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First Case of Multiple Resistance to EPSPS and PSI in *Eleusine indica* (L.) Gaertn. Collected in Rice and Herbicide-Resistant Crops in Colombia

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Abstract: *Eleusine indica* is a highly competitive and difficult-to-control plant in annual and perennial crops. In Colombia, broad-spectrum herbicides, such as paraquat and glyphosate, have begun to present poor levels of control for this weed. The multiple resistance to glyphosate and paraquat, the increase in herbicide performance with adjuvants (Retenol® and Trend® 90), and alternative herbicides were evaluated in a resistant (R) population of *E. indica* collected in rice fields, which is rotated with herbicide-resistant (HR) crops. Based on plant mortality, the R population was 9.8 and 7.2 times more resistant than susceptible (S) plants to glyphosate and paraquat, respectively. R plants accumulated 4.2 less shikimic acid and had at least 70% less electrolyte leakage than S plants when they were exposed to glyphosate and paraquat, respectively. Both adjuvants increased the foliar retention of herbicides. In addition, adjuvants also increased the performance of glyphosate effectiveness between 22% and 58% and that of paraquat from 61% to 100%. Alternative herbicides (atrazine, clethodim, imazamox, diuron, flazasulfuron, glufosinate, oxyfluorfen, quizalofop, and tembotrione) provided high levels of control in both populations of *E. indica*. This is the first case of multiple resistant *E. indica* confirmed in Colombia. Adjuvants improved the leaf retention and efficacy of glyphosate and paraquat. In summary, the alternative herbicides evaluated in this study should be adopted by Colombian farmers and provide additional herbicide modes-of-action to combat future resistance.

Keywords: adjuvants; electrolyte leakage; foliar retention; glyphosate; integrated weed management; paraquat; shikimate

1. Introduction

In Colombia, as in many other Latin American countries, rice, corn, and cotton are important crops for food and fiber production [1,2]. Among strategies to increase the productivity of these crops are the technification of cultivated areas by using certified seeds in which most of them have herbicide resistance traits [3,4], and, consequently, their management programs. The weed species found in the Colombian agricultural areas are highly diverse [5,6]. However, the over-reliance on glyphosate in-crop weed control or paraquat for weed control before crop establishment has led to the selection of herbicide resistant weed biotypes. One of the first weeds that selected resistance in these crops was *E. indica* [7].

Eleusine indica (L.) Gaertn. (goosegrass) is an introduced-naturalized species in Colombia, widely distributed throughout the country [8]. It is a common weed in rice producing areas and is present in 70% of irrigated rice fields [6]. *Eleusine indica* is one of the most important weeds world that is highly competitive with a C_4 metabolism. [9]. This species is present in more than 42 countries with a high grow and reproductive capacity [10]. *Eleusine indica* is a weed that has a propensity for herbicide resistance from different modes of action (up to eight in 2020), occurring in both annual and perennial crops in Asia, America, and Australia [7]. In the world, *E. indica* has been confirmed with herbicide resistance in Argentina, Australia, Bolivia, Brazil, China, Colombia, Costa Rica, United States, Japan, Malaysia, and Mexico [7].

Glyphosate and paraquat are non-selective herbicides with unique mechanisms and modes of action widely used for total weed control, mainly in perennial crops, but their use in annual crops has become common due to the adoption of herbicide-resistant (HR) crops [11,12]. Glyphosate is a systemic herbicide that inhibits 5-enolpyrivyl-shikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimic acid pathway, responsible for biosynthesis of 5-enolpyrivyl-shikimate-3-phosphate (EPSP), interrupting the production of essential aromatic amino acids [13].

Paraquat belongs to the bipyridiniums and, due to its low mobility in light conditions, it is generally considered a contact herbicide [14]. This herbicide acts within chloroplasts as an electron acceptor of photosystem I (PSI) and, depending on the light, generates reactive oxygen species that destroy membranes, leading to the rapid death of plant cells [11]. Currently, 51 unique cases (weed $\times$ herbicide) of glyphosate resistance and 32 to bipyridiniums are known [7].

