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STUDIES ON MAIZE INBRED LINES SUSCEPTIBILITY TO HERBICIDES

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This paper presents the analysis of results obtained during long-term studies on the response of maize inbred lines to herbicides. Under the agroecological conditions of Zemun Polje the response (reaction of maize inbred lines to herbicides of different classes was investigated. Biological tests were performed and some agronomic, morphological, physiological and biochemical parameters were determined when the response of maize inbred lines to herbicides was estimated. The use of active ingredients of herbicides from triazine, acetanilide, thiocarbamate to new chemical groups (sulfonylurea etc.), have been resulted in changes in weed suppression and susceptibility of inbred lines. Obtained results show that effects of

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herbicides on susceptible maize genotypes can be different: they can slowdown the growth and development and affect the plant height; they can also affect the stages of the tassel and ear development and at the end they can reduced grain yield of the tested inbreds. Numerous studies confirmed the existence of differences in susceptibility level of maize genotypes in relation to herbicides. According to gained results the recommendations for growers are made on the possibility of the application of new herbicides in the hybrid seed production.

Key words: maize inbred lines; herbicides; yield; fresh weight; EWRC estimation

INTRODUCTION

Since the beginning of the introduction of herbicides into the technological process of maize growing, a great attention has been paid not only to their efficiency in weed suppression, but also to their selectivity in relation to the crop. Effects of selective herbicides depend, to a great extent, on disruption of the process of the transport of applied compound in the plant organism and on the capacity of the plant to decompose herbicide components as foreign substances. However, the herbicide selectivity is not an absolute property but depends on many factors, such as: the type of the active ingredient, amounts and the time of the application, morphological and physiological plant traits, environment conditions, which, in combination directly or indirectly, affect plants (WYCH and SCHOPER, 1987).

Maize reaction to the herbicides applied in the soil is relatively good and is connected to the plant capacity to decompose the herbicide as early as in the root system. The alteration of active ingredients used in the maize crop from triazine, acetanilide, thiocarbamate to sulfonylurea, resulted in changes of mechanism in related to weed suppression. Results observed in numerous studies aimed for the observation of effects of selective herbicides on maize hybrids and inbreds confirmed the existence of differences in susceptibility levels of different maize genotypes. The increase of inbred line susceptibility was observed when new classes of herbicides for the grass weed (fam. *Poaceae*) control were produced and introduced into the practice. Maize inbreds became more susceptible if these herbicides were applied during the growing season of maize. As the consequence, weed suppression in maize seed production turned out to be very difficult. At the same time, all these effects are directly dependent on weather conditions of the year; they are interrelated and specific for each habitat. Under unfavourable environmental conditions at the beginning of the growing season (the low temperatures with the increased humidity), herbicides can cause stronger of weaker damages and can contribute to a faster decay of susceptible maize inbred plants. The absorption of herbicides increases at higher temperatures and higher humidity. Under such conditions, very susceptible and moderately susceptible inbreds mainly are affected (ZARIĆ *et al*., 1998; STEFANOVIĆ *et al*., 1997, 2000). The growth and development of maize inbreds are very important in the maize seed production. Flowering of parental pairs has to be consistent, and delay in the development and lagging in the growth of one component due to susceptibility to the herbicide is not desired.

Since knowing that the response of genotypes to applied herbicides is important in the evaluation of general efficiency, permanent studies aimed at this direction have been carried out in the world as well as in our country too. Different biological tests were applied and different agronomic, morphological, physiologic and biochemical parameters have been measured when the plant response to used herbicides was estimated.

EFFECTS OF HERBICIDES ON THE MAIZE PLANT - A HISTORICAL VIEW

Although maize plants are generally resistant to herbicides of the triazine group at the beginning of their application, some authors observed the differences in tolerance to simazine and atrazine in a smaller number of maize inbred lines (GROGAN *et al.,* 1963; ANDERSEN, 1964; EASTIN *et al*., 1964). Resistance to herbicides of these group is based on the maize plant capacity to decompose atrazine molecules soon after their absorption (SHIMABUKURO *et al*., 1970). Under conditions of Zemun Polje, the growth inhibition was observed in plants treated with atrazine and also chlorosis of the above ground plant parts in susceptible inbreds at the beginning of the growing season, (MIRŽINSKI-STEFANOVIĆ and PLESNIČAR, 1976; MIRŽINSKI STOMA-STEFANOVIĆ, 1977).

