Wild oat (*Avena fatua* L.) biotypes resistant to acetolactate synthase and acetyl-CoA carboxylase inhibitors in Poland

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ABSTRACT

The aim of the study was to collect seeds of wild oat from the fields where, in spite of the applied herbicides, the weed is very poorly controlled, and to determine under greenhouse conditions if any resistant biotypes are present. In the years 2008–2011, 34 samples of wild oat were collected from fields where the weed was poorly controlled. The biotypes were analyzed in greenhouse experiments to determine if they are resistant to herbicides. Among five resistant biotypes three of them (R3, R4 and R5) were resistant only to iodosulfuron and mesosulfuron, and biotype R2 – only to propoxycarbazone-sodium. Biotype R1 exhibited multiple resistance to iodosulfuron + mesosulfuron and pinoxaden. The use of sulfometuron proves that the mechanism of resistance of two biotypes of wild oat (R1 and R4) to acetolactate synthase inhibitors is associated with target-site mutation. The curve of biotypes R3 and R5 controlled with iodosulfuron + mesosulfuron shows a relatively low resistance index and control of those biotypes with sulfometuron indicates a metabolic resistance.

Keywords: herbicides; resistance index; multiple resistance; target-site mutation

Wild oat (*Avena fatua* L.) holds the leading position in world literature on weeds and occurs world-wide, even in Alaska and Iceland. In Poland the weed can be found in large numbers in many regions of the country, especially in soils rich in nutrients and moderately moist. It infests mainly spring cereals, sugar beet and leguminous plants as well as winter cereals in Żuławy region and north-western and south-eastern Poland (Kieć 1998). Infestation with wild oat has been growing every year. The reason is that some farmers find it difficult to identify the weed. A characteristic trait of wild oat is that its leaves curl to the left, i.e. counter-clockwise, which facilitates distinguishing it from other cereals.

Its mass occurrence reduces grain yield of cereals even by 40%, of pea by 30%, and sugar beet even by 90% (Belles et al. 2000). Control of *A. fatua* in winter cereal cultivation has been involving herbicides inhibiting acetyl-CoA carboxylase and acetolactate synthase for many years. The chemical control of wild oat, especially in cereals, is one of the most expensive measures due to the cost of herbicides. Assessment of the occurrence of wild oat biotypes resistant to herbicides is complicated by the fact of occurrence of several botanical varieties of wild oat differing in sensitivity to used herbicides. Therefore it is a clear signal that there is a problem of resistant biotypes of wild oat and one may assume it will become more significant in the following years. Wild oat belongs to the group of weeds that are the most susceptible to the development of resistance (Bourgeois et al. 1997, Beckie et al. 1999, De Prado and Franco 2004). Such traits are affected by: genetic diversity of the species, great fertility, common occurrence and very high competitiveness in comparison with cultivated plants. Additionally, its control usually involves the use of herbicides of the group of ACCase inhibitors (inhibitors of acetyl-CoA carboxylase), characterized by a single site of action in a plant, which influences selection of resistant plants in the weed population. Such a combination of the mechanism of action of the herbicide and biological properties of a weed susceptible to development of resistance results in fast selection in the population, which may cause

sudden development of the phenomenon of wild oat resistance. According to the list provided by HRAC (www.weedscience.com), at present there are 43 biotypes of wild oat resistant to herbicides documented in the world. Most resistant biotypes were noted in the USA and Canada. Wild oat was the most often resistant to inhibitors of acetyl-CoA carboxylase and acetolactate synthase. Research on resistance of wild oat in Poland to diclofop-methyl and fenoxaprop-P-ethyl was carried out in the Agricultural Academy in Kraków (at present – the University of Agriculture) by Stokłosa and Kieć (2006). The results of the research showed that there are biotypes of wild oat resistant to fenoxaprop-P-ethyl and diclofop-methyl in the fields of south-eastern Poland.

The mechanism of weed resistance to acetolactate synthase (ALS) is quite well known. Target-site resistance of these herbicides is the most often connected with Pro-197 mutation. As a result of the mutation, amino acid proline at position 197 may be replaced with other amino acids. Pro197-Ser mutation is the most prevalent in resistant weed biotypes. Researches carried out by Jander et al. (2003), Hull and Moss (2007) and Yu et al. (2010) demonstrate that owing to the use of sulfometuron (Oust 75 WG) on weed biotypes resistant to ALS, it is possible to carry out selection of weeds and answer the question if the resistance is mutational or non-mutational. Sulfometuron is a sulfonylurea herbicide, a non-selective preparation controlling all weeds except for those resistant to ALS inhibitors and having a mutation at position Pro197.

