Advances in Weed Science
formerly Planta Daninha
RESEARCH ARTICLE

First report of multiple resistance of goosegrass to herbicides in Brazil

Núbia M. Correia[a*], Lucas da S. Araújo[b], Roni A. Bueno Júnior[c]

[a] Brazilian Agricultural Research Corporation, Embrapa Cerrados, Planaltina, Brasília, DF, Brazil. [b] Phytus Institute, Planaltina, Brasília, DF, Brazil. [c] Agronomist, Local Consultant, Luziânia, GO, Brazil.

Abstract: Background: This manuscript presents data of screening and dose-response curves of a goosegrass population from Primavera do Leste, MT, Brazil, which results in the first official report of multiple resistance of goosegrass to ACCase and EPSPs inhibiting herbicides in Brazil.

Objective: Evaluate the control of a goosegrass population from Primavera do Leste, MT, Brazil, suspected of resistance to the glyphosate, fenoxaprop-p-ethyl, and haloxyfop-methyl herbicides, using dose-response curves.

Methods: The study was carried out in two stages, the first in Brasília, DF, Brazil, and the second in Piracicaba, SP, Brazil. The first stage consisted in the initial evaluation and identification of resistance, which was confirmed in the second. In the first stage, the herbicides glyphosate, fenoxaprop-p-ethyl, and haloxyfop-methyl were applied to susceptible and resistant goosegrass populations (F1 and F2 generations) with plants with 2 and 3 tillers, using nine rates (0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the recommended rate). In the second stage, the herbicides were applied to susceptible and resistant populations with plants with 4 leaves, using 12 rates (0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the recommended rate).

Results: The high sensitivity of susceptible plants combined with the high resistance level of resistant ones resulted in high resistance factors for fenoxaprop-p-ethyl and haloxyfop-methyl, with values greater than 27.8. The resistance factor for glyphosate ranged from 3.3 to 11, depending on growth stage at application time.

Conclusions: The goosegrass population from Primavera do Leste, MT, Brazil, presented resistance to EPSPs (glyphosate) and ACCase (haloxyfop-methyl and fenoxaprop-ethyl) inhibiting herbicides.

Keywords: *Eleusine indica*; Fenoxaprop-p-ethyl; Glyphosate; Haloxyfop-p-methyl

Journal Information:
ISSN - 2675-9462
Website: http://awsjournal.org
Journal of the Brazilian Weed Science Society

How to cite: Correia NM, Araújo LS, Bueno Júnior RA. First report of multiple resistance of goosegrass to herbicides in Brazil. Adv Weed Sci. 2022;40:e020220007.
https://doi.org/10.51694/AdvWeedSci/2022;40:00012

Approved by:
Editor in Chief: Anderson Luis Nunes
Associate Editor: Bianca Assis Barbosa Martins

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Received: January 26, 2022
Approved: June 6, 2022

* Corresponding author:
<nubia.correia@embrapa.br>

1. Introduction

Eleusine indica, popularly known as goosegrass, is an important grass species in agricultural areas, especially in those with center pivot irrigation in the Cerrado biome in Brazil. This is an autogamous annual or perennial plant, with C4 photosynthetic metabolism, form clumps, which is propagated by seeds (Ganeshaiah, Umashaanker, 1982; Kissmann, Groth, 1997). The plants can develop in compacted and poor soils, produce up to 120,000 seeds per plant, and have slow initial growth (Kissmann, Groth, 1997).

In 2003, there was the first report of resistance of goosegrass to Acetil-CoA Carboxylase (ACCase) inhibiting herbicides in Brazil (Heap, 2021). The mechanism of resistance identified was a mutation in the site of action, the replacement of the asparagine amino acid by glycine in the 2078 position (Asp-2078-Gly) (Osuna et al., 2012). In 2016, there was a report of resistance of goosegrass to the glyphosate herbicide, which belongs to the substituted glycine chemical group and inhibits the enol-piruvil-shiquimato-phosphate synthase enzyme (EPSPs). The mechanism of resistance was attributed to a change in the enzyme, the replacement of the proline amino acid by serine in the 106 position (Pro-106-Ser) (Takano et al., 2018).

Resistance of weeds to herbicides is defined as the inherent or inheritable capacity of some biotypes within a population to survive and reproduce after exposure to herbicide rates that would be lethal to a normal population (susceptible) of the same species (Christoffoleti, López-Ovejero, 2008). Resistance is a natural phenomenon that occurs spontaneously in plant populations; therefore, the herbicide is not the agent that causes, but the selector of resistant plants, which present low initial frequency (Christoffoleti, López-Ovejero, 2008). Thus, the exclusive and frequent use of herbicides with the same mechanism of action contribute to increase the selection pressure of resistant biotypes of plant species. In this case, the control is ineffective even when increasing the herbicide rate or spraying it on small plants.

In 2017, there was the first report in Brazil of multiple resistance of goosegrass to glyphosate and ACCase (fenoxaprop-p-ethyl and haloxyfop-methyl) inhibiting herbicides (Heap, 2021). Multiple resistance is the individual capacity to survive herbicide applications with two or more different mechanisms of action. The selection pressure increases as the use of these herbicides is increased in the agricultural area up to the selection of biotypes with multiple resistance in the population. In this case, the losses for agriculture are high, since the weed control is more difficult and expensive. This manuscript presents data of screening and dose-response curves of a goosegrass population, which results in the first official report of multiple resistance of goosegrass to ACCase and EPSPs inhibiting herbicides in Brazil. Thus, the objective of this study was to evaluate the control of a goosegrass population from Primavera do Leste, MT, Brazil, suspected of resistance to the glyphosate, fenoxaprop-p-ethyl, and haloxyfop-methyl herbicides, using dose-response curves.

