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PREVALENCE OF HERBICIDE-RESISTANT WEED SPECIES IN UKRAINE

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Introduction

Crop production in Ukraine is an important sector of the economy and has accounted for the dominant share of budget revenues from exports in recent years. However, the dominance of a limited list of crops in crop production – sunflower, wheat, maize, soybeans – leads to a reduction in the biodiversity of agrophytocenoses and complicates weed control [3, 5]. The vast majority of herbicides in Ukraine for use on crops of cultivated plants belong to acetolactate synthase inhibitors (pyruvate:pyruvate acetaldehyde transferase (decarboxylating), EC No. 2.2.1.6; ALS, or AHAS [2, 4]. ALS is a key enzyme in the synthesis of branched-chain amino acids such as isoleucine, leucine, and valine. The class of ALS inhibitors includes more than 50 herbicides – derivatives of sulfonylureas, imidazolinones, pyrimidinyl benzoates, sulfonylaminocarbonyl triazolinones and triazole pyrimidines. The widespread use of herbicides with one mode of action and significant restrictions on the use of herbicides with other modes of action creates the threat of herbicide-resistant weeds emergence. The emergence and widespread of herbicide-resistant weed biotypes can significantly increase the cost of growing crops, to the point where farming becomes unprofitable. Since the 1950s, herbicides with new modes of action have been discovered, and in recent decades the number of weeds that have shown resistance to herbicides has increased, as has the number of cases of cross- and multiple resistance. Triazine resistance in *Senecio vulgaris* L. was identified in the United States as early as 1968. To date, 533 unique cases of resistant weed biotypes are known worldwide, including 273 plant species (156 dicotyledons and 117 monocots). Weeds have evolved resistance to 21 of the 31 known herbicide sites of action and to 168 different herbicides. Herbicide resistant weeds have been reported in 101 crops in 72 countries. Among the herbicide-resistant biotypes in the world, those resistant to ALS inhibitors are the most common [1]. In Ukraine, in recent years, crops of farms with large areas, more than 50-100 thousand hectares, and reduced crop rotations, including sunflower, wheat, maize, soybean, rapeseed etc., have been particularly at risk from resistant weed biotypes prevalence.

Therefore, the purpose of the study was to identify ALS resistance in harmful weed species on the crops of leading agricultural enterprises in the regions of Ukraine and to identify ways to counteract the emergence of resistant weed biotypes.

Materials and methods

The study was carried out under production conditions and in vegetation experiments. The plants *Echinochloa crus-galli* var. *crus-galli* (L.) P. Beauv., *Amaranthus retroflexus* L., and *Chenopodium album* L., collected on the arable lands of the Experimental Agricultural Station of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine; *Ambrosia artemisiifolia* L. in the M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine were used as controls. The collected control seeds and samples of plant seeds from the regions of Ukraine were dried and kept at different storage temperatures: +4 ^{o}C for 2 weeks, then at -18 ^{o}C for 2 weeks; 1-5 cycles. Weed seedlings were obtained in vegetative

conditions, in the BBCH12 phase, at the temperature of 23-25°C, treated with aqueous solutions of herbicides with a professional hand-held sprayer Gloria (Germany). Treatments were arranged in a randomized complete block design with six replications. Phytotoxicity of herbicides was assessed by changes in dry matter weight [1, 5]. Statistical data processing was performed by analysis of variance in the program StatPlus, AnalystSoft Inc. Version v.7.

Results and discussions

ALS resistance was detected in the biotype of the monocotyledonous weed *E. crus-galli*, whose seeds were collected in the Skadovsk and Kalanchak districts of Kherson region in 2015-2021 (tabl.). *E. crus-galli* is a dominant weed on rice crops and is also highly competitive with other crops in the crop rotation: sunflower, maize, and cereals. Numerous cases of weed biotypes resistant to ALS herbicides have been reported worldwide in rice crops [1, 5]. This is due to the high efficacy and therefore widespread use of the relatively inexpensive, selective and phytotoxic penoxsulam, as well as reduced doses of the herbicide in aerial applications.

