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Research Article

Glyphosate-resistant goosegrass population shows impaired translocation patterns

População de capim-pé-de-galinha resistente ao glyphosate apresenta padrões prejudicados de translocação

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ABSTRACT

Several reports of herbicide resistance in goosegrass have been confirmed worldwide. This work aimed to study a putative glyphosate-resistant goosegrass and investigate some non-target site mechanisms involved. Dose-response studies were conducted in greenhouse conditions. Visual control, and biomass reduction were evaluated 28 days after application. Absorption and translocation studies of ^{14}C-glyphosate were conducted in a growing chamber. The ^{14}C-glyphosate was applied to the third fully expanded leaf and evaluated at 4, 8, 24, 48, and 96 hours after treatment - HAT. The study results showed that the biotype 115-R was identified as resistant to glyphosate with a resistance factor of 6.6 based on GR_{50}. The absorption and translocation studies show different responses among the populations evaluated. The standard susceptible population 18-S has absorbed 58% of ^{14}C-glyphosate while the 115-R absorbed 44% at 96 HAT. Also, the translocated ^{14}C-glyphosate was higher in 18-S (34%) than 115-R (17%). The results indicate impaired absorption and translocation involved in the resistance of 115-R goosegrass population to glyphosate.

KEYWORDS: *Eleusine indica*. Herbicides. Weeds. Resistance. Non-target site mechanisms.

RESUMO

Vários relatos de resistência a herbicidas em capim-pé-de-galinha foram confirmados em todo o mundo. Este trabalho teve como objetivo estudar a possível resistência da população de capim-pé-de-galinha ao glyphosate e investigar alguns mecanismos de resistência não relacionados ao sítio de ação. Estudos de dose-resposta foram conduzidos em condições de casa de vegetação. O controle e a redução de biomassa foram avaliados aos 28 dias após a aplicação. Estudos de absorção e translocação do ^{14}C-glyphosate foram realizados em câmara de crescimento. O ^{14}C-glyphosate foi aplicado na terceira folha completamente expandida e avaliado às 4, 8, 24, 48 e 96 horas após o tratamento (HAT). Os resultados do estudo mostraram que o biótipo 115-R foi identificado como resistente ao glyphosate, com um fator de resistência de 6,6 com base no GR_{50}. Os estudos de absorção e translocação apresentaram respostas diferentes entre as populações avaliadas. A população considerada suscetível, 18-S, absorveu 58% do ^{14}C-glyphosate, enquanto a 115-R absorveu 44% às 96 HAT. Além disso, a translocação do ^{14}C-glyphosate foi maior na 18-S (34%) do que na 115-R (17%). Os resultados indicam absorção e translocação comprometidas envolvidas na resistência da população 115-R de capim-pé-de-galinha ao glyphosate.

PALAVRAS-CHAVE: *Eleusine indica.* Herbicidas. Plantas daninhas. Resistência. Mecanismos não relacionados ao sítio de ação.

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INTRODUCTION

One of the biggest challenges in the future's agriculture to increase the crop yield is the weed resistance management. The use of herbicides has exerted selection pressure on weeds, leading to the development of resistance to at least one herbicide in 273 species globally. To date, 59 cases have been reported in Brazil (HEAP 2025). Goosegrass (*Eleusine indica)* has been considered one of the most important weeds in Brazil in soybean, corn, and cotton areas. Three official reports of resistance were done, involving two mechanisms of action: acetyl-CoA carboxylase inhibitors (ACCase, HRAC Group 1), 5-enolpyruvylshikimate-3-phosphate synthase inhibitor (EPSPS, HRAC group 9) (OSUNA et al. 2012, TAKANO et al. 2017).

Goosegrass is native to the Asian continent, however, the species has spread to most tropical and subtropical regions worldwide (MUELLER et al. 2011). The annual life cycle, rapid growth, and development, combined with a high seed production capacity, make this species a significant weed in grain production areas, and it was found in 42% of the agricultural fields across the country in Brazil (ULGUIM et al. 2013, LUCIO et al. 2019). Under conditions of soil temperature fluctuation, goosegrass seeds achieved 80% germination 12 days after sowing, tillering begins nine days after emergence (DAE), with intense tillering and dry matter accumulation occurring between 38 and 43 DAE; maximum leaf area is reached at 71 DAE; it produces 120,000 seeds at 108 DAE; and completes its life cycle at 120 DAE (TAKANO et al. 2017).

