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Multiple resistance of silky windgrass to acetolactate synthase- and acetyl-CoA synthase–inhibiting herbicides

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Research Article

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Abstract

Field and pot experiments were conducted in Greece to study the occurrence of resistance in silky windgrass to acetolactate synthase (ALS)- and acetyl-CoA synthase (ACCase)-inhibiting herbicides. Twenty-four populations of silky windgrass were examined in whole-plant response experiments. High levels of field-evolved resistance to chlorsulfuron (0% to 28% control in terms of fresh weight reduction) with the recommended field rates were confirmed in most silky windgrass populations. However, other ALS inhibitors, such as pyroxsulam and a premix of mesosulfuron-methyl and iodosulfuron, provided adequate control (76% to 100% in terms of fresh weight reduction) of most populations, except eight silky windgrass populations that were found to be cross-resistant to all ALS-inhibiting herbicides tested (i.e., chlorsulfuron, commercial mixture of mesosulfuron-methyl plus iodosulfuron, and pyroxsulam). Conversely, most silky windgrass populations were controlled effectively (90% to 100% in terms of fresh weight reduction) with the recommended field rates of ACCase inhibitors cycloxydim, clethodim, and pinoxaden, but five populations were also found to be resistant to clodinafop-propargyl (10% to 68% control in terms of fresh weight reduction). The *ALS* gene sequencing of the eight silky windgrass populations, with cross-resistance to ALS inhibitors, revealed a point mutation at the Pro-197 position, causing amino acid substitution by Ser or Thr in the ALS enzyme. Overall, chlorsulfuron and clodinafop-propargyl were selecting agents of field-evolved multiple resistance to ALS- and ACCase-inhibiting herbicides in five silky windgrass populations. As the available postemergence-applied chemistries/modes of action registered for grass weed control in cereals are rather limited, adopting integrated management practices and implementing proactive and reactive measures to delay the evolution of resistant populations is essential.

Introduction

Silky windgrass is a winter annual grass occurring as a weed in cereal production systems in several parts of the world (Warwick et al. 1985; USDA-NRCS 2016). In particular, silky windgrass is a common grass weed infesting arable crops in central and eastern Europe (e.g., Germany, Czech Republic, Poland, Switzerland, Slovakia, and Hungary), but it also grows in Asia and North America (Krysiak et al. 2011). This grass is a problematic weed in winter cereals in the Czech Republic, often found in 80% of the fields in the west and central parts of the country (Soukup et al. 2006). Recently, this species has become a major weed in Scandinavia (Melander et al. 2008; Babineau et al. 2017). It infests mainly winter cereals, but it is also found in oilseed rape, forage crops, and spring cereals (Massa and Gerhards 2011). Silky windgrass represents a growing threat to arable crops in extended areas of northwestern Greece, given that the dominant sterile oat (*Avena sterilis* L.) is replaced by other grasses, such as spring milletgrass (*Milium vernale* M. Bieb.) (Grevena and Ptolemaida Counties), brome (*Bromus* spp.) (Florina County), or silky windgrass (infesting large areas of Ptolemaida County).

Abandonment of herbicide rotation and repeated application of herbicides with same modes of action (MOAs) exerted greater levels of selection pressure to silky windgrass populations, resulting in field-evolved resistance in many agricultural areas (Balgheim et al. 2007; Delabays et al. 2006; Novakowa et al. 2006; Massa and Gerhards 2011). The first case of herbicide resistance in silky windgrass was documented for the substituted urea isoproturon (Mayor and

Mallard 1997; Niemann 2000). Since then, numerous populations of silky windgrass from central and eastern Europe have evolved resistance to several acetolactate synthase (ALS) inhibitors (e.g., chlorsulfuron, flupyrsulfuron, sulfosulfuron, and a commercial mixture of mesosulfuron-methyl + iodosulfuron) (Hamouzova et al. 2011; Krysiak et al. 2011; Massa and Gerhards 2011). Herbicide resistance to ALS inhibitors in silky windgrass is likely to spread rapidly, causing significant economic losses (Gerhards and Massa 2011). Recent research confirmed resistant populations of silky windgrass to iodosulfuron and fenoxaprop ethyl ester in Denmark (Babineau et al. 2017). In Lithuania, several silky windgrass populations were found resistant to sulfosulfuron (Auskalniene et al. 2020). In Czech Republic, silky windgrass populations with varying levels of resistance to herbicides inhibiting ALS, acetyl-CoA carboxylase (ACCase), and photosystem-II (PSII) were confirmed (Kosnarova et al. 2021). However, cases of silky windgrass resistance in Greece have not previously been reported.

Field-selected resistance to ALS- or ACCase-inhibiting herbicides in most weed populations of silky windgrass involved mostly target-site (Krysiak et al. 2011; Massa et al. 2011; Hamouzova et al. 2014) and probably non-target-site resistance (NTSR) mechanisms (Massa and Gerhards 2011; Babineau et al. 2017). More specifically, Babineau et al. (2017) reported NTSR due to detoxification in silky windgrass populations at different levels of herbicides and underlined that NTSR mechanisms do not always confer broad resistance to different herbicide subclasses, hence suggesting complex relationships to resistant phenotypes. Target-site resistance typically occurs after a single nucleotide change in the gene encoding the ALS enzyme, which affects the strength of herbicide binding to the target protein and thus renders the enzyme insensitive to ALS-inhibiting herbicides (Tranel and Wright 2002; Yu and Powles 2014b). Six amino acid substitutions of the *ALS* gene (Trp-574-Leu, Arg-377-His, Pro-197-Thr, Pro-197-Asn, Pro-197-Ser, and Ala-122-Val) have been so far identified in ALS-resistant silky windgrass populations (Krysiak et al. 2011; Massa et al. 2011). By contrast, although it is not currently known for silky windgrass, NTSR is related to different factors than the gene encoding the ALS enzyme, such as reduced herbicide penetration to the foliage, reduced translocation into the target site, or enhanced metabolism mainly by cytochrome P450 mixed-function oxidases, Family 1 UDP-glucose-dependent glycosyltransferases, and glutathione transferases (Cummins et al. 2013; Yu and Powles 2014a). NTSR mechanisms in grasses usually cause multiple resistance to ALS inhibitors and other herbicides with different MOAs (Délye et al. 2013).

