Mesotrine use for selective post-emergence control of glyphosate-resistant *Conyza* spp. in black oats

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**Abstract:** Background: Achieving satisfactory weed control levels in black oat (*Avena strigosa*) fields is often difficult, owing to the limited number of registered herbicide molecules.

**Objective:** To determine novel options for selective, post-emergence chemical weed control in this crop.

**Methods:** Eight herbicide treatments were sprayed in the field onto black oat plants at the tillering stage to evaluate crop safety and control of *Conyza* spp. and *Gamochaeta americana*, two major weeds of Brazilian black oats. A separate trial was conducted to further assess herbicide safety in a controlled-environment setting, and mesotrine selectivity was then re-evaluated separately in the greenhouse during the following growing season. Crop phytotoxicity was determined using biomass production both in the field and in greenhouse trials.

**Keywords:** U46; *Avena strigosa* Schreb; bentazon; herbicides; HPPD; metsulfuron-methyl

**Results:** Mesotrine, bentazon, 2,4-D, and a 2,4-D+bentazon tank-mix produced light symptoms of crop phytotoxicity, from which plants quickly recovered. *Conyza* spp. control was achieved via applications of mesotrine (192 g a.i. ha⁻¹), metsulfuron-methyl (3.9 g a.i. ha⁻¹), and a 2,4-D + bentazon tank-mix (502.5 g a.e. ha⁻¹ + 720 g a.i. ha⁻¹, respectively), whereas proper *G. americana* control was only achieved via applications of either mesotrine or metsulfuron-methyl. Biomass accumulation by black oat plants in the greenhouse was similar across mesotrine-treated and untreated plants.

**Conclusions:** Mesotrine could become an option for selective, post-emergence weed control in black oat fields, aiding in the fight against multiple herbicide-resistant *Conyza* spp. populations which are widespread across major black oat-growing areas in Brazil.

**1. Introduction**

Herbicide usage represents an inexpensive, efficient option for weed management in crops and pastures, delaying the onset of the critical period of weed interference and increasing productivity (Hussain et al., 2011). Weed allelopathy and competition for resources are known to decrease biomass production in forage species, from establishment to regrowth stages. The elimination of toxic weed species in pastures is also a major advantage related to herbicide applications, hence lowering the risk of intoxication or even death which could follow biomass ingestion by farm animals (Russell et al., 2018).

Black oats (*Avena strigosa* Schreb), also referred to as Japanese or bristle oats, are an annual, C₃-type grass species which resemble common oats (*Avena sativa* L., also referred to as white or yellow oats); however, plants of the former are larger and deeper rooting (Martins et al., 2016). This cold-adapted crop is commonly grown in southern Brazil in late autumn and winter months and primarily used for forage purposes, grown either singular (as a single crop) or in consortium with other forage species, or as cover cropping to provide biomass mulch for direct seeding of summer crops. Black oats have become increasingly popular in direct-seeded systems due to its elevated tillering capacity, low soil fertility requirements, and large production of slow-decaying biomass which greatly suppress growth of several weed species (Oliveira Neto et al., 2010). A combined total of nearly 430,000 ha were cultivated in 2020 with either common oats, black oats, short oats (*Avena brevis* Koch) in Brazil, which represents a 400% increase in just 13 years (Companhia Nacional de Abastecimento, 2021).

In Brazil, three troublesome species in the *Conyza* genus (syn. *Erigeron*; horseweed or fleabane) constitute major weeds of summer crops such as soybeans (*Glycine max* (L.) Merr.) and maize (*Zea mays* L.: *Conyza canadensis* (L.) Cronquist, *C. bonariensis* (L.) Cronquist, and *C. sumatrensis* (Retz.) E. Walker. The latter, commonly named Sumatran fleabane or tall fleabane (Figure 1D), is native to Brazil and South America but has spread to all continents (Borges et al., 2015; Pruski, Sancho, 2006), suggesting strong invasiveness capabilities which might be related to its elevated production of small, easily-dispersed seeds which germinate rapidly (Hao et al., 2009). *C. sumatrensis* has recently developed resistance to multiple herbicides in this country, including a
unique, worrisome case of resistance to 2,4-D due to rapid necrosis (Heap, 2021; Queiroz et al., 2020).