Since the 1990's decade, the goosegrass control was frequently based on ACCase inhibitors in post-emergence applications. However, in 2003 the first goosegrass resistance investigations demonstrated a biotype from Primavera do Leste (MT) resistant to ACCase herbicides cyhalofop-butyl, fenoxaprop p-ethyl, and sethoxydim (OSUNA et al. 2012). Some years later, the Roundup Ready® (RR®) technology was adopted in Brazil, and chemical control was extensively performed with the use of glyphosate in soybean, corn, and cotton. As a result of selection pressure, several species, including goosegrass in 2016, have been reported as resistant to glyphosate in Brazil (HEAP 2025, TAKANO et al. 2017). Furthermore, more complex cases were confirmed in the following years, in which goosegrass plants survived applications of glyphosate and the ACCase-inhibiting herbicides fenoxaprop-p-ethyl and haloxyfop-methyl (CORREIA et al. 2022), and later, some populations were found with resistant to clethodim, haloxyfop-methyl and glyphosate (NUNES et al. 2022).

Resistance can be endowed by two types of mechanisms: target-site resistance (TSR) and non-target-site resistance (NTSR) (SAMMONS & GAINES 2014). The most frequent mechanism of TSR are mutations at the herbicide target site gene, preventing the herbicide from effectively obstructing the target site. On the other hand, NTSR commonly results in impaired patterns of herbicide absorption, translocation, and/or metabolism, converting them into non-toxic compounds. The recurring instances of resistance to herbicides in goosegrass are generally attributed to target-site resistance (TAKANO et al. 2020). Mutations such as Asp-2078-Gly and Trp-2027-Cys in the ACCase enzyme are significant mechanisms of resistance found in goosegrass (OSUNA et al. 2012, CHA et al. 2014, JALALUDIN et al. 2015, MCCULLOUGH et al. 2016). Additionally, the metabolism of herbicides can also contribute to resistance to

herbicides, as verified in species goosegrass and Italian ryegrass (*Lolium rigidum)*, and also, impaired translocation in smooth pigweed (*Amaranthus hybridus*) (NALIN et al. 2023, MCCULLOUGH et al. 2016, POWLES et al. 2010, WANG et al. 2017).

We have been investigating a goosegrass population that could be resistant to glyphosate. Besides, TSR predominantly evolves in GR goosegrass around the globe (TAKANO et al. 2019), it has been rare NTSR studies in populations from Brazil. Hence, it is necessary further investigations including absorption and translocation methods. This manuscript aims to investigate how is the absorption and translocation of glyphosate in some surviving goosegrass populations from the main agronomic systems in Brazil.

MATERIAL AND METHODS

Plant Material for the Dose–Response Test

Seeds samples came from a weed resistance monitoring program (SIMON - Bayer Crop Science) performed over 10 years, where researchers collect the seeds after verifying a control failure after glyphosate spray in the field. The goosegrass seeds were obtained from an agricultural production area in the municipality of Campo Verde, MT, Brazil (15°31'05.9" S, 55°11'59.3" W). These samples were then homogenized to form a composite sample consisting of at least 1,000 seeds (BURGOS et al. 2013). The seeds were stored in brown paper bags, properly labeled with the geographic coordinates, municipality, and state where the collection was conducted, and remained in these conditions until the experiment was initiated.

Dose–Response assay

The experiments were conducted in a greenhouse at the Experimental Station of Agro do Mato Soluções Agronômicas Ltda, located in the Santa Bárbara D'Oeste, São Paulo state (22° 48' 47" S; 47° 28' 45" W; 601 m altitude). The seeds were placed in 2 L plastic containers filled with commercial substrate. When the plants had two leaves, they were transplanted into 200 mL pots filled with a mixture of substrate and soil in a 3:1 ratio, where they remained until the end of the experiment, with an average density of one plant per pot, without water deficiency, and properly fertilized. When the plants reached the early tillering stage they were subjected to a dose-response curve experiment to assess the level of resistance of the species to the aforementioned herbicides. The experimental design was a randomized complete block design with 13 treatments and four replications. The treatments consisted of thirteen doses of glyphosate (0, 30, 60, 120, 240, 480, 960, 1.920, 3.840, 7.680, 15.360, 30.720, and 61.440 g ae ha^{-1}).