Unsatisfactory control of silky windgrass populations has lately occurred in winter cereal–growing areas of northwestern Greece with continuous and exclusive use of chlorsulfuron and clodinafop-propargyl without integration with nonchemical methods, thus causing an alarming situation among farmers and field practitioners. However, there is no information whether the cause of this situation is due to evolution of resistance. The objective of this research was (1) to investigate whether the reported unsatisfactory control of silky windgrass claimed by some cereal farmers in northwestern Greece was due to the occurrence of resistance to either ALS and/or ACCase inhibitors, (2) to determine the molecular mechanism of silky windgrass cross-resistance to ALS-inhibiting herbicides, and (3) to evaluate other postemergence herbicides for silky windgrass control in winter cereals (or broadleaf crops).

Materials and Methods

Seed Source

Seeds were collected in early summer 2013 and 2014 from wheat fields located in northwestern Greece, in the West Macedonia region, where failure of silky windgrass control with chlorsulfuron, clodinafop-propargyl, and other ALS- or ACCase-inhibiting herbicides had been reported. Seeds were collected from the locations Olympiada (40°34′N, 21°36′E), Galateia (40°33′N, 21°35′E), Anarahi (40°29′N, 21°34′E), and Tripotamos (40°49′N, 21°30′E). All sampling locations were rural areas devoted to continuous wheat monoculture for more than 10 yr, accompanied by repeated use of herbicides with the same MOAs, especially chlorsulfuron and clodinafop-propargyl. Mature seeds were collected from different patches and from several plants across representative fields before winter wheat harvest. Seeds from each field were pooled together and regarded as a distinct population. A susceptible population originated from the margins of a field that had never been treated with any herbicide was included in the study. The seeds were initially collected in plastic bags and then transported to the laboratory. After air-drying, seeds were stored for 4 mo in paper bags at 5 to 7 C for use in the whole-plant response experiments. No dormancy was observed after the cool storage of seeds.

Whole-Plant Response Experiments (Amaliada)

Two identical pot trials at different times were conducted outdoors at the farm of the Technological Educational Institute of Western Greece in Amaliada during winter 2013 to spring 2014, where one susceptible (S) population never treated with herbicides and 16 putative resistant (A1 to A16) silky windgrass populations were evaluated. Experiments were conducted in 0.9-L plastic pots filled with a 1:1:1 (v/v/v) mixture of clay loam soil with peat and sand. Each pot was surface seeded with approximately 35 seeds of silky windgrass seeds and covered by a thin layer of sand. At the 2-leaf growth stage, seedlings were carefully thinned to six per pot. All pots were moved outdoors, irrigated, and fertilized with a liquid fertilizer (Bayfolan® 11:8:6, Bayer Crop Science, Athens, Greece) as and when required for optimum plant growth. Mean air temperature during the experimental period in Amaliada ranged from 9.2 to 19.7 C in 2013 and from 9.9 to 20.4 C in 2014.

The silky windgrass plants of the putative resistant populations were sprayed at the 4- to 5-leaf growth stage with the recommended (X), 4X, 8X, and 16X field rates of the following herbicides: chlorsulfuron (Glean® 75 WG, Dupont Hellas, Athens, Greece; 15, 60, 120, 240 g ai ha^{-1}), mesosulfuron-methyl + iodosulfuron (Atlantis® WG, Bayer Crop Science Hellas, Athens, Greece; 15 + 3, 60 + 12, 120 + 24, 240 + 48 g ai ha^{-1}), pyroxsulam (Senior® 75 WG, Dow Elanco Hellas, Acharnes, Greece; 18.75, 75, 150, 300 g ai ha^{-1}), pinoxaden (Axial® 100 EC, Syngenta Hellas, Oinofyta, Greece; 45, 180, 360, 720 g ai ha^{-1}), clodinafop-propargyl (Topik® 240 EC, Syngenta Hellas; 41, 164, 328, 656 g ai ha^{-1}), and clethodim (Clethodim Arysta® 24 EC, Arysta LifeScience, Athens, Greece; 240, 960, 1,920, 3,840 g ai ha^{-1}). The recommended rates of these herbicides were also co-applied with the recommended field rate (1,000 g ai ha^{-1}) of the systemic organophosphate (OP) insecticide chlorpyrifos (Aspida® 480 EC, Bayer Crop Science Hellas), applied 1 h before the application of herbicides. This application was made to examine the possible existence of NTSR mechanisms involved in resistance in silky windgrass populations. Chlorpyrifos is a cytochrome P_{450} inhibitor that has been used previously to detect indirect evidence of

enhanced metabolism resistance (Liu et al. 2016). Because only cytochrome P450s and not glutathione transferases are inhibited by chlorpyrifos, a lack of suppression of weed control in response to chlorpyrifos does not indicate the potential presence of a different metabolic resistance mechanism, such as from glutathione transferases. An untreated control for each putative resistant and the susceptible populations was included in the experiments. A field plot sprayer (AZO Sprayer, Ede-Konstrukties, Ede, the Netherlands) with a 2.4-m-wide boom was used for herbicide applications. The sprayer carried six 8002 flat-fan nozzles and delivered 300 L ha^{-1} of water at 280 kPa. The application of herbicides was performed when silky windgrass plants were at GS21 to GS22 (3 to 4 leaves, 1 to 2 tillers) of the Zadoks scale (Zadoks et al. 1974).

Each of the two identical pot experiments was established in a completely randomized design with treatments replicated three times. Pot randomization within each population was made weekly to ensure uniform growth conditions for all plants. Silky windgrass control was evaluated by determining the fresh weight of surviving plants at 35 days after treatment (DAT). Then, fresh weight data were turned to a percent reduction of the nontreated control (fresh weight suppression over the nontreated control) and subjected to analysis of variance (ANOVA).