Herbicides belonging to the class of chloroacetamide and thiocarbamate (alachlor and metolachlor), affecting the emergence and the growth of grass coleoptiles, can damage plants of susceptible maize inbreds and hybrids (ZAMA and HATZIOS, 1986). Herbicides of these classes inhibit many physiological processes, especially the protein synthesis in the emergency period. Considering changes observed in susceptible plants, detailed studies were carried out related to the response of maize inbred lines to effects of these herbicides (DEAL and HESS, 1980). Tests of genotypes with different types of endosperm showed that standard maize hybrids have good tolerance to herbicides belonging to the class of acetanilide (FUERST, 1987). However, maize inbred lines can express differences in the extent of susceptibility. Many authors determined different responses of inbreds to alachlor (NARSAIAH and HARVEY, 1977; FRANCIS and HAMIL, 1980; BENET and GORSKI, 1989; acetochlor (LANDI *et al*., 1990); metolachlor (BOLDT and BARETT, 1989; ROWE *et* al., 1990); thiocarbamate and propachlor (BURT and AKINSOROTAN, 1976; SAGAREL and FOY, 1982) and to other herbicides (NICCUM, 1970; FLEMING *et al*., 1988). Under unfavourable climatic conditions for the maize development, these herbicides can damage plants and have phytotoxic effects.

Since different stress conditions affect maize inbred lines and thereby quality of the maize seed production, the response of maze genotype to herbicides has been investigated under conditions of the experimental field of Maize Research Institute in Zemun Polje for a long time period. Our results confirm the existence of differences in susceptibility of inbreds (MIRŽINSKI-STEFANOVIĆ, 1978; STEFANOVIĆ and ZARIĆ, 1989) (Tab.1).

Table 1. Effects of the herbicide application on the yield of maize inbred lines, the average for the 1986-87 period

Inbred lines	EPTC-dichlormid Yield (t ha^{-1})	EPTC-dichlormid Rank	Metolachlor Yield (t ha^{-1})	Metolachlor Rank	Control (t ha^{-1})	Average
F'7	2.37	M	2.47	R	2.56	2.47
CM'7MV	0.96	R	1.04	R	0.98	0.99
K-1	1.69	M	2.45**	R	1.96	2.04
HD-120-2-6	1.94	S	2.90	M	3.17**	2.67
A-654	3.69**	R	3.17	R	3.26	3.37
L155	4.16	R	3.95	R	3.86	3.99
R-59 rfc	3.21	S	3.65	S	4.45**	3.77
R-59 cms	4.41	M	5.06	R	5.07**	4.84
R-59-nrc	4.20	R	4.37	R	4.45	4.34
SP-8	4.02	R	4.24	R	4.20	4.15
V-158RFC	4.68*	R	4.71	R	4.35	4.58
L 70/9	5.19	M	5.86**	R	5.63	5.56
A 632Ht	4.57**	R	3.83	M	4.37	4.26
Mo17NRC	2.67**	R	2.03	S	2.62	2.44
B-73	4.10	R	4.78**	R	4.02	4.33
Average	3.46		3.63**		3.66**	

	Inbred lines	Herbicides	Inbred lines x Herbicides
$LSD_{5\%}$	2.9	1.3	5.0
$LSD_{1\%}$	3.8	1.7	6.6

R-resistant; M-medium resistant; S-susceptible; *P=5%; **P=1%

Results obtained in studies under field conditions show that herbicides inhibited the growth, i.e. they affected the plant height of investigated inbred lines (STEFANOVIĆ, 1986). These results also point out that herbicides affected phases in the tassel and ear development in susceptible inbred lines. These phases were shifted and normal ear fertilisation was not possible in three out of 15 inbreds observed under field conditions, which all interfered with the seed production (STEFANOVIĆ and ZARIĆ, 1989, 1991). A lag in developmental stages in certain inbreds directly resulted in reduced average yields and increased grain moisture of observed maize inbred lines treaded with a combination of atrazine and EPTC-dichlormid, as well as with metolachlor. Herbicides also affected the plant dry matter content, leaf area, as well as the chlorophyll content in maize leaves treated with metolachlor (BOJIČIĆ *et al.*, 1995). Furthermore, it was observed that parental components of hybrids of early maturity groups expressed greater susceptibility to herbicides (STEFANOVIĆ *et al.,* 1995). Phytotoxic effects of herbicides on maize inbred plants can be initiated by different causes. A greater amount of herbicides can cause lagging in the growth of maize seed crops in the initial developmental stages, reducing of inbred plant height, fresh biomass and grain yield (STEFANOVIĆ, 1992). Negative effects are not caused only by genotype susceptibility, but also by the herbicide application in the inappropriate developmental stage of inbred lines, caused by climatic conditions.

