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Multiple resistance in goosegrass to clethodim, haloxyfop-methyl and glyphosate

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Abstract: Background: Goosegrass is one of the most troublesome weed species in Brazil due to its wide dispersion and the ability to evolve herbicide resistance.

Objective: Investigate the response of goosegrass accessions from Mato Grosso, Brazil to glyphosate, clethodim, and haloxyfop.

Methods: Goosegrass seed samples were collected from seventeen production fields (accessions) in Mato Grosso and screened with the recommended label rates of clethodim, haloxyfop-methyl, and glyphosate. Six accessions withstood the label rate of the evaluated herbicides and were subjected to a dose-response study. Out of the six accessions, two were selected for further F1 dose-response investigations due to their ability to survive the label rate of the three herbicides individually. All studies were conducted under greenhouse conditions in a completely randomized design with four replications, and the F1 dose-response study was replicated in time.

Results: All six accessions investigated in the dose-response study presented ED_{50} values higher than susceptible plants for control and biomass reduction. Haloxyfop-methyl had the highest resistance ratios, followed by clethodim and glyphosate. The two accessions investigated in the F1 dose-response study were confirmed to be cross-resistant to clethodim and haloxyfop-methyl and showed low-level resistance to glyphosate.

Conclusions: The continuous reliance of POST herbicides for weed management in Mato Grosso cropping systems has selected goosegrass accessions that can withstand high rates of ACCase-inhibiting herbicides, particularly haloxyfop-methyl. Moreover, two accessions with resistance to ACCase-inhibiting herbicides, clethodim and haloxyfop-methyl, and low-level resistance to glyphosate were identified, suggesting the presence of multiple resistance in goosegrass accessions from Mato Grosso, Brazil.

Keywords: *Eleusine indica;* Graminicides; Cyclohexanediones; Aryloxyphenoxypropionates

1. Introduction

Weed resistance is defined as *"the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type"* (Heap, 2005). This evolutionary process has been favored with the continuous selection pressure imposed by the overreliance on herbicides for weed control (Neve et al., 2009). Since the first confirmed herbicide weed resistance case in the fifties, more than 500 new cases have been reported worldwide (Heap, 2021). Herbicide resistance poses a major challenge to cropping systems due to an increase in inputs and management costs, losses to weed interference, difficulty in controlling resistant populations, and threatening the future of herbicide options for weed management (Délye et al., 2013; Lucio et al., 2019). In Brazil, 53 resistance cases have been reported, affecting mainly soybean, corn, rice, and cotton production systems (Heap, 2021). Moreover, amongst the several herbicide resistance cases that have been confirmed in Brazil, goosegrass appears as one of the most troublesome species prone to evolve resistance (Oliveira et al. 2020; Lucio et al. 2019).

Goosegrass (*Eleusine indica* (L.)) is an annual grass species from the Poaceae family that reproduces exclusively through seeds and is characterized by its rapid growth and tiller development (Takano et al., 2016). During its 120 days life cycle, it can reach 30 - 50 cm in height, and one single plant can produce up to 120,000 seeds (Takano et al., 2016). It is considered one of the five most troublesome weed species in the world due to its wide occurrence and numerous resistance cases reported worldwide (Heap, 2021; Holm et al., 1977). Furthermore, goosegrass was considered the second most common weed species in Brazil, infesting a broad range of cropping systems (Lucio et al., 2019).

Among the 37 goosegrass resistance cases reported worldwide (Heap, 2021), some have drawn the attention of the weed science community due to their occurrence and complexity. For instance, goosegrass was the first species to evolve the single (Pro106Ser), and later on, the double (Tre102Ile e Pro106Ser) mutations in the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) enzyme, conferring low to moderate and high levels of resistance to glyphosate, respectively (Baerson et al., 2002; Yu et al., 2015). Furthermore, goosegrass was the first species to develop two

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target-site mechanisms in the same population conferring resistance to glyphosate. Biotypes found in Mexico were confirmed to be resistant due to both the Pro106Ser mutation and the EPSPS gene overexpression acting simultaneously against glyphosate (Gherekhloo et al., 2017). In Malaysia, goosegrass was also confirmed as the first species to develop multiple resistance to the three non-selective herbicides glyphosate, glufosinate, and paraquat (Jalaludin et al., 2014). Altogether, these reports indicate the likelihood of goosegrass to evolve resistance to herbicides used for its management.

