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FIRST REPORT OF IMIDAZOLINONE-RESISTANT *Bassia scoparia* IN ARGENTINA: EVIDENCE FROM DOSE-RESPONSE BIOASSAYS AND ALS GENE SEQUENCING

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ABSTRACT

Kochia (*Bassia scoparia* (L.) A. J. Scott.) is a problematic weed. In Argentina, farmers have recently experienced poor control over imidazolinone-resistant sunflowers, specifically the sensitivity of putative kochia resistant plants to imidazolinone herbicides. Seeds from putative resistant populations from Pellegrini (P), De Bary (DB), and 30 de Agosto (A) and a known susceptible (S) population from Col. Martín Fierro were sown in pots. A mixture of imazapyr (Clearsol® WG 80%) and imazamox (Trigosol WG 70%) was sprayed when plants reached approximately 6 cm in height and 10-leaf stage using a boom sprayer. A trial was conducted to determine the response of kochia to the recommended doses (1x) of imazapyr 80.00 g ai ha^{-1} + imazamox 20.30 g ai ha^{-1}. Plants were harvested 30 days after treatment to determine fresh shoot weight. A dose–response experiment was performed using imazapyr + imazamox at concentrations of 0x, 1/4x, 1/2x, 1x, 2x, 4x, and 8x. Partial ALS gene amplification was performed using leaves of plants that survived an 8x dose. The 1x fresh weight of the putative populations did not differ from 0x. LD_{50} of imazapyr+imazamox (g ai ha^{-1}) for each biotype was: S, 18.86 + 4.79; DB, 82.48 + 20.93; A, 179.36 + 45.51; and P, 550.88 + 139.79. The three resistant populations exhibited a single-point mutation from guanine to thymine, detected in codon 574 of the ALS gene. This mutation confers resistance to ALS inhibitors. This is the first reported case of kochia resistance in Argentina, providing valuable insights that extend beyond sunflower weed management.

Keywords: biotype, control, herbicides, imazapyr, imazamox, resistant, sunflower, weed.

INTRODUCTION

Kochia *[Bassia scoparia* (L.) A. J. Scott. syn. *Kochia scoparia*] is a member of the Amaranthaceae family, subfamily Camphorosmoidea, and belongs to the *Bassia*/*Camphorosma* group (Kadereit and Freitag, 2011; Grabowska et al., 2023). It is widespread in temperate and subtropical regions of the world. Although its origin has not been confirmed, it may be traced to Central Asia and South Siberia, where the greatest diversity of morphological forms is present (Sukhorukov et al., 2025). Kochia, a C4 species, is highly competitive, germinates at low soil temperatures and emerges early, grows

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rapidly, tolerates heat, drought, and salinity, and exerts allelopathic effects on neighboring species (Friesen et al., 2009). It is a problematic weed present in sandy and saline soils (Marzocca, 1993; Brignone and Denham, 2021).

In Argentina, 24 weed species distributed across the main extensive crops, including soybeans, maize, wheat, barley, oilseed rape, sunflowers, chickpeas, and peanuts, have evolved herbicide resistance. The highest number of herbicide-resistant species was identified in soybeans, followed by maize, wheat, barley, and fallow. Weed species with the highest number of resistant individuals included *Amaranthus hybridus*, *Amaranthus palmeri*, *Lolium multiflorum*, and *Raphanus sativus* (Oreja et al., 2024). At that time, kochia populations had not been documented as herbicide-resistant weeds in the country. However, the International Herbicide Resistant Weeds Database reports 56 cases of herbicide-resistant kochia; some exhibiting multiple resistance to two or three sites of action. Most cases were reported in the United States, followed by Canada, with only one report was from the Czech Republic. Three reports specifically referred to resistance to imidazolinones; two documented imazethapyr resistance in Canada in 1988 and in the United States in 1994; and one reported imazamox resistance in the United States in 2005 (Heap, 2025). Another study reported resistance to 1,000 g ai ha^{-1} of imazapyr in a kochia population from the Czech Republic (Chodová and Mikulka, 2001). Geddes and Sharpe (2022) highlighted the rapid increase in herbicide-resistant kochia populations over the past few decades.

