Received: 8 December 2023 Revised: 11 August 2024

(wileyonlinelibrary.com) DOI 10.1002/ps.8399

Resistance to acetyl-CoA carboxylase (ACCase)-inhibiting herbicides in *Lolium multiflorum* Lam. populations of Argentina

Mara B. Depetris,[a,b†] Esteban Muñiz Padilla,[c,d] Fabián Ayala,[c] Daniel Tuesca[a] and Gabriela Breccia[a,b*]

Abstract

BACKGROUND: Italian ryegrass (*Lolium multiflorum* Lam.) is one of the most troublesome grass weeds in Argentina. The extensive and repetitive use of acetyl-CoA carboxylase (ACCase)-inhibiting herbicides has induced resistance in this weed species. The objectives of this study were to quantify the resistance levels to ACCase-inhibiting herbicides in two resistant populations and to identify the target-site mutations associated with their resistance.

RESULTS: Two resistant Italian ryegrass populations, Roldán and H2, were studied. Roldán was a suspected haloxyfop-resistant population, located in a wheat field from Santa Fe province with a history of ACCase-inhibiting herbicide use. The H2 population was obtained from the susceptible Hernandarias population (H0) after two cycles of selection with the herbicide quizalofop-ethyl. Whole-plant dose–response assays revealed that the resistant populations exhibited a high resistance to haloxyfop, with resistance factors (RF) exceeding 97-fold. Additionally, both populations showed a moderate resistance to pinoxaden (RF > 7), while maintaining susceptibility to clethodim. Partial chloroplastic ACCase sequences revealed isoleucine-to-asparagine substitution at position 2041 (Ile-2041-Asn) in both resistant populations.

CONCLUSION: This work provides a better understanding of cross-resistance to ACCase-inhibiting herbicides in *L. multiflorum* populations and represents the first report of the target-site mutation Ile-2041-Asn conferring resistance in populations from Argentina.

Keywords: ACCase; Italian ryegrass; clethodim; haloxyfop; pinoxaden

1 INTRODUCTION

Lolium grasses are globally distributed problematic weeds causing significant yield losses in field crops.[1] *Lolium multiflorum* Lam. (Italian ryegrass), is an annual or biennial diploid winter species native to north-west Africa, temperate Europe and south-west Asia.[2] It is characterized as a very adaptable species (obligate outbreeding), with high genetic diversity and phenotypic plasticity[3] that can evolve different herbicide resistance mechanisms very quickly presenting difficulties to chemical control.[4–6]

Acetyl-CoA carboxylase (ACCase) inhibitor herbicides (Group 1), can be grouped into three chemical families with limited residual activity in the soil, comprising cyclohexanodiones (DIMs), aryloxyphenoxypropionates (FOPs), and phenylpyrazole (DENs), which include clethodim, haloxyfop, and pinoxaden, respectively.[7] These herbicides inhibit the carboxylation of acetyl-CoA into malonyl-CoA, preventing the formation of lipid and secondary metabolites by blocking fatty acid biosynthesis producing a loss of cell membrane integrity, metabolite leakage, rapid necrosis and ultimately, cell death.[7] ACCase inhibitor herbicides provide a limited number of active ingredients for use in monocot crops, playing a key role in one of the greatest weed challenges, controlling glyphosate-resistant grasses in South America.[8]

Over the last 30 years, the control of *Lolium* grasses has exerted high selection pressure on their populations due to the exclusive application of PRE- and POST-emergence herbicides causing resistance to most herbicide sites of action. The first ACCase herbicide resistance case in *L. multiflorum* was found in 1987 in

---

[*] *Correspondence to: G Breccia, Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR, UNR, CONICET), Campo Experimental "José Villarino", CC14, S2125ZAA, Zavalla, Santa Fe, Argentina, E-mail:* gbreccia@unr.edu.ar *(Breccia)*

[†] *Present address: AgriBio, Centre for AgriBioscience, Agriculture Victoria, Bundoora, Victoria, Australia*

[a] *Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Zavalla, Argentina*

[b] *Instituto de Investigaciones en Ciencias Agrarias de Rosario, Universidad Nacional de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas (IICAR, UNR, CONICET), Zavalla, Argentina*

[c] *Facultad de Ciencias Agropecuarias, Universidad Nacional de Entre Ríos, Oro Verde, Argentina*

[d] *Facultad de Ciencia y Tecnología, Universidad Autónoma de Entre Ríos, Oro Verde, Argentina*

Oregon, United States.[9] Since then, herbicide resistance in this species has increased up to 74 cases recorded in the database of the International Survey of Herbicide Resistant Weeds.[9] In small grain cereal crops, ACCase inhibitor herbicides are one of the remaining options for the post-emergence control of *Lolium* species.[6]

In Argentina, Italian ryegrass densities of 100 plants m^2 reduced wheat yield by 20%–30%.[10] ACCase-inhibiting herbicides such as haloxyfop and clethodim are used as post-emergent herbicides in broadleaf crops to control grass weeds like Italian ryegrass. Pinoxaden combined with cloquintocet-methyl (crop safener) is commonly used for the control of wild oats and annual ryegrass in wheat and barley crops.[11]

Both target-site resistance and non-target-site resistance mechanisms have evolved in response to ACCase inhibitors.[12] Target-site ACCase point mutations conferring resistance were described at eight codon positions 1781, 1999, 2027, 2041, 2078, 2088, 2096, and 2097.[13] Moreover, higher ACCase gene copy number or over-expression was involved in target-site resistance.[14,15] Non-target-site resistance mechanisms like enhanced metabolism were also found for these herbicides.[16–18] Unfortunately, only a very limited number of weeds resistant to ACCase inhibitors in South America have had the resistance mechanism elucidated.[8]

Developing effective weed management strategies and ensuring the long-term sustainability of this mode of action requires a thorough understanding of cross-resistance patterns and resistance mechanisms, alongside other factors.[19] The aim of this study was to investigate the resistance to three ACCase inhibitors and the target-site resistance mechanism involved in two Argentinian *L. multiflorum* populations. One of the resistant populations exhibited significant shortcomings in control efficacy when haloxyfop was applied under field conditions near Roldán, in Santa Fe province. The other resistant population was artificially selected from an initially susceptible population sourced from a field, with a documented record of exposure to graminicides, located near Hernandarias, in Entre Ríos province.

