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CHANGES IN BRANCHED CHAIN AMINO ACIDS CONTENT IN LEAVES OF *APERA SPICA-VENTI* BIOTYPES RESISTANT AND SUSCEPTIBLE TO CHLORSULFURON

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Abstract: One of the negative aspects of the intensive use of herbicides is related to the selection of resistant biotypes (Gasquez 2001). Of all biotypes resistant to herbicides, 93 species do not respond to sulfonylurea herbicides (ALS-inhibiting herbicides). The acetolactate synthase (ALS) enzyme is the first step in biosynthesis of a branched chain amino acids (valine, leucine, isoleucine). In Poland the problem of resistance to sulfonylurea herbicides has been discussed since 2001 (Rola and Marczewska 2002). Resistance tests of *Apera spica-venti* biotypes were conducted in the greenhouse conditions. Chlorsulfuron was applied at the four-leaf stage of development at rates ranging from 11.25 to 360 g/ha. In confirmation of resistance to chlorsulfuron as identified in biological tests, the chemical analyses were performed. The analyses investigated the influence of different doses of chlorsulfuron on free amino acids content in the aboveground part of resistant and susceptible *Apera spica-venti.* The analyses were carried out applying high performance liquid chromatography method (HPLC). The resistance of the biotype was confirmed in amino acids analysis. In the resistant biotype followed the increase of valine, leucine and isoleucine concentration in comparison with untreated plants and those susceptible to chlorsulfuron biotype.

Key words: biological tests, resistance, sensitivity, chlorsulfuron, branched chain amino acids, valine, leucine, isoleucine, liquid chromatography

INTRODUCTION

Generally, recommended herbicides in cereals protection against weeds are sulfonylureas. One of the negative aspects of the intensive use of these preparations is related to the selection of resistant weeds (Gasquez 2001). The number of species resistant to ALS-inhibitors keeps increasing. Currently the number of biotypes resistant to these herbicides is higher than weed species resistant to the other groups. Numerous

reports indicate that 93 species do not respond to sulfonylurea herbicides. The first active ingredient from this group is chlorsulfuron. The researches carried out throughout the world confirm occurrence of resistance to this active ingredient in such species: *Stellaria media* (Canada, Denmark, Norway), *Raphanus raphanistrum* (Australia), *Papaver rhoeas* (Italy, Greece), *Kochia scoparia* (Canada, the Czech Republic, USA), *Sonchus oleracea* (Australia), *Lolium rigidum* (Australia), *Galium spurium* (Canada).

Sulfonylureas are rapidly taken up by roots and shoots. After penetration to the plant the herbicides are transferred by xylem and phloem. In susceptible plants growth is inhibited within a few hours upon the uptake by the plant. Visual symptoms appear in 1–2 days in the form of enhanced anthocyanin formations, terminal bud death, chlorosis and necrosis (Smith 1991). The physiological effect of sulfonylureas is manifested by inhibition of cell division in connection with disturbance of DNA synthesis as the consequence of changes in the biosynthesis of free amino acids (Chodova and Mikulka 2000).

The mechanism of sulfonylurea herbicides' affecting consists in disturbance of biosynthesis of free amino acid, 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 group. Formation of weed species resistant to these herbicides is connected with modification of the enzyme activity (ALS). Activity of the ALS enzyme in biotypes resistant to sulfonylureas is higher than in case of susceptible plants. Therefore, the increased ALS enzymatic activity (that takes part in biosynthesis of branched chain amino acids) results in more intensive synthesis of free amino acids in the resistant biotypes. Thus the content of such amino acids in the resistant biotypes is higher than in the susceptible plants (Chodova and Mikulka 2000a, b; Drye 1993).

The objective of the research was identification of the degree of resistance to chlorsulfuron of *Apera spica-venti* biotypes and defining the changes in the free amino acids content in aboveground parts of plants resistant and susceptible to chlorsulfuron.