For treatments applications, a precision backpack sprayer pressurized by CO_2 was used, equipped with a boom with two XR TeeJet 110.02 nozzles, positioned 0.50 m above the targets, with a spray volume of 200 L ha^{-1}. Applications were always held in the morning in suitable weather conditions.

Visual control evaluations were performed 28 days after application (DAA), as well as the percentage of residual dry mass. For the visual control rates, 0% was assigned in the absence of herbicide symptoms and 100% for plant death, according to the method proposed by the Brazilian Society of Weed Science (VELINI et al. 1995). Plant biomass was obtained by harvesting the remaining plant material in the plots,

followed by drying in an oven at 70 °C for 72 hours. Dry mass was corrected to percentage values by comparing the mass obtained in the herbicide treatments with that of the untreated control, considered as 100%.

ABSORPTION, TRANSLOCATION STUDY

Preparation of ^{14}C-Glyphosate Solution

These experiments were developed at the Laboratory of Ecotoxicology of the CENA/USP, in Piracicaba, SP, Brazil. The study used ^{14}C-glyphosate (98% purity) with a specific activity of 55 mCi mmol^{-1}, sourced from American Radiolabeled Chemicals, Sant Louis, MO, USA. The ^{14}C-glyphosate solution was prepared by combining 200 µL of the radiolabeled compound with the conventional formulation (Roundup Transorb R, 480 g ae kg^{-1}) to achieve a total activity of 66.6 KBq. In all experiments, approximately 200,000 dpm per plant (3.3 KBq per plant) of ^{14}C-glyphosate were applied.

Absorption and Translocation of ^{14}C-Glyphosate

The experiment was conducted using a completely randomized design within a factorial scheme, involving two goosegrass populations (18-S - susceptible and 115-R - resistant) and five times of evaluation (4, 8, 24, 48, and 96 hours after treatment) with three biological replicates for both absorption and translocation studies. To prepare for herbicide application, the third youngest leaf of each plant was covered with plastic. Non-radiolabeled commercial formulations of glyphosate were sprayed using a CO_2-pressurized backpack calibrated to deliver 200 L ha^{-1} at 250 kPa, with a flat fan spray nozzle (model XR 11002) positioned 50 cm from the plants. Subsequently, radiolabeled ^{14}C-glyphosate was applied on the same covered leaf from the last step, at rates of 1440 g ae ha^{-1}. After removing the plastic covers, the radiolabeled herbicides were applied using an automatic microsyringe (Hamilton PB6000 Dispenser, Hamilton, CO, USA). Ten 1-µL droplets were carefully placed on the upper surface of each plant's leaf.

The treated leaves underwent a washing procedure with 3 mL of a methanol solution (50:50 v/v). Two 500 µL aliquots of the wash solution were then collected to quantify radioactivity through liquid scintillation spectrometry (LSS), enabling the determination of the proportion of herbicide that remained unabsorbed from the total amount applied. The plants were subsequently uprooted, and their roots were thoroughly washed. Following this, the entire plants were dried at 45 °C for 24 hours in an oven. Autoradiography images were captured using a radio scanner (Packard-Cyclone, Perkin Elmer, Shelton, CT, USA) for qualitative analysis.

The dried plants were then sectioned into three parts for quantitative analysis: treated leaf, root, and the rest of the shoot. Each section was combusted in a biological oxidizer (OX 500—R.J. Harvey Instrument Co., Tappan, NY, USA) at 950 °C for 3 minutes, producing ^{14}CO2, which was captured in scintillation vials for radioactivity measurement via LSS. The absorbed percentages of ^{14}C-glyphosate were calculated as the ratio of absorbed radioactivity to the total applied radioactivity. The percentage of herbicide translocation was determined by summing the radioactivity of each plant section, excluding the treated leaf, and expressing this as a percentage of the total absorbed radioactivity.