Simazine-resistant biotypes of *E. crus-galli* were first identified on corn in the USA. Herbicide-resistant biotypes of common rust have now been identified on crops in 25 countries. The discovery in the last decade of numerous weed biotypes with multiple resistance to herbicides with different modes of action, particularly to ALS herbicides and graminicides – acetyl-CoA carboxylase (ACC) inhibitors – is extremely dangerous. In 2008, several multiple biotypes resistant to the following herbicides were identified in South Korea – to azimsulfuron, bensulfuron-methyl, bispiribac-sodium, cyhalofop-butyl, fenoxaprop-P-ethyl, flucetosulfuron, halosulfuron-methyl, imazosulfuron, metamifop, pyrazosulfuron-methyl, pyribenzoxime and pyriminobac-methyl; in 2009 in Italy – to azimsulfuron, bispiribac sodium, cyhalothop butyl, imazamox, penoxsulam and profoxidim; in 2009 in Turkey – to bispiribac-sodium, cyhalofop butyl and penoxsulam [1]. It should be noted that grass weed species have significantly increased their presence in agrophytocenoses in Ukraine and in the world since the 50s of the last century with the widespread introduction of aryloxyphenoxyacetic, propionic and benzoic acid derivatives selective against dicotyledonous species. This trend has continued until recently. Therefore, the identification of an ALS-resistant biotype of *E. crus-galli* complicates weed control in the following crops in rotations in the southern regions of the country: in maize crops with cross-resistance of *E. crus-galli* to nicosulfuron, in sunflower – to imidazolinones (imazamox, imazapyr), in wheat and barley – to numerous ALS herbicides, and also makes the use of penoxsulam in rice production inappropriate [5].

In 2019 and 2020, a number of farms in the Chernihiv and Cherkasy regions found that the composite ALS herbicide mayster power (Bayer Crop Science Ukraine; foramsulfuron, 31.5 g/L + iodosulfuron-methyl-sodium, 1.0 g/L + thiencarbazone-methyl, 10 g/L + cyprosulfamide antidote, 15 g/L) was not effective on maize crops. By 2019, ALS-inhibitor herbicides had been used annually on wheat, soybean, sunflower and corn for more than 7 years. In 2021, the same fields were treated with the composite herbicide euro-lightning (BASF; imazamox, 33 g/L + imazapyr, 15 g/L) of the imidazolinone class in sunflowers, which proved to be ineffective in controlling the highly damaging biotype of *A. retroflexus* in 2022. In 2022, the ineffectiveness of double applications of tribenuron-methyl (express 75, FMS) at a dose of 50 g/ha twice or euro-lightning at the maximum registered dose (1.2 L/ha) for control of *C. album* in Vinnytsia and Chernihiv regions was revealed. The level of control of the dicotyledonous species *A. retroflexus* and *C. album* with the application of herbicides in the maximum doses registered in Ukraine - imidazolinone derivatives, imazamox or the composition imazapyr + imazamox, did not differ from the control plants in terms of inhibition of weed development. Similarly, no phytotoxicity to this weed species was observed with sulfonylurea derivatives (tribenuron-methyl). Thus, the absence of phytotoxicity of imidazolinones at these doses indicates the formation of ALS resistance in the populations of *A. retroflexus* and *C. album* in fields [3].

When triazolpyrimidine sulfonamides (florasulam, flumetsulam) were applied, an initial weak inhibition of plant development was observed, but with time, effective control of the species

was also absent. A similar dependence was observed for the efficacy of the ALS herbicide compositions foramsulfuron + iodosulfuron + thiencarbazone-methyl and tribenuron-methyl + thifensulfuron-methyl – weak burns of dicotyledonous plant species were observed after spraying with no proper weed control one month after treatment. In 2022-2023, multiple resistant *A. artemisiifolia* to acetolactate synthase inhibition HRAC group 2 (Legacy B), protoporphyrinogen oxidase inhibition HRAC group 14 (Legacy E) was identified. This species is problematic in sunflower crops in the southern and central parts of the country.

Table

Herbicide-resistant weed species identified in Ukraine [1]

Year	Species	Mode of action(s)	Actives	Situation
2017	*Echinochloa crus-galli var. crus-galli*	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)	imazapyr, nicosulfuron, imazamox, penoxsulam	Rice
2020	*Amaranthus retroflexus*	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)	imazethapyr, thifensulfuron-methyl, tribenuron-methyl, flumetsulam, imazamox, florasulam, iodosulfuron-methyl-Na, foramsulfuron, thiencarbazone-methyl	Corn (maize), Sunflower
2022	*Chenopodium album*	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)	thifensulfuron-methyl, tribenuron-methyl, flumetsulam, imazamox, florasulam, iodosulfuron-methyl-Na, thiencarbazone-methyl	Corn (maize), Soybean, Wheat, Sunflower
2023	*Ambrosia artemisiifolia*	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B), Inhibition of Protoporphyrinogen Oxidase HRAC Group 14 (Legacy E)	imazapyr, amidosulfuron, nicosulfuron, flazasulfuron, flumetsulam, carfentrazone-ethyl, imazamox, iodosulfuron-methyl-Na, foramsulfuron, thiencarbazone-methyl	Sunflower

Note: Acknowledgments from the website: The Herbicide Resistance Action Committee, The Weed Science Society of America, and weed scientists in Ukraine have been instrumental in providing this information to the International Herbicide-Resistant Weed Database.