In Brazil, the first goosegrass resistance case was reported in 2003, where biotypes evolved an ACCase (Acetyl coenzyme A carboxylase) mutation conferring resistance to the herbicides cyhalofop-butyl, fenoxaprop-p-ethyl e sethoxydim (Osuna et al., 2012). At that time, these herbicides were commonly used for POST emergence grass control in soybean since the Roundup Ready® (RR®) technology was yet to arrive in Brazil. Once the RR® technology became available, glyphosate also became the main POST emergence herbicide option for weed management in RR crops (Duke, Powles, 2011). Consequently, due to its overreliance, several species, amongst them goosegrass in 2016, have been reported resistant to glyphosate in Brazil (Takano et al., 2017; 2018a; Heap, 2021). Moreover, a more complex case was confirmed a year later, where goosegrass plants survived applications of glyphosate and the ACCase-inhibiting herbicides fenoxaprop-p-ethyl and haloxyfop-methyl, representing the first multiple resistance case of goosegrass in Brazil (Heap, 2021). Glyphosate and ACCase-inhibiting herbicides play a critical role in POST-emergence grass control in soybean and cotton cropping systems due to their high efficacy and selectivity (Andrade Junior, 2018; Takano et al., 2018b; 2021). Herbicide resistance monitoring plays a crucial role in the timely identification, management, and mitigation of weed resistance cases under field conditions. Hence, this study aimed to investigate the response of several goosegrass accessions from Mato Grosso, Brazil to clethodim, haloxyfop, and glyphosate.

2. Material and Methods

2.1 Plant material

Goosegrass seeds were collected in commercial fields planted with either corn or cotton in a double-crop system following soybean harvest during March and April of 2019 near Primavera do Leste, Mato Grosso. Seeds were collected in areas with a history of high adoption of glyphosate and ACCase-inhibiting herbicides where producers reported recent goosegrass control escapes after using these chemistries (Nunes, personal communication). In total, seeds were collected from 20 to 30 mature plants per field from a total of seventeen fields (each field was treated as an accession). When seeds were collected, each field had received at least one POST-emergence herbicide application in crop (corn or cotton). Therefore, the selected goosegrass plants had likely survived an application of either glyphosate or ACC-inhibiting herbicide. During sampling, seeds from each field were stored in paper bags and adequately identified with the geographical coordinates and the number of the accession (A1 to A17). Seeds were then air-dried, manually cleaned, and stored in paper bags under room temperature until the beginning of the studies.

2.2 Experimental Design

All studies were conducted under greenhouse conditions in a completely randomized design with four replications. Each experimental unit consisted of a plastic pot (5 dm^3) filled with soil (clay: 36.5%; sand: 56.5%; silt: 7.0%; organic matter: 2.3%; and pH: 6.2), except for the first 3 cm of the pot surface that was filled with comercial potting mix and changed at the end of each experimental run to prevent seeds of different species and accessions from germinating and contaminating the experimental unit. Fifteen seeds of each accession were planted directly into the pots, and after emergence, pots were thinned to four uniform seedlings selected to be sprayed and evaluated.

2.3 Herbicide Applications

Herbicides treatments were sprayed when plants reach one to three tillers, using a CO_2-pressurized backpack sprayer equipped with a 3 m hand-boom with six AXI 110.02 flat fan nozzles on 50 cm spacing, calibrated to deliver 150 L of spray solution per hectare at a pressure of 2.5 kgf cm^{-2}. In all studies, crop oil concentrate at 0.5% v/v was added to each spray solution for every herbicide treatment. After spraying, pots were returned to the greenhouse and were watered daily to keep adequate growing conditions.

2.4 Studies

2.4.1 Study 1 – Preliminary Screening

The preliminary study was conducted to identify the accessions completely susceptible to the field rate of the herbicides of interest. The seventeen accessions were sprayed with the recommended label rates of clethodim (96 g ha^{-1} ai), haloxyfop-methyl (60 g ha^{-1} ae), and glyphosate (960 g ha^{-1} ae). Each herbicide was evaluated separately, and according to visual control assessments, the accessions that showed high tolerance to these herbicides were then selected and submitted to a subsequent dose-response study. The screening was carried out from July to September of 2019.