Imidazolinones represent a class of herbicides that can be used either pre- or post-emergence for the control of a wide range of weeds in broadleaf and cereal crops as well as in non-crop areas. In addition, these herbicides are used with imidazolinone-tolerant crops (Clearfield® crops) (Tan et al., 2005). In Argentina, in 2003, BASF SA registered the herbicide imazapyr, becoming one of the most representative members of this herbicide family. The Clearfield system continued to evolve, leading to the introduction of the Clearsol Plus technology, a mixture of imazapyr and imazamox. Both herbicides belong to the acetolactate synthase (ALS) inhibitor group, targeting an enzyme essential for the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine. Inhibition of ALS disrupts protein synthesis, halts cell division and plant growth, and ultimately causes plant death.

Imidazolinone-tolerant sunflowers have provided farmers with an alternative method for managing weeds. A significant sunflower-based economy characterizes the west of Buenos Aires Province (Argentina). In the 2019/2020 season, about 60% of the sunflowers sown at the country level corresponded to hybrids tolerant to imidazolinones (RETAA, 2020).

Kochia commonly interferes with sunflower crops, resulting in losses of around 20% (Lewis and Gulden, 2014; Durgan et al., 1990). High seedling emergence occurs very early in the spring, while continued emergence into midsummer emphasizes the need for extended periods of kochia management (Dille et al., 2017). In Argentina, kochia was declared an 'agricultural pest' in 1946 (Decree No. 3383/46), and was well managed for many years. Recently, however, its presence and density have increased, particularly in farmland in the west-central Pampas region of Argentina (Montes et al. 2024). In recent years, several farmers reported poor control of kochia in imidazolinone-resistant sunflowers.

The objective of this study was to evaluate imidazolinone herbicide resistance in *Bassia scoparia* populations from western Buenos Aires province (Argentina) through dose–response bioassays under controlled conditions and molecular analysis of the ALS gene.

MATERIALS AND METHODS

Plant material

To verify the resistance of kochia to imidazolinone herbicide, two experiments were conducted under greenhouse conditions. Seeds samples were collected during the winter of 2021 from different farms where failure to control with post-emergence imidazolinone herbicides was reported. Seeds of three putative resistant to imidazolinone populations were harvested from plants located in the west of Buenos Aires province: Pellegrini (P) (-36°19′ 40.2′′, -63° 0′ 24.7′′), De Bary (DB) (-36°30′23.5″, -63°18′12.8″) and 30 de Agosto (A) (-36° 13′ 40.8″, -62° 33′ 4.9″). The putative resistant populations were compared with a known susceptible (S) population from the livestock fields of Col. Martín Fierro, with no history of herbicide use (-35° 43′ 50.2536″, -62° 51′ 2.1564″).

Plant growth

Seed germination was evaluated on moistened tissue paper with distilled water. Three replicates of 25 seeds per Petri dish were used for each population, and the assay was conducted in a growth chamber at a constant temperature of 25 °C. Germination and radicle emergence were observed as early as six hours after preparation of the Petri dishes, and maximum germination capacity was reached within 24 h: A, 96%; DB, 96%; P, 97%; and S, 92%.

The experimental units consisted of plastic pots measuring 11 cm in diameter and 11 cm in depth, each filled with 700 g of air-dried sandy-loamy soil (clay 4.7%, silt 17.0%, sand 78.3%, organic matter 0.6%, electrical conductivity 0.28 dS m^{-1} and pH 6.61). Six seeds per pot were sown at a depth of 0.5 cm. Pots were maintained under greenhouse conditions throughout the growing period and were periodically irrigated to keep soil moisture near field capacity. After emergence, the number of plants per pot was recorded.

