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STUDY OF THE SUSCEPTIBILITY OF MAIZE LINES TO SOME SULFONYLUREA HERBICIDES

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Maize lines are susceptible to different herbicides, what makes seed production more complicate. The susceptibility is depending in high extent on meteorological conditions. The objective of the study was to investigate genetic variability and correlation between phytotoxicity (EWRC evaluation), alterations in dry matter (DM), phenolics and soluble proteins (SP) in sets of 19 ZP lines, to nicosulfuron and foramsulfuron, with the aim to determine sensitivity of individual lines and the potential tolerance patterns during period 2006-2009. The negative impact of both herbicides reflects through DM increase, but in higher degree in nicosulfuron

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treatment. They also induced in average SP increase and decrease of phenolic's content. According to different meteorological conditions present during examined four years, SP increase was followed by increased EWRC values at same lines, opposite to lines in which decrease of SP and increase of phenolic's level could be tied to potential tolerance, what gives importance to testing of each individual maize line. Special attention must be given to application time (according to meteorological conditions and level of weed infestation). The decrease in SP level and increase of phenolics, together with lower EWRC values observed at some lines could be associated to tolerance patterns what was emphasized particularly during 2009, when visible injuries were absent.

Key words: EWRC, maize lines, phenolics, soluble proteins, sulfonylurea herbicides

INTRODUCTION

The suppression of weed plants in maize seed crop is essential for achieving of high yields. Owing to the reduced tolerance of maize lines, the majority of herbicides that are applied after emergence are not registered for application in the seed production under conditions in Serbia. However, a high level of weed infestation emerged these herbicides as necessary in the hybrid maize seed production (STEFANOVIĆ *et al*, 2010).

STEFANOVIĆ *et al* (2000; 2007) underlined that weed control became more efficient when sulfonylurea herbicides were introduced into practice. Meanwhile, the efficient and safe application of sulfonylurea herbicides is reduced in maize lines due to their different responses. According to HUND *et al* (2012) maize lines are susceptible to different herbicides due to low vigor, slower growth and smaller habitus. Tolerance to sulfonylurea herbicides is not always a function of plant metabolism, but also other factors can contribute to the different degree of the selectivity expression. Genotypes susceptible to the application of sulfonylurea herbicides lag in the growth and deform. Symptoms are expressed in a form of various malformations, twists, color changing, plant height reduction and at the end in a form of grain yield reduction (STEFANOVIĆ *et al*, 2010). According to de CARVALHO *et al* (2009) phytotoxicity can be interpreted as an overcoming of the maximum protection capacity offered by the mechanisms of selectivity, or when considering metabolism as the main factor, the overcoming of the inherent plant ability to detoxify a particular molecule.

The seed crop susceptibility is depending in high degree on meteorological conditions. BONIS *et al* (2006) emphasized greater phytotoxic damage due to cool and wet weather which retarded the metabolic processes of maize. Similar results were obtained by STEFANOVIĆ *et al* (2001) with higher toxicity effects noticed in PL (inbred) lines, than in KL (combined) lines. The intensity of phytotoxic response could be strong at sensitive lines, leading to drying and dying, while at lesser sensitive lines recovery was observed after some time (STEFANOVIĆ and SIMIĆ, 2008). It is emphasizing the necessity to test the reaction of every line to new herbicides, what will improve technological process of seed production.

Since the herbicides could be considered as oxidative agents, by increasing of free radical's level in plant tissues (ŠTAJNER *et al*, 2003), wide range of reaction could be considered. The susceptible maize lines were characterised with lower bound water content and a higher phenol content (STEFANOVIĆ *et al*, 2010). What's more, RAO (2000); SACALA *et al* (2003); DRAGICEVIC *et al*. (2010); SIMIĆ *et al*. (2010) reported that stress factor induced by herbicide increased content of amino compounds and dry matter content, also with parallel increase of the effects of some other stress, like salinity.

The objective of this study was to investigate genetic variability and correlation between phytotoxicity, alterations in dry matter, phenolics and soluble proteins in sets of 19 ZP lines, to two common used sulfonylurea herbicides, with the aim to determine sensitivity of individual lines and the potential tolerance patterns.

