of bulbs with lateral shoot growth and even so there was no difference concerning the productivity of commercial bulbs of the seminoble group garlic cultivars. According to the authors, a genetic variability between

the genotypes of the noble group garlic to those with lateral shoot growth varied between 16.2% (São Valentim cultivar) to 99.2% (Bergamota cultivar).

In total bulb productivity (TBP), no difference was found for the herbicide, but in average a 17.62% was found for a higher herbicide dose (20%), in relation to the witness without application. There was a TBP reduction of 5, 15 and 10% doses for paraquat, ammonium glufosinate and glyphosate, respectively; the behavior found in the underdose-response description adjusted with high precision ( $R^2 \geq 0.84$ ) to the polynomial square model (Table 1). However, lower losses were remarked by the formation of abnormal bulbs with lateral shoot growth LSG from underdoses of 5% paraquat and ammonium glufosinate, instead of glyphosate, which did not show the same behavior (Table 1). For this variable, the adjustment to the polynomial square model with glyphosate was superior to that of paraquat ( $R^2 \geq 0.48$ ) and inferior to ammonium glufosinate ( $R^2 \geq 0.81$ ).

A higher productivity was remarked for commercial bulbs (CBP), the 5% underdoses of paraquat and glyphosate, along with 10% for ammonium glufosinate, which resulted in enhancement in relation to the control, around 41.25; 44.09 and 34.46%, respectively. Research results with stimulating effects of herbicide underdoses for a desirable phenotypical change, although still restricted in garlic cultures, have already been investigated for a large variety of cultures, and this phenomenon has been named "hormesis" or "hermetic effect" (Duke *et al.*, 2006). Velini *et al.* (2008) reported a stimulus of a glyphosate underdose to the growth of five species of plants and an absence of this effect to plants resistant to glyphosate (RR soy), showing that hormesis may be probably related to the site of action of the aforementioned herbicide.

Lateral shoot growth is considered a physiological genetic anomaly that may cause the garlic culture to produce defective bulbs (Resende *et al.*, 2017). The importance of new research geared towards the selection of genotypes better-adapted to the edaphoclimatic

conditions of the Center-South region of the state of Paraná, in addition to promising handling techniques, such as chemical stress induced by herbicides, becomes clearer than ever. In this sense, we also highlight that the investigation of the use of herbicides to reduce lateral shoot growth, will be necessary to know the minimum period of latency between application and harvest of the garlic culture, in a way that the Maximum Residual Limit (MRL) is safely below the levels established by Brazil's regulatory agencies, such as ANVISA [Agência Nacional de Vigilância Sanitária (National Sanitary Inspection Agency)].

The chemical stress of herbicides paraquat, ammonium glufosinate and glyphosate in the 25, 40 and 26 i.a. ha<sup>-1</sup> underdoses, respectively, was efficient in reducing the electron transport rate and lateral shoot growth of the vernalized Chonan garlic culture, as well as technically viable in increasing the productivity of the Chonan garlic cultivar commercial bulbs.

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