2 MATERIALS AND METHODS

2.1 Plant material

Seeds from the *L. multiflorum* were collected in Roldán (32° 55′04.8′ S, 60°55′06.0′ W), Santa Fe province, Argentina, with suspected resistance to glyphosate and haloxyfop were sourced. This population originated from a field that has been under agricultural cultivation for over 20 years, with annual rotations of wheat–soybean or corn. In the last 5 years, glyphosate resistant weeds were controlled using haloxyfop and clethodim.

Two susceptible populations were collected from the Entre Ríos province, Argentina. They were Empalme, from a field with no agricultural history (31°32′4.45′ S, 59°56′15.43′ W), and Hernandarias (31°18′22.71′ S, 59°47′54.42′ W). The last population belonged to a field with annual wheat–soybean rotation with FOPs and DIMs applications for weed control and to a lesser extent, acetohydroxyacid synthase (AHAS) inhibitor and hormonal-type herbicides. Hernandarias (H0) population was demonstrated to be resistant to glyphosate.[20] The population H2 was obtained from the susceptible H0 after two cycles of selection with the herbicide quizalofop-ethyl.[21]

2.2 Dose–response assays

The sensitivity to haloxyfop, clethodim and pinoxaden in two resistant ryegrass populations (H2 and Roldán) and the susceptible populations Empalme and H0 were evaluated. Plants from seeds were grown at 1 cm deep in 40 × 30 × 10 cm plastic trays containing a mixture of soil/perlite/organic substrate in a 60/20/20 ratio. One seedling with two leaves per pot was transplanted into a 1600 cm^3-pot filled with the mixture. Pots were placed in a glasshouse under natural light with average day/night temperatures ranging from 22 to 15 °C.

The experiment was arranged as a completely randomized design with six treatments and six replications for each population. Herbicide application was conducted on plants with three tillers. The rates evaluated were 0×, 1/4×, 1/2×, 1×, 2×, 4×, 8× per each herbicide, x being the commercial rate of haloxyfop (GALANT® HL 54 EC, CORTEVA, Argentina) 93.6 g a.e. ha^{-1}; clethodim (LORD 36 EC®, UPL, Argentina) 216 g a.i. ha^{-1} and pinoxaden (AXIAL®, pinoxaden 50 g a.i. L^{-1} plus cloquintocet-mexyl 12.5 g L^{-1}, SYNGENTA, Argentina) 40 g a.i. ha^{-1}. Herbicide rates for clethodim included 0.4% (v/v) methylated soybean oil (DASH® MSO MAX, BASF, Argentina). Pinoxaden and clethodim were applied using a 2 m manual boom section with four air-induction flat fan spray tips 11 001 (AirMix®, Agrotop, Obertraubling, Bavaria, Germany). The application rate was 120 L ha^{-1}, with a pressure of 340 kPa and an application speed was 4.0 km h^{-1}. The meteorological conditions during the applications were monitored using the Skywatch® Atmos thermo-hygro-anemometer (JDC Electronic SA, Yverdon-les-Bains, Switzerland) which registered temperature, relative air humidity, and wind speed values ranging from 17.5 to 22 °C, 49%, and 3.5 km h^{-1}, respectively. Herbicide rates for haloxyfop included 0.8 L ha^{-1} mineral oil (NIMBUS®, SYNGENTA, Argentina) and were applied using a cabinet sprayer with a spray volume of 140 L ha^{-1} at a pressure of 300 kPa.

Twenty-eight days after application, the above plant tissue was harvested and dried at 60 °C for 72 h. Dry weights were recorded. Finally, biomass reduction was fit to a three-parameter log-logistic model using R and the *drc* package.[22] Resistance factor (RF) was calculated as the ratio of GR_{50} values of resistant population relative to that of the mean of the susceptible populations.

2.3 DNA extraction and ACCase gene sequencing

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) from leaf segments of three plants from each susceptible population (Empalme y H0) and haloxyfop-surviving plants of each resistant population (Roldán and H2). Two primer pairs, 1f (5′ AGCAACCCTGAACGTGGATT 3′), 1r (5′ GTCTTCGCCCATCCTCCAAA 3′), and 2f (5′ TGGCTGGGTGGTATGTTTGA 3′), 2r (5′ ATGCTTTGCTCCCTGGAGTT 3′) were designed to amplify the carboxyl transferase (CT) domain of the ACCase gene that participates in resistance to ACCase inhibitors. These primer pairs were designed using the ACCase gene sequences from *L. multiflorum* (AY710293) and *A. myosuroides* (accession number AJ310767) as reference. The primer pair 1f and 1r amplifies a 674 bp fragment, while the second primer pair 2f and 2r amplifies a 621 bp fragment. PCR amplifications were conducted in a volume of 25 μL reaction mixture consisting in 1.25 U/μl GoTaq® G2 DNA Polymerase (Promega Corporation, Madison, USA), 0.01 μg/μl DNA extract, 0.2 μM forward and reverse primers, GoTaq® Reaction Buffer 1×, and 0.2 mM dNTP mix. The amplification reaction was conducted under the following thermal conditions: 94 °C for 5 min, followed by 34 cycles of 94 °C for 30 s; 60 °C for 30 s and 72 °C for 30 s, followed by a final extension step of 72 °C for 1 min. The DNA profiles were determined by electrophoresis on a 2% agarose gel supplemented with a 1/10,000 dilution of SYBR® Safe and were sent for sequencing to Macrogen Inc. (Korea). The sequences were aligned using BioEdit software[23] to identify mutations responsible for conferring herbicide resistance.