A combined over-the-two-experiments ANOVA was conducted for each evaluated silky windgrass population, using a 6 (herbicides) × 5 (herbicide rates, X-recommended rate, X+OP, 4X, 8X, and 16X) factorial approach. Differences between herbicide × rate means presented within each population were tested using the Bonferroni adjusted least significant difference (LSD) value at $P < 0.05$ (Steel et al. 1997). In addition, a combined over-the-two-experiments ANOVA was conducted for each herbicide, using a 17 silky windgrass population × 5 herbicide rate factorial approach, while the differences between population means within each herbicide rate were tested using the Bonferroni adjusted LSD value at $P < 0.05$.

Whole-Plant Response Experiments (Florina)

Two identical pot experiments at different times were conducted outdoors at the farm of the Technological Educational Institute of West Macedonia (Florina County), Greece, during winter 2013 to spring 2014, where one susceptible population never treated with herbicides and eight putative resistant (A17 to A24) populations were evaluated. In these experiments, clethodim was replaced with cycloxydim (Focus® 10 EC, BASF Hellas, Athens, Greece) applied at the recommended (X), 2X, 4X, 8X, and 16X field rates (200, 400, 800, 1,600, 3,200 g ai ha^{-1}). Also, the same proportions of the recommended rates (X, 2X, 4X, 8X, and 16X) were used for the other five herbicides. The silky windgrass plants of the putative resistant populations were sprayed at the 4- to 5-leaf growth stage. Each of the two identical pot experiments was established in a completely randomized design with treatments replicated three times, as described previously for the experiments carried out in Amaliada. Mean air temperature during the experimental period in Florina ranged from 6.3 to 16.8 C in 2013 and from 4.2 to 20.4 C in 2014.

A combined over-the-two-experiments ANOVA was conducted for each evaluated silky windgrass population using a 6 (herbicides) × 5 (herbicide rates, X-recommended rate, X+OP, 4X, 8X, and 16X) factorial approach. Differences between herbicide × rate means presented within each population were tested using the Bonferroni adjusted LSD value at $P < 0.05$ (Steel et al. 1997). In addition, a combined over-the-two-experiments ANOVA was conducted for each herbicide, using a 9 silky windgrass population × 5 herbicide rate factorial approach, while the differences between population means within each herbicide rate were tested using the Bonferroni adjusted LSD value at $P < 0.05$. All statistical analyses were performed with SPSS version 23 (SPSS Inc., Chicago, IL, USA) statistical software.

Amplification and Sequencing of the ALS Gene Fragment from Silky Windgrass Plants

The S and the eight R silky windgrass populations A1, A2, A3, A4, A5, A6, A23, and A24, with cross-resistance to chlorsulfuron, mesosulfuron-methyl + iodosulfuron, and pyroxsulam, were the only ones selected for sequencing of the *ALS* gene due to limited resources. Sequencing of the *ACC* gene for R populations to ACCase inhibitors was not conducted. The plant material for the amplification of the *ALS* gene was taken from plants grown in separate pots for this purpose, as described earlier. Three pots with six plants per pot from each of the S and eight R populations, at the 3- to 4-leaf stage (1 to 2 tillers), were sprayed with the recommended rate of chlorsulfuron (15 g ai ha^{-1}), whereas three pots with six plants per pot of the S population were left untreated. This procedure was followed to eliminate possible individual susceptible plants from the R populations and to confirm the susceptibility of the S population. Leaf samples of individual surviving plants from the eight R populations were harvested, stored at −28 C, and subsequently used for DNA extraction. Also, leaf samples of individual plants from the untreated S population were harvested. Six leaf samples from plants of the R and the S populations were sequenced. DNA was extracted from 100-mg leaf tissue using a NucleoSpin® Plant II kit (MACHEREY-NAGEL GmbH, Düren, Germany) according to the manufacturer's protocol. The amplification of the *ALS* gene fragment (375 bp) from genomic DNA of the S and R populations, which includes the Pro-197 codon, was performed using the primers ALS375for 5′-CGAGCCCCGCAAGGGC GCCGACAT-3′ (forward) and ALS375rev 5′-GTGATGGAG CGGGTGACCTCTA-3′ (reverse), following Massa et al. (2011). The amplification focused on the Pro-197 codon, following the response of the populations in the whole-plant response experiments and also because mutations at Pro-197 are by far the most commonly reported in the literature, with 11 amino acid substitutions endowing resistance to ALS-inhibiting herbicides (Powles and Yu 2010). These primers were designed on the basis of the nucleotide and amino acid sequence of the *ALS* gene reported by Massa et al. (2011) for silky windgrass. The polymerase chain reaction (PCR) test consisted of 0.2 mM deoxyribonucleotide triphosphate, 1.5 mM $MgCl_2$, 10 μM of each forward and reverse primer, 2 μL of the supplied 10X thermophilic buffer, 1 μL of genomic DNA diluted at 20 ng/uL, and 1 enzyme unit (U) of standard *Thermus aquaticus* (Taq) polymerase in 20 μL mixture. Amplification was conducted in a Veriti™ thermocycler (Applied Biosystems, Foster City, CA, USA) using the following cycles: DNA denaturation for 5 min at 95 C, 40 cycles of 30-s denaturation at 95 C, 30 s annealing at 50 C, and 1 min elongation at 72 C. The samples were submitted in a final step of elongation at 72 C for 5 min, and the PCR products were separated in 1% agarose gel. The purified product was sent immediately for sequencing to the School of Medicine, Department of Immunology and Histocompability, University of Thessaly. Each PCR product was sequenced once. The sequencing chromatograms were edited with ChromasPro software, and the nucleotide sequences were aligned using Blast®

(https://blast.ncbi.nlm.nih.gov/Blast.cgi). The EMBOSS suite of bioinformatics tools (https://www.ebi.ac.uk/Tools/st/emboss_transeq/) was used to translate the nucleotide to peptide sequence.