Early scouting and detection of herbicide resistant weeds is crucial for management [15]. Conversely, optimizing the effectiveness of herbicides is a constant concern for farmers. The use of adjuvants that reduce the surface tension and increase wettability, retention, and persistence of the active substances, improves the performance of the herbicides [16]. These products may improve control of herbicide resistance weeds since there is evidence that adjuvants increase foliar retention, absorption, and translocation of glyphosate, diminishing the resistance level in several weeds [17,18].

In Colombia, *E. indica* was found to be resistant to glyphosate in the central coffee region in 2006 [19], but, in this particular study, it was not possible to make a reliable comparison of dose-response curves. Control failures of *E. indica* were observed in rice fields in the HR crop rotation from Colombia. Glyphosate and paraquat were widely used during various times throughout the season. It was suspected that this weed has evolved resistance to these herbicides. The objectives of this research were to (1) confirm glyphosate and paraquat resistance in *E. indica* populations from Colombia, (2) determine susceptibility/resistance levels relative to herbicide performance with adjuvants, and (3) evaluate alternative chemical control options of both populations in greenhouse trials.

2. Materials and Methods

2.1. Herbicides and Adjuvants Used

Glyphosate (Roundup Energy®, 450 g ae L^{-1} as potassium salt of N-(phosphonomethyl)-glycine, Monsanto Agricultura, Madrid, Spain) and paraquat (Gramoxone 200 g L^{-1} of 1,1′-dimethyl-4,4′-bipyridinium, Syngenta, Edo. de México, México) were used in all assays described below. In addition, two non-ionic adjuvants were used: Trend 90 (90% isodecyl ethoxylated alcohol w/v, FMC, Valencia, Spain) and Retenol (66.5% terpenic alcohols w/v, Daymsa, Zaragoza, Spain), which decrease the surface tension of droplets and, thus, intend to increase wettability, foliar retention, and persistence of the active substances [20]. Alternative herbicides are listed in Section 2.8.

2.2. Plant Material

Eleusine indica seeds with suspected herbicide resistance (R) were collected in a commercial field with a history of crop rotation with rice, HR corn, and HR cotton, located in

Central Colombia (4°10′03.2″ N, 74°55′32.4″ W), where glyphosate and paraquat did not provide acceptable levels of weed control. Each population was defined as the mixture of seeds collected in a single batch. Conversely, seeds of a susceptible (S) population were collected in a vacant lot with no history of herbicide use (5°6′51″ N, 75°50′8″ W).

The seeds were mechanically scarified, put into trays, and mixed with a mechanical scarifying device with sandpaper [18]. Subsequently, seeds were put in trays containing peat moss. The trays were placed in a cold chamber at 4 °C for 48 h and then taken to a growth chamber with 26/18 °C day/night with 70% relative humidity and a photoperiod of 16 h at a light density of 850 mmol m^{-2} s^{-1}.

Seedlings with the first leaf were transplanted into pots (one plant pot^{-1}) of 7 × 7 × 5 cm, with 240 g of substrate (soil:peat moss (1:1)). Pots were placed in a greenhouse and irrigated daily, as necessary, for use in the assays. Plants with four true leaves, counted from the bottom, were used in the different experiments.

2.3. Dose-Response Assays

The R and S *E. indica* populations were treated with the following doses of glyphosate: 0, 62.5, 125, 250, 500, 1000, 1500, 3000, g ae ha^{-1} plus 4500 and 6000 g ae ha^{-1} only in R, and paraquat: 0, 50, 100, 200, 400, 800, 1200, 2400, and 4800 g ai ha^{-1}. Ten plants were treated for each dose of herbicide. Spraying was done in a laboratory chamber equipped with an 8002 flat fan nozzle, delivering 200 L ha^{-1} at a constant pressure of 200 kPa. Twenty-one days after treatment (DAT), plant response, and dry weight (growth) per plant were evaluated. Data were expressed as a percentage in relation to the untreated control.