3 RESULTS

3.1 Whole-plant dose–response analysis

The dose–response curves of the resistant populations (Roldán and H2) and the susceptible populations (Empalme and H0) have shown differences for haloxyfop, decreasing the dry weight of shoots in the susceptible populations but not affecting that of the resistant plants (Figs 1(A), (B) and 2(A)). Roldán and H2 populations had GR_{50} values >749 g a.i. ha^{-1} for haloxyfop (Table 1), corresponding to a RF of >97.

Resistant populations also presented some resistance to pinoxaden (Figs 1(C), (D) and 2(B), Table 1). The GR_{50} values of the Roldán and H2 populations were 15- and 7-fold higher than the susceptible populations, respectively. However, GR_{50} values for H2 population were lower or similar than the normal field application rate (40 g a.i. ha^{-1}).

On the other hand, the four evaluated populations showed sensibility to clethodim (Figs 1(E), (F) and 2(C)). The estimated GR_{50} values of the resistant and susceptible populations ranged between 14.9 and 48.4 g a.i. ha^{-1} (Table 1), which are much lower than the normal field application rate in Argentina (216 g a.i. ha^{-1}).

3.2 ACCase gene sequencing

The aligned sequences of the ACCase gene fragments with *A. myosuroides* reference sequence did not reveal any point mutation at codon positions Ile-1781, Trp-2027, Ile-2041, Asp-2078, Cys-2088, Gly-2096 in the two susceptible populations Empalme and H0. However, the resistant populations Roldán and H2 showed the same 2041-Asn mutation, responsible for conferring ACCase herbicide resistance (Table 1, Fig. 3). The nucleotide T-to-A transversion at the second position of the codon 2041 (ATT to AAT) of the plastid ACCase gene determined the

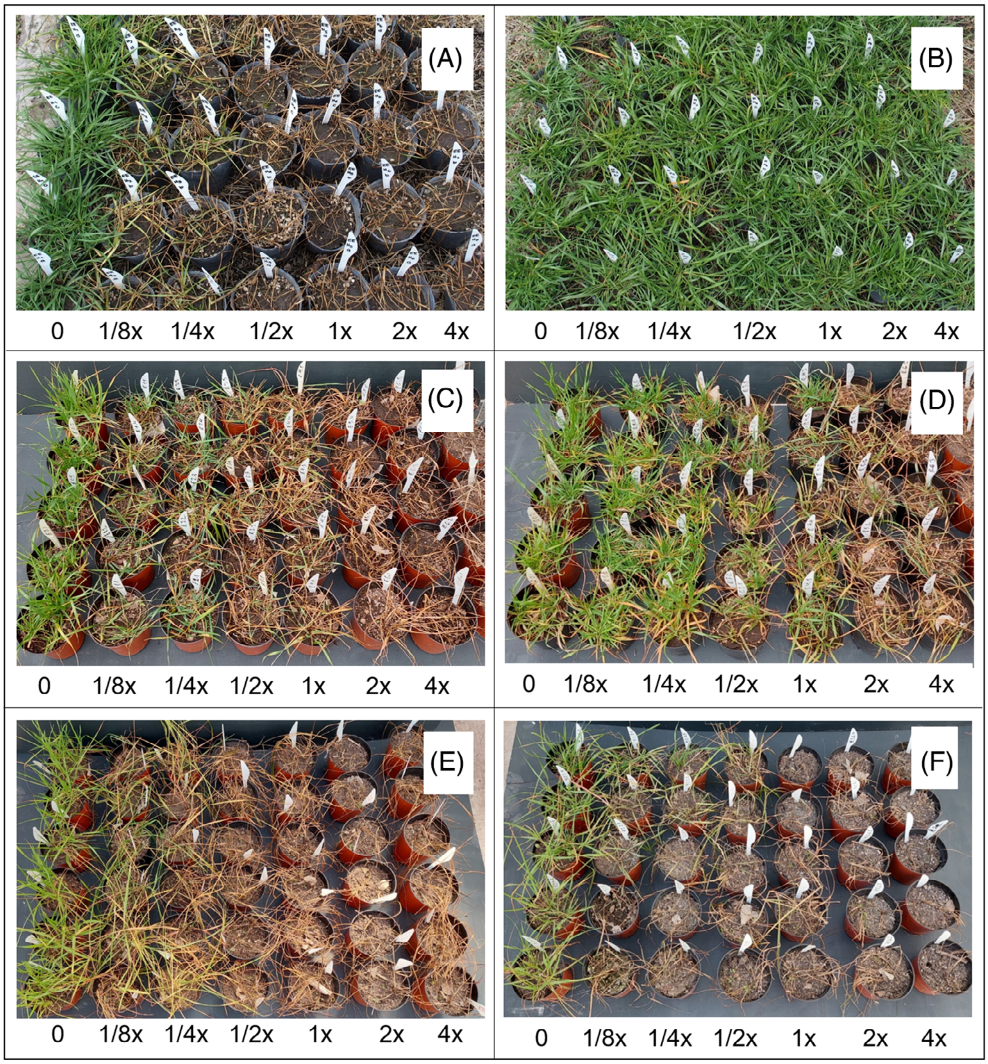

Figure 1. Plants from susceptible Empalme (A, C, and E) and resistant Roldán (B, D, and F) populations 28 days after haloxyfop (A, B), pinoxaden (C—D) and clethodim (E, F) application. The normal field application rate (x) of haloxyfop, pinoxaden, and clethodim are 93.6 g a.e. ha^{-1}, 40 g a.i. ha^{-1}, and 216 g a.i. ha^{-1}, respectively.

