



## Identification of a herbicide-resistant biotype of *Echinochloa crus-galli* in Ukraine

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Ukraine is one of the world's guarantors of food security and has the potential to further increase agricultural production. However, the vast majority of herbicides used on crops are acetolactate synthase (ALS) inhibitors, which poses a threat of herbicide-resistant weed species. The emergence and spread of herbicide-resistant weed biotypes can significantly increase the cost of growing crops to the point of loss of profitability. Herbicide resistance in barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) has been studied in long-term field and greenhouse experiments. Resistance of *E. crus-galli* to the ALS herbicide triazolopyrimidine derivative – penoxsulam was identified. Expressed resistance was observed in weed plants grown from seeds collected under production conditions in Kherson region in 2015–2016 and 2020–2021. Cross resistance was observed for imidazolinone and sulfonylurea derivatives. It should be noted that the level of cross resistance to ALS herbicides was slightly higher for plants derived from weed seeds harvested in 2020–2021 compared to those harvested in 2015–2016. The introduction of a herbicidal composition of a herbicide mixture – an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) (an enzyme in plants in the chain of carotenoid synthesis) – mesotrione with an ALS-inhibitor (nicosulfuron) allowed effective control of the weed, which indicates the absence of multiple resistance to herbicides – inhibitors of carotenoid synthesis. The high efficiency of *E. crus-galli* control was established by the application of herbicides – inhibitors of fatty acid synthesis (graminicides). The highest level of efficiency in the experiments was observed with the application of fluzifop-butyl and somewhat less – with pinoxaden. A tendency to reduce phytotoxicity to barnyardgrass from the south of Ukraine was observed with the introduction of tepraloxydim and quizalofop-ethyl. A lower level of phytotoxicity of fenoxaprop-p-ethyl on *E. crus-galli* should be noted compared to the effect of pinoxaden. No multiple resistance was observed with glyphosate (5-enolpyruylshikimate-3-phosphate synthase inhibitor) and reglone (photosystem I inhibitor), allowing control of vegetative weeds at the beginning and end of the growing season. It has been established that monocot weed species have significantly increased their presence and harmfulness in agrophytocenoses in Ukraine and in the world since the 1950s with the widespread introduction of selective dicotyledonous species control with aryloxyphenoxyacetic, propionic and benzoic acid derivatives. This trend has been maintained until recently – barnyardgrass is one of the dominant weed species in modern agrophytocenoses of Ukraine. Therefore, the identification of the ALS-resistant biotype of barnyardgrass complicates weed control in the following crops in rotations in the southern regions of the country: in maize crops with cross resistance to nicosulfuron, in sunflower – to imidazolinones (imazamox, imazapyr), and also makes it impossible to use penoxsulam in rice production. Traditionally, the use of synthetic auxins, phenoxyacetic acid derivatives, etc. is used to control ALS-resistance. However, in Kherson region of Ukraine, already in the third year of application of rinskor (florporauxifen-benzyl), some weed plants were found on rice fields affected by ALS-resistant barnyardgrass, which recovered after the use of synthetic auxin. Therefore, the control of ALS-resistance (penoxsulam, etc.) in *E. crus-galli* with the application florporauxifen-benzyl in the Kherson region of Ukraine is already limited. An obvious and economically feasible preventive measure against the emergence of resistant weed biotypes is the implementation of GAP (Good Agricultural Practice, FAO) approaches: in particular, the use of high quality seeds without 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 rotations, and using herbicides with different modes of action in crops separately or in mixtures. At the same time, agrotechnical measures and the preservation of biodiversity in agrophytocenoses should be the main factor in controlling resistance in weeds. The use of herbicides and their mixtures with different modes of action is of secondary importance. The identification of highly damaging ALS-resistant *E. crus-galli* in southern Ukraine indicates the insufficient effectiveness of weed control exclusively with herbicides with a single 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 for the preservation of Ukraine's potential as one of the guarantors of global food security.

**Keywords:** barnyardgrass; ALS-resistance; penoxsulam; imidazolinones; sulfonylureas; florporauxifen-benzyl.

### Introduction

Crop production in Ukraine is an important sector of the economy, accounting for more than 40% of budget revenues from exports in recent years. The country is one of the guarantors of food security in the world and has the potential to further increase agricultural production (Morgun et al., 2010). However, the dominance of a limited list of crops in crop production – sunflower, wheat, maize, soybean – leads to a reduction in the biodiversity of agrophytocenoses and complicates weed control (Mykhalska & Schwartau, 2022; Schwartau & Mykhalska, 2022). The vast majority of herbicides in Ukraine for use on crops are acetolactate syntha-

se (ALS) inhibitors (pyruvate:pyruvate acetaldehyde transferase (decarboxylating), EC No. 2.2.1.6; ALS, also called AHAS) (Duggibley et al., 2008; Powles & Yu, 2010), 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 (SU, 36 derivatives are known), imidazolinones (IMI, 6 derivatives), pyrimidyl benzoates (PYB, 5 derivatives), sulfonylaminocarbonyl triazolines or triazolinones (SCT or SACT, 4 derivatives), and triazolopyrimidines (TP, 7 derivatives) (Garcia et al., 2017; Heap, I. The International Herbicide-Resistant Weed Database, 2023, [www.weedscience.org](http://www.weedscience.org)). The exact mechanism of phytotoxic action of ALS herbicides is still under

debate. The widespread use of herbicides with one mode of action and significant restrictions on the use of herbicides with other modes of action creates a threat of herbicide-resistant weed species. The emergence and widespread spread of herbicide-resistant weed biotypes can significantly increase the cost of growing crops, to the point where crop production becomes unprofitable.