2.4. Shikimic Accumulation Assay

Leaf discs (4-mm in diameter) were taken from the second or third fully expanded young leaves for 50-mg plant tissue samples. Shikimic acid accumulation (mg of shikimic acid g^{-1} fresh tissue) was determined according to Shaner et al. [21]. The glyphosate concentrations were: 0, 100, 250, 500, and 1000 µM. The absorbance of samples was measured in a spectrophotometer (Beckman DU-640, Beckman Instruments Inc., Fullerton, CA, USA) at a 380-nm wavelength. The experiment had a completely random design, using five tissue samples from each R and S *E. indica* populations per glyphosate concentration.

2.5. Electrical Conductivity Test

Four untreated S and R plants were reserved as a control and another four plants of each population were treated with 200 g ai ha^{-1} of paraquat. Fully expanded leaves were harvested, rinsed with distilled water to remove electrolytes present on the surface, and cut with a scalpel into small pieces at 4, 8, and 12 h after treatment (HAT). Leaf segments were incubated (900 µmol m^{-2} s^{-1} at 27 °C) in 4 mL of distilled water in test tubes for 4 h. Samples were frozen for 24 h to determine the total electrolytes [22]. Conductivity was measured using a conductivity meter (Crison CM 35+, Hach Lange Spain, Barcelona, Spain). Four biological samples and two technical replicates per sample were evaluated.

2.6. Foliar Retention

This assay was performed following the methodology described by Vázquez-García et al. [18]. Glyphosate (360 g ae ha^{-1}) and paraquat (150 g ai ha^{-1}), without and with adjuvants (1 mL L^{-1} Trend 90; 2 mL L^{-1} Retenol), plus a labeling reagent (100 mg L^{-1} of fluorescein of 5 mM NaOH), were applied on seven plants of each *E. indica* populations. HAT plants, once the herbicides were dried, were cut at the soil level and washed individually in 50 mL of 5 mM NaOH by shaking vigorously for 30 s to eliminate possible residues of herbicide and dye that could remain in the leaf tissue. The absorbance of wash solutions was measured in a spectrofluorometer (F-2500, Hitachi, Japan) at a wavelength of 490 nm for excitation and 510 nm for emission. Finally, cut tissues were packed in paper bags and dried in an oven at 80 °C for 72 h for weighing. The retention was expressed in µL of herbicide solution per g of dry matter.

2.7. Increase in Herbicide Effectiveness with Adjuvants

Resistant and Susceptible plants were treated with paraquat or glyphosate solution containing adjuvants (1 mL L^{-1} Trend 90, 2 mL L^{-1} Retenol). The herbicide doses applied corresponded (approximately) to the concentration that reduced plant growth by 50% (1500 and 125 g ae ha^{-1} glyphosate for R and S populations, respectively, and 400 and 150 g ai h^{-1} of paraquat for R and S, respectively). Herbicide applications were done using the same media as in the dose response experiments. Ten plants of each *E. indica* population were treated per treatment (herbicide, herbicide + Retenol, or herbicide + Trend 90). In addition, one set of 10 plants of each population was reserved as an untreated control. After herbicide treatment, plants were kept under greenhouse conditions and, at 21 DAT, fresh weight reduction was evaluated to estimate the percentage of increase in herbicide efficacy.

2.8. Alternative Control with POST Herbicides

Greenhouse experiments were performed to find the effectiveness of alternative POST emergence herbicides on *E. indica* populations. Ten herbicides of six different mechanisms of action were tested (Table 1). Ten plants per population were treated as described in the dose-response experiments. Plants were kept in greenhouse conditions until evaluation of visual control and plant survival at 21 DAT. Experiments were conducted in a randomized complete design with 10 replications (one plant pot^{-1}). Visual control (%) was based on plant vigor and chlorosis, compared with the untreated plants. In addition, 0% corresponded to no injuries and 100% when the herbicide had a lethal effect on the plants. Shoot fresh weight of treated plants were expressed as a percent reduction relative to that of the nontreated plants. Control values above 85% were considered satisfactory.

