

First case of resistance to bispyribac-sodium in barnyardgrass (*Echinochloa crus-galli*) from Ecuador

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Abstract: Background: *Echinochloa crus-galli* is a weed that has developed resistance to multiple herbicides. In Ecuador, the selection pressure caused by the continued use of the acetolactate synthase-inhibiting herbicide bispyribac-sodium would have favored the selection of resistant accessions.

Objective: The current study aimed to analyze the sensitivity to bispyribac-sodium of *E. crus-galli* populations collected in the province of Guayas, the main rice-growing region of Ecuador. **Methods:** A total of 35 *E. crus-galli* populations were assessed. These were sprayed with bispyribac-sodium at a commercial dose of 40 g a.i. ha⁻¹. The four most resistant accessions and the most susceptible population were submitted to dose-response curves. Data were adjusted using a log-logistic model and the mean lethal dose (LD₅₀)

for each population was calculated. Finally, response of the populations to the herbicide and their interaction with malathion, a cytochrome P450 monooxygenase inhibitor, was studied. **Results:** Seventy-two percent of the 35 accessions showed poor to deficient control (<70%) by bispyribac-sodium. Neither population showed 100% mortality in response to the recommend dose the herbicide. The LD₅₀ values for the R populations were at least 16.1 times higher than those for the S population. The interaction of bispyribac-sodium with malathion indicated that, for at least one of the R populations, resistance may be determined by xenobiotic metabolism. **Conclusions:** *E. crus-galli* populations collected from rice fields showed high level of resistance to bispyribac-sodium, even at the highest doses evaluated.

Keywords: Herbicide Resistance; Dose-Response; Detoxification; Metabolism, ALS Inhibitors

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1. Introduction

Rice (*Oryza sativa* L.) is one of the main crops worldwide. In Ecuador, this crop is one of the non-perennial crops with the largest cultivated area (312,900 ha⁻¹), along with corn. In this country, about 65% is produced in Guayas province, 25% in Los Ríos province and about 10% in other provinces (Fuentes, 2021). Rice cultivation is affected by *E. crus-galli*, a troublesome weed due to its resistance to herbicides of different modes of action, including propanil and acetolactate synthase (ALS)-inhibiting herbicides in many populations (Riar et al., 2013). Also, this weed is highly efficient and competitive against rice due to its adaptation to flooded environments and rapid growth, producing up to 34,000 seeds per m² in ideal environments (Bosnic, Swanton, 1997). In conditions of high temperatures and limited water availability, i.e. possible scenarios associated with climate change, it is considered that *E. crus-galli* will be able to show even better competitive ability against rice (Rodenburg et al., 2011).

ALS-inhibiting herbicides have been employed in rice cultivation to control *E. crus-galli*, owing to the ineffectiveness of propanil. However, excessive use of these herbicides has led to the emergence of resistant biotypes in rice crops (Valverde, 2007). ALS-inhibiting herbicides are distinguished by having had the greatest participation in the chronological increase in herbicide resistance at a global level. Evidence has shown that *E. crus-galli* has developed resistance to most ALS-inhibiting herbicides used in rice, including a pyrimidinylbenzoate (bispyribac-sodium) and triazolopyrimidine (penoxsulam) herbicides (Kaloumenos et al., 2013), as well as to imazethapyr and imazamox, which are used in rice resistant to imidazolinones (Bonow et al., 2018).

In 2002, as a result of the development of the technology called Clearfield® and the commercialization of imidazolinone-resistant rice lines, the development of ALS-resistant *E. crus-galli* biotypes due to the indiscriminate use of ALS-inhibiting herbicides became of high risk globally. In the USA, some populations became resistant to imazethapyr in Arkansas and Mississippi (Bagavathiannan et al., 2014). In addition, reports of cross-resistance and multiple resistance are becoming more frequent (Choudhary et al., 2023). These types of resistance are more complex to manage because different resistance mechanisms may coincide in a population or biotype (Yang et al., 2021). In this regard, the selection for important detoxifying genes with

the potential to metabolize herbicides in unpredictable patterns is already a threat (Rigon et al., 2023).

The mechanisms of herbicide resistance exhibited by weeds can be dichotomized into two primary categories: target-site resistance (TSR), wherein mutations or modifications occur at the herbicide's molecular binding site, and non-target-site resistance (NTSR), which encompasses resistance mechanisms that operate independently of the target site (Gaines et al., 2020). The replacement of at least nine residues codified by *ALS* gene has been evidenced as TSR mechanisms in several weed species (Powles, Yu 2010). In NTSR, the main enzymes responsible for herbicide metabolism, even in the case of alternative herbicides or molecules that have not yet been discovered (Takano et al., 2023), seem to be cytochrome P450 monooxygenases (P450s), glucosyl transferases (GTs), and glutathione S-transferases (GSTs) (Feng et al., 2022; Yannicari et al., 2020). Compounds as malathion, piperonyl butoxide or aminobenzothiazole inhibit P450s and can reverse the metabolic resistance of weeds to certain ALS-inhibiting herbicides (Yu, Powles, 2014). Genes that confer resistance through P450s -metabolizing activity (*CYP71AK2* and *CYP72A254*) have been associated with conferring resistance to bispyribac-sodium in *Echinochloa phyllopogon* (Iwakami et al., 2014). NTSR involves plant mechanisms that reduce herbicide levels at the target site. Herbicide detoxification is a multi-step process involving transformation of parent molecules into hydrophilic metabolites (Phase I), conjugation with sugar molecules or glutathione (Phase II), and transport and compartmentalization of non-toxic metabolites (Phase III), facilitated by enzymes such as P450s, carboxylesterases, and transporters (Carvalho-Moore et al., 2024).

Both the timely detection of herbicide resistance and the appropriate choice of herbicide are necessary to prevent increases in production costs due to the increase in the number of applications (Kaundun et al., 2011). Consequently, further research is necessary to improve the management of herbicide resistance in *E. crus-galli*. Currently, there are no publications on herbicide-resistant weeds in Ecuador. However, the suspicions of agronomists and researchers regarding the ineffective control of ALS-inhibiting herbicides on *E. crus-galli* in rice cultivation in Ecuador are growing. In this country, bispyribac-sodium is marketed under several trademarks, and it is frequently used to control *Echinochloa* species in rice crops. Thus, the aim of the current study was to analyze the sensitivity to bispyribac-sodium of *E. crus-galli* populations collected in the main rice-growing region of Ecuador.

2. Material and Methods

2.1 Plant material

During November 2018 to February 2019 *E. crus-galli* panicles were collected at physiological maturity from rice fields in the northeast and southwest cantons of the Guayas

province, called Daule and Yaguachi regions, respectively. Based on random procedures (Burgos et al., 2013) and suspicions of weed control failures reported by technicians, 35 accessions were selected: 17 from Daule and 18 from Yaguachi. The panicles were threshed and labeled, identifying the site of collection by using the Global Positioning System (Figure 1). They were named by the acronym of the species (ECG), the locality (Daule (D) and Yaguachi (Y)) and the number of accession (ECG-D01 and ECG-Y01), and stored in a refrigerator at 4 °C and 65% relative humidity to preserve seed viability. The experiments were carried out in 2019 under greenhouse conditions at the National Institute of Agricultural Research of Ecuador (INIAP), at 32/22 (day/night) $\pm$ 2 °C and 12 h of photoperiod, with a light intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.2 Screening of *E. crus-galli* sensitivity to bispyribac-sodium

To determine *E. crus-galli* sensitivity to bispyribac-sodium, the seeds were immersed in a 0.25% sodium hypochlorite solution to disinfect the seeds, and then placed in 168-well germination trays, which contained a substrate consisting of soil:peat:sand (2:1:1). When seedlings had between one and two leaves, which occurred around 7 days after planting, they were removed from the trays. Subsequently, they were transplanted to 1-L plastic pots (two seedlings per pot), and, when the plants showed three to four fully expanded leaves were sprayed with bispyribac-sodium (Grammya 400, ADAMA Ltd., Guayaquil, Ecuador) at the dose recommended by the manufacturer 40 g a.i. ha^{-1} . Spraying was performed with a back sprayer equipment pressurized with CO_2 at 30 PSI, equipped with TJ8002 fan nozzles, calibrated for a consumption of 200 L ha^{-1} together with non-ionic surfactant Nonylphenol ethoxylated: 920 g/l (Agral 90, Syngenta Crop Protection S.A. Ecuador) at a concentration of 0.1% (1 $\text{cm}^3 \text{L}^{-1}$) and a pH regulator (Fixer plus, Agripac S.A., Ecuador), under conditions of 65% relative humidity and a wind speed of 3.5 km h^{-1} .

Eight hours after herbicide application, the soil surface was kept saturated for three days, and then plants were periodically watered with 100 mL water every 48 h. The accessions remained on the soil until 21 days after the application (DAA) of the herbicide, date on which survival was determined by recording plants that presented general symptoms of chlorosis, wilting and necrosis, without evidence of new tissues. Plants were then cut at ground level, placed in paper bags, and taken to an oven at 65 °C for 72 h for drying and shoot dry weight (SDW) determination.

Putatively resistant (R) and susceptible (S) populations were identified using a completely randomized design, which consisted of the following factors: 1) *E. crus-galli* populations and 2) application of bispyribac-sodium at the commercial dose 40 g a.i. ha^{-1} , with five replications per population, including a control without herbicide application. Each replication consisted of five pots and each pot had two plants (i.e. 50 individuals per population), with each group of five pots being the experimental unit.

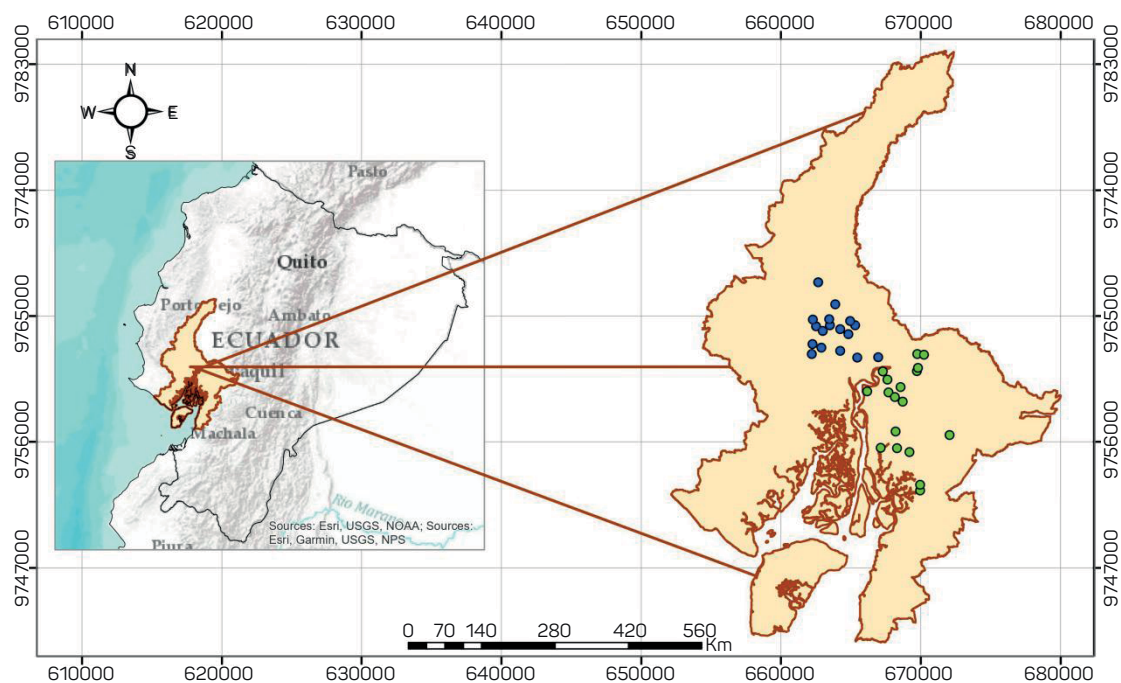


Figure 1 - Localization of thirty-five populations of *Echinochloa crus-galli* sampled from Daule (blue dots) and Yaguachi (green dots) region, Ecuador

2.3 Dose-response to bispyribac-sodium of R *E. crus-galli* populations

The screening study allowed identifying four populations with the highest frequency of R plants (100%) and it was selected as S the population with the highest mortality rate. (Table 1). The seeds used for the experiments were taken from the stored samples, and the sowing, establishment, herbicide application, harvesting and equipment used were similar to those described in the screening (item 2.2). The herbicide doses were increased according to a common multiplier, being 0, 20, 40, 80, 160, 320, 640 g a.i. ha⁻¹ for the R populations and 0, 5, 10, 20, 40, 80, 160 g a.i. ha⁻¹ for the S population. The design was completely randomized with five replications. The factorial arrangement for treatments included: 1) *E. crus-galli* populations selected for resistance and susceptibility to bispyribac-sodium and 2) application of bispyribac-sodium at different doses.

Herbicide effects on the plants was evaluated at 14 DAA, by estimating the percentage of weed control, where zero was absence of the visual effect of the herbicide and 100% was complete death of the weed, using the scale proposed by the Latin American Weed Association (Vera Ojeda et al., 2023). According to this variable, C₅₀ was calculated as the herbicide dose that promote 50% of control. At 21 DAA, weed survival was determined by calculating the bispyribac-sodium mean

Table 1 - Identification and geo-referenced location of five populations of <i>Echinochloa crus-galli</i> resistant and susceptible to bispyribac-sodium from two regions (Yaguachi and Daule) of Ecuador			
Condition	Locality	Population	Location (GPS)
Resistant	YAGUACHI	ECG-Y12	2°29'36.4"S 79°37'38.9"W
Resistant	YAGUACHI	ECG-Y18	2°20'01.6"S 79°42'50.5"W
Resistant	DAULE	ECG-D07	1°58'03.9"S 79°55'38.3"W
Resistant	DAULE	ECG-D11	1°52'12.7"S 79°57'56.3"W
Susceptible	YAGUACHI	SY10	1°58'43.0"S 79°38'18.8"W

lethal dose (LD₅₀), i.e. the dose at which the number of surviving plants decreases to 50%. Next, the mean biomass production inhibition dose (GR₅₀), i.e. the dose at which the biomass of treated plants decreases by 50% compared to untreated plants, and the resistance index (RI) were determined.

A set of S and R accession plants (n=5) treated with 320 and 640 g a.i. ha⁻¹ were grown up until the end of the plant life cycle. Surviving plants were harvested and manually threshed. The seeds obtained were counted and 100 seeds were germinated as was described above in order to confirm the capacity of surviving plants to generate descendants.

2.4 Bispyribac-sodium and its interaction with malathion

Finally, the response to herbicide and malathion was evaluated by using a completely randomized design with five replications, and the factorial arrangement included: 1) R and S *E. crus-galli* biotypes with and without application of malathion and 2) application of bispyribac-sodium at the commercial dose 40 g a.i. ha⁻¹. Malathion was sprayed 6 h before herbicide at a dose of 1,000 g ha⁻¹ (Yasuor et al., 2009). All the tasks and equipment used were similar to those described in the previous assays.

