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Characterisation of iodo- plus mesosulfuron resistance in an *Alopecurus myosuroides* Huds. population from the Czech Republic

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Abstract: The intensification of *Alopecurus myosuroides* Huds. (black-grass) is becoming a major problem due to its growing resistance to a broad spectrum of acetolactate synthase (ALS)-inhibiting herbicides. Hence, the present study was conducted to evaluate the resistance level of a black-grass population to iodosulfuron plus mesosulfuron and to identify the underlying resistance mechanism. Dose-response studies revealed that the resistance population is 22 times less sensitive to iodosulfuron plus mesosulfuron than the susceptible population. The probable resistance mechanism identified was the target-site substitution of proline (Pro) by threonine (Thr) at the 197th position of the ALS enzyme. Furthermore, whole plant response bioassay experiments demonstrated that this population is also resistant to pinoxaden, chlorotoluron, diflufenican plus pendimethalin plus chlorotoluron, fenoxaprop and flufenacet plus diflufenican. In summary, the current findings recommend using alternative herbicides in integrated weed management to interrupt the possible evolution of herbicide resistance in these species.

Keywords: ALS-inhibitors resistance; weed control; molecular mechanisms; Pro197 mutations; target-site resistance

Alopecurus myosuroides Huds. (black-grass) is one of the most challenging annual grass weeds in cereal-based rotation fields in Western Europe, with increasing densities of this weedy species (Délye et al. 2011, Rosenhauer and Petersen 2015). Some factors responsible for this intensification might be due to rotations with high proportions of winter cereals and reduced tillage (Lutman et al. 2013, Messelhäuser et al. 2021). Black-grass is a very competitive and economically important weed and might cause almost 15–20% (at densities of 100 plants/m^2) yield losses in winter wheat production (Gerhards et al. 2016, Messelhäuser et al. 2021). The evolution of herbicide resistance to five herbicide mode of action in *A. myosuroides* has risked their management in Europe where acetolactate synthase (ALS)-inhibitors (HRAC Group 2), acetyl-CoA carboxylase (ACCase)-inhibitors (HRAC Group 1) and PSII-inhibitors (HRAC Group 5) are mainly used, to manage this species (Moss et al. 2007, Cummins et al. 2013, Klauk and Petersen 2023).

Since their commercialisation, ALS-inhibiting herbicides have been widely used for weed management due to their broad-spectrum weed control efficiencies (Mazur and Falco 1989). Some commonly used ALS-inhibiting herbicides against *A. myosuroides* include pyroxsulam, mesosulfuron-methyl, mesosulfuron plus iodosulfuron, etc. These herbicides

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are known to inhibit the ALS enzyme, an important enzyme which catalyses the first step in the synthesis of the branched-chain amino acids. However, their unintended overuse and, in some cases, sub-lethal doses have resulted in the evolution of resistance in a substantial number of weed species worldwide. The resistance mechanisms might be target-site-based (TSR) or non-target site-based (NTSR); while the TSRs include single nucleotide substitutions and target gene overexpression, the NTSRs comprise of enhanced metabolism, reduced absorption, and translocation, etc. (Gaines et al. 2020). In some cases, there is a co-existence of both TSRs and NTSRs, such as in *Lolium multiflorum* involvement of cytochrome P450 and presence of single point mutation, enhanced metabolism, and target gene overexpression in *Bromus sterilis* and P450s-involved enhanced metabolism and target-site gene mutation in ACCase resistant *Rapistrum rugosum* (Hatami et al. 2016, Sen et al. 2021, Wu et al. 2022). The coexistence of TSR and NTSR mechanisms in black grass has also been anticipated by Petit et al. (2010). Among the TSR mutations, to date, eight functional sites within the ALS enzyme (Ala122, Pro197, Ala205, Asp376, Arg377, Trp574, Ser653 and Gly654) have been identified in weed species that are known to confer resistance against ALS-inhibiting herbicides (Powles and Yu 2010). Among these, to the best of our knowledge, only Pro197 and Trp574 mutations have been detected in black-grass (https://www.weedscience.org/pages/MutationDisplayAll.aspx).