Statistical Analysis

The dose-response curves were fitted to a non-linear logistic regression model. The control variable was fitted to the model of three parameters (STREIBIG 1988). The model parameters were estimated using the drc package in R software.

$$y = \frac{a}{[1+\left(\frac{x}{b}\right)c]} \quad (1)$$

Where: *y* denotes the dependent variable, either control or dry mass, while *x* corresponds to the independent variable, specifically the herbicide dose. The parameter *a* represents the maximum response value, *b* indicates the slope of the curve near *c*, and *c* signifies the dose required to achieve a 50% response (LD_{50} or GR_{50}). The LD_{50} (control by 50%) and GR_{50} (growth reduction by 50%) are herbicide doses that result in 50% weed control or 50% reduction in weed biomass, respectively (CHRISTOFFOLETI 2002). The resistance factor (RF) was determined by calculating the ratio of the LD_{50} and GR_{50} values between the putative-resistant and sensitive populations.

The data regarding ^{14}C-glyphosate absorption and translocation were subjected to analysis of variance (ANOVA). When the population (18-S and 115-R) x HAT time interaction was significant, the interaction was sliced, and means were compared within each time point using the Tukey test at a 5% significance level. The data were also assessed with a non-linear regression exponential rise to a maximum with two parameters.

$$f = a * (1 - exp\ exp\ (-b * x)\ ) \quad (2)$$

Where: *a* represents the asymptote or the maximum value that the function can reach, *b* is a constant rate which controls the rate at which the function rises toward its maximum value, and *x* is an independent variable represented for the time of evaluation.

RESULTS

Plant Dose-Response Curve

Both populations showed increased control and biomass reduction as the glyphosate dose increased ($p < 0.05$). However, dose-response analysis revealed a different pattern of increased symptoms among 18-S and 115-R goosegrass populations (Figure 1a, b). The needed dose to control 50%, and to reduce 50% of mass to 115-R was higher than 18-S population, resulting in a RF between 5.9 (DL50) and 6,68 (GR50) (Table 1), and based in LD_{90} and GR_{90} values, the glyphosate doses required to control the resistant biotype were 12.5 and 8.3 times higher, respectively, than those needed for the susceptible population.

The highest dose applied in this study (61440 g a.e ha^{-1}) effectively controlled the 115-R population. The model has an estimated 24731.88 and 14631.05 g ae ha^{-1} for LD_{90} and $GR_{90,}$ respectively, which means a satisfactory control level. However, those doses are 25x and 15x times higher than the field-recommended dose (960 g ae ha^{-1}) (Table 1).

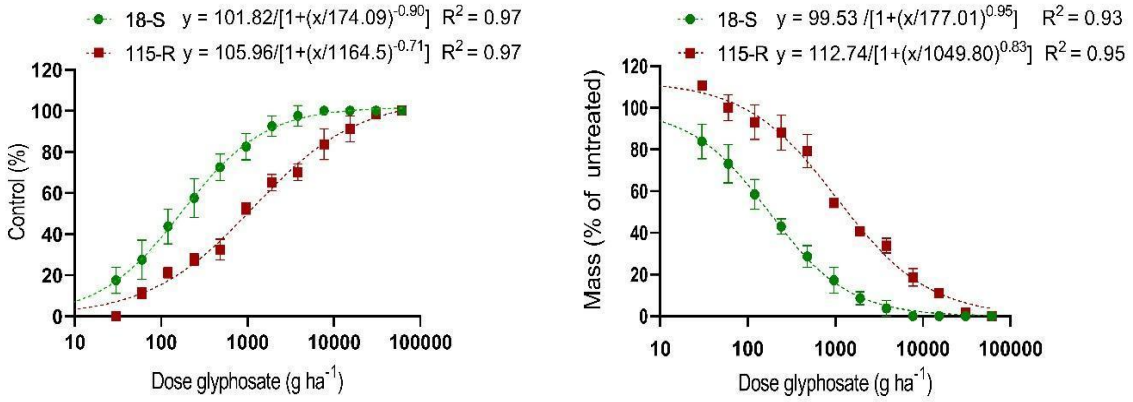


Figure 1. Dose-response curves for the 115-R and 18-S populations at 28 DAA. (a) Control levels and (b) mass reduction under glyphosate doses. Model fit: y = a/[1+(x/c)b]. Error bars represent the standard error of the means (n = 4).