Generally, maize hybrids with standard seed quality are tolerant to the majority of herbicides that are applied in this crop. Hybrids with specific traits (sweet corn, popcorn, white corn, etc.), and particularly maize inbred lines, can differently react to herbicides of different classes, which limits their efficient and safe application (MONKS *et al.,* 1992; MORTON *et al.,* 1992; O'SULIVAN *et al.*, 1995, 1998; STEFANOVIĆ *et al.*, 2002, 2005; PAJIĆ, 2007). Parental components (inbred lines), belonging to the FAO early maturity groups, expressed greater susceptibility to herbicides (STEFANOVIĆ *et al.*, 1995). The choice of herbicides to be applied in the maize seed production has to be such that it provides good efficiency for the majority of herbicides and sufficient safety for the crop.

Since knowing that the response of genotypes to applied herbicides is important for maize breeders and seed growers, many studies have been carried out in this area. Due to the development of new inbred lines through the process of breeding and selection, as well as, the production of new herbicides with the possibility to be applied in different maize developmental stages, the studies of inbred responses to herbicides of new classes are still actual (STEFANOVIĆ and SIMIĆ, 2007, 2008). The analysis of long-term results show that the introduction of herbicides of new chemical groups led to the changes in the number of maize inbred lines susceptible to herbicides.

RESPONSE OF MAIZE INBRED LINES TO POST-EM HERBICIDES

The introduction of herbicides of the class sulfonylurea in the maize production process widen the possibilities for the efficient suppression of perennial and annual weeds of the family *Poaceae* and weeds resistant to atrazine, due to the fact that these herbicides can be applied during the growing season of maize (KUPATT and MAURER, 1989; FOY and WITT, 1990). On the other hand, the introduction and the intensive application of herbicides of the sulfonylurea class, led to the increase of susceptibility of inbred lines (STEFANOVIĆ *et al.,* 1995, 2007; MALIDŽA *et al.,* 1996, MALIDŽA, 2007). Due to the reduced tolerance of maize inbreds, the majority of herbicides that are applied after emergence are not permitted in the seed production under conditions in Serbia. However, a strong weediness resulted in the application of these herbicides in the hybrid maize seed production. Therefore, studies on the response of maize plants to sulfonylurea herbicides are becoming actual.

Obtained results confirmed that the number of inbreds susceptible to herbicides had increased. EBERLEIN *et al.* (1989) established differences in resistance to rimsulfuron among inbreds and pointed to the fact that the herbicide metabolism was much faster in the resistant than in the susceptible maize inbreds. Based on the studies of response of 20 maize inbred lines, LANDI *et al.,* (1989, 1990) determined a broad genetic variability in maize resistance to sulfonylurea herbicides. Results indicate that differences in enzymes activities are probably the main factor of greater tolerance of maize hybrids to nicosulfuron (GREEN and URLICH, 1993; GREEN, 1998). 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 (CAREY *et al.,* 1997). Differences among treated inbreds were determined by measuring different parameters of growth (STEFANOVIĆ *et al*., 2004), (Tab.2).

Table 2. Statistical analysis of observed parameters (average for 20 inbreds)

Observed Parameters	Control	Herbicides			$LSD_{1\%}$
		nicosulfuron	primisulfuron-methyl	rimsulfuron	
Plant height	174.20 a	173.00 a	165.90 b	166.50 b	3.334
Ear height	59.44 a	57.45 ab	54.30 c	54.44 bc	2.74
Leaf width at the ear bottom	8.05 a	8.01 a	7.91 ab	7.76 b	0.21
Tassel length	38.26 a	38.10 ab	36.83 c	37.13 bc	0.97
Number of primary branches	8.31 b	8.92 ab	9.35 a	9.08 a	0.75
Grain yield	4.40 a	4.09 a	4.14 a	4.21 a	0.31
Grain moisture percentage	34.62 b	35.55 a	34.8 b	34.96 ab	0.63

