

POTENTIAL FOR RESISTANCE EVOLUTION TO RICE HERBICIDE MIXTURES: A RECURRENT SELECTION STUDY IN *Echinochloa crus-galli*

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Introduction

The diverse herbicide resistance mechanisms reported in *Echinochloa crus-galli* challenge the effective use of herbicides and complicates the understanding of the herbicide resistance evolution in this species. Recurrent selection with low herbicide rates to investigate resistance evolution has been used to study resistance evolution, yielding valuable insights across several weed species. In grasses such as *Lolium rigidum*, *Avena fatua* and *Echinochloa crus-galli*, the use of recurrent selection using different herbicides on biotypes possessing different resistant mechanisms have produced variable results, ranging from non-shift in sensitivity to an 8-fold increase in resistance (Busi et al., 2016, Rigon et al., 2023). Additionally, some of these studies have reported increased survival to herbicides to which that populations had not been previously exposed.

The use of herbicide mixtures is a common practice to broaden the spectrum of weed control and to delay the evolution of herbicide resistance. In *Lolium rigidum*, the use of herbicide mixtures delays the onset of resistance and mitigate the existing levels of herbicide resistance and cross-resistance (Busi and Beckie, 2021). In barnyardgrass, the recurrent use of mixtures at low doses of imazethapyr and fenoxaprop-p-butyl increased the GR50 at 1.6- and 2.6-fold in susceptible and imazethapyr-resistant biotypes, respectively, and increase cross-resistance to diclofop, cyhalofop, sethoxydim, and quinclorac (Rigon et al., 2023). Therefore, the effectiveness of herbicide mixtures in preventing the evolution of resistance can be compromised by the presence of multiple resistance mechanisms, whether through stacked mutations in target-site genes or through non-target-site resistance. The herbicide florpyrauxifen-benzyl represents a valuable option for controlling both widespread ALS-resistant and emerging ACCase-resistant barnyardgrass populations in Brazil. This study aimed to evaluate the effect of recurrent applications of florpyrauxifen-benzyl, in combination with other grass herbicides commonly used in rice fields, on the evolution of resistance in a susceptible barnyardgrass population.

Material and methods

In preliminary studies, one biotype of *Echinochloa crus-galli* named as MOST was characterized as susceptible to all herbicide modes of action. Seeds of the initial parental line (Generation 0, G0) were subjected to dormancy breaking by immersion in 96% sulfuric acid for 5 minutes, followed by rinsing under running water for 2 minutes. This method was consistently used for all subsequent generations. Seeds were sown in germination trays filled with soil, and seedlings were later transplanted into trays containing between 30 to 50 plants each. Each tray represents one replicate, and a treatment consisted of three replicates, totaling 90 to 150 plants. All experiments were conducted in a greenhouse maintained at 27 °C ± 3 °C. The schematic view of the experiment is showed at Figure 1. Herbicide treatments and the respective reference label

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dose ($\text{g} \cdot \text{ha}^{-1}$) were florpyrauxifen-benzyl (Loyant) - 30, cyhalofop-butyl (Clincher) – 360 with the adjuvant Assit at $1,5 \text{ L} \cdot \text{ha}^{-1}$, profoxydim (Aura 200) - 100 with Assist $1,5 \text{ L} \cdot \text{ha}^{-1}$, penoxsulam (Ricer) – 72 with Veget Oil at $1 \text{ L} \cdot \text{ha}^{-1}$, and the mixtures of Florpyrauxifen-benzyl + Cyhalofop-butyl, Florpyrauxifen-benzyl + Profoxydim, Florpyrauxifen-benzyl + Penoxsulam, Cyhalofop-butyl + Profoxydim, Cyhalofop-butyl + Penoxsulam, Profoxydim + Penoxsulam, and a non-treated control. Plants were treated at the three- to four-leaf stage and remained in the greenhouse until final evaluations, conducted 28 days after treatment (DAT). The treatments were applied in an automated spray chamber (greenhouse spray chamber, model Generation III), using a TJ8002E nozzle, calibrated to deliver $200 \text{ L} \cdot \text{ha}^{-1}$ at a pressure of 280 kPa and speed of $1.2 \text{ m} \cdot \text{s}^{-1}$.

Herbicide doses were initially selected by testing a wide range of concentrations. The dose chosen for seed production was the lowest one that allowed a sufficient number of surviving plants to produce enough seeds for the next generation. In each subsequent selection cycle (G1, G2), at least three low doses were tested for each herbicide treatment. The lowest dose used in each cycle corresponded to the dose selected in the previous generation. The range of doses tested varied among treatments, depending on the response of each line. For herbicide mixtures, the ratio between individual herbicides was maintained when dose levels were increased.