2. Material and Methods

The study was carried out in two stages, the first in Brasília, DF, Brazil, and the second in Piracicaba, SP, Brazil. The first stage consisted in the identification and initial evaluation of resistance, which was confirmed in the second stage. In Brasília, the experiments were conducted using pots maintained in a greenhouse of the Brazilian Agricultural Research Corporation (Embrapa Vegetables). A screening experiment was carried out to evaluate the response of two goosegrass populations, one suspected of resistance todifferent herbicides , and one susceptible. The results were used to conduct dose-response experiments for goosegrass plants, using the herbicides glyphosate, fenoxaprop-p-ethyl e haloxyfop-methyl.

Goosegrass seeds from plants suspected of resistance to glyphosate and ACCase inhibiting herbicides were obtained in an agricultural area in Primavera do Leste, MT, Brazil (15°22'32.57''S, 54°26'03.53''W, and 631 m of altitude). The area had been used for the growth of annual crops (soybean, maize, cotton, and common bean) with history of applications of glyphosate and ACCase inhibiting herbicides for the control of goosegrass. Seeds from plants susceptible to the herbicides, used as a standard population, were obtained from the Agrocosmos company (Engenheiro Coelho, SP, Brazil), which is specialized in weed seed production.

In all experiments (screening and dose-response curve), each experimental unit consisted of a plastic pot with capacity for 2 dm^3 of soil. The substrate used consisted of a mixture of soil, sand, and organic compost at the ratio of 3:1:1, fertilized with 100 mg of nitrogen, 200 mg of phosphorus, and 150 mg potassium per kilogram of substrate. The goosegrass seeds were sown in expanded polystyrene trays and then transplanted to the pots when the seedlings had 2 to 3 leaves, maintaining two plants per pot. Each pot was placed in a plastic container of larger diameter without holes to maintain the water regime in the plots. The soil moisture was controlled daily by applying water to the containers when required.

In the screening experiment, a completely randomized design was used, with four replications, in a 2 x 10 factorial arrangement. Plants of the two goosegrass populations with 3 to 4 tillers were subjected to applications of clethodim (108 g ha^{-1}), fenoxaprop-p-ethyl (110 g ha^{-1}), haloxyfop-p-methyl (62.35 g ha^{-1}), and quizalofop-p-tefuryl (72 g ha^{-1}), alone and combined with glyphosate (1.0 kg a.e. ha^{-1}), and glyphosate alone (1.0 kg a.e. ha^{-1}). A treatment without application was used as a control.

In the dose-response experiments, a completely randomized design with four replications was used, in a 3 x 9 factorial arrangement; one experiment was conducted for each herbicide. Plants of the susceptible and resistant populations (F1 and F2 generations) with 2 to 3 tillers were subjected to applications of herbicides with increasing rates: 0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the recommended rate, which was 62.35 g ha^{-1} for haloxyfop-methyl (applied with 0.5% oil mineral), 110 g ha^{-1} for fenoxaprop-p-ethyl, and 1.0 kg a.e. ha^{-1} for glyphosate, respectively. The seeds of the resistant population F2 (R_{-F2}) were obtained from plants that survived the herbicide applications in the step before the screening experiment; they were collected and stored for the dose-response curve experiments.

The herbicide applications were carried out using a CO_2-pressurized (2.0 kgf cm^{-2}) backpack sprayer equipped with a spray boom containing two nozzles (TTI 110015) spaced 0.5 m apart; the solution rate applied was equivalent to 150 L ha^{-1}. The specifications of herbicides used in the experiments, commercial product name, formulation type, concentration, and product supplier in Brazil are shown in Table 1.

The weed control was visually evaluated at 15, 30, and 40/45 (40 for the screening, and 45 for the dose-response experiments) days after application (DAA) of the herbicides, using a scale of grades from 0% to 100%, in which zero represents absence of visual injuries and 100 represents the death of the plant, according to the *Sociedade Brasileira da Ciência das Plantas Daninhas* (Sociedade Brasileira da Ciência das Plantas Daninhas, 1995). The aerial part of the plants was collected at 40 or 45 DAA to determine the shoot dry matter (g pot^{-1}). The material was dried in a forced air circulation and renewal oven at 50°C until constant matter.

The control and dry matter data were subjected to analysis of variance by the F test. Analysis of variance was carried out in the screening experiment, using the Sisvar 5.7 program (Ferreira, 2011), and when the effects of treatments or interaction between factors were significant, they were compared by the Scott-Knott test at 5% probability level.