The aim of the study was to collect seeds of wild oat from the fields where, in spite of the applied herbicides, the weed is very poorly controlled, and to determine under greenhouse conditions if any resistant biotypes are present.

MATERIAL AND METHODS

Sample collection. Samples were collected from the fields where farmers have signaled poor effectiveness of herbicide use. The samples were collected from many sites so that they represented the whole field or sites where herbicide effect was poor. One panicle was collected per each plant, and about 50–60 panicles of wild oat were collected from each field. During sample collection, blanks and headland were omitted. In the years 2008–2011, altogether 34 samples of wild oat seeds were gathered.

Greenhouse experiments. The seeds were separated from panicles and purified under laboratory conditions. Then they were placed in a fridge at ca. –5°C for one week to disrupt the seed-dormancy period. Thus prepared seeds were examined under greenhouse conditions to determine their resistance to herbicides.

Greenhouse experiments were conducted in four repetitions in plastic pots of the volume of 0.5 L and the diameter of 9 cm. Garden soil mixed with sand in a ratio of 3:1 was used for the experiments. About 15 seeds were sown into each pot, and after emergence the seedlings were thinned so that 7 plants were left in each pot. Temperature in the greenhouse was 20–25°C, and the length of day and night was 16/8 h.

In the first stage of the research, 4 herbicides were used at recommended doses propoxycarbazone-sodium (Attribut 70 SG – 100 g/ha, Bayer Crop Science), iodosulfuron-methyl + mesosulfuron-methyl (Atlantis 04 WG – 0.4 g/ha, Bayer Crop Science), pendimethalin + isoproturon (Maraton 375 SC – 4 L/ha, Bayer Crop Science) and pinoxaden (Axial 100 EC – 0.75 L/ha, Syngenta). Spraying was carried out in the 3–4 leaf stage of wild oat. On this basis, biotypes which were not controlled or very poorly controlled were selected. In the second experiment, iodosulfuron-methyl + mesosulfuron-methyl (Atlantis 12 OD), pinoxaden (Axial 100 EC), propoxycarbazone-sodium (Attribut 70 WG) and sulfometuron (Oust 75 WG, Du Pont) were used to plot regression curve, calculate resistance index and to assess the mechanism of resistance. The examined preparations were used at 7 doses: iodosulfuron-methyl + mesosulfuron-methyl were used at doses: 2.4, 4.8, 9.6, 19.2, 38.3, 76.8 and 115.2 g; pinoxaden at doses: 10, 20, 40, 80, 120, 180 and 300 g; propoxycarbazone-sodium at doses: 17.5, 35, 70, 140, 280, 560 and 1120 g; and sulfometuron at doses: 18.75, 37.5, 75, 150, 300, 600 and 1200 g per ha. Herbicides Atlantis 12 OD and Oust 75 WG were used for the experiment with 4 biotypes: R1 from Kąsinowo/Szamotuły (52°36'40''N, 16°34'44''E), R3 from Karolin/Szamotuły (52°42'23''N, 16°31'45''E), R4 from Paluzy/Bisztynek (Mazury) (54°8'0''N, 20°58'0''E), R5 from Psary/Trzebnica (51°11'5''N, 17°1'53''E).

Herbicide Axial 100 EC was used with one biotype R1 from Kąsinowo/Szamotuły (52°36'40''N, 16°34'44''E), and Attribut 70 WG herbicide was used with biotype R2 from Kluczewo-Huby (52°38'7''N, 16°29'27''E). Biotype from Winna Góra (52°12'0''N, 17°27'0''E) sensitive (S) to all of tested herbicides was

used as a standard. All the herbicides used in the experiment except of sulfometuron are registered in Poland. Herbicide spraying was conducted with a greenhouse sprayer at the 3–4 leaf stage of wild oat with TeeJet TT 11002 sprayers, the used pressure was 3 bar, and the amount of water for the treatment was 250 L/ha. Assessment of effect of the used herbicides was made 3 weeks after the treatment by evaluation of green matter of aboveground plant parts. Percentage of plant green matter loss was assessed in comparison with the control. The results were statistically analyzed with analysis of variance, and regression curve was plotted for each biotype with confidence interval of 0.05. Statistical analysis was carried out with the use of Polo Plus software and the logit model was used (Robertson et al. 2002). The application automatically plots the curve and makes calculations of the effective dose (ED_{50}) causing a 50% reduction in green matter. On that basis, resistance index (R/S) was determined, which is a ratio of the dose causing a 50% reduction in green matter of resistant biotype plants and the dose causing a similar effect in sensitive biotype plants.