Similarly to Conyza spp., Gamochaeta americana (Mill.) Wedd. (syn. Gnaphalium coarctatum Willd. or Gamochaeta coarctata (Willd.) Kerguelen), commonly named gray everlasting (Figure 1B), is also a member of the Asteraceae (sunflower) family. Such species is native to the Americas, occurring in the Central Brazilian Savanna, Atlantic Rainforest, and Pampa regions (Loeuille, 2020). Despite being present in carrots (Daucus carota L.) and sugarcane (Saccharum officinarum L.)-growing fields, G. americana represents a more serious threat to soybeans, maize, common oats, and wheat (Triticum aestivum L.) production in Brazil (Piza et al., 2016).

Currently, farmers who choose to grow black oats in Brazil have only one post-emergent herbicide active ingredient at their disposal, i.e. the acetolactate synthase (ALS)-inhibitor metsulfuron-methyl. Pre-plant burndown using glyphosate is the only other remaining chemical weed control option currently available (Ministério da Agricultura, Pesca e Abastecimento, 2021). This fact complicates proper weed control in this crop, as some weed species evade control following metsulfuron-methyl spraying, potentially causing large yield losses. Additionally, herbicide mode of action rotation is affected severely. Among many herbicide modes of action available for use in the Brazilian market, the so-called 4-HPPD inhibitors (4-hydroxyphenylpyruvate dioxygenase, EC 1.13.11.27; WSSA group 27), which are commonly referred to as bleachers (Figure 1A) and act by inhibiting a key enzyme in the carotenoid biosynthetic pathway, have received great attention and scrutiny in recent years due to their wide use and broad weed control spectrum (Grossmann, Ehrhardt, 2007). 4-HPPD inhibitors such as mesotrione (2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione) might also display selectivity to major crops such as sugarcane and maize (Pannacci, Covarelli, 2009). Importantly, to date no case of resistance to 4-HPPD inhibitors have been reported in Brazil (Heap, 2021).

Spraying herbicides earlier into the winter growing season, when most Conyza spp. seedlings emerge, has been shown to facilitate summer crop sowing practices and improve early-growth conditions due to significantly lower weed infestation levels (Oliveira Neto et al., 2010). It also allows for proper herbicide mode of action rotation, potentially delaying or even preventing herbicide resistance evolution. In the present study, we aimed at evaluating crop injury caused by different herbicide treatments (sprayed either alone or as tank-mixtures) as well as G. americana and glyphosate-resistant Conyza spp. control efficacy in the field, such that novel chemical control options for Brazilian black oats can be determined.

2. Material and Methods

Three studies (one field and two greenhouse trials) were conducted in 2016 and 2017 in order to determine crop phytotoxicity levels as well as weed control efficacy in real field conditions. All field and greenhouse trials were conducted at an experimental station located in Itaara, RS/Brazil (29°35′06.69″S, 53°48′28.53″W; 460 m elevation). The municipality’s climate is classified as a Cfa (humid subtropical) according to the Köppen-Geiger classification, and soil analysis performed at the experimental area indicated average soil organic matter and clay percentages of 3.4 and 27.0, respectively, as well as <15% sand. Conyza species in the experimental site were identified following Lazaroto et al. (2008) as a 50:50 mix of Conyza sumatrensis and C. canadensis; such populations were mostly composed of glyphosate-resistant individuals as demonstrated in a study conducted in the previous year in which glyphosate spraying at 1,800 g a.e. ha⁻¹ resulted in 85% plant survival (data not shown).

Black oat seeds were purchased at a local seed supply store; the actual cultivar is referred to as “common” or regular black oats. The crop was drill-seeded on July 10th, 2016 in a no-till system with a row spacing of 0.17 m, requiring nearly 80 kg ha⁻¹ for a harvest density of 3,000,000 plants ha⁻¹. Soybeans were grown in the experimental area in the previous summer (late November 2015 through March 2016). Crop fertilization was performed during sowing at a rate of 300 kg ha⁻¹ of 08-18-28 (NPK ratio), and 150 kg ha⁻¹ of urea (46% N) were broadcast-applied at the tillering stage.

Eight herbicide treatments (Table 1) plus an untreated control check consisting of distilled water-only were sprayed in the field onto black oat plants in order to determined crop phytotoxicity and weed control efficacy. Rates for all herbicides except saflufenacil and flumioxazin were chosen based on labelled rates at which these herbicides are sprayed onto other crops in order to ensure optimum weed control performance. Since saflufenacil and flumioxazin are commonly sprayed as part of burndown treatments and hence not as postemergence, selective herbicides, their rates were at least 50% lower than recommended burndown use rates instead (Ministério da Agricultura, Pesca e Abastecimento, 2021) in order to allow for the study of crop selectivity. Conyza spp. density averaged 3.1 ± 0.8 plants m⁻² (mean±SE) at the time of herbicide spraying in the field. A separate trial was conducted on October 2016 in a controlled-environment setting in order to further assess herbicide safety, and did not include 2,4-D and bentazon sprayed alone as well as an imazamox+bentazon premix formulation. Mesotrione was then re-evaluated separately in the second greenhouse study during the following growing season; these trials are thereafter referred to as greenhouse trials #1 and #2, respectively. The actual number of herbicide treatments in greenhouse trial #1 was decreased relative to the field study since the latter was conducted roughly 10 weeks prior, allowing for the elimination of treatments producing unwanted results (such as severe phytotoxicity or low weed control efficacy).

A CO₂-pressurized backpack sprayer calibrated to deliver 150 L ha⁻¹ at 210 kPa and equipped with a spray
Table 1 - List of all herbicides (common and trade names) employed in the field trial, as well as application rates (g a.i.1 or a.e.2 ha−1), and adjuvant concentration (% v/v) in the spray mixture. An untreated control check consisting of distilled water-only was also included to allow for herbicide phytotoxicity-related impacts onto black oat plants to be determined.

| Common name          | Trade name | Application rate1 | Adjuvant (% v/v) |
|----------------------|------------|------------------|-----------------|
| 2,4-D amine2         | U46 Prime  | 502.52           | -               |
| Bentazon2            | Basagran 600 | 720             | Assist 10.3     |
| 2,4-D+Bentazon2      | DMA 806+Basagran | 502.5 + 720 | -               |
| Flumioxazin          | Flumyzin 500 WP | 25             | Dash 0.25       |
| Mesotrione           | Callisto   | 192              | Assist 0.5      |
| Metsulfuron-methyl   | Ally       | 3.9              | Assist 0.1      |

¹a.i., active ingredient; a.e., acid equivalent; ²these treatments were only employed in field trials; ³rate expressed in L ha−1 rather than % v/v.

3. Results and Discussion

ANOVA results indicated that crop phytotoxicity and dry aboveground biomass levels differed significantly (p≤0.05) across treatment means, indicating herbicides impacted black oat plants’ growth differently (Tables 2-4). Despite being the only post-emergent herbicide registered for use in black oats, spraying metsulfuron-methyl has been shown to reduce biomass accumulation of black oats (Dellazen et al., 2015). Here, it resulted in mild crop phytotoxicity which lasted throughout the course of the trial (Table 2). Crop plants appeared stunted and mild leaf chlorosis developed 8-10 days after spraying, which are known ALS inhibitor-related symptoms (Roman et al., 2007). However, biomass production was not impacted (Table 3), indicating crop plants had recovered from the treatment.

Surprisingly, spraying mesotrione resulted in low crop phytotoxicity levels in the field (Table 2) and greenhouse (Table 4) trials, and allowed for crop biomass production levels which matched those of the untreated control check (Table 3), even though it was used at 192 g a.i. ha−1. This corresponds to 0.4 L ha−1 of Callisto®, which is, in turn, the highest labelled rate and the one expected to allow for the greatest weed control level attainable by this molecule. To further explore mesotrione’s selectivity to black oats, the herbicide was then re-evaluated in the next growing season (e.g. greenhouse trial #2), yielding crop phytotoxicity and biomass production results which resemble those of the untreated control check (Table 5; Figure 1B). Reports in the literature indicate that mesotrione applied either pre- or post-emergence was also selective to common oats and barley (Hordeum vulgare L.) plants, whereas only applications at pre-emergence displayed sufficient selectivity to wheat (Soltani et al., 2011).

The only herbicide treatment found to significantly decrease black oat biomass production levels was an imazamox + bentazon premix formulation (Table 3), whereas such results paralleled visual crop phytotoxicity levels, at which widespread plant death was recorded at 34...
positive impact on weed control efficacy. Thus, their use in black oats as post-emergence treatments seems unlikely, given that the actual rates needed for efficient weed control are significantly above those employed at the present work.

