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**THE MANIFESTATION OF SYMPTOMS OF HERBICIDE
(SULFONYLUREA) PHYTOTOXIC EFFECTS IN THEIR
APPLICATION IN SEED MAIZE CROP**

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Effects of three sulfonylurea herbicides on 82 commercial maize inbreds, developed by the Maize Research Institute, Zemun Polje, were encompassed by the present study. Fifty six of the total number of inbreds were self-pollination ones derived in spatial isolation (PL), while the rest of 26 were inbred combinations (KL). The trial under production conditions was set up on degraded chernozem in Zemun Polje during 1997 and 1998. In 1997, the percentage of malformed plants was established for each inbred and each treatment. In 1998, phytotoxicity was determined only by the EWRC estimate. The highest percent of plants with leaf colour change (45.63 %), occurring ten days upon the herbicide application (1st estimate), was observed in the treatment with rimsulfuron in 1997. The percent of injured plants in a sense of colour change was on the average lower in inbred combinations (KL) than in PL inbred lines. The number of plants characterised by twisting and bending of the above ground parts was also on the average significantly lower. Injures of plants treated with primisulfuron-methyl, although the mildest,

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remained till the end of the growing season. The levels of phytotoxicity in PL inbreds, determined by the EWRC estimate, were higher in 1998 than 1997 and on the average ranged from 4.78 to 4.92, pointing out that plants were slightly to moderately injured.

Key words: maize inbred lines, sulfonylurea herbicides, effects of herbicides, symptoms, alteration of the above ground plants parts

INTRODUCTION

Amounts and combination of herbicides applied in seed maize production equal those applied in commercial maize production. It is, however, known that slow growing and lower habitus of plants significantly change microclimatic conditions, suiting grass weeds (fam. *Poaceae*), which have favourable conditions for emergence and growth in this crop during the whole vegetation period. Therefore, it is necessary to apply additional cultivation measures to seed maize crop during the vegetation period, such as hoeing and the herbicide application. Herbicides of the sulfonylurea group are the first selective herbicides used during the maize growing season (post-emergence) (MAURER *et al.*, 1987). They effectively control perennial and annual grasses, as well as, some broad leafed weed species in maize (FOY and WITT, 1990). Nicosulfuron and primisulfuron-methyl, as members of the sulfonylurea family, have similar chemical composition, rate of application and the same mode of action. On the other hand, it is known that plants of maize inbred lines are susceptible to impacts of many stress factors including herbicides (FUERST, 1987; LANDI *et al.*, 1990; ZARIĆ *et al.*, 1998).

The introduction of herbicides of the sulfonylurea group, resulted in susceptibility of a greater number of maize inbred lines to effects of herbicides in comparison with the number of inbreds when only soil applied herbicides had been used (GREEN and URLICH, 1994; LEVIĆ *et al.*, 1995). These herbicides are powerful inhibitors of cell division and plant growth. They have no effects on seed germination, but vigorously inhibit root and stalk growth in susceptible seedlings. EBERLINE *et al.* (1989) observed differences in susceptibility among maize inbred lines to the herbicide rimsulfuron and pointed out that herbicide degradation had been much faster in the resistant (A671) than in susceptible inbred (A619), what had been related to weather conditions. Results obtained in Yugoslavia by STEFANOVIĆ (1995); STEFANOVIĆ *et al.* (1996); MALIDŽA *et al.*, (1996); IVANOVIĆ *et al.*, (1997) are similar to those above mentioned.

All these phenomena are directly related to weather conditions of a year (STEFANOVIĆ *et al.*, 1997). Environmental factors significantly affect weed and maize development, but also plant uptake of herbicides and their effects. Herbicides can contribute to rapider decay of susceptible maize inbreds under unfavourable environmental conditions, especially under stress conditions of low temperatures at the beginning of the vegetation period when seedling emergence is slower.

Effects of three herbicides of the sulfonylurea group on changes of leaves and above ground plant parts of commercial PL and KL inbreds of ZP maize hybrids were observed in the present study with the aim to determine resistant inbreds in whose seed production the application of these herbicides is possible.