Table 1. Alternative herbicides (active ingredients), mode of action (MOA), trade name, and field doses (g ai ha^{-1}) evaluated to control susceptible and resistant *Eleusine indica* populations from Colombia.

Active Ingredient	MOA [a]	Trade Name	Field Doses
Flazasulfuron	ALS	Terafit 25%	50
Imazamox	ALS	Pulsar 40	40
Clethodim	ACCasa	Centurion Plus 12%	100
Quizalofop	ACCasa	Leopard 5%	100
Glufosinate	GS	Finale 15%	500
Tembotrione	HPPD	Laudis 20%	120
Oxyfluorfen	PPO	Goal Supreme 24%	480
Atrazine	PSII	Atazinax-FLO 47.5%	2000
Diuron	PSII	Diuron 80%	1800

[a] Mode of action: Inhibitors of acetolactate synthase (ALS), acetyl-CoA carboxylase (ACCase), glutamine synthetase (GS), 4-hydroxyphenylpyruvate dioxygenase (HPPD), protoporphyrinogen oxidase (PPO), and photosystem II (PSII).

2.9. Statistical Analyses

Non-linear regression analysis was conducted using growth reduction (%) and plant survival (%) to estimate the amount of herbicide needed to reduce the growth by 50% (GR_{50}) and the lethal dose 50 (LD_{50}) of each *E. indica* population. The log-logistic model of three-parameters $Y = ((d)/(1 + (x/g)^b))$ was conducted using a "*drc*" statistical analysis of the R package [23]. In the equation, Y represents the plant response (dry weight or mortality) to the dose x of the herbicide. Furthermore, d is the lower limit of the curve, b is the slope at the inflection point (i.e., GR_{50}, LD_{50}), and x is the herbicide dose. The resistance factor (RF) was calculated to the relationship between the GR_{50}/LD_{50} of the population R and the GR_{50}/LD_{50} of the population S (RF = R/S).

The experiments were conducted twice. Since no interaction was observed between the treatments and the experiments, analysis of variance (ANOVA) was conducted for shikimic acid, electrical conductivity, alternative herbicides, foliar retention, and use of adjuvants data. For the statistical analysis, model assumptions of a normal distribution of errors and homogeneous variance were graphically inspected. Significant differences ($p \leq 0.05$) were compared using Tukey's test at the 95% probability level.

3. Results

3.1. Dose-Response Assay

The GR_{50} and LD_{50} values of the R *E. indica* population were 1574.4 and 3204.44 g ae ha^{-1} glyphosate, respectively. The resistance factors (RFs), based on dry weight and plant survival, were 11.6 and 9.8 times higher compared to the S population. Paraquat GR_{50} and LD_{50} values were 449.5 and 1450.81 g ai ha^{-1}, respectively. The RFs of the R population were 3.3 and 7.2 times higher than population S (Table 2, Figure 1).

Table 2. Parameters of the log-logistic equations [a] used to estimate the glyphosate (g ae ha^{-1}) and paraquat (g ai ha^{-1}) rates required to reduce growth by 50% (GR_{50}) and lethal doses (LD_{50}) in susceptible and resistant *Eleusine indica* populations from Colombia.

Population	Dry Weight Reduction				Plant Survival			
	b	d	GR_{50}	RF [b]	b	d	LD_{50}	RF [b]
Glyphosate								
R	1.8	100.4	1574.4 ± 54.4	13.3	3.09	99.4	3204.44 ± 129.8	9.8
S	1.5	99.82	118.43 ± 6.7		2.09	95.1	327.3 ± 24.7	
Paraquat								
R	1.3	101.6	449.5 ± 28.2	3.3	2.09	100.1	1450.81 ± 61.5	7.2
S	1.9	99.3	135.6 ± 7.5		2.17	102.1	199.80 ± 9.71	

[a] $Y = d/1 + (x/g)^b$: Y = response by 50% with respect to the control, *b* = slope of the curve, *d* = upper limit, g = inflection point of the curve (GR_{50} or LD_{50}), and x = herbicide concentration. [b] Resistance factor (RF = GR_{50}-R population/GR_{50}-S population).

