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Reaction of Bulgarian varieties of perennial ryegrass (*Lolium perenne* L.) to glyphosate

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Abstract

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The aim of this study was to determine and compare the sensitivity of new high yield Bulgarian varieties of perennial ryegrass (*Lolium perenne* L.) – Harmoniya, Tetrany and Tetramis to glyphosate in laboratory conditions. The effect of three doses of glyphosate – 360, 720 and 1440 g a.i./ha applied in the growth stage tillering (BBCH – 22-23) on perennial ryegrass on the dynamics of plant growth and formed fresh biomass in g per plant was studied.

Genotypic differences were observed between the studied varieties of perennial ryegrass to glyphosate. With a relatively high sensitivity to glyphosate conditionally can be determined diploid variety Harmoniya in which the depression coefficients (B) on the dynamics of growth and accumulation of fresh biomass are in the range from -5.7 to – 4.7, while for tetraploids Tetrany (B) (average) is in the range of -3.3 to -3.9 and for Tetramis (B) (average) is within limits from -3.2 to -3.9, they have relatively high tolerance to glyphosate and can be used as components in future programs.

Keywords: Bulgarian varieties; perennial ryegrass; sensitivity; glyphosate

Introduction

Perennial ryegrass (*Lolium perenne* L.) is used as a permanent stand in temperate zone climates for multifunctional uses – highly productive potential for forage and seed production including amenity grassland, sports turf and over seeding dormant bermuda grass (*Cynodon dactylon* L.) in subtropical climates (Humphreys et al., 2010; Baldwin & Blythe, 2015; Tironi et al*.,* 2014). Perez-Jones et al. (2005) discovered that ryegrass cultivated in a competitive environment with weeds reduces the crop productivity by around 60% depending on the cultivar and weed density.

Lolium perenne L. is characterized with slow growth and development during the year of stand establishment and seed production years. During these periods of development is very sensitive to the competitive influence of weeds (Dimitrova & Katova, 2010; Sanna et al., 2014; Katin-Grazzini, 2018).

Although weed control in the perennial ryegrass (*Lolium perenne* L.) are extremely limited in Bulgaria and abroad we had established herbicides that can be used to control broadleaf weeds in the during establishing and seed production years (Dimitrova, 1984; 1995; 2002; Mueller-Warrant & Rosato, 2002a; 200b; Dimitrova & Katova, 2010). According to studies Dimitrova (2007), Stoeva & Vateva (2008; 2010) and Gunnarsson et al. (2017) grass weed species control in cereal crops is difficult because they are very similar by morphology and physiology, irrespective of which some herbicides had been identified to the grass weed control in the perennial ryegrass.

Limited weed control at perennial ryegrass and incorrect used of herbicides, such as intensive of the same compound, doses below the recommended rate applied at incorrect weed growth stage has selected resistant ryegrass plants of the different herbicides diclofop, clodinafop, mesosulfuron and others (Michael et al., 2010).

Over the last two decades in Bulgaria, herbicides based on active ingredient glyphosate are the most widely used, mainly for weed control in fallow in non-cropped areas and stubbles.

Glyphosate (N- [Phosphonomethyl] glycine) is the active ingredient of the systemic and non-selective (or broad-spectrum) that controls most annual and perennial weeds (Pesticide Information Profiles, 1996). The intensive and prolonged use of glyphosate in weed control has led to the selection of persistent ryegrass biotypes (Sammons & Gaines, 2014).

According to generalized studies by Yanniccari et al. (2012) the first glyphosate-resistant ryegrass biotype was reported in Australia (Pratley et al.,1996), in USA (Perez-Jones et al., 2005), Spain, France, South Africa, Israel, Italy, but there are no such studies in Bulgaria.

The aim of this work was to evaluate and compare the sensitivity to glyphosate 360 g/kg (Buggy 360 SG) on Bulgarian varieties perennial ryegrass (*Lolium perenne* L.) and determine a donor for the tolerance of them for the purpose of the breeding.