In the dose-response experiments, the data were fitted to four-parameter logistic regression model $y=A_2+(A_1-A_2)/(1+(x/x0^p)$, in which A_2 is the highest rate with no control or decrease in dry matter; A_1 is the lowest rate that cause absolute damages; p is the slope; and x0 is the median lethal dose (LD_{50}), which is the herbicide rate that results in 50% control or decrease in dry matter (Seefeldt et al., 1995). However, a linear model y=ax + b was chosen to fit the data of some variables, considering the best biological

Table 1 - Names of the active ingredients and commercial products, formulation, concentration, and supplier of the herbicides used in the experiments

Herbicide		Formulation[1]	Concentration (g L^{-1})	Supplier
Active ingredient	Commercial product			
Clethodim	Select 240 EC	EC	240	UPL
Fenoxaprop-p-ethyl	Podium EW	EW	110	Bayer
Glyphosate	Zapp QI 620	SL	620	Syngenta
Haloxyfop-p-methyl	Verdict R	EC	124.7	Corteva
Quizalofop-p-tefuryl	Panther 120 EC	EC	120	UPL

[1] EC: emulsifiable concentrate, EW: oil in water emulsion, SL: soluble concentrate
Source: Rodrigues and Almeida (2018)

explanation, statistical significance, and coefficient of determination; the LD_{50} was calculated manually by replacing the values in the equation. The Origin software was used for the analysis and development of graphics. The resistance factor was estimated by LD_{50} resistant / LD_{50} susceptible (Burgos et al., 2013).

Other three dose-response experiments were conducted one for each herbicide, in the Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ/USP), in Piracicaba, SP, to confirm the results obtained in the first stage. The methodology was similar to the experiments carried out in Brasília, with weed control and dry matter evaluations of plants at 21 DAA. A completely randomized design was used, with four replications, in a 3 x 12 factorial arrangement. Plants from susceptible and resistant populations (generations F1 and F2) with 4 leaves were subjected to herbicide applications, using increasing rates: 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the recommended rate, which corresponded to 62.35 g ha^{-1} for haloxyfop-methyl (applied with 0.5% oil mineral), 110 g ha^{-1} for fenoxaprop-p-ethyl, and 1.0 kg a.e. ha^{-1} for glyphosate.

The data obtained were the data were fitted to four-parameter logistic regression model. The SigmaPlot software was used for the analysis and development of graphics. The resistance factor was estimated by LD_{50} resistant / LD_{50} susceptible (Burgos et al., 2013).

3. Results and Discussion

In the first stage, the interaction between population and herbicide treatments in the screening experiment was significant ($p<0.01$). All evaluated herbicides, applied alone or combined with glyphosate controlled the susceptible goosegrass population (Table 2). Contrastingly, the population from of Primavera do Leste was controlled only by clethodim and quizalofop applied alone, which resulted in the death of the plants, with 100% of control, differing from the other herbicide treatments, which were ineffective at 40 days after application (DAA).

Control of resistant plants was not maintained when clethodim and quizalofop were mixed with glyphosate. Herbicide performance decreased, denoting an antagonistic effect between the ACCase inhibiting herbicides and glyphosate. The combinations resulted in losses in efficiency of 76% for clethodim and 44% for quizalofop at 40 DAA, when compared to the herbicides applied alone. This effect may have occurred also in susceptible plants, but in this case, it was masked by the action of glyphosate in the mixture, which is efficient for these plants. Several phenomena could explain the loss of action of clethodim and quizalofop when combined with glyphosate, including the chemical or even physiological incompatibility between these products, which may be connected to the absorption, translocation, and metabolization of ACCase inhibiting herbicides in the presence of glyphosate.

These results contradict a study with combinations of clethodim and glyphosate for the control of glyphosate resistant and susceptible *Digitaria insularis* populations, in which the mixtures had synergistic effect, with satisfactory control of resistant plants (Bianchi et al., 2020). In another study on the control of *D. insularis*, the effect of mixtures of glyphosate with ACCase-inhibiting herbicides was classified as antagonist, synergic, or additive, depending on the glyphosate formulation (isopropylamine salt, ammonium salt, and potassium salt) and the ACCase-inhibiting herbicide formulation (clethodim, haloxyfop, sethoxydim, and quizalofop) evaluated (Barroso et al., 2014). The glyphosate formulation used in the present study was potassium salt. Thus, studies on mixtures of glyphosate with ACCase-inhibiting herbicides are complex and depend on many factors, mainly the weed species evaluated.

Regarding the resistant goosegrass population of Primavera do Leste, MT, the use of glyphosate combined with clethodim or quizalofop is not recommended for the control of these plants due to the partial loss of action of ACCase inhibiting herbicides. However, these mixtures are used in field crops in one single application since glyphosate is used for the control of other weed species and clethodim or quizalofop is added to the solution for goosegrass control. However, integrated weed control strategies should be used for goosegrass with multiple resistance. These strategies include chemical control, which is complemented with cultural or mechanized

Table 2 - Control (%) of two goosegrass populations, one suspected of resistance and other susceptible to herbicides at 15, 30 and 40 days after application (DAA); shoot dry matter (SDM) at 40 DAA; and control without herbicide application