The experimental units consisted of four pots with three plants each (60 individuals per population); four R populations and one S population were used. The experiment was replicated twice and, when there were no significant differences between the data sets ($p < 0.05$), data from both experiments were combined.

2.5 Response to alternative herbicides

The response of S and R *E. crus-galli* populations (Table 1) to two alternative herbicides was evaluated. The seeds used were taken from the stored samples. The methodology, growth conditions and equipment employed were similar to those described in the screening (item 2.2). Penoxsulam (2.5%, Bengala™ 25 OD) and cyhalofop butyl ester (18%, Clincher™ EC) were applied at the dose recommended by the manufacturer, 40 and 270 g a.i. ha⁻¹ respectively. The first herbicide is an ALS inhibitor as bispyribac-sodium but from a different chemical family (triazolopyrimidine) and cyhalofop is an acetyl-CoA carboxylase (ACCase) inhibitor; both are postemergence herbicides for selective control of weeds in rice. A treatment without herbicide application was used as control. A completely randomized design with five replications was employed. Each replication consisted of five pots and each pot had two plants (i.e. 50 individuals per population), with each group of five pots being the experimental unit. Plant survival was recorded at 21 DAA.

2.6 Statistical analysis

Data obtained in the experiments for screening *E. crus-galli* sensitivity to bispyribac-sodium, the interaction with malathion and the response to alternative herbicides were subjected to analyses of variance (ANOVA) and comparisons were made using a Fisher's least significant differences test ($p < 0.05$). Data of dose-response experiment were used to build curves following a log-logistic nonlinear regression model of four parameters using the GraphPadPrism® v10 software (Graphpad Software, Inc.). The LD₅₀ and GR₅₀ values were calculated from the parameters of the equation proposed by Seefeldt et al. (1995) (Equation 1), which expresses the response of the plant to the herbicide dose:

$$Y = C + (D - C) / [1 + (x/LD_{50})^b]$$

where Y is the response to bispyribac-sodium; x is the herbicide concentration; C is the asymptote of the lower limit; D is the upper limit or mean response when the herbicide concentration is zero; b is the slope of the curve; and LD₅₀ is the dose that provides 50% of the response to the variable evaluated (Ritz et al., 2015).

The fit of the model was evaluated by studying the variance of the error. In addition, the parameters of the adjusted models of the populations were compared by performing F-tests ($p < 0.05$). The LD₅₀ values were used to obtain the RI, which corresponds to the relationship between the LD₅₀ of the R accession and the LD₅₀ of the S accession. As a confirmatory criterion, in the R populations, the two highest doses tested (320 and 640 g a.i. ha⁻¹ bispyribac-sodium) were repeated to determine the ability of these populations to generate offspring and the viability of their seeds.

3. Results and Discussion

3.1 Screening of *E. crus-galli* sensitivity to bispyribac-sodium

The screening performed to evaluate *E. crus-galli* sensitivity to bispyribac-sodium showed that 22 of the 35 *E. crus-galli* populations evaluated (62.8%) were not satisfactorily controlled (plant survival > 70%) by bispyribac-sodium at the commercial dose (40 g ha⁻¹), according to the percentage scale proposed, and that ten of these showed 100% survival. These included ECG-Y12 and ECG-Y18 from Yaguachi and ECG-D07 and ECG-D11 from Daule, which also showed the highest SDW values and were selected as R populations (Figure 2). Neither population showed 100% of mortality in response to the recommend dose of herbicide (Figure 2), however, one accession (ECG-Y10) showed the lowest survival values found in the survey (17.5%) and SDW (14.4%), and it was selected as S and later identified as SY10.

3.2 Dose-response to bispyribac-sodium of R *E. crus-galli* populations

According to the visual control performed, there was interaction ($p < 0.05$) between the accessions and doses at 14 DAA. The log-logistic nonlinear regression model adjusted the data for the SY10 and the R accessions (ECG-D11, ECGY12 and ECG-Y18) (Table 2). In contrast, the curve of accession ECG-D07 was not adjusted above 70%, even at the highest bispyribac-sodium doses due to poor control (Figure 3). The data revealed that SY10, designated as population S, exhibited differences compared to the other populations. The LD₅₀ value for SY10 revealed that an herbicide dose of 21.7 g a.i. ha⁻¹ reduced the plant survival in 50%, however the effects on 50% of visual control (C₅₀) and 50% of biomass production (GR₅₀) inhibition were obtained with around 11 g a.i. ha⁻¹ of bispyribac-sodium. Furthermore, the commercial dose of 40 g a.i. ha⁻¹ resulted in > 89% weed control (Table 2). On the other hand, control higher than 70% was achieved for the R accessions except