2.4.2 Study 2 – Dose-response

Out of the seventeen accessions initially collected, six were selected to the dose-response study for being the most tolerant to one or more herbicides evaluated (refer to Table 1 for accessions and target herbicides). Besides the six accessions selected from the initial screening, seeds of goosegrass plants found in the urban area of Primavera do Leste – MT, Brazil were also collected to be evaluated as the susceptible accession during the dose-response studies. For each of the three studied herbicides, ten rates (up to 16X the label rate) were adopted to investigate the response of the accessions: clethodim: 0, 6, 12, 24, 48, 96, 192, 384, 768, and 1,536 g ha^{-1}, haloxyfop-methyl: 0, 3.75; 7.5, 15, 30, 60, 120, 240, 480, and 960 g ha^{-1}, and glyphosate: 0., 60, 120, 240, 480, 960, 1,920, 3,840, 7,680, and 15,360 g ha^{-1}. Due to space limitations in the greenhouse, the accessions were studied by pairs. Therefore, the susceptible accession was repeated and evaluated at each experiment. The dose-response was carried out from October 2019 to April 2020.

2.4.3 Study 3 – F1 Dose-response

During the dose-response study, two accessions (A11 and A15) survived the label rates of clethodim, haloxyfop-methyl, and glyphosate, each applied separately. Therefore, seeds of these two accessions (A11 and A15) were collected from additional herbicide-treated plants during the previous study and submitted to another dose-response with the F1 seeds for further investigations. The F1 dose-response study aimed to confirm a new goosegrass multiple resistance case in Brazil. The only multiple resistance case of the species in the country had been reported to affect glyphosate, fenoxaprop-P-ethyl, and haloxyfop-methyl, but not clethodim or any other DIM herbicide. The F1 study followed the same range of rates as the previous study. However, to obtain more robust conclusions, two separate experimental runs were carried out from October 2020 through January 2021. Furthermore, since only three accessions were being evaluated (A11, A15 and susceptible), all were evaluated simultaneously.

Table 1 - Accessions and the respective herbicides evaluated in the dose-response study

Accession	Target Herbicide
A1	Clethodim and haloxyfop-methyl
A4	Clethodim and haloxyfop-methyl
A11	Clethodim, haloxyfop-methyl, and glyphosate
A14	Clethodim, haloxyfop-methyl, and glyphosate
A15	Clethodim, haloxyfop-methyl, and glyphosate
A16	Haloxyfop-methyl and glyphosate
S[1]	Clethodim, haloxyfop-methyl, and glyphosate

[1] Susceptible accession, included in all experiments.

2.5 Data Collection

At 28 days after treatment (DAT), visual control was assessed (0% - no control to 100% - complete plant death), and aboveground green biomass was harvested. Biomass was dried to constant weight in a forced-air oven at 60°C and weight (g of dry biomass per pot) was converted to % of biomass reduction (BR) according to the non-treated check of each accession, as follows:

$$BR = 100 - \frac{\textit{Dry biomass of treated plants (g per pot)}}{\textit{Dry biomass of untreated check (g per pot)}} \times 100$$

2.6 Data Analyses

Non-linear regression models were fitted to visual control and biomass reduction data using the four-parameter log-logistic model in the *'drc'* package (Ritz et al., 2015) in R:

$$f(x) = c + \frac{d - c}{1 + exp\,(b(log(x) - log(e)))}$$

where *'f'* is the percentage of visual control or biomass reduction; *'x'* is the herbicide rate in a log scale; *'b'* is the relative slope at the inflection point; *'e'* is the herbicide rate that causes 50% response of the dependent variable; and *'c'* and *'d'* are the lower and upper limits of the curve, which for these analyses were fixed at 0 and 100, respectively.

The effective herbicide doses needed for 50% (ED_{50}) and 80% (ED_{80}) visual control or biomass reduction were calculated using the *ED()* function. The ED_{50} resistance ratio (ED_{50} of the resistant divided by the ED_{50} of the susceptible accession) was estimated using the *EDcomp()* function. Both functions are from the *'drc'* package. All statistical analyses were performed in R software version 4.0.2 (R Core Team, 2021).

3. Results and Discussion

As the goal of the preliminary screening (study 1) was to select the most tolerant accessions to the evaluated herbicides, its results will not be presented and the focus of the results and discussion will be on the two dose-response studies (studies 2 and 3).