Ploidy levels of silky windgrass were not considered in this study, following a common practice of previous studies concerning resistance of certain weed species with known ploidy level, such as shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.] (Jin et al. 2011; Cui et al. 2012) and littleseed canarygrass (*Phalaris minor* Retz.) (Gherekhloo et al. 2012). Ploidy levels may influence the isolation of target genes of interest due to existence of more than one copy of the *ALS* gene in the genome (Scarabel et al. 2010); thus, in the case of a polyploid, one ancestral genome could have a mutation and not the other. This point should be considered in future research, but the most important objective of the present study was to explain the reported unsatisfactory control of grasses claimed by some cereal farmers in northwestern Greece and not to explore the ploidy levels of silky windgrass.

Wheat Field Experiments

Two field experiments were conducted during 2013 and 2014 in Florina County, a main winter cereal–growing area of northwestern Greece, to evaluate the response of the A6 silky windgrass (cross-resistant to ALS-inhibiting herbicides) population to a range of postemergence herbicides. The efficacy evaluation was performed in a natural silky windgrass population of relatively uniform density that was previously evaluated in the pot experiments (A6 population). The field trials were established in Tripotamos District of Florina County (40°49′N, 21°30′E) on a grower's farm where common agronomic practices (e.g., soil tillage, selection of wheat cultivar, sowing date, sowing density) were implemented. The two trials were established in heavily and uniformly infested fields, with high silky windgrass densities ranging between 60 and 90 plants m^{-2}. The following commercial herbicide formulations were used at the recommended and two times the recommended field rate: clodinafop-propargyl (Topik® 240 EC) applied at 41 and 82 g ai ha^{-1}, fenoxaprop-p-ethyl (Puma® S 6.9 EW, Bayer Crop Science Hellas) applied at 82.5 and 165 g ai ha^{-1}, pinoxaden (Axial® 60 EC, Syngenta Hellas) applied at 45 and 90 g ai ha^{-1}, pyroxsulam (Senior® 75 WG) applied at 18.75 and 37.5 g ai ha^{-1}, and mesosulfuron-methyl + iodosulfuron (Atlantis® WG) applied at 15 + 3 and 30 + 6 g ai ha^{-1}. Chlorsulfuron was not included in the field experiments because it imposes serious restrictions on crop rotation, while clethodim also was not included because it is not registered for use in wheat. A field plot sprayer (AZO Sprayer) with a 2.4-m-wide boom was used for herbicide applications. The sprayer carried six 8002 flat-fan nozzles and delivered 300 L ha^{-1} of water at 280 kPa. Herbicides were applied as previously noted, when silky windgrass plants were at GS22 to GS23 (4 to 5 leaves, 2 to 3 tillers) of the Zadoks scale. Treatments were applied in plots laid out in a randomized complete block design, with four replicates for each treatment. Plot size was 6 × 3 m. An untreated control (four replicate plots) was used for the necessary comparisons. Mean air temperature during the experimental period ranged between 6.9 C and 19.6 C in 2013 and between 4.1 C and 16.1 C in 2014. Visual phytotoxicity assessments by three scientists were done 15, 30, and 45 d following herbicide treatments by comparison with plots that received no herbicide treatment (controls). Herbicide efficacy was assessed on a scale from 0 to 100, with 0 corresponding to no plant injury (as compared with the nontreated control) and 100 corresponding to no plant survival (dead plants). To confirm visual assessments, aboveground biomass data of the silky windgrass were collected from 1 m^2 in the center of each plot. Only visual assessments were reported, reflecting weed control levels more accurately.

The homogeneity of variances of the two field trials was checked using Bartlett's test (Snedecor and Cochran 1989), and therefore silky windgrass control data were combined from the two experiments prior to conducting ANOVA, using a 5 × 2 factorial approach (five herbicides × two rates). Differences of treatment means were compared at $P < 0.05$ using Fisher's protected LSD test.

Results and Discussion

Whole-Plant Response Experiments

The S population of silky windgrass was completely controlled with all rates of postemergence-applied chlorsulfuron examined (Tables 1 and 2). None of the suspected R populations were adequately controlled at the chlorsulfuron labeled rate. The recommended (X) rate of postemergence-applied chlorsulfuron, 15 g ha^{-1}, provided only 0% to 10% control of 16 putative R populations (A1, A2, A3, A4, A5, A6, A9, A10, A11, A12, A13, A15, A16, A17, A18, A19) and 14% to 30% control of seven silky windgrass populations (A7, A8, A14, A20, A21, A22, A23) (Tables 1 and 2). Co-application of the OP insecticide chlorpyrifos only slightly increased the overall poor level of control (Table 1). The increase in fresh weight reduction from 0% to 33% (population A5) was statistically significant yet offered practically poor control of this population. Similar control was documented with the application of 4X (60 g ha^{-1}) and 8X (120 g ha^{-1}) chlorsulfuron rate, whereas the application of 16X (240 g ha^{-1}) rate reduced fresh weight by 0% to 58% in 21 putative R populations (Tables 1 and 2).

Acceptable to excellent control (73% to 100%) of 13 putative R populations (A8, A10, A12, A13, A14, A15, A16, A17, A18, A19, A20, A21, A22) was achieved with the X rate (15 + 3 g ha^{-1}) of mesosulfuron-methyl + iodosulfuron, whereas the remaining populations were insufficiently controlled (5% to 71%) (Tables 3 and 4). The overall control level was not significantly increased with co-application of chlorpyrifos, although a 12% to 19% control increase was documented against some silky windgrass populations (A3, A4, A9, A10, A11) when mesosulfuron-methyl + iodosulfuron was co-applied with chlorpyrifos (Table 3). It is also noteworthy that the OP treatment had a major effect on the control of some populations with mesosulfuron-methyl + iodosulfuron; for example, the control of population A10 increased from 73% to 94% after the OP treatment, bringing its level from just unacceptable to excellent, and the control of population A13 increased from 75% (acceptable) to 86% (good). Mesosulfuron-methyl + iodosulfuron at the 4X rate (60 + 12 g ha^{-1}) resulted in excellent control (94% to 100%) of 15 populations, while its efficacy against seven populations (A1, A2, A3, A4, A6, A23, A24) ranged from 31% to 78% (Tables 3 and 4). In addition, application of the 16X rate (240 + 48 g ha^{-1}) provided 44% to 77% control of four silky windgrass populations (A1, A2, A6, A23).