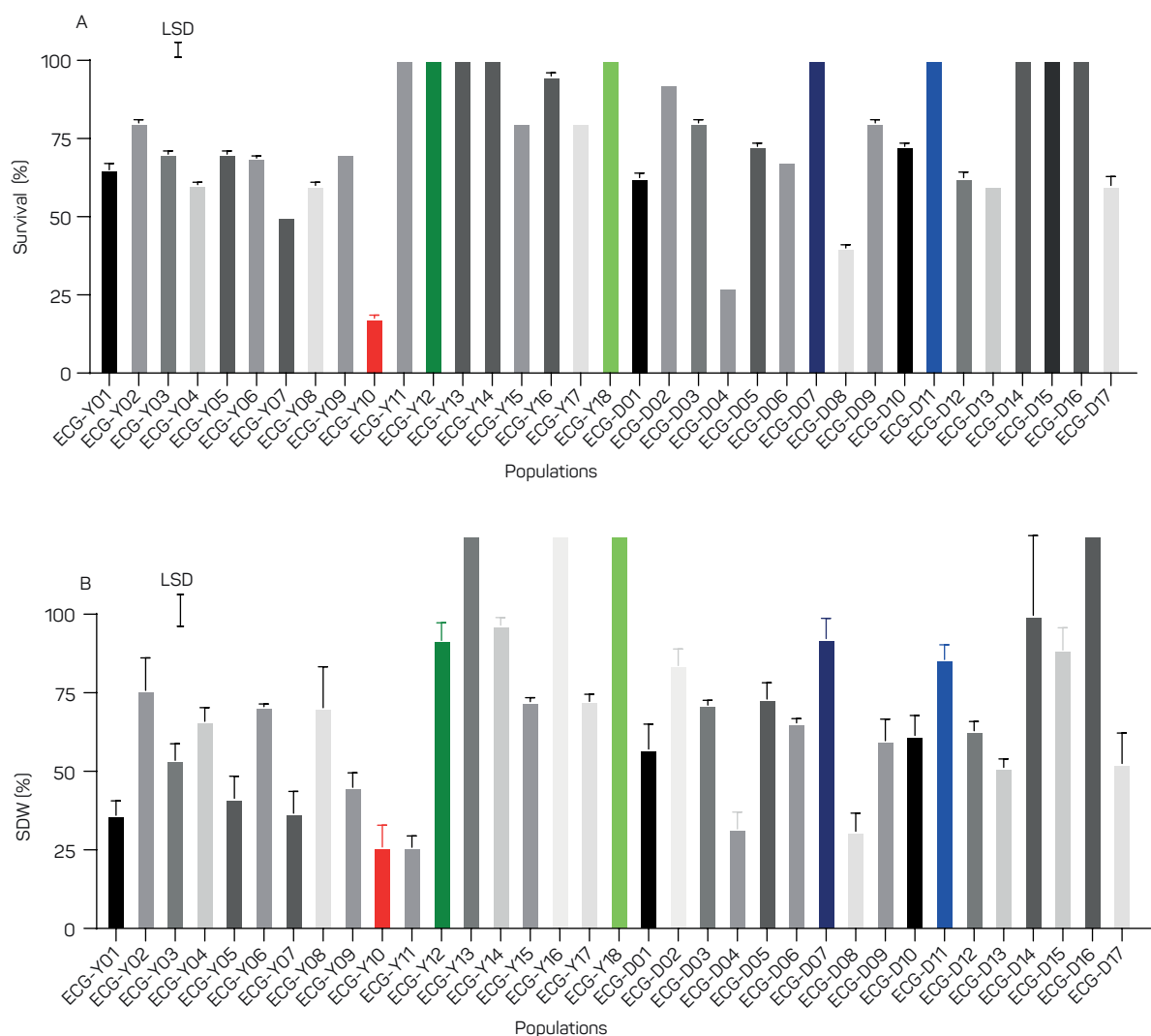


Figure 2 - Response of *Echinochloa crus-galli* populations to the recommended dose of bispyribac-sodium (40 g a.i. ha⁻¹): survival (A) and shoot dry weight (SDW) as percentages from the untreated control of each population (B). Columns and bars represent mean values and ± 1 standard error of the mean, respectively. Vertical bars represent LSD ($p < 0.05$) for comparison

for ECG-D07 when increasing the dose between 3 and 13 times to obtain the same control as in the *S. E. crus-galli* population (Figure 3A).

The relationship between increasing doses of bispyribac-sodium and *E. crus-galli* plants that survived after spraying allowed determining the LD₅₀, which revealed that the survival of the S population depended on the dose, with only half of the commercial dose being necessary to generate 50% control. In this sense, SY10 evidenced an LD₅₀ of 22.7 g a.i. ha⁻¹. In contrast, the four R populations showed very low mortality; ECG-D11 and ECG-Y12 presented LD₅₀ values of 364.3 and 494.7 g a.i. ha⁻¹ respectively and RI was 16.1 and 21.8, respectively (Figure 3). In the two remaining R populations analyzed (ECG-D07 and ECG-Y18), the maximum herbicide dose did not decrease survival below 50%; therefore, the RI of these populations at the maximum dose evaluated was

