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Response of *Sorghum halepense* (L.) Pers. populations to nicosulfuron, rimsulfuron and cycloxidim in the Bosnia and Herzegovina

Biljana Kelečević*[1], Siniša Mitrić[1], Maja Simić[1], Renata Dejanović[2]
[1]University of Banja Luka, Faculty of Agriculture, Republic of Srpska, Bosnia and Herzegovina
[2]Bayer d.o.o., Bayer Crop Science, Republic of Croatia
e-mail: biljana.kelecevic@agro.unibl.org

SUMMARY

Resistance of *Sorghum halepense* (L.) Pers. to acetolactate synthase (ALS) inhibitors has been reported worldwide, but has not previously been confirmed in Bosnia and Herzegovina. In this study, four populations were evaluated for their response to nicosulfuron, rimsulfuron, and cycloxydim using rhizome-derived plants. Resistance was assessed through a bioassay and molecular techniques based on PCR. Nicosulfuron and rimsulfuron were applied at 0.5×, 1×, 1.5×, and 2× the recommended dose, while cycloxydim was applied at recommended dose (1×). Phytotoxicity was assessed 20 days after treatment. Resistance to nicosulfuron and rimsulfuron was confirmed in two populations, whereas all tested populations remained susceptible to cycloxydim. Molecular analysis identified the Trp574-Leu mutation at frequencies of 14% and 50% in two populations. Given the extensive maize production and reliance on ALS inhibitors in weed control, early detection and the implementation of integrated weed management strategies are essential to delay and mitigate the spread of resistant johnsongrass.

Keywords: ALS-inhibitors, resistance, *Sorghum halepense,* weed control.

INTRODUCION

The development of some agricultural scientific disciplines, such as entomology and phytopathology, began before the development of weed science (Zimdahl, 2010). The primary goal of these early disciplines was not control, but rather the understanding of the underlying biological phenomena. In contrast, weed science began to develop when weeds could be controlled (Zimdahl, 2025). Initially, herbicide-based weed control was somewhat rudimentary—relying primarily on the identification and location of weed species, without a deeper understanding of their biology or ecology (Buhler, 2006). Over time, as both weeds and cultivated plants co-evolved, weeds adapted to various agrotechnical and chemical practices applied to arable land. One major outcome of this adaptation is the development of resistance. Resistant weed populations represent a challenge for their control (Konstantinović et al., 2008; Simić et al., 2012; Vrbničanin, 2020; Ilić et al., 2022). The phenomenon of reduced weed sensitivity to herbicides was first documented in 1954 (Abel, 1954), and the first cases of resistance to ALS inhibitors were recorded shortly thereafter, in the early 1980s (Heap, 2025). The widespread use of monoculture and repeated application of herbicides with the same mode of action significantly accelerated the development and spread of resistance—a phenomenon now observed globally (Vrbničanin, 2020).

Sorghum halepense - johnsongrass is a highly competitive and economically the most significant weed species, particularly in disturbed and agricultural areas of Bosnia and Herzegovina (B&H). In the territory of the Republic of Srpska, it is more prevalent in intensively cultivated agricultural regions, especially where corn is predominantly grown, and where *S. halepense* cause a big problem (Kovačević and Mitrić, 2013). ALS herbicides (acetolactate synthase inhibitors) are widely used in a variety of cultivated crops, such as small grains, corn, soybeans, peas, beans, alfalfa, potatoes, grapevines, sunflowers, and imidazolinone-tolerant sugar beets, as well as in non-agricultural areas. Frequent and intensive use of herbicides from this group of herbicides has resulted in the development of resistance in numerous weed species. Resistance to ALS inhibitors in *S. halepense* has already been documented across all countries in the region, including Serbia (Bozic et al., 2007; Malidža et al., 2014; Malidža and Rajković, 2017; Vrbničanin et al., 2017), Croatia (Dejanović et al., 2018), as well as a Chile, Hungary, Israel, Italy, Mexico, Spain, United States, and Venezuela (Heap, 2025). Therefore, it was only a matter of time before such cases appear in the territory of B&H. The causes of reduced sensitivity of *S. halepense* to herbicides, in addition to resistance, may be: the quality of herbicide application, agrometeorological conditions, phenophase of weed development, etc. (Božić et al., 2010). To confirm weed resistance to herbicides, clear scientific research is necessary, primarily involving molecular studies and/or bioassay methods.