Iodosulfuron and mesosulfuron are one of the commonly used herbicides in winter wheat fields of the central European countries, including the Czech Republic. These herbicidal active ingredients (a.i.) belong to the sulfonylureas group of herbicides, one of the most widely used groups since their commercialisation (HRAC group 2). Presently, the field-recommended dose of iodosulfuron and mesosulfuron cannot control black-grass in some winter wheat fields of the Czech Republic. Hence, the current study was performed to estimate the resistance level of the *A. myosuroides* population to iodosulfuron plus mesosulfuron, understand the mechanism of resistance, and investigate the sensitivity to other herbicidal modes of action.

MATERIAL AND METHODS

Plant material and whole-plant dose-response assays. The susceptible (S) population was collected from the organic farm field 49.1234N, 14.6291E and the resistant (R) population was collected from a winter wheat field in the southern Bohemian region of the Czech Republic 49.1709N, 14.6030E shortly before crop harvest in July. Seeds were dried and stored in paper bags at room temperature until use. Approximately 5–8 seeds were sown in pots (~343 cm^3), filled with chernozem soil [high fertility property and moisture storage capacity, clay content 46% (loamy soil), soil pH (potassium chloride) 7.5, cation exchange capacity (CEC) of soil: 209 mmol$_+$/kg), 87 mg/kg phosphorus, 203 mg/kg potassium, 197 mg/kg magnesium, 8 073 mg/kg calcium] (Harova and Spejra 2014). Seedlings were thinned to 5 individuals per pot. The dose-response experiments were conducted in an open-air vegetation hall (with a rooftop to avoid rain). The seedlings were regularly watered, and fertilisers were applied as required. Herbicide was sprayed using a laboratory spray chamber equipped with a Lurmark 015F80 nozzle with a spray volume of 250 L/ha and pressure of 120 kPa. All the herbicide doses were sprayed at a three-leaf stage. Herbicide Atlantis OD (Bayer CropScience AG, 2 g/L iodosulfuron plus 10 g/L mesosulfuron (methyl 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl] benzoate + methyl 2-[(4,6-dimethoxypyrimidin-2-yl) carbamoylsulfamoyl]-4-(methanesulfonamidomethyl) benzoate), Axial Plus (Syngenta Crop Protection AG, 50 g/L pinoxaden ([8-(2,6-diethyl-4-methylphenyl)-7-oxo-1,2,4,5-tetrahydropyrazolo[1,2-d][1,4,5]oxadiazepin-9-yl] 2,2-dimethylpropanoate) and Lentipur 500 FW (Nufarm GmbH and Co KG, 500 g/kg chlorotoluron (3-(3-chloro-4-methylphenyl)-1,1-dimethylurea) were sprayed at the rates shown in Table 1. The field recommended rates (1N) for iodosulfuron + mesosulfuron, pinoxaden and chlorotoluron are 14.4 g/ha, 45 g/ha and 1 000 g/ha, respectively. Four-pot replicates were done for each rate. In preliminary experiments, doses of 0N, 1N, 2N and 4N were used to justify the rates for dose-response experiments. The dose-response experiment has been repeated in space and time.

Partial *ALS* gene sequencing. Leaves from 25 individual plants of R and S black-grass populations were harvested for the extraction of genomic DNA (gDNA). Following the manufacturer's instructions, the gDNA extraction was done with shock frozen leaf tissues (± 80 mg per sample) from R and S plants using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Primer pairs were designed to amplify the *ALS* gene at