MATERIAL AND METHODS

The effects of three herbicides of the sulfonylurea group (primisulfuron-methyl-Tell, rimsulfuron-Tarot and nicosulfuron-Motivell) on the occurrence of symptoms of leaf colour change and malformation of above ground parts of commercial ZP maize inbreds were studied (Table 1). Fifty six of the total number of inbreds (82) were self-pollination ones derived in spatial isolation (PL), while the rest of 26 were inbred combinations (KL). The trial under production conditions was set up on degraded chernozem in Zemun Polje during 1997 and 1998. The elementary plot size amounted to 10 m². The whole plot was once hoed. The herbicides were applied on the average in the 6-8 and 8-9 leaf stage of PL, i.e. KL inbreds, respectively.

Table 1. - Herbicides applied in the trial

Herbicides	Commercial product	Quantity g.a.m. ha ⁻¹	Dates of application	
			1997	1998
Primisulfuron-methyl(75 %)	Tell 75 WG	3000	May 19	June 1
Rimsulfuron (25 %)	Tarot 25-DF	1500	"	"
Nicosulfuron (40 %)	Motivell	4800	"	"

The effects of herbicides on the above plant parts were monitored on all trial variants in 1997. Numerous plants with symptoms of leaf colour change and malformation of the above parts were detected. The percent of malformed plants for each inbred and each treatment was estimated on the basis of the relation between the number of plants with detected changes and the total number of the plants. The symptoms were scored two days, ten days and a month upon the herbicide application. The levels of phytotoxicity were estimated on the 1-9 EWRC scale (FELDVERSUCHE MANUAL, 1975). In 1998, phytotoxicity was estimated on the EWRC scale only twice and the dates of performed operations are presented in the Table 2.

Data obtained for each inbred and each treatment were processed and the number of malformed plants (leaf colour change or leaf rolling) for each estimate (%), average values of obtained data per each treatment and estimate and deviation from the mean - standard deviation (SD) were calculated.

Weather conditions for the growing season in 1997 and 1998 are presented in the Table 3. Since weather conditions varied over investigation years, the time of performing certain operations was adjusted to them.

Table 2. - Time-table of performed operations

Performed operations	Year	
	1997	1998
Sowing	April 29	April 23
1 st estimate of injured plants	10 days upon herbicide application	-
2 nd estimate of injured plants	39 days upon herbicide application	-
1 st EWRC estimate	15 days upon herbicide application	11 days upon herbicide application
2 nd EWRC estimate	-	30 days upon herbicide application

Table 3. - Weather conditions during growing season over investigation years

Months	1997		1998	
	Temperature (°C)	Precipitation (mm)	Temperatures (°C)	Precipitation (mm)
April	8.2	87.0	14.9	31.0
May	18.6	51.0	17.0	51.0
June	21.7	31.0	25.0	62.0
July	26.3	131.0	23.8	33.0
August	20.4	113.0	23.7	45.0
September	17.7	31.0	17.6	96.0
October	14.5	107.0	14.4	91.0
\bar{X}/Σ	18.2	551.0	19.5	409.0

The 1997 growing season was, on the average, characterised by lower air temperatures and significantly higher precipitation (551.0 mm) in relation to 1998 (409.0 mm). Moreover, significant differences in precipitation distribution over years were observed. Hence, in June 1997, precipitation was significantly lower upon the herbicide application than in the same month in the following year. Also, precipitation was abundant in the months of maize intensive development (July and August) in 1997 in contrast to 1998.

RESULTS AND DISCUSSION

Injuries of inbred line plants treated with sulfonylurea herbicides were manifested through different symptoms. Approximately ten days upon the herbicide application (1st estimate), changes on leaves of certain genotypes, depending on an applied herbicide, were observed. They occurred as colour alternation and leaf rolling. The above parts of treated plants mainly turned red (anthocyanin), while their leaves turned yellow (chlorosis). The intensity of these changes in susceptible genotypes was so strong that whole plants dried up. Results presented in Table 4 show that on the average the highest number of plants (11.7) with altered leaf

colour was in the injury category of 30-40 %. The greatest number of plants (16) with this symptom was detected in the treatment with rimsulfuron. In the second estimate, however, the majority of plants (26.7) were classified within the category of 0-10%, pointing out that plants had been recovered to a certain extent.