Table 2 - Mean control of the weed (C ₅₀), mean lethal dose (LD ₅₀), mean shoot dry weight (SDW) (GR ₅₀) and index of resistance to bispyribac-sodium (RI) in <i>E. crus-galli</i> populations						
Populations	Control		Survival		SDW	
	C ₅₀		LD ₅₀		GR ₅₀	
	g ai ha ⁻¹	RI	g ai ha ⁻¹	RI	g ai ha ⁻¹	RI
ECG-D07	41.1	2.9	>640.0	>28.2	30.4	3.1
ECG-D11	42.4	3.0	364.3	16.0	160.5	16.7
ECG-Y12	31.0	2.2	494.7	21.8	31.8	3.3
ECG-Y18	49.3	3.5	>640.0	>28.2	108.7	11.3
SY10	13.9	-	22.65	-	9.5	-

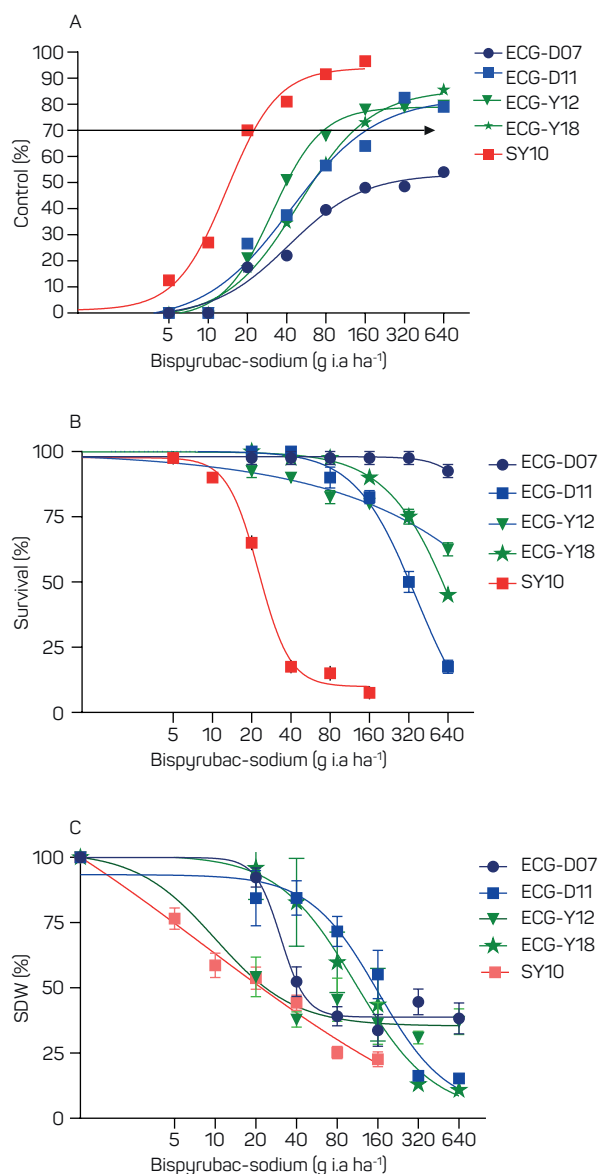


Figure 3 - Response of resistant and susceptible *Echinochloa crus-galli* populations to different doses of bispyribac-sodium: A. Weed control at 14 days after the application of the treatment (DAA). B. Survival at 21 DAA. C. Dry weight at 24 DAA. Symbols and bars represent mean values and ± 1 standard error of the mean, respectively. The predicted responses are shown by lines according to the adjusted models

higher (RI > 28.3) (Table 2 and Figure 3). Similar experiments in whole plants have shown that the RI of resistant biotypes to ALS-inhibiting herbicides, including bispyribac-sodium, was up to 17 times higher than the RI of susceptible ones, and that, in addition to a mutation in the gene, these populations had improved rates of GST metabolism (Feng et al., 2022).

Regarding SDW, results also showed differences between the R and S populations. The control values of the various accessions harvested at 21 DAA were around 1.2 and 1.5 (g per plant). The R populations ECG-D11

and ECG-Y18 showed the highest RI for this variable and greater dry matter accumulation, with RI values 16.7 and 11.3 times higher than that of the SY10 population. ECG-D07 and ECG-Y12 were at an intermediate range with an average RI 3.2. This effect was evidenced with a bispyribac-sodium dose ≤ 40 g a.i. ha^{-1} , but the trend was no longer observed at higher doses (Table 2 and Figure 3). These results are in agreement with previous studies of the C_{50} , which showed two to four times greater differences among resistant populations than among susceptible ones (Choudhary et al., 2023).

By definition, the herbicide resistant plants should be able to survive and reproduce following exposure to a dose of herbicide that would normally be lethal (Weed Technology, 1998). Current results show that R populations survived to 8 and 16-folds the recommended dose (320 and 640 g a.i. ha^{-1}) of bispyribac-sodium and produced viable seeds. No statistically differences were observed among these populations or between treatments (Figure 4).