https://doi.org/10.17221/377/2023-PSE

Table 1. List of herbicides used in the current study and their respective doses

Herbicide	Active ingredient (a.i., surfactant)	Doses used (g a.i./ha)
Atlantis OD	iodosulfuron + mesosulfuron + mefenpyr-diethyl (methylated rape seed oil)	0, 3.6, 7.2, 10.8, 14.4, 21.6, 32.4, 43.2, 129.6, 360 and 480 (1 460)
Axial	pinoxaden (methylated rape seed oil)	0, 5, 10, 15, 20, 30, 45, 60 and 120 (1 460)
Lentipur 500 FW	chlorotoluron	0, 62.5, 125, 250, 500, 1 000, 2 000, 4 000 and 8 000
Trinity	diflufenican + pendimethalin + chlorotoluron	40 + 300 + 250
Puma Extra	fenoxaprop-P-ethyl + mefenpyr-diethyl	69 + 75
Battle Delta	flufenacet + diflufenican	400 +200

the seven previously reported mutation sites (Table 2). The primers were designed using Primer-BLAST and Primer3 software based on AJ437300.2 (GenBank accession number for *Alopecurus myosuroides ALS* gene). C1000 thermocycler (Bio-Rad, Hercules, USA) was used for polymerase chain reaction (PCR). 50 ng of total gDNA per reaction was used. The PCR cycling profile consists of an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of 5 s at 95 °C, 10 s at 60 °C and 30 s at 72 °C and a final elongation step for 10 min at 72 °C. After that, the amplicons were resolved in the 1.5% agarose gel and were subsequently purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, USA) following the manufacturer's instructions. The gel-purified amplicons were sent for custom DNA Sequencing at Eurofins, Germany. BioEdit (version 7.2) software was used for single nucleotide substitution detection (https://bioedit.software.informer.com/7.2/).

Efficacy of other herbicides. For efficacy-testing experiments, diflufenican plus pendimethalin plus chlorotoluron, fenoxaprop and flufenacet plus diflufenican were applied to the black-grass plants at the two-leaf stage. The herbicides were applied at their recommended doses: diflufenican plus pendimethalin plus chlorotoluron, fenoxaprop and flufenacet plus diflufenican (Table 1). In the case of two or three herbicides, we used tank-mix application. Four pot replicates were used for each of the herbicides mentioned above. Herbicide efficacy was calculated 28 days after treatment (DAT) as biomass decreased in treated pots compared with untreated control.

Statistical analysis. The data analysis and dose-response curves were calculated using the non-linear regression four-parameter log-logistic model by the R-Studio program (https://www.r-project.org/) described by Hamouzová et al. (2014). The 50% growth inhibition or GR50 values were calculated for each population, and after that, based on its resistance factor (RF) ratios, were calculated. For the multiple-resistance studies with diflufenican plus pendimethalin plus chlorotoluron, fenoxaprop and flufenacet plus diflufenican, the chosen herbicides were sprayed at their recommended field doses (Table 1), and the

Table 2. List of primers used for *acetolactate synthase* gene sequencing. The mutation points shown in the table correspond to acetolactate synthase from *Arabidopsis thaliana*

Primer name	Sequence (5' to 3')	Annealing temperature	Mutation points	Amplicon size (bp)
F1	ATCAGGTGCTCAGCGGTGT	60 °C	Ala-122	210
R1	GAAAAGGTGGTTGGTGATGG			
F2	CATCACCAACCACCTTTTCC		Pro-197 & Ala-205	245
R2	AGCGGGTGACCTCTACAATG			
F3	CAGGTGTCACGGTTGTTGAC		Trp-574	162
R3	ATTTTCTGGGTTCCCAAGGT			
F4	AAGCAGGTCCAAGATTGTGC		Asp-376 & Arg-377	465
R4	CTGCCATCCCCATCAATGTC			
F5	TTGGGAACCCAGAAAATGAG		Ser-653 & Gly-654	248
R5	TGCCATCACCTTCCATGATA			

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Table 3. Result from the non-linear regression analysis of iodosulfuron plus mesosulfuron dose-response experiment

Population	b	d	GR_{50}	RF
Resistant	11.05 ± 2.74	10.43 ± 0.21	182.81 ± 20.14	> 24
Susceptible	8.15 ± 1.87	9.42 ± 0.28	7.53 ± 0.344	

b – slope around the GR_{50}; ± standard error; d – upper limit; GR_{50} – herbicide rate (g a.i./ha) required to reduce the above-ground dry biomass by 50%; RF – resistance factor, which is computed as the ratio of GR_{50} of the resistant and susceptible populations

results were evaluated by one-way analysis of variance (ANOVA) in R-Studio program. The normality was checked using the Shapiro-Wilk statistical test. Tukey's post hoc analysis (at a 5% significance level) was used for the statistical analysis.