Table 4. - The number of plants with changed colour within 56 PL maize inbred lines in 1997

Injury category (%)	1 st estimate				2 nd estimate			
	Tell	Tarot	Motivell	Mean	Tell	Tarot	Motivell	Mean
0	0	0	0	0	20	20	15	18.3
0-10	15	0	5	6.7	25	28	27	26.7
10-20	14	4	10	9.3	7	6	8	7.0
20-30	7	9	12	9.3	1	0	2	1.0
30-40	7	16	12	11.7	0	0	1	0.3
40-50	5	11	8	8.0	0	0	0	0
50-60	2	7	4	4.3	1	0	2	1.0
60-70	1	2	3	2.0	0	0	0	0
0-80)	3	2	1	2.0	0	2	0	0.7
80-90	0	0	0	0	2	0	1	1.0
90-100	2	5	1	0	0	0		0
Total				56				

The average percentage of injured PL inbred plants (leaf colour change) ten days upon the herbicide application in 1997, in dependence on the applied herbicide, indicates that the highest percent of plants with altered leaf colour (45.63%) was detected in the treatment with rimsulfuron (Table 5).

Table 5. - The average percentage of leaf colour change in PL inbred plants caused by herbicide application in 1997 (%)

Applied herbicides	Number of plants with changed colour			
	1 st estimate		2 nd estimate	
	Average	SD	Average	SD
Primisulfuron-metil	26.97	23.21	18.66	32.12
Rimsulfuron	45.63	22.63	7.40	15.34
Nicosulfuron	31.80	19.62	12.01	21.51
Mean	34.80		12.69	

The mean percent of injured plants for all treatments amounted to 34.80%. In a month upon the herbicide application (2nd estimate) this percent decreased in all treatments (12.69%), pointing to the fact that the majority of inbreds had recovered.

The corresponding percent in inbred combinations (KL) was, on the average, lower (29.87), meaning that this inbreds were more resistant than the ones

derived in spatial isolation. The lowest percent of plants with altered colour of the above ground parts was, on the average, observed in the treatment with nicosulfuron (25.15%). Such a trend remained till the end of the growing season. A month later, the majority of plants recovered (33.91%).

Table 6. - Leaf colour change (%) in KL maize inbred lines caused by herbicides in 1997

Applied herbicides	Number of plants with changed colour			
	1 st estimate		2 nd estimate	
	Average	SD	Average	SD
Primisulfuron-methyl	25.77	16.59	3.24	8.15
Rimsulfuron	35.86	19.50	2.98	7.33
Nicosulfuron	25.15	12.85	2.50	5.98
Mean	29.87		2.91	

Fasciation type of malformation of the above ground parts is a second typical symptom caused by effects of sulfonylurea herbicides. Table 7 presents the frequency of the number of plants over injury categories from 0 (no injuries) to over 30%.

Table 7. - Average injuries of the fasciation type in PL maize inbred lines caused by herbicide application in 1997 (%)

Applied herbicides	Number of plants with malformed above ground parts			
	1 st estimate		2 nd estimate	
	Average	SD	Average	SD
Primisulfuron-methyl	4.09	9.62	0.77	2.64
Rimsulfuron	8.22	16.88	0	
Nicosulfuron	5.22	11.66	0	
Mean	5.84		0.26	

The number of plants with such a malformation was on the average significantly lower than the number of plants with changed colour (5.84%), while the deviations from the mean (SD) point to great variations. The highest percent of these changes in PL inbreds in the 1st estimate was on the average detected in the treatment with rimsulfuron (8.22%). However, a month later (2nd estimate), above ground part malformations and leaf rolling disappeared in the treatments with rimsulfuron and nicosulfuron, indicating a complete recovery of plants, in contrast to the treatment with primisulfuron-methyl, in which a low percent (0.77%) of malformed plants were observed. In respect to the malformation of this type, the greatest number of plants (17.7) at the beginning of the growing season belonged to the injury category 0. The average values of the number of plants with this symptom indicate that the greatest number of injured plants were in the variant treated with rimsulfuron (Table 8).

This type of malformation was much less presented in the inbred combinations (KL) than in the PL inbreds and a month later the majority of plants of these inbreds recovered.

The percent of malformed plants of these inbreds amounted to 5.80% in the treatment with rimsulfuron, which is higher than a mean for all treatments in the 1st estimate (3.63). Injuries of plants treated with primisulfuron-methyl, although the least significant, remained till the end of the growing season.