3.1 Study 2 - Dose-response

3.1.1 Clethodim

As displayed in Table 2, all five goosegrass accessions treated with clethodim resulted in higher ED_{50} values for visual control and biomass reduction than the susceptible ($p<0.05$). However, despite the significant difference between the accessions collected from the fields to the susceptible accession, only A11 and A15 required a higher dose than the recommended field rate of clethodim (96 g ha^{-1}) to achieve 80% visual control and biomass reduction. Hence, it is expected that producers can still achieve effective control of the accessions A1, A4, and A14 if applications are made under the label recommendations, especially to the appropriate weed size, which is an essential factor in herbicide efficacy for goosegrass control (Takano et al., 2017; 2018b).

Even though the accessions A1, A4, and A14 were effectively controlled by the recommended rate of clethodim their ED_{50} values were 7.7 up to 12.3 times higher than the susceptible accession (Table 2) revealing a significant difference between field accessions, frequently exposed to herbicides, compared to the wild type from the urban area. Evidence in the literature suggests that when there is a mutation in the ACCase enzyme conferring resistance to clethodim in this species, the ED_{50} values observed were higher than those obtained with these three accessions (Osuna et al., 2012; Andrade Junior, 2018). Therefore, other resistance mechanisms, for instance, non-target site mechanisms, may be evolving due to the frequent exposure of goosegrass to ACCase-inhibiting herbicides (Gaines et al., 2020).

The first goosegrass resistance case confirmed in Brazil to ACCase-inhibiting herbicides was also found in soybean fields in Mato Grosso. The studied population showed an ED_{50} of 737.1 g ha^{-1} of clethodim due to the Asp2078Gly mutation in the ACCase enzyme, conferring resistance to clethodim and other ACCase-inhibiting herbicides (Osuna et al., 2012). Besides soybean, ACCase-inhibiting herbicides are also fundamental for POST-emergence grass control in cotton production systems in the state. During a screening by Andrade Junior (2018), 68.4% of 57 goosegrass populations had less than 80% control when treated with the double recommended rate of clethodim (216 g ha^{-1}) and tepraloxydim (208 g ha^{-1} ai). Moreover, Andrade Junior (2018) also reported the Asp2078Gly mutation in the ACCase enzyme resulting in ED_{50} of 96.7, 104.1, and 190.1 g ha^{-1} of clethodim to control three resistant goosegrass populations which are values closer to the present study (Table 2) than the ones obtained by Osuna et al. (2012).

3.1.2 Haloxyfop-methyl

Out of the three evaluated herbicides, haloxyfop-methyl showed the lowest efficacy with the highest resistance levels across accessions. Similar to clethodim, all accessions treated with haloxyfop-methyl also resulted in higher ED_{50} values than the susceptible accession for both visual control and biomass reduction ($p<0.05$). Moreover, none of the six accessions were effectively controlled by the recommended rate of haloxyfop-methyl (60 ga ha). Accessions A4 and A11 presented a very low response to haloxyfop-methyl (ED_{50} values of 607.2 and 1224.3 g ha^{-1} of haloxyfop-methyl, respectively). Due to their low response, the log-logistic equation failed to provide reliable ED_{80} values for visual control and biomass reduction. In both cases, even with 16 times the recommended rate of haloxyfop-methyl (960 g ha^{-1}), none of these two accessions reached such levels (80%) to both response variables (Table 3). In contrast, Andrade Junior (2018) observed ED_{50} values of 49.8, 166.8, and 180.9 of haloxyfop-methyl to control three resistant goosegrass populations. Moreover, Osuna et al. (2012) needed 185.3 g ha^{-1} of haloxyfop-methyl to achieve 50% control of another resistant goosegrass population.