RESULTS

Whole plant dose-response experiment. Weed resistance determination using greenhouse bioassays provides evidence of the effect of herbicide application on the whole plant. Two runs were pooled together since the results between them were not statistically significant ($P > 0.05$). Based on our dose-response experiments, the GR_{50} value of the R population was found to be ~182 g a.i./ha (higher than the recommended dose). In contrast, the GR_{50} value of the S population was found to be ~8 g a.i./ha (lower than the recommended dose). The resulting resistance index was > 24 (Table 3). The whole-plant dose responses of the S and R populations to iodosulfuron plus mesosulfuron are shown in Figure 1. These results imply high resistance to iodosulfuron plus mesosulfuron in the R population.

Partial *ALS* gene sequencing. The target site mechanism of resistance was investigated using partial amplification of the *ALS* gene. Five primers covering all the reported mutation points (such as Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653 and Gly-654) were used for the amplification. Comparison among the R and S populations confirmed no mutations for all the positions except the 197^{th} position. At the 197^{th} position, we detected a single nucleotide change, leading to proline (Pro) to threonine (Thr) mutation.

Multiple herbicide resistance experiments to other herbicidal modes of action. Whole-plant response bioassays were conducted to investigate the possibilities of multiple resistance to pinoxaden (ACCase-inhibiting herbicide) and chlorotoluron (PSII-inhibiting herbicide). The GR_{50} of the R population to both pinoxaden (31.05 g a.i./ha) and chlorotoluron (385.35 g a.i./ha) were higher than the susceptible standard population, hence giving a resistance index of ~1.6 and ~4.7, respectively (Table 4, Figure 2). Therefore, the iodosulfuron plus mesosulfuron resistant population from the Czech Republic is also resistant to pinoxaden and chlorotoluron. However, this resistance is not strong as compared to iodosulfuron plus mesosulfuron. In addition to these, field-rate test (for efficacy) also showed the probable resistance of this population against diflufenican plus pendimethalin plus chlorotoluron (average efficacy 23.5%), fenoxaprop (average efficacy 54%) and flufenacet plus diflufenican (average efficacy 33.75%) (Figure 3). The susceptible population was controlled from > 90% by all herbicides tested.

DISCUSSION

Our dose-response and efficacy experiments have shown that the R population is resistant against

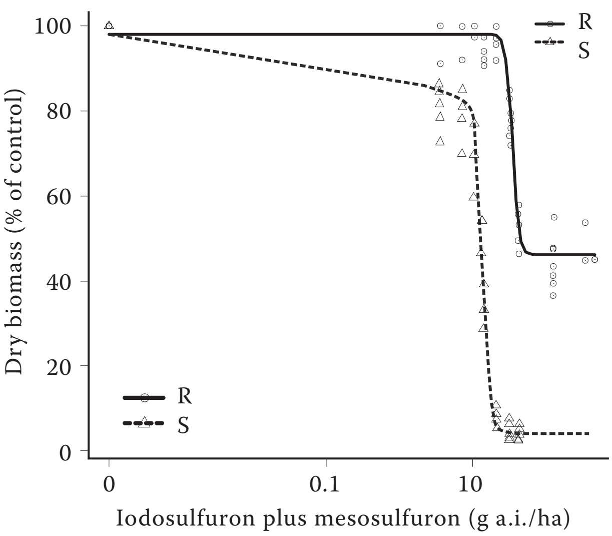

Figure 1. Fitted logarithmic dose-response curves for the susceptible (S) and the resistant (R) population. The current figure represents the whole-plant dose responses of the S and R populations to iodosulfuron plus mesosulfuron. These results indicate high resistance to this herbicidal active ingredient (a.i.) in the R population