Populations	Herbicides	Control (%)			SDM
		15 DAA	30 DAA	40 DAA	(g pot^{-1})
Suspected of resistance	Clethodim	85.0 a[1]	98.3 a	100.0 a	0.0 a
	Fenoxaprop-ethyl	15.0 d	15.0 d	10.0 d	26.0 c
	Haloxyfop-p-methyl	5.0 d	0.0 d	0.0 d	27.7 c
	Quizalofop-p-tefuryl	83.7 a	99.2 a	100.0 a	0.0 a
	Glyphosate	83.1 a	36.2 c	15.0 d	24.2 c
	Clethodim+glyphosate	82.5 a	63.1 b	23.8 c	15.7 b
	Fenoxaprop+glyphosate	76.8 b	18.8 c	5.0 d	26.7 c
	Haloxyfop+glyphosate	76.2 b	20.0 c	11.2 d	29.0 c
	Quizalofop+glyphosate	86.9 a	77.5 b	56.2 b	12.8 b
	Control	0.0 d	0.0 d	0.0 d	29.6 c
Susceptible	Clethodim	92.5 a	98.2 a	100.0 a	0.0 a
	Fenoxaprop-ethyl	91.9 a	99.2 a	100.0 a	0.0 a
	Haloxyfop-p-methyl	95.0 a	100.0 a	100.0 a	0.0 a
	Quizalofop-p-tefuryl	97.5 a	100.0 a	100.0 a	0.0 a
	Glyphosate	98.1 a	100.0 a	100.0 a	0.0 a
	Clethodim+glyphosate	100.0 a	100.0 a	100.0 a	0.0 a
	Fenoxaprop+glyphosate	98.1 a	100.0 a	100.0 a	0.0 a
	Haloxyfop+glyphosate	95.6 a	98.9 a	100.0 a	0.0 a
	Quizalofop+glyphosate	100.0 a	100.0 a	100.0 a	0.0 a
	Control	0.0 b	0.0 b	0.0 b	29.4 b

[1] Means followed by the same letters in the columns within each population are not significantly different from each other by the Scott-Knott test at 5% probability level

practices, mainly in the autumn-winter period, with inclusion of cover crops, winter crops, or plowing and harrowing operations, in the case of conventional soil preparation. The objective is to avoid the full development of goosegrass plants, which is beneficial for the control before the implementation of the summer crop.

Post-emergence control of resistant goosegrass plants is compromised by the loss of action of EPSPs and ACCase inhibiting herbicides, due to the absence of other herbicides that have efficacy plants with up to 4 tillers. Ammonium-glufosinate is an option for post-emergence weed control, which is selective for transgenic glufosinate-tolerant cotton and maize, and for the pre-sowing weed control (burndown) (Rodrigues, Almeida, 2018). However, ammonium-glufosinate efficacy is when applied to plants with up to one tiller only and with the addition of adjuvant to the herbicide solution.

Regarding the dose-response experiments, the interaction between population and rates was significant ($p<0.01$) for the three herbicides tested, thus, the rates were evaluated within each population (susceptible, R-$_{F1}$, and R-$_{F2}$). The data were fitted to four- [y=A2+(A1–A2)/(1+(x/x0^p)] or two-parameter (y= ax +b) models, which were chosen considering the best biological explanation, statistical significance, and coefficient of determination. All equations were significant by the F test at 1% significance level and had coefficients of determination higher than 85%. In addition, the models met the requirements to calculate the LD_{50}, which is the herbicide rate needed to cause 50% control or decrease in shoot dry matter of plants, and the values were used to determine the resistance factor.

The high sensitivity of susceptible plants associated to the high resistance level of resistant plants resulted in high resistance factors for the fenoxaprop and haloxyfop herbicides. The application of 32 times the recommended rate of these herbicides was not enough to cause the death of plants with 2 to 3 tillers at the time of application, which is the recommended growth stage for the applications. The application of 64 times the recommended rate would probably not cause the death of all plants, since the action of high rates of fenoxaprop and haloxyfop has a contact effect, causing total necrose of the plants followed by their regrowth.

Considering there was no death of all plants, part of the data of fenoxaprop and haloxyfop was fitted to linear model, since they did not fit satisfactory (without statistical significance) to three- or four-parameter non-linear models. Probably, fenoxaprop and haloxyfop should be applied to smaller plants, without tillers, for the fitting of the data to these models. However, the objective was to prove the resistance and resistance heritability through LD_{50} and use the LD_{50} to estimate the resistance factor; thus, the linear model was adequate and met the proposed objectives. The data for glyphosate were fitted to the four-parameter model, with statistical significance and coefficients of determination higher than 90%.

The resistance factors of fenoxaprop ranged from 38.6 to 53.6 for $R\text{-}_{F1}$ and from 40.0 to 49.2 for $R\text{-}_{F2}$ (Table 3). The resistance factors were even higher for haloxyfop, ranging from 65.4 to 138.9 for $R\text{-}_{F1}$ and from 96.1 to 159.7 for $R\text{-}_{F2}$ (Table 4). Resistance to ACCase inhibiting herbicides had already been recorded in 2003 for goosegrass populations in soybean areas in the state of Mato Grosso (MT), Brazil (Heap, 2021). This first report confirmed the cross-resistance of goosegrass to herbicides of the aryloxyphenoxypropionate (FOP) and cyclohexanedione (DIM) chemical groups (Vidal et al., 2006). Another goosegrass population in MT developed cross-resistance, with high resistance levels for fenoxaprop (RF=143), haloxyfop (RF=126), sethoxydim (RF=84), and fluazifop (RF=58) (Osuna et al., 2012).