MATERIALS AND METHODS

Biological tests

Biological tests were conducted in the greenhouse conditions. Natural sunlight was supplemented with light from sodium lamps which provided a photosynthetic photon flux density of 500 μmol/m^{2}s at plant height during a 14-hour photoperiod. In the greenhouse temperature was maintained in the 20–22°C range and humidity reached the level of *ca.* 70%. The experimental material was collected from fields with winter wheat monoculture and intensive chemical weed control with chlorsulfuron used for a long time. The seed samples came from fields where the efficacy of chlorsulfuron was unsatisfactory. The seeds were sown in plastic pots containing 2:1 mixture of peat and sand. After emergency the number of plants were reduced to 3. Chlorsulfuron was applied at the four-leaves stage of development at rates ranging from 1 to 32 times higher than the recommend field dose (11.25–360 g a.i./ha). Herbicide was applied using a stationary chamber sprayer with mobile nozzle type TeeJet XR 11003-VS and calibrated to deliver 250 L/ha at the pressure of 200 kPa. After 6 weeks upon the application of the herbicide the fresh and dry weight of plants was established. The experiments were conducted using a completely randomized design

with 3 reapplications. ED_{90} was used to evaluate the herbicide efficacy. The plants that have been weed-controlled to such degree are numbered among the herbicide sensitive ones. At the same time lack of effective weed-killing proves the herbicide resistance of plants.

Laboratory analysis

Sulfonylurea herbicides inhibit synthesis of branched chain amino acids (valine, leucine and isoleucine). Evaluation of the free amino acids content in plants allowed to confirm resistance of weeds to chlorsulfuron. The analyses were carried out in the chemical laboratory in Institute of Soil Science and Plant Cultivation in Wrocław with the application of high performance liquid chromatography method (HPLC). The analyses of free amino acids were based on the method described by Bayer (1976), Schmidt (1979) and Wilkinson (1978) with the certain modifications. The amino acids were examined in the dansyl derivatives form.

Experimental material used for the analysis was the aboveground part of resistant (from Lubcz) and susceptible (from Stradomia) biotypes of *Apera spica-venti* from biological tests. In the resistant biotype the chlorsulfuron was applied at rates ranging from 1 to 32 times the recommended field dose (11.25–360 g a.i/ha). Comparatively, the analysis of susceptible biotype was made. Rates of herbicide corresponded to 1, ½, ¼, ⅛, 1/16, 1/32 multiplication of the recommended dose applied in the field. After 4 weeks the leaves were collected for the purposes of the chemical analysis.

RESULTS

In the susceptible biotype of *Apera spica-venti,* the recommended dose of chlorsulfuron caused the fresh and dry weight reduction of about 90–93% in comparison with the untreated plants. The weeds were in a bad condition. The shoot top withering was observed as well as the leaves wilting and growth retardation. The highest active ingredient doses (180 and 360 g/ha) caused complete weed control of this biotype (Table 1).

Table 1. Influence of different chlorsulfuron doses on fresh and dry weight of susceptible *Apera spica-venti* biotype

Object	Dose of chlorsulfuron [g/ha]	Fresh weight [g]	Dry weight [g]
1	control	0.77	0.129
2	11.25	0.08	0.009
3	22.5	0.04	0.006
4	45.0	0.04	0.004
5	90.0	0.03	0.001
6	180.0	–	–
7	360.0	–	–
		LSD (0.05) = 0.06	LSD (0.05) = 0.003

Seeds sampling place: Stradomia

After treatment with chlorsulfuron (11.25 g/ha) the decrease in valine and leucines (leucine + isoleucine) content by over 40% compared to the control (untreated plants) was observed. After the application of chlorsulfuron in 0.68 and 0.38 g/ha doses the decrease in valine content followed by 4 and 3% respectively, and leucines' content in the 8–4% range. It was found that the valine' and total of leucines content in these cases reached almost the control plants value (Fig. 1). Probably these were such little quantities of chlorsulfuron that they did not affect the modification of free amino acids content in this *Apera spica-venti* biotype.