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Table 4. Result from the non-linear regression analysis of pinoxaden and chlorotoluron dose-response experiment

Active ingredient (a.i.)	Population	b	d	GR_{50}	RF
Pinoxaden	R	0.42 ± 0.15	10.98 ± 0.55	30.95 ± 5.64	1.63
	S	5.11 ± 1.33)	10.24 ± 0.61	18.97 ± 1.03	
Chlorotoluron	R	4.13 ± 1.21	10.78 ± 0.41	405.41 ± 40.46	4.80
	S	3.12 ± 0.58	11.35 ± 0.28	84.42 ± 3.98	

b – slope around the GR_{50}; ± standard error; d – upper limit; GR_{50} – herbicide rate (g a.i./ha) required to reduce the above-ground dry biomass by 50%; RF – resistance factor, which is computed as the ratio of GR_{50} of the resistant and susceptible populations; R – resistant; S – susceptible

iodosulfuron plus mesosulfuron and is multiple-resistant to pinoxaden and chlorotoluron. Low efficacy has also been detected against diflufenican plus pendimethalin plus chlorotoluron, fenoxaprop and flufenacet plus diflufenican. Usually, TSR to ALS inhibitors is mediated by single-point mutations, which subsequently lead to amino acid substitutions at the herbicide-binding site of the ALS enzyme, and finally, the sensitivity to the herbicides is reduced. Eight different substituted positions of ALS (Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653 and Gly-654) have been identified in a variety of grass weed species, which are reported to confer TSR (Yu et al. 2008, Rey-Caballero et al. 2017). In general, amongst these mutation sites, mutation at Pro-197 and Trp-574 are the most commonly linked with a strong resistance to ALS-inhibiting herbicides, especially against sulfonylurea herbicides. However, this can vary within species, within the mutation points, within haplotypes and also even within the ALS-inhibiting active ingredients.

In this study, TSR was tested for partial amplification of the *ALS* gene. Five primer pairs covering the previously reported mutation points were used. Based on the sequencing analysis results, Pro-197 substitution by Thr is discovered here. Similar mutation has been found in many other weeds, such as in *Alopecurus myosuroides* (Huang et al. 2021), *Beckmannia syzigachne* (Wang et al. 2020), *Echinochloa phyllopogon* (Liu et al. 2019), *Descurainia sophia* (Deng et al. 2017) and can be considered as the most frequent mutation of *ALS* genes in different weeds. In a study by Liu et al. (2019), the authors conducted a computational analysis to study the putative impacts of Pro-197 mutation on ALS binding affinity for ALS inhibitors (Liu et al. 2019).