MATERIALS AND METHODS

The field experiment was conducted during 2006-2009 in the field of the Maize Research Institute, in Zemun Polje on a slightly calcareous chernozem type of soil under rain-fed conditions. The influence of sulfonylurea herbicides: nicosulfuron and foramsulfuron on sets of 19 ZP lines, 48 hours after application was examined. Most of the examined lines were PL-s (inbred lines), while L3, L4, L5, L9 and L11 were KL-s (combined lines). The experiment was conducted by RBCD design with four replications: main plots encompassed 4 rows of each line, while subplots included two herbicides and control, without herbicide application.

The sowing was performed on 29.04.2006 (L1-L19), 18.04.2007 (L1-L16), 12.04.2008 (L1-L19) and 27.04.2009 (L1-L16), while the herbicides were applied in the 4–6-leaf phase: nicosulfuron – T1 (preparation Motivell) in a quantity of 50 g ha^{-1} a.i., foramsulfuron – T2 (preparation Equip) in a quantity of 50.0 g ha^{-1} a.i. Subsequently, 48 h after herbicide application plant shoots were collected (4x5), weighted and dried at 40°C for dry matter (DM) determination. The content of soluble proteins (SP) by LOWRY *et al* (1951) and phenolics by SIMIĆ *et al* (2004) were determined. Fitotoxicity evaluation (EWRC) of maize plants was conducted 21 days after herbicide application, according to WEED RESEARCH COUNCIL, Feldfersuche (1975).

The experimental data were statistically processed by analysis of the variance (ANOVA) and analysed by the LSD-test (5 %) and regression analysis with the MINITAB 14 software.

Meteorological conditions. The study was performed during starting period of maize vegetation April-May (Table 1). During that period, average temperature had increasing trend in April and May from 2006 to 2009 (from 13.4 to 15.8 and from 16.9 to 19.8 °C, respectively). On the other hand, higher level of precipitation characterised 2007 and 2008 (almost double, in regard to 2006 and 2009).

Table 1. Average daily temperatures and precipitation sum during April and May of 2006, 2007, 2008 and 2009

	Temperature (°C)					Precipitation (mm)				
	2006	2007	2008	2009	Aver.	2006	2007	2008	2009	Aver.
April	13.4	13.8	14.1	15.8	14.3	19.4	11.0	27.3	7.3	16.3
May	16.9	18.9	19.3	19.8	18.7	15.2	52.6	39.7	27.4	33.7
Aver./Sum.	15.2	16.4	16.7	17.8		34.6	63.6	67.0	34.7	

RESULTS AND DISCUSSION

Obtained results point that beginning of vegetation of 2006 was the unfavourable (lower temperature and precipitation, Table 1), than 2007, 2008 and 2009, with significantly higher content of average DM (Table 2) and EWRC values (Table 3), similarly to results of STEFANOVIC *et al* (2001).

Table2. The dry matter in maize shoot, 48 h after application of herbicides in: control (C), nicosulfuron (T1) and foramsulfuron (F2) treatments; PL lines: L1, L2, L6, L7, L8, L10, L12-L19; KL lines: L3, L4, L5, L9 and L11

	2006				2007			
	C	T1	T2	Aver.	C	T1	T2	Aver.
L1	18.11	20.14	20.84	**19.70**	10.75	12.39	14.67	**12.60**
L2	18.52	19.49	21.72	**19.91**	12.94	12.34	11.94	**12.40**
L3	19.12	18.61	20.44	**19.39**	10.73	11.28	12.89	**11.64**
L4	22.66	28.11	24.19	**24.98**	10.12	10.74	11.06	**10.64**
L5	23.72	31.82	22.59	**26.04**	9.70	10.23	11.92	**10.62**
L6	19.59	29.82	19.63	**23.01**	9.61	10.89	11.50	**10.67**
L7	17.28	7.26	17.70	**14.08**	9.68	10.53	11.58	**10.59**
L8	15.23	17.21	29.35	**20.60**	10.56	11.59	12.21	**11.45**
L9	19.74	18.18	15.01	**17.64**	10.87	11.02	10.63	**10.84**
L10	17.86	20.67	21.94	**20.16**	12.08	12.57	11.53	**12.06**
L11	18.49	19.81	22.16	**20.15**	13.19	13.86	13.08	**13.38**
L12	16.54	20.43	18.70	**18.56**	16.31	14.00	12.55	**14.29**
L13	15.92	20.05	20.74	**18.90**	10.32	10.43	10.47	**10.41**
L14	15.55	17.60	19.83	**17.66**	11.30	11.08	10.90	**11.09**
L15	16.85	23.73	20.58	**20.39**	10.80	11.24	10.36	**10.80**
L16	15.98	16.16	17.04	**16.40**	14.35	12.24	12.11	**12.90**
L17	16.13	16.20	18.40	**16.91**				
L18	14.54	16.28	16.00	**15.61**				
L19	14.71	18.32	15.94	**16.32**				
Av.	17.71	20.00	20.15	**19.11***	11.46	11.65	11.84	**11.65**