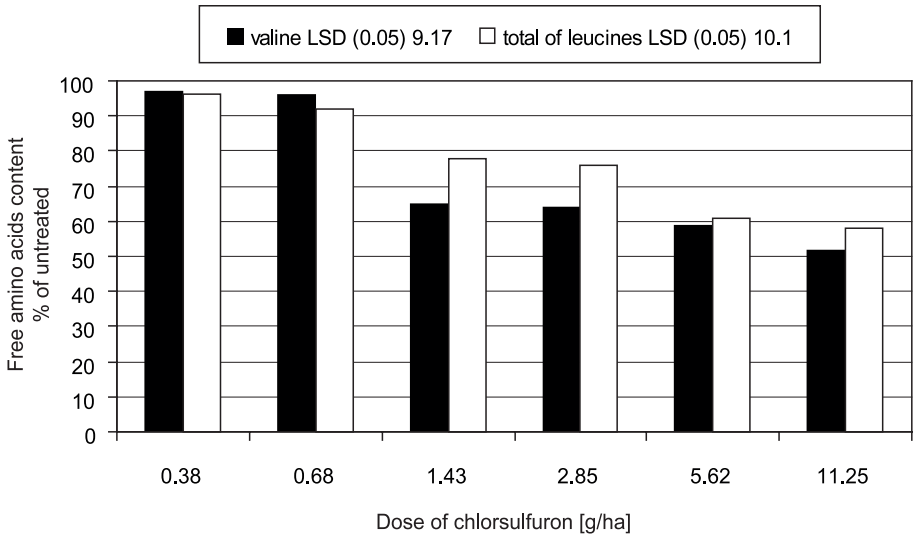

Fig. 1. Changes of free amino acids content in aboveground part of sensitive to chlorsulfuron *Apera spica-venti* biotype

As far as the resistant *Apera spica-venti* biotype was concerned, the application of chlorsulfuron at doses 11.25 and 22.5 g/ha did not result in reduction of fresh and dry weight of the aboveground part of plants. Increase in the active ingredient doses to 45 g/ha reduced fresh and dry weight of plants by approx. 5%. After the chlorsulfuron application in the highest dose (360 g/ha) weights of plant decreased by *ca.* 25% in comparison with the untreated plants (Table 2).

Table 2. Influence of different chlorsulfuron doses on fresh and dry weight of resistant *Apera spica-venti* biotype

Object	Dose of chlorsulfuron [g/ha]	Fresh weight [g]	Dry weight [g]
1	control	0.70	0.106
2	11.25	0.80	0.112
3	22.5	0.72	0.106
4	45.0	0.66	0.101
5	90.0	0.60	0.097
6	180.0	0.57	0.094
7	360.0	0.53	0.080
		LSD (0.05) = 0.046	LSD (0.05) = 0.004

Seeds sampling place: Lubcz

The weeds were in a good condition and leaves' wilting was not observed. Chlorsulfuron in the highest dose caused only inconsiderable growth retardation of *Apera spica-venti* biotype.

The analysis of free amino acids content in the biological material showed that the level of valine and leucines content increased irrespective of chlorsulfuron doses applied (Fig. 2). Under the influence of recommended dose, the valine and leucines content increased by 4–8% in comparison with the control object. Further increase in the active ingredient doses resulted in an increase in free amino acids content. The highest dose of chlorsulfuron (360 g/ha) resulted in the increase in the aforesaid amino acids by 80% in comparison with untreated plants.