Table 1. Glyphosate dose of 50% and 90% of the control (LD), mass reduction (GR), and resistant factor (RF) in *goosegrass*.

Glyphosate	Biotypes	
	18-S	115-R
LD_{50}	174.09 ± 11	1164.50 ± 144
[1]RF (LD_{50})	-	6.68
[1]GR_{50}	177.01 ± 27	1049.80 ± 146
[1]RF (GR_{50})	-	5.9
LD_{90}	1966.50 ± 346	24731.88 ± 7138
GR_{90}	1746.19 ± 374	14631.05 ± 2938

[1] Calculated by the ratio of LD 50 and GR 50 of 115-R/18-S. Model fit: y = a[/1+(x/b)c].

Absorption and translocation of ^{14}C-Glyphosate

The average of total radioactivity recovery (washed and absorbed) was 79 ± 2 (ranging from 75% to 85%) of the total applied dose across all treatments.

The analysis of variance (ANOVA) revealed a significant interaction between biotypes and evaluation times ($p < 0.001$). Comparison at each time point is summarized in Table 2. Although that, the absorption and translocation behavior of ^{14}C-glyphosate is represented in Figure 2. There were different patterns of absorption among the 18-S and 115-R goosegrass populations. Both populations had the absorption increasing to 24 HAT, and at 48 and 96 HAT the values were similar. In the last time of evaluation, at 96 HAT the maximum absorbed was 58% and 44% for 18-S and 115-R, respectively. The translocation of ^{14}C-glyphosate also shows different patterns among the evaluated populations (Figure 2b). The 18-S presented higher translocation (34%) when compared to the 115-R (17%) *goosegrass* population. While the 115-R population reaches a stabilized translocated value at 96 HAT, the 18-S

shows an increased behavior at 96 HAT, where the estimated model shows that the values will continue increasing after this time.

Table 2. Summary of *p-values* for the comparison between susceptible (18-S) and resistant population (115-R) at each time point.

Variable	4 HAT	8 HAT	24 HAT	48 HAT	96 HAT
Absorption	<0.001	<0.001	0.17	0.05	0.23
Translocation	0.62	0.61	0.42	<0.001	<0.001

P-values were calculated using the F-test for the interaction, followed by means comparison within each time point using the Tukey (*p-value 0.05*).