	2008				2009			
	C	T1	T2	Aver.	C	T1	T2	Aver.
L1	14.14	13.35	16.14	**14.54**	12.45	11.23	11.41	**11.69**
L2	13.41	12.18	12.49	**12.69**	13.07	12.06	11.12	**12.08**
L3	12.92	11.29	12.93	**12.38**	12.98	11.45	10.48	**11.64**
L4	13.34	14.87	12.78	**13.66**	12.58	11.86	10.62	**11.69**
L5	13.99	13.04	13.46	**13.50**	12.87	12.41	10.85	**12.04**
L6	12.80	12.18	11.57	**12.19**	13.00	14.26	10.25	**12.50**
L7	13.64	13.22	14.08	**13.65**	13.54	12.33	12.14	**12.67**
L8	12.98	11.40	12.88	**12.42**	12.08	10.78	10.88	**11.25**
L9	12.60	11.45	11.01	**11.69**	12.81	11.00	10.62	**11.48**
L10	14.39	12.70	13.80	**13.63**	14.40	12.96	12.47	**13.28**
L11	13.47	11.02	13.15	**12.55**	14.30	11.19	12.31	**12.60**
L12	13.26	11.55	14.21	**13.01**	12.99	12.72	11.39	**12.37**
L13	13.13	13.16	13.67	**13.32**	12.55	12.82	13.49	**12.95**
L14	13.22	12.02	12.03	**12.42**	13.70	10.88	11.38	**11.98**
L15	12.54	12.51	13.70	**12.92**	12.96	12.17	12.06	**12.40**
L16	14.01	10.85	10.08	**11.65**	14.49	12.95	13.19	**13.54**
L17	13.84	11.09	11.55	**12.16**				
L18	12.76	11.47	12.56	**12.26**				
L19	11.66	11.31	11.05	**11.34**				
Av.	13.27	12.14	12.80	**12.73**	13.17	12.07	11.54	**12.26**
		Treatment	Genotype		Year			
$LSD_{0.05}$		4.16	4.24		2.64			

Average DM content had increasing trend in both herbicide treatments in 2006 and 2007 (Table 2), opposite to 2008 and 2009, when herbicides induced DM decrease, regardless to present variations between lines. Generally, the negative impact of both herbicides reflects through DM increase (Figure 3) and in higher degree in nicosulfuron treatment (R^2 = 0.102). Between lines, the highest alterations in DM were obtained under influence of nicosulfuron at L6, L7 and L17 during 2006, while the both herbicide had similar influence at L12, during 2007, L16 and L17 during 2008, as well as L11 and L14 during 2009. Variations in DM content were also reported by SACALA *et al* (2003), who emphasized that lower doses of rimsulfuron induced DM increase, while the mutual stress induced by herbicide and salinity decreased DM, equally to common influence of herbicide and meteorological factors. DM in L1, emphasized as the most sensitive line, according to highest EWRC values present during 2006-2008 wasn't altered significantly during examined seasons.

Generally, both applied herbicides induced decrease in average SP content, compared to control (Figure 1), as well as foramsulphron in higher extent to nicosulfuron. On the other hand, mechanism of action of sulfonylurea herbicides is based on restraining of polypeptide polymerization, so as the increased SP level could be achieved, as a consequence. Such results were confirmed by SACALA *et al* (2003) and STEFANOVIĆ *et al* (2010). The specific mode of actions of sulfonylurea herbicides is blocking of cell division, as they are initially applied in a certain concentrations (RAO, 2000). Also, this can be connected to the increased content of soluble proteins in susceptible maize plants 48 hours after the application of herbicides. According to different meteorological conditions present during examined four years, observed trend of SP increase was noticed at the most of lines during 2006 (with highest value at L1); L1, L3, L4, L8, L13 and L14 during 2007; at L10, L11 and L13 during 2008, under the influence of both herbicides. Moreover, increased EWRC values were observed at same lines (Table 3). Nicosulfuron induced average increase in SP level of majority of lines, indicating significant and negative correlation between SP and EWRC values (Figure 3). Other than that, foramsulfuron treatment increased SP level only at L1, L2 and L17. The decrease in SP level, observed at some lines, could be tied to lower EWRC values, what was emphasized during 2009, when visible injuries were absent.