Nine putative (A12, A13, A14, A15, A16, A17, A18, A20, A21) R silky windgrass populations were controlled 86% to 100% after application of the X rate (18.75 g ha^{-1}) of pyroxsulam, whereas the remaining populations were less controlled (Tables 5 and 6). A slight increase of control was documented for most populations with co-application of chlorpyrifos (Table 5). Pyroxsulam applied

Table 1. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with chlorsulfuron (Amaliada trials).[a,b]

Population	Chlorsulfuron rate (g ai ha^{-1})					
	15	15 + OP	60	120	240	LSD[c]
	% control					
A1	10	17	18	26	28	8
A2	0	1	1	4	20	6
A3	6	13	15	20	26	7
A4	7	16	23	34	38	6
A5	0	33	32	33	40	8
A6	0	0	0	0	20	7
A7	14	17	22	26	32	5
A8	23	26	34	36	40	5
A9	0	0	0	0	0	6
A10	7	11	29	32	38	7
A11	0	0	0	0	0	6
A12	2	12	22	29	31	5
A13	4	14	21	24	33	4
A14	17	24	37	42	52	6
A15	0	0	0	9	26	3
A16	0	0	0	0	2	3
S	100	100	100	100	100	NA
LSD[d]	7					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 2. Fresh weight reduction over the nontreated control of silky windgrass populations (A17 to A24) with chlorsulfuron (Florina trials).[a,b]

Population	Chlorsulfuron rate (g ai ha^{-1})					
	15	30	60	120	240	LSD[c]
	% control					
A17	8	18	21	25	42	4
A18	0	0	14	23	58	4
A19	0	0	1	23	29	4
A20	30	33	41	73	76	3
A21	26	35	38	47	50	4
A22	22	28	31	36	51	3
A23	21	48	63	82	93	4
A24	42	49	52	94	97	3
S	100	100	100	100	100	NA
LSD[d]	4					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 3. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with mesosulfuron-methyl plus iodosulfuron (Amaliada trials).[a,b]

Population	Mesosulfuron-methyl plus iodosulfuron rate (g ai ha^{-1})					
	15 + 3	15 + 3 + OP	60 + 12	120 + 24	240 + 48	LSD[c]
	% control					
A1	14	15	35	42	44	8
A2	21	27	36	71	77	6
A3	48	67	78	88	91	7
A4	35	47	67	82	88	6
A5	32	34	91	100	100	8
A6	54	55	58	65	66	7
A7	62	68	100	100	100	5
A8	76	72	100	100	100	5
A9	42	58	93	100	100	6
A10	73	94	94	100	100	7
A11	60	72	94	100	100	6
A12	83	78	100	100	100	5
A13	75	86	100	100	100	4
A14	95	94	100	100	100	6
A15	89	100	100	100	100	3
A16	95	100	100	100	100	3
S	100	100	100	100	100	NA
LSD[d]	8					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 4. Fresh weight reduction over the nontreated control of silky windgrass populations (A17 to A24) with mesosulfuron-methyl plus iodosulfuron (Florina trials).[a,b]

Population	Mesosulfuron-methyl plus iodosulfuron rate (g ai ha^{*1})					
	15 + 3	30 + 6	60 + 12	120 + 24	240 + 48	LSD[c]
	% control					
A17	88	95	99	100	100	4
A18	90	95	98	100	100	4
A19	96	99	100	100	100	4
A20	100	100	100	100	100	3
A21	97	97	99	100	100	4
A22	95	99	100	100	100	3
A23	5	16	31	51	59	4
A24	71	72	75	77	85	3
S	100	100	100	100	100	NA
LSD[d]	<1					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

at the 4X rate (75 g ha^{-1}) caused 80% to 100% reduction of fresh weight in 13 populations (A7, A10, A11, A12, A13, A14, A15, A16, A17, A18, A19, A20, A21) and 13% to 68% reduction in 10 populations (A1, A2, A3, A4, A5, A6, A9, A22, A23, A24) (Tables 5 and 6). The 8X rate (150 g ha^{-1}) controlled populations A1, A2, A3, A4, A6, A22, A23, and A24 15% to 72%, whereas populations A1, A2, A3, A6, A22, and A23 were controlled 33% to 70% with pyroxsulam at the 16X rate (300 g ha^{-1}).

Pinoxaden at the X rate (45 g ha^{-1}) controlled 14 putative R populations 91% to 100%, whereas populations A7 and A11 were controlled 87% (Table 7). In addition, pinoxaden applied at the 4X rate (180 g ha^{-1}) controlled 13 populations 100%, whereas its co-application with chlorpyrifos did not increase the level of control, except for population A9, for which control increased from 91% to 97% (Table 7). Finally, excellent control (100%) of all populations was observed with pinoxaden in the Florina trials (data not shown).

Table 5. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with pyroxsulam (Amaliada trials).[a,b]

	Pyroxsulam rate (g ai ha^{-1})					
Population	18.75	18.75 + OP	75	150	300	LSD[c]
	% control					
A1	33	34	36	43	48	8
A2	26	51	57	67	69	6
A3	38	44	59	62	70	7
A4	43	33	59	72	87	6
A5	55	59	64	83	100	8
A6	16	41	46	47	50	7
A7	37	47	80	89	100	5
A8	31	34	70	85	91	5
A9	36	45	59	100	100	6
A10	53	56	88	94	98	7
A11	35	39	81	100	100	6
A12	96	100	100	100	100	5
A13	92	91	100	100	100	4
A14	86	91	99	100	100	6
A15	100	100	100	100	100	3
A16	87	89	96	100	100	3
S	100	100	100	100	100	NA
LSD[d]	9					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 6. Fresh weight reduction over the nontreated control of silky windgrass populations (A17 to A24) with pyroxsulam (Florina trials).[a,b]