Table 8. - Number of malformed plants within the PL inbreds

Injury category	1 st estimate			Average	2 nd estimate			Average
	Tell	Tarot	Motivell		Tell	Tarot	Motivell	
0	22	16	15	17.7	47	0	0	15.7
0-1	9	2	7	9.3	2	0	0	0.7
1-2	9	9	11	9.7	2	0	0	0.7
2-3	3	4	1	2.7	1	0	0	0.3
3-4	2	7	8	5.7	1	0	0	0.3
4-5	1	2	2	1.7	0	0	0	0
5-10	4	8	4	5.3	1	0	0	0.3
10-30	3	4	6	4.3	2	0	0	0.7
>30	3	4	2	3.0	0	0	0	0

Table 9. - Number of crinkled plants within the KL maize inbreds caused by herbicides in 1997 (%)

Applied herbicides	Number of plants with malformed above ground parts			
	1 st estimate		2 nd estimate	
	Average	SD	Average	SD
Primisulfuron -methyl	1.78	3.43	0.04	0.23
Rimsulfuron	5.80	15.25	0	
Nicosulfuron	3.31	4.55	0	
Mean		3.63		0.01

Table 10. - The average EWRC estimate of phytotoxicity of PL and KL inbreds in relation to applied herbicides in 1997

Observed inbreds	Applied herbicides					
	primisulfuron -methyl		rimsulfuron		nicosulfuron	
	Estimate	SD	Estimate	SD	Estimate	SD
PL	3.36	1.26	3.75	1.40	3.25	1.01
KL	2.81	0.69	3.15	0.78	2.35	0.63
Mean		3.08		3.45		2.30

The EWRC estimate of toxic effects, used in this study as a method, confirms the stated data. The average values of the EWRC estimate of phytotoxicity for PL inbreds in 1997 was uniform ranging from 3.75 (rimsulfuron) to 3.25 (nicosulfuron) implying slight to moderate plant injuries (Table 10). The

average EWRC estimate in KL inbreds had similar trends with the difference that the detected values were lower (3.15 , i.e. 3.25, respectively). The highest average EWRC estimate of phytotoxicity of PL and KL inbreds in 1997 was detected in the treatment with rimsulfuron, which was confirmed by the 1st average ERWRC estimate (3.45) (Table10).

Since both, EWRC estimates and the percent of malformed plants, showed the identical trend in 1997, phytotoxicity was estimated in the subsequent year only by the EWRC method. In 1998, the average EWRC estimates of phytotoxicity of PL inbreds were higher than those in 1997 and they ranged on the average from 4.78 to 4.92, indicating that plant injuries were slight to moderate (Table 11). The higher average estimate of phytotoxicity in 1998 in relation to the preceding year, was probably a result of lower temperatures in the time of the herbicide application. Nonetheless, it is interesting that the average estimates determined a month later did not lag behind significantly (4.32- 4.62).

On the other hand, the average EWRC estimates were lower (3.69-2.92) in KL inbreds. Furthermore, these inbreds did not recover as the plants in 1997 did (2nd estimate). The lowest average EWRC estimate in both inbreds, PL and KL, at the beginning of the growing season was determined in the variant treated with primisulfuron-methyl. A faster recovery was observed in inbred combinations (KL) in comparison with PL inbreds, pointing to their resistance.

Higher temperatures, as well as, precipitation in June of 1998 favoured rapider growth and development of plants of both, inbreds and weeds. The herbicide application under such conditions stimulated a stronger manifestation of herbicide phytotoxic effects, contributing to the existence of a greater percentage of susceptible PL and KL inbreds in 1998 than in 1997 on the basis of the average EWRC estimate (Table 11).

Table 11. The average EWRC phytotoxicity of PL and KL inbreds in relation to applied herbicides in 1998

Observed inbreds		Applied herbicides					
		primisulfuron -methyl		rimsulfuron		nicosulfuron	
		No. of plants	SD	No. of plants	SD	No. of plants	SD
PL	1 st estimate	4,78	1,33	4,92	1,47	4,91	1,86
	2 nd estimate	4,34	1,56	4,62	1,76	4,32	2,10
KL	1 st estimate	2,92	1,62	3,69	1,09	3,46	1,75
	2 nd estimate	2,15	1,69	2,50	1,56	2,15	1,93

Symptoms caused by herbicides of the sulfonylurea group on maize are expressed as chlorosis or redness, as well as, various malformations and leaf rolling. Weather conditions have a great influence on herbicide effects, also reflecting, on plant growth and development. Thus, differences in expressing effects of these herbicides on plants occur each year. All this creates new problems in weed control in maize crop.