In addition to fenoxaprop and haloxyfop resistance, the $R\text{-}_{F1}$ and $R\text{-}_{F2}$ populations were not controlled by the recommended rate of glyphosate (1.0 kg a.e. ha^{-1}) (Table 5). The shoot dry matter data showed that the LD_{50} for the susceptible population was 0.21 kg a.e. ha^{-1}, and 2.34 and 1.17 kg a.e. ha^{-1} for the $R\text{-}_{F1}$ and $R\text{-}_{F2}$ populations, respectively. The resistance factors found for the populations $R\text{-}_{F1}$ and $R\text{-}_{F2}$ were 11.1 and 5.6, respectively, for percentage of control (45 DAA), and 9.4 and 5.7 for shoot dry matter, respectively.

The resistance levels found in the present study were higher than those found in the first report of goosegrass resistant to glyphosate in Brazil, which were 3.9 and 6.8 for shoot dry matter of $R\text{-}_{F1}$ plants from two selected resistant populations (Takano et al., 2017). The resistance level is partly connected to the resistance mechanism in plants; however, plant size at the time of application should be considered. In both studies, treated plants were at the same growth stage, that is, 2 to 3 tillers.

In the USA and Philippines, two- to four-fold resistance to glyphosate in goosegrass populations have been reported (Kaundun et al., 2008; Janel et al., 2016; Molin et al., 2013); four- to eight-fold resistance in Malaysia, China, and USA (Tennessee) (Lee, Ngim, 2000; Mueller et al., 2011; Chen et al., 2015); and more than 180-fold resistance to glyphosate was found in a goosegrass population in Malaysia (Jerantut) (Yu et al., 2015).

Goosegrass resistance to glyphosate has been often connected to target-site based resistance (TSR) mechanisms, where Pro106Ser or Pro106Tre mutations (Baerson et al.,

Table 3 - Fitting of the data to four-[(1)] or two-parameter [(2)] regression equations, coefficient of determination (R^2), and resistance factor (RF) for susceptible (S) and resistant ($R\text{-}_{F1}$ and $R\text{-}_{F2}$) goosegrass populations to the fenoxaprop-p-ethyl herbicide, for control at 15, 30, and 45 days after application (DAA) and shoot dry matter at 45 DAA (experiment in Brasília, DF, Brazil)

Populations	A1	A2	LD_{50} (kg ha^{-1})	p	R^2	RF
	Control - 15 DAA					
Susceptible	0.0000012	96.26	0.022	4.05	0.99	-
$R\text{-}_{F1}$	y= 47.00x + 9.96		0.850	-	0.88	38.64
$R\text{-}_{F2}$	y= 45.17x + 10.21		0.880	-	0.89	40.00
	Control - 30 DAA					
Susceptible	-0.00000041	97.66	0.026	24.17	0.99	-
$R\text{-}_{F1}$	6.50	87.78	1.280	5.41	0.92	49.23
$R\text{-}_{F2}$	y= 40.33x + 3.84		1.140	-	0.94	43.85
	Control - 45 DAA					
Susceptible	-0.00000061	98.09	0.027	25.11	0.99	-
$R\text{-}_{F1}$	5.77	87.92	1.310	4.81	0.92	48.52
$R\text{-}_{F2}$	y= 37.04x + 3.64		1.250	-	0.94	46.29
	SDM - 45 DAA					
Susceptible	31.85	1.08	0.025	6.94	0.99	-
$R\text{-}_{F1}$	31.20	0.96	1.340	3.77	0.90	53.60
$R\text{-}_{F2}$	30.24	1.94	1.230	2.49	0.93	49.20

[(1)] Model y=A2+(A1–A2)/(1+(x/x0^p), where x0 = LD_{50}, rate of fenoxaprop-p-ethyl that resulted in 50% control or decrease in shoot dry matter. [(2)] Model y= ax+b, with manual calculation of LD_{50}. RF calculated by the ratio between LD_{50} $R\text{-}_{F1}$ (or $R\text{-}_{F2}$) and LD_{50} S

Table 4 - Fitting of the data to four-[1] or two-parameter[2] regression equations, coefficient of determination (R^2), and resistance factor (RF) for the susceptible (S) and resistant (R_{-F1} and R_{-F2}) goosegrass populations to the haloxyfop-p-methyl herbicide, for control at 15, 30, and 45 days after application (DAA) and shoot dry matter at 45 DAA (experiment in Brasília, DF, Brazil)

Populations	A1	A2	LD_{50} (kg ha^{-1})	p	R^2	RF
			Control - 15 DAA			
Susceptible	0.00019	100.15	0.0026	0.60	0.99	-
R_{-F1}	2.76	82.08	0.1700	4.15	0.97	65.38
R_{-F2}	-1.34	91.60	0.2500	1.50	0.97	96.15
			Control - 30 DAA			
Susceptible	0.00000105	100.01	0.0088	4.14	1.00	-
R_{-F1}	y= 48.98x + 5.43		0.9100	-	0.91	103.41
R_{-F2}	y= 46.72x + 3.06		1.0000	-	0.96	113.64
			Control - 45 DAA			
Susceptible	-0.000000028	100.00	0.0100	5.47	1.00	-
R_{-F1}	y= 48.79x + 3.98		0.9400	-	0.96	94.00
R_{-F2}	y= 46.44x + 0.40		1.0700	-	0.99	107.00
			SDM - 45 DAA			
Susceptible	32.24	0.61	0.0072	4.17	0.99	-
R_{-F1}	y= -16.44x + 33.04		1.0000	-	0.92	138.89
R_{-F2}	y= -16.03x + 36.87		1.1500	-	0.87	159.72