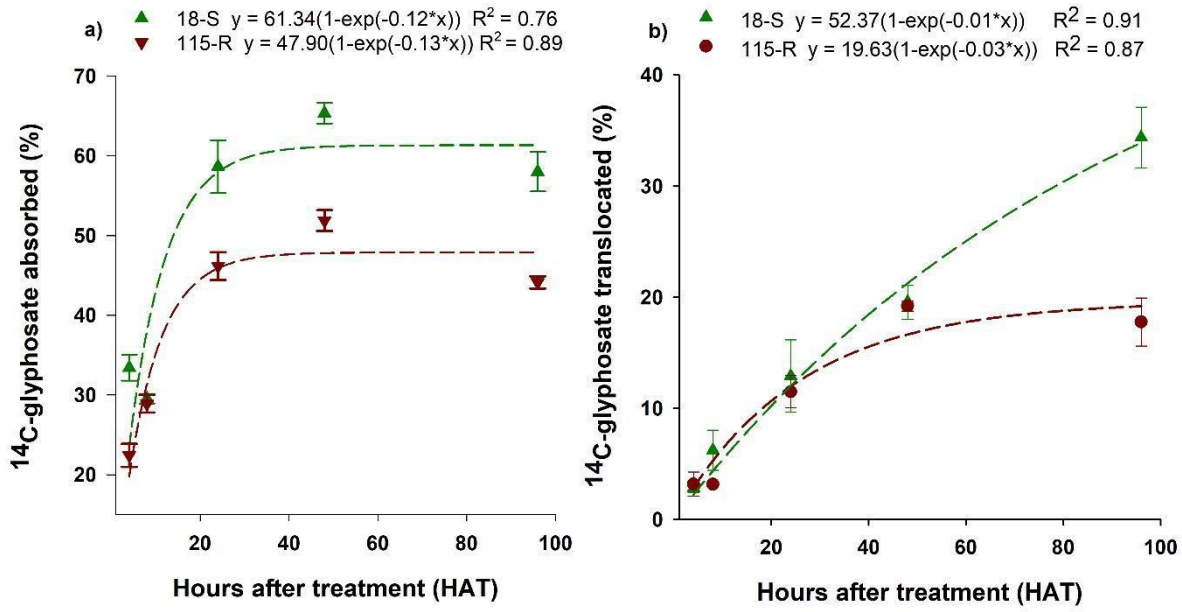


Figure 2. Patterns of absorption (a) and translocation (b) after ^{14}C-glyphosate sprayed in 18-S and 115-R goosegrass populations. Error bars represent the standard error of the means (n = 3).

DISCUSSION

Plant Dose-Response Curve

Weed resistance has been a complex and growing issue in modern agriculture. Its occurrence poses a substantial threat to global food security and sustainable farming systems. Quickly identifying resistant weed species and understanding the evolutionary mechanism that drives their resistance is crucial for developing effective alternative strategies to achieve successful control within integrated weed management programs (OWEN 2016). Monitoring systems for weed resistance and the use of dose-response curves are fundamental tools in assessing the efficacy of herbicides and the susceptibility of weed populations (CARVALHO et al. 2011, OVEJERO et al. 2017, CORREIA et al. 2020, MENDES et al. 2021).

Goosegrass can rapidly develop resistance to multiple herbicides, and it presents significant challenges for effective weed management. The first report of glyphosate-resistant goosegrass in Brazil was in 2016, with an RF of 7.19 (HEAP 2025, TAKANO et al. 2017). After that, other research found biotypes with cross and multiple herbicide resistance involving ACCase inhibitors and EPSPs inhibitor. In the present study, the resistance factors (RF) obtained were 5.9 (GR_{50}) and 6.68 (LD_{50}). These values align

with those reported by CORREIA et al. (2022), who observed RFs ranging from 5.57 to 11.14. Furthermore, while VIDAL et al. (2006) and KAUNDUN et al. (2008, 2011) suggest that RFs between 2.0 and 5.0 are common across various species, our results slightly exceed this range, yet remain consistent with the 'low level of glyphosate resistance' classification proposed by NUNES et al. (2022).

The effectiveness of post-emergence chemical control for resistant goosegrass is reduced due to the loss of efficacy of herbicides that inhibit EPSPs and ACCase enzymes. The ammonium-glufosinate herbicide can be a good tool to control these populations. However, it just shows a good level of control when applied to small plants (CORREIA et al. 2022). To overcome the challenges of post-emergence control, a robust management strategy must incorporate the use of pre-emergence herbicides. Applying residual herbicides to the soil allows for the control of goosegrass before emergence, significantly reducing the initial population density and providing a wider window for subsequent interventions.

Furthermore, the integration of non-chemical inputs is crucial for a sustainable Integrated Weed Management (IWM) program. Practices such as maintaining straw mulch and utilizing cover crops are effective in suppressing germination through physical barriers and allelopathy. By combining the residual effect of pre-emergence chemicals with cultural and mechanical practices, growers can decrease the selection pressure on post-emergence herbicides and improve the long-term management of resistant biotypes.

Absorption and translocation of ^{14}C-Glyphosate

Reduced absorption and impaired translocation represent key non-target site resistance (NTSR) mechanisms contributing to weed resistance (GHANIZADEH et al. 2017). The data analyzed indicate that the patterns of herbicide absorption and translocation differed between the susceptible and resistant populations over the 96 hours. Regarding absorption, significant differences were observed only at the early stages of the experiment (4 and 8 HAT; $p<0.001$), where the resistant biotype (115-R) exhibited a slower initial uptake compared to the susceptible one (15-S). However, this gap narrowed over time, with absorption levels becoming statistically similar from 24 HAT onwards ($p>0.05$), indicating that both populations eventually absorbed comparable amounts of the herbicide.