3.3 Bispyribac-sodium and its interaction with malathion

Among the R populations pretreated with malathion as a P450s inhibitor, ECG-D11 evidenced an increase in the sensitivity to bispyribac-sodium. This suggests that at least one of the resistance mechanisms could be enhanced herbicide metabolism. The malathion + bispyribac-sodium treatment decreased ECG-D11 survival by 62.5% compared to the treatment where only herbicide was applied. The remaining R populations showed no differences between treatments, maintaining a survival close to 100% (Table 2 and Figure 5). The patterns of inhibition by malathion would be associated with various metabolisms surely influenced by different P450 isoenzymes (Yannicari et al., 2020). Unlike mutations in the herbicide active site (TSR), currently, it is more common to find R populations with NTSR mechanisms (Rigon et al., 2020). Isoenzymes CYP81A were able to metabolize 18 herbicides inhibiting ALS, PDS, PSII, PPO, HPPD, and DXS (6 MoAs), through demethylation or hydroxylation reactions, demonstrating the potential of Cyt-P450 enzymes in conferring broad cross-resistance to herbicides in *E. pollyginum* (Dimaano et al., 2020). However, in the genus *Echinochloa*, given the complexity of the biochemical processes involved and the genomic information of the species, further research is still necessary (Suda et al., 2023). In this case, the effect observed on the control of the R population ECG-D11 demonstrated the existence of synergism between malathion and bispyribac-sodium by inhibiting P450s. These results are related to other cases that evidenced that gene polymorphisms positively regulate the response to malathion treatment prior to the application of bispyribac-sodium (Iwakami et al., 2014). For the remaining three accessions reported as R, the mechanism involved is still unknown, but could be related to other P450 inhibitors or TSR mechanisms.

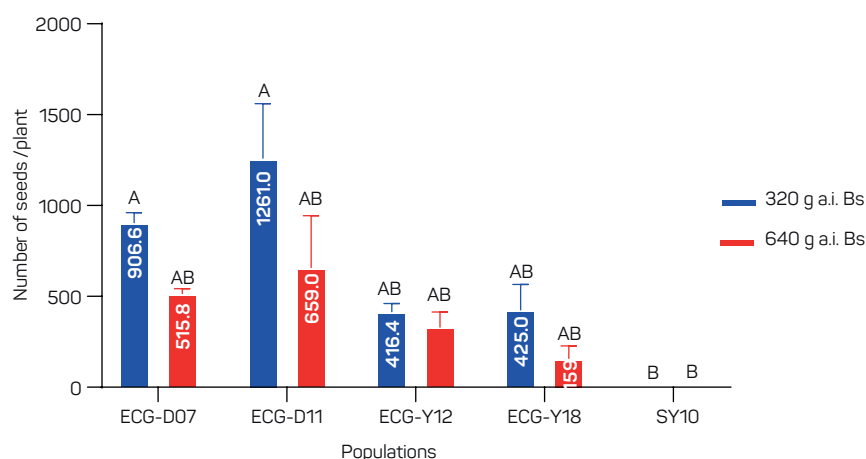


Figure 4 - Number of seeds per plant obtained from resistant (ECH-D07, ECG-D11, ECG-Y12 and ECG-Y18) and susceptible (SY10) *Echinochloa crus-galli* plants treated with bispyribac-sodium. Columns and bars represent mean values and ± 1 standard error of the mean, respectively. Similar letters indicate non-significant differences among mean values

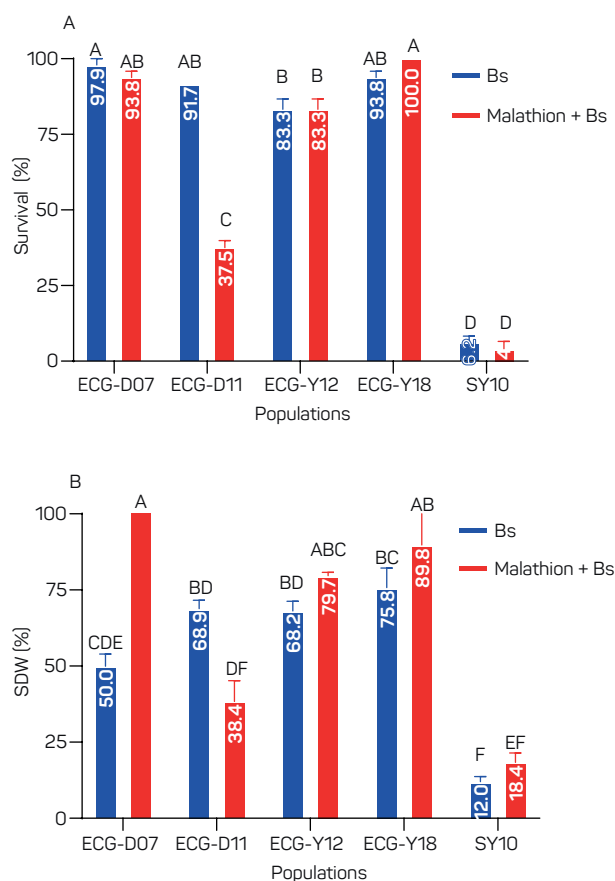


Figure 5 - Response of resistant and susceptible *Echinochloa crus-galli* populations to bispyribac-sodium and Malathion + bispyribac-sodium. A. Survival, B. Shoot dry weight (SDW). Columns and bars represent mean values and ± 1 standard error of the mean, respectively. Similar letters indicate non-significant differences among mean values