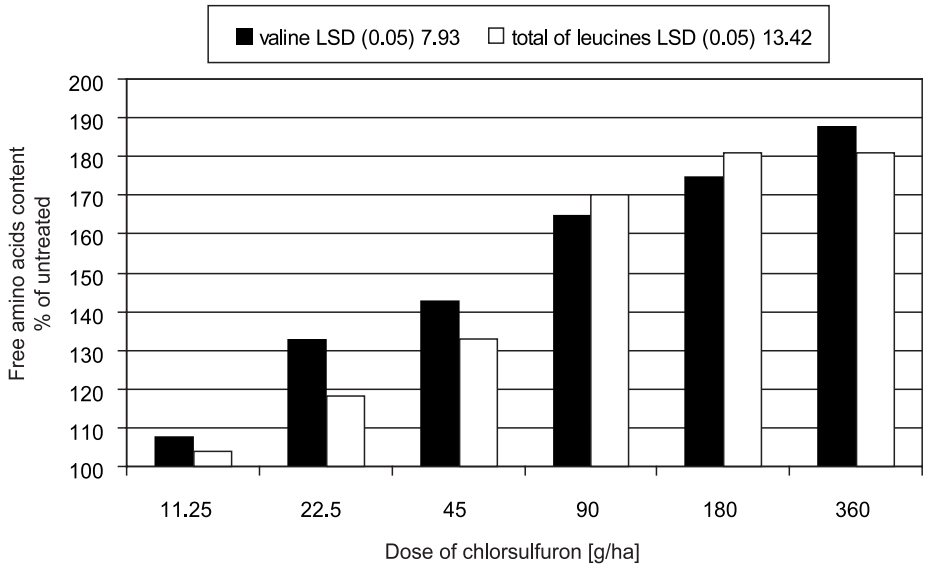

Fig. 2. Changes of free amino acids content in aboveground part of resistant to chlorsulfuron *Apera spica-venti* biotype

DISCUSSION

The biological tests are a popular and effective method of identifying resistance to herbicides of different weed species (Rola 2001; Mikulka and Chodova 2002). In experiments of resistance, the chlorsulfuron was applied at the doses ranging from 11.25 g/ha (recommended) to 360 g/ha (32 times higher than the field one). The aim of the experiments was to define the herbicide dose that causes fresh and dry weight reduction of weeds by 90% in comparison with untreated plants. The weeds that respond to herbicide in such degree are numbered among the sensitive ones. But lack of weed-killing ability proves that plants are herbicide resistant.

Similar researches were conducted by Chodova and Mikulka (2000a, b). In the experiments chlorsulfuron applied at 22.5 and 67.5 g/ha doses generally did not cause fresh weight reduction of *Kochia scoparia* compared to the control plants. Such result may prove herbicide resistance of this species. But reaction of *Kochia scoparia* against chlorsulfuron was differentiated and depended on a place of origin. Among testing

biotypes this was such that did not react to chlorsulfuron, irrespective of the dose applied.

The resistant biotype of *Apera spica-venti* that came from winter wheat field in Lubcz responded to chlorsulfuron only to an inconsiderable degree. After applying herbicide in a 32 times higher dose than the recommended field dose, little growth retardation and only a 25% reduction of fresh and dry weight of this plant followed (Table 2). A high level of resistance to chlorsulfuron of *Apera spica-venti* may indicate the occurrence of target site. According to Jordan (1996) such species are tolerant irrespective of the herbicide dose applied.

Mechanism of formation of resistance to sulfonylurea herbicides is connected with the target site of the preparations. Acetolactate synthase (ALS) is the first step in the synthesis of the branched chain amino acids: valine, leucine and isoleucine. This enzyme is modified in resistant biotypes conferring resistance (Eberlein 1997; Rosst 1985; Saari 1994). The Authors emphasize that the enzyme activity is higher in the resistant biotypes than in those susceptible. In addition, ALS enzyme takes part in biosynthesis of free amino acids, and therefore many scientists all over the world think that the free amino acids concentration is higher in case of the resistant biotype than in case of these sensitive, too (Eberlein 1997; Saari 1990; Chodova and Mikulka 2000a). In confirmation of this argument the plant materials (that came from resistant and susceptible to chlorsulfuron biotypes of *Apera spica-venti*) were subject to the analysis of free amino acids content. In the sensitive biotype of *Apera spica-venti* the decrease in valine and leucine plus isoleucine concentration were observed compared to the untreated plants. The clear drop of the free amino acids level (even by over 40% in comparison with the control object) followed under the influence of 1 and ½ of the recommended field dose (Fig. 1). Also, Chodova and Mikulka (2000b) stated in their research that valine and leucines content in the susceptible biotype of *Kochia scoparia* was lower than in the control plant.