Table 3. The EWRC evaluation, performed three weeks after herbicide application in: nicosulfuron (T1) and foramsulfuron (F2) treatments. EWRC values for 2009 were absent, because of missing of visible signs of injuries; PL lines: L1, L2, L6, L7, L8, L10, L12-L19; KL lines: L3, L4, L5, L9 and L11

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	Av.
											2006									
T1	3.0	2.3	1.7	1.7	1.7	3.3	2.0	2.7	2.7	2.0	2.3	2.3	2.7	2.0	2.3	3.0	2.0	2.3	3.3	2.4
T2	4.3	2.3	2.0	1.7	2.0	3.0	2.3	2.3	2.3	2.0	2.3	2.7	2.3	2.0	2.3	3.3	2.3	2.7	3.3	2.5
Aver.	3.7	2.3	1.8	1.7	1.8	3.2	2.2	2.5	2.5	2.0	2.3	2.5	2.5	2.0	2.3	3.2	2.2	2.5	3.3	2.4
											2007									
T1	6.7	1.3	1.0	1.0	1.0	1.3	1.3	1.3	1.3	1.0	1.3	2.3	1.3	1.7	2.7	1.3				1.8
T2	6.7	1.0	1.3	1.3	1.3	1.3	1.3	1.3	1.7	1.3	1.3	2.3	1.3	1.7	2.3	1.7				1.8
Aver.	6.7	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.5	1.2	1.3	2.3	1.3	1.7	2.5	1.5				1.8
											2008									
T1	2.7	1.0	1.2	1.2	1.7	1.5	1.7	1.8	1.3	1.0	1.2	1.5	1.7	1.0	1.7	0.3	1.5	1.5	2.0	1.4[a]
T2	6.8	2.2	2.5	2.3	2.0	2.8	1.7	1.7	2.5	1.8	1.5	1.8	2.2	1.7	2.0	1.2	1.7	2.0	2.2	2.2[b]
Aver.	4.8	1.6	1.8	1.8	1.8	2.2	1.7	1.8	1.9	1.4	1.3	1.7	1.9	1.3	1.8	0.8	1.6	1.8	2.1	1.8
Aver.	5.0[c]	1.7[a]	1.6[a]	1.5[a]	1.6[a]	2.2[b]	1.7[a]	1.9[a]	2.0[a]	1.5[a]	1.7[a]	2.2[b]	1.9[a]	1.7[a]	2.2[b]	1.8[a]	1.9[a]	2.1[a]	2.7[b]	
$LSD_{0.05}$			Treatment 1.01				Genotype 0.74				Year 0.99									