The application of glyphosate, an inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, resulted in a high level of control of *A. retroflexus*, *C. album*, and *A. artemisiifolia*. A high level of control of dicotyledonous weeds was also achieved by application of the diphenyl ether derivative aclonifen, which by its mechanism of action interferes with chlorophyll synthesis in plants by causing blinding (discoloration). The grass species was also effectively controlled by graminicide, an acetyl-CoA carboxylase inhibitor.

The effectiveness of controlling ALS-resistant weed biotypes is important given the limited time intervals for additional herbicide applications after resistance is identified and the corresponding ineffectiveness of pretreatment. It has been shown that for herbicides with an acidic fragment in the structure (derivatives of phenoxyacetic acid, benzoic acid – dicamba, etc.), the addition of ammonium sulfate (5 kg/ha) to the spraying solution can increase the phytotoxicity of the composition. The ammonium pool increases the effectiveness of control of ALS-resistant *A. retroflexus* sprayed with herbicides – a benzoic acid derivative (dianate) and a benzoic acid derivative with a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor (stellar – dicamba + topramezone). The mechanism of this increase in phytotoxicity of the herbicide may be the activation of protonation of the plasma membrane H^+-ATPase under the influence of ammonium cation. The addition of ammonium salts to herbicide spraying solutions (dicamba, probably 2.4-D derivatives) can achieve higher levels of control of ALS-resistant weeds and reduce the time of phytotoxicity of the compositions. Important and economically feasible preventive measures against the emergence and spread of resistant weed biotypes include the use of high quality seeds free of

weed impurities, increasing the proportion of agrotechnical weed control measures, restoring and expanding crop rotations with mandatory rotation of herbicides with different modes of action, introducing dicotyledonous/leguminous crops into crop rotations, and using herbicides with different modes of action individually or in mixtures in production crops. The development of digital technologies is also promising for improving weed management systems.

Conclusions

In the period from 2015 to 2024, ALS-resistant weed species were identified in Ukraine. It was found that the imidazolinone-resistant biotype of the grass species *E. crus-galli* is cross-resistant to widely used herbicides of the sulfonylurea and triazole pyrimidine classes. This significantly limits the ability to chemically control the species in rice, wheat, corn, sunflower, soybean, etc. No multiple resistance was found in the *E. crus-galli* biotype to herbicides – inhibitors of 5-enolpyruvylshikimate-3-phosphate synthase (non-selective glyphosate) and acetyl-CoA carboxylase (post-emergence graminicides).

ALS resistance has been identified in common dicotyledonous species of *A. retroflexus*, *C. album* to imidazolinone herbicides – imazapyr and imazamox. There is cross-resistance to herbicides - ALS inhibitors of the sulfonylurea class, as well as to triazolinone derivatives, triazolpyrimidine derivatives. No resistance of *E. crus-galli* to pinoxaden was found, and no resistance of *A. retroflexus*, *C. album*, *A. artemisiifolia* to herbicides of the class of glycine derivatives – glyphosate, phenoxycarboxylates – 2.4-D, benzoic acid – dicamba; tricetones – topramezone; biphenyl ethers – aclonifen; pyridine carboxylates.

Multiple resistance of *A. artemisiifolia* to acetolactate synthase inhibition HRAC group 2 (Legacy B) + protoporphyrinogen oxidase (PPO) inhibition HRAC group 14 (Legacy E) was identified in 2022-2023. *A. artemisiifolia* has been problematic in sunflower crops in Southern and Central Ukraine in recent years.

For the first time, it was shown that herbicide formulations under the influence of the fertilizing with ammonium sulfate can increase the level of efficiency in controlling resistant weed biotypes. Thus, the addition of ammonium sulfate increases the effectiveness of control of ALS-resistant dicotyledon plants with herbicides – a benzoic acid derivative (dianate) and a benzoic acid derivative with a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor (stellar – dicamba + topramezone).

Identification of ALS-resistant biotypes of *E. crus-galli* var. *crus-galli*, *A. retroflexus*, *C. album*, *A. artemisiifolia* in the Southern and Central part of the "grain belt" of Ukraine indicates the limited effectiveness of weed control by herbicides with only one mechanism of action and requires a significant revision of the principles of crop rotation and ways of weed control in the country to maintain high levels of profitability and productivity of agrophytocenoses. Solving this problem is urgent to preserve the potential of Ukraine as one of the guarantors of global food security.

Information on the identification of ALS-resistant, and ALS-PPO-resistant weed biotypes was entered into the International Herbicide-Resistant Weed Database (https://www.weedscience.org/ /Ukraine).

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