Since the 1950s, studies of the effects of herbicides on plants have identified new sites of their phytotoxic action, but in recent decades the number of herbicides with new modes of action introduced to the market has been insignificant. In addition, the number of weeds that are resistant to pesticides has increased in recent decades, and there is a noticeable trend toward an increase in the number of cases of cross and multiple resistance. Triazine resistance in common fireweed (*Senecio vulgaris* L.) was identified in the United States as early as 1968. To date, there are 522 unique cases of resistant weed biotypes worldwide, including 269 plant species (154 dicotyledons and 115 monocots). Weeds have developed resistance to 21 of the 31 known herbicide sites of action and to 165 different herbicides. Herbicide-resistant weed biotypes have been reported in 99 crops in 72 countries (International survey of herbicide resistant weeds, 2023, [www.weedscience.com](http://www.weedscience.com)). Among herbicide-resistant biotypes worldwide, those resistant to ALS inhibitors are the most common (Carranza et al., 2023; Damalas et al., 2023): a total of 693 ALS resistance events in weeds have been identified to date.

It should be noted that monocot weed species have significantly increased their presence and harmfulness in agrophytocenoses in Ukraine and worldwide since the 1950s due to the widespread long-term introduction of selective phenoxyacetic, propionic and benzoic acid derivatives against dicotyledonous species. The tendency of cereals to dominate in agrophytocenoses has been maintained until recently. In addition, the reduction of agrotechnical measures for soil management has shifted the burden of weed control to herbicides alone, which, with the dominance of ALS inhibitors, creates conditions for the emergence of resistance in weed biotypes in rice, sunflower, etc. crops, primarily cereals.

In Ukraine, information on the presence of resistant weed biotypes in crops is still limited. In recent years, crops of farms with large areas (more than 50 thousand hectares) and reduced crop rotations, including sunflower, wheat, corn, soybeans, rapeseed, and much less often other crops, have been particularly threatened by resistant weed biotypes. First of all, these areas have formed phytocenoses with limited biodiversity, where selected weeds are dominant and difficult to control. First of all, it is worth mentioning a highly damaging species – barnyardgrass (*Echinochloa crus-galli* (L.) Pal.). It is believed that this species originated in Europe and India, but drawings from China in 1590 clearly show plants of barnyardgrass (Mitich, 1990). Today, the species is found in the region from latitude 50° N to 40° S, in both temperate and tropical climates (Mitich, 1990; Ivaschenko & Ivaschenko, 2019). In addition to *Echinochloa crus-galli* var. *crus-galli*, *E. colona*, *E. crus-galli* var. *formosensis*, *E. crus-galli* var. *zeylanica*, *E. crus-pavonis*, *E. erecta*, *E. oryzoides*, *E. phyllospadix* (= *E. oryzicola*) are found on major crops in the world.

*Echinochloa crus-galli* is the most common annual monocot weed in all regions of Ukraine. The species is mainly found on black and fertile soils. *Echinochloa crus-galli* includes a number of morphologically distinct biotypes, which have been described in detail (Mitich, 1990; Ivaschenko & Ivaschenko, 2019). Also, almost annually, waves of emergence of plants of the species are observed in early spring, in the middle or at the end of the growing season, which are probably related to weed's phenotypic differences in agrophytocenoses. The presence of a sufficient amount of nitrogen compounds in the soil significantly increases the germination rate of barnyardgrass seeds, which allows the species to be classified as a typical nitrophilous plant. In chernozem soils with minimal moisture, seeds germinate better when the topsoil is loosened, and under optimal moisture, seeds germinate well in dense soil.

A better understanding of weed biology and ecology is crucial for the development of effective weed management practices for modern crop production systems. Classical reviews consider some views on the biology and control of *Echinochloa* species (Bajwa et al., 2015; Arslan Masood et al., 2016); however, there is no information on the control of this species in Ukraine. Therefore, the aim of our research was to identify ALS resistance in the dominant monocot weed in Southern Ukraine, barnyardgrass

(*E. crus-galli*), in the crops of leading agricultural companies and to identify ways to counteract the emergence of resistant weed biotypes.

## Materials and methods

The study was carried out under field conditions and in green-house experiments. Plants of *E. crus-galli* var. *crus-galli* (L.) P. Beauv. (synonym *E. muricata* (L.) P. Beauv.), family Poaceae, were used in research. The weed seeds were collected from the fields of the State Enterprise "Research Agricultural Production" of Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine in Kyiv region and from the fields of PodillyaLatInvest LLC in Vinnytsia region.

Seeds of the experimental biotype of the weed were collected on rice fields at Ukrainian Rice Systems LLC (Kherson region) near Kalanchak and Skadovsk in 2015–2016, and near Kalanchak in 2020–2021. The collected seeds were dried and stored at different temperatures: +4 °C for 2 weeks, then at -18 °C for 2 weeks; 4–5 cycles. In green-house experiments weed seedlings in the BBCH13-15 phase at the temperature of 23–25 °C were treated with aqueous solutions of herbicides using a professional hand-held sprayer Gloria (Germany).