Materials and Methods

The study was conducted under greenhouse conditions at the Institute of Forage Crops, Pleven, in 2018 and 2019. Two factors were studied: Factor A – the applied rate of glyphosate 360 g a.i./kg (Buggy 360 SG, a water soluble granule containing 360 g/kg glyphosate (acid equivalent), manufacturer Sipcam, Iberia): a_1 – control, a_2 – 360 g a.i./ha, a_3 – 720 g a.i./ha and a_4 – 1440 g a.i./ha; Factor B – new a high productive Bulgarian varieties of perennial ryegrass (*Lolium perenne* L.) b_1 – Harmoniya – diploid (Katova, 2011), b_2 – Tetrany (Katova, 2017a) and b_3 – Tetramis (Katova, 2017a) – tetraploids.

In order to determine and compare the sensitivity on Bulgarian varieties of perennial ryegrass from glyphosate in the laboratory, were sown (by twenty-five plants) of each variety in plastic containers of 0.5 l with five replications for factor A. Untreated plants sown in the same pot volume were used as a control. Fertilizing was conducted with P_2O_5 – 100 kg/ha and N – 120 kg/ha the day of seeding. Irrigation was provided as needed to prevent wilt stress and no additional, fungicide, or insecticide applications were necessary.

At the growth stage tillering (BBCH – 22-23) the tested varieties of perennial ryegrass were sprayed with different rates of glyphosate 360 g a.i./kg (Buggy 360 SG) and with distilled water in the control treatments, according to test Factor B. Treatments were conducted with a knapsack sprayer "ptp 18" with conic nozzle, pressure P_{max} 3 bar, V_{max} 1.66 l, and Q_{max} 0.64 l/min, with a working solution quantity of 500 l/ha. The following characteristics were studied based on factors *A* and *B*.

Herbicide phytotoxicity, depending on application of different rates of glyphosate 360 g a.i./kg (Buggy 360 SG) in the varieties of perennial ryegrass was determined according to EWRS logarithmic scale (score 1 – no damage; score 9 – completely killed plants), the recording being performed on the 7th, 14th and 21th day after treatment (DAT). The percentage of surviving plants (%), plant length (root + stem) (cm) and formed fresh biomass in g per plant at the 21th day after treatment (DAT) were noted. Percent leaf firing (PLF) was rated on a scale of 0% to 100% (were 0% no damage, 20% = acceptable level of damage, 100% = brown, killed plants) at the 21st day after treatment (DAT).

The rate of growth (*K*) of the studied perennial ryegrass genotypes was determined with regard to height of growth (K/*cm*), as well as for formed fresh biomass ($K/g_{/fb}$) in all variants of the trial depending on the studied factors – glyphosate dose (A) and kind of genotype (B) by the equation (1) Mamonov and Kim (1978):

$$K = (w_2 - w_1)/(t_2 - t_1) \qquad (1)$$

where W_1 – initial stage of recording of the parameter; W_2 – final stage of recording of the parameter; $t_2 - t_1$ – period of time in the interval between W_1 and W_2.

Inhibition effect (*IE)* was determined by the Equation (2).

$$IE = \left[\frac{C - T}{C}\right].100 \qquad (2)$$

where *C* – characteristic in the control variants; *T* – characteristics in each treatment with different rates glyphosate.

The Equation (3) of Shabanov et al. (1982) was used to determine the depression coefficients (B) depending on the studied factors.

$$\begin{cases} n\,lg\,Y_0 + B.\sum_{i=1}^{n} lg\,x_i = \sum_{i=1}^{n} lg\,Y_i \\ lg\,Y_0 \sum_{i=1}^{n} lg\,x_i + B.\sum_{i=1}^{n} (lg\,x_i)^2 = \sum_{i=1}^{n} (lg\,x_i - lg\,Y_i) \end{cases} \qquad (3)$$

where •*n* – volume of pair samplings depending on the studied factors – *A* and *B* of the used doses of glyphosate 360 g/kg; •x_i – the used g a. i. ha^{-1} glyphosate for each of the studied perennial ryegrass varieties were transformed in a four-score scale: •$Y_{i=4}$ – studied characteristics for perennial ryegrass varieties: ($x_{I=1}$ – plant length; $x_{I=2}$ – formed fresh biomass in g per plant, %; •$Y_{0=3}$ – tested characteristics for all perennial ryegrass varieties ($Y_{0=1}$ – plant length; $Y_{0=2}$ – formed fresh biomass in g per plant in the control variants taken for 100%).