Table 2 - Estimated doses of clethodim needed for 50% (ED_{50}) and 80% (ED_{80}) control or biomass reduction at 28DAT

Accession	Dose of clethodim g ha^{-1} ai							
	Visual control (%)				Biomass reduction (%)			
	ED_{50}(±SE)[1]	p-value[2]	ED_{80}(±SE)	RR[3]	ED_{50}(±SE)	p-value	ED_{80}(±SE)	RR
S1[4]	4.6(0.6)	-	8.7(0.9)	-	5.0(0.7)	-	6.3(0.7)	-
A4	53.6(2.1)	<0.001	76.8(5.7)	11.7	52.3(1.9)	<0.001	70.8(6.4)	10.5
A11	86.2(3.7)	<0.001	124.3(7.8)	18.9	73.8(4.7)	<0.001	147.3(12.6)	14.8
S2	4.1(1.2)	-	6.1(0.4)	-	4.3(1.8)	-	7.4(0.6)	-
A1	50.5(2.4)	<0.001	79.0(5.7)	12.3	35.1(1.7)	<0.001	51.6(3.5)	8.1
S3	5.0(0.5)	-	8.6(0.8)	-	4.2(0.9)	-	6.7(0.6)	-
A14	49.5(2.4)	<0.001	80.0(5.5)	9.9	31.8(2.0)	<0.001	66.3(6.0)	7.7
A15	112.6(7.1)	<0.001	253.4(23.5)	22.6	101.9(6.1)	<0.001	200.1(18.7)	24.5

[1] Values between parentheses represent the standard error of the parameters ED_{50} and ED_{80}.
[2] Statistical comparison between the ED_{50} values of each accession against the susceptible accession. P-value >0.05 indicates non-significant difference between accessions.
[3] Resistance ratio estimated by dividing the ED_{50} value of the accession of interest by the susceptible accession.
[4] Due to space limitations in the greenhouse, the accessions were studied by pairs. Therefore, S1, S2, and S3 represent the same susceptible accession evaluated three times. The accessions A4 and A11 should be compared to S1, A1 to S2, and A14 and A15 to S3.

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The accessions A11 and A15 were not effectively controlled by clethodim nor haloxyfop-methyl (Tables 2 and 3). The same was also observed by Osuna et al. (2012) and Andrade Junior (2018), which obtained populations with cross-resistance to the same ACCase inhibiting herbicides. Even though ACCase-inhibiting herbicides target the same enzyme, the binding site is specific to each family (DIMs cyclohexanediones, FOPs aryloxyphenoxypropionates, and DEN phenylpyrazoline). Therefore, the specificity affects the impact of target-site mutations conferring resistance to these herbicides (Yu et al., 2007; Jang et al., 2013; Kaundun 2014; Gaines et al., 2020). The eight known point mutations in the ACCase enzyme have been reported to cause resistance to FOPs. Thus, this chemical family is the most affected by target-site mutations conferring resistance to ACCase-inhibiting herbicides (Yu et al., 2007; Jang et al., 2013; Kaundun 2014; Gaines et al., 2020). Furthermore, clethodim is considered the graminicide with the lowest risk of target-site resistance. Since only substitutions at the positions Asp2078 and Cys2088 seem to cause resistance to this DIM herbicide (Beckie, Tardif, 2012). Altogether, these may help to explain why all six accessions survived high rates of haloxyfop-methyl while only two accessions

Table 3 - Estimated doses of haloxyfop-methyl needed for 50% (ED_{50}) and 80% (ED_{80}) control or biomass reduction at 28DAT

Accession	Dose of haloxyfop-methyl g ha^{-1} ae							
	Visual control (%)				Biomass reduction (%)			
	ED_{50}(±SE)[1]	p-value[2]	ED_{80}(±SE)	RR[3]	ED_{50}(±SE)	p-value	ED_{80}(±SE)	RR
S1[4]	3.1(0.4)	-	6.5(0.7)	-	2.9(0.5)	-	6.8(1.0)	-
A4	607.2(120.6)	<0.001	>960	194.0	2705.6(2488.5)	<0.001	>960	933.0
A11	1224.3(346.5)	<0.001	>960	391.2	2299.8(1775.0)	<0.001	>960	793.0
S2	3.4(0.4)	-	7.2(0.8)	-	3.4(0.5)	-	7.2(1.0)	-
A1	58.7(4.3)	<0.001	145.8(19.3)	17.3	40.7(2.6)	<0.001	56.7(5.5)	12.0
A16	140.6(14.6)	<0.001	727.9(129.5)	41.5	109.3(12.7)	<0.001	398.5(75.0)	32.1
S3	3.6(0.3)	-	6.7(0.7)	-	1.9(0.5)	-	4.8(1.2)	-
A14	164.9(11.9)	<0.001	354.4(35.9)	45.5	96.2(12.8)	<0.001	433.2(84.6)	50.6
A15	334.4(36.2)	<0.001	1610.2(373.5)	92.4	182.9(26.1)	<0.001	1091.5(320.5)	96.3