This study aimed to determine occurence of resistent populations of *S. halepense* to ALS herbicides rimsulfuron and nicosulfuron in Bosnia and Herzegovina, as well as the potential resistance to acetyl-CoenzymeA carboxylase (ACC-ase) inhibitors cycloxydim.

MATERIAL AND METHODS

S. halepense rhizomes were collected from three populations (P_1-P_3) suspected of resistance to ALS-inhibiting herbicides and one population (P_4) never before exposed to herbicides. The rhizomes were sampled from corn fields in the northern part of B&H (Table 1). Rhizomes from plants that survived the application of nicosulfuron at a rate of 80 g a.s. ha^{-1} were collected in the autumn of 2019.

Table 1. Overview of the studied populations.

Population	Locations	Latitude (N)	Longitude (E)	Herbicide applied in 2019	Crop
P_1	Laktaši 1	44°54'52.64"	17°18'57.78"	nicosulfuron	maize
P_2	Srbac	45° 6'11.68"	17°31'11.57"	nicosulfuron	maize
P_3	Laktaši 2	44°56'42.92"	17°22'1.90"	nicosulfuron	maize
P_4	Novi Grad	45° 2'46.99"	16°22'6.05"	-	maize

Populations P_1, P_2 and P_3 were subjected to PCR analysis to detect target-site substitution of Pro197-Thr and Trp574-Leu at the ALS gene, and Ile1781-Leu, Trp2027-Cys, Ile2041-Asn, Asp2078-Gly and Gly2096-Ala at the ACCase gene. Rhizomes were collected from each of these three populations that survived the treatments and were sent to Bayer's research center for resistance in Germany. Mutations in the genes responsible for resistance at the target site of ALS herbicides (target site resistance) were analyzed using molecular biology techniques based on PCR technology (polymerase chain reaction). DNA sequence analyses were performed using a pyrosequencer.

Four populations (P_1-P_4) were used for a bioassay. Rhizomes were cleaned and stored at 4°C for 14 days. Rhizomes about 5 cm long, each with two to three nodules were planted in pots filled with commercial humus. The pots were maintained under greenhouse conditions until the plants reached a 20-25 cm in height. Plants (20-25 cm tall) were sprayed with diferent nicosulfuron and rimsulfuron rates, as well as cycloxydim (Table 2) using a research spray chamber calibrated to deliver 400 L ha^{-1}. Each treatment was set up in four replicates, along with untreated plants per population, used as control. Following herbicide drift exposure, plants were returned and kept under greenhouse conditions. Phytotoxicity was evaluated at 20 days after application (DAA), by visual assessment (0-100%).

Table 2. Herbicide application rates.

Herbicide	Product	Active substance content	Rate of herbicide application (g a.s. ha^{-1})				
			0×	0.5×	1×	1.5×	2×
Nicosulfuron	Talisman	40 g L^{-1}	0	25	50	75	100
Rimsulfuron	Tarot 25-WG	250 g kg^{-1}	0	7.5	15	22.5	30
Cycloxydim	Focus ultra	100 g L^{-1}	0	-	200	-	-

The obtained data were statistically processed using the software package STATISTICA 12. The effective-dose to cause 50% of phytotoxicity (ED_{50}) was estimated for each progeny using a four-parameter log-logistic model:

$$Y = C + (D - C) / \{1 + \exp [B(\log X - \log E)]\} \qquad [1]$$

In which Y corresponds to the phytotoxicity (%),
B is the slope at the inflection point,
C is the lower limit of the model (fixed to 0%),
D is the upper limit (fixed to 100%), and
E is the inflection point (effective dose to cause phytotoxicity in 50%).
This functional dependency was measured using the statistical program Origin®Pro 2016.

RESULTS AND DISCUSSIONS

Molecular analysis results. Using PCR technology, a mutation in one gene (Trp574-Leu) was detected in P_1 and P_2 populations, at a frequency of 14% and 50% respectively (Table 3). Although Pro197-Thr mutation is the most common (occures in 51% cases) in *Lolium multiflorum* and *S. halepense*, Trp574-Leu mutation occurs in about 23% of cases, compared to the total number of mutations reported in the ALS gene so far (Saavedra-Avila et al., 2025). In this case Trp574-Leu mutation was confirmed. Resistance to ACCase inhibitors (cycloxydim) was not detected, which was confirmed by the bioassay method (Table 4).

Table 3. Results of gene mutation analysis in *S. halepense*.