DAS (Table 2; Figure 1C). This effect was not observed when bentazon was sprayed alone, strongly suggesting lack of imazamox selectivity to black oats. Interestingly, a similar result was reported by Harker et al. (2007) while studying wild oats (Avena fatua L.). Such results are not unexpected, given that the imazamox + bentazon premix formulation is commonly utilized for grassy weed control in broadleaved crops such as common beans (Phaseolus vulgaris L.) and alfalfa (Medicago sativa L.) (Hekmat et al., 2008).

The protoporphyrinogen oxidase (PPO) inhibitors flumioxazin and saflufenacil caused mild to severe early phytotoxicity levels (Table 2), which declined around 28 DAS and did not impact aboveground biomass production (Table 3). However, as previously mentioned, saflufenacil mand flumioxazin rates used in the present study (Table 1) were at least 50% lower than recommended use rates in order to allow for the study of crop selectivity. Based on these results, one can expect that higher rates of both herbicides would damage the crop even further, despite a

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### Table 2 - Herbicide phytotoxicity levels (%) displayed by black oat plants recorded at 7, 13, 21, 28, and 34 days after spraying (DAS) in the field.

| Treatment                  | Evaluation date (days after spraying) |
|----------------------------|---------------------------------------|
|                            | 7 DAS       | 13 DAS    | 21 DAS    | 28 DAS    | 34 DAS    |
| Untreated check            | 0.0         | d          | 0.0       | e          | 0.0       | d          |
| Imazamox+Bentazon          | 43.7 b      | 82.0 a     | 96.0 a    | 96.2 a    | 100.0 a   |
| 2,4-D amine                | 10.0 cd     | 0.0 d      | 17.5 c    | 10.0 c    | 11.0 cd   |
| Saflufenacil               | 37.5 b      | 14.2 c     | 5.5 de    | 0.0 d     | 0.0 d     |
| Bentazon                   | 4.5 d       | 0 d        | 0.0 e     | 0.0 d     | 0.0 d     |
| 2,4-D+Bentazon             | 8.7 cd      | 0.0 d      | 12.2 cd   | 8.0 cd    | 13.7 cd   |
| Flumioxazin                | 73.7 a      | 28.7 b     | 12.2 cd   | 0.0 d     | 0.0 d     |
| Mesotrione                 | 12.5 cd     | 0.0 d      | 0.0 e     | 0.0 d     | 0.0 d     |
| Metsulfuron-methyl         | 27.5 bc     | 28.7 b     | 43.7 b    | 27.5 b    | 33.7 b    |

1Numbers indicate mean values calculated from four replications; 2Means followed by the same lower-case letter within columns are not statistically different according to Tukey’s HSD test (p≤0.05).

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### Table 3 - Dry aboveground biomass (DAB, expressed in kg ha⁻¹) accumulated in the field at each treatment.

| Treatment                  | DAB (kg ha⁻¹) |
|----------------------------|---------------|
| Untreated control          | 5062.5 a      |
| Imazamox+Bentazon          | 0.0 b         |
| 2,4-D amine                | 46875 a       |
| Saflufenacil               | 55625 a       |
| Bentazon                   | 54375 a       |
| 2,4-D+Bentazon             | 41875 a       |
| Flumioxazin                | 38125 a       |
| Mesotrione                 | 50625 a       |
| Metsulfuron-methyl         | 36500 a       |

1Means followed by the same lower-case letter within columns are not statistically different according to Tukey’s HSD test (p≤0.05).

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### Table 4 - Phytotoxicity levels (%) of treated and untreated black oat plants, recorded at 7, 14, and 21 days after spraying (DAS) at greenhouse trial #1.

| Treatment                  | Crop Phytotoxicity (%) | Greenhouse Trial #1 |
|----------------------------|------------------------|---------------------|
|                            | 7 DAS      | 14 DAS    | 21 DAS    |
| Untreated check            | 0.0        | d          | 0.0       | ns        |
| Saflufenacil               | 40.0       | b          | 9.2       | 175       |
| 2,4-D+Bentazon             | 20.8 bcd   | 5.0        | 3.3       |
| Flumioxazin                | 80.8 a     | 27.5       | 16.7      |
| Mesotrione                 | 17.5 cd    | 7.5        | 3.3       |
| Metsulfuron-methyl         | 25.5 bc    | 6.7        | 6.7       |

1Numbers indicate mean values calculated from six replications; 2Means followed by the same lower-case letter within columns are not statistically different according to Tukey’s HSD test (p≤0.05); 3ns indicates non-significant values (p>0.05) according to ANOVA results obtained for assessments performed at 14 and 21 DAS (p=0.081 and p=0.67, respectively).