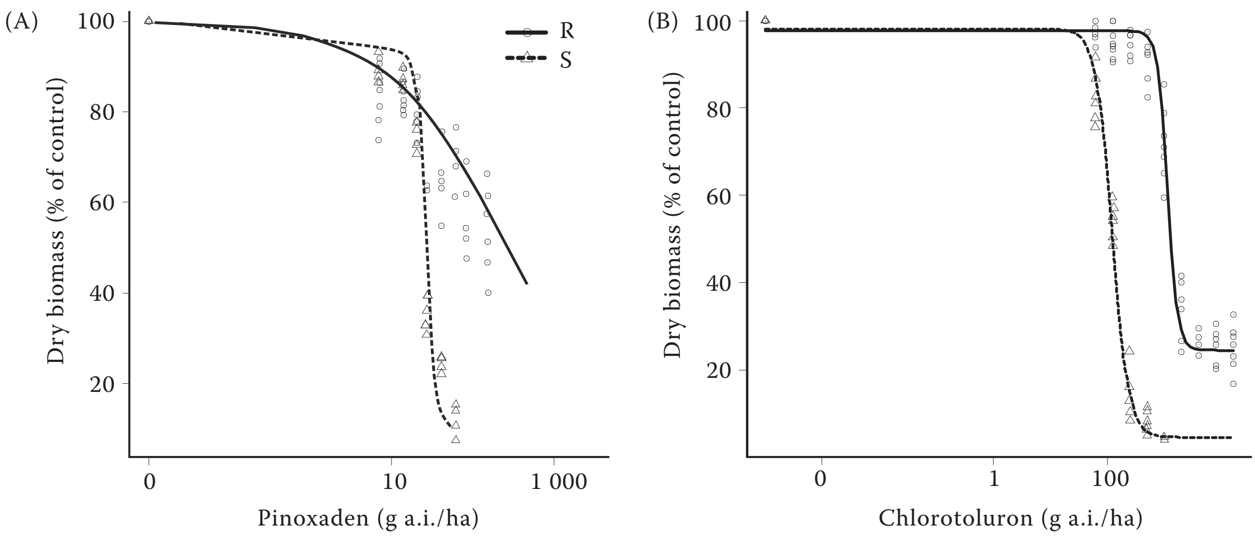

Figure 2. Fitted logarithmic dose-response curves for the susceptible (S) and the resistant (R) population for (A) pinoxaden and (B) chlorotoluron. The current figure represents the whole-plant dose responses of the S and R populations to pinoxaden and chlorotoluron. These results indicate high resistance to these herbicidal active ingredients (a.i.) in the R population

https://doi.org/10.17221/377/2023-PSE

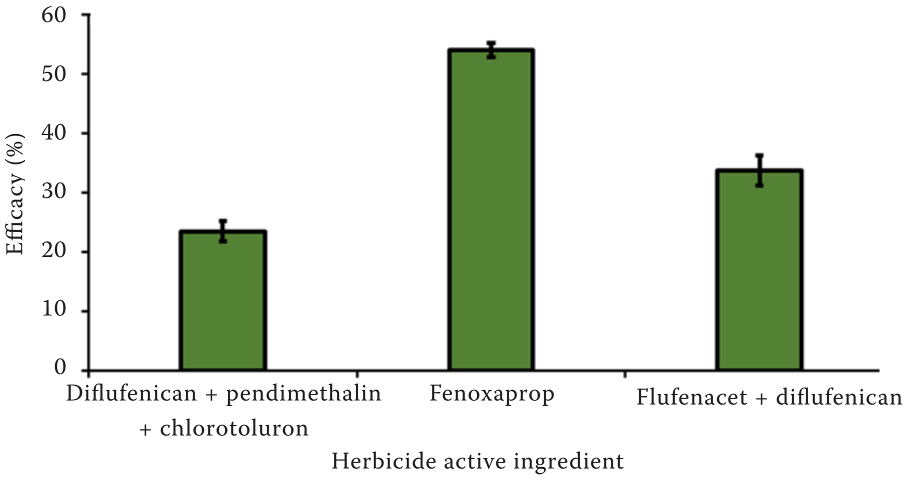

Figure 3. Efficacy of other herbicides. The herbicides were sprayed at their field-recommended doses, and the results were evaluated by one-way analysis of variance (ANOVA) in the R-Studio program

According to their results, despite not being located at the binding site of the substrate, the substitution of amino acid at position 197 of the ALS might affect the spatial structure of the substrate centre and create a positional offset of the side chain. In our case, this mechanism might be the same. However, further detailed experimental validations (such as site-directed mutagenesis, X-ray crystallography and molecular dynamic studies) are required in the near future. In addition to this, Pro-197-Thr substitution has been found to be associated with cross and multiple resistance in many species, such as *Beckmannia syzigachne* (Wang et al. 2020) and *Bromus japonicus* (Lan et al. 2022a). In the current study, we identified cross-resistance. However, further investigation is required in the near future to explore any possible connection between Pro-197-Thr and cross-resistance in our specific species. Nevertheless, cross and multiple herbicide resistance in black-grass has also been reported by Lan et al. (2022b). They found a clodinafop-propargyl-resistant population to be cross-resistant to fenoxaprop-P-ethyl and multiply resistant to pyroxsulam and mesosulfuron-methyl.