RESULTS AND DISCUSSION

Results of the first test showed resistance of 5 biotypes to the used herbicides at the recommended doses. The biotypes were further analyzed to plot the control curve and calculate resistance index.

Biotypes R1, R3, R4 and R5 of wild oat were very poorly controlled by iodosulfuron + mesosulfuron (Atlantis 12 OD) (Figures 1a and 2). Only after using very high doses, above 20 g/ha (5N), the weed was efficiently controlled. Obtaining 50% destruction of resistant biotype plants of wild oat required application of iodosulfuron + mesosulfuron at the dose from 19.3 g/ha for biotype R5 from Psary to 46.8 g/ha for biotype R1 from Kąsinowo (Table 1). Sensitive biotype (S) was efficiently controlled already after using the dose of 3.4 g/ha of these two active substances (Figures 1a and 4). Resistance to iodosulfuron + mesosulfuron for resistant biotypes was from 5.7 (biotype R5) to 13.8 (biotype R1). After application of sulfometuron on wild oat plants resistant to ALS herbicides, it turned out that the preparation did not affect biotypes R1 and R4 (Figures 1b and 4), while biotypes R3 and R5 were well controlled. Therefore the mechanism of resistance of wild oat biotypes resistant to ALS was diverse. The mechanism of resistance of biotypes R1 and R4 was mutational (Pro197) (Yu et al. 2008). Sulfometuron is included as an indicator of ALS target site resistance and most populations tested i.e. in UK give similar results with mesosulfuron + iodosulfuron and sulfosulfuron (Hull and Moss 2007). Burnet et al. (1994) also proves that survival after sulfometuron treatments indicates the presence of a resistant ALS. Several different point mutations were identified to be responsible for target-site resistance to ALS inhibitors. These mutations include amino acid substitutions at Ala122, Pro197, Val205, Asp376,

Table 1. Parameters of resistance of *Avena fatua* biotypes

Biotype, location	Iodosulfuron + mesosulfuron		Sulfometuron		Propoxycarbazone -sodium		Pinoxaden	
	ED_{50}	R/S**	ED_{50}	R/S**	ED_{50}	R/S**	ED_{50}	R/S**
R1 Kąsinowo	46.8 (41.3–52.3)*	13.8	365.1 (340.7–389.5)*	23.0	–	–	132.9 (121.5–144.2)*	8.4
R2 Kluczewo-Hyby	–	–	–	–	354.0 (327.3–380.1)*	13.6	–	–
R3 Karolin	20.7 (16.1–25.34)*	6.1	16.9 (12.9–20.6)*	1.1	–	–	–	–
R4 Paluzy	35.3 (29.9–42.3)*	10.4	228.2 (202.8–253.7)*	14.3	–	–	–	–
R5 Psary	19.3 (14.5–24.1)*	5.7	17.6 (11.3–23.8)*	1.1	–	–	–	–
S Standard (6/2010)	3.4 (2.4–4.7)*	–	15.9 (11.2–22.1)*	–	26.0 (21.6–30.9)*	–	15.8 (14.2–17.5)*	–

*confidence interval; **R/S – resistance index; ED_{50} – effective dose causing a 50% reduction in green matter