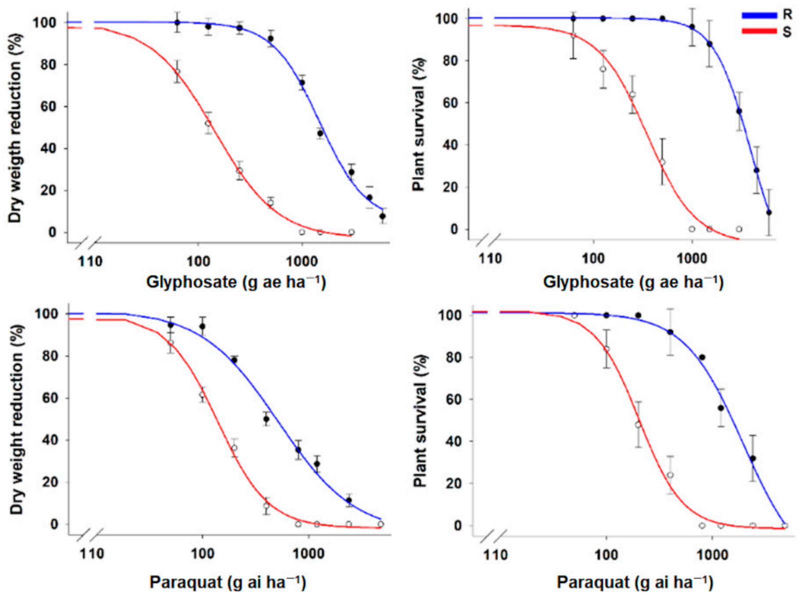

Figure 1. Dose response curves to glyphosate and paraquat on the fresh weight reduction and plant survival rate of susceptible (S) and resistant (R) *Eleusine indica* populations from Colombia. Vertical bars represent the standard error (n = 20).

3.2. Shikimic Acid Accumulation

Both populations, R and S, accumulated shikimic acid but accumulation was more pronounced in the S population as glyphosate concentration increased. At 100 µM glyphosate, S plants accumulated 2.5 times more shikimic acid than R plants and, at 1000 µM, the accumulation ratio became 4.2 greater in the S plants in relation to the R ones (Figure 2).

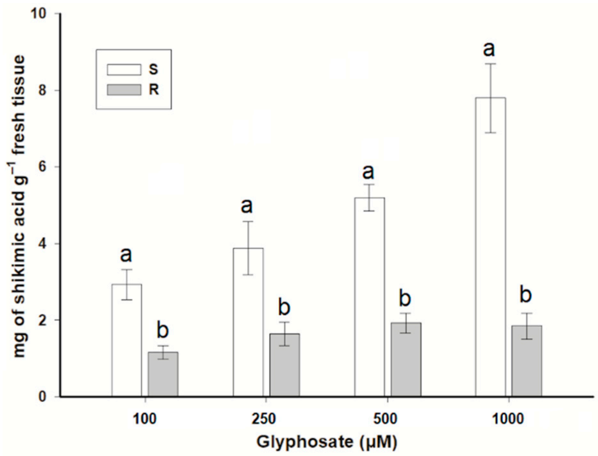

Figure 2. Shikimic acid accumulation in susceptible (S) and resistant (R) *Eleusine indica* plants at different glyphosate concentrations. Histograms represent the means and vertical bars of the standard error (n = 10). Different letters within each group of bars did not differ statistically at the 95% Tukey test.