Recurrent cropping of winter crops, along with minimum tillage, early drilling, and the fast evolution of herbicide resistance, have caused *A. myosuroides* to spread extensively (Keshtkar et al. 2015, Huang et al. 2021). Corresponding to this study, the Pro-197-Thr mutation is most likely the cause of resistance to iodosulfuron plus mesosulfuron in this black-grass population from the Czech Republic. The rotation of herbicides with different sites of action or different herbicide mixtures is an important option for a sustainable weed management program (Bajwa 2014, Nakka et al. 2019). Unfortunately, low efficacies and multiple resistance have also been identified, thus making the overall black-grass management scenario in the Czech Republic and other European countries very complicated. Hence, in the current scenario, we recommend screening more black-grass populations for herbicide resistance and endorse using integrated weed management strategies. As an additional recommendation to the farmers, we would suggest rotating the herbicides strategically and also using multiple herbicides in tank mixes and intercropping practices. While mechanical control can be an effective weed management strategy, it may not always be practical. Well-planned crop rotations will aid the germination of dormant weeds and hence might contribute to the effective controlling of the major weeds. For example, rotation with an alternation of spring and winter crops can be considered as the most efficient solution against *Alopecurus myosuroides* (Chauvel et al. 2009).

REFERENCES

Bajwa A.A. (2014): Sustainable weed management in conservation agriculture. Crop Protection, 65: 105–113.

Chauvel B., Guillemin J.P., Colbach N. (2009): Evolution of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment. Crop Protection, 28: 343–349.

Cummins I., Wortley D.J., Sabbadin F., He Z., Coxon C.R., Straker H.E., Sellars J.D., Knight K., Edwards L., Hughes D., Kaundun S.S., Hutchings S.-J., Steel P.G., Edwards R. (2013): Key role for a glutathione transferase in multiple-herbicide resistance in grass weeds. Proceedings of the National Academy of Sciences, 110: 5812–5817.

Délye C., Gardin J.A.C., Boucansaud K., Chauvel B., Petit C. (2011): Non-target-site-based resistance should be the centre of attention for herbicide resistance research: *Alopecurus myosuroides* as an illustration. Weed Research, 51: 433–437.

Deng W., Yang Q., Zhang Y., Jiao H., Mei Y., Li X., Zheng M. (2017): Cross-resistance patterns to acetolactate synthase (ALS)-inhibiting herbicides of flixweed (*Descurainia sophia* L.) conferred by different combinations of ALS isozymes with a Pro-197-Thr mutation or a novel Trp-574-Leu mutation. Pesticide Biochemistry and Physiology, 136: 41–45.

Gaines T.A., Duke S.O., Morran S., Rigon C.A., Tranel P.J., Küpper A., Dayan F.E. (2020): Mechanisms of evolved herbicide resistance. Journal of Biological Chemistry, 295: 10307–10330.

Gerhards R., Dentler J., Gutjahr C., Auburger S., Bahrs E. (2016): An approach to investigate the costs of herbicide-resistant *Alopecurus myosuroides*. Weed Research, 56: 407–414.

Hamouzová K., Košnarová P., Salava J., Soukup J., Hamouz P. (2014): Mechanisms of resistance to acetolactate synthase-inhibiting herbicides in populations of *Apera spica-venti* from the Czech Republic. Pest Management Science, 70: 541–548.

Harova L.V.M., Spejra R.K.R. (2014): Soil analysis using Mehlich 3 extractant techniques for sample preparation. California, Teledyne Technologies.

Hatami Z.M., Gherekhloo J., Rojano-Delgado A.M., Osuna M.D., Alcántara R., Fernández P., Sadeghipour H.R., De Prado R. (2016): Multiple mechanisms increase levels of resistance in *Rapistrum rugosum* to ALS herbicides. Frontiers in Plant Science, 7: 169.

Huang Z., Lu X., Wei S., Zhang C., Jiang C., Li W., Saeed M., Huang H. (2021): The target-site based resistance mechanism of *Alopecurus myosuroides* Huds. to pyroxsulam. Crop Protection, 147: 105707.