[1] Model y=A2+(A1–A2)/(1+(x/x0^p), where x0 = LD_{50}, rate of haloxyfop-p-methyl that resulted in 50% control or decrease in shoot dry matter. [2] Model y= ax+b, with manual calculation of LD_{50}. RF calculated by the ratio between LD_{50} R_{-F1} (or R_{-F2}) and LD_{50} S

Table 5 - Parameters of regression equations[1], coefficient of determination (R^2), and resistance factor (RF) for susceptible (S) and resistant (R_{-F1} and R_{-F2}) goosegrass populations to the glyphosate herbicide, for control at 15, 30, and 45 days after application (DAA) and shoot dry matter at 45 DAA (experiment in Brasília, DF, Brazil)

Populations	A1	A2	LD_{50} (kg ha^{-1})	p	R^2	RF
			Control - 15 DAA			
Susceptible	0.0048	100.32	0.08	1.32	0.99	-
R_{-F1}	-4.44	98.00	0.62	2.05	0.98	7.75
R_{-F2}	4.55	97.16	0.82	3.56	0.99	10.25
			Control - 30 DAA			
Susceptible	0.000063	100.03	0.18	3.64	1.00	-
R_{-F1}	-1.32	103.17	1.16	1.65	0.97	6.44
R_{-F2}	4.20	99.75	1.12	3.49	0.99	6.22
			Control - 45 DAA			
Susceptible	0.000012	100.01	0.21	4.76	1.00	-
R_{-F1}	12.41	101.27	2.34	5.04	0.92	11.14
R_{-F2}	1.55	99.58	1.17	3.96	0.99	5.57
			SDM - 45 DAA			
Susceptible	31.46	0.81	0.19	5.38	0.99	-
R_{-F1}	32.97	-0.82	1.79	1.84	0.93	9.42
R_{-F2}	30.22	1.12	1.09	6.74	0.96	5.74

[1] Model y=A2+(A1–A2)/(1+(x/x0^p), where x0 = LD_{50}, rate of haloxyfop-p-methyl that resulted in 50% control or decrease in shoot dry matter. RF calculated by the ratio between LD_{50} R_{-F1} (or R_{-F2}) and LD_{50} S

2002; Ng et al., 2004; Kaundun et al., 2008), Tre102Iso + Pro106Ser [TIPS] double mutation (Yu et al., 2015), or genetic amplification of EPSPs (Chen et al., 2015) have been reported. TSR is also a common mechanism of resistance for many ACCase inhibiting herbicides, with occurrence of two mutations in goosegrass (Trp-2027-Cys and Asp-2078-Gly), which is found in resistant populations outside Brazil (Cha et al., 2014; McCullough et al., 2016). The non-target-site resistance (NTSR) mechanism is also important for the resistance to fluazifop-p-butyl, confirming the capacity of goosegrass in degrade this herbicide into non-toxic substances (Wang et al., 2017).

Resistance of goosegrass populations to ACCase or EPSPs inhibiting herbicides in Brazil have been attributed exclusively to TSR. The Asp2027Gly mutation was detected in goosegrass with cross resistance to aryloxyphenoxypropionate (FOP) and cyclohexanedione (DIM) herbicides, and the Pro106Ser mutation was responsible for resistance to glyphosate (Osuna et al., 2012; Takano et al., 2018). However, in these cases, the populations developed resistance to only one mechanism of action.

The dose-response experiments carried out in the second stage of the study confirmed the goosegrass resistance to the fenoxaprop, haloxyfop, and glyphosate herbicides, with variations in resistance factors due to the size of resistant and susceptible plants at the time of application (Table 6). The resistance factors found for fenoxaprop ranged from 640 to 568 for percentage of control of populations R-F1 and R-F2, respectively, and 318 and 395 for shoot dry matter of R-F1 and R-F2 plants, respectively. However, haloxyfop data for the susceptible population did not fit any regression model (two, three or four parameters), due to the high susceptibility of plants,

Table 6 - Parameters of regression equations[1], coefficient of determination (R^2), and resistance factor (RF) for susceptible (S) and resistant (R_{-F1} and R_{-F2}) goosegrass populations to the glyphosate herbicide, for control and shoot dry matter reduction (in percentage) at 21 days after application in plants with four leaves (experiment in Piracicaba, SP, Brazil)

Populations	A1	A2	LD_{50} (kg ha^{-1})	p	R^2	RF
			Fenoxaprop-p-ethyl - control			
Susceptible	-0.74	100.71	0.00098	-0.10	0.95	-
R_{-F1}	-1.19	115.47	0.63000	-2.17	0.94	640.20
R_{-F2}	-0.83	124.92	0.56000	-2.41	0.96	568.33
			Fenoxaprop-p-ethyl - SDM			
Susceptible	1.03	100.09	0.00014	0.49	0.96	-
R_{-F1}	1.50	96.23	0.43000	-0.12	0.90	318.45
R_{-F2}	0.65	101.06	0.53000	-31.97	0.93	395.03
			Haloxyfop-p-methyl - control			
Susceptible	NA	NA	<0.00019	NA	-	-
R_{-F1}	-2.55	101.78	0.05000	8.88	0.99	>27.84
R_{-F2}	-4.32	100.39	0.05000	8.66	0.99	>27.84
			Haloxyfop-p-methyl - SDM			
Susceptible	NA	NA	<0.00019	NA	-	-
R_{-F1}	9.01	101.79	0.06000	-0.28	0.98	>33.65
R_{-F2}	18.11	112.04	0.06000	0.32	0.99	>31.03
			Glyphosate - control			
Susceptible	-2.90	100.01	0.14000	-1.02	0.99	-
R_{-F1}	-2.49	100.28	0.58000	-1.85	0.98	4.05
R_{-F2}	-2.94	99.41	0.52000	-1.80	0.99	3.62
			Glyphosate - SDM			
Susceptible	4.31	102.14	0.14000	0.80	0.97	-
R_{-F1}	2.86	104.37	0.47000	0.57	0.99	3.33
R_{-F2}	6.84	100.49	0.46000	2.23	0.98	3.28