In fresh plant samples according to factor A and B, the total content of photosynthetic pigments (chlorophylls a and b and carotenoids) (mg/100 g fresh weight) was determined spectrophotometrically according to the method of Zelenski & Mogileva (1980). The collected data were evaluated using analysis of variance with means separation based on Fisher's least significant difference test at a = 0.05 with the software Statgraphics Plus for Windows Ver. 2.1 and Statistica Ver. 10.

Results and Discussion

The doses of 360, 720 and 1440 g a.i.ha^{-1} of glyphosate 360 g l^{-1} applied at the growth stage tillering (BBCH – 22-23) the tested varieties of perennial ryegrass had from indifferent to an depressed effect on survival and initial development of the studied Bulgarian varieties of perennial ryegrass .

A specific genotype response was also observed with regard to phytotoxicity of the used glyphosate 360 g/kg to the studied perennial ryegrass varieties. The herbicide applied at the doses of 360 g a.i.ha did not induce phytotoxicity (score 1) until the 7th, 14th and 21th day after treatment (DAT) for all varieties of perennial ryegrass, except for variety Harmoniya where at the 21th day after treatment there were chlorotic spots (score 2) and percent leaf firing (PLF 10) (Table 1).

With increase of the doses to 720 and 1140 g a.i.ha, the phytotoxicity of the studied varieties Tetrany and Tetramis was low to moderate (score 1.5 – 2.0) (PLF 10%) with expressed chlorotic spots between the leaf veins. Applied doses of 720 and 1140 g a.i.ha in Harmoniya variety showed stronger phytotoxicity (score from 3.0 to 4.5) (PLF from 35 to 50%) with expressed chlorotic spots on the top of the leaves and anthocyanin coloring at the base of the leafs.

At the 14th day after treatment, the Tetrany and Tetramis varieties showed low to moderate phytotoxicity (score 2-3) (PLF from 10 to 30%) only at the higher applied doses -720 and 1140 g a.i.ha. The newly appearing leaves had no visible changes due to the herbicide effect, but the Harmoniya variety responding with higher sensitivity has a high phytotoxic effect – score from 6 to 8 (PLF from 70 to 90%).

At 21th DAT, the phytotoxicity effect was significantly different between tested varieties of perennial ryegrass. The lower application doses (360 and 720 g a.i.ha) glyphosate applied at Tetrany and Tetramis varieties had a weak phytotoxic effect (score 2 – 2.5) (*PLF* from 10 to 25%) and relatively higher (score from 4.5 to 5) (PLF from 45 to 50%) the highest – 1140 g a.i.ha therefore this varieties can be conditionally determined, as glyphosate tolerant. The herbicide effect on the more sensitive variety Harmoniya increased at 21th DAT where plants no showed re-growth at 21th days after application, where had a high phytotoxicity (720 g a.i.ha) and lethal effect, after treatment with glyphosate – 9.0 (PLF 100%) is established.

The results of the analysis of the total content of plastid pigments and chlorophyll for all treatments of the experiments are presented in Table 2.

Table 1. Results of visual determination of the phytotoxicity and percent leaf firing for Bulgarian perennial ryegrass varieties after treatment with different doses glyphosate