Keshtkar E., Mathiassen S.K., Moss S.R., Kudsk P. (2015): Resistance profile of herbicide-resistant *Alopecurus myosuroides* (blackgrass) populations in Denmark. Crop Protection, 69: 83–89.

Klauk B., Petersen J. (2023): Evaluation of different weed management strategies including non-chemical tools and cinmethylin for control of multiple herbicide-resistant *Alopecurus myosuroides*. Weed Research, 63: 246–260.

Lan Y., Zhou X., Lin S., Cao Y., Wei S., Huang H., Li W., Huang Z. (2022a): Pro-197-Ser mutation and cytochrome P450-mediated metabolism conferring resistance to flucarbazone-sodium in *Bromus japonicus*. Plants, 11: 1641.

Lan Y., Li W., Wei S., Huang H., Liu Z., Huang Z. (2022b): Multiple resistance to ACCase-and ALS-inhibiting herbicides in black grass (*Alopecurus myosuroides* Huds.) in China. Pesticide Biochemistry and Physiology, 184: 105127.

Liu J., Fang J., He Z., Li J., Dong L. (2019): Target site-based resistance to penoxsulam in late watergrass (*Echinochloa phyllopogon*) from China. Weed Science, 67: 380–388.

Lutman P.J.W., Moss S.R., Cook S., Welham S.J. (2013): A review of the effects of crop agronomy on the management of *Alopecurus myosuroides*. Weed Research, 53: 299–313.

Mazur B.J., Falco S.C. (1989): The development of herbicide resistant crops. Annual Review of Plant Biology, 40: 441–470.

Messelhäuser M., Saile M., Sievernich B., Gerhards R. (2021): Effect of cinmethylin against *Alopecurus myosuroides* Huds. in winter cereals. Plant, Soil and Environment, 67: 46–54.

Moss S.R., Perryman S.A., Tatnell L.V. (2007): Managing herbicide-resistant blackgrass (*Alopecurus myosuroides*): theory and practice. Weed Technology, 21: 300–309.

Nakka S., Jugulam M., Peterson D., Asif M. (2019): Herbicide resistance: development of wheat production systems and current status of resistant weeds in wheat cropping systems. The Crop Journal, 7: 750–760.

Petit C., Bay G., Pernin F., Delye C. (2010): Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. Pest Management Science, 66: 168–177.

Powles S.B., Yu Q. (2010): Evolution in action: plants resistant to herbicides. Annual Review of Plant Biology, 61: 317–347.

Rey-Caballero J., Menéndez J., Osuna M.D., Salas M., Torra J. (2017): Target-site and non-target-site resistance mechanisms to ALS inhibiting herbicides in *Papaver rhoeas*. Pesticide Biochemistry and Physiology, 138: 57–65.

Rosenhauer M., Petersen J. (2015): Bioassay development for the identification of pre-emergence herbicide resistance in *Alopecurus myosuroides* (Huds.) populations. Gesunde Pflanzen, 67: 141–150.

Sen M.K., Hamouzová K., Mikulka J., Bharati R., Košnarová P., Hamouz P., Roy A., Soukup J. (2021): Enhanced metabolism and target gene overexpression confer resistance against acetolactate synthase-inhibiting herbicides in *Bromus sterilis*. Pest Management Science, 77: 2122–2128.

Wang J., Chen J., Li X., Li D., Li Z., Cui H. (2020): Pro-197-Ser mutation in ALS and high-level GST activities: multiple resistance to ALS and ACCase inhibitors in *Beckmannia syzigachne*. Frontiers in Plant Science, 11: 572610.

Wu C., Song M., Zhang T., Zhou C., Liu W., Jin T., Zhao N. (2022): Target-site mutation and cytochrome P450s confer resistance to multiple herbicides in Italian ryegrass (*Lolium multiflorum* Lam.) from China. Crop Protection, 161: 106068.

Yu Q., Han H., Powles S.B. (2008): Mutations of the ALS gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. Pest Management Science, 64: 1229–1236.

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