[1] Model y=A2+(A1–A2)/(1+(x/x0^p), where x0 = LD_{50}, rate of fenoxaprop-p-ethyl, of haloxyfop-p-methyl or of glyphosate that resulted in 50% control or decrease in the dry matter. RF calculated by the ratio between LD_{50} R_{-F1} (or R_{-F2}) and LD_{50} S. NA = data not fitted to the model

since the lowest tested rate. The resistance factor for glyphosate varied from 3.3 to 4.1 for the R-F1 population and from 3.3 to 3.6 for the R-F2 population.

A proactive action to manage ACCase- and glyphosate-resistant goosegrass is to delay or prevent the selection of resistance. It is important to resume the use of graminicide herbicides in pre-emergence, such as clomazone, isoxaflutole, s-metolachlor, trifluralin etc., whether in no-tillage or conventional soil preparation systems. These herbicides should be chosen considering the selectivity for the crops and efficacy against goosegrass plants. The main grain (soybean, maize, and bean), fiber (cotton), and energy (sugarcane) crops in Brazil have, in general, at least one grass selective and residual herbicide choice for the control of goosegrass (Rodrigues, Almeida, 2018).

The soil seed bank should also be managed, which can be done by avoiding weed seed production in the field, and thus, preventing the addition of new diaspores to the soil. Although the goosegrass cycle is annual in the conditions of Brazil, the control of this weed should focus not only on avoiding the interference of weeds with the crop, but also in preventing goosegrass plants to produce seeds and increment the soil seed bank.

The control of resistant plants is difficult and requires changes in the choice of herbicides and in the management of the area in the medium- and long-term. Therefore, the resistance problem should be identified at its beginning to implement proper resistance management strategies to contain resistance spread. The production sector (farmers, agronomists, technicians etc.) should act proactively to prevent the introduction or selection of resistant biotypes in agricultural areas without record of resistance. Rotation of herbicides from the cyclohexanodione e aryloxyphenoxypropionate chemical groups are a good strategy for farmers that use ACCase inhibiting herbicides, such as common bean and vegetable growers, to minimize or delay the selection of plants resistant to these herbicides, when compared to the exclusive use of herbicides of the same chemical group. However, resistance management should focus not only on rotating different herbicide mechanisms of action, but also integrating diversity to the overall weed management strategy.

Conclusion

Resistance to EPSPs (glyphosate) and ACCase (haloxyfop-methyl and fenoxaprop-ethyl) inhibiting herbicides was confirmed in the studied goosegrass population from Primavera do Leste, MT, Brazil.

Authors' contributions

NMC: planned and conducted part of the experiments, and wrote and submitted the manuscript for publication. LSA: conducted part of the experiments and contributed to the interpretation of the data and writing of the manuscript. RABJ: contributed to the greenhouse step, supporting the first author in the conduction of the experiments.

References

Baerson SR, Rodriguez DJ, Tran M, Feng Y, Biest NA, Dill GM. Glyphosate-resistant goosegrass: identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiol. 2002;129(3):1265-75. Available from: https://doi.org/10.1104/pp.001560

Barroso AAM, Albrecht AJP, Reis FC, Filho RV. [Accase and glyphosate diferent formulations herbicides association interactions on sourgrass control]. Planta Daninha. 2014; 32(3):619-27. Portuguese. Available from: https://doi.org/10.1590/S0100-83582014000300018

Bianchi L, Anunciato VM, Gazola T, Perissato SM, Dias RC, Tropaldi L et al. Effects of glyphosate and clethodim alone and in mixture in sourgrass (*Digitaria insularis*). J. Crop Prot. 2020;138. Available from: https://doi.org/10.1016/j.cropro.2020.105322

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK et al. Review: confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci. 2013;61(1):4-20. Available from: https://doi.org/10.1614/WS-D-12-00032.1

Cha TS, Najihah MG, Sahid IB, Chuah TS. Molecular basis for resistance to ACCase-inhibiting fluazifop in *Eleusine indica* from Malaysia. Pestic Biochem Physiol. 2014;111:7-13. Available from: https://doi:10.1016/j.pestbp.2014.04.011

Chen J, Huang H, Zhang C, Wei S, Huang Z, Chen J et al. Mutations and amplification of EPSPS gene confer resistance to glyphosate in goosegrass (*Eleusine indica*). Planta. 2015;242(4):859-68. Available from: https://doi:10.1007/s00425-015-2324-2

Christoffoleti PJ, López-Ovejero RF. [Weed resistance to herbicides: definitions, bases and situation in Brazil and worldwide]. In: Christoffoleti PJ, Coordenador. [Aspects of weed resistance to herbicides]. 3th ed. Piracicaba: Associação Brasileira de Ação a Resistência de plantas daninhas; 2008. p. 9-29. Portuguese.