Fresh weight in response to the recommended dose of imazapyr-imazamox

A trial was conducted to determine the response of *Bassia scoparia* to the recommended doses of a mixture of imazapyr (Clearsol® WG 80%) 80.00 g of active ingredients (ai) ha^{-1} and imazamox (Trigosol WG 70%) 20.30 g ai ha^{-1} compared with the control treatment, 0x. Methylated soybean oil adjuvant (Dash®) was added at 0.250 L ha^{-1}. Herbicides were sprayed using a JACTO DBJ-2.0 boom sprayer equipped with a JDF 04 flat fan spray nozzle, operating at constant pressure. The sprayer delivered a volume of 99 L ha^{-1}. Applications were conducted outdoors under the following environmental conditions: mean temperature of 14.8 °C, wind speed of 3.8 m s^{-1} from the east, and relative humidity of 87%. Treatments were applied at post-emergence, when plants reached the 10-leaf stage and 6 cm in height. After treatment, plants were returned to the greenhouse.

Shoot fresh weight (FW) was evaluated. Plants were harvested 30 days after treatment (DAT). The total FW per pot was divided by the number of plants to calculate percentage values, comparing herbicide-treated plants to the untreated control, which was considered 100%. The experiment followed a completely randomized design with five replicates per treatment per population. Data were analyzed using ANOVA and treatment means were compared with Fisher's Least Significant Difference (LSD) test (SAS Institute Inc., 2015).

Plant survival in response to imazapyr-imazamox doses

The lethal dose 50 (LD_{50}) is the amount of herbicide required to cause 50% mortality in a target population. In weed resistance studies, such as those involving kochia, LD_{50} is generally estimated through non-linear regression analysis of survival data. The response (e.g., % survival) is expressed as a percentage relative to the untreated control (100% survival). Increasing doses of a mixture of imazapyr (Clearsol® WG 80%) and imazamox (Trigosol® WG 70%) were applied: 0x, ¼x, ½x, 1x, 2x, 4x, and 8x, where x represents the field rate recommended by the manufacturer (80.00 + 20.30 g ai ha^{-1} of each herbicide, respectively). Methylated soybean oil adjuvant (Dash®) was added at 0.250 L ha^{-1}. Applications were performed outdoors using a JACTO DBJ-20 boom sprayer and equipped with a JDF 04 flat-fan nozzle, delivering 9 L ha^{-1} spray volume and operating at constant pressure. The treatments were applied at post-emergence, when plants reached the 10-leaf stage and approximately 6 cm in height. After application, plants were returned to the greenhouse.

Mortality was assessed visually at 30 DAT by counting the surviving plants per pot. Survival values were expressed as percentages relative to the control (0x), which was considered 100%. The experiment was conducted using a completely randomized design with five replicates per treatment and population. Data from survival counts were subjected to analysis of variance (ANOVA) in a "dose × population" arrangement. When the interaction was significant, the SLICE statement in SAS was used to partition and examine simple effects within the levels of the interacting factors. Survival data were then analyzed using nonlinear regression by fitting them to a three-parameter log-logistic dose–response model (Equation 1) (Streibig et al., 1993):

$$\text{Survival (\%)} = C+((D - C)/(1 + (x/LD_{50})^{b})) \qquad \text{Equation 1}$$

where Y is the survival percentage, C the lower bound, D the upper bound, b the slope, and LD_{50} the dose producing 50% survival. Model fitting was performed using PROC NLIN in SAS (SAS Institute Inc., 2015). Goodness of fit was evaluated by calculating the coefficient of determination (R^2) using GraphPad.

ALS gene sequencing

Leaves of five plants that survived an 8x dose of imazapyr-imazamox of P, DB, and A populations, and three untreated S plants were selected. The total DNA was isolated from the leaves following the method described by Doyle and Doyle (1990) at Chacra Experimental Integrada Barrow. DNA integrity and quantification were spectrophotometrically determined.