Days after treatment (DAT)	Dose glyphosate g a.i. ha	Varieties					
		Harmoniya		*Tetrany*		*Tetramis*	
		EWRS	*PLF*	*EWRS*	*PLF*	*EWRS*	*PLF*
7 DAT	Control	1.0	0.0	1.0	0.0	1.0	0.0
	360	2.0	10.0	1.0	0.0	1.0	0.0
	720	3.0	35.0	1.5	10.0	1.5	10.0
	1440	4.5	50.0	2.0	10.0	1.5	10.0
14 DAT	Control	1.0	0.0	1.0	0.0	1.0	0.0
	360	5.0	50.0	1.0	0.0	1.0	0.0
	720	6.0	70.0	2.0	10.0	2.0	10.0
	1440	8.0	90.0	3.0	30.0	3.0	30.0
21 DAT	Control	1.0	0.0	1.0	0.0	1.0	0.0
	360	5.5	60.0	1.0	0.0	1.0	0.0
	720	7.5	85.0	2.5	25.0	2.0	10.0
	1440	9.0	100.0	4.5	45.0	5.0	50.0

Legend: EWRS – Herbicide phytotoxicity according to EWRS logarithmic scale (score 1 – no damage; score 9 – completely killed plants); Percent leaf firing (PLF) was rated on a scale of 0 to 100% (0% no damage; 100% = brown, killed plants)

Table 2. Plastid pigments content in Bulgarian perennial ryegrass varieties after treatment with different doses glyphosate 21th day after application

Varieties	Dose glyphosate g a.i. ha	Plastid pigments mg/100g fresh weight						
		Chlorophyll			Carotenoids	Total	% Ct	IE
		a	b	a+b				
Harmoniya	Control	45.9^{c}	41.6^{b}	87.5^{d}	13.6^{b}	101.1^{d}	100.0	0.0
	360	40.3^{b}	32.0^{b}	72.3^{c}	10.3^{ab}	82.7^{c}	81.8	18.2
	720	30.2^{b}	21.7^{ab}	51.9^{b}	13.9^{b}	65.8^{b}	65.1	34.9
	1440	2.7^{a}	5.1^{a}	7.8^{a}	2.7^{a}	10.5^{a}	10.4	89.6
Tetrany	Control	26.5^{b}	24.0^{b}	50.4^{b}	10.9^{b}	61.4^{b}	100.0	0.0
	360	27.4^{b}	24.0^{b}	51.4^{b}	10.7^{b}	62.1^{b}	101.2	-1.2
	720	23.9^{b}	19.1^{ab}	43.0^{b}	9.3^{b}	52.3^{ab}	85.3	14.7
	1440	6.9^{a}	12.7^{a}	19.6^{a}	8.1^{ab}	27.7^{a}	45.2	54.8
Tetramis	Control	16.8^{b}	14.7^{b}	31.4^{b}	7.4^{b}	38.8^{bc}	100.0	0.0
	360	15.7^{b}	13.4^{b}	29.2^{b}	7.2^{b}	36.3^{bc}	93.7	6.3
	720	9.5^{ab}	9.2^{ab}	18.7^{ab}	6.3^{ab}	25.0^{b}	64.6	35.4
	1440	1.8^{a}	2.6^{a}	4.4^{a}	2.9^{a}	7.3^{a}	18.9	81.1

Legend: %Ct – Percentage compared to the control treatment, %; IE – Inhibition effect*; a, b, c and d – statistically significant differences in P = 0.05*

Low values of chlorophyll a were reported after treatment for all tested perennial ryegrass varieties (from 1.8 to 32.0 mg/100g FW) as compared to control treatments (16.8 to 41.6 mg/100g FW). The analysis of the data shows that the content of chlorophyll b is relatively less in the comparison to the established for chlorophyll a. This dependence can be explained by the phytotoxic effect of the glyphosate and the biological characteristics of the tested perennial ryegrass varieties. According to the studies of Kannangara & Hanson (1998) and Nacheva et al. (2012) at the stress, plants increase the synthesis of anthocyanins. For the conditions of the study, it was found that decreasing the content of photosynthetic plastid pigments as an indicator of phytotoxicity of herbicides confirms the observed visible symptoms of chlorosis in tested perennial ryegrass varieties. The results obtained are similar in terms of content of the total chlorophyll "a + b" in the tested perennial ryegrass varieties on the 21st day after treatment with the different doses glyphosate ranged from 10.4 to 101.2%, compared to control variants. The carotenoid content was relatively lower reduced from 2.1 to 80.1% at the tested varieties of perennial ryegrass, according to control treatments disproportionate to the increased applied doses of glyphosate.