The branched chain amino acids content in resistant biotype changed in comparison with untreated plants (Fig. 2). The recommended dose of chlorsulfuron (11.25 g/ha) caused the increase in these amino acids by *ca.* 8%. But further increase in chlorsulfuron doses did not negatively affect valine and leucines content. Chlorsulfuron in 32 – multiplication field dose (360 g/ha) caused the increase in these amino acids even by *ca.* 90% in comparison with untreated plants.

Similar experiments were made by Chodova and Mikulka (2000a, b). They investigated the influence of chlorsulfuron on valine and leucines content in the *Kochia scoparia* biotype resistant to chlorsulfuron. The results of their experiments showed that chlorsulfuron had an effect on amino acids concentration. Increasing by 26% valine and leucine content in the aboveground part of the resistant biotype of *Kochia scoparia* followed after chlorsulfuron doses 22.5 and 67.5 g/ha were applied.

CONCLUSIONS

By application of biological tests resistant and sensitive to chlorsulfuron biotypes of *Apera spica-venti* were selected. Resistance of *Apera spica-venti* occurring in winter wheat in Lubcz was identified. The biotype resistance was confirmed in the amino acids analysis. In the biotype an increase in valine, leucine plus isoleucine concentration was found in comparison with untreated plants and those susceptible to chlorsulfuron biotype.

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POLISH SUMMARY

ZMIANY ZAWARTOŚCI AMINOKWASÓW O ŁAŃCUCHACH ROZGAŁĘZIONYCH W CZĘŚCIACH NADZIEMNYCH ODPORNEGO I WRAŻLIWEGO NA CHLOROSULFURON BIOTYPU *APERA SPICA-VENTI*

Jednym z negatywnych aspektów stosowania herbicydów jest zjawisko odporności chwastów na te związki. Spośród wszystkich biotypów chwastów odpornych na różne herbicydy, 93 to gatunki które przestały reagować na inhibitory ALS. Enzym

– syntetaza acetylomleczanowa (ALS) jest pierwszym etapem biosyntezy aminokwasów o łańcuchach rozgałęzionych: waliny, leucyny i izoleucyny.

W Polsce pierwszy przypadek odporności *Apera spica-venti* na chlorosulfuron zidentyfikowano w 2001 roku na plantacji pszenicy ozimej (Rola i Marczewska 2002). Testy odporności prowadzono w warunkach szklarniowych. Chlorosulfuron aplikowano w dawkach od zalecane do odchwaszczania pszenicy ozimej (11,25 g/ha) do 32 – krotnie wyższej (360 g/ha). Dla potwierdzenia odporności, którą zidentyfikowano w testach biologicznych, wykonano analizy chemiczne. Badano wpływ różnych dawek chlorosulfuronu na zawartość wolnych aminokwasów w częściach nadziemnych odpornego i wrażliwego biotypu *Apera spica-venti*. Analizy wykonano metodą chromatografii cieczowej.

U odpornego biotypu *Apera spica-venti* stwierdzono wzrost zawartości wolnych aminokwasów w porównaniu do roślin kontrolnych (nie traktowanych herbicydem) oraz do biotypu wrażliwego na ten związek, co potwierdza występowanie u tego osobnika odporności na chlorosulfuron.