Mutations		Samples from location P_1		Samples from location P_2		Samples from location P_3	
		Target site resistance	Mutations	Target site resistance	Mutations	Target site resistance	Mutations
ACCase	Ile1781- Leu	no	0.0%	no	0.0%	no	0.0%
	Trp2027-Cys	no	0.0%	no	0.0%	no	0.0%
	Ile2041- Asn	no	0.0%	no	0.0%	no	0.0%
	Asp2078- Gly	no	0.0%	no	0.0%	no	0.0%
	Gly2096- Ala	no	0.0%	no	0.0%	no	0.0%
ALS	Pro197-Thr	no	0.0%	no	0.0%	no	0.0%
	Trp574-Leu	yes	50.0%	yes	14.0%	no	0.0%

As *S. halepense* is a tetraploid (4N) plant, according to Dejanović et al. (2018), mutation in one of these gene copies confers weak resistance, which is not significant in field conditions. However, the presence of a mutation on two copies of the gene out of four implies a high resistance. Moreover, the Trp574-Leu mutation is among the most common ALS gene mutations responsible for a high level and broad spectrum of resistance to ALS-inhibiting herbicides (Božić et al., 2024). In this study, although only a mutation in one gene copy (Trp574-Leu) was observed, it caused a significant decrease in sensitivity in the P_1 and P_2 populations (Figure 1 and 2).

Bioassay responses of *S. halepense* populations. The bioassay under controlled conditions shows almost no effect to nicosulfuron (Figure 1) and rimsulfuron (Figure 2) in P_1 and P_2 populations. When these populations were treated with two times the recommended rate of nicosulfuron (100 g a.s. ha^{-1}), % of phytotoxicity were 10% and 25% at 20 days, respectively. We noted plants that survived a 2-fold dose with almost no damage in both of these populations (Picture 1).

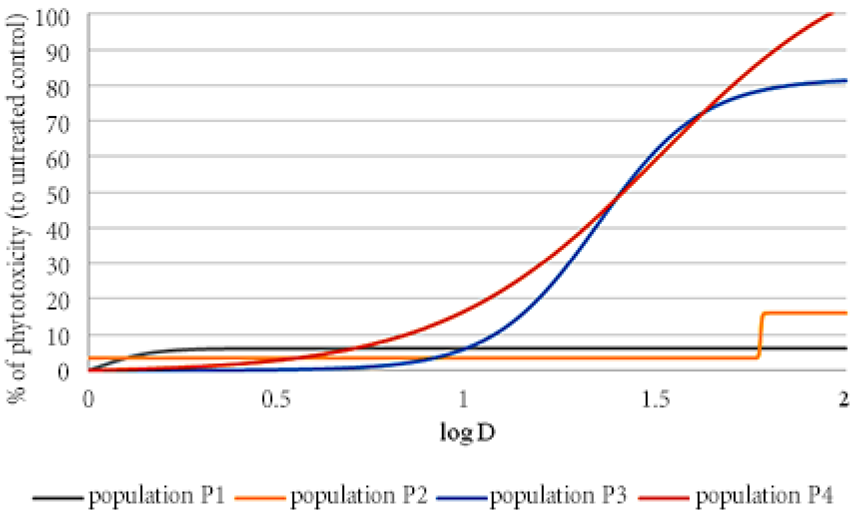


Figure 1. Response of *S. halepense* (P_1-P_4) populations to increasing nicosulfuron rates.

Given that even the highest applied amount of herbicide (nicosulfuron = 100 g a.s. ha^{-1}) did not cause significant phytotoxic effects on the treated plants from P_1 and P_2 populations, it was impossible to fit the data with the log–logistic equation and ED_{50} could not be calculated. However, Panozzo et al. (2017) characterized populations with LD_{50} values ranging from 109-514 g a.s. ha^{-1} of nicosulfuron as medium to highly resistant. Given that the P_1 and P_2 populations in our study exhibited no substantial injury even at 100 g a.s. ha^{-1}, it can be concluded that they fall within the resistant category, likely exhibiting at least a moderate level of resistance. The mean survival rates of P_3 and P_4 were 76.75% and 76.76%, respectively. The amount of nicosulfuron required to cause phytotoxic effects of 50% in P_3 and P_4 populations was 22.11 a.s. g ha^{-1} and 32.09 g a.s. ha^{-1}, respectively.