The values followed by the same letters are not significantly different at the 0.01 level

Genotypes susceptible to the application of sulfonylurea herbicides lag in the growth and deform. Symptoms are more pronounced than symptoms caused by the previous herbicide class and are expressed in a form of various malformations, twists, colour changing, plant height reducing and at the end in a form of grain yield reduction. These changes are noticeable at the beginning of the growing season, and later on they significantly decrease (STEFANOVIĆ *et al*., 2000, STEFANOVIĆ and SIMIĆ, 2007, 2008). As already stated, different factors can contribute to differences in the degree of the selectivity expression. Environmental factors significantly affect the plant development, the herbicide absorption by plants and effects of herbicides on plants (STEFANOVIĆ *et al*., 1996, 1997). Previous studies confirmed that unfavourable conditions and under effects of herbicides plant biomass changes, i.e. pheno - phases of a generative organ development shift in maize inbred lines (STEFANOVIĆ *et al.,* 1993, 1999; MILIVOJEVIĆ *et al.,* 1998; 2003), (Tab. 3).

Table 3. Fresh biomass of maize inbred line plants treated with herbicides (average 1996-1998)

Inbreds	*H e r b i c i d e s*						Average
	H1	H2	H3	H4	H5	H6	
A 634M	854.44	877.78	776.67	661.11	663.33	762.20	765,93
B 84M	1120.00	1071.11	1167.78	1122.22	894.44	1126.67	1083,71
L-92	458.89	581.11	534.44	568.89	496.67	806.67	574,44
L-1325	413.33	443.33	575.56	533.33	328.89	562.22	476,11
L-217	571.11	617.78	690.0	615.56	528.89	681.11	617,41
L-70/9	460.0	542.22	497.78	448.89	488.89	693.33	521,85
L-105	352.22	404.44	227.78	360.00	306.67	694.44	390,93
L-3/452su	813.35	932.22	764.44	991.11	587.88	946.67	839,26
L-773M1	696.67	720.00	714.44	770.0	548.89	780.0	705,00
Va-35nrc	447.78	585.56	468.89	513.33	415.56	481.11	490,37
Average	621.78	677.56	641.78	658.44	526.00	753.44	$LSD_{5\%}$ =54.52
$LSD_{5\%}$ H = 42.23							

H1-Tell 20+20 g; H2-Tell 40 g; H3-Tarot 30+30 g; H4 - Tarot 60 g; H5- Motivell 1 l; H6-Control

Recent results show that the values of investigated parameters varied over years of investigation (Table 4). The treatment with herbicide H1 caused a negative response in the majority of inbreds in 2006. The susceptible inbreds were characterised with a lower bound water content and a higher phenol content. According to the EWRC scale, the highest response estimations on the average for all inbreds were registered in treatments with H5 in 2006 and H4 in 2007. The herbicide application resulted in significantly lower yields.

The soluble protein content in the shoots of treated inbreds significantly varied from the control (Figure 1). Depending on a genotype and herbicide, the protein content in the shoots was generally higher in variants treated with herbicides than in the control, especially in 2007. Results obtained in previous studies show that the protein content increased in susceptible genotypes as a results of the herbicides application such as atrazine and metolachlor (HIRANPRADIT *et al.,* 1972; PILLAI *et al*., 1979). On the other hand, sulfonylurea herbicides have a very specific mode of action; initially and applied in small doses they do not affect photosynthesis, respiration, synthesis of proteins, lipids and RNA, but if applied in certain concentrations they strongly block the cell division (RAY, 1982). This can be explained by the increased content of soluble proteins in susceptible maize plants 48 hours after the application of herbicides (RAY, 1980).

Table 4. Average values of investigated parameters in dependence on herbicide treatments

	Bound water (%)		Phenolics (mg g DM^{-1})		EWRC		Grain yield (t ha^{-1})	
	2006	2007	2006	2007	2006	2007	2006	2007
Control	39.00	5.86	80.17	354.92	-	-	7.66	3.93
H1	32.13	6.42	87.26	330.26	2.16	1.72	6.87	4.22
H2	34.98	6.33	65.88	306.75	2.42	1.92	7.00	4.09
H3	31.24	6.06	65.81	282.28	2.55	2.14	7.67	3.95
H4	40.18	5.99	77.50	274.60	2.48	2.83	7.51	4.00
H5	28.63	6.12	87.29	284.00	3.16	2.64	7.58	4.07

H1-Motivell; H2-Equip; H3-Tarot plus; H4-Maister; H5-Grid

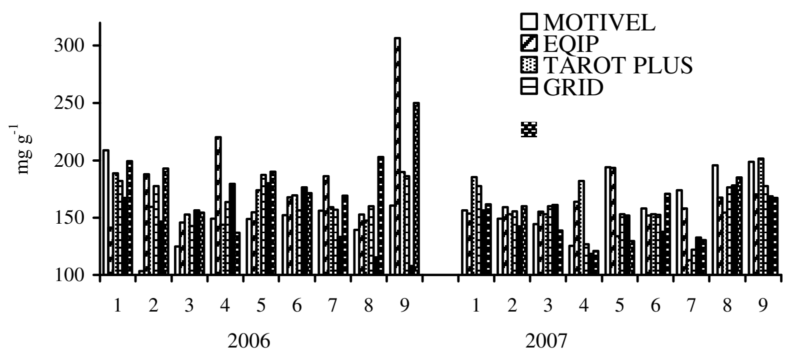

Figure 1. The effects of applied herbicides on the level of soluble proteins in maize plants

The development of maize growing practices has been resulted in the introduction of new herbicides that are more selective and have fewer limits regarding to the date of their application during the growing season. These herbicides are more adapted and safe for weed suppression and thereby the seed maize. The application of herbicides with new active ingredients, such as mezotrion, foramsulfuron, sulcotrione, etc., with new effects and wider possibility of application has been increased in the seed crop production. This imposes the need of permanent studying of effects of herbicides on maize inbred lines. The response to new herbicide classes was observed over 40 maize inbred lines PL and KL in the 2004-2007 period. As in previous studies, the obtained results vary in dependence on a genotype, an applied herbicide and meteorological conditions of the year. The average EWRC estimate of phytotoxicity of observed inbred lines treated with herbicides over years ranged from 2.36 to 3.08. The average values of this estimation (3.5) over herbicides did not also exceed the susceptibility limits, pointing out to very mild and mild damages (STEFANOVIĆ and SIMIĆ, 2007). The average seed yield was good over years and treatments with the exception of 2007, when it was lower due to weather conditions (tab. 5).

Table 5. Average EWRC estimations of PL inbred lines in dependence on herbicide and year

Treatments	Years							
	2004.		2005.		2006.		2007.	
	EWRC	S.D.	EWRC	S.D.	EWRC	S.D.	EWRC	S.D.
T1	2.42	2.71	3.0	0.67	2.60	0.45	2.0	1.62
T2	2.71	0.77	2.9	0.43	-		-	
T3	2.52	0.98	3.0	0.79	2.69	0.64	2.0	1.59
T4	3.45	0.83	3.3	0.52	2.86	0.54	2.3	1.76
T5	2.60	0.54	3.5	0.64	2.75	0.41	2.8	1.81
T6	2.60	0.93	2.8	0.34	-		-	
T7	-		-		3.39	0.97	2.70	1.22
Average	2.72		3.08		2.89		2.36	

T-nicosulfuron; T-isoxaflutole; T3-foramsulfuron; T4-dicamba +rimsulfuron; T5-mezotrion;T6-rimsulfuron+ thifensulfuron-methyl; T7-foramsulfuron + iodosulfuron.

Table 6. Response of the inbred PL 38 to applied herbicides expressed by the EWRC estimate and grain yield (t ha^{-1})

Treatments	Years							
	2004		2005		2006		2007	
	EWRC	t ha^{-1}	EWRC	t ha^{-1}	EWRC	t ha^{-1}	EWRC	t ha^{-1}
T1	4.33	1.82	5.3	0	3.0	2.04	6.7	1.7
T2	2.67	1.73	2.5	0	-	-	-	
T3	6.5	1.54	6.2	0	4.3	1.54	6.7	0
T4	6.17	0.9	4.9	0	4.0	1.9	6.3	1.16
T5	3.17	1.4	5.6	0	-		-	
T6	5.5	1.38	2.8	0	3.3	2.3	7.0	0.79
T7	-	-	-		6.3	0.92	6.0	0.73
Average	4.72	1.46	4.55	0	4.18	1.74	6.54	0.88
S.D.	1.59	0.33	1.53	0	1.29	0.53	0.39	0.62

T1-nicosulfuron; T2- isoxaflutole; T3-foramsulfuron; T4-dicamba +rimsulfuron; T5-mezotrion;T6-rimsulfuron+ thifensulfuron-methyl;T7-foramsulfuron + iodosulfuron.