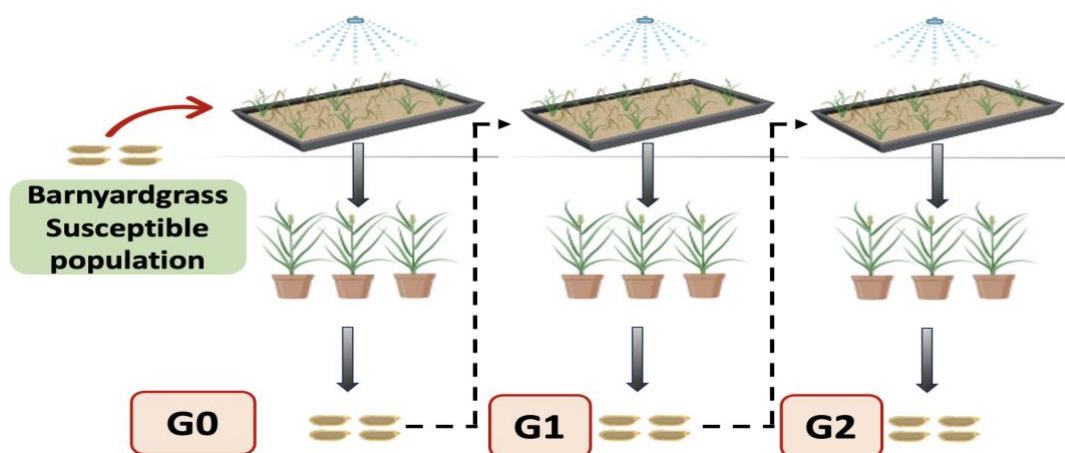


Figure 1. Representative diagram of the application cycles of sub-doses of each herbicide, survival assessments and selection of surviving plants of the MOST biotype.

Results and discussion

An increase in the dose for selection was observed from G0, G1 and G2 for the treatments penoxsulam (150%), florypyrauxifen-benzyl + penoxsulam (60%), and profoxydim + penoxsulam (93%) (Figure 2, Table 1). There was also a dose increase for the treatments cyhalofop + penoxsulam (60%), profoxydim (50%), cyhalofop (50%), profoxydim + cyhalofop (30%), and florypyrauxifen-benzyl + profoxydim (25%). The increase of herbicide dose that result in plant survival was lower to herbicide used in mixtures in comparison with the isolated use. In another study with barnyardgrass, recurrent selection using one susceptible and one resistant population to imazethapyr showed that the efficacy of an herbicide mixture (fenoxaprop-p-ethyl + imazethapyr) decreased in the progeny after two selection cycles. The progeny became 1.6- to 2.3-fold less sensitive to the mixture in the susceptible and ALS-resistant biotypes, respectively (Rigon et al., 2023).

Table 1. Survival of G0, G1 and G2 generations of the MOST biotype in relation to herbicide application.

Herbicide	Generation	Dose (g ha ⁻¹) (% label dose)	Treated plants	Survival (%)	Selected plants
Penoxsulam	G-0	2.16 (3%)	147	17.0	9
	G-1	4.32 (6%)	143	79.0	9
	G-2	5.4 (7.5%)	98	88.0	6
Florpirauxyfen-benzyl	G-0	18 (60%)	151	15.9	9
	G-1	18 (60%)	150	16.7	9
	G-2	18 (60%)	96	26.0	9
Profoxydim	G-0	15 (15%)	152	49.3	9
	G-1	22.5 (22.5%)	136	11.8	9
	G-2	22.5 (22.5%)	100	3.0	3
Cyhalofop	G-0	28.8 (8%)	151	41.1	9
	G-1	43.2 (12%)	150	11.3	9
	G-2	43.2 (12%)	94	1.1	1
Florpirauxyfen-benzyl + Profoxydim	G-0	9 + 8 (30% + 8%)	150	16.0	9
	G-1	9 + 8 (30% + 8%)	139	56.1	9
	G-2	11.25 + 10 (37.5% + 10%)	93	8.6	8
Florpirauxyfen-benzyl + Cyhalofop	G-0	7.5 + 21.6 (25% + 6%)	150	47.3	9
	G-1	7.5 + 21.6 (25% + 6%)	148	4.1	6
	G-2	7.5 + 21.6 (25% + 6%)	96	3.0	3
Florpirauxyfen-benzyl + Penoxsulam	G-0	7.5 + 1.08 (25% + 1.5%)	150	16.0	9
	G-1	9.48 + 1.37 (31.6% + 1.9%)	149	12.8	9
	G-2	12 + 1.73 (40% + 2.4%)	72	30.6	9
Profoxydim + Cyhalofop	G-0	5 + 18 (5% + 5%)	150	70.0	9
	G-1	5 + 18 (5% + 5%)	145	20.0	9
	G-2	6.5 + 23.4 (6.5% + 6.5%)	72	6.9	5
Profoxydim + Penoxsulam	G-0	35 + 5.4 (35% + 7.5%)	150	35.3	9
	G-1	46.6 + 7.2 (46.6% + 10%)	118	55.9	9
	G-2	67.6 + 10.44 (67.6% + 14.5%)	96	6.0	6
Cyhalofop + Penoxsulam	G-0	36 + 3.6 (10% + 5%)	150	63.3	9
	G-1	36 + 3.6 (10% + 5%)	126	11.9	9
	G-2	57.6 + 5.76 (16% + 8%)	96	32.0	9
No treated control	G-0	-	5	100.0	5
	G-1	-	5	100.0	5
	G-2	-	5	100.0	5

