

RESEARCH ARTICLE

# Proliferative capacity in relation to metamifop resistance in *Echinochloa glabrescens*: A case study

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## ABSTRACT

Knowing the proliferative capacity of herbicide-resistant weeds is the basis for the integrated management. We collected seeds of two *Echinochloa glabrescens* Munro ex Hook. f. populations (TS and WH) from rice (*Oryza sativa* L.) fields in eastern China (F<sub>0</sub> populations), and those from individuals surviving the initial metamifop treatment (F<sub>1</sub> lines) were tested for their responses to metamifop, and common garden experiments were conducted to compare their productivities. We found that TS was highly resistant to metamifop, and WH was susceptible. After treated with metamifop at the label dose (120 g ai ha<sup>-1</sup>), averagely 56% and 16% of TS and WH F<sub>1</sub> lines contained surviving seedlings, and averagely 24% and 1% F<sub>1</sub> seedlings survived among total seedlings of the same population, respectively. After treated with metamifop at a dose of 0, 60, 120 or 240 g ai ha<sup>-1</sup>, surviving F<sub>0</sub> plants escaped from higher doses reproduced higher proportions of metamifop-resistant F<sub>1</sub> progenies in both populations. At 120 d after sowing (DAS), TS individuals surviving 2 × label dose of metamifop showed nonsignificant difference in plant heights, biomass and seed production, compared with relative control treatment; while WH plants surviving the label dose of metamifop decreased seed production by 25%-39%. One WH and TS plant individual surviving metamifop treatment at the label dose may generate 10-21 and 490-701 progenies resistant to the label dose of metamifop, respectively.

**Key words:** F<sub>0</sub> population, F<sub>1</sub> line, metamifop resistance, productivity, surviving seedling.

## INTