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Target-site cross-resistance to ALS inhibitors in johnsongrass originating from Greek cornfields

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Research Article

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Abstract

Five johnsongrass populations collected from corn grown in northern Greece were studied to elucidate the levels and mechanisms of resistance to acetolactate synthase (ALS)- and acetyl-CoA carboxylase (ACCase)-inhibiting herbicides. Whole-plant response assays indicated that two populations were highly cross-resistant to all ALS inhibitors tested (foramsulfuron, nicosulfuron, rimsulfuron, and imazamox) but were effectively controlled by the recommended rate of the ACCase-inhibiting herbicides propaquizafop and clethodim. The *ALS* gene sequence revealed a point mutation that resulted in the substitution of Trp574 by Leu in the ALS enzyme, suggesting that the resistance mechanism is target-site mediated. These findings highlight a serious threat against the sustainable use of the ALS-inhibiting herbicides in controlling johnsongrass and other grass weeds in cornfields, suggesting rotational use of herbicides with different modes of action, along with the use of nonchemical methods, for viable Johnsongrass management.

Introduction

Johnsongrass is an erect tetraploid ($2\times = 40$), perennial, predominately self- and partially cross-pollinated grass weed that reproduces sexually by seeds and asexually by a below-ground rhizome system (Fernandez et al. 2013; Holm et al. 1977; Warwick and Black 1983). It is native in the Mediterranean areas of Africa, Asia, and Europe (especially Syria and Turkey) and has invaded new agricultural areas of the world between latitudes 55°N and 45°N (Follak and Essl 2013). It spreads mainly through cropping practices, including the movement of machinery during soil tillage, which will break and spread rhizomes and allow johnsongrass to thrive (Barroso et al. 2012; Panozzo et al. 2017; Reichmann et al. 2016).

Johnsongrass is ranked as the world's sixth worst weed, infesting 30 different crops in 53 countries (Peerzada et al. 2017). It is a noxious weed responsible for severe infestations in many economically important crops, such as corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), sugarcane (*Saccharum officinarum* L.), vegetables, pastures, alfalfa (*Medicago sativa* L.), orchard trees, and vineyards (Chirita et al. 2007; Jensen et al. 2011). Moreover, owing to its close ancestry and risks associated with gene flow, it represents a serious threat to grain sorghum grown for seed (Ohadi et al. 2018).

The ability of this species to persist and compete with crops as a serious weed problem is related to its remarkable accelerated growth; its high biomass accumulation, particularly during warm periods (C4 plant species); and its enormous reproductive ability (vigorous rhizome system and high seed production) (Klein and Smith 2020; Reichmann et al. 2016; Rout et al. 2013; Schwinning et al. 2017; Travlos et al. 2019). More specifically, a single johnsongrass plant can produce up to 90 m of rhizomes and 80,000 seeds in one growing season (Riar et al. 2011; Ryder et al. 2018), making its control very difficult (Panozzo et al. 2017). Plants from rhizomes exhibit higher growth rates and are more competitive than plants originating from seeds (Acciaresi and Guiamet 2010; Karkanis et al. 2020; Mitskas et al. 2003).

Control of johnsongrass in corn grown in Greece mainly relies on three postemergence-applied acetolactate synthase (ALS)-inhibiting herbicides, foramsulfuron, nicosulfuron, and rimsulfuron, which provide effective control of this weed in sensitive plants originating from both seed and rhizomes (Eleftherohorinos and Kotoula-Syka 1995; Travlos et al. 2019). These herbicides inhibit ALS, also referred to as acetohydroxyacid synthase (AHAS), which is the key enzyme in the biosynthetic pathway of branched chain amino acids valine, leucine, and isoleucine. However, their intensive use has imposed a strong selection pressure that has led to the evolution of 169 resistant weed species globally (Heap 2022). These resistant populations are a severe threat to the sustainability of intensive cropping systems and endanger food security for the ever-increasing world population. Among the resistant species, johnsongrass is particularly important because some of its populations have developed multiple resistances to different families of ALS- and acetyl-CoA carboxylase (ACCase)-inhibiting herbicides (Heap 2022).

Johnsongrass control in broadleaf crops (cotton, soybean, sugarbeets (*Beta vulgaris* L.), and vegetable crops) is mainly based on postemergence application of two chemically distinct classes of grass-selective, ACCase-inhibiting herbicides (aryloxyphenoxypropionates and cyclohexanediones) (Haitas et al. 1995; Scarabel et al. 2014). These herbicides inhibit the ACCase enzyme and consequently de novo fatty acid synthesis in sensitive grass weeds, leading to necrosis and plant death (Kaundun 2014). A johnsongrass population in Greece has developed cross-resistance to ACCase-inhibiting herbicides (Kaloumenos and Eleftherohorinos 2009), whereas in Argentina, many johnsongrass populations have evolved multiple resistances to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and ACCase inhibitors (Heap 2022).

Johnsongrass in Greece is one of the most abundant and harmful weeds in summer crops, such as corn, cotton, sunflower, tomato (*Solanum lycopersicum* L.), and vegetables, where its control during the last 35 years has relied on the extensive use of ALS and ACCase inhibitors. However, recently, growers from northern Greece have complained about unsatisfactory control of this weed grown in corn after application of the ALS inhibitor foramsulfuron, which had been continuously used in the area for at least ten consecutive years. Based on this information, the aims of this study were (1) to test the putatively resistant (R) populations for resistance evolution to foramsulfuron and other ALS-inhibiting herbicides, (2) to elucidate the possible presence of a point mutation in the ALS gene, and (3) to evaluate the efficacy of other postemergence herbicides registered in broadleaf crops for effective johnsongrass control.

Materials and Methods

Seed Source of ALS Putative R Johnsongrass Populations

A roadside survey was conducted during spring and early summer of the 2018 growing season in corn monoculture fields located in two widely distributed counties in northern Greece (Kavala in northeastern Macedonia, Greece, and Florina in northwestern Macedonia, Greece), where failure of johnsongrass control with foramsulfuron and other ALS-inhibiting herbicides had been reported. After conducting the survey, fields with johnsongrass escapes after herbicide application were noted, the associated farmers were contacted and interviewed, and fields that farmers had anticipated to have poor control were marked. Among the marked fields with poor control of johnsongrass, seeds were collected from two fields located in Kavala and three in Florina before corn harvest (September 2018); these fields were chosen as they exhibited the most notable Johnsongrass escapes. Mature seeds were collected by hand from 70 to 80 individual johnsongrass plants of each field, pooled together, and characterized as a putatively resistant population. During seed collection, care was taken to obtain a representative sample (500 to 600 g of seeds) from each field. Seeds were also collected from johnsongrass plants grown in a noncultivated area at the Aristotle University farm, which had no history of exposure to herbicide applications (these seeds were considered as the susceptible population). The collected seeds were transferred to the laboratory, where they were air-dried, threshed, placed in paper bags, and stored at room temperature to be used in the subsequent experiments.

Whole-Plant Preliminary Screening Assays for Putative Resistance

Two johnsongrass populations originating from Kavala (P1, P2) and three from Florina (P3, P4, P5) were evaluated in 2019 for possible evolution of cross-resistance to ALS-inhibiting herbicides. A sensitive johnsongrass population (PS), originating from the Aristotle University farm, was also included in the preliminary screening. The experiment was conducted in $22 \times 22 \times 25$ cm plastic pots filled with a 1:1:1 (v/v/v) mixture of clay loam soil with peat and sand. Johnsongrass seeds were initially exposed to concentrated H_2SO_4 for approximately 4 to 5 min and were subsequently immersed in 1.5% solution of KNO_3 for 2 h (Balicevic et al. 2016). Seeds were then placed on filter paper inside petri dishes, which were placed on laboratory benches for seed germination (21 to 24 C). Each pot was seeded at a depth of 1 cm with ten pregerminated johnsongrass seedlings. When johnsongrass seedlings reached the two-leaf stage, they were carefully thinned to five per pot. Johnsongrass plants were irrigated and fertilized as and when required to maintain optimum plant growth throughout the experiment.

The PS and the five putative R johnsongrass populations were tested for resistance to sulfonylurea herbicide foramsulfuron and for cross-resistance to sulfonylurea herbicide rimsulfuron (Table 1). The herbicide applications were performed when johnsongrass plants of both putative R and PS populations reached the three- to four-leaf stage (25 to 35 cm tall). A nontreated control for the putative R and the PS population was also included. A portable field plot sprayer with a 2.4-m-wide boom was used for herbicide applications. The sprayer boom had six 8002 flat-fan nozzles and was calibrated to deliver 300 L ha^{-1} of water at a pressure of 280 kPa. The whole-plant screening experiment was conducted twice.

Each of the two identical pot experiments was established in a completely randomized design with three replications for each treatment. Pot randomization within each population was made weekly to ensure uniform growth conditions for all plants. No strong rainfall events or high temperatures were noted in the period of the experiments. Johnsongrass control was evaluated by determining the aboveground fresh weight of surviving plants at 35 d after treatment (DAT) for all herbicides. Fresh weight data were expressed as a percentage reduction of the nontreated control (fresh weight suppression over the nontreated control) and subjected to analysis of variance (ANOVA). The data were analyzed over the two experiments because the homogeneity of variances checked by Bartlett's test (Snedecor and Cochran 1989) indicated no significant departure of normality. Therefore a combined ANOVA over two experiments was performed for the johnsongrass populations evaluated, using a $6 \times 2 \times 2$ split-plot approach, where the six johnsongrass populations were the main plots and the two herbicide by two herbicide rates were the subplots. Significant means were separated using Fisher's protected LSD test ($P = 0.05$). Because the comparison of means showed that the populations P1 and P2 were not controlled with foramsulfuron and rimsulfuron, they were considered as R populations and used in subsequent dose–response assays.

Table 1. Source of materials for the products used in the screening test experiments against the putative resistant and the reference johnsongrass population.[a]

Herbicide	Trade name	Form	Rate	Manufacturer
			g ai ha^{-1}	
Foramsulfuron	Equip®	OD	60	Bayer Crop Science
			120	
Rimsulfuron[b]	RUSH®	WDG	15	Corteva Agriscience Hellas
			30	

[a]Abbreviations: OD, oil dispersion; WDG, water-dispersible granule.
[b]Rimsulfuron treatments were applied with the surfactant iodecyl alcohol ethoxylate 90% w/v (Trend® 90 SL, Corteva Agriscience Hellas, Athens, Greece) at 0.1% vol/vol.

Whole-Plant Dose–Response Assays

The P1 and P2 populations were treated with four rates of the ALS inhibitors foramsulfuron, nicosulfuron, rimsulfuron, and imazamox and with four rates of the ACCase-inhibiting herbicides propaquizafop and clethodim (Table 2). The PS johnsongrass population was also exposed to four rates of the ALS-inhibiting herbicides foramsulfuron, nicosulfuron, rimsulfuron, and imazamox and of the ACCase inhibitors propaquizafop and clethodim (Table 3). An untreated control for the two R and one S population was included. Johnsongrass plants of both R and S populations (two resistant and one susceptible populations) were exposed to herbicide applications when they reached the three- to four-leaf growth stage (25 to 35 cm height), as recommended in the Greek product label for these herbicides. The herbicides were applied in a similar way to that described in the previously mentioned screening experiments. Pot randomization within each population was made weekly to ensure uniform growth conditions for all plants. No strong rainfall events or high temperatures were noted in the period of the experiments. Each of the two identical dose–response pot experiments was established in a completely randomized design with three replications for each herbicide treatment. Johnsongrass control was assessed by determining the aboveground fresh weight of surviving plants at 35 DAT. The assessment was made at 35 instead of 21 DAT to study the possible regrowth of the treated plants.

Fresh weight data were expressed as a percentage reduction of the untreated control (fresh weight suppression over the untreated control) and subjected to ANOVA. An ANOVA combined over two experiments was performed to evaluate the two selected johnsongrass populations used in the dose–response experiment, using a 2 × 6 × 4 split-plot approach, where the two weed populations were the main plots and the six herbicide by four herbicide rates were considered as the subplots. The data were analyzed over the two experiments because the homogeneity of variances checked by using Bartlett's test (Snedecor and Cochran 1989) indicated no significant departure from normality. Differences between means were compared using Fisher's protected least significant difference (LSD) test ($P = 0.05$).

The combined growth response data were also fit to a four-parameter log-logistic curve for nonlinear regression analysis (Seefeldt et al. 1995):

$$y = c + (d - c)/\{1 + exp[b(logx - logGR_{50})]\}$$

where c is the lower limit, d is the upper limit, and b is the relative slope around the herbicide dose resulting in 50% growth reduction (GR_{50}). The herbicide dose was the independent variable (x), and the growth response (percentage of the untreated control) was the dependent variable (y) in the regression equation. This equation was used to estimate the dose causing a 50% fresh-weight growth reduction (GR_{50}).

Table 2. Source of materials for the products used in the whole-plant rate–response experiments against the P1 and P2 ALS-resistant johnsongrass populations.[a]

Herbicide[a]	Trade name	Form	Rate	Manufacturer
			g ai ha^{-1}	
Foramsulfuron	Equip®	OD	60	Bayer Crop Science
			120	
			240	
			480	
Nicosulfuron	Samson Extra	OD	45	Alpha Agricultural Supplies SA
			90	
			180	
			360	
Rimsulfuron[b]	RUSH®	WDG	15	Corteva Agriscience Hellas
			30	
			60	
			120	
Imazamox	Pulsar®	SL	50	BASF Hellas
			100	
			200	
			400	
Propaquizafop	AGIL®	EC	18.8	Alpha Agricultural Supplies SA
			37.5	
			75	
			150	
Clethodim	VETRI	EC	37.5	Arysta Hellas
			75	
			150	
			300	

[a]Abbreviations: OD, oil dispersion; WDG, water-dispersible granule; SL, soluble liquid; EC, emulsifiable concentrate.
[b]Rimsulfuron was applied with the iodecyl alcohol ethoxylate 90% w/v (Trend® 90 SL) at 0.1% vol/vol; imazamox was applied with the 37.5% w/w fatty acid esters + 22.5% w/w alkoxylated alcohols-phosphate esters (Dash® HC, BASF Hellas, Athens, Greece) at 0.4% vol/vol; clethodim was applied with the paraffin oil 60% w/v (Atplus™, Croda International Plc, East Yorkshire, UK) at 0.5% vol/vol.

Amplification and Sequencing of the ALS Gene Fragment

For the amplification of the *ALS* gene, plant material was collected from P1 and P2 individual plants, grown in six pots per population, and treated with the labeled rate of foramsulfuron and in four untreated-with-herbicide pots of the PS population. This treatment was made for eliminating individual susceptible plants from the resistant population and for ensuring the susceptibility of the PS population. Leaf tissues from surviving P1 and P2 johnsongrass plants and from the untreated PS plants were harvested, immediately stored at −28 C, and subsequently used for DNA extraction.

Genomic DNA was isolated from three PS, four P1, and five P2 plants, using 40 to 50 mg of young leaf tissue (one leaf per plant) and according to the NucleoSpin® Plant II DNA extraction kit protocol (MACHEREY-NAGEL, Düren, Germany). The quality and quantity of the isolated DNA were checked using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the DNA concentration of each sample was adjusted to 20 ng/μl through dilution with ultrapure water.

Table 3. Source of materials for the products used in the whole-plant rate–response experiments against the PS johnsongrass population.[a]

Herbicide	Trade name	Form	Rate	Manufacturer
			g ai ha^{-1}	
Foramsulfuron	Equip®	OD	7.5	Bayer Crop Science
			15	
			30	
			60	
Nicosulfuron	Samson Extra	OD	5.6	Alpha Agricultural Supplies SA
			11.2	
			22.5	
			45	
Rimsulfuron[b]	RUSH®	WDG	1.88	Corteva Agriscience Hellas
			3.75	
			7.5	
			15	
Imazamox	Pulsar®	SL	6.25	BASF Hellas
			12.5	
			25	
			50	
Propaquizafop	AGIL®	EC	18.8	Alpha Agricultural Supplies SA
			37.5	
			75	
			150	
Clethodim	VETRI	EC	37.5	Arysta Hellas
			75	
			150	
			300	

[a]Abbreviations: OD, oil dispersion; WDG, water-dispersible granule; SL, soluble liquid; EC, emulsifiable concentrate.
[b]Rimsulfuron was applied with the iodecyl alcohol ethoxylate 90% w/v (Trend® 90 SL) at 0.1% vol/vol; imazamox was applied with the 37.5% w/w fatty acid esters + 22.5% w/w alkoxylated alcohols-phosphate esters (Dash® HC) at 0.4% vol/vol; clethodim was applied with the paraffin oil 60% w/v (Atplus™) at 0.5% vol/vol.

The forward, ECH-5F (5′-AGG TCA CSC GCT CCA TCA CCA-3′), and reverse, ECH-3R (5′- TCC TGC CAT CAC CHT CCA KGA-3′), primers were used to produce a genomic fragment of 1,364 base pairs (bp), harboring F, B, and E domains of the *ALS* gene (Panozzo et al. 2013, 2017). Primers were designed for amplification of conserved domains where mutation sites (e.g., Trp574) endowing cross-resistance to ALS inhibitors have been previously identified. Cycling conditions consisted of an initial denaturation step of 95 C for 2 min followed by 35 cycles of 95 C for 30 s, 58 C for 30 s, and 72 C for 1 min and 40 s, with a final extension at 72 C for 10 min. PCR was performed in 10 μl volumes containing 8 μl (1×) of One*Taq*® 2X master mix (New England Biolabs, Ipswich, MA, USA), 0.5 μl of each forward and reverse primer (0.5 μM each), and 1 μl of template DNA (20 ng).

A volume of 3 μl of each PCR product was electrophoresed on a 1.5% agarose gel stained with MIDORI Green DNA stain (NIPPON Genetics Europe, Düren, Germany). The quality and quantity of the fragments were checked against a FastGene 100 bp DNA ladder (NIPPON Genetics Europe). When PCR products showed a clear and single band of the correct expected length, the whole of the PCR products was purified with the microCLEAN DNA cleanup reagent (Gel Company, San Francisco, CA, USA) according to the manufacturer's protocol. Finally, the purified PCR products were single-strand sequenced with BigDye™ Terminator v3.1 (Life Technologies, Waltham, MA, USA) cycle sequencing methodology on an ABI3500 Genetic Analyzer (Applied Biosystems™, Waltham, MA, USA), using the same primers as for PCR (reverse primer). To detect the presence or absence of point mutations at the aforementioned domains of the ALS gene, johnsongrass sequences were manually checked and aligned using BioEdit v7.2.6 software (Hall 1999), following comparison with existing johnsongrass sequences for the *ALS* gene in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results and Discussion

Whole-Plant Assays for Putative R Johnsongrass Populations to ALS Inhibitors

The fresh weight of the PS population in the preliminary screening assays was reduced by 100% with 60 and 15 g ai ha^{-1} (recommended rate) of foramsulfuron and rimsulfuron, respectively (Table 4). However, fresh weight reduction for P1 and P2 johnsongrass populations ranged from 1% to 2% after the application of the recommended rate of foramsulfuron and rimsulfuron, while the respective reduction by the 2-fold rate was 18% and 2% for foramsulfuron and 22% and 15% for rimsulfuron. In addition, the fresh weight of the P3, P4, and P5 johnsongrass population treated with the recommended rate of foramsulfuron was reduced by 52%, 43%, and 43%, respectively, whereas the respective reduction with the 2-fold rate was 87%, 82%, and 72%. By contrast, their fresh weight was reduced by 100% with rimsulfuron applied at recommended and 2-fold rates.

The 60, 45, 15, and 50 g ai ha^{-1} (recommended rate) of foramsulfuron, rimsulfuron, nicosulfuron, and imazamox, respectively, in the whole-plant dose–response assays reduced the fresh weight of the P1 population by 1%, 11%, 14%, and 0%, respectively, whereas, averaged over the rates of each herbicide, the order of fresh weight reduction was nicosulfuron > rimsulfuron > foramsulfuron > imazamox (Table 5). A similar resistance profile was documented for the P2 population, where the recommended rate of foramsulfuron, rimsulfuron, nicosulfuron, and imazamox provided 0%, 5%, 5%, and 0% fresh weight reduction, respectively, whereas, averaged over the rates of each herbicide, the order of fresh weight reduction was nicosulfuron > rimsulfuron > foramsulfuron > imazamox. By contrast, 37.5 and 75 g ai ha^{-1} (one-fourth the recommended rate) of the ACCase inhibitors propaquizafop and clethodim, respectively, reduced the fresh weight of both ALS herbicide–resistant P1 and P2 populations by 100% (data not shown). Moreover, the application of all ALS (foramsulfuron, rimsulfuron, nicosulfuron, and imazamox) and ACCase (propaquizafop and clethodim) inhibitors at lower than the recommended rates resulted in 96% to 100% control of the PS population (data not shown).

The calculated GR_{50} value (herbicide rate [g ai ha^{-1}] required for 50% reduction of fresh weight) for the P1 population was 295, 75, and 104 g ai ha^{-1} for foramsulfuron, nicosulfuron, and rimsulfuron, respectively, whereas the respective GR_{50} values for the P2 population were 284, 104, and 79 (Table 6). The GR_{50} value for imazamox was greater than the highest rate tested, whereas the GR_{50} value of all herbicides for the PS population was lower than their lowest rates tested. Therefore the resistance index (RI), as the ratio of the GR_{50} of the R population to the GR_{50} of the PS population, was not calculated, because the GR_{50} of the PS population was not estimated for the reason reported.

The unsatisfactory control of P1 and P2 populations with foramsulfuron, nicosulfuron, rimsulfuron, and imazamox applied at higher than the recommended rates supports the evidence of cross-resistance to these ALS-inhibiting herbicides. The limited use of

Table 4. Fresh weight reduction (% of control) of one sensitive (PS) and five putative R johnsongrass populations (P1, P2, P3, P4, P5) in a screening assay with the recommended and 2-fold the recommended rate of foramsulfuron and rimsulfuron.[a]

Herbicide	Rate	Population					
		P1	P2	P3	P4	P5	PS
	g ai ha^{-1}	——— % of control ———					
Foramsulfuron	60	52	43	43	1	1	100
	120	87	82	72	18	2	100
Rimsulfuron	15	100	100	100	2	1	100
	30	100	100	100	22	15	100
$LSD_{0.05}$				3			

[a]Values of each herbicide rate are means of six replicates.

Table 5. Fresh weight reduction (% of control) of the R P1 and P2 johnsongrass populations as affected by the ALS-inhibiting herbicides foramsulfuron, rimsulfuron, nicosulfuron, and imazamox.[a]

Herbicide	Rate	Population	
		P1	P2
	g ai ha^{-1}	— % of control —	
Foramsulfuron	60	1	0
	120	4	1
	240	49	56
	480	68	66
Nicosulfuron	45	14	5
	90	67	52
	180	90	83
	360	100	100
Rimsulfuron	15	11	5
	30	21	13
	60	67	29
	120	89	77
Imazamox	50	0	0
	100	2	0
	200	11	15
	400	42	22
$LSD_{0.05}$		3	

[a]Values of each herbicide rate are means of six replicates.

crop rotation and the inevitable high reliance of corn farmers on the intense and repeated postapplied sulfonylurea herbicides foramsulfuron, rimsulfuron, and nicosulfuron for effective control of johnsongrass could account for its cross-resistance. Similar results were reported by Panozzo et al. (2017), who found that Italian johnsongrass populations were highly cross-resistant to ALS-inhibiting herbicides nicosulfuron, foramsulfuron, imazamox, and byspiribac-sodium, although the Hungarian johnsongrass populations that they tested were resistant to sulfonylureas and bispyribac-Na but susceptible to imazamox. The fact that these two populations were effectively controlled with all rates (as low as ×/8) of the ACCase-inhibiting herbicides propaquizafop and clethodim shows clearly that these populations had not yet evolved multiple resistances to these herbicides. These results agree with those reported by Johnson et al. (2014), who found that one johnsongrass population from Arkansas with resistance to ALS inhibitor imazethapyr was sensitive to the ACCase-inhibiting herbicides fluazifop and clethodim. Regarding the excellent efficacy of all herbicide treatments against the PS population, it supports its selection as a reference population. However, the reduced control of the P3, P4, and P5 johnsongrass populations when exposed to

Table 6. Estimated GR_{50} values of foramsulfuron, nicosulfuron, and rimsulfuron for two resistant johnsongrass populations.[a]

Populations	GR_{50}[b] (95% CI)	b Slope (SE)	Res. MS	R^2
	g ai ha^{-1}			
Foramsulfuron				
P1	295 (247–331)	4.9 (0.9)	1,376	0.93
P2	284 (233–364)	5.0 (1.6)	3,181	0.86
Nicosulfuron				
P1	75 (72–78)	7.7 (0.3)	182	0.99
P2	104 (88–125)	5.9 (0.8)	13,423	0.57
Rimsulfuron				
P1	47 (44–49)	5.7 (0.3)	305	0.98
P2	79 (70–84)	5.8 (0.4)	418	0.97

[a]Abbreviations: GR_{50}, herbicide concentration for 50% reduction of the johnsongrass fresh weight; Res. MS, residual mean of squares.
[b]The GR_{50} value for imazamox was not estimated because none of its rates caused less than 50% reduction of fresh weight. The GR_{50} value for the S population was not estimated because the lower rate of all herbicides caused more than 50% reduction of fresh weight.

the foramsulfuron recommended rate only supports the hypothesis that its repeated applications against these populations may have started to select plants with reduced sensitivity.

The intermediate resistance of P1 and P2 populations to nicosulfuron is partially in agreement with results found by Panozzo et al. (2017), who reported that two Hungarian johnsongrass populations exhibited lower resistance indexes (2.3, 2.8) to this sulfonylurea herbicide as compared with the 47 estimated index for one population. By contrast, Hernández et al. (2015) found a johnsongrass population with very high resistance to nicosulfuron, and Werle et al. (2016) reported that 5 and 3 out of 59 johnsongrass populations were highly resistant to imazethapyr and nicosulfuron, respectively. The different resistance profiles of the johnsongrass populations to different ALS inhibitors support the occurrence of different resistance mechanisms in the populations, which could be attributed in part to different herbicides used in the corresponding fields (i.e., the selection history).

Amplification and Sequencing of the ALS Gene Fragment

The comparison of *ALS* gene fragment sequences in the nine R and three PS johnsongrass plants with the coding sequence of *Arabidopsis thaliana* revealed a point mutation at the second base of codon Trp-574 (TGG) in the R plants only, which resulted in a substitution of Trp-574 by Leu (TTG) in the ALS enzyme (Figure 1). The fact that all nine R plants have TKG (K = G or T) at the codon Trp-574 indicates the tetraploidy of the johnsongrass plants and establishes the existence of two *ALS* gene copies, one of which is probably homozygous for the R allele (Hernández et al. 2015). The lack of any point mutation on all three sequenced PS plants confirms their susceptibility to ALS inhibitors.

The detected Trp574-Leu is one of the most common field-evolved ALS amino acid substitutions and confers high levels and broad-spectrum ALS target-site cross-resistance across all chemically dissimilar classes of ALS-inhibiting herbicides in many other weed species (Beckie and Tardif 2012; Heap 2022; Yu and Powles 2014). This mutation has been frequently identified in many field-evolved ALS inhibitor R johnsongrass populations in continuous corn cropping systems around the world (Hernández et al. 2015; Panozzo et al. 2017; Werle et al. 2017). The Trp574 substitution was also identified in the ALS enzyme of late watergrass [*Echinochloa oryzicola* (Ard.) Fritch] populations, conferring broad-spectrum cross-resistance to penoxsulam, imazamox, bispyribac-sodium, and nicosulfuron (Kaloumenos

P1 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P1 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P1 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P1 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P2 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P2 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P2 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P2 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
P2 CACCTGGGGATGGTGGTGCAG**TKG**GAGGACAGGTTCTAT
H L G M V V Q **W/L** E D R F Y
PS CACCTGGGGATGGTGGTGCAGTGGGAGGACAGGTTCTAT
H L G M V V Q W E D R F Y
PS CACCTGGGGATGGTGGTGCAGTGGGAGGACAGGTTCTAT
H L G M V V Q W E D R F Y
PS CACCTGGGGATGGTGGTGCAGTGGGAGGACAGGTTCTAT
H L G M V V Q W E D R F Y
574

Figure 1. Alignment of johnsongrass ALS sequences using BioEdit v7.2.6 software. The first four and the following five samples represent the DNA sequences of the P1 and P2 resistant johnsongrass plants originating from cornfields, whereas the last three DNA sequences correspond to PS johnsongrass plants. Observed polymorphisms (TKG) are marked in bold and correspond to the Trp-574 position of the *A. thaliana ALS* gene (X51514).

et al. 2013). In addition, the Asp376Glu substitution in the ALS enzyme was found in four johnsongrass populations to confer resistance to ALS inhibitors (Panozzo et al. 2017).

Mutations in the *ALS* gene conferring herbicide resistance are generally inherited as partially dominant nuclear genes, suggesting that resistance can be spread by both seed and pollen (Tsuji et al. 2003). However, because studies on ALS resistance evolution in johnsongrass do not exist, further research is needed to elucidate the number of genes involved in resistance of this weed tetraploid species, their expression, and their inheritance (Riar et al. 2011). In addition, given the inconsistent or negligible impact of this mutation on ALS functionality and plant fitness in other species, for example, rigid ryegrass (*Lolium rigidum* Gaudin) (Yu et al. 2010) and wild mustard (*Sinapis arvensis* L.) (Ntoanidou et al. 2020), further studies are needed to investigate the growth rate and fitness of ALS herbicide–resistant johnsongrass and their final impact on competitive ability.

On the basis of the preceding findings, it could be concluded that the two johnsongrass populations originating from corn monoculture fields in northern Greece have evolved cross-resistance to the ALS-inhibiting herbicides foramsulfuron, nicosulfuron, rimsulfuron, and imazamox due to a point mutation in the *ALS* gene, which resulted in the substitution of Trp574 by Leu in the ALS enzyme. The field expression of this ALS enzyme alteration precludes the use of the registered sulfonylurea herbicides foramsulfuron, nicosulfuron, and rimsulfuron on the corn farms where the R biotypes originated and also the use of the herbicide imazamox in imidazolinone-tolerant Clearfield sunflower. However, the fact that both populations were effectively controlled by the recommended rate of the ACCase-inhibiting herbicides propaquizafop and clethodim supports the potential use of these herbicides for their effective management in broadleaf rotational crops. Nevertheless, these results highlight a serious threat against the sustainable use of the ALS-inhibiting herbicides in controlling johnsongrass and other weed species in corn monoculture fields. Therefore the rotational use of herbicides with different modes of action, along with the complementary use of nonchemical methods like tillage and crop rotation, is crucial for effective and sustainable johnsongrass management.

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References

Acciaresi HA, Guiamet JJ (2010) Below- and above-ground growth and biomass allocation in maize and *Sorghum halepense* in response to soil water competition. Weed Res 50:481–492

Balicevic R, Ravlic M, Balic A (2016) Dormancy and germination of Johnsongrass seed [*Sorghum halepense* (L.) Pers.]. J Central Eur Agric 17(3):725–733

Barroso J, Andújar D, San Martin C, Fernández-Quintanilla C (2012) Johnsongrass (*Sorghum halepense*) seed dispersal in corn crops under Mediterranean conditions. Weed Sci 60:34–41

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Prot 35:15–28

Chirita R, Grozea I, Sarpe N, Lauer KF (2007) Control of *Sorghum halepense* (L.) in western part of Romania. Commun Agric Appl Biol Sci 73:959–964

Eleftherohorinos IG, Kotoula-Syka E (1995) Influence of herbicide application rate and timings for post-emergence control of *Sorghum halepense* (L.) Pers. in maize. Weed Res 35:99–103

Fernandez L, de Haro LA, Distefano AJ, Martinez MC, Lia V, Papa JC, Olea L, Tosto D, Hopp HE (2013) Population genetics structure of glyphosate-resistant Johnsongrass (*Sorghum halepense* L. Pers.) does not support a single origin of the resistance. Ecol Evol 3:3383–3400

Follak S, Essl F (2013) Spread dynamics and agricultural impact of *Sorghum halepense*, an emerging invasive species in Central Europe. Weed Res 53:53–60

Haitas VC, Kotoula-Syka E, Eleftherohorinos IG (1995) Influence of propaquizafop application rate and time on *Sorghum halepense* (L.) Pers. control and cotton (*Gossypium hirsutum*) yield. Weed Res 35:1–6

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Heap I (2022) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/. Accessed: March 31, 2022

Hernández MJ, León R, Fischer AJ, Gebauer M, Galdames R, Figueroa R (2015) Target-site resistance to nicosulfuron in Johnsongrass (*Sorghum halepense*) from Chilean corn fields. Weed Sci 63:631–640

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977) The World's Worst Weeds: Distribution and Biology, 2nd Edn. Malabar, FL: Krieger. 610 p

Jensen PK, Bibard V, Czembor E, Dumitru S, Foucart G, Froud-Williams RJ, Jensen JE, Saavedra M, Sattin M, Soukup J, Palou AT, Thibord JB, Voegler W, Kudsk P (2011) Survey of weeds in maize crops in Europe. Paper 149. Aarhus, Denmark: Department of Integrated Pest Management, Aarhus University. 44 p

Johnson DB, Norsworthy JK, Scott RC (2014) Distribution of herbicide-resistant Johnsongrass (*Sorghum halepense*) in Arkansas. Weed Technol 28:111–121

Kaloumenos NS, Chatzilazaridou SL, Mylona PV, Polidoros AN, Eleftherohorinos IG (2013) Target-site mutation associated with cross-resistance to ALS-inhibiting herbicides in late watergrass (*Echinochloa oryzicola* Vasing.). Pest Manag Sci 69:865–873

Kaloumenos NS, Eleftherohorinos IG (2009) Identification of a Johnsongrass (*Sorghum halepense*) biotype resistant to ACCase-inhibiting herbicide in northern Greece. Weed Technol 23:470–476

Karkanis A, Athanasiadou D, Giannoulis K, Karanasou K, Zografos S, Souipas S, Bartzialis D, Danalatos N (2020) Johnsongrass (*Sorghum halepense* (L.) Pers.) interference, control and recovery under different management practices and its effects on the grain yield and quality of maize crop. Agronomy 10:266

Kaundun SS (2014) Resistance to acetyl-CoA carboxylase-inhibiting herbicides. Pest Manag Sci 70:1405–1417

Klein P, Smith CM (2020) Invasive Johnsongrass, a threat to native grasslands and agriculture. Biologia 76:413–420

Mitskas BM, Tsolis CE, Eleftherohorinos IG, Damalas CA (2003) Interference between corn and Johnsongrass (*Sorghum halepense*) from seed or rhizomes. Weed Sci 51:540–545

Ntoanidou S, Madesis P, Menexes G, Eleftherohorinos I (2020) Growth rate and genetic structure of *Sinapis arvensis* susceptible and herbicide resistant populations originating from Greece. Euphytica 216(12):185

Ohadi S, Hodnett GL, Rooney W, Bagavathiannan M (2018) Gene flow and its consequences in *Sorghum* spp. Crit Rev Plant Sci 36(5–6):367–385

Panozzo S, Milani A, Scarabel L, Balogh A, Dancza I, Sattin M (2017) Occurrence of different resistance mechanisms to ALS inhibitors in European *Sorghum halepense*. J Agric Food Chem 65:7320–7327

Panozzo S, Scarabel L, Tranel PJ, Sattin M (2013) Target-site resistance to ALS inhibitors in the polyploid species *Echinochloa crus-galli*. Pestic Biochem Physiol 105:93–101

Peerzada AM, Ali HH, Hanif Z, Bajwa AA, Kebaso L, Frimpong D, Iqbal N, Namubiru H, Hashim S, Rasool G, Manalil S, van der Meulen A, Chauhan BS (2017) Eco-biology, impact, and management of *Sorghum halepense* (L.) Pers. Biol Invasions. https://doi.org/10.1007/s10530-017-1410-8

Reichmann LG, Schwinning S, Polley WH, Fay PA (2016) Traits of an invasive grass conferring an early growth advantage over native grasses. J Plant Ecol 9:672–681

Riar DS, Norsworthy JK, Johnson DB, Scott RC, Bagavathiannan M (2011) Glyphosate resistance in a Johnsongrass (*Sorghum halepense*) biotype from Arkansas. Weed Sci 59:299–304

Rout ME, Chrzanowski TH, Smith WK, Gough L (2013) Ecological impacts of the invasive grass *Sorghum halepense* on native tallgrass prairie. Biol Invasions 15:327–339

Ryder N, Dorn KM, Huitsing M, Adams M, Ploegstra J, DeHaan L, Larson S, Tintle NL (2018) Transcriptome assembly and annotation of Johnsongrass (*Sorghum halepense*) rhizomes identify candidate rhizome-specific genes. Plant Direct 2(6):e00065

Scarabel L, Panozzo S, Savoia W, Sattin M (2014) Target-site ACCase-resistant Johnsongrass (*Sorghum halepense*) selected in summer dicot crops. Weed Technol 28:307–315

Schwinning S, Meckel H, Reichmann LG, Polley HW, Fay PA (2017) Accelerated development in Johnsongrass seedlings (*Sorghum halepense*) suppresses the growth of native grasses through size-asymmetric competition. PLoS ONE 12(5):e0176042

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose–response relationships. Weed Technol 9:218–227

Snedecor GW, Cochran WG (1989) Statistical Methods, 8th Edn. Ames, IA: Iowa State University Press

Travlos IS, Montuli JM, Kukorelli G, Malidza G, Dogan MN, Cheimona N, Antonopoulos N, Kanatas PJ, Zannopoulos S, Peteinatos G (2019) Key aspects on the biology, ecology and impacts of Johnsongrass [*Sorghum halepense* (L.) Pers.] and the role of glyphosate and non-chemical alternative practices for the management of this weed in Europe. Agronomy 9:717

Tsuji R, Fischer AJ, Yoshimo M, Roel A, Hill JE (2003) Herbicide-resistant late watergrass (*Echinochloa phyllopogon*): similarity in morphological and amplified fragment length polymorphism traits. Weed Sci 51:740–747

Warwick SL, Black LD (1983) The biology of Canadian weeds. 61. *Sorghum halepense* (L.) Pers. Can J Plant Sci 63:997–1014

Werle R, Begcy K, Yerka MK, Mower JP, Dweikat I, Jhala AJ, Lindquist JL (2017) Independent evolution of acetolactate synthase-inhibiting herbicide resistance in weedy *Sorghum* populations across common geographic regions. Weed Sci 65:164–176

Werle R, Jhala AJ, Yerka MK, Dille JA, Lindquist JL (2016) Distribution of herbicide-resistant shattercane and Johnsongrass populations in sorghum production areas of Nebraska and northern Kansas. Agron J 108:321–328

Yu Q, Han H, Vila-Aiub MM, Powles SB (2010) AHAS herbicide resistance endowing mutations: effect on AHAS functionality and plant growth. J Exp Bot 61:3925–3934

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest Manag Sci 70:1340–1350