

Changes in Free Amino Acids Content in Kochia (*Kochia scoparia*) Resistant to ALS-Inhibitors

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Abstract

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The content of free amino acids was determined in aboveground parts of selected *Kochia scoparia* s.l. populations after treatment with chlorsulfuron and imazapyr. In the population from the locality Kolín (27% resistant individuals) the total content of free amino acids rose to 155% and 245% of the control 24 h after chlorsulfuron treatment (using doses of 22.5 g and 67.5 g/ha), while imazapyr treatment (1000 g/ha) increased the content to 215% of the control. In populations from the locality Prague-Vršovice (78% of resistant individuals) the same treatments resulted in a level of 115% and 94% (for chlorsulfuron) and 131% (for imazapyr) of free amino acids compared to the control. The plants from the two localities differed in the content of the amino acids valine and leucine. A significant decrease of these amino acids in plants from Kolín in contrast to those from Prague-Vršovice substantiates the opinion of a higher sensitivity of acetolactate synthase (ALS) which is responsible for the biosynthesis of amino acids with branched chains. A significant increase of arginine content was determined in both populations after all herbicide treatments.

Key words: *Kochia scoparia*; resistance to chlorsulfuron and imazapyr; content of amino acids

Resistance of the weed kochia, *Kochia scoparia* s.l., to sulfonylureas was first observed on wheat fields in the U.S.A. in 1987 (PRIMIANY *et al.* 1990). In 1993, resistant kochia was detected in 832 habitats of 11 states of the U.S.A. and three Canadian provinces (SAARI *et al.* 1994). Cross resistance to imidazolinones in some weed species was confirmed later (THILL *et al.* 1989).

The physiological effect of sulfonylureas and imidazolinones is manifested by inhibition of cell division in connection with a disturbance of DNA synthesis (ROST *et al.* 1990) as the consequence of changes in the biosynthesis of free amino acids, especially those with branched chains. The enzyme acetolactate synthase (ALS), which takes part in biosynthesis of these amino acids, can be inhibited by herbicides of the sulfonylurea and imidazolinone groups.

As kochia is expected to spread in some geographical regions including our country (TONKS & WESTRA 1997; JEHLÍK 1998), it is necessary to pay attention to all pertinent studies of the weed. In our laboratory the differences in growth of kochia populations with different sensitivity to imazapyr and chlorsulfuron (CHODOVÁ & MIKULKA

1998) and sensitivity to other selected herbicides (CHODOVÁ & MIKULKA 1997) have been studied.

Our present work continues the earlier ones, by studying the differences of free amino acids content in populations with different levels of resistant individuals.

MATERIAL AND METHODS

Tested Biotypes of Kochia (*Kochia scoparia* s.l.)

Seeds of kochia were collected at the localities Kolín and Prague-Vršovice in autumn 1997. In preliminary experiments the percentage of susceptible and resistant individuals within both populations were verified. Plants

Table 1. Percentage of resistant individuals of *Kochia scoparia* after treatment with chlorsulfuron and imazapyr

Herbicide	Dose [g/ha]	% resistant individuals	
		Vršovice	Kolín
Chlorsulfuron	45	78	27
Imazapyr	2000	65	12

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3 to 5 cm high were treated with herbicides. Individuals which did not die even 3 weeks after treatment were rated as resistant (Table 1). Sometimes they were of a lighter yellow color, had more compact growth, and broader leaves that after chlorsulfuron treatment were reddish in color.

Growing of plants: Seeds were sown in August 1998 into 10 × 10 cm sized plastic containers (0.1 g of seeds per container). After emergence the number of plants per container was reduced to 10. The plants were grown under natural weather conditions, though protected against rain and watered by soaking.

Application of herbicides: Three weeks after sowing the plants were treated with herbicides. They were sprayed with a hand sprayer at an amount of 50 ml of water per 1 m² or herbicide solution of a concentration given in Table 2.

Table 2. Herbicides used in the experiments

Herbicide	Active ingredient
Arsenal	Imazapyr 250 g/l
Glean 75 WG	Chlorsulfuron 75%

Five containers were treated with each herbicide and dose. The experiments were repeated three times.

Free amino acid content determination: 24 h after treatment with herbicides, 3 g of fresh matter of the aboveground parts of both treated and untreated plants were harvested and inactivated by hot steam for 5 min. The specimens were cut into small parts, homogenized in a mortar and extracted with 80% ethanol in a refrigerator. After 24 h the homogenates were filtered, the absorbent cotton washed twice with 10 ml of 80% ethanol and 5 ml of water and the solution then evaporated. The residue was dissolved in 5 ml of 80% ethanol and 2 ml of water and centrifuged 10 min at 10 000 g. The supernatant was evaporated again at 50°C and the residue dissolved in 2 ml of 80 % ethanol.

The analysis of free amino acids was done in a gas analyser at the Institute of Organic Chemistry. The data

presented are the average of analyses of the three repetitions. The contents of amino acids in 1 g of dry or fresh weight are given. Part of the fresh material was used to determine the content of dry matter at 78°C.

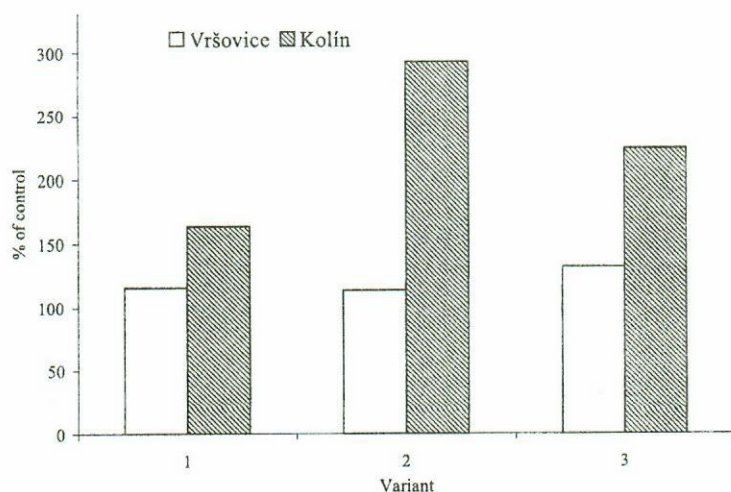
RESULTS AND DISCUSSION

The herbicides chlorsulfuron or imazapyr when applied to a leaf are very quickly transported throughout the plant system, and within 24 h most of the active substance is distributed, causing significant metabolic changes in the plant (SHANER & ROBSON 1985; SHANER & MALLIPUDI 1991).

In this study we determined the contents of free amino acids in the aboveground parts of the two kochia populations that differ in the proportions of individuals resistant to herbicides. At the locality Kolín the percentage of individuals resistant to chlorsulfuron was 27%, and those resistant imazapyr was 12%. That locality had never been treated with herbicides before, in contrast to the locality Vršovice which over the past several years had been treated irregularly with the herbicide Arsenal. At this locality the percentage of individuals resistant to chlorsulfuron was 78%, and those resistant to imazapyr 65% (Table 1). The percentage of chlorsulfuron resistant individuals was higher at both localities. That corresponds with the higher sensitivity of acetolactate synthase from kochia to chlorsulfuron, as reported e.g., by SAARI *et al.* (1990) from Canada.

The total amino acids contents in the aboveground parts of kochia populations treated with herbicides is presented in Table 3. Their content in fresh matter expressed as percentage of the untreated control is shown in Fig. 1.

After treatment with chlorsulfuron and imazapyr the total contents of free amino acids in the aboveground parts of kochia from the locality Kolín (with a high number of susceptible individuals) increased compared to the control. After chlorsulfuron treatment with doses of 22.5 and 67.5 g/ha the values reached 155 and 245%, and after imazapyr treatment 214% of that of the control.



- 1 – chlorsulfuron (22.5 g/ha)
- 2 – chlorsulfuron (67.5 g/ha)
- 3 – imazapyr (1000 g/ha)

Fig. 1. Effects of chlorsulfuron and imazapyr on the free amino acids content in fresh matter of *Kochia scoparia* expressed as the percentage of control

Table 3. Effects of chlorsulfuron and imazapyr on the free amino acids contents of *Kochia scoparia* from two localities

Concentration of herbicides	μmol/g dry matter of aboveground parts	
	Vršovice	Kolín
Control	19.793	26.961
Chlorsulfuron (22.5 g/ha)	22.802	41.937
Chlorsulfuron (67.5 g/ha)	18.637	66.080
Imazapyr (1000 g/ha)	25.955	57.873

Plants from Vršovice (with a high percentage of resistant individuals) showed after treatment with the same doses of chlorsulfuron levels of 115% and 94%, and after imazapyr treatment one of 131% compared with the control.

Twenty-four hours after imazapyr treatment a 30% increase in total amino acids content of maize was reported by SHANER & REIDER (1986). In sensitive biotypes this effect is probably caused by a disturbed function of acetolactate synthase (EBERLAIN *et al.* 1997; STIDHAM 1991).

In a sensitive line of maize, SHANER (1991) found an increase of total amino acids content (370% of the control) after application of 10 g/ha imazapyr into the soil, while in a resistant line the level remained at that of the control. This increase is not caused by a new synthesis

of free amino acids but by conversion of current protein (RHODES *et al.* 1987).

The levels of individual amino acids in the aboveground parts of *kochia* are given in Table 4.

In the population from Kolín treated with a chlorsulfuron dose of 22.5 g/ha the valine and leucine content reached only 33% and 23% of that of the control, while after a dose of 67.5 g/ha it was even lower at 20% and 13%. After imazapyr treatment valine disappeared completely, while the level of leucine was 18% of the control.

In the Vršovice population treated with either dose of chlorsulfuron the level of valine reached 124% of the control, but it could not be detected after application of imazapyr. The leucine content after either dose of chlorsulfuron was 129%, and after imazapyr treatment 75% of that of the control.

In the population with a high percentage of susceptible individuals (Kolín) the content of valine and leucine was decreased proportionally with the increased dose of chlorsulfuron (Table 4). In the population with a high percentage of resistant individuals (Vršovice) such relation was not found. This is in accordance with results of SHANER & REIDER (1986).

In most plant species, treatment with these herbicides depresses most the level of leucine amongst the amino acids. The decrease of leucine plus valine together shows that the total decrease is greater than the decrease of

Table 4. The content of single amino acids in percent of the free amino acid pool of the aboveground parts of *Kochia scoparia* after treatment with chlorsulfuron and imazapyr

Amino acid	Vršovice				Kolín			
	control	herbicide and dosage [g/ha]			control	herbicide and dosage [g/ha]		
		chlorsulfuron	imazapyr			chlorsulfuron	imazapyr	
		22.5	67.5	1000		22.5	67.5	1000
Aspartate + asparagine	7.8	6.83	6.83	4.40	5.8	4.87	4.61	5.62
Threonine	1.4	2.09	2.09	2.52	1.56	1.79	2.35	2.83
Serine	12.3	0	0	0	11.5	32.32	35.83	39.80
Glutamate + glutamine	37	37.6	37.66	28.9	43.00	20.19	16.98	24.00
Glycine	0.59	0.65	0.65	0.82	0.73	0.72	0.62	1.19
Alanine	20.9	24.9	24.90	31.00	20.50	20.83	20.81	0
Cysteine	1.18	1.44	1.44	5.97	0.60	0.53	0.79	5.17
Valine	3.44	4.27	4.27	0	3.60	1.17	0.73	0
Methionine	2.76	3.06	3.06	4.60	0.28	0.61	0.68	2.33
Isoleucine	1.69	2.61	2.61	3.45	2.50	2.81	3.10	2.94
Leucine	2.74	3.54	3.54	2.06	2.89	0.66	0.39	0.54
Tyrosine	0.94	1.25	1.25	1.09	0.92	0.78	1.17	1.50
Phenylalanine	1.63	2.39	2.39	1.10	1.78	1.92	2.77	2.78
Histidine	0.3	0.69	0.69	1.30	0.23	1.30	1.49	1.61
Lysine	0.43	0.65	0.65	0.80	0.55	0.11	0.38	0.74
Tryptophane	0	0.25	0.27	0.50	0	0.32	0.21	0.23
Arginine	0.3	1.82	1.82	7.95	0.30	5.05	4.04	6.17

Table 5. Total content of valine plus leucine in the aboveground parts of *Kochia scoparia* after treatment with chlorsulfuron and imazapyr

Herbicide	Dose [g/ha]	% of control	
		Kolín	Vršovice
Chlorsulfuron	22.5	28	126
Chlorsulfuron	67.5	17.2	126
Imazapyr	1000	8	33

either amino acid alone (STIDHAM 1991). The combined effect on leucine plus valine is shown in Table 5.

In contrast to valine and leucine, there is no uniform opinion amongst authors on isoleucine. Some of them showed a decreased level of isoleucine after chlorsulfuron treatment (ROST *et al.* 1990), and an increased level of leucine after three doses of chlorsulfuron (RHODES *et al.* 1987). The results of our experiments with both populations confirmed an increased level of isoleucine compared with the control.

However, treatment of *Lemna* with 10 and 100 g/ha of chlorsulfuron decreased the concentration of valine and leucine and increased the concentration of isoleucine. Addition of valine and isoleucine into a cell culture in the presence of chlorsulfuron decreased the herbicide effect (SINGH & SHANER 1986).

SHANER (1991) reported the accumulation of threonine and serine in susceptible maize after treatment with the herbicide imazaquin from the imidazolinone group. In the population from Kolín treated with imazapyr and chlorsulfuron we found an increase in serine content (over 700% of the control), whereas it had completely disappeared in the Vršovice population treated in the same way.

Interesting phenomena are the increase of arginine in both treated populations, and the disappearance of alanine in treated plants from Kolín. These effects can not be satisfactorily explained. It is possible to think of inhibition of some processes. SINGH and SHANER (1995) explained the disappearance of alanine by a disturbed glutamine synthase. RHODES *et al.* (1987) found in *Lemna minor* treated with five concentrations of chlorsulfuron a significant decrease of alanine.

The highest percent of dry matter in both populations was found 24 h after treatment with the higher dose of chlorsulfuron (Table 6).

Table 6. Percent dry matter in the aboveground parts of *Kochia scoparia* 24 h after treatment with chlorsulfuron and imazapyr

Herbicide	Dose [g/ha]	% of control	
		Kolín	Vršovice
Chlorsulfuron	22.5	8.2	7.7
Chlorsulfuron	67.5	9.3	9.3
Imazapyr	1000	8.1	7.7
Control		7.8	7.6

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Souhrn

CHODOVÁ D., MIKULKA J. (2000): Změny v obsahu volných aminokyselin u bytlu metlatého (*Kochia scoparia*) rezistentního vůči inhibitorům ALS. Plant Protect. Sci., 36: 15–19.

V pokusech byl stanoven obsah volných aminokyselin v nadzemních částech vybraných populací bytlu metlatého (*Kochia scoparia* s.l.) po ošetření chlorsulfuronem a imazapyrem. V populaci z Kolína (27 % rezistentních jedinců) byl celkový obsah volných aminokyselin 24 h po ošetření chlorsulfuronem v dávkách 22,5 a 67,5 g/ha 155 % a 245 % kontroly, po ošetření imazapyrem 1 000 g/ha 215 % kontroly. Na lokalitě Praha-Vršovice (78 % rezistentních jedinců) byl celkový obsah aminokyselin po ošetření chlorsulfuronem v dávkách 22,5 a 67,5 g/ha 115 % a 94 %, po ošetření imazapyrem 131 % kontroly. Rostliny z obou lokalit se po ošetření herbicidy lišily v obsahu aminokyselin valinu a leucinu. Výrazné snížení těchto aminokyselin v lokalitě Kolín na rozdíl od lokality Praha-Vršovice potvrzuje názor o vyšší citlivosti acetolaktátsyntasy (ALS), která je zodpovědná za biosyntézu těchto aminokyselin s větveným řetězcem. Výrazné zvýšení obsahu argininu bylo stanoveno u obou populací při všech ošetřeních herbicidy.

Klíčová slova: *Kochia scoparia*; rezistence vůči chlorsulfuronu a imazapyru; obsah aminokyselin

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XV. Česká a Slovenská konference o ochraně rostlin (Brno, 12. – 14. září 2000)

Ústav ochrany rostlin Mendelovy zemědělské a lesnické univerzity v Brně ve spolupráci s dalšími univerzitami a výzkumnými ústavy v České i Slovenské republice pořádá již XV. Českou a Slovenskou vědeckou konferenci o ochraně rostlin.

První den konference budou předneseny vyžádané referáty předních našich i zahraničních odborníků. Další den bude věnován jednáním v sekcích – virologie, bakteriologie, mykologie, entomologie a herbologie.

Účastníci, kteří nechtějí přednést referát, mohou na konferenci přijet i nepřihlášení a konferenční poplatek zaplatit na místě. Je pro ně zajištěna dostatečná rezerva ubytování, nebudou však mít zajištěno stravování.

K účasti zveme nejen vědecké a odborné pracovníky, ale všechny zájemce. Organizátoři si považují za čest pozvat i všechny bývalé kolegy, se kterými jsme se dlouhá léta setkávali, ale kteří již nejsou v pracovním poměru. Proto od nich nepožadujeme zaplacení vložného. Bližší informace a přihlášku si můžete vyžádat na adrese:

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