Populations resistant to nicoslfuron showed low sensitivity against another ALS inhibitor – rimsulfuron. Resistance to rimsulfuron was further evident in the P_1 and P_2 populations (Figure 2), which exhibited only 10% and 30% phytotoxicity, respectively, even when treated with double the recommended rate (30 g a.s. ha^{-1}) (Picture 1).

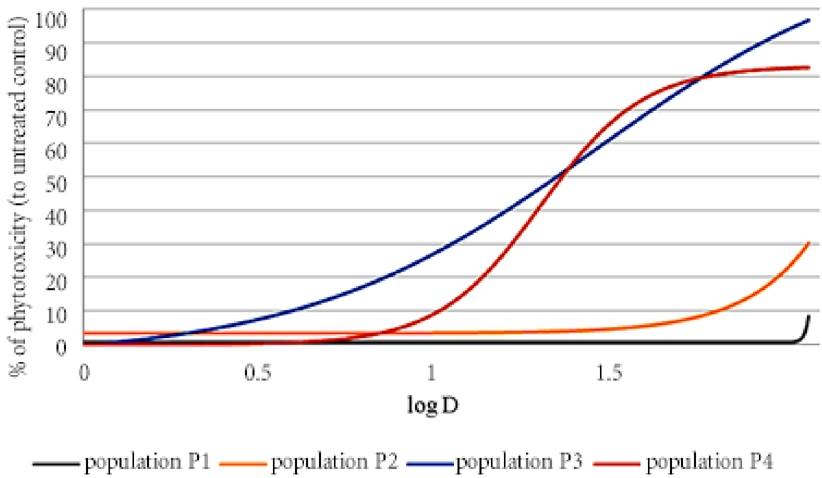


Figure 2. Response of *S. halepense* (P_1-P_4) populations to increasing rimsulfuron rates.

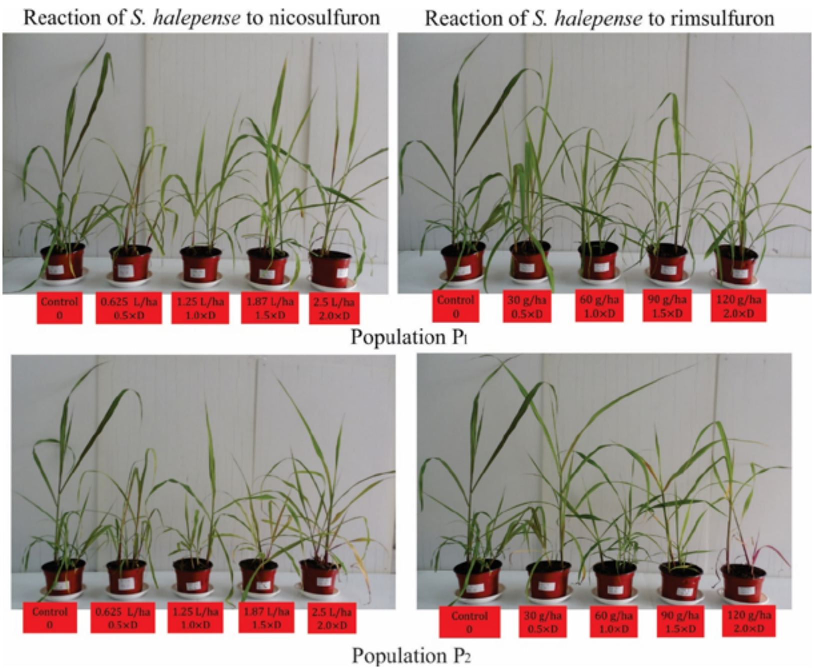
Table 4. Phytotoxicity of nicosulfuron, rimsulfuron and cycloxidim on *S. halepense* populations (P_1-P_4).

Population	Rate	Mean (%) ± σ (nicosulfuron)	Mean (%) ± σ (rimsulfuron)	Mean (%) ± σ (cycloxydim)
P_1	0.5×	9.25 ± 2.21	2.25 ± 1.89	86.75 ± 4.72
	1.0×	4.00 ± 0.81	4.50 ± 1.91	
	1.5×	5.25 ± 0.96	6.00 ± 1.83	
	2.0×	5.75 ±1.50	8.25 ± 2.36	
P_2	0.5×	8.00 ± 0.81	6.25 ± 0.96	96.25 ± 4.79
	1.0×	4.50 ± 1.00	8.00 ± 2.16	
	1.5×	20.00 ± 3.56	12.25 ± 1.71	
	2.0×	15.25 ± 4.57	30.50 ± 4.20	
P_3	0.5×	48.75 ± 4.35	52.75 ± 2.21	94.50 ± 3.11
	1.0×	76.75 ± 5.38	76.25 ± 3.40	
	1.5×	77.75 ± 5.43	81.00 ± 4.97	
	2.0×	82.75 ± 6.40	99.25 ± 0.95	
P_4	0.5×	49.50 ± 4.20	33.25 ± 8.50	97.00 ± 2.45
	1.0×	76.76 ± 3.95	82.00 ± 5.72	
	1.5×	99.25 ± 0.96	83.25 ± 5.91	
	2.0×	99.50 ± 1.00	86.00 ± 7.75	

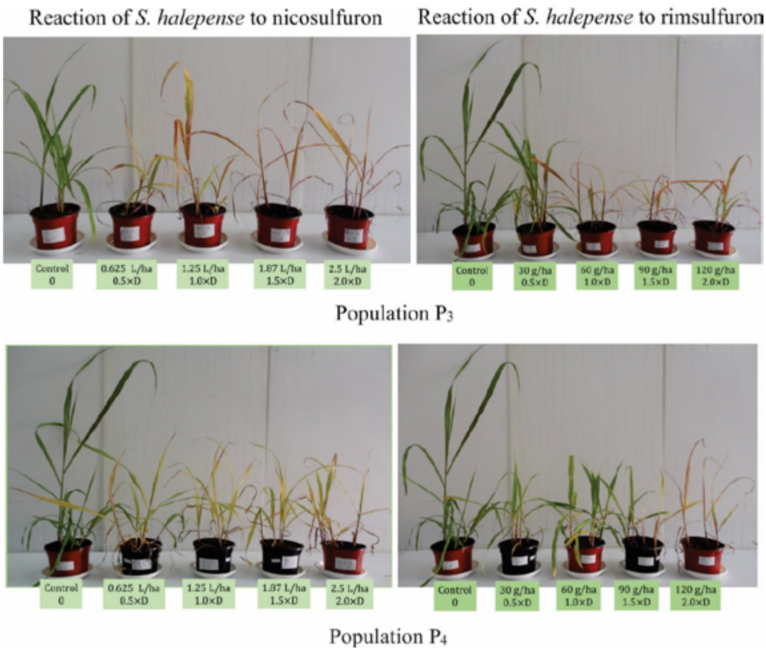
Conversely, the P_3 and P_4 populations showed a substantial growth reduction of 76.25% and 82.00%, respectively, when exposed to the recommended rate of rimsulfuron (15 g a.s. ha^{-1}).

With the treated plants of populations P_1 and P_2, no symptoms of phytotoxicity were observed, whereas phytotoxicity, as a significant reduction in plant height, was recorded in populations P_3 and P_4 (Picture 1 and 2). At the end of the experiment (approximately one month after the treatment), rhizomes of susceptible plants were destroyed, whereas new shoots emerged from the rhizomes of resistant plants (picture not shown).

ACC-ase inhibitor (cycloxydim), used in the registered dose, showed very high efficacy in all the tested populations in our experiment. The average efficacy values for cycloxydim ranged from 86.75-97.00% at the recommended dose (Table 4). The resistance of *S. halepense* to this herbicide group has not been described in the study area, including our study; all tested samples in our study were proven sensitive (Figure 3). This result could be very useful for herbicide treatment in the management of ALS-resistant *S. halepense*. Nevertheless, relying only on this group of herbicides in weed control can lead to new problems. The first case of *S. halepense* resistant to ACCase inhibitors (HRAC 1) in Serbia was confirmed in 2017, followed by the first recorded case of resistance to cycloxydim in 2021 (Malidža and Krstić, 2022). This phenomenon is most likely due to the excessive reliance on ACC-ase inhibitor herbicides in perennial monocultures, particularly under conditions where resistance to ALS inhibitors has already been established. Since the emergence of *S. halepense* biotypes resistant



Picture 1. Effect of increasing rates of nicosulfuron and rimsulfuron on resistant populations of *S. halepense* to ALS inhibitors (20 DAA).



Picture 2. Effect of increasing rates of nicosulfuron and rimsulfuron on sensitive populations of *S. halepense* to ALS inhibitors (20 DAA).

to acetyl-CoenzymeA carboxylase (ACC-ase) inhibitor herbicides can cause significant economic damage (Malidža et al., 2022), this highlights the need to develop an economically sustainable strategy for delaying resistance emergence and managing it.