	Pyroxsulam rate (g ai ha^{-1})					
Population	18.75	37.5	75	150	300	LSD[c]
	% control					
A17	93	97	99	100	100	4
A18	87	95	97	98	100	4
A19	85	93	98	100	100	4
A20	99	100	100	100	100	3
A21	92	97	99	99	100	4
A22	22	28	31	36	51	3
A23	8	10	13	15	33	4
A24	55	61	68	72	82	3
S	100	100	100	100	100	NA
LSD[d]	4					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 7. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with pinoxaden (Amaliada trials).[a,b]

	Pinoxaden rate (g ai ha^{-1})					
Population	45	45 + OP	180	360	720	LSD[c]
	% control					
A1	94	92	100	100	100	8
A2	91	90	100	100	100	6
A3	95	92	97	100	100	7
A4	97	94	100	100	100	6
A5	93	94	100	100	100	8
A6	99	100	100	100	100	7
A7	87	86	100	100	100	5
A8	91	96	100	100	100	5
A9	91	97	100	100	100	6
A10	93	98	100	100	100	7
A11	87	87	98	100	100	6
A12	98	95	100	100	100	5
A13	100	100	100	100	100	4
A14	93	95	98	100	100	6
A15	98	99	100	100	100	3
A16	100	100	100	100	100	3
S	100	100	100	100	100	NA
LSD[d]	3					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Clodinafop-propargyl efficacy was reduced against some putative R silky windgrass populations as compared with the other ACCase inhibitors, clethodim and pinoxaden (Tables 8 and 9). However, excellent control (93% to 100%) was obtained with clodinafop-propargyl at the X rate (41 g ha^{-1}) against seven populations (A4, A6, A12, A15, A16, A23, A24), whereas moderate (60% to 77%) to good (83% to 90%) control was evident against five (A7, A8, A10, A18, A20) and nine populations (A1, A2, A3, A9, A11, A13, A14, A17, A22), respectively. By contrast, control with the X rate (41 g ha^{-1}) of clodinafop-propargyl was 10% and 26% against population A19 and A5, respectively (Tables 8 and 9). The control level increased by 9% to 29% in certain silky windgrass populations when clodinafop-propargyl was co-applied with chlorpyrifos (Table 8). Significant increases in control with clodinafop-propargyl were noted in populations A3 (85% to 90%), A5 (26% to 49%), A7 (72% to 89%), A8 (77% to 96%), A9 (84% to 98%), and A13 (89% to 100%) (Table 8) and also in populations A17 (83% to 91%), A18 (68% to 76%), A19 (10% to 32%), and A20 (62% to 78%) (Table 9). Overall, control increase following OP treatment was the highest for clodinafop-propargyl. For these populations, NTSR cannot be ruled out. Application of the 4X rate (164 g ha^{-1}) of clodinafop-propargyl provided excellent control (90% to 100%) of 19 populations; moderate control (79% and 88%) of the A10 and A21 populations, respectively; and poor control (56% and 42%) of the A5 and A19 populations, respectively (Tables 8 and 9). The 8X (328 g ha^{-1}) and 16X (656 g ha^{-1}) rates of clodinafop-propargyl controlled silky windgrass plants of all putative R populations, with the exception of two populations (A5 and A19), whose fresh weight was reduced by 74% to 91%.

Excellent control (90% to 100%) of 13 out of 16 putative R silky windgrass populations studied in Amaliada pot experiments was achieved with the clethodim X (240 g ai ha^{-1}) rate, whereas three populations (A1, A2, A7) were effectively controlled by 84% to 87% (Table 10). No significant differences in the level of control were documented when clethodim was applied together with chlorpyrifos, except in population A1 (87% to 95%) (Table 10). All other clethodim rates (4X, 8X, and 16X) killed all plants of each silky windgrass population (Table 10). Finally, all cycloxydim rates controlled all silky windgrass populations 100% (data not shown).

Table 8. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with clodinafop-propargyl (Amaliada trials).[a,b]

	Clodinafop-propargyl rate (g ai ha^{-1})					
Population	41	41 + OP	164	328	656	LSD[c]
	% control					
A1	83	94	100	100	100	8
A2	88	97	97	100	100	6
A3	85	90	96	100	100	7
A4	95	98	100	100	100	6
A5	26	49	56	74	91	8
A6	94	99	100	100	100	7
A7	72	89	91	100	100	5
A8	77	96	100	100	100	5
A9	84	98	100	100	100	6
A10	68	73	79	99	100	7
A11	85	90	93	100	100	6
A12	100	100	100	100	100	5
A13	89	100	100	100	100	4
A14	89	94	100	100	100	6
A15	97	100	100	100	100	3
A16	100	100	100	100	100	3
S	100	100	100	100	100	NA
LSD[d]	3					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Table 9. Fresh weight reduction over the nontreated control of silky windgrass populations (A17 to A24) with clodinafop-propargyl (Florina trials).[a,b]

	Clodinafop-propargyl rate (g ai ha^{-1})					
Population	41	82	164	328	656	LSD[c]
	% control					
A17	83	91	98	100	100	4
A18	68	76	90	100	100	4
A19	10	32	42	80	100	4
A20	62	78	89	100	100	3
A21	81	82	88	97	100	4
A22	90	95	100	100	100	3
A23	94	99	100	100	100	4
A24	97	100	100	100	100	3
S	100	100	100	100	100	NA
LSD[d]	3					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Amplification and Sequencing of the *ALS* Gene Fragment from Silky Windgrass Plants

The 352 bp fragment of the sequenced *ALS* gene from all silky windgrass plant samples was aligned with the *ALS* gene from an *Arabidospis thaliana* sequence obtained from GenBank (accession number X51514). The DNA sequence of eight of the R populations revealed a single point mutation at the first base of the codon Pro-197 (Table 11). More specifically, substitution of the first cytosine (C) of the codon 197 (CCT) by thymine (T) resulted in substitution of Pro-197 by Ser (TCC) in 42 individual plants belonging to 7 R populations, whereas substitution of the first C by adenine (A) resulted in substitution of Pro-197 by Thr (ACC) in 6 plants of one R (R2) population. All R plants were heterozygous (RS), as they had the point mutation in only one allele.