Ferreira DF. Sisvar: a computer statistical analysis system. Cienc Agrotec. 2011;35(6):1039-42. Available from: https://doi.org/10.1590/S1413-70542011000600001

Ganeshaiah KN, Umashaanker R. Evolution of reproductive behavior in the genus *Eleusine*. Euphytica. 1982;3(2):397-404. Available from: https://doi.org/10.1007/BF00021656

Heap I. The international survey of herbicide resistant weeds. Weedscience. 2021[access Sept 23, 2021]. Available from: http://www.weedscience.org/

Janel LH, Riggins CW, Steckel LE, Tranel PJ. The EPSPS Pro106Ser substitution solely accounts for glyphosate resistance in a goosegrass (*Eleusine indica*) population from Tennessee, United States. J Integr Agric. 2016;15(6):1304-12. Available from: https://doi.org/10.1016/S2095-3119(15)61220-5

Kaundun SS, Zelaya IA, Dale RP, Lycett AJ, Carter P, Sharples KR, et al. Importance of the P106S target-site mutation in conferring resistance to glyphosate in a goosegrass (*Eleusine indica*) population from the Philippines. Weed Sci. 2008; 56(5):637-46. Available from: https://doi:10.1614/ws-07-148.1

Kissmann KG, Groth D. [Weeds and harmful plants]. 2nd ed. São Paulo: Basf; 1997. Portuguese.

Lee LJ, Ngim J. A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. Pest Manag Sci. 2000;56(4):336-9. Available from: https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<336::AID-PS123>3.0.CO;2-8

McCullough PE, Yu J, Raymer PL, Chen Z. First report of ACCase-resistant goosegrass (*Eleusine indica*) in the United States. Weed Sci. 2016;64(3):399-408. Available from: https://doi.org/10.1614/WS-D-15-00203.1

Molin WT, Wright AA, Nandula VK. Glyphosate-resistant goosegrass from Mississippi. Agronomy. 2013;3(2):474-87. Available from: https://doi.org/10.3390/agronomy3020474

Mueller TC, Barnett KA, Brosnan JT, Steckel LE. Glyphosate-resistant goosegrass (*Eleusine indica*) confirmed in Tennessee. Weed Sci. 2011;59(4):562-6. Available from: https://doi.org/10.1614/WS-D-11-00063.1

Ng CH, Wickneswary R, Salmijah S, Teng YT, Ismail BS. Glyphosate resistance in *Eleusine indica* (L) Gaertn from different origins and polymerase chain reaction amplification of specific alleles. Austr J Agric Res. 2004;55(4):407-14. Available from: https://doi.org/10.1071/AR03155

Osuna MD, Goulart ICGR, Vidal RA, Kalsing A, Ruiz Santaella JP, Prado R. Resistance to ACCase inhibitors in *Eleusine indica* from Brazil involves a target site mutation. Planta Daninha. 2012;30(3):675-81. Available from: https://doi.org/10.1590/S0100-83582012000300025

Rodrigues BN, Almeida FLS. [Herbicide guide]. 7th ed., Londrina: Produção Independente; 2018. Portuguese.

Sociedade Brasileira da Ciência das Plantas Daninhas - Sbcpd. [Procedures for installing, evaluating and analyzing herbicide experiments]. Londrina: Sociedade Brasileira da Ciência das Plantas Daninhas; 1995. Portuguese.

Seefeldt SS, Jensen JE, Fuerst EP. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 1995; 9(2):218-225. Available from: https://doi.org/10.1017/S0890037X00023253

Takano HK, Mendes RR, Scoz LB, Lopez Ovejero RF, Constantin J, Gaines TA et al. Proline-106 EPSPS mutation imparting glyphosate resistance in goosegrass (*Eleusine indica*) emerges in South America. Weed Sci. 2018;67(1):48-56. Available from: https://doi.org/10.1017/wsc.2018.71

Takano HK, Oliveira Jr. RS, Constantin J, Braz GBP, Gheno EA. Goosegrass resistant to glyphosate in Brazil. Planta Daninha. 2017;35:1-9. Available from: https://doi.org/10.1590/S0100-83582017350100013

Vidal RA, Portes ES, Lamego FP, Trezzi MM. [Eleusine indica resistance to ACCase inhibitors]. Planta Daninha. 2006;24(1):163-71. Portuguese. Available from: https://doi.org/10.1590/S0100-83582006000100021

Wang CS, Lin WT, Chiang YJ, Ching-Yuh Wang CY. Metabolism of fluazifop-p-butyl in resistant goosegrass (*Eleusine indica*) in Taiwan. Weed Sci. 2017;65(2):228-38. Available from: https://doi.org/10.1017/wsc.2016.35

Yu Q, Jalaludin A, Han H, Chen M, Sammons RD, Powles SB. Evolution of a double amino acid substitution in the 5-enolpyruvylshikimate-3-phosphate synthase in *Eleusine indica* conferring high-level glyphosate resistance. Plant Physiol. 2015;167(4):1440-7. Available from: https://doi.org/10.1104/pp.15.00146