The data on biometric measurements of the plant length allows estimating objectively the herbicide phyto-

Table 3. Effect of the herbicide glyphosate 360 g/kg on the height and accumulation of fresh biomass of the Bulgarian perennial ryegrass varieties

Varieties	Dose glyphosate g a.i. ha	length of growth per plant, cm						formed fresh biomass per plant, g					
		radix	*IE*	stem	*IE*	plant	*IE*	radix	*IE*	stem	*IE*	plant	*IE*
Harmoniya	Control	11.7^{c}		8.6^{c}		20.3^{d}		0.053^{d}		0.034^{d}		0.087^{d}	
	360	9.9^{b}	15.4	4.4^{ab}	48.9	14.3^{c}	29.6	0.034^{c}	35.8	0.032^{c}	5.9	0.066^{c}	24.1
	720	7.5^{b}	35.9	3.5^{ab}	59.3	11.0^{b}	45.8	0.030^{b}	43.4	0.020^{b}	40.9	0.050^{b}	42.4
	1440	3.6^{a}	69.2	2.8^{a}	68.1	6.4^{a}	68.7	0.002^{a}	96.2	0.003^{a}	91.2	0.005^{a}	94.3
Tetrany	Control	8.1^{b}		7.8^{b}		15.9^{b}		0.036^{a}		0.047^{b}		0.083^{bc}	
	360	8.2^{b}	-1.2	6.8^{b}	13.2	15.0^{b}	5.8	0.038^{a}	-5.6	0.047^{b}	0.0	0.085^{bc}	-2.4
	720	7.5^{b}	7.4	5.1^{ab}	34.9	12.6^{ab}	20.9	0.037^{a}	-2.8	0.042^{b}	10.6	0.079^{b}	4.8
	1440	5.2^{a}	35.8	2.9^{a}	63.0	8.1^{a}	49.2	0.037^{a}	-2.8	0.032^{a}	31.9	0.069^{a}	16.9
Tetramis	Control	10.0^{c}		7.3^{b}		17.3^{b}		0.050^{a}		0.031^{b}		0.081^{bc}	
	360	8.9^{bc}	11.0	7.1^{b}	2.7	16.0^{b}	7.5	0.047^{a}	6.0	0.029^{b}	6.5	0.076^{b}	6.2
	720	8.4^{bc}	16.0	6.6^{b}	10.3	15.0^{ab}	13.6	0.046^{a}	7.2	0.024^{a}	24.2	0.070^{b}	13.7
	1440	6.5^{a}	34.9	4.2^{a}	42.5	10.7^{a}	38.1	0.042^{a}	17.0	0.020^{a}	35.5	0.062^{a}	24.1

Legend: a*, b, c, d – statistically significant differences at P = 0.05, IE – Inhibition effect* toxicity at the initial developmental stages of the perennial ryegrass (*Lolium perenne* L.) varieties (Table 3). With increase doses of glyphosate from 360.0 to 1440.0 g a.i./ha, the plants height decreased disproportionately to the increase in the rates of herbicide, the differences with the control variants are statistically significant at P = 0.05.

Glyphosate applied at the dose 432.0 and 1440.0 g a.i./ha disproportionately decreased the plants height with to the increase in the rates of herbicide, the differences with of the control variants are statistically significant at P = 0.05 only for Harmoniya variety.

An inhibitory effect (15.3 to 69.2%) was observed for all applied doses in Harmoniya variety being statistically significant at P = 0.05, an exception was observed for Tetrany and Tetramis varieties, where the differences were statistically no significant only at the lower applied concentrations 360 and 720 g a.i.ha of glyphosate.

The formation of fresh biomass in g per plant varied in the studied variants depending on the genotype and on quantity of applied glyphosate 360 g/kg. At the lowest glyphosate concentration (360 g a.i.ha) the perennial ryegrass varieties formed a greater quantity of fresh biomass in g per plant on average. With increase of the glyphosate dose the formed fresh biomass decreased disproportionately to the increase of its concentration (Table 3).

There was a negative correlation (*r*) between quantity of applied glyphosate and accumulated fresh biomass for the studied perennial ryegrass varieties, as follows: Harmoniya – root -0.985, stem -0.983 and plant -0.998; Tetrany root -0.739, stem -0.966 and plant -0.994 and Tetramis root -0.353, stem -0.755 and plant -0.996. An exception to the described relationship was observed only for Tetrany variety where the lowest glyphosate dose (360 g a.i./ha) had a statistically insignificant stimulatory effect.

The obtained experimental data was unidirectional with the published results of Cedergreen (2008), Velini et al. (2008), Goggin et al. (2012) and Matthew et al (2012) according to which the applied glyphosate at low doses had a stimulatory effect on dynamics of growth and accumulation of fresh biomass in different agricultural plants. Therefore, the observed differences between the perennial ryegrass (*Lolium perenne* L.) varieties with regard to their sensitivity to glyphosate can be explained by genetic differences, because the comparisons between them were made under the same conditions and doses of the applied herbicide.

The obtained results when observing the dynamics of accumulation of fresh biomass in g per plant were analogous (Table 3).

The rate of growth (K/cm) and accumulation of fresh (K/g/fb) biomass in the studied perennial ryegrass varieties depended on the dose of applied glyphosate. Applied doses glyphosate had a inhibitory effect, which is more pronounced

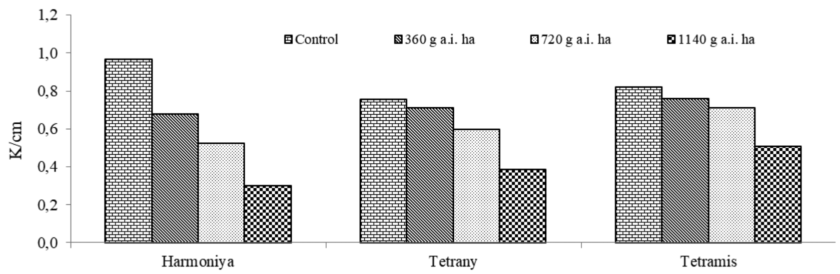

Fig. 1. Rate of growth (K/cm) of the studied perennial ryegrass varieties depending on the dose of the applied glyphosate

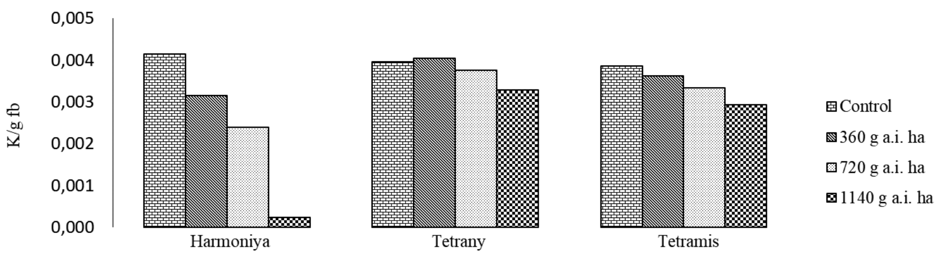

Fig. 2. Rate of accumulation of fresh (K/g/fb) biomass of the studied perennial ryegrass varieties depending on the dose of the applied glyphosate

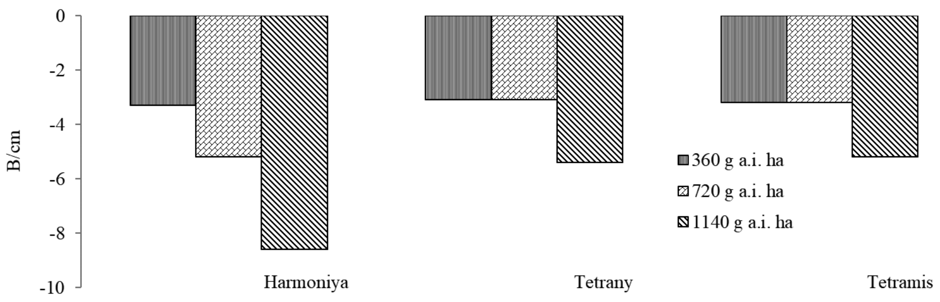

Fig. 3. Depression coefficients (B) in the length of growth in studied perennial ryegrass varieties depending on the doses of the applied glyphosate