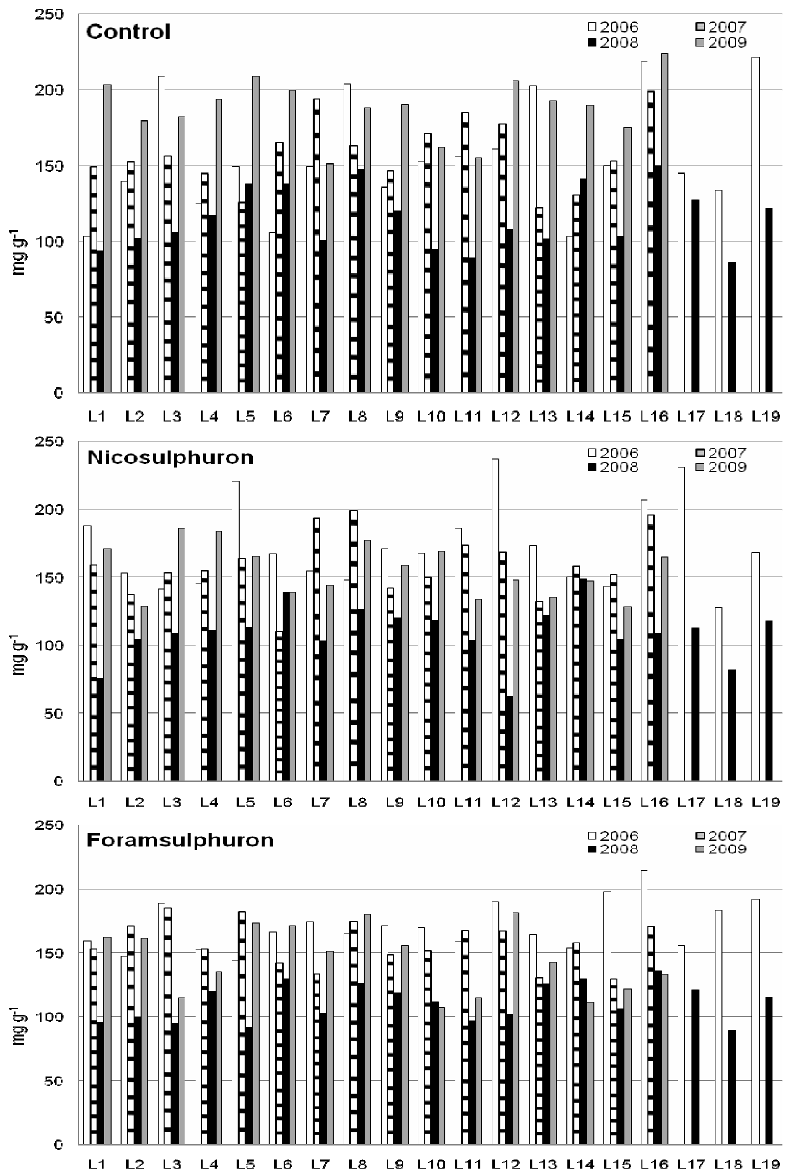

Figure 1. The alterations of soluble protein content in maize lines in control, nicosulfuron and foramsulfuron treatments; PL lines: L1, L2, L6, L7, L8, L10, L12-L19; KL lines: L3, L4, L5, L9 and L11 (Analysis of variance - $LSD_{0.05}$ Treatment = 33.91; $LSD_{0.05}$ Year = 25.20; $LSD_{0.05}$ Genotype = 33.48)