Partial ALS gene amplification was performed using the following primers: 5′-TCCTCTTCATTTCGCAACCT-3′ and 5′-TCAACCATCGATACGAACAT-3′. The PCR reaction consisted of an initial denaturation at 95 °C for 3 min, followed by 45 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 2 min, with a final extension at 72 °C for 8 min. The reaction mix included 300 ng DNA template, 1X reaction buffer (Inbio Highway), 1.5 mM $MgCl_2$, 0.8 mM of dNTPs, 0.5 μM of each primer, and 1 U Taq polymerase (Inbio Highway) in a 25 μL reaction mix. A 1912-bp fragment encompassing 122, 197, 205, 376, 377, 574, 653, and 654 codons was obtained as a single PCR product (Annex 1). It was detected in agarose gel (1%) using DNA lambda/hind III ladder (Inbio Highway) as marker (125- to 23130-bp). The amplicons were purified and sequenced from both ends by Macrogen Inc. (Seoul, Korea). Chromatograms obtained were viewed using Chromas v.2.6.4 (Technelysium Pty Ltd, South Brisbane, Australia). The sequence data were aligned and compared using Bioedit v.7.2 (North Carolina State University, Raleigh, USA).

RESULTS AND DISCUSSION

Fresh weight response to 1-x imazapyr imazamox dose

The use of 1x doses significantly reduced ($p < 0.05$) the shoot fresh weight (FW, g $plant^{-1}$) of the susceptible population S (13.83%) compared with the untreated control, 0x (100%). In contrast, the putative resistant populations DB (42.77%) and A (69.32%) did not differ from 0x. The P population also showed no reduction; in fact, its FW was markedly greater (160.76%) than that of the control, suggesting a growth stimulation response at the 1x imidazolinone rate (Fig. 1). Although no evidence supports a general improvement in kochia performance following herbicide field-rate application, numerous studies confirm that the response to herbicide treatment is highly population-specific. This is attributed to the species' wide genetic diversity and the documented presence of multiple and variable resistance mechanisms across populations (Kumar et al., 2019; Dhanda et al., 2024). For instance, Dhanda et al. (2024) reported populations with differential resistance levels–some surviving herbicide doses up to tenfold higher than the recommended rate–depending on their origin within the Southern Great Plains. These findings indicate that responses to field rates cannot be generalized across kochia and highlight the importance of assessing resistance and herbicide efficacy at the population level rather than the species level. Such stimulation in biomass could provide a competitive advantage to resistant plants, potentially contributing to their persistence and spread in the field. This behavior could exacerbate the problem if producers continue relying on Clearfield® technology for kochia management.

Dose responses

The S population of kochia was very well controlled at the half-recommended dose, reaching 6.9% survival, but never achieved complete control (1/4: 24.0%; 1/2: 6.9%; 1: 10.3%; 2: 7.4%; 4: 6.9%; and 8: 13.8%). While the putative resistant P population would have required higher rates than those evaluated in this trial, 8x barely reached a 50% mortality rate. Significantly higher herbicide dose would be required to control this population. The surviving plants from the resistant populations completed their cycle and produced viable offspring.

A significant "population x dose" interaction was observed ($p<0.05$). Plants belonging to the populations P, DB, and A survived doses of imazapyr + imazamox ≥ 1x (Imazapyr 80,00 + Imazamox 20.30 g ai ha^{-1}) (Fig. 2). Comparing the

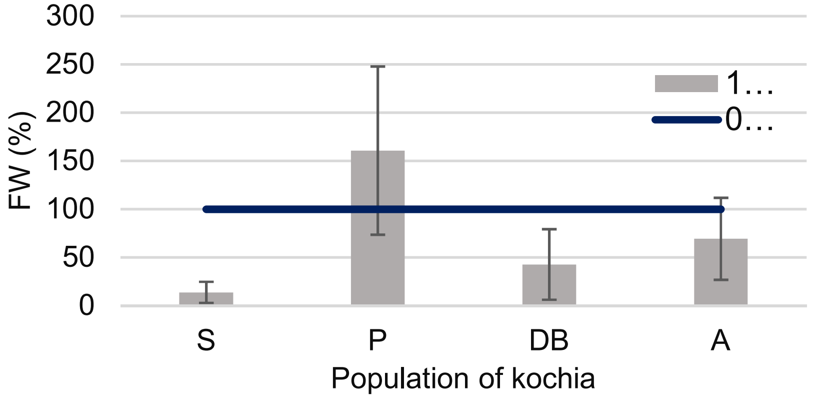

Fig. 1. Shoot fresh weight (FW) expressed in % to control (0x) for each population of kochia (*Bassia scoparia*) (Pellegrini, P; De Bary, DB; 30 de Agosto, A; and susceptible population, S).