The variants were replicated 6 times. Experiments were repeated two times. Herbicide phytotoxicity was assessed by changes in dry matter weight, and results were expressed as a percentage relative to the control (Burgos, 2015).

Statistical data processing was performed by analysis of variance in the program StatPlus (AnalystSoft Inc. Version v. 7), followed by Tukey's test. Data were considered reliable at a significance level of  $P < 0.05$ .

## Results

To date, herbicide-resistant biotypes of barnyardgrass have been found on crops in 25 countries (Table 1). Herbicide-resistant biotypes of *E. crus-galli* var. *crus-galli* to triazine derivatives were first identified on maize crops in the United States. The widespread use of triazine derivatives – PSII inhibitors – led to the emergence of resistant biotypes of barnyardgrass in the period from 1978, in the 1990s and later, mainly in rice and corn fields.

In the early 1990s, barnyardgrass resistance to mitotic cycle inhibitors (pendimethalin) and fatty acid synthesis inhibitors (butachlor, etc.) was first identified. In the late 1990s, multiple resistance was first identified in barnyardgrass to herbicides with two different modes of action: inhibition of PSII-serine 264 binding HRAC group 5 (legacy C1 C2) + inhibition of very long chain fatty acid synthesis HRAC group 15 (legacy K3 N). In 2011, it was found that a biotype of barnyardgrass had acquired multiple resistance to 4 different herbicide sites of action: inhibition of acetyl-CoA carboxylase HRAC Group 1 (Legacy A) + inhibition of acetolactate synthase HRAC Group 2 (Legacy B) + inhibition of PSII – serine 264 binders HRAC Group 5 (Legacy C1 C2) + inhibition of cellulose synthesis HRAC Group 29 (Legacy L). At the same time, 11 cases of invention of multiple resistant barnyardgrass biotypes have already been identified.

To date in the world, barnyardgrass biotypes have been identified as resistant to the vast majority of herbicides registered for use in major crops to control monocot weeds. The International Survey of Herbicide-resistant Weeds, 2023 ([www.weedscience.com](http://www.weedscience.com)) does not mention the identification of resistance to arylpicolinate derivatives: rinskor (florpyrauxifen-benzyl), etc. that have been introduced in recent years.

In order to determine the resistance of barnyardgrass plants from the central and southern parts of Ukraine, seeds were selected on the territory of leading agricultural enterprises. Then, under the conditions of vegetative experiments, the differences in the reaction of barnyardgrass seedlings in the phase from BBCH13 to 15 to the action of selected herbicides registered in Ukraine were determined.

It was found that no resistance to herbicides – ALS inhibitors was detected in plants of *E. crus-galli* from farms of Kyiv and Vinnytsia regions (Table 2). High levels of resistance to penoxsulam and cross resistance to imidazolinone and sulfonylurea derivatives were found in plants grown from seeds from the south of Ukraine. The combination of mesotrione with an ALS-inhibitor (nicosulfuron) allowed for effective control of

*E. crus-galli*. The high efficiency of control of *E. crus-galli* was observed by the application of herbicides – acetyl-CoA carboxylase inhibitors (graminicides). At the same time, the highest level of efficiency was observed in the experiments when fluazifop-p-butyl was applied, while the phytotoxicity of pinoxaden was somewhat slower. A tendency to reduce phytotoxicity to *E. crus-galli* from southern Ukraine was observed with the introduction of tepraloxoym, quizalofop-p-ethyl, and especially fenoxaprop-p-ethyl.

The non-selective herbicides glyphosate and diquat effectively controlled *E. crus-galli* var. *crus-galli*.

**Table 1**

Identification of herbicide-resistant biotypes of *Echinochloa crus-galli* var. *crus-galli* in the world [www.weedscience.com, 2023; with amendments]

No.	Country	Resistance identification year	Crop/Situation	Active Ingredients	Site of Action
1978 – resistance to photosynthesis inhibitor herbicides was identified for the first time, and cross resistance is observed					
1	United States (Maryland)	1978	Corn (maize), and Cropland	atrazine, cyanazine, and simazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
2	Canada (Ontario)	1981	Corn (maize), and Cropland	atrazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
3	France	1982	Corn (maize)	atrazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
4	Greece	1986	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
5	United States (Arkansas)	1990	Cropland, and Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
6	United States (Texas)	1991	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
1992 – Resistance to mitotic cycle inhibitors was identified					
7	Bulgaria	1992	Orchards	pendimethalin	Inhibition of Microtubule Assembly 2HRAC Group 3 (Legacy K1)
8	Spain	1992	Corn (maize)	atrazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
1993 – resistance to fatty acid synthesis inhibitors identified					
9	China	1993	Rice	butachlor	Very Long-Chain Fatty Acid Synthesis inhibitors HRAC Group 15 (Legacy K3 N)
10	China	1993	Rice	thiobencarb/benthiocarb	Very Long-Chain Fatty Acid Synthesis inhibitors HRAC Group 15 (Legacy K3 N)
11	Czech Republic	1994	Corn (maize)	atrazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
12	United States (Missouri)	1994	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
13	Poland	1995	Orchards	atrazine	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
14	United States (Louisiana)	1995	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
15	Sri Lanka	1997	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
1998 – multiple resistance in a weed biotype identified for the first time					
16	Thailand	1998	Rice	butachlor, and propanil	Multiple Resistance: 2 Sites of Action Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2) Very Long-Chain Fatty Acid Synthesis inhibitors HRAC Group 15 (Legacy K3 N)
1998 – resistance to auxin mimetics identified for the first time					
17	United States (Louisiana)	1998	Rice	quinclorac	Auxin Mimics HRAC Group 4 (Legacy O)
18	Brazil	1999	Rice	quinclorac	Auxin Mimics HRAC Group 4 (Legacy O)
19	United States (Arkansas)	1999	Rice	propanil, and quinclorac	Multiple Resistance: 2 Sites of Action PSII inhibitors-Serine 264 Binders HRAC Group 5 (Legacy C1 C2) Auxin Mimics HRAC Group 4 (Legacy O)
20	China	2000	Rice	quinclorac	Auxin Mimics HRAC Group 4 (Legacy O)
21	Italy	2000	Rice	propanil	Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2)
2000 – in addition to multiple resistance, biotype identified as resistant to acetyl-CoA carboxylase inhibitors					
22	United States (California)	2000	Rice	cyhalofop-butyl, fenoxaprop-p-ethyl, molinate, and thiobencarb/benthiocarb	Multiple Resistance: 2 Sites of Action Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Very Long-Chain Fatty Acid Synthesis inhibitors HRAC Group 15 (Legacy K3 N)
23	Thailand	2001	Rice	cyhalofop-butyl, fenoxaprop-p-ethyl, and quizalofop-p-ethyl	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)
2005 – biotype resistance to acetolactate synthase inhibitors identified for the first time					
24	Italy	2005	Corn (maize), and Rice	azimsulfuron, bispuryribac-sodium, imazamox, nicosulfuron, and penoxsulam	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
25	Philippines	2005	Rice	butachlor, and propanil	Multiple Resistance: 2 Sites of Action Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2) Very Long-Chain Fatty Acid Synthesis inhibitors HRAC Group 15 (Legacy K3 N)
26	South Korea	2008	Rice	azimsulfuron, bensulfuron-methyl, bispuryribac-sodium, cyhalofop-butyl, fenoxaprop-p-ethyl, flurotosulfuron, halosulfuron-methyl, imazosulfuron, metamifop, pyrazosul-	Multiple Resistance: 2 Sites of Action Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)

No.	Country	Resistance identification year	Crop/Situation	Active Ingredients	Site of Action
27	United States (Arkansas)	2008	Rice	furon-ethyl, pyribenoxim, and pyniminobac-methyl clomazone bispyribac-sodium, imazethapyr, penoxsulam, and quinclorac	Inhibition of Microtubule Assembly HRAC Group 13 (Legacy F4) <i>Multiple Resistance: 2 Sites of Action</i> Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B) Auxin Mimics HRAC Group 4 (Legacy O)
28	Brazil	2009	Rice	fenoxaprop-p-ethyl azimsulfuron, bispyribac-sodium, cyhalofop-butyl, imazamox, penoxsulam, and profoxydim	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) <i>Multiple Resistance: 2 Sites of Action</i> Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
29	Egypt	2009	Rice	bispyribac-sodium, cyhalofop-butyl, and penoxsulam	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)
30	Italy	2009	Rice	fenoxaprop-p-ethyl azimsulfuron, bispyribac-sodium, cyhalofop-butyl, and penoxsulam, imazamox, penoxsulam, and profoxydim	<i>Multiple Resistance: 2 Sites of Action</i> Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
31	Turkey	2009	Rice	bispyribac-sodium, cyhalofop-butyl, and penoxsulam	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
32	China	2010	Rice, and Soybean	fenoxaprop-p-ethyl, and quizalofop-ethyl	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)
33	Japan	2010	Rice	cyhalofop-butyl	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)
34	Austria	2011	Corn (maize)	nicosulfuron	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
35	China	2011	Rice	penoxsulam	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
36	Italy	2011	Rice	cyhalofop-butyl, and profoxydim	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) <i>Multiple Resistance: 4 Sites of Action</i> Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)
37	United States (Mississippi)	2011	Rice	fenoxaprop-p-ethyl, imazamox, imazethapyr, propanil, and quinclorac (MOA in monocots)	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B) Inhibition of PSII – Serine 264 Binders HRAC Group 5 (Legacy C1 C2) Inhibition of Cellulose Synthesis HRAC Group 29 (Legacy L)
38	Germany	2012	Corn (maize)	nicosulfuron	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
39	France	2013	Corn (maize), and Rice	foramsulfuron, and penoxsulam	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
40	United States (Louisiana)	2013	Rice	imazethapyr	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
41	Uruguay	2013	Rice	quinclorac	Auxin Mimics HRAC Group 4 (Legacy O)
42	Uruguay	2013	Rice	imazapic, and imazapyr	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
2015 – in addition to multiple resistance, biotype identified which is resistant to cellulose synthesis inhibitors					
43	Brazil	2015	Rice	cyhalofop-butyl, penoxsulam, and quinclorac (MOA in monocots)	<i>Multiple Resistance: 3 Sites of Action</i> Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A) Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B) Inhibition of Cellulose Synthesis HRAC Group 29 (Legacy L)
44	Spain	2015	Corn (maize)	nicosulfuron	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
45	India	2017	Rice	bispyribac-sodium	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
46	Ukraine	2017	Rice	imazamox, imazapyr, nicosulfuron, and penoxsulam	Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B)
47	Vietnam	2017	Rice	bispyribac-sodium, penoxsulam, and quinclorac (MOA in monocots)	<i>Multiple Resistance: 2 Sites of Action</i> Inhibition of Acetolactate Synthase HRAC Group 2 (Legacy B) Inhibition of Cellulose Synthesis HRAC Group 29 (Legacy L)
2019 – a biotype resistant to 5-enolpyruvylshikimate-3-phosphate synthase inhibitor (glyphosate) was identified					
48	Argentina	2019	Corn (maize)	glyphosate	Inhibition of Enolpyruvyl Shikimate Phosphate Synthase HRAC Group 9 (Legacy G)
49	Brazil	2020	Soybean	glyphosate	Inhibition of Enolpyruvyl Shikimate Phosphate Synthase HRAC Group 9 (Legacy G)
50	Australia (New South Wales )	2021	Rice	cyhalofop-butyl, and profoxydim	Inhibition of Acetyl CoA Carboxylase HRAC Group 1 (Legacy A)

**Table 2**Efficacy of herbicides in controlling *Echinochloa crus-galli* var. *crus-galli* (greenhouse experiments in 2017–2022)

Herbicide	Active ingredients	Herbicide doses, mg/0.5 kg soil	<i>Echinochloa crus-galli</i> var. <i>crus-galli</i>			
			control – 1*	control – 2	3	4
Without herbicide application	–	–	0 <sup>a</sup> **	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Herbicides – acetolactate synthase inhibitors						
Cytadel 25 OD, Corteva Agriscience	penoxsulam, 25 g/L	0.25 0.50	100 <sup>b</sup> 100 <sup>b</sup>	100 <sup>b</sup> 100 <sup>b</sup>	0 <sup>a</sup> 0 <sup>a</sup>	0 <sup>a</sup> 0 <sup>a</sup>
Euro-Lightning, BASF	imazamox, 33 g/L + imazapyr, 15 g/L	1.00 2.00	70 ± 5 <sup>d</sup> 90 ± 7 <sup>b</sup>	80 ± 6 <sup>d</sup> 100 <sup>b</sup>	10 ± 2 <sup>e</sup> 0 <sup>a</sup>	0 <sup>a</sup> 0 <sup>a</sup>

Herbicide	Active ingredients	Herbicide doses, mg/0.5 kg soil	<i>Echinochloa crus-galli</i> var. <i>crus-galli</i>			
			Central part of Ukraine		The South of Ukraine	
			control – 1*	control – 2	3	4
Euro-Lightning Plus, BASF	imazamox, 16.5 g/L + imazapyr, 7.5 g/L	1.00	70 ± 7 <sup>d</sup>	75 ± 5 <sup>d</sup>	5 ± 3 <sup>c</sup>	0 <sup>a</sup>
		2.00	90 ± 5 <sup>b</sup>	90 ± 5 <sup>c</sup>	0 <sup>a</sup>	0 <sup>a</sup>
		4.00	100 <sup>b</sup>	100 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Pulsar 40, BASF	imazamox, 40 g/L	1.00	80 ± 5 <sup>d</sup>	80 ± 4 <sup>d</sup>	0 <sup>a</sup>	0 <sup>a</sup>
	foramsulfuron, 31.5 g/L + iodosulfuron-methyl-Na, 1.0 g/L + thiencarbazone-methyl, 10 g/L + antidote cyprosulfamide, 15 g/L	2.00	90 ± 6 <sup>c</sup>	90 ± 4 <sup>c</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MaisTer Power, Bayer Crop Science Ukraine	Basis 75 DF, Corteva Agriscience	1.00	70 ± 4 <sup>d</sup>	80 ± 5 <sup>d</sup>	10 ± 3 <sup>c</sup>	0 <sup>a</sup>
		2.00	100 <sup>b</sup>	100 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>
		0.25	70 ± 5 <sup>d</sup>	75 ± 7 <sup>d</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Milagro 40 SC, Syngenta	nicosulfuron, 40 g/L	0.50	90 ± 5 <sup>b</sup>	90 ± 5 <sup>c</sup>	5 ± 2 <sup>c</sup>	0 <sup>a</sup>
		1.00	95 ± 4 <sup>c</sup>	100 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>
		1.00	100 <sup>b</sup>	100 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Composition of a bleaching herbicide - an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and an inhibitor of acetolactate synthase						
Elumis 105 OD, Syngenta	mesotriion, 75 g/L + nicosulfuron, 30 g/L	0.50	95 ± 3 <sup>b</sup>	100 <sup>b</sup>	85 ± 5 <sup>cd</sup>	80 ± 4 <sup>d</sup>
		1.00	100 <sup>b</sup>	100 <sup>b</sup>	90 ± 5 <sup>c</sup>	85 ± 6 <sup>d</sup>
Herbicides – acetyl-CoA carboxylase inhibitors (graminicides)						
Puma Super 144 EW, EB, Bayer Crop Science	fenoxaprop-p-ethyl, 69 g/L + antidote mefenpyr-diethyl	0.50	85 ± 4 <sup>c</sup>	85 ± 5 <sup>c</sup>	75 ± 7 <sup>d</sup>	70 ± 5 <sup>c</sup>
		0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Axial 050 EC, Syngenta	pinoxaden, 50 g/L + antidote cloquintocet-mexyl	0.50	100 <sup>b</sup>	100 <sup>b</sup>	90 ± 6 <sup>c</sup>	90 ± 6 <sup>c</sup>
		0.50	100 <sup>b</sup>	100 <sup>b</sup>	95 ± 4 <sup>c</sup>	95 ± 3 <sup>c</sup>
Aramo, BASF	tepraloxydin, 45 g/L	0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
		0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Targa Super, Summit Agro Ukraine	quizalofop-p-ethyl, 50 g/L	0.50	100 <sup>b</sup>	100 <sup>b</sup>	90 ± 6 <sup>c</sup>	90 ± 6 <sup>c</sup>
		0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Fusilade Forte 150 EC, Syngenta	fluazifop-p-butyl, 150 g/L	0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
		0.50	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Inhibitor of 5-enol-pyruvylshikimate-3-phosphate synthase						
Roundup Max, Bayer Crop Science	glyphosate, 450 g/L glyphosate in acid equivalent (551 g/L in the form of potassium salt of glyphosate)	2.00	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
		4.00	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>
Inhibitor of photosystem I						
Reglone Forte 200 SL, Syngenta	diquat, 200 g/L	1.00	90 ± 4 <sup>c</sup>	90 ± 5 <sup>c</sup>	90 ± 4 <sup>c</sup>	90 ± 4 <sup>c</sup>
		2.00	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>

Notes: \* – weed seeds were collected: 1 – on the non-arable lands of the State Enterprise "Research Agricultural Production" of Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine (Kyiv region) in 2020; 2 – on the fields of PodillyaLatInvest LLC (Vinnytsia region) in 2018–2020; 3 – on rice fields near Kalanchak and Skadovsk, Kherson region, in 2015–2016; on rice fields near Kalanchak, Kherson region, in 2020–2021; \*\* – on the 30th day after treatment: 0% – no weed damage, 100% – plants dead; herbicides registered for use in Ukraine were tested; letters are used to compare samples (Tukey's test, P < 0.05): variants without statistically significant differences are marked with the same letters.

## Discussion

Crop production in southern Ukraine was faced with the emergence of ALS resistance in the biotype of barnyardgrass in 2015–2017. ALS resistance was found in the biotype *E. crus-galli* var. *crus-galli*, whose seeds were collected in Skadovsk and Kalanchak districts of Kherson region in 2015–2021 (Figs. 1, 2; Table 2). On rice crops, barnyardgrass is a dominant weed, and it is also widespread on other crops in crop rotation: it is highly damaging to maize and spiked cereals, as well as sunflower (Mykhalska & Schwartau, 2022; Schwartau & Mykhalska, 2022). In rice, numerous cases of weed biotypes resistant to ALS herbicides have been reported worldwide (Comont et al., 2020; Takano et al., 2023; Heap, I. The International Herbicide-Resistant Weed Database, 2023, [www.weedscience.org](http://www.weedscience.org)). This is due to the high efficacy and thus widespread use of the relatively inexpensive, selective, and phytotoxic penoxsulam on sedges (Cyperaceae spp.), and other monocot species. In addition, until recently, herbicide application in rice fields was mainly done by jets, and consequently a significant part of the fields were treated with reduced doses of herbicides. In recent years, reduced-rate herbicide application using drones, etc. has also become increasingly popular, which also makes it difficult to control the level of herbicide doses. In recent years, growers have also reduced herbicide doses over large areas due to economic factors, which is an additional factor in the rapid emergence of resistant weed biotypes.

Another strong factor in the emergence of ALS resistance in Ukraine is the widespread use of herbicides from the imidazolinone and sulfonylurea classes for weed control. In 2019 and 2020, a number of farms in the Chernihiv and Cherkasy regions found that the composite ALS herbicide MaisTer Power (Bayer Crop Science Ukraine; foramsulfuron, 31.5 g/L + iodosulfuron-methyl-Na, 1.0 g/L + thiencarbazone-methyl, 10 g/L + cyprosulfamide antidote, 15 g/L) was not effective on maize crops. Until 2019, ALS inhibitor herbicides were used annually on wheat, soybeans, sunflower and corn for more than 7 years. In 2018–2023, numerous cases

of ineffectiveness of the imidazolinone herbicide composition Euro-Lightning (BASF; imazamox, 33 g/L + imazapyr, 15 g/L) and, in recent years, Euro-Lightning Plus (imazapyr, 7.5 g/L + imazamox, 16.5 g/L) were identified on sunflower crops, which proved to be ineffective in controlling a number of highly harmful weeds. In 2022, the double application of tribenuron-methyl (Express 75, FMC) at a dose of 50 g/ha twice or Euro-Lightning at the maximum registered dose (1.2 L/ha) was found to be ineffective in controlling a number of weeds in Vinnytsia, Chernihiv, Mykolaiv and Cherkasy regions.



Fig. 1. ALS-resistant biotype of *Echinochloa crus-galli* var. *crus-galli* dominates in rice crop after penoxsulam application, South of Kherson region, 2015–2021

In long-term field and vegetation experiments, we established ALS resistance of the biotype *E. crus-galli* to the ALS inhibitor triazolopyrimidine sulfonamide derivative penoxsulam. Expressed resistance levels were observed in weed plants grown from seeds collected under production

conditions in 2015–2016 and 2021. Cross resistance was observed for imidazolinone and sulfonylurea derivatives. It should be noted that the level of cross resistance to ALS herbicides was slightly higher for plants derived from seeds harvested in 2021 compared to those harvested in 2015–2016. In Ukraine, in the period 2016–2021, differences in the efficacy of control of two phenotypes of *E. crus-galli* var. *crus-galli* ("red" and "green" phenotypes) were observed when penoxsulam was applied at the beginning of weed plant development – up to BBCH10-12 (Fig. 3).



**Fig. 2.** Rice crop infected with ALS-resistant *Echinochloa crus-galli* var. *crus-galli*, Kherson region, near Kalanchak, 2016



**Fig. 3.** Phenotypic differences in the population of the ALS-resistant biotype of *Echinochloa crus-galli* var. *crus-galli* on rice crops: the "red" phenotype is slightly more resistant to penoxsulam in the BBCH10-12 phase (foreground) compared to the "green" phenotype (background); Kherson region, near Skadovsk, 2018

The introduction of a herbicidal composition of a mixture herbicide – an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) – mesotrione with an ALS inhibitor (nicosulfuron) allowed the weed to be effectively controlled, which indicates that it does not have multiple resistance to the HPPD inhibitor. HPPD is an enzyme involved in the synthesis of carotenoids in plants. In warm-blooded organisms, the function of HPPD is tyrosine catabolism (Ahrens et al., 2013; Jhala et al., 2023).

We note the high efficiency of control of *E. crus-galli* with the introduction of graminicides – inhibitors of fatty acid synthesis. At the same time, the highest level of efficiency was observed in the experiments when fluazifop-p-butyl was applied, while the phytotoxicity of pinoxaden was somewhat slower. With the introduction of tepraloxydim, and quizalofop-ethyl, a tendency to reduce phytotoxicity to cereal species from the South of Ukraine was observed. A lower level of phytotoxicity of fenoxaprop-p-ethyl on *E. crus-galli* should be noted in comparison with the effect of pinoxaden. We observed a moderate level of weed control by fenoxaprop-p-ethyl in vegetation experiments and under production conditions in rice. At the same time, rice plants tolerated up to 0.5–0.7 L/ha of the herbicide with a reduction in productivity of 1–3 t/ha. Plants of *E. crus-galli* var. *crus-galli* and rice under production conditions could not withstand the effect of pinoxaden (Axial) at a dose of 0.5–1.0 L/ha.

The discovery in recent decades, for the first time since 1998, of numerous weed biotypes with multiple resistance to herbicides with different

mechanisms of action, in particular to ALS herbicides and graminicides that are acetyl-CoA carboxylase (ACC) inhibitors, is extremely dangerous. In 2008, biotypes resistant to azimsulfuron, bensulfuron-methyl, bispyribac-sodium, cyhalofop-butyl, fenoxaprop-p-ethyl, flucetsulfuron, halo-sulfuron-methyl, imazosulfuron, metamifop, pyrazosulfuron-methyl, pyribenoxim, and pyriminobac-methyl were identified. In 2009 in Italy biotypes resistant to azimsulfuron, bispyribac-sodium, cyhalothop-butyl, imazamox, penoxsulam and profoxydim were found. In 2009 in Turkey biotypes resistant to bispyribac-sodium, cyhalofop-butyl, and penoxsulam were identified. Herbicide-resistant *Echinochloa* species – *E. crus-galli*, *E. crus-pavonis*, *E. hispidula*, *E. phyllopogon*, *E. oryzicola* and *E. oryzoides* – have been identified in rice.

Multiple resistance was not observed when glyphosate (5-enolpyravylshikimate-3-phosphate synthase inhibitor) and reglone (photosystem I inhibitor) were applied, allowing control of vegetative weeds at the beginning and end of the growing season.

The effectiveness of herbicide formulations with different modes of action in controlling weed resistance requires further research (Yukhymuk et al., 2022; Turra et al., 2023). According to numerous reports, the number of multiple resistant weed biotypes is gradually increasing worldwide (Heap, I. The International Herbicide-Resistant Weed Database, 2023, [www.weedscience.org](http://www.weedscience.org)). It has also been shown that the use of herbicide complexes can reduce the level of sensitivity to active ingredients in plants derived from seeds of plants previously treated with the herbicide compositions under study (Takano et al., 2023). The traditional way to control ALS resistance is to use synthetic auxins, phenoxyacetic acid derivatives, etc. Therefore, an important step in achieving high levels of control of ALS (penoxsulam) resistant barnyardgrass was the introduction of rinskor (florpyrauxifen-benzyl), which belongs to the arylpicolinate herbicides. Florpyrauxifen-benzyl belongs to a new class of herbicides – synthetic auxins with a peculiarity in the mechanism of action: fast and strong binding to auxin receptors AFB5 versus TIR1. Rinskor is being proposed for the control of resistance to widely used herbicides in the following classes: ALS, ACCase, HPPD, and propanil, quinclorac, glyphosate, triazine target site resistant species (Herrera et al., 2021).

However, in the Kherson region of Ukraine, in the third year of rinskor application, individual weed plants reaching a height of 180–230 cm were found in rice fields affected by ALS-resistant barnyardgrass (Fig. 4, 5). At the same time, a characteristic stem elongation was observed along the first internode of barnyardgrass plants, which was formed after the application of a plant growth regulator – synthetic auxin rinskor. It is likely that the barnyardgrass plants will fully recover during the growing season of the plants – no morphological effects were observed on parts of plants that were formed in the subsequent vegetation (Fig. 6). In addition, cross resistance to rinskor was identified prior to commercialization of the compound (Takano et al., 2023).



**Fig. 4.** Sampling of plants of ALS-resistant *Echinochloa crus-galli* var. *crus-galli*, Kherson region, near Kalanchak, 2021

## Conclusion

A biotype of the monocot weed barnyardgrass (*E. crus-galli* var. *crus-galli*) resistant to penoxsulam (a triazolopyrimidine sulfonamide

herbicide, an ALS inhibitor) has been identified in Ukraine. The ALS-resistant biotype of barnyardgrass was found to be cross resistant to widely used herbicides of the imidazolinone class (imazamox, imazapyr) and the sulfonylurea class (nicosulfuron, foramsulfuron, iodosulfuron-methyl-Na, rimsulfuron, thifensulfuron-methyl, and thiencarbazone-methyl). This significantly limits the options for chemical weed control in rice, wheat, corn, sunflower, soybeans, etc. No multiple resistance was found in the barnyardgrass biotype to herbicides – inhibitors of 5-enolpyruvylshikimate-3-phosphate synthase (non-selective glyphosate) and acetyl-CoA carboxylase (post-emergence graminicides), as well as to reglone (photosystem I inhibitor) and mesotrione (HPPD inhibitor).



**Fig. 5.** Sampling of herbicide resistant *Echinochloa crus-galli* var. *crus-galli* plants on rice after application of penoxsulam and florporauxifen-benzyl, Kherson region, rice paddies near Kalanchak, 2021



**Fig. 6.** Characteristic curve of the *Echinochloa crus-galli* var. *crus-galli* plant stem above the first-second internode after the phytotoxic action of the synthetic auxin florporauxifen-benzyl; after treatment with a synthetic auxin herbicide the ALS-resistant plant recovered and formed powerful generative organs; Kherson region, rice paddies near Kalanchak, 2021

It is known that monocot weed species have significantly increased their presence and harmfulness in agrophytocenoses in Ukraine and worldwide since the 1950s with the widespread introduction of selective against dicotyledonous species of phenoxyacetic, propionic and benzoic acid derivatives. This trend has been maintained until recently, with barnyardgrass being one of the dominant species in modern agrophytocenoses of Ukraine.

Therefore, the identification of ALS-resistant barnyardgrass biotype complicates weed control in the following crops in rotations in the southern regions of the country: in maize with cross resistance of barnyardgrass to nicosulfuron, sunflower – to imidazolinones (imazamox, imazapyr), and also makes it impossible to use penoxsulam in rice production.

High levels of control of *E. crus-galli* were achieved with the use of graminicides – inhibitors of fatty acid synthesis. The highest level of efficiency was observed in the trials when fluazifop-p-butyl was applied, and the phytotoxicity of pinoxaden was somewhat slower. With the introduction of tepraloxydin and quizalofop-ethyl, a tendency to reduce phytotoxicity to monocot species from the South of Ukraine was observed. A lower

level of phytotoxicity of fenoxaprop-p-ethyl on *E. crus-galli* should be noted in comparison with the effect of pinoxaden.

The effectiveness of herbicide formulations with different modes of action in controlling weed resistance requires further research. A large body of data indicates that the number of multiple resistant weed biotypes is gradually increasing worldwide. It has also been shown that the use of herbicide complexes can reduce the level of sensitivity to active substances in plants grown from seeds of plants previously treated with the herbicide compositions under study. The traditional method of controlling ALS resistance is the use of synthetic auxins. Therefore, an important step in achieving a high level of control of ALS-resistant barnyardgrass was the introduction of rinskor (florporauxifen-benzyl), which belongs to the class of arylpicolinate herbicides, and is a synthetic auxin by its mechanism of action. However, in the Kherson region of Ukraine, in the third year of application of rinskor in rice fields affected by ALS-resistant rice barnyardgrass, some slightly damaged weeds were found in the rice fields. Control of ALS resistance (penoxsulam, etc.) in barnyardgrass with the introduction of the synthetic auxin florporauxifen-benzyl is already limited.

Thus, an obvious and economically feasible preventive measure against the emergence of resistant weed biotypes is the introduction of GAP (Good Agricultural Practice) approaches: in particular, the use of high quality seeds without weed impurities, an increase in the share of agrotechnical weed control measures, the restoration and expansion of crop rotations with mandatory rotation of herbicides with different mechanisms of action, and the introduction of crop rotations dominated by dicotyledonous, and cereals/legumes. At the same time, agrotechnical measures and the preservation of biodiversity in agrophytocenoses should be the main factor in controlling resistance in weeds. The use of herbicides and herbicide mixtures with different modes of action is important, but only of subsidiary value.

The identification of highly damaging ALS-resistant barnyardgrass in Southern Ukraine indicates the limited effectiveness of weed control exclusively by herbicides with a single 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 for the preservation of Ukraine's potential as one of the guarantors of global food security.

For the first time from Ukraine, information on the identification of the ALS-resistant biotype of *E. crus-galli* var. *crus-galli* was entered into the International Herbicide-Resistant Weed Database ([weed.sci.org/](http://weed.sci.org/) Ukraine).

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The authors declare no conflicts of interest.

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