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### Table 5 - Herbicide-related crop phytotoxicity levels (%) determined at 7, 14, and 21 days after spraying (DAS), and dry aboveground biomass (expressed in g plant⁻¹) measured at the end of greenhouse trial #2.

| Treatment                  | Crop Phytotoxicity (%) | Greenhouse Trial #2 | DAB (g plant⁻¹) |
|----------------------------|------------------------|---------------------|-----------------|
|                            | 7 DAS      | 14 DAS    | 21 DAS    | 7 DAS      | 14 DAS    | 21 DAS    |
| Untreated control          | 0.0        | ns²       | 0.0       | ns²       | 0.0       | ns²       |
| Mesotrione                 | 12.0       | 2.0       | 2.5       | ns²       | 66.0      | ns²       |
|                          | 63.4       | ns²       | 63.4      | ns²       | ns²       | ns²       |

1Numbers indicate mean values calculated from four replications; 2ns indicates non-significant values (p>0.05) according to ANOVA results obtained for both crop phytotoxicity and DAB variables.
Figure 1 - (A) Mesotrione-treated black oat plant alongside goosegrass (Eleusine indica [L]. Gaertn.) plant demonstrating typical bleaching symptoms set forth by applications of 4-HPPD inhibitors such as mesotrione; (B) Similar-looking treated and untreated black oat plants 21 days after mesotrione spraying in greenhouse trial #2; (C) Overall appearance of a field plot which had been sprayed with an imazamox + bentazon premix formulation 28 days earlier; (D and E) Glyphosate-resistant Conyza sp. (D) and Gamochaeta americana (E) plants found growing in untreated control plots, 28 days after treatment spraying was performed. At the lower right-hand side, a close-up photo of G. americana’s inflorescence.

| Treatment                  | Glyphosate-resistant Conyza spp. Control (%) |
|----------------------------|---------------------------------------------|
|                            | 7 DAS | 13 DAS | 21 DAS | 28 DAS | 34 DAS |
| Untreated control          | 0\(^1\) | c\(^2\) | 0 | d | 0 | d | 0 | d | 0 | c |
| Imazamox+Bentazon          | 99 | a | 80 | ab | 45 | c | 19 | cd | 6 | c |
| 2,4-D amine                | 99 | a | 83 | ab | 84 | ab | 73 | ab | 73 | b |
| Saflufenacil               | 99 | a | 85 | ab | 83 | ab | 67 | b | 15 | c |
| Bentazon                   | 85 | a | 64 | bc | 56 | c | 33 | c | 20 | c |
| 2,4-D+Bentazon             | 99 | a | 96 | a | 90 | ab | 91 | ab | 72 | b |
| Flumioxazin                | 65 | b | 19 | cd | 9 | d | 0 | d | 0 | c |
| Mesotrione                 | 99 | a | 92 | a | 96 | a | 94 | a | 99 | a |
| Metsulfuron-methyl         | 92 | a | 22 | cd | 75 | b | 71 | ab | 77 | b |

\(^1\)Numbers indicate mean values calculated from four replications; \(^2\)Means followed by the same lower-case letter within columns are not statistically different according to Tukey’s HSD test (\(\alpha = 0.05\)).

Table 7 - Gamochaeta americana control levels (%) recorded from 7 to 34 days after herbicide spraying in the field.