Table 10. Fresh weight reduction over the nontreated control of silky windgrass populations (A1 to A16) with clethodim (Amaliada trials).[a,b]

	Clethodim rate (g ai ha^{-1})					
Population	240	240 + OP	960	1,920	3,840	LSD[c]
	% control					
A1	87	95	100	100	100	8
A2	84	84	100	100	100	6
A3	93	93	100	100	100	7
A4	90	90	100	100	100	6
A5	92	94	100	100	100	8
A6	100	100	100	100	100	7
A7	85	86	100	100	100	5
A8	90	91	100	100	100	5
A9	95	95	100	100	100	6
A10	96	97	100	100	100	7
A11	97	95	100	100	100	6
A12	97	97	100	100	100	5
A13	99	98	100	100	100	4
A14	96	94	100	100	100	6
A15	97	99	100	100	100	3
A16	96	92	100	100	100	3
S	100	100	100	100	100	NA
LSD[d]	2					

[a]Values are means of two experiments (3 + 3 replicates per treatment).
[b]Abbreviations: S, susceptible population; OP, organophosphate insecticide chlorpyrifos (1,000 g ai ha^{-1}); NA, not applicable; LSD, least significant difference.
[c]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the herbicide rate means presented within each population.
[d]Common Bonferroni LSD (at $P < 0.05$) value that allows performing all interesting pairwise comparisons between the populations means within each herbicide rate.

Wheat Field Experiments

The A6 silky windgrass population evaluated in the two field experiments was controlled 15% and 36% with the X and 2X field rate of pyroxsulam (18.75 and 37.7 g ha^{-1}), respectively, whereas the respective rates of the prepackaged mixture mesosulfuron-methyl + iodosulfuron (15 + 3 and 30 + 6 g ha^{-1}) controlled the A6 silky windgrass population 55% and 71%, respectively (Table 12). However, application of the X and 2X rates of fenoxaprop-P-ethyl (82.5 and 165 g ha^{-1}) controlled silky windgrass 98% and 100%, respectively, which was similar (99% and 100% control) with percentages obtained by the application of the X and 2X rates (45 and 90 g ai ha^{-1}) of pinoxaden, respectively (Table 12). Finally, the X and 2X rates of clodinafop-propargyl (41 and 82 g ai ha^{-1}) controlled silky windgrass 88% and 95%, respectively. Regarding herbicide selectivity, none of the herbicides caused any wheat phytotoxicity. There was a correlation between the control values at the X rate in the field study versus the pot study (88% vs. 93%, 99% vs. 99%, 15% vs. 16%, and 55% vs. 54% for clodinafop, pinoxaden, pyroxsulam, and mesosulfuron-methyl + iodosulfuron, respectively).

Implications for Field Management

The present study indicated that the unsatisfactory control of both silky windgrass populations claimed by some cereal growers in northwestern Greece was due to the occurrence of resistance to

Table 11. Nucleotide and deduced amino acid sequence alignment of *ALS* gene fragments, originating from one susceptible and eight resistant silky windgrass populations indicating different point mutations at the first nucleotide of codon Pro-197.[a]

Population	Susceptibility	Pro-197 codon[b]	Genotype	Plants with a specific genotype
S	S	CCC[c]	Pro-197/Pro-197	6
A1	R	YCC	Pro-197/Ser-197	6
A2	R	MCC	Pro-197/Thr-197	6
A3	R	YCC	Pro-197/Ser-197	6
A4	R	YCC	Pro-197/Ser-197	6
A5	R	YCC	Pro-197/Ser-197	6
A6	R	YCC	Pro-197/Ser-197	6
A23	R	YCC	Pro-197/Ser-197	6
A24	R	YCC	Pro-197/Ser-197	6

[a]Abbreviations: S, susceptible; R, resistant.
[b]The codon positions refer to the standard *Arabidopsis thaliana ALS* gene (GenBank: X 51514).
[c]IUPAC-IUB nucleotide codes (YCC, Y = C + T, CCC + TCC = Pro-197/Ser-197; MCC, M = A + T, CCC + ACC = Pro-197/Thr-197).

Table 12. Control of silky windgrass population (Florina field experiment).[a,b]

		Control (nontreated)		
Herbicide	Rate	Experiment 1	Experiment 2	Mean[c]
	g ai ha^{-1}	%		
Clodinafop-propargyl				
X	41	88	88	88
2X	82	95	95	95
Fenoxaprop-P-ethyl				
X	82.5	98	98	98
2X	165	100	100	100
Pinoxaden				
X	45	99	100	99
2X	90	100	100	100
Pyroxsulam				
X	18.75	10	20	15
2X	37.5	34	38	36
Mesosulfuron-methyl + iodosulfuron				
X	15 + 3	52	58	55
2X	30 + 6	69	73	71
LSD (0.05)		8	9	9

[a]The silky windgrass population examined in the field experiments was A6.
[b]Abbreviation: LSD, least significant difference.
[c]Mean value of two experiments (4 + 4 replicates per treatment).

ALS and/or ACCase inhibitors. In particular, the current study confirmed a high level of field-evolved resistance to chlorsulfuron in terms of biomass reduction in all silky windgrass populations studied. However, despite the high resistance levels to chlorsulfuron, other ALS inhibitors (i.e., the prepackaged mixture of mesosulfuron-methyl + iodosulfuron, pyroxsulam) provided excellent control of 10 out of 24 populations, with major differences between the two in populations A13 and A22. Some populations were moderately susceptible, and a few were highly resistant and cross-resistant to chlorsulfuron. These findings are in line with those of previous studies in other countries (Novakowa et al. 2006; Krysiak et al. 2011; Massa and Gerhards 2011), which showed a widespread high level of silky windgrass resistance mainly to chlorsulfuron and less pronounced resistance to other sulfonylureas (e.g., sulfosulfuron, flupyrsulfuron, mesosulfuron-methyl + iodosulfuron).