Treatments with florpyrauxifen-benzyl alone and florpyrauxifen-benzyl + cyhalofop showed no change in the selected dose from G0 to G2. Similarly, repeated exposure to sublethal doses of glufosinate over five generations did not reduce susceptibility in *Echinochloa colona* under normal conditions. However, under heat stress, plants recovered more rapidly from glufosinate injury, suggesting that environmental factors may influence herbicide response (Velasquez and Roma-Burgos, 2024). These results highlight the necessity to further evaluate the different patterns on herbicide sensitivity for each herbicides mixtures combination to potentialize the mitigation of resistance evolution.

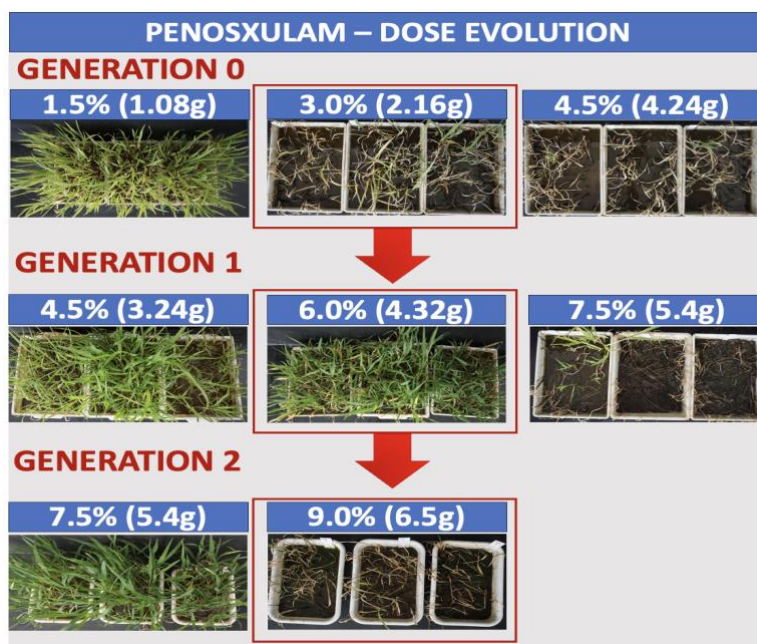


Figure 2. Representation the recurrent selection procedure of the survival of barnyardgrass generations at 28 days after treatment using the herbicide Penoxsulam (Ricer). Red box indicated the selected treatments for the next generation evaluation.

Conclusions

The low dose recurrent evaluation indicates that the utilization of herbicide in mixtures results in lower increase of tolerance in comparison with the herbicides used alone. The minimum dose that results in plant survival increases for the treatments penoxsulam, profoxydim, cyhalofop and several mixtures of these compounds. Treatments with florpyrauxifen-benzyl alone and florpyrauxifen-benzyl + cyhalofop showed no change in the selected dose across the studied generations. These results highlight the necessity to further evaluate the different patterns on herbicide sensitivity for each herbicides mixtures, to potentialize the mitigation of resistance evolution. The use of full recommended herbicide rates of herbicide mixtures is fundamental to reduce the risk of resistance evolution.

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