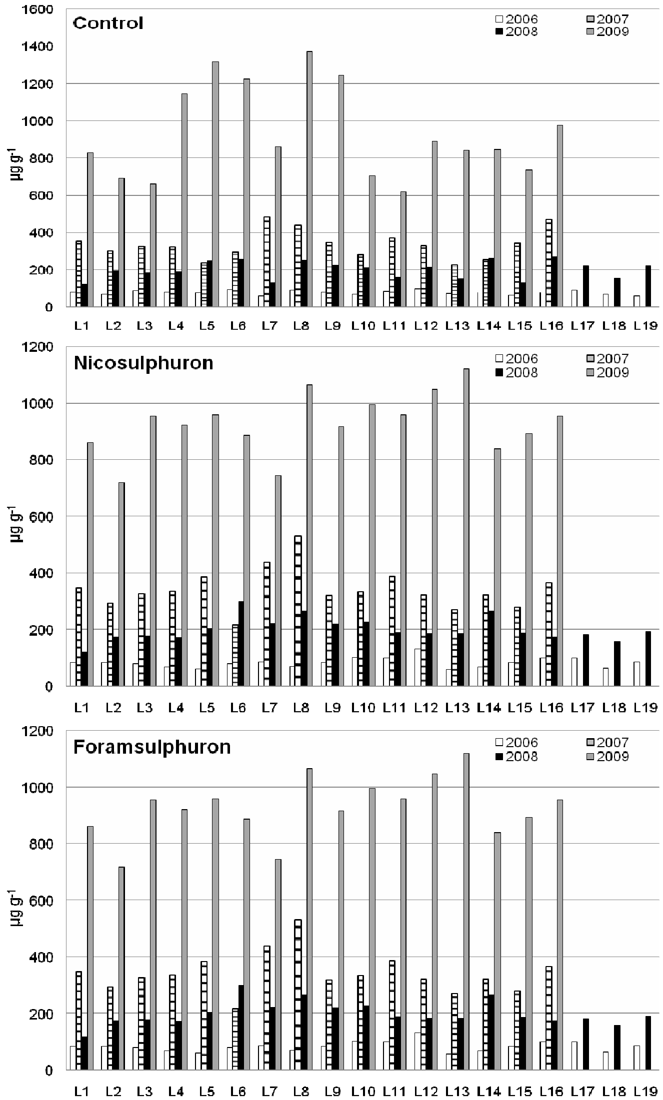

Figure 2. The alterations of pheniolic's content in maize lines in control, nicosulfuron and foramsulfuron treatments; PL lines: L1, L2, L6, L7, L8, L10, L12-L19; KL lines: L3, L4, L5, L9 and L11 (Analysis of variance - $LSD_{0.05}$ Treatment = 326.10; $LSD_{0.05}$ Year = 101.50; $LSD_{0.05}$ Genotype = 328.70)

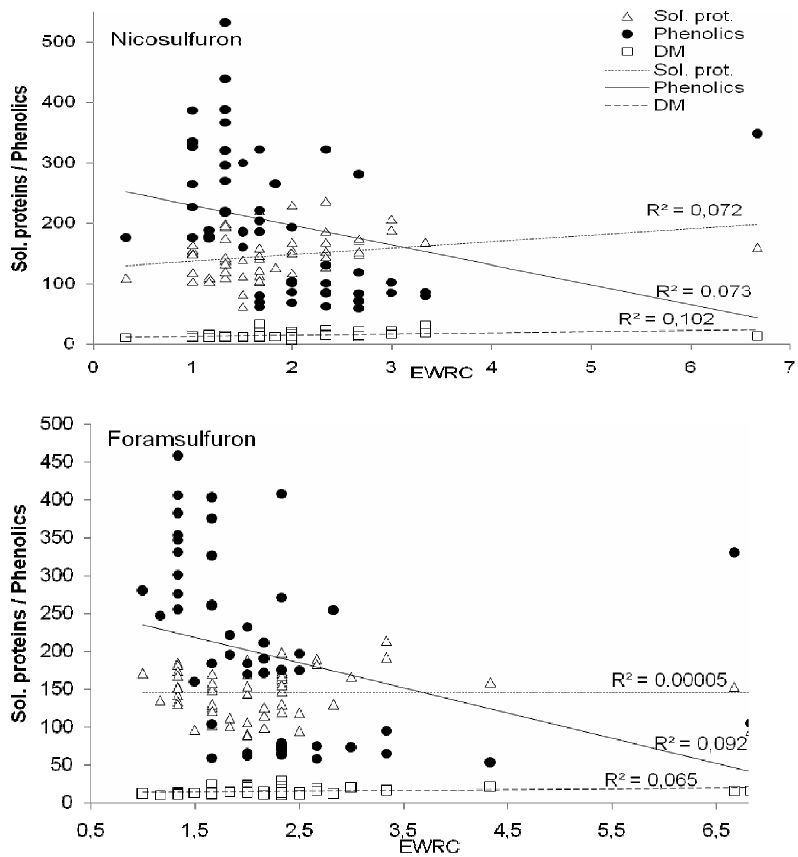

Figure 3. Correlation between EWRC values, soluble proteins and phenolics, influenced by nicosulfuron and foramsulfuron

Significant and negative correlation between EWRC values and phenolics' content (Figure 3) indicated their importance as a protective factor against stress induced oxidation. Their content also varied according to meteorological conditions, having lowest values in 2006 (Figure 2), as unfavourable season, while the highest content was obtained in 2009, season with highest average temperature and moderate precipitation level during May (Table 1). Nicosulfuron slightly increased average content of phenolics in relation to control (0.7 µg g^{-1} for all examined years), while foramsulfuron decreased it (25.2 µg g^{-1} for all examined years). Both herbicide treatments induced decrease in phenolics' content in most of examined lines (Figure 3), what was particularly underlined at foramsulfuron treatment in 2008 and 2009. Similarly to previous results of STEFANOVIĆ *et al* (2010), the increase in phenolics' content was observed mainly at lines, where EWRC values were relative low, i.e. in nicosulfuron treatment, the highest values of phenolic's content was obtained at L10 and L12 in 2006, L5 in 2007, L7 in 2008 and L3, L10, L11 and L13 in 2009. At foramsulfuron treatment, the highest increase in content of phenolics was noticed at L15 and L16 in 2006, L5 in 2007, L15 in 2008 and L2 in 2009. According to results of DRAGICEVIC *et al*. (2010); SIMIĆ *et al*. (2010) dry weight reduction appeared to coincide with the changes in the parameters of maize metabolism, such are proteins and phenolics, suggesting a regulatory role of secondary metabolism on growth of maize and soybean seedlings.