3.3. *Electrical Conductivity Test (EC)*

Untreated R and S *E. indica* plants showed similar low electrical conductivity values (electrolyte leakage ≥ 5%) in leaves. However, paraquat-treated R and S plants showed high electrolyte leakage. The S population exhibited the highest electrolyte leakage ranging from 76% to 99.3% between 4 to 12 HAT, while those of the R population were between 23% and 30% in the same period (Figure 3).

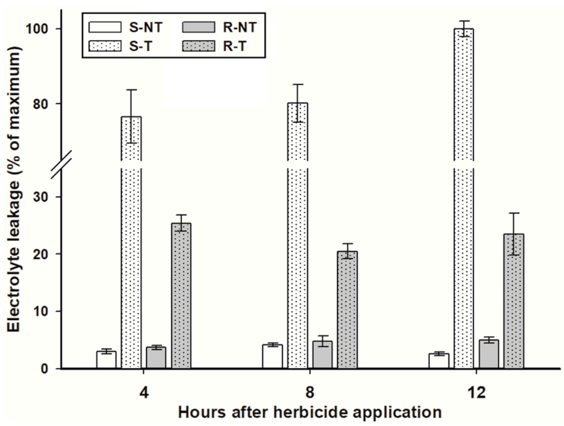

Figure 3. Percentage of electrical conductivity in leaf segments with respect to total electrolytes of susceptible (S) and resistant (R) *Eleusine indica* plants from Colombia, non-treated (NT), and treated (T) with 200 g ai ha^{-1} paraquat. Histograms represent the means and vertical bars of the standard error (n = 8).

3.4. *Foliar Retention*

Leaf retention between the R and S *E. indica* populations was similar within each treatment (herbicide × adjuvant). Glyphosate retention was slightly higher than paraquat (407 vs. 322 µL herbicide solution g^{-1} of dry matter). The addition of adjuvants increased the leaf retention of both herbicides. Retenol® increased glyphosate retention moderately (232 µL), whereas, paraquat retention more than doubled (408 µL). Trend® 90 improved glyphosate and paraquat retention 2.1 and 2.7 times, respectively, in relation to treatment without adjuvants (Figure 4).

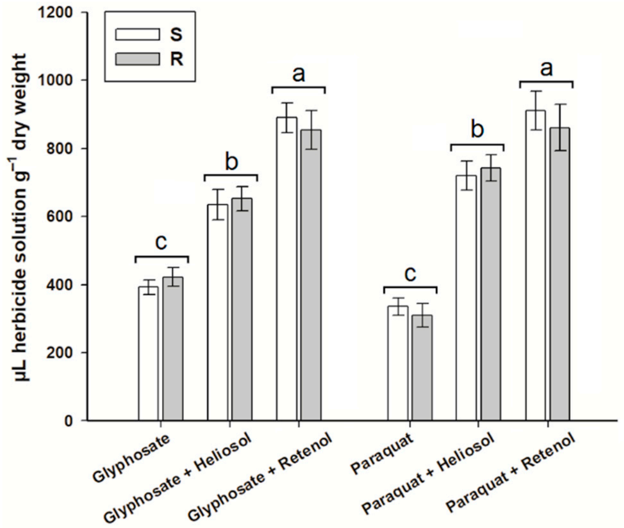

Figure 4. Foliar retention of glyphosate and paraquat in susceptible and resistant *Eleusine indica* plants from Colombia, using two adjuvants, Retenol® and Trend® 90. Histograms represent the means and vertical bars of the standard error (n = 14). Different letters denote significant differences between treatments of the same herbicide by the 95% Tukey test.

3.5. Increase in Herbicide Effectiveness

Herbicides without adjuvants caused a fresh weigh reduction close to 50%, as expected since the R and S *E. indica* populations were treated with the herbicide doses of glyphosate or paraquat corresponding to their GR_{50} values (approximately). Adjuvants, in addition to improving leaf retention, increased the efficacy of herbicides. Retenol and Trend increased glyphosate efficacy by 37% and 58%, respectively, in the S population, while, for the R population, this increase was less than 30% for both adjuvants. In the case of paraquat, both adjuvants increased the efficacy by 90%, while, in the R population, the Trend presented the best performance (77%) (Table 3).

Table 3. Fresh weight (Fw) and percentage in increasing herbicide effectiveness (IE) of glyphosate (Gly) and/or paraquat (Par.) with and without adjuvants in resistant (R) and susceptible (S) *Eleusine indica* populations from Colombia in relation to the absolute controls (C) and treatments without adjuvants (T).

Treatment [a]	S			R		
	Fw (g)	**IE-C**	**IE-T**	**Fw (g)**	**IE-C**	**IE-T**
Control	2.40	-	-	1.72	-	-
Gly	1.14 ± 0.12 a	52	-	0.94 ± 0.07 a	46	-
Gly + Retenol	0.76 ± 0.08 b	68	37	0.68 ± 0.06 b	61	28
Gly + Trend	0.49 ± 0.05 c	79	58	0.74 ± 0.07 b	57	22
Par	1.04 ± 0.07 a	57	-	0.87 ± 0.07 a	51	-
Par + Retenol	0.12 ± 0.03 b	95	89	0.34 ± 0.06 bc	80	61
Par + Trend	0 ± 0 c	100	100	0.24 ± 0.05 c	86	77

[a] Herbicide doses applied were the corresponding GR_{50} values determined for each population and herbicide. Different letters per column refer to treatments that are significantly different based on the Tukey test at the 95% probability. Mean values ± standard errors of the mean (n = 20).

3.6. Alternative Chemical Control

Eleusine indica control was satisfactory with most of the herbicides assessed. Herbicides, such as glufosinate, controlled both R and S populations at seven DAT and tembotrione and oxyfluorfen injured R and S plants from four DAT. However, plant mortality was observed at 21 DAT. Additionally, clethodim, imazamox, quizalofop, atrazine, and diuron provided excellent control of both *E. indica* populations, which died at 21 DAT.

Flazasulfuron was the only one that did not cause 100% fresh weight reduction, but it was also considered satisfactory using the 85% threshold discussed earlier (Table 4).

Table 4. Percentage of visual control and fresh weight reduction in susceptible and resistant *Eleusine indica* populations from Colombia with alternative herbicides.

Herbicide	Visual Control [a]		Fresh Weight Reduction	
	S	R	S	R
Control	0 b	0 b	0 c	0 c
Flazasulfuron	100 a	95 a	93.3 ± 5.9 b	90.4 ± 3.9 b
Clethodim	100 a	100 a	100 a	100 a
Quizalofop	100 a	100 a	100 a	100 a
Glufosinate	100 a	100 a	100 a	100 a
Tembotrione	100 a	100 a	100 a	100 a
Oxyfluorfen	100 a	100 a	100 a	100 a
Atrazina	100 a	100 a	100 a	100 a
Diuron	100 a	100 a	100 a	100 a

[a] Visual evaluation was based on plant vigor and chlorosis. Compared with the untreated plants, 0% corresponded to no injuries and 100% when plants were killed. Mean values ± standard errors of the mean (*n* = 20).

4. Discussion

The high GR_{50} and LD_{50} values of the R population confirmed resistance to glyphosate and paraquat in *E. indica* from Colombia. Glyphosate and paraquat resistance is widely distributed worldwide [7]. The resistance levels were quite variable. For example, *E. indica* populations characterized as being glyphosate resistance showed RFs (based on GR_{50}) of 4.2 (Brazil), 4.9 to 13.4 (China), 3 to 16 (Mexico), and up to 32 (Malaysia) [24–27], among other cases. Paraquat resistance seems to be less variable, and resistant *E. indica* populations from Malaysia showed RFs ranging from 2 to 3.5 times [28,29]. However, a population from Florida, USA, was 30 times more resistant than its S counterpart [30].

Glyphosate behaves like a phosphoenolpyruvate analog in the reaction of this substrate with shikimate-3-phosphate, mediated by EPSPS, impeding the biosynthesis of EPSP. The interruption of this reaction by glyphosate induces the accumulation of shikimic acid [31], which explains the high levels of shikimate in the S *E. indica* population as glyphosate concentration increased. Conversely, since the amount of glyphosate that arrives and interacts with EPSPS is not sufficient in resistant plants, the accumulation of shikimic acid is relatively low [21], confirming the resistance observed in the glyphosate dose-response assays.

High paraquat-induced electrolyte leakage in the S *E. indica* population was due to the disruption of cell membranes caused by rapid superoxide production and other reactive oxygen species [11]. Low electrolyte leakage in the R population followed patterns similar to those observed in paraquat-resistant *Lolium multiflorum* from California [22] and *E. indica* from China [32], confirming resistance to paraquat.

Herbicide formulation design is based on bringing the active ingredient to its target site in sufficient quantity to control weeds at a label dose [33]. Although glyphosate and paraquat retention was lower without adjuvants, both herbicides were efficient in controlling the S *E. indica* population with lower doses than indicated on the label, i.e., formulations met their objective. Glyphosate retention varies depending on the type of salt (ammonium, potassium, or isopropylamine) in the formulation, as well as the specific weeds treated [34]. In contrast, some adjuvants improve the performance of pesticides [35] and, in this research, both Retenol and Trend improved the foliar retention of glyphosate and paraquat, and increased the efficacy of the herbicides on R and S *E. indica* populations. This increase in herbicide efficacy is due to the fact that some adjuvants also improve absorption and translocation patterns [17,36]. Although Retenol and Trend are nonionic adjuvants, glyphosate and paraquat presented better performance (increased retention and efficacy) with Trend. These differences could be due to the concentration of the active ingredient of each adjuvant (90% in Trend and 66% in Retenol), highlighting that the choice of the most suitable adjuvant may contribute to reducing environmental impacts [37], while the level of control is maintained.

Experiments evaluating alternative modes of action of herbicides showed several effective herbicides to manage resistant *E. indica*. Experiments have shown that tembotrione, atrazine, and diuron could be used in HR-corn, clethodim, and quizalofop in HR-cotton, clethodim, and imazamox in rice (Clearfield), and glufosinate as an alternative in pre-sowing. In addition, the response of S and R *E. indica* showed sensitivity to ALS (flazasulfuron) and PPO (oxyfluorfen) inhibitors, ruling out multiple resistance at these sites of action. The control of glyphosate-resistant populations has been observed when grass weed herbicides were applied over different species, such as *Hordeum murinum* [18] or *Chloris distichophylla* [38], which were controlled with ACCase, GS, and PSII inhibiting herbicides. Additional works in *Echinochloa colona* and *Chloris virgata* reported, like glyphosate resistant, were perfectly controlled using herbicides with a different mode of action and they emphasized that a rotation and mixture with different mode of actions (MOAs) is a good tool in integrated weed management [39].

Within integrated weed management, crop sequence diversification, i.e., crop rotation, is among some advantages, allowing the rotation of herbicide choices [40]. This and other strategies, if managed with an integrated approach, can greatly reduce the management of herbicide resistance, as well as the appearance of new herbicide resistant weeds [15,41].

5. Conclusions

Resistance to glyphosate and paraquat observed in *E. indica* across rice and HR fields in Colombia was confirmed. This is the first confirmed case of multiple resistance in this country. To control this herbicide resistant weed, different chemical alternatives are still available, either by the use of adjuvants that improve leaf retention and the efficacy of glyphosate and paraquat, as well as herbicides with different modes of action (ACCase, ALS, GS, HPPD, PPO, and PSII inhibitors), since no other multiple resistance pattern was observed in the addition of resistance to EPSPS and PSI inhibitors.

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