Favourable ecotoxicological properties of mezotrion provide the application of this herbicide in the seed maize crops. According to the statistical analysis of the observed parameters, the rate of treated plant damage expressed by the EWRC estimate of phytotoxicity in the average of six hybrids significantly varied in dependence on a year and an observed genotype. The exception was the inbred PL 38, which was the most susceptible among observed inbreds. The average values of the EWRC estimate for this inbred ranged from 4.72 to 6.54 and the yield was low. These results point to a fact that observed herbicides should not be applied in the hybrid combinations that include this inbred (tab.6).

CONCLUDING OBSERVATIONS

Considering problems we face in weed suppression with herbicides in seed maize crops, and in order to provide more efficient and safe weed control, a combined application of several measures within the integrated weed management system is recommend (SWANTON and MURPHY, 1996; KOVAČEVIĆ and MOMIROVIĆ, 1996; SIMIĆ et al., 2004; 2009).

Due to a high-cost and sensitivity of the seed production, it is very important to perform all preventive actions in due time from the selection of the plot, preceding crops, primary tillage to pre-sowing. Sowing in the seed production is one of a key cropping practice, since parental components of the majority of hybrids can be sown on two or tree dates that are related to a certain developmental and growth stage. In such a way, smaller amounts of herbicides would be used in order to alleviate risk and crop damages. The introduction of the crop rotation that would encompass rotation of crops and herbicides would also be one of measures of this system. Weed infestation will be easily controlled and herbicide requirements will be lowered if the crop rotation with the adequate tillage is professionally performed.

Studies confirmed that maize tolerance to herbicides is very complex (STEFANOVIĆ and ZARIĆ, 1990, 1991; ZARIĆ *et al*., 1998; STEFANOVIĆ and SIMIĆ, 2008). Considering that new genotypes have been permanently derived by the process of breeding and selection and that the chemical companies have been constantly producing new herbicides, these studies are still relevant. Knowing different responses of maize inbred lines, i.e. the degree of their susceptibility to certain herbicides, is extremely important in the estimation of the general efficiency of herbicides in weed suppression and in the selection of new hybrids. Such information will improve the technological process of hybrid seed maize production. The results point out to a possibility of the application of new herbicide classes in the seed maize production. Since maize inbred susceptibility to herbicides greatly depends on the climatic conditions of a year, when herbicides are applied during the growing season, it is necessary to be aware of the previous inbred responses and also of developmental stages of both, cultivated and weed plants. Based on comprehensive studies and experimental results recommendations related to the obtainment of high maize yields in the seed production are made.

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REZULTATI ISPITIVANJA OSETLJIVOSTI LINIJA KUKURUZA NA HERBICIDE

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I z v o d

U radu se, na osnovu višegodišnjih ispitivanja, daje analiza rezultata koji se odnose na reakciju linija kukuruza prema herbicidima. U višegodišnjem periodu u uslovima Zemun Polja ispitivana je reakcija samooplodnih linija kukuruza u odnosu na herbicide raznih grupa, primenjenih u raznim rokovima. Za ocenu reakcije biljaka prema herbicidima korišćeni su razni biološki testovi i mereni različiti pokazatelji: agronomski, morfološki i fiziološko – biohemijski. Sa promenom aktivnih materija koje su se koristile u usevima kukuruza od triazina, acetanilida, tiokarbamata do uvođenja novih hemijskih grupa (sulfonilurea i dr.), menjali su se i problemi u vezi suzbijanjem korova i osetljivošću samooplodnih linija kukuruza. Rezultati istraživanja pokazuju da herbicidi kod osetljivih genotipova kukuruza mogu da uspore rastenje, deluju na visinu biljaka, utiču na proticanje etapa razvića metlice i klipa i na kraju smanje prinos zrna ispitivanih linija. Brojnim istraživanjima potvrđeno je postojanje razlika u nivou osetljivosti raznih genotipova kukuruza u odnosu na herbicide. Na osnovu dobijenih rezultata se daju preporuke proizvođačima o mogućnosti primene novog herbicida u proizvodnji hibridnog semena.

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