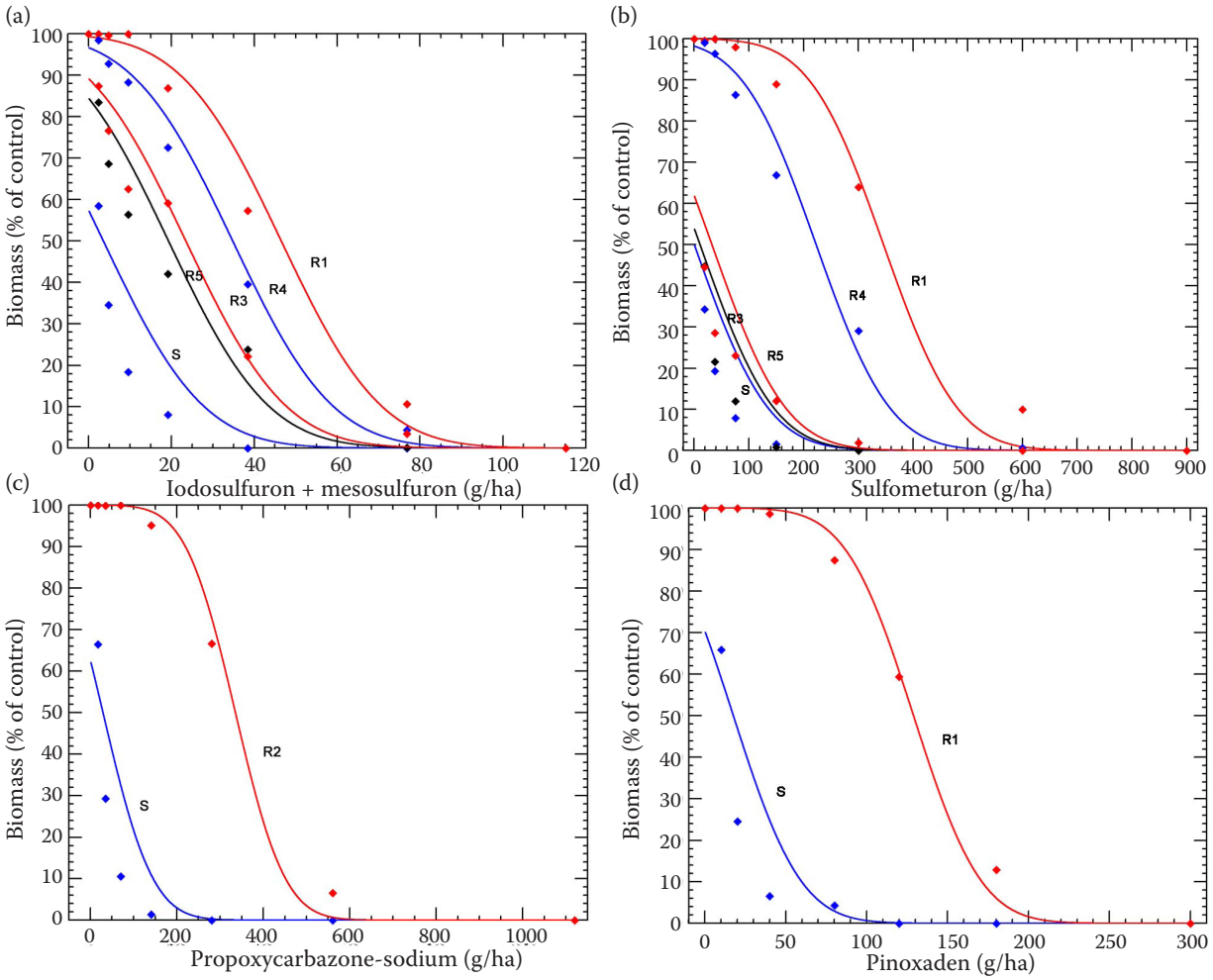

Figure 1. (a) The effect of iodosulfuron + mesosulfuron on fresh weight reduction of resistant (R1, R3, R4, R5) and susceptible (S) biotypes of *Avena fatua;* (b) the effect of sulfometuron on fresh weight reduction of resistant (R1, R3, R4, R5) and susceptible (S) biotypes of *A. fatua;* (c) the effect of propoxycarbazone-sodium on fresh weight reduction of resistant (R2) and susceptible (S) biotypes of *A. fatua*, and (d) the effect of pinoxaden on fresh weight reduction of resistant (R1) and susceptible (S) biotypes of *A. fatua*

Arg377, Trp574, Ser653 and Gly654. In most cases Pro197 mutation confers a high level of resistance to SU herbicides (Yu et al. 2008, Park et al. 2012). Mutation Pro197, i.e. missing of proline at that position, prevented involvement of iodosulfuron + mesosulfuron in the metabolic pathway, and therefore the herbicide could not have affected wild oat plants. In our experiment the resistance index to sulfometuron for biotypes was as follows: for R1 – 23.0 and for R4 – 14.3 (Table 1). However, resistance of biotypes R3 and R5, affected by sulfometuron, was different than mutational. Presumably it is metabolic resistance (Table 1, Figure 1b). Obtaining 50% destruction of wild oat plants required application of sulfometuron in the amount of 365.1 g/ha for biotype R1 and 228.2 g/ha for biotype R4, and 16.9 g/ha and 17.6 g/ha respectively for biotypes R3 and R5 (Table 1). On the other hand, sensitive biotype S required using only 15.9 g/ha of sulfometuron. Wild oat biotype R2 coming from Kluczewo-Huby was resistant only to propoxycarbazone-sodium (Attribut 70 WG) (Figure 1c). Fifty-percent destruction of this biotype required application of 354 g/ha of propoxycarbazone-sodium, while the resistance index was 13.6 (Table 1). Among five resistant biotypes of wild oat, only R1 from Kąsinowo was characterized by multiple resistance to two herbicide chemical groups, to inhibitors of acetolactate synthase and acetyl-CoA carboxylase. Obtaining 50% destruction of biotype R1 required using 132.9 g/ha of pinoxaden (Axial 100 EC) (Table 1, Figures 1d and 3). Resistance index to pinoxaden for biotype R1 amounted to 8.4.