[1] Values between parentheses represent the standard error of the parameters ED_{50} and ED_{80}.
[2] Statistical comparison between the ED_{50} values of each accession against the susceptible accession. P-value >0.05 indicates non-significant difference between accessions.
[3] Resistance ratio estimated by dividing the ED_{50} value of the accession of interest by the susceptible accession.
[4] Due to space limitations in the greenhouse, the accessions were studied by pairs. Therefore, S1, S2, and S3 represent the same susceptible accession evaluated three times. The accessions A4 and A11 should be compared to S1, A1 and A16 to S2, and A14 and A15 to S3.

Table 4 - Estimated doses of glyphosate needed for 50% (ED_{50}) and 80% (ED_{80}) control or biomass reduction at 28DAT

Accession	Dose of glyphosate g ha^{-1} ae							
	Visual control (%)				Biomass reduction (%)			
	ED_{50}(±SE)[1]	p-value[2]	ED_{80}(±SE)	RR[3]	ED_{50}(±SE)	p-value	ED_{80}(±SE)	RR
S1[4]	164.6(10.3)	-	342.6(28.6)	-	136.0(10.5)	-	290.5(30.2)	-
A11	598.0(37.0)	<0.001	1,319.6(129.2)	3.6	358.9(29.6)	<0.001	872.4(96.6)	2.6
A14	796.7(41.5)	<0.001	1,407.9(129.6)	4.8	303.7(32.0)	<0.001	1,194.6(165.6)	2.2
S2	132.5(9.0)	-	306.3(28.2)	-	81.3(8.9)	-	228.3(29.0)	-
A15	743.1(50.8)	<0.001	1930.3(194.1)	5.6	300.3(19.5)	<0.001	548.5(65.9)	3.7
A16	332.1(21.0)	<0.001	731.0(73.8)	2.5	175.3(9.1)	<0.001	248.4(19.7)	2.2

[1] Values between parentheses represent the standard error of the parameters ED_{50} and ED_{80}.
[2] Statistical comparison between the ED_{50} values of each accession against the susceptible accession. P-value >0.05 indicates non-significant difference between accessions.
[3] Resistance ratio estimated by dividing the ED_{50} value of the accession of interest by the susceptible accession.
[4] Due to space limitations in the greenhouse, the accessions were studied by pairs. Therefore, S1 and S2 represent the same susceptible accession evaluated two times. The accessions A11 and A14 should be compared to S1 and A15 and A16 to S2.

were not effectively controlled by the recommended rate of clethodim (Tables 2 and 3).

3.1.3 Glyphosate

Similar to what was observed with clethodim and haloxyfop-methyl (Tables 2 and 3), all four accessions treated with glyphosate presented higher ED_{50} values than the susceptible accession for visual control and biomass reduction ($p<0.05$). However, only three accessions (A11, A14, and A15) needed higher doses than the recommended field rate of glyphosate to achieve 80% goosegrass control (Table 4). A similar trend was reported by Takano et al. (2017) where although 24 out of 25 studied populations yielded resistance ratios higher than 1.0, four were not effectively controlled by the recommended rate of glyphosate (960 g ha^{-1}). Moreover, one of the four populations not controlled by the recommended rate of glyphosate was confirmed to carry the Pro106Ser mutation in the EPSPS enzyme as the glyphosate resistance mechanism (Takano et al., 2018a).

Besides not being controlled by the recommended rate of glyphosate, accessions A11 and A15 were also not effectively controlled by clethodim and haloxyfop-methyl (Table 4). Thus, representing a potential new multiple resistance case of goosegrass in Brazil since the only multiple resistance case confirmed in the country confers resistance to glyphosate, haloxyfop-methyl, and fenoxaprop-p-ethyl but not to clethodim (Heap, 2021). A similar case was reported in a goosegrass population from China resistant to glyphosate and cyhalofop-butyl (Deng et al., 2020). Furthermore, an even more complex multiple resistance case has been found in Malaysia, where a goosegrass population evolved resistance to herbicides from four sites of action: glyphosate, glufosinate, paraquat, and butroxydim, fluazifop-p-butyl, and haloxyfop-methyl (Jalaludin et al., 2014). Combined, these findings demonstrate that goosegrass can evolve multiple resistance to herbicides from multiple sites of action adopted for its management.