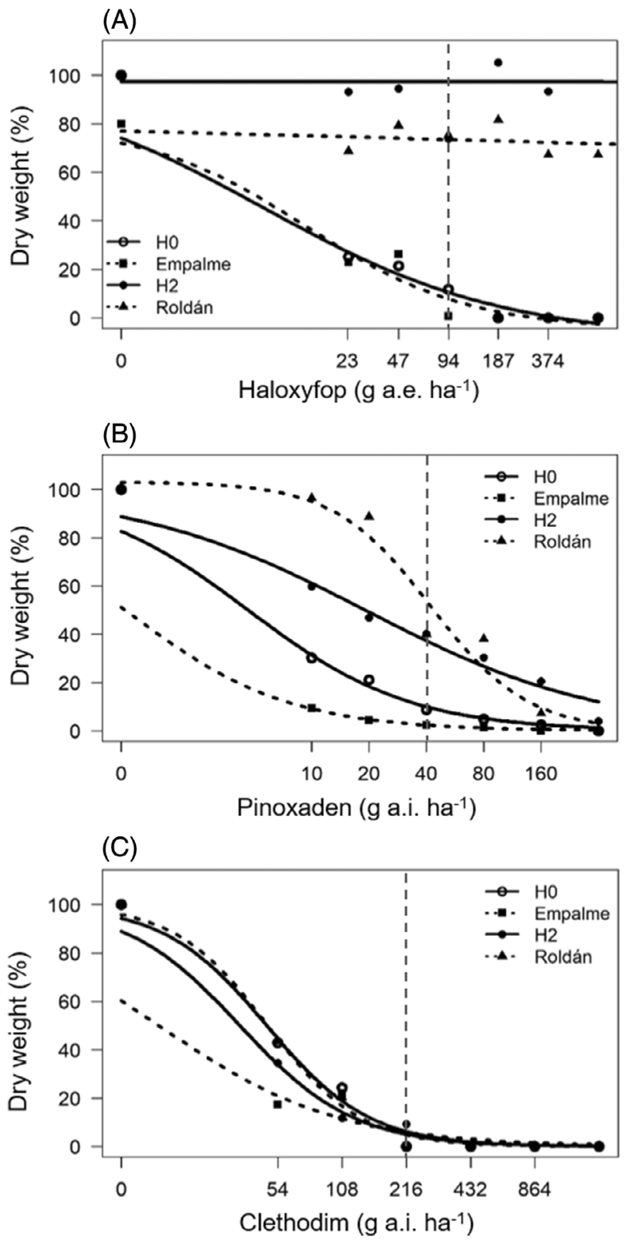

Figure 2. Whole-plant dose–response curves of the resistant H2 and Roldán populations and the susceptible Empalme and H0 populations for (A) haloxyfop, (B) pinoxaden, and (C) clethodim. Vertical dashed line in each graphic represents the herbicide commercial rate (x).

isoleucine to asparagine amino acid substitutions (Ile-2041-Asn mutation).

4 DISCUSSION

Given the persistent selection pressures arising from the repeated use of herbicides and the swift evolution of resistance observed in out-crossing species,[24] Italian ryegrass has evolved as a major weed in agricultural contexts across Europe, Asia, and both North and South America.[1] In Argentina, glyphosate-resistant *L. multiflorum* populations have been reported since year 2007. A recent survey in Uruguay showed that 80% of Italian ryegrass populations are resistant to glyphosate,[25] and the situation is similar in Argentina.[26]

A diclofop-methyl-resistant *L. multiflorum* biotype found in the south of Buenos Aires province was reported in 2009 but its mechanism of resistance is unknown.[9] Recent studies of *L. perenne* populations from the south of Buenos Aires reported resistance to these herbicides, with resistance involving both target-site and non-target-site mechanisms.[17,27] The resistant populations evaluated herein provides the first evidence of Italian ryegrass with high resistance to haloxyfop and moderate resistance to pinoxaden from Argentina.

Different mutations at ACCase gene conferring target-site resistance were identified in several weed species. Different reports found large ACCase variability in *L. multiflorum* populations collected from agricultural fields.[28,29] In this study, the same Ile-2041-Asn was found in both resistant populations. This mutation has been reported to confer a high level of resistance to FOPs and susceptibility to DIMs for several species including *Avena fatua*,[18] *Alopecurus aequalis*,[30] *Alopecurus japanicus*,[31] *Alopecurus myosuroides*,[32,33] *Beckmannia syzigachne*,[34] *Hordeum glaucum*,[35] *Polypogon fugax*,[36] and *Sorghum halepense*.[37] Reduced sensitivity to pinoxaden was associated with this mutation, but results were inconsistent among species.[13] Our results showed similarity with previous findings in a *Lolium sp*. populations from Italy,[29] Spain,[4] China,[38] and New Zealand.[39]

Previous studies suggest that point mutations causing resistance to ACCase inhibitors arise from standing genetic variation in weed populations.[40,41] In populations exposed to herbicide selection pressures, the frequency of resistance alleles has been observed to undergo a marked increase.[42] The repeated applications of the same FOP herbicide in the H0 population from Entre Ríos province has led to the fast selection of plants with resistant alleles, which were initially at low frequency in the susceptible population H0. The progression of resistance in H2 population

Table 1. Estimation of GR_{50} for the resistant (R) and susceptible (S) populations in the whole-plant assays and the acetyl-CoA carboxylase (ACCase) mutations found in the R populations

	GR_{50}[†]			
Population	Haloxyfop (g a.e. ha^{-1})	Pinoxaden (g a.i. ha^{-1})	Clethodim (g a.i. ha^{-1})	ACCase amino acid change
Empalme (S)	8.4 ± 3.3	1.0 ± 0.7	48.4 ± 5.6	–
Roldán (R)	>749	42.2 ± 5.6*	35.8 ± 14.2	Ile-2041-Asn
H0 (S)	6.9 ± 2.3	4.5 ± 1.5	14.9 ± 8.7	–
H2 (R)	>749	19.3 ± 4.7*	47.9 ± 9.4	Ile-2041-Asn

*Significant differences between resistant and susceptible populations at $P < 0.05$.
[†] GR_{50} is the herbicide rate that reduces dry weight by 50%.

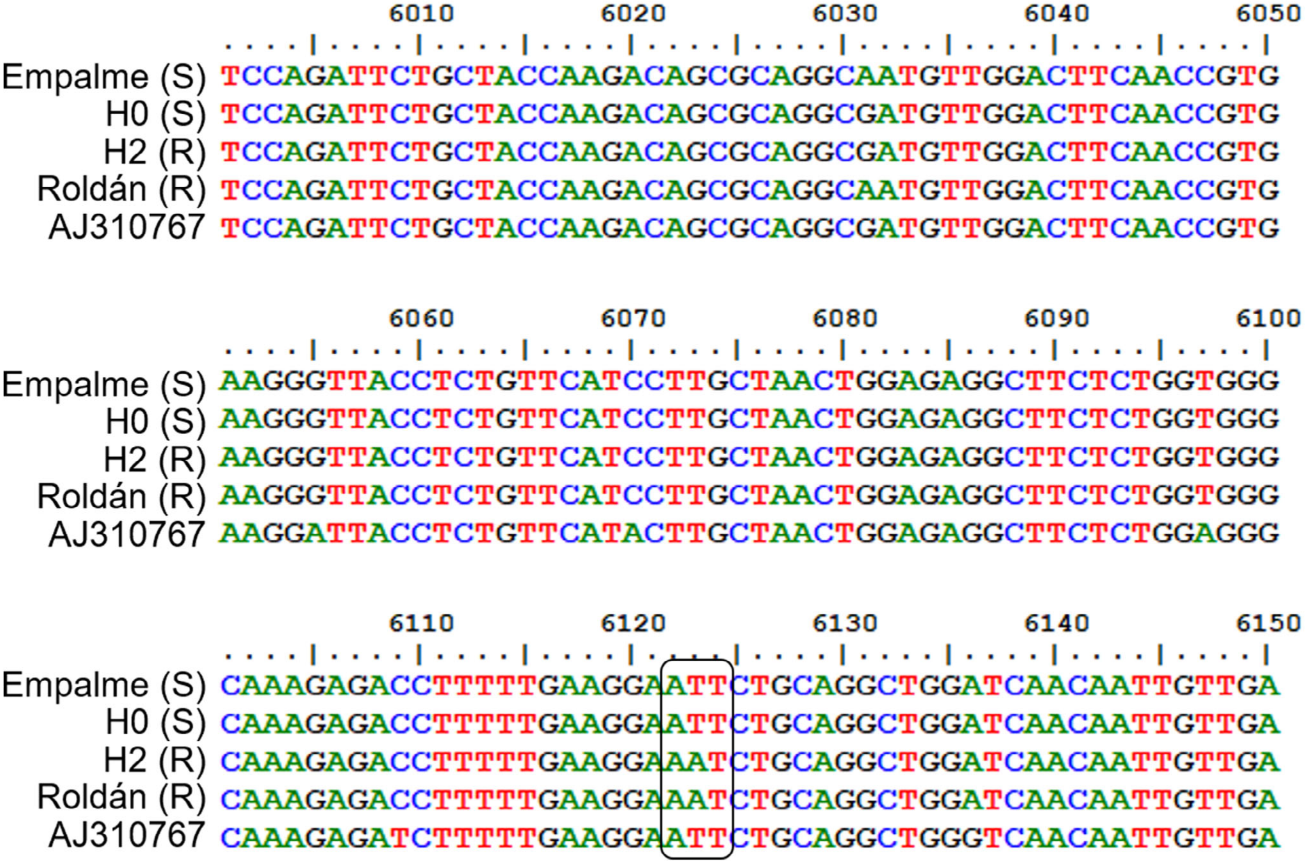

Figure 3. Sequence alignment of the partial plastid ACCase gene in the susceptible (S) and resistant (R) populations. ACCase sequence from *Alopecurus myosuroides* accession AJ310767 was included in the alignment as reference sequence. The nucleotide transversion at the second position of the codon (ATT to AAT) in R populations determines the isoleucine to asparagine amino acid substitutions (Ile-2041-Asn mutation).

highlights the direct impact of selection pressures from application of herbicides with the same mode of action, thus underscoring the complex interplay between agricultural practices and the adaptive evolution of weed populations.

In the south region of the Argentinian Pampas, the selection pressure associated with clethodim and haloxyfop pre-plant application, and pinoxaden use on wheat and barley crops, favored the propagation of *L. perenne* with the Asp-2078-Gly mutation.[27] The resistant *L. multiflorum* populations evaluated in this work derive from the north Pampas, where pinoxaden utilization is comparably limited. Regional variations in herbicide application practices could explain the observed differences in resistance patterns, highlighting the impact of agronomic strategies on the emergence and dynamics of resistance mechanisms.

Mutations at the 2041 position in the ACCase gene caused some fitness costs in *L. rigidum*,[43] *Hordeum glaucum*[44] and *Beckmannia syzigachne*.[45] Consequently, the 2041-Asn allele may experience a reduction in frequency in the absence of ACCase-inhibiting herbicide selection pressure. Nonetheless, the main recommendations for delaying the evolution of herbicide resistance include the integration of non-chemical methods of weed management with chemical practices. Examples of chemical management practices include the rotation of herbicides with differing modes of action and the utilization of herbicide mixtures. Several alternative management strategies for pre-emergent herbicides, recognized for their efficacy in controlling ryegrass, may vary according to the timing of use (fallow or pre-sowing) and the subsequent crop in rotation. Some of these alternatives include trifluralin (dinitroaniline group, HRAC group 3), recommended to prevent the germination of ryegrass,[46] pyroxasulfone, s-metolachlor, a mixture with flufenacet (HRAC Group 15), flumioxazin (HRAC Group 14), imazapic, imazapyr, a mixture of sulfometuron-methyl + chlorimuron-ethyl (HRAC Group 2),[47] and bixlozone (HRAC Group 13), an isoxazolidinone herbicide recently introduced in Argentina and effective for controlling annual ryegrass.

Among alternative non-selective post-emergent herbicides for controlling ryegrass with different modes of action, excluding ACCase inhibitors, are those in HRAC Group 2 (e.g., imazapic, imazapyr), HRAC Group 27 (e.g., mesotrione), HRAC Group 22 (e.g., paraquat), HRAC Group 14 (e.g., epyrifenacil). For selective herbicides registered for wheat and barley to control ryegrass, there are several alternatives in HRAC Group 2, including iodosulfuron-methyl-sodium + mesosulfuron-methyl, pyroxsulam, sodium flucarbazone, and imazamox for wheat with CLEARFIELD® technology.[11] For plants at advanced growth stages, a chemical alternative is the double knock technique. This method involves applying glyphosate or graminicides at the recommended dose first to control most weeds, followed by paraquat[48] or glufosinate[49] to eliminate any surviving weeds.

On the other hand, the implementation of non-chemical strategies encompasses the adoption of cover crops such as *Avena sativa* or *Secale cereale*, which compete effectively with ryegrass, reducing its density and growth,[50] diversification of crop rotations alternating between summer and winter crops can disrupt the life cycle of ryegrass.[51] Additionally, rotating with perennial pastures such as *Medicago sativa* or *Trifolium repens* can reduce ryegrass pressure through resource competition and shading.[52] Other effective strategies include the application of mechanical control measures, variation in sowing times, adjustment of row spacing and crop density, and the use of competitive cultivars.[53] In Argentina's agricultural regions, chemical weed control remains the dominant strategy, despite the existence of low-cost and effective non-chemical alternatives such as cover crops or cleaning tillage and harvesting equipment. These non-chemical methods are, however, underutilized.[54]

Our investigation focused on target-site resistance mechanisms in two Italian ryegrass populations. However, it is important to note that multiple resistance mechanisms, both target-site and non-target-site based, can be simultaneously expressed in individual plants of genetically diverse, cross-pollinated *L. multiflorum*.[5,17,55–57] Dose–response experiments with quizalofop-p-ethyl on plants from the H2 population pretreated with the cytochrome P450 inhibitors malathion and piperonyl butoxide did not show differences compared to those without pretreatment. This suggests that this metabolic system is not linked to the resistance observed in H2 (García IE, unpublished). Further experiments using cytochrome P450 and gluthation-S-transferases inhibitors and other biochemical or metabolic approaches are required to fully understand the resistance mechanisms involved in the Roldán resistant population.

5 CONCLUSION

This study contributes to our understanding of ACCase-inhibiting herbicide resistance patterns and mechanisms in *L. multiflorum* populations from the Argentinian provinces of Santa Fe and Entre Ríos. Results from whole-plant dose–response experiments elucidate that the resistant Italian ryegrass populations, namely Roldán and H2, have developed pronounced resistance to haloxyfop, moderate resistance to pinoxaden, and retained sensitivity to clethodim. The observed resistance profiles were associated with a specific point mutation, Ile-2041-Asn, localized within the CT domain of the ACCase gene. This work provides valuable insights into herbicide resistance dynamics in this agricultural region, and highlights the importance of rotation of modes of action and other agricultural practices to mitigate the risk of further resistance development.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1 Beckie HJ and Jasieniuk M, Chapter 12 *Lolium rigidum* and *Lolium multiflorum*, in *Biol Manage Prob Crop Weed Species*, ed. by BS Chauhan, Academic Press, New York, pp. 261–283 (2021).

2 Beddows AR, *Lolium multiflorum* Lam. *J Ecol* **61**:587 (1973).

3 Matzrafi M, Preston C and Brunharo CA, Review: evolutionary drivers of agricultural adaptation in *Lolium* spp. *Pest Manag Sci* **77**:2209–2218 (2021).

4 Torra J, Montull JM, Taberner A, Onkokesung N, Boonham N and Edwards R, Target-site and non-target-site resistance mechanisms confer multiple and cross- resistance to ALS and ACCase inhibiting herbicides in *Lolium rigidum* from Spain. *Front Plant Sci* **12**:625138 (2021).

5 Zhu G, Wang H, Gao H, Liu Y, Li J, Feng Z *et al.*, Multiple resistance to three modes of action of herbicides in a single Italian ryegrass (*Lolium multiflorum* L.) population in China. *Agronomy* **13**:216 (2023).

6 Tehranchian P, Nandula V, Jugulam M, Putta K and Jasieniuk M, Multiple resistance to glyphosate, paraquat and ACCase-inhibiting herbicides in Italian ryegrass populations from California: confirmation and mechanisms of resistance. *Pest Manag Sci* **74**:868–877 (2018).

7 Wenger J, Niderman T, Mathews C and Wailes S, Acetyl-CoA carboxylase inhibitors, in *Modern Crop Prot Compd*, ed. by WP Jeschke, M Witschel, W Krämer and U Schirmer, pp. 501–528 (2019).

8 Takano HK, Ovejero RFL, Belchior GG, Maymone GPL and Dayan FE, ACCase-inhibiting herbicides: mechanism of action, resistance evolution and stewardship. *Sci Agric* **78**:e20190102 (2021).

9 Heap I, The International Herbicide-Resistant Weed Database (2023). www.weedscience.org [accessed 23 November 2023].

10 Scursoni JA, Palmano M, De Notta A and Delfino D, Italian ryegrass (*Lolium multiflorum* lam.) density and N fertilization on wheat (*Triticum aestivum* L.) yield in Argentina. *Crop Prot* **32**:36–40 (2012).

11 Gigón R, Vigna M, Yanniccari M and Privado A, Manejo de malezas problema. Raigrás (*Lolium* spp.) Bases para su manejo y control en sistemas de producción, REM - AAPRESID. Rosario (2017).

12 Gaines TA, Duke SO, Morran S, Rigon CAG, Tranel PJ, Küpper A *et al.*, Mechanisms of evolved herbicide resistance. *J Biol Chem* **295**: 10307–10330 (2020).

13 Murphy BP and Tranel PJ, Target-site mutations conferring herbicide resistance. *Plants* **8**:382 (2019).

14 Laforest M, Soufiane B, Simard M, Obeid K, Page E and Nurse RE, Acetyl-CoA carboxylase overexpression in herbicide-resistant large crabgrass (*Digitaria sanguinalis*). *Pest Manag Sci* **73**:2227–2235 (2017).

15 González-Torralva F and Norsworthy JK, Overexpression of acetyl CoA carboxylase 1 and 3 (ACCase1 and ACCase3), and CYP81A21 were related to cyhalofop resistance in a barnyardgrass accession from Arkansas. *Plant Signal Behav* **18**:2172517 (2023).

16 Suzukawa AK, Bobadilla LK, Mallory-Smith C and Brunharo CACG, Non-target-site resistance in *Lolium* spp. globally: a review. *Front Plant Sci* **11**:609209 (2021).

17 Yanniccari M, Gigón R and Larsen A, Cytochrome P450 herbicide metabolism as the main mechanism of cross-resistance to ACCase- and ALS-inhibitors in *Lolium* spp. populations from Argentina: a molecular approach in characterization and detection. *Front Plant Sci* **11**:600301 (2020).

18 Han Y, Sun Y, Ma H, Wang R, Lan Y, Gao H *et al.*, Target-site and non-target-site based resistance to clodinafop-propargyl in wild oats (*Avena fatua* L.). *Pest Biochem Physiol* **197**:105650 (2023).

19 Peterson MA, Collavo A, Ovejero R, Shivrain V and Walsh MJ, The challenge of herbicide resistance around the world: a current summary. *Pest Manag Sci* **74**:2246–2259 (2018).

20 Muñiz Padilla E, *Evaluación de la resistencia a glifosato en poblaciones naturalizadas de Lolium multiflorum Lam en la provincia de Entre Ríos*, Master's Thesis. Universidad Nacional de Tucumán, San Miguel de Tucuman, p. 128. (2018).

21 García E, Ayala F, Ahumada M, Carballo V, Breccia G, Depetris M *et al.*, Evaluación de supervivencia en poblaciones de *Lolium multiflorum* seleccionadas en forma recurrente con diferentes dosis de quizalofop-p-etil, in *Actas del III Congreso Argentino Malezas*, Buenos Aires, pp. 264–265. (2021).

22 Ritz C, Baty F, Streibig JC and Gerhard D, Dose-response analysis using R. *PLoS One* **10**:1–13 (2015).

23 Hall T, BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser* **41**:95–98 (1999).

24 Ghanizadeh H and Harrington KC, Perspectives on non-target site mechanisms of herbicide resistance in weedy plant species using evolutionary physiology. *AoB Plants* **9**:plx035 (2017).

25 Marques Hill S, Vila-Aiub M, Hernández M, Kaspary TE and García MA, Cross- and multiple herbicide resistance in *Lolium multiflorum* across Uruguay. *Weed Res* **62**:296–305 (2022).

26 AAPRESID, REM, Mapa de malezas (2023). https://www.aapresid.org.ar/rem-malezas/mapa-malezas/ [accessed 23 November 2023].

27 Yanniccari M and Gigón R, Cross-resistance to acetyl-CoA carboxylase–inhibiting herbicides conferred by a target-site mutation in perennial ryegrass (*Lolium perenne*) from Argentina. *Weed Sci* **68**:116–124 (2020).

28 Brunharo CACG and Tranel PJ, Repeated evolution of herbicide resistance in *Lolium multiflorum* revealed by haplotype-resolved analysis of acetyl-CoA carboxylase. *Evol Appl* **16**:1969–1981 (2023).

29 Scarabel L, Panozzo S, Varotto S and Sattin M, Allelic variation of the ACCase gene and response to ACCase-inhibiting herbicides in pinoxaden-resistant *Lolium* spp. *Pest Manag Sci* **67**:932–941 (2011).

30 Zhao N, Yan Y, Ge L, Zhu B, Liu W and Wang J, Target site mutations and cytochrome P450s confer resistance to fenoxaprop-P-ethyl and mesosulfuron-methyl in *Alopecurus aequalis*. *Pest Manag Sci* **75**:204–214 (2019).

31 Chen G, Wang L, Xu H, Wu X, Pan L and Dong L, Cross-resistance patterns to acetyl-coa carboxylase inhibitors associated with different mutations in japanese foxtail (*Alopecurus japonicus*). *Weed Sci* **65**: 444–451 (2017).

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

33 Délye C, Matéjicek A and Michel S, Cross-resistance patterns to ACCase-inhibiting herbicides conferred by mutant ACCase isoforms in *Alopecurus myosuroides* Huds. (black-grass), re-examined at the recommended herbicide field rate. *Pest Manag Sci* **64**:1179–1186 (2008).

34 Pan L, Li J, Zhang T, Zhang D and Dong LY, Cross-resistance patterns to acetyl coenzyme a carboxylase (ACCase) inhibitors associated with different ACCase mutations in *Beckmannia syzigachne*. *Weed Res* **55**:609–620 (2015).

35 Shergill LS, Malone J, Boutsalis P, Preston C and Gill G, Basis of ACCase and ALS inhibitor resistance in *Hordeum glaucum* Steud. *Pest Manag Sci* **73**:1638–1647 (2017).

36 Tang W, Zhou F, Chen J and Zhou X, Resistance to ACCase-inhibiting herbicides in an Asia minor bluegrass (*Polypogon fugax*) population in China. *Pest Biochem Physiol* **108**:16–20 (2014).

37 Papapanagiotou AP, Loukovitis D, Damalas CA and Eleftherohorinos IG, Identification of an acetyl-CoA carboxylase-resistant johnsongrass (*Sorghum halepense* L.) population from a cotton field in northern Greece. *Weed Biol Manag* **22**:88–93 (2022).

38 Li W, Wu C, Wang M, Jiang M, Zhang J, Liao M *et al.*, Herbicide resistance status of italian ryegrass (*Lolium multiflorum* lam.) and alternative herbicide options for its effective control in the Huang-Huai-Hai plain of China. *Agronomy* **12**:2394 (2022).

39 Ghanizadeh H, Harrington KC and Mesarich CH, The target site mutation Ile-2041-Asn is associated with resistance to ACCase-inhibiting herbicides in *Lolium multiflorum*. *N Z J Agric Res* **63**:416–429 (2020).

40 Délye C, Deulvot C and Chauvel B, DNA analysis of herbarium specimens of the grass weed *Alopecurus myosuroides* reveals herbicide resistance pre-dated herbicides. *PLoS One* **8**:e75117 (2013).

41 Boutsalis P, Gill GS and Preston C, Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technol* **26**:391–398 (2012).

42 Délye C, Clément JAJ, Pernin F, Chauvel B and Le Corre V, High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. *Basic Appl Ecol* **11**:504–512 (2010).

43 Sabet Zangeneh H, Mohammaddust Chamanabad H, Zand E, Alcántara-de la Cruz R, Travlos I, De Prado R *et al.*, Clodinafop-propargyl resistance genes in *Lolium rigidum* Guad. Populations are associated with fitness costs. *Agronomy* **8**:106 (2018).

44 Shergill LS, Boutsalis P, Preston C and Gill GS, Fitness costs associated with 1781 and 2041 ACCase-mutant alleles conferring resistance to herbicides in *Hordeum glaucum* Steud. *Crop Prot* **87**:60–67 (2016).

45 Du L, Qu M, Jiang X, Li X, Ju Q, Lu X *et al.*, Fitness costs associated with acetyl-coenzyme a carboxylase mutations endowing herbicide resistance in American sloughgrass (*Beckmannia syzigachne* Steud.). *Ecol Evol* **9**:2220–2230 (2019).

46 Shaner DL, Lessons learned from the history of herbicide resistance. *Weed Sci* **62**:427–431 (2014).

47 Metzler MJ and Ahumada M, Manejo de Lolium multiflorun (raigrás anual) resistente a glifosato mediante el uso de herbicidas preemergentes. https://malezascrea.org.ar/download/manejo-lolium-multiflorum-resistente-a-glifosato-m-metzler-y-m-ahumada/ (2016).

48 Walsh MJ and Powles SB, Management strategies for herbicide-resistant weed populations in Australian dryland crop production systems. *Weed Technol* **21**:332–338 (2007).

49 Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM *et al.*, Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed Sci* **60**:31–62 (2012).

50 Mirsky SB, Ryan MR, Teasdale JR, Curran WS, Reberg-Horton CS, Spargo JT *et al.*, Overcoming weed management challenges in cover crop–based organic rotational no-till soybean production in the eastern United States. *Weed Technol* **27**:193–203 (2013).

51 Powles SB and Yu Q, Evolution in action: plants resistant to herbicides. *Annu Revi Plant Biol* **61**:317–347 (2010).

52 Daugovish O, Thill DC and Shafii B, Modeling competition between wild oat (*Avena fatua* L.) and yellow mustard or canola. *Weed Sci* **51**:102–109 (2003).

53 Beckie HJ, Busi R, Lopez-Ruiz FJ and Umina PA, Herbicide resistance management strategies: how do they compare with those for insecticides, fungicides and antibiotics? *Pest Manag Sci* **77**:3049–3056 (2021).

54 Scursoni JA, Vera ACD, Oreja FH, Kruk BC and de la Fuente EB, Weed management practices in Argentina crops. *Weed Technol* **33**:459–463 (2019).

55 Han H, Yu Q, Owen MJ, Cawthray GR and Powles SB, Widespread occurrence of both metabolic and target-site herbicide resistance mechanisms in *Lolium rigidum* populations. *Pest Manag Sci* **72**:255–263 (2016).

56 Scarabel L, Panozzo S, Loddo D, Mathiassen SK, Kristensen M, Kudsk P *et al.*, Diversified resistance mechanisms in multi-resistant *Lolium* spp. in three European countries. *Front Plant Sci* **11**:608845 (2020).

57 Kaundun SS, Downes J, Jackson LV, Hutchings S-J and Mcindoe E, Impact of a novel W2027L mutation and non-target site resistance on acetyl-CoA carboxylase-inhibiting herbicides in a French *Lolium multiflorum* population. *Genes* **12**:1838 (2021).