LD_{50} of imazapyr + imazamox (g ai ha^{-1}) for each biotype, the sensitivity of the populations was: S (18.86 + 4.79) > DB (82.48 + 20.93) > A (179.36 + 45.51) > P (550.88 + 139.79). In P population, 50% survival was achieved with a dose close to the maximum one studied (8x = 640.00 + 162.40) (Table 1; Fig. 3). Primiani et al. (1990) found that 4 g ha^{-1} of imazapyr was required to inhibit growth by 50% in a susceptible biotype, and 10 g ha^{-1} for the resistant biotype. Kochia has been shown to have a high susceptibility to evolving resistance to herbicides (Kumar et al., 2019). In Argentina, populations resistant to herbicides had not yet been detected. Although Clearfield technology is often used in combination with residual pre-emergent applications, it is widely used in sunflower cultivation for weed management. The resistance observed in this study to the imazapyr -imazamox mixture confirms the challenges reported by sunflower producers.

ALS gene sequencing

In the three resistant populations analyzed, a single-point mutation from guanine to thymine was detected in codon 574 of the ALS gene. This transversion mutation implies a Trp-574-Leu substitution in ALS as the source of the phenotype in the imidazolinone-resistant plants (Fig. 4). It is known that this mutation confers resistance to imidazolinones and other ALS-inhibitor herbicides of different chemical families such as pyrimidinyl benzoates, triazolinones, sulfonylureas, and triazolopyrimidine (Powles and Yu, 2010; Deng et al., 2017; Nandula et al., 2020). Various biotypes with AHAS mutations have been reported in field populations of kochia

Table 1. Nonlinear regression parameters for survival (%) plants, p-value for model accuracy and the goodness of fit (R^2) were calculated in a dose-response trial for each population of kochia (*Bassia scoparia*) (Pellegrini, P; De Bary, DB; 30 de Agosto, A; and susceptible population, S).

Population	C (%)	D (%)	LD_{50} imazapyr+imazamox (g ai ha^{-1})	b	p-value	R^2
S	8.93	100.00	18.86 + 4.79	33.83	<0.001	0.99
P	33.38	97.25	550.88 + 139.79	4.31	<0.001	0.97
DB	35.15	76.82	82.48 + 20.93	17.91	<0.001	0.85
A	34.54	79.23	179.36 + 45.51	3.21	<0.05	0.85

The model fitted corresponded to: Survival (%) = $C+((D - C)/(1 + (x/LD_{50})^b))$.

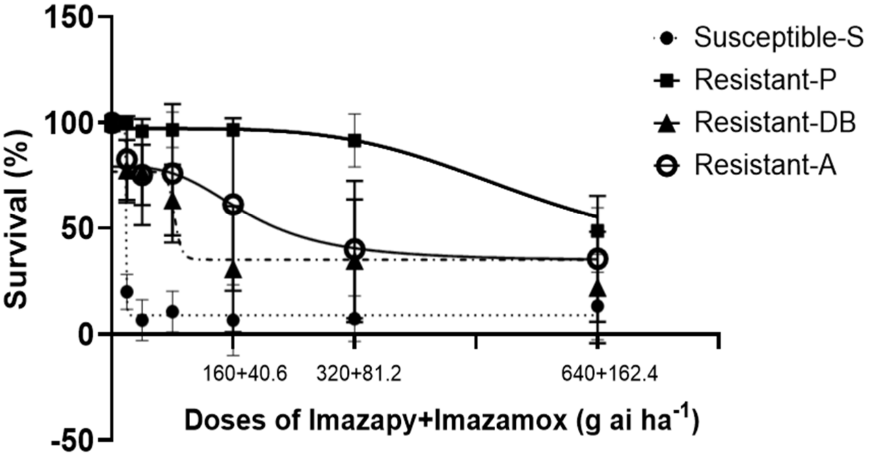

Fig. 2. Percentage survival plants of kochia (*Bassia scoparia*) at different doses of imazapyr + imazamox (g ai ha^{-1}). Symbols = Observed data. Lines = Estimated data. Pellegrini (P), De Bary (DB), 30 de Agosto (A), Susceptible (S).