Figure 2. The effect of iodosulfuron + mesosulfuron (Atlantis) on susceptible and resistant *Avena fatua* biotypes. Denotations: 1–3 – susceptible biotype; 4–6 – resistant biotype; 1, 4 – untreated; 2, 5 – Atlantis 12 OD 0.4 L (1N); 3, 6 – Atlantis 12 OD 1.6 L (4N)

Figure 3. The effect of pinoxaden (Axial 100 EC) on susceptible and resistant *Avena fatua* biotypes. Denotations: 1–3 – susceptible biotype; 4–6 – resistant biotype; 1, 4 – control; 2, 5 – Axial 100 EC 0.4 L (1N); 3, 6 – Axial 100 EC 1.6 L (4N)

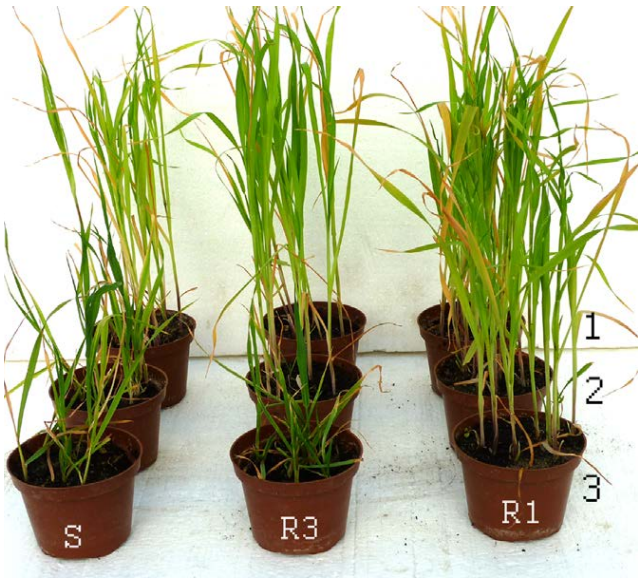

Figure 4. The effect of ALS-inhibiting herbicides on susceptible and resistant *Avena fatua* biotypes. Denotations: S – sensitive biotype; R1, R3 – biotypes resistant to sulfonylurea herbicides: 1 – untreated; 2 – iodosulfuron + mesosulfuron-methyl; 3 – sulfometuron

Biotypes of wild oat are most often resistant to inhibitors of acetolactate synthase and acetyl-CoA carboxylase (Seefeldt et al. 1996, Cavan et al. 1998, Beckie et al. 1999). The results of the present research and those obtained by Stokłosa and Kieć (2006) demonstrate that in Poland resistance of wild oat is related also to herbicides with that mechanism of action. The data obtained in our research show that 3 (R3, R4 and R5) resistant biotypes of wild oat exhibited simple resistance to iodosulfuron + mesosulfuron (Atlantis 12 OD), and one biotype (R2) was resistant to propoxycarbazone-sodium (Attribut 70 WG). The mechanism of resistance of biotypes R1 and R4, not controlled by sulfometuron, proves that the mechanism of resistance of these biotypes is presumably target-site. The curve of biotypes R3 and R5 controlled with iodosulfuron + mesosulfuron (Atlantis 12 OD) shows a relatively low resistance index and control of those biotypes with sulfometuron indicate a metabolic resistance.

Increased weed infestation of many crops with wild oat was observed for several years. Information reaching the Institute of Plant Protection in Poland from services for plant protection demonstrates worse herbicide effect of controlling this weed. The phenomenon is alarming and may indicate the process of wild oat resistance to herbicides. As it is stated by Cavan et al. (1998), fast distribution of wild oat resistance to herbicides results also from the fact of transmission of resistance through pollen.

In conclusion, three biotypes (R3, R4 and R5) of wild oat, among 34 analyzed, were resistant to iodosulfuron + mesosulfuron (Atlantis 12 OD); biotype R2 was resistant only to propoxycarbazone-sodium, while biotype R1 was characterized by multiple resistance to iodosulfuron + mesosulfuron and pinoxaden. The use of sulfometuron shows that the mechanism of resistance of two biotypes of wild oat (R1 and R4) to inhibitors of acetolactate synthase is related to a target-site mutation.

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