On the other hand, most silky windgrass populations were effectively controlled with the ACCase inhibitors cycloxydim, clethodim, and pinoxaden at the recommended field rates, except for five populations (A5, A10, A18, A19, A20) with resistance to ALS-inhibiting herbicides that were also resistant to ACCase-inhibiting herbicide clodinafop-propargyl. This trend could be attributed to the less frequent use of clodinafop-propargyl as compared with the frequent and repeated applications of chlorsulfuron and other ALS inhibitors. By looking at the complete cross-resistance pattern, it is confirmed that the later-introduced products showed less resistance. In fields with a long-term monoculture of winter wheat together with the use of chlorsulfuron, resistance of silky windgrass biotypes to this active ingredient was ascertained (Marczewska 2006). In addition, the considerable plasticity of silky windgrass as well as its genetic diversity, coupled with high fecundity, may account for the selection of multiple and cross-resistant populations bearing mutations, impairing or reducing effectiveness of both ALS- and ACCase-inhibiting herbicides (Massa and Gerhards 2011). In agreement with our multiple resistance findings, Dicke et al. (2016) reported that a silky windgrass population, originating from spring/winter-sown cereals in Germany, evolved multiple resistance across ACCase-, ALS-, and PSII-inhibiting herbicides.

Apart from the repeated and widespread use of herbicides with same MOAs, some other factors, such as monoculture and reduced tillage practices applied in winter cereals grown in Greece, may influence selection pressure. In addition, the good adaptation of this weed to current agricultural production systems, along with its similarity to the wheat crop in terms of phenology and physiology, could account for this prevalence (Barberi 2003). Adoption of long-term reduced or no tillage practices allows increased weed seedbank in the top layers of the soil (Murphy and Lemerle 2006), which eventually may lead to higher weed seed germination, seedling establishment, and higher weed competition compared to conventional tillage systems (Massa et al. 2013). Moreover, as silky windgrass has a short seed dormancy period and early seedling emergence (Rola 1990), early winter crop sowing enhances infestations and increases the risk of field-evolved herbicide resistance (Massa et al. 2013). Also, high silky windgrass densities (>40 plants m^{-2}) significantly increase the risk of selection and spread of herbicide resistance (Murphy and Lemerle 2006; Massa et al. 2013).

The presence of Pro-197-Ser or Pro-197-Thr mutation in the analyzed *ALS* sequences from the eight R silky windgrass populations suggests that the cross-resistance to ALS-inhibiting herbicides detected in the whole-plant response experiments was due to mutant *als* alleles encoding an amino acid replacement at codon 197. Similar results were reported by other researchers (Krysiak et al. 2011; Massa and Gerhards 2011; Hamouzova et al. 2014), who also found resistant target-site enzyme to ALS-inhibiting herbicides in most of their studied silky windgrass populations. Nevertheless, because the populations were not tested for ACCase mutations, the presence of TSR due to this mechanism cannot be ruled out. In addition, the change in fresh weight reduction was relatively large (>25%) for both chlorsulfuron and clodinafop-propargyl with OP application, which suggests the presence of a metabolic pathway capable of degrading both compounds or the presence of two separate metabolic pathways, in addition to the *ALS* mutation. The Pro-197 mutation that was detected in one of the two alleles provides evidence that all plants of the R sequenced populations were heterozygous (RS). The lack of point mutations

at codon 197 of the sequenced *ALS* gene from the S silky windgrass population supports our findings in the whole-plant response experiments. Concerning the metabolic degradation of ALS-inhibiting herbicides in silky windgrass, this process has not thus far been thoroughly studied (Massa et al. 2011). Our findings show that co-application of the OP insecticide chlorpyrifos only slightly increased the overall poor level of control, except for some populations in which a significant increase in fresh weight reduction was noted with both chlorsulfuron and clodinafop-propargyl. Thus a NTSR mechanism based on increased metabolic degradation cannot be ruled out in some of the studied silky windgrass populations. Nevertheless, additional studies may shed more light on this aspect.

It is worth mentioning that in some winter cereal–producing areas of northwestern Greece, sterile oat is replaced by spring milletgrass (Grevena and Ptolemaida Counties), brome (Florina County), or silky windgrass (infesting large areas of Ptolemaida County). Therefore silky windgrass represents a growing threat to arable crop production in extended areas of northwestern Greece. In these specific production systems, mostly dryland arable crops (predominantly winter wheat) are cultivated repeatedly, because limited water availability does not allow for cultivation of more profitable row crops. Only recently, growers have started to adopt crop rotation, principally with winter legumes, attempting to mitigate problems closely associated with monoculture, such as evolution of herbicide resistance.

The results of this study suggest that chlorsulfuron and clodinafop-propargyl were the selecting agents of field-evolved multiple resistance to ALS- and ACCase-inhibiting herbicides in five silky windgrass populations. Although cases of herbicide-resistant silky windgrass populations to several ALS inhibitors have been reported in northwestern, central, and eastern Europe (Hamouzova et al. 2011; Krysiak et al. 2011; Massa and Gerhards 2011; Babineau et al. 2017), no cases of silky windgrass resistance have been reported in Greece so far. Moreover, our findings confirm multiple resistance of silky windgrass to ALS- and ACCase-inhibiting herbicides in some of the tested populations. As the available postemergence-applied chemistries/MOAs registered for grass weed control in cereals are rather limited, the risk for field-evolved resistance in grass weeds is inevitably aggregated in the absence of crop and herbicide MOA rotation. Sustainable wheat production is threatened by the expanding occurrence of herbicide-resistant weed populations, with limited efforts to discover new herbicide molecules (Nakka et al. 2019). Therefore it is important to adopt integrated management practices, implementing proactive and reactive measures to delay the evolution of herbicide-resistant populations and manage their established presence. More specifically, systematic crop and herbicide rotation, reduced reliance on herbicides as the exclusive mean of weed management, adoption of diversified production systems, and implementation of alternative or complementary measures for effective weed control should be systematically practiced.

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