3.2 Study 3 - F1 dose-response

In the study conducted with F1 seeds collected from herbicide treated plants during the previous study (study 2), accessions A11 and A15 were confirmed to tolerate higher than the recommended field rates of clethodim (96 g ha^{-1}) and haloxyfop-methyl (60 g ha^{-1}; Table 5). These results corroborate to Osuna et al. (2012) and Andrade Junior (2018), who had previously reported goosegrass accessions with cross-resistance to ACCase inhibiting herbicides in Mato Grosso. Moreover, even 16 times the label rate of haloxyfop-methyl failed to provide effective control (>80%) of accession A11, as observed in the previous dose-response study, thus, confirming a high resistance level of this accession to haloxyfop-methyl. Similarly, Takano et al. (2020) observed a high resistance ratio (613-fold) in a sourgrass (*Digitaria insularis*) population resistant to haloxyfop-methyl due to the target-site mutation Trp2027Cys in the ACCase enzyme. However, the population was not resistant to clethodim. Therefore, resistance to ACCase-inhibiting herbicides, and consequently, its management should not be addressed as a

Table 5 - Estimated doses of clethodim, haloxyfop-methyl, and glyphosate needed for 50% (ED_{50}) and 80% (ED_{80}) control or biomass reduction in the F1 dose-response study at 28DAT

Accession	Dose of clethodim g ha^{-1} ai							
	Visual control (%)				Biomass reduction (%)			
	ED_{50}(±SE)[1]	p-value[2]	ED_{80}(±SE)	RR[3]	ED_{50}(±SE)	p-value	ED_{80}(±SE)	RR
S	4.5(0.4)	-	12.3(0.8)	-	2.9(1.2)	-	5.4(0.7)	-
A11 - F1	102.8(3.9)	<0.001	235.9(12.5)	22.8	60.4(4.5)	0.02	245.2(25.1)	20.9
A15 - F1	93.1(3.1)	<0.001	181.5(9.1)	20.6	45.1(2.7)	0.02	123.1(11.0)	15.6
	Dose of haloxyfop-methyl g ha^{-1} ae							
S	2.9(0.2)	-	5.1(0.4)	-	3.5(0.2)	-	5.0(0.5)	-
A11 - F1	437.2(282.3)	<0.001	>960	150.7	245.4(57.8)	<0.001	>960	71.0
A15 - F1	128.2(7.3)	<0.001	380.2(0.4)	44.1	46.9(5.5)	<0.001	401.4(74.5)	13.6
	Dose of glyphosate g ha^{-1} ae							
S	354.8(12.2)	-	676.2(32.0)	-	146.5(11.1)	-	465.4(49.1)	-
A11 - F1	467.9(14.7)	<0.001	806.2(36.3)	1.3	233.6(19.4)	0.001	898.2(95.8)	1.6
A15 - F1	525.9(16.2)	<0.001	920.2(43.9)	1.4	349.7(25.1)	<0.001	1122.3(114.5)	2.4

[1] Values between parentheses represent the standard error of the parameters ED_{50} and ED_{80}.
[2] Statistical comparison between the ED_{50} values of each accession against the susceptible accession. P-value >0.05 indicates non-significant difference between accessions.
[3] Resistance ratio estimated by dividing the ED_{50} value of the accession of interest by the susceptible accession.

general occurrence process. Instead, management practices and herbicide recommendations should address resistance on a case-by-case basis. Given that resistance to ACCase-inhibiting herbicides might not affect their chemical groups (FOPs, DIMs, and DEN) simultaneously.

As for glyphosate resistance, the ED_{50} values observed in the F1 study were lower than those reported in the parental generation for both accessions (study 2; A11 and A15; Table 5). Likewise, Takano et al. (2017) also reported that a goosegrass population treated with glyphosate yielded an ED_{50} of 802.4 g ha^{-1} of glyphosate but when the F1 was submitted to a dose-response study, the ED_{50} lowered almost half of the parental ED_{50} rate (419.6 g ha^{-1}). Studies indicate that goosegrass populations with the Pro106Ser mutation in the EPSPS enzyme conferring resistance to glyphosate needed 868.0 g ha^{-1} (Mueller et al., 2011) and 741.9 g ha^{-1} (Takano et al., 2018a) of glyphosate to achieve 50% control, yielding resistance ratios of 7.4-fold and 4.2-fold, respectively.

Even though the ED50 values and resistance ratios observed herein are lower than those described in the literature in the confirmed presence of a target-site mutation conferring resistance to glyphosate, it should not eliminate the possibility of a resistance case. Given that, besides the significant difference in the glyphosate ED_{50} between accessions A11 and A15 compared to the susceptible, the herbicide resistance literature supports that weed resistance is not an entirely black and white process. Instead, it is an evolutionary process in which weeds have shown to be constantly adapting, evolving, and accumulating new resistance mechanisms as the selection pressure increases (Jalaludin et al., 2014; Yu et al., 2015; Deng et al., 2020; Gaines et al., 2020). For example, a goosegrass population has been described to be multiple resistant to glyphosate and cyhalofop-butyl in China. As the resistance mechanism, the target-site mutation Asp2078Gly resulted in resistance to the ACCase-inhibiting herbicide. However, for glyphosate, no amino acid substitution was detected in the EPSPS enzyme. Instead, the overexpression of the EPSPS gene seemed to contribute to glyphosate resistance (Deng et al., 2020). Furthermore, herbicide-resistant weed populations carrying target-site mutations have been shown to likely have non-target site resistance mechanisms in conjunction (Han et al., 2016). Therefore, the fact that ACCase mutation conferring resistance to group one herbicides have been previously described in Mato Grosso (Osuna et al., 2012; Andrade Junior, 2018), combined, with dose-response results supporting the cross-resistance of accessions A11 and A15 to clethodim and haloxyfop-methyl, indicate the occurrence of low-level resistance to glyphosate in these two accessions.

The low-level resistance might not be faced as a big challenge, given that the recommended rate of glyphosate was enough to provide 80% control of the accessions A11 (806.2 g ha^{-1}) and A15 (920.2 g ha^{-1}) in the F1 dose-response study (Table 5). However, goosegrass is characterized by its rapid growth and development (Takano et al., 2016). That associated with the need for higher herbicide rates to control plants out of the recommended growth stage (Takano et al., 2017; 2018b) will likely result in poor goosegrass control under field conditions if producers do not follow label specifications. Furthermore, it has been a common practice by soybean and cotton producers to spray glyphosate in association with ACCase inhibiting herbicides to control troublesome grass weed species in Brazil (Takano et al., 2021). However, the low response of accessions A11 and A15 to clethodim and haloxyfop will result in stronger selection pressure from glyphosate, the herbicide with the highest efficacy in the mixture, worsening a low-level resistance case (Délye et al., 2013).

Given the role that glyphosate and ACCase inhibiting herbicides play in cropping systems across Brazil (Oliveira et al., 2020; Takano et al., 2021), integrated weed management practices should be put into place to create diversity and prolong the life of the limited POST-emergence herbicide options available for grass control. Producers should adopt effective crop rotation, adoption of cover crops, use of PRE-emergence herbicides, frequent field scouting, and timely applications of herbicide mixtures in rotation. Moreover, academics should continue to monitor for herbicide resistance and devote energy to better understand the impact of non-target site resistance mechanisms, which is considered the new frontier of weed resistance, on weed management to better assist producers in mitigating further resistance issues.

4. Conclusions

In conclusion, the continuous adoption of herbicides for weed management in Mato Grosso cropping systems has selected goosegrass accessions that can withstand the recommended label rates of the ACCase-inhibiting herbicides clethodim and haloxyfop-methyl, and the EPSPS-inhibitor glyphosate. Moreover, two accessions with resistance to the ACCase-inhibiting herbicides, clethodim and haloxyfop-methyl, and low-level resistance to glyphosate were identified as having multiple resistance to these two modes of action.

Authors' contributions

All authors read and agreed to the published version of the manuscript. JJN, and PCRC: Conceptualization of the manuscript and development of the methodology. JJN: data collection and curation, data analysis, resources acquisition, writing the original draft of the manuscript. JJN, RW, MAMF, and PCRC: data interpretation, writing, review and editing. JJN, RW, and PCRC: project administration. RW, and PCRC: supervision.

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