In contrast, the translocation profile followed a different trajectory. While no differences were detected during the first 24 hours ($p>0.05$), a sharp divergence occurred at 48 and 96 HAT ($p<0.001$). At these later time points, the susceptible biotype showed substantially higher herbicide movement, whereas the resistant biotype demonstrated impaired translocation. These results suggest that, although absorption was initially slower in the resistant population, it was the restricted translocation rather than total absorption that served as the primary physiological barrier contributing to the glyphosate resistance in the 115-R biotype.

Glyphosate resistant goosegrass is frequently associated with TSR mechanisms, including mutations such as Pro106Ser or Pro106Thr (KAUNDUN et al. 2008, BAERSON et al. 2002), the Tre102Iso + Pro106Ser (TIPS) double mutation (YU et al. 2015), or the genetic amplification of EPSPS (CHEN et al. 2015). In Brazil, the resistance of goosegrass populations to EPSPS inhibiting herbicides has been

attributed exclusively to Thr-102 and Pro-106 mutations (TAKANO et al. 2019, ARAÚJO et al. 2023). Even though is still necessary to investigate TSR mechanism for 115-R, this work illustrated that NTSR mechanism can evolve independently in some goosegrass population.

The use of radiolabeled herbicides in experimental studies provides a definitive method for identifying reduced absorption and impaired translocation of herbicides within plants (GOGGIN et al. 2016). Using the radiolabeled herbicides some researchers have found impaired translocation contributing to the weed resistance in sourgrass (*Digitaria insularis*) (CARVALHO et al. 2012), waterhemp (*Amaranthus tuberculatus*) (NANDULA et al. 2013), ryegrass species -*Lolium rigidum*and *Lolium perenne* (ADU-YEBOAH et al. 2014, GHANIZADEH et al. 2015), horseweed (*Conyza bonariensis*) (KLEINMAN et al. 2017), palmer amaranth (*Amaranthus palmeri*) (PALMA-BAUTISTA et al. 2019), and smooth pigweed (NALIN et al. 2023).

However, the resistance landscape for goosegrass in South America involves distinct pathways. TAKANO et al. (2019) demonstrated that glyphosate resistance in Brazilian goosegrass populations can be primarily attributed to a target-site resistance (TSR) mechanism, specifically a Pro-106-Ser substitution in the EPSPS gene. In their study, no significant differences were observed in ^{14}C-glyphosate uptake, translocation, or metabolism between resistant and susceptible biotypes. This distinction is crucial, as it suggests that while some populations rely on enzymatic mutations (TSR), others may evolve physiological barriers (NTSR), highlighting the complex and independent evolution of resistance mechanisms in this species.

This study demonstrated that the resistant goosegrass population (115-R) possesses the ability to reduce glyphosate translocation. However, it is important to note that resistance in goosegrass can involve multiple pathways; for instance, non-target-site resistance (NTSR) mechanisms have already been reported to play a role in resistance to fluazifop-P-butyl, enabling this species to metabolize the herbicide into non-toxic compounds (WANG et al. 2017). Although our findings confirm physiological barriers to glyphosate, other NTSR or target-site resistance (TSR) mechanisms that may coexist and contribute to the overall resistance level were not investigated in this biotype. Therefore, future research involving the 115-R population is essential, focusing on identifying potential TSR mechanisms, screening for cross-resistance to other modes of action, and evaluating integrated weed management alternatives.

CONCLUSION

The results confirmed that the 115-R goosegrass population is resistant to glyphosate*.* Impaired translocation has contributed to resistance in the 115-R population.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, A.G.N, D.N, V.L.T and G.J.P.J; software and validation, D.N, and A.G.N; investigation, A.G.N, D.N, and G.J.P.J; resources and data curation, D.N, A.G.N, and V.F.V.S; writing-original draft

preparation, D.N, A.G.N, V.F.V.S, and G.J.P.J; writing-review and editing, D.N, A.G.N and G.J.P.J; visualization, D.N, A.G.N and G.J.P.J; supervision, M.N, V.L.T, and R.F.L.O; project administration, G.J.P.J, V.F.V.S and A.G.N; funding acquisition, R.F.L.O, and M.N. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT
Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT
Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT
The data can be made available under request.

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CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

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