

**MANIFESTATION OF SYMPTOMS OF HERBICIDE
(SULFONYLUREA) PHYTOTOXIC EFFECTS AFTER
TREATMENT OF SEED MAIZE CROP**

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Effects of three sulfonyleurea herbicides on 82 commercial
maize inbreds, developed at the Maize Research Institute, Zemun
Polje, were investigated. Fifty- six of the total number of inbreds
were self-pollinating ones obtained in spatial isolation (PL), while
the remaining 26 were inbred combinations (KL). The trial was set up
in production conditions on a degraded chernozem soil at 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 percentage of plants with leaf colour change (45.63 %),
occurring ten days after herbicide application (1st estimate), was
found in the treatment with rimsulfuron in 1997. The percentage of
plants undergoing colour change was lower on the average in inbred
combinations (KL) than in PL inbred lines. The number of plants
characterized by twisting and bending of the above- ground parts
was also significantly lower on the average. Injuries of plants
treated with primisulfuron-methyl, although slightest, remained till

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

Key words: maize inbred lines, sulfonylurea herbicides, effects of herbicides, symptoms, change in above-ground plants parts

INTRODUCTION

Amounts and combinations of herbicides applied in seed maize production are the same as those applied in commercial maize production. It is, however, known that slow growing and lower habitus of maize plants significantly change the micro-climatic conditions, which suits grass weeds (fam. *Poaceae*) and provides favourable conditions for their emergence and growth in this crop during the whole vegetation period. It is therefore necessary to apply additional cultivation measures to seed maize crop during the vegetation period, such as hoeing and herbicide treatment. Herbicides of the sulfonylurea group are the first group of 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-leaved 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 mode of action. On the other hand, maize inbred lines are known to be 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 has resulted in susceptibility of a greater number of maize inbred lines to the 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 effect on seed germination but strongly 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), which depended on weather conditions. The results obtained in Yugoslavia by STEFANOVIĆ (1995); STEFANOVIĆ *et al.* (1996); MALIDŽA *et al.*, (1996); IVANOVIĆ *et al.* (1997) are similar to those mentioned above.

All these phenomena are directly related to weather conditions over the year (STEFANOVIĆ *et al.*, 1997). Environmental factors significantly affect weed and maize development, as well as the uptake of herbicides and their effects on plants. Herbicides may contribute to faster 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 on leaves and above-ground plant parts of commercial PL and KL inbreds of ZP maize hybrids were investigated aiming to determine resistant inbreds, whose seed production would allow the application of these herbicides.

MATERIAL AND METHODS

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-pollinating ones obtained in spatial isolation (PL), while the remaining 26 were inbred combinations (KL). The trial was set up under production conditions and on a degraded chernozem soil at Zemun Polje during 1997 and 1998. The elementary plot size was 10 m². The whole plot was hoed once. The herbicides were generally applied at the 6-8 and 8-9 leaf stages for PL and KL inbreds, respectively.

Table 1. Herbicides applied in the trial

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

Effects of herbicides on the above-ground plant parts were monitored in all trial variants in 1997. Numerous plants with symptoms of leaf colour change and malformation of the above-ground parts were detected. The percentage of malformed plants for each inbred and each treatment was estimated based on the relation between the number of plants with detected changes and the total number of plants. Symptoms were evaluated two days, ten days and a month after herbicide treatment. 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 shown in 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.

Table 2. Timetable of performed operations

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

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

Table 3. Weather conditions over the growing seasons of the investigation years

Month	1997		1998	
	Temperature (°C)	Precipitation on (mm)	Temperature (°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
$\bar{X} \pm \Sigma$	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) compared with 1998 (409.0 mm). Moreover, significant differences in precipitation distribution over the years were observed. In June 1997, precipitation was significantly lower after herbicide treatment than in the same month 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 to inbred plants treated with sulfonylurea herbicides were manifested by different symptoms. Approximately ten days after herbicide application (1st estimate), changes were observed on leaves of certain genotypes, depending on the herbicide applied. They occurred as colour change and leaf rolling. The above-ground parts of the treated plants generally turned red (anthocyanin), while leaves turned yellow (chlorosis). The intensity of such 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 changed leaf colour was in the injury category of 30-40 %. The highest 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 in the category of 0-10%, showing that plants had recovered to a certain extent.

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

Injury category (%)	1 st estimate			2 nd estimate				Mean
	Tell	Tarot	Motivell	Mean	Tell	Tarot	Motivell	
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 after herbicide treatment in 1997, depending on the applied herbicide,

indicates that the highest percentage of plants with changed 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 treatment in 1997 (%)

Applied herbicide	Number of plants with changed colour			
	1 st estimate		2 nd estimate	
	Average	SD	Average	SD
Primisulfuron-methyl	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 percentage of injured plants for all treatments amounted to 34.80%. A month after herbicide application (2nd estimate), this percentage decreased in all treatments (12.69%), showing that the majority of inbreds had recovered.

The corresponding percentage of inbred combinations (KL) was lower on the average (29.87), meaning that these inbreds were more resistant than the ones obtained in spatial isolation. The lowest percentage of plants with changed colour of the above-ground parts was, on the average, observed in the treatment with nicosulfuron (25.15%). Such a trend remained until 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 herbicide	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 another typical symptom caused by effects of sulfonylurea herbicides. Table 7 shows its frequency in 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 treatment in 1997 (%)

Applied herbicide	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 malformation was significantly lower on the average than the number of plants with colour change (5.84%), while deviations from the mean (SD) indicate considerable variations. The highest percentage of these changes in PL inbreds in the 1st estimate was on the average detected in rimsulfuron treatment (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 percentage (0.77%) of malformed plants was observed. Concerning 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 present in the inbred combinations (KL) than in the PL inbreds and a month later the majority of plants of these inbreds recovered.

The percentage of malformed plants of these inbreds amounted to 5.80% in the treatment with rimsulfuron, which is higher than the 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.

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, except 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) (Table 10).

Table 8. The number of malformed plants of PL inbreds

Injury category	1 st estimate				2 nd estimate			
	Tell	Tarot	Motivell	Average	Tell	Tarot	Motivell	Average
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. The number of crinkled plants of KL maize inbreds caused by herbicides in 1997 (%)

Applied herbicide	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

Inbred	Herbicide					
	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	

Since both EWRC estimates and the percentage of malformed plants showed identical trends 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 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 at the time of herbicide treatment. 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, those 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 and precipitation in June 1998 were favourable for faster growth and development of plants of both inbreds and weeds. 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 based on the average EWRC estimate (Table 11).

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

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

The acquired results indicate that inbred resistance to herbicides is a very complex matter. The basis of selectivity of sulfonylurea herbicides consists primarily of 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 of 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, manifestations of herbicide effects differ over the years. The degree of 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) indicate that tolerance to sulfonylurea herbicides is not always a function of plant metabolism. Other factors may also contribute to differences in the degree of selectivity. The complex of several interacting factors can determine the degree of susceptibility of certain plant species to any of sulfonylurea herbicides.

The findings indicate a possibility to apply sulfonylurea herbicides to resistant maize inbred lines. The fact that inbred susceptibility to herbicides strongly depends on weather conditions of the habitat shows the need for cautiousness in the application of these herbicides in this crop's seed production. Permanent monitoring of responses of new maize genotypes to the effects of new herbicides is necessary. Information acquired in such a way will improve the 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 černozem 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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