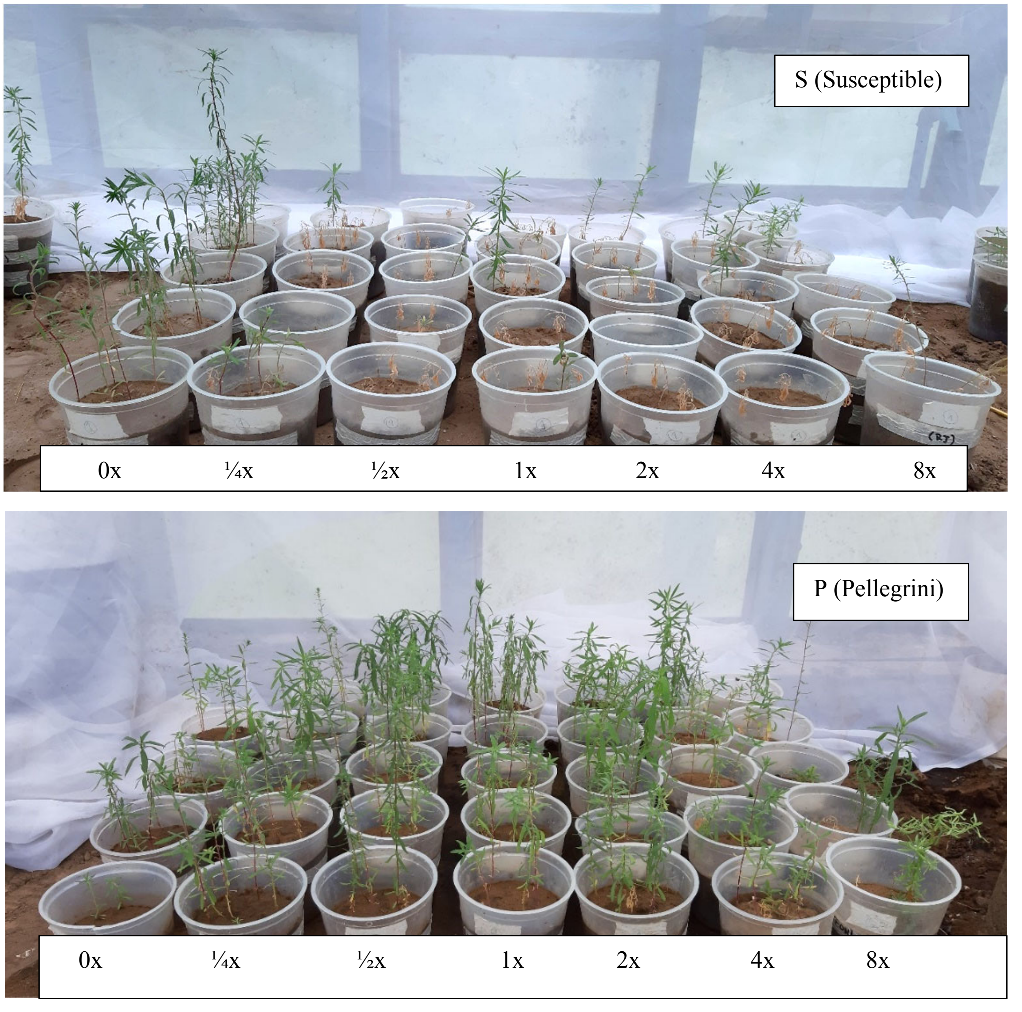

Fig. 3. Plants of kochia (Bassia scoparia) from the susceptible (S) and Pellegrini (P) populations, 30 days after treatment with the herbicide combination imazapyr + imazamox.