The phytotoxicity of applied herbicides reflected through EWRC values ranged from 6.7 (L1 at nicosulfuron and foramsulfuron treatments in 2007) to 0.3 (L16 at nicosulfuron treatment in 2008) (Table 3), with no observed phytotoxic effects in 2009. Significantly higher average EWRC was at L6, L12, L15 and L19. L1, as a KL line was determined as a highly sensitive line to both herbicides (EWRC was in range 2.7- 6.8), with more pronounced sensitivity to foramsulfuron, what was in agreement with results of STEFANOVIC *et al*. (2001; 2007). STEFANOVIĆ and SIMIĆ (2008) noticed that PL38, as the most susceptible line among other PL-s, like L1 in this research, show high sensitivity to sulfonylurea herbicides, while the KL lines were more tolerant to sulfonylurea herbicides, similar to our results. Such situation, with increased sensitivity of the most susceptible line (L1) was particularly underlined in 2007 and 2008, when precipitation level was higher during April-May (Table 1), similarly to results of BONIS *et al*. (2006), who ascertained that cool wet spring is retarding metabolic processes at maize, leading to greater injuries. Relative low average EWRC values (under 2) were obtained at majority of lines, in 2007 and 2008, when temperature was higher and precipitations were moderate during April.

Obtained data suggest that stress induced by nicosulfuron and foramsulfuron herbicides was mainly followed by increase in DM and SP and decrease in phenolic's content. That was particularly underlined during seasons with cold and wet period when herbicides were applied. In highly sensitive line crop, like L1 is nicosulfuron show lower toxicity, compared to foramsulfuron. Special attention must be given to application time (according to meteorological conditions and level of weed infestation). Lines, which could decrease SP content and increase level of phenolics, fast after application of sulfonylurea herbicides could be characterised as potentially tolerant, what gives importance to testing of each individual maize line. Moreover, alterations of SP and phenolic's content, according to obtained results could be used as a test in determination of tolerance of every individual maize line.

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ISPITIVANJE OSETLJIVOSTI LINIJA KUKURUZA PREMA NEKIM SULFONILUREA HERBICIDIMA

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Linije kukuruza su osetljive prema različitim herbicidima, što otežava semensku proizvodnju. Osetljivost zavisi u velikoj meri od meteoroloških uslova. Objekat ispitivanja je utvrđivanje genetičke varijabilnosti i korelacije između fitotoksičnosti (EWRC ocena) i promena suve materije (DM), fenola i rastvorljivih proteina (SP) kod 19 ZP linija prema nikosulfuronu i foramsulfuronu, sa ciljem da se utvrdi osetljivost pojedinačnih linija, kao i model potencijalne tolerantnosti, tokom perioda 2006-2009. Negativan uticaj oba herbicida se odrazio ne povećanje DM, ali u većem stepenu je na to uticao nikosulfuron. Primenjeni herbicidi su takođe uticali na povećanje prosečnog sadržaja SP i smanjenje sadržaja fenola. Zahvaljujući različitim meteorološkim uslovima prisutnim tokom četiri godine ispitivanja, povećanje SP je bilo praćeno povećanjem EWRC vrednosti kod istih linija,suprotno od linija kod kojih bi se pad SP i povećanje nivoa fenola mogli vezati za potencijalnu tolerantnost, što naglašava važnost testiranja svake pojedinačne linije. Posebna pažnja bi se trebala posvetiti vremenu upotrebe herbicida (vezano za na meteorološke uslove i zakorovljenost).

Smanjenje SP i povećanje sadržaja fenola kod pojedinih linija zajedno sa nižim EWRC vrednostima bi moglo biti povezano sa njihovom tolerantnošću, što je posebno bilo naglašeno tokom 2009, kada nisu bila zabeležena vidljiva oštećenja kod biljaka.

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