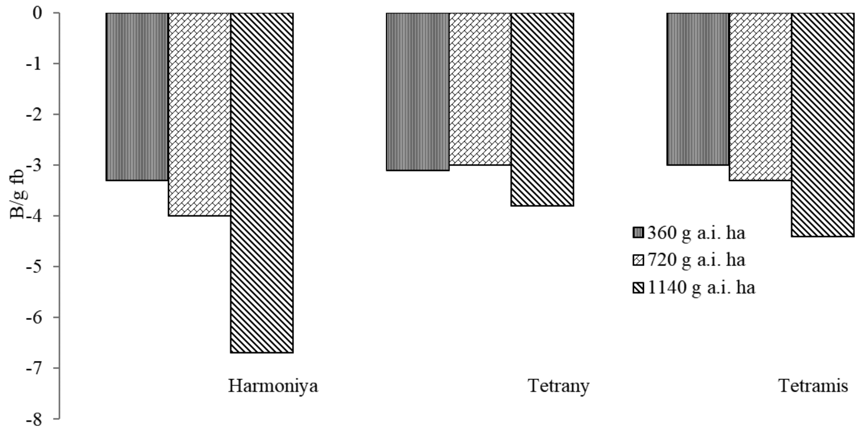

Fig. 4. Depression coefficients (B) in the formed fresh biomass in g per plant in studied perennial ryegrass varieties depending on the doses of the applied glyphosate

in diploid Harmoniya variety and less so in tetraploid varieties Tetrany and Tetramis, with increase of the dose to 360, 720 and 1440 g a.i.ha, the rate of growth on K/cm and K/g/fb decreased (Figure 1 and 2).

Similar results were obtained by Cedergreen (2008) and Simarmata & Penner (2008). According to the authors, the responses of the plants after their treatment to glyphosate can have variable degree and direction of manifestation expressed in change of dynamics of growth and rate of accumulation of fresh and dry biomass.

There was a strong negative correlation – *r* from -0.932 to – 0.998 between the quantity of the applied doses glyphosate and root, stem and plant height and formed fresh biomass in g per plan in the tested Bulgarian perennial ryegrass varieties.

The depression coefficients (B) in the studied characteristics depended mainly on the perennial ryegrass varieties and on the applied doses glyphosate. It is evident from Figure 3 and 4 that the depression coefficients (B) were relatively the lowest at the smallest studied dose of 360 g a.i.ha and with its increase to720 and 1440 g a.i.ha the depression coefficient (B) also increased.

The results analysis showed that the depression coefficients (B) were in negative correlation (r) with regard to length of growth plant, r varied from – 0.983 to – 0.998 and from – 0.990 to – 0.992 with regard to growth and formation of fresh biomass in g per plant.

Conclusions

Genotypic differences were observed between the studied Bulgarian varieties of perennial ryegrass (*Lolium perenne* L.) by glyphosate 360 g/kg (Buggy 360 SG). With a relatively high sensitivity by glyphosate conditionally can be determined diploid Harmoniya variety in which the depression coefficients (B) on the dynamics of growth and accumulation of fresh biomass are in the range from -5.7 to – 4.7, while for tetraploids Tetrany (B) (average) is in the range of -3.3 to -3.9) and for Tetramis (B) (average) is within limits from -3.2 to – 3.9) they have relatively high tolerance from glyphosate and can be used as components in future breeding programs.

Equivalence has been established between the phytotoxicity of herbicides glyphostate 360 g/kg (Buggy 360 SG) as measured by EWRS logarithmic scale, percent leaf firing (PLF) and depression coefficients (B) of the studied the newest high productive Bulgarian varieties of perennial ryegrass (*Lolium perenne* L.).

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