(Kumar et al., 2019). However, comparison of sequences from susceptible and resistant plants in these populations revealed no changes at condons 122, 197, 205, 376, 377, 653, or 654.

The Trp-574-Leu mutation has been reported in resistant kochia populations from western Canada, where this allele is the most frequent source of resistance to ALS-inhibitor herbicides (Warwick et al., 2008; Beckie et al., 2011). No penalties or little impact of Trp-574-Leu mutation on kochia growth were found in Canadian kochia populations (Légère et al., 2013). This allele was also detected in *B. scoparia* populations from the Czech Republic (Salava and Chodová, 2007). These results represent the first report of the Trp-574-Leu mutation in kochia populations from South America.

The confirmation of imidazolinone resistance in kochia poses a new challenge to sunflower farmers. Literature indicates that kochia emerges early at the end of winter, during the fallow period, which represents a critical window for implementing effective weed management in fields intended for sunflower cultivation (Geddes and Sharpe, 2022). Additionally, the extended emergence pattern continuing into midsummer demands prolonged and integrated control strategies (Dille et al., 2017). Torbiak et al. (2021) evaluated herbicide strategies for managing kochia in spring wheat. Some herbicides that proved effective in this study could be adapted for the management of this species in sunflower fields. During the fallow period, strategies based on tank mixtures of fluroxypyr and 2,4-D, or fluroxypyr/halauxifen combined with MCPA, applied at the early-season peaks of kochia emergence,