As in survival, ECH-D11 showed a decrease in SDW, presenting the same trend (64.6%). The susceptible SY10 population showed no differences between treatments, and the decreases were around 90%. On the other hand, malathion did not affect the response of the other three R populations (ECG-D07, ECGY12 and ECG-Y18), and showed an antagonistic effect by increasing their SDW by up to 45%, as in the case of ECG-D07. The same was true of the other two R accessions, with ECG-Y12 having the lowest increase in SDW (16.9%) (Figure 5). A decrease in SDW has also been reported in *E. crus-galli* accessions with enhanced metabolism favoring herbicide detoxification (Chiapinotto et al., 2023), due to the effect caused by the synergism between malathion and bispyribac-sodium. However, the existence of overexpression of the transcription of genes encoding P450 enzymes needs to be ruled out.

3.4 Response to alternative herbicides

All R populations demonstrated a high survival rate to penoxsulam between 87.5 and 93.8%, but in response to cyhalofop these populations showed different herbicide sensitivity. The plant survival of ECG-D07 and ECG-D11 accessions to the ACCase inhibitor was 87.5 and 83.3%, respectively. ECG-Y12 and ECG-Y18 populations were most sensitive to this herbicide and the survival was around 30 and 6%, respectively. Penoxsulam as cyhalofop controlled around 95% of the plants of the S population (Figure 6). The multiple resistance of *Echinochloa* populations to ALS inhibitors and ACCase inhibitor herbicides has been well documented (Bonow et al., 2018. Dimaano et al., 2020). Although this is the first report of *E. crus-galli* resistance to bispyribac-sodium in Ecuador,

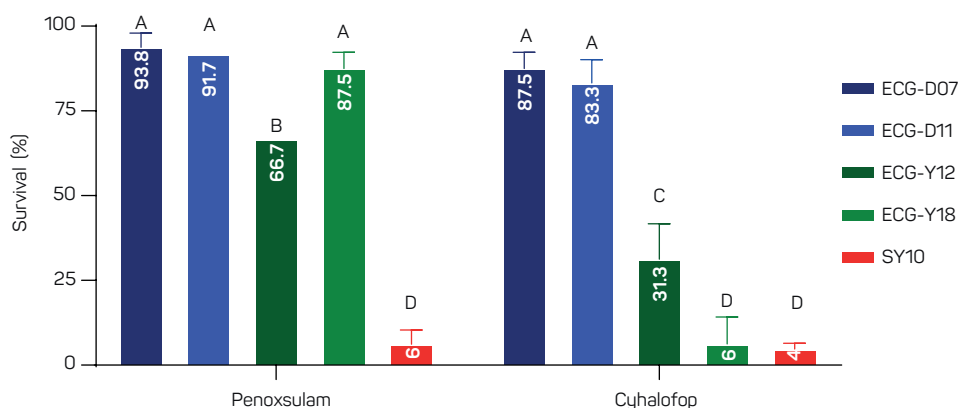


Figure 6 - Survival 21 DDA, response of resistant and susceptible *Echinochloa crus-galli* populations to penoxsulam (40 g a.i. ha⁻¹) and cyhalofop (270 g a.i. ha⁻¹) at the dose recommended by the manufacturer. Columns and bars represent mean values and ± 1 standard error of the mean, respectively. Similar letters indicate non-significant differences among mean values

more than one mechanism of resistance seem be involved and it would be linked to multiple resistance in several populations. The finding of resistance based on enhanced metabolism of P450s could indicate that this weed would have developed resistance as a generalist adaptation to the herbicides commonly used in rice (Riar et al., 2013). Currently, since bispyribac-sodium shows an erratic behavior for the control of weeds in rice production in Ecuador, it is necessary to develop strategies to protect the usefulness of these molecules in the long term by avoiding the selection of herbicides with shared resistance mechanisms (Duggleby et al., 2008; Yu, Powles, 2014).

4. Conclusions

The results of the present study confirm resistance to the herbicide bispyribac-sodium in four populations of *E. crus-galli* from the main rice-growing region of Ecuador. The resistance mechanism involved seems to be cytochrome P450 monooxygenase-mediated metabolism (NTSR), at least in one of the biotypes evaluated. This is the first report confirming the presence of herbicide-resistant weeds in Ecuador and highlighting the need of new management alternatives.

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Author's contributions

All authors read and accepted the published version of the manuscript. PDF, LAP, and MEY: conceptualization of the manuscript and development of the methodology. PDF, and LAP: data collection and curation. PDF, LAP, MEY, and LAR: data analysis. PDF, LAP, RDB, LAR, and MEY: data interpretation. PDF, and LAP: acquisition of financing and resources. PDF: project management.

PDF, and MEY: supervision. PDF, LAP, MEY, RDB, and LAR: writing of the original draft of the manuscript. PDF, LAP, and MEY: writing, revision and editing of the manuscript.

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