Obtained results indicate that inbred resistance to herbicides is a very complex problem. A primary basis of selectivity of sulfonylurea herbicides consists of, first of all, a different degree and speed of herbicide metabolising (HARMS *et al.*, 1990). Sulfonylurea herbicides inhibit the enzyme acetolactate synthase (ALS) in susceptible plants, while resistant species swiftly break them down to inactive products (RAY, 1984). According to GREEN and URLICH (1993) these differences in enzyme susceptibility are probably the essential factor in increased tolerance of maize hybrids to nicosulfuron. Weather conditions affect not only plant growth and development, but also, plant uptake of herbicides and their effects. Consequently, the manifestation of herbicide effects differs over years. The degree of a metabolised herbicide regulates resistance of maize and other observed species to nicosulfuron and primisulfuron-methyl. Hence, Johnson grass, for instance, did not show tolerance to these two herbicides. Results obtained by CAREY *et al.* (1997) point to the fact that tolerance to sulfonylurea herbicides is not always a function of a plant metabolism. Other factors could also contribute to differences in the degree of selectivity. A complex of the interaction of several factors can determine a degree of susceptibility of certain plant species to any of sulfonylurea herbicides.

Gained results indicate to a possibility to apply sulfonylurea herbicides to resistant maize inbred lines. The fact that inbred susceptibility to herbicides is strongly depended on weather conditions of the habitat, point out to compulsory cautiousness in the application of these herbicides in this crop seed production. A permanent monitoring of a response of new maize genotypes to effects of new herbicides is necessary. Information acquired in such a way will improve a technological process of hybrid maize seed production.

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ISPOLJAVANJE SIMPTOMA FITOTOKSIČNOG DELOVANJA HERBICIDA (SULFONILUREA) KOD PRIMENE U SEMENSKOM USEVU KUKURUZA

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I z v o d

Ispitivan je uticaj tri herbicida grupe sulfonilurea na 82 komercijalne linije kukuruza Instituta za kukuruz. Od ukupnog broja ispitivanih linija, 56 su samooplodne linije iz prostorne izolacije (PL) i 26 kombinacije linija (KL). Ogljed je postavljen u proizvodnim uslovima, na zemljištu tipa degradirani černoze u Zemun Polju tokom 1997 i 1998. godine. U 1997. godini na svim varijantama ogleđa utvrđivan je procenat deformisanih u odnosu na ukupan broj biljaka, za svaku liniju i svaki tretman. U 1998. godini korišćena je samo EWRC ocena fitotoksičnosti. Za svaki tretman obračunate su prosečne vrednosti dobijenih rezultata i odstupanje od proseka (SD).

Simptomi oštećenja biljaka linija tretiranih sulfonilurea herbicidima ispoljavala su se u pojavi različitih simptoma, koji su se sastojali u promeni boje listova i pojavi uvrnuća nadzemnog dela. Promena boje nadzemnog dela se najčešće manifestovala u vidu crvene ili žute boje listova tretiranih biljaka. Kod osetljivih genotipova, intenzitet ovih promena je tako jak da dolazi do sušenja cele biljke. Najveći procenat biljaka sa promenom boje nadzemnog dela deset dana po primeni herbicida (prva ocena) uočen na tretmanu rimsulfuronom (45,63 %). Procenat oštećenja biljaka tipa promene boje kod kombinacija linija (KL) je u proseku manji u odnosu na samooplodne PL linije.

Broj biljaka kod kojih su utvrđene deformacije tipa uvrnuća nadzemnog dela biljaka je u proseku bio znatno manji. Oštećenja biljaka tretiranih sa primisulfuron-metilom, iako najmanja, zadržavaju se do kraja vegetacionog perioda. Vrednosti EWRC ocena fitotoksičnosti kod PL linija u 1998. godini su veće u odnosu na 1997. godinu i u proseku su se kretale od 4,78 do 4,92, što ukazuje na to da su oštećenja biljaka laka do umerena.

Rezultati ukazuju da postoji mogućnost primene sulfonilurea herbicida kod otpornih linija kukuruza. Činjenica da je osetljivost linija u odnosu na herbicide u jakoj zavisnosti i od klimatskih uslova staništa, ukazuje na obaveznu opreznost pri primeni ovih herbicida u semenskoj proizvodnji ovog useva.

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