| Treatment                  | Gamochaeta americana Control (%) |
|----------------------------|----------------------------------|
|                            | 7 DAS | 13 DAS | 21 DAS | 28 DAS | 34 DAS |
| Untreated check            | 0\(^1\) | c\(^2\) | 0 | d | 0 | b | 0 | b | 0 | c |
| Imazamox+Bentazon          | 94 | ab | 91 | a | 80 | a | 64 | a | 100 | a |
| 2,4-D amine                | 45 | bc | 20 | cd | 14 | b | 0 | b | 0 | c |
| Saflufenacil               | 99 | a | 86 | ab | 74 | a | 81 | a | 57 | b |
| Bentazon                   | 95 | ab | 81 | ab | 77 | a | 58 | a | 0 | c |
| 2,4-D+Bentazon             | 99 | a | 87 | ab | 91 | a | 75 | a | 28 | bc |
| Flumioxazin                | 75 | ab | 25 | cd | 10 | b | 0 | b | 0 | c |
| Mesotrione                 | 87 | ab | 46 | a | 93 | a | 100 | a | 100 | a |
| Metsulfuron-methyl         | 51 | abc | 46 | bc | 56 | a | 98 | a | 100 | a |

\(^1\)Numbers indicate mean values calculated from four replications; \(^2\)Means followed by the same lower-case letter within columns are not statistically different according to Tukey’s HSD test (\(\alpha = 0.05\)).
The remaining treatments (i.e. 2,4-D, bentazon, and a 2,4-D + bentazon tank-mix) resulted in light crop phytotoxicity and did not impact biomass accumulation (Tables 2 and 3, respectively), and hence could become broadleaf weed control tools. These results corroborate Vargas and Roman (2005) indicating 2,4-D and bentazon selectivity to winter cereals such as barley, black oats and even Italian ryegrass (Lolium perenne L. spp. multiflorum) used for forage purposes in Southern Brazil.

Mesotrione was the only herbicide treatment achieving satisfactory (>80%) levels of glyphosate-resistant Conyza spp. control at 34 DAS. This was followed by metsulfuron-methyl and 2,4-D applied either alone or tank-mixed with bentazon, which resulted in control levels above 70% while also preventing weed regrowth. Interestingly, all metsulfuron methyl-based products sold in Brazil are not labelled for Conyza spp. control (Ministério da Agricultura, Pesca e Abastecimento, 2021). Moreover, weed control efficacy following flumioxazin or saflufenacil applications were likely impaired by lower-than-recommended rates employed in this study, and might not reflect their true ability to control these species. However, satisfactory G. americana control was only realized via applications of mesotrione, metsulfuron-methyl, or an imazamox + bentazon premix formulation suggesting some degree of tolerance to 2,4-D herbicide which was not previously identified in the literature and remains to be elucidated.

2,4-D is widely used to improve glyphosate-resistant Conyza spp. control during summer crop pre-plant burndown or during winter months, when some fields are left fallow (Oliveira Neto et al., 2010). However, due to recent reports of simultaneous resistance to 2,4-D, glyphosate, PPO, and ALS inhibitors in Brazilian C. sumatrensis biotypes (Heap, 2021), growers are advised to spray herbicides with different modes of action, as well as implementing integrated weed management control practices. In this scenario, post-emergence applications of the 4-HPPD inhibitor mesotrione could constitute a new option for weed management in black oats, aiding in the fight against multiple-herbicide resistant Conyza spp. which grow during late autumn and winter months. Its use, however, is pending further studies for registration as required by the country’s law.

To our knowledge, this is the first report of selectivity to mesotrione in black oats. Given that results by Cargnin et al. (2015) pointed toward different levels of tolerance to herbicides among common oat genotypes, ongoing research efforts are aimed at determining responses of black oat cultivars to a wide range of herbicides as well as understanding the mechanism(s) of mesotrione tolerance demonstrated by this crop.

4. Conclusions

Applications of mesotrione as well as 2,4-D, either alone or tank-mixed with bentazon, are safe for black oat plants when performed during mid-tillering stages while also allowing for excellent glyphosate-resistant Conyza spp. control; with the former also effective for controlling G. americana. In the wake of a troubling case of resistance to multiple herbicides including 2,4-D and PPO inhibitors in Conyza sumatrensis, growers have been strongly advised to spray herbicides with different modes of action and alternate these. In this scenario, the 4-HPPD inhibitor mesotrione might represent an option for post-emergence weed control in black oat fields, aiding in the fight against multiple-herbicide resistant Conyza spp. which grow during late autumn and winter months.

Author’s contributions

RMP and RCAN: conceptualization of the manuscript and development of the methodology; data collection and curation; data analysis; data interpretation; funding acquisition and resources: not related to this project; writing the original draft of the manuscript. RMP: project administration; supervision. RMP, RVF, DDN, and RCAN: writing, review and editing: All authors read and agreed to the published version of the manuscript.

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