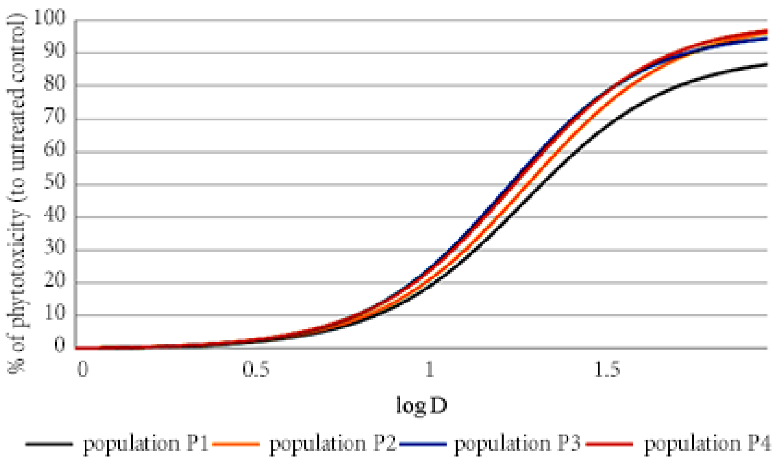


Figure 3. Response of *S. halepense* (P_1-P_4) populations to cycloxydim.

CONCLUSION

Resistance of johnsongrass to acetolactate synthetase (ALS) inhibitors has been confirmed in many cases. In the study area, although suspected, the presence of resistant biotypes has not been previously confirmed. Consistent bioassay and molecular analysis results confirm the presence of ALS-resistant *S. halepense* biotype in the studied populations. In populations in which the resistance to ALS inhibitors was confirmed by molecular analysis, ED_{50} could not be determined. However, even the highest applied dose of nicosulfuron (100 g a.s. ha^{-1}) and rimsulfuron (30 g a.s. ha^{-1}) did not cause phytotoxic reactions on treated plants. The results of molecular analysis and bioassays clearly indicate that the plants from the studied populations are susceptible to cycloxydim, and no resistance to acetyl-CoA carboxylase (ACC-ase) inhibitors was observed. The presence of a resisvtant biotype of *S. halepense* to ALS inhibitors greatly restricts the choice of herbicides available for weed control. These findings underscore the necessity for more extensive field research aimed at assessing the scope and prevalence of the issue. Such efforts are essential for informing the implementation of effective preventive measures and for identifying sustainable and context-appropriate solutions for the affected sites. All that significantly increases production costs and creates numerous challenges for agricultural management.

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Reakcija populacija *Sorghum halepense* (L.) Pers. na nikosulfuron, rimsulfuron i cikloksidim u Bosni i Hercegovini

REZIME

Rezistentnost *Sogrhum halepense* L. (Pers.) na herbicide iz grupe inhibitora acetolaktat sintetaze (ALS inhibitore) konstatovana je u mnogim slučajevima, međutim na teritoriji Bosne i Hercegovine do sada nije zvanično potvrđena. Za ispitivanje reakcije četiri populacije na aktivne supstance: nikosulfuron, rimsulfuron i cikloksidim korišćeni su rizomi. Rezistentnost je procijenjena korišćenjem molekularne tehnike zasnovanoj na PCR tehnologiji i biotesta. Nikosulfuron i rimsulfuron su primijenjeni u različitim količinama (0,5×, 1×, 1,5× i 2× od količine koja je preporučena za primjenu u poljskim uslovima), dok je cikloksidim primjenjen u preporučenoj količini (1×). Ocjena fitotoksičnosti je urađena 20 dana nakon tretmana, vizuelnom ocjenom (0-100%). Rezistentnost na nikosulfuron i rimsulfuron potvrđena je kod dvije populacije, dok su sve analizirane populacije bile osjetljive na cikloksidim. Molekularnom analizom je konstatovana mutacija na mjestu Trp574-Leu, u frekvenciji 14% i 50%. S obzirom na obim proizvodnje kukuruza i značaj primjene herbicida iz grupe ALS inhibitora u kontroli korova, rano otkrivanje i primjena integralnih mjera u suzbijanju korova ključni su za odgađanje i spriječavanje širenja rezistentnog divljeg sirka.

Ključne reči: ALS inhibitori, rezistentnost, *Sorghum halepense*, suzbijanje korova.