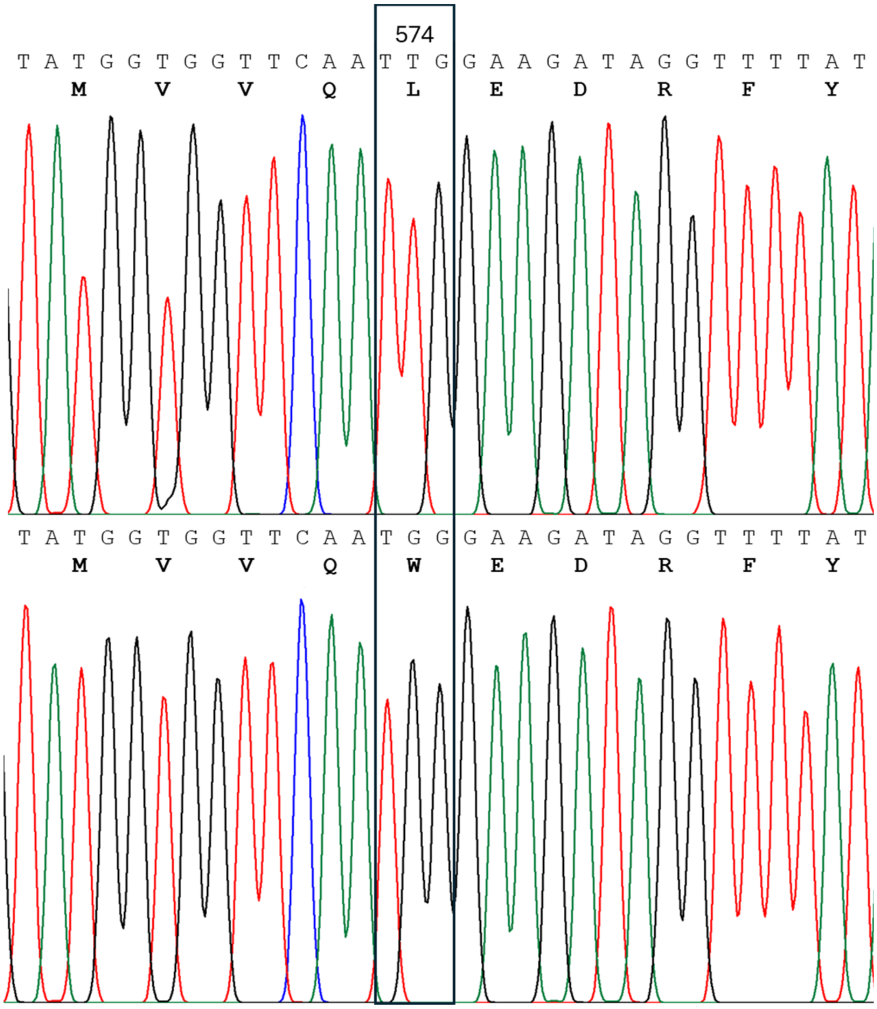

Fig. 4. Sequence and chromatograms of the ALS gene obtained from the imidazolinone-resistant (upper panel) and -susceptible (lower panel) plants of kochia (*Bassia scoparia)* and the conceptual translation of the amino acid sequence. The resistance-conferring 574 codon is shown in the box.

are critical to prevent its establishment and reduce seedbank replenishment. Furthermore, the use of sulfentrazone in combination with S-metolachlor (Reddy et al., 2012) as a residual treatment could provide extended control, limit subsequent flushes, and enhance the overall effectiveness of the program. Combining these chemical strategies with cultural and mechanical practices would strengthen an integrated weed management approach, improve long-term control sustainability, and reduce the risk of further herbicide resistance evolution.

In this context, it becomes critical to avoid the exclusive use of ALS-inhibiting herbicides, which are already compromised due to widespread resistance. Instead, it is recommended to apply residual herbicides effective against kochia, and to use mixtures involving different modes of action to reduce selection pressure. Monitoring of late-emerging plants and control escapes is also essential to prevent seedbank replenishment and ensure the effectiveness of the overall program.

Moreover, the finding of the Trp-574-Leu mutation that confers resistance to multiple ALS-inhibiting herbicides presupposes broader challenges in other crops that rely on this mode of action. This highlights the need for continued research into the biology of resistant kochia populations, as well as the development of diversified technologies and best management practices to sustain sunflower yield and weed control in the region.

CONCLUSIONS

The evidence confirms the resistance to imidazolinones in kochia collected in western Buenos Aires Province. Our results are the first case of evolved herbicide resistance in kochia in Argentina. This study provides valuable insights into the resistance of kochia to different herbicides, including ALS inhibitors, which are not only applicable to sunflower weed management.

Author contributions

Jorgelina C. Montoya participated actively in bibliographic review, development of the dose-response experiments, analysis of data and discussion of results. Esteban E. Fabressi carried out dose-response experiments and participated in the bibliographic review. Marcos E. Yanniccari carried out the molecular analysis, reviewed and approved the final version of the article.

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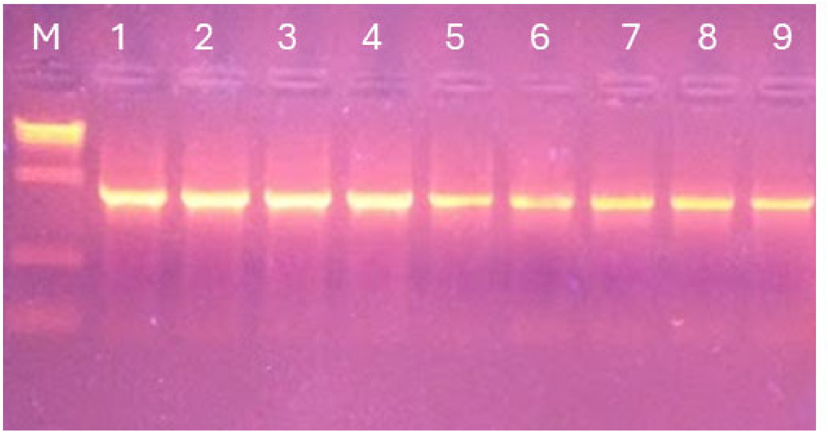

Annext 1. Ampliacions (1912-bp) obtained from plants of kochia (*Bassia scoparia*) that survived to an 8x dose of imazapyr-imazamox of P (1 and 2), DB (3 and 4) and A (5 and 6) populations, and untreated S plants (7, 8 and 9). A DNA lambda/hind III ladder (Inbio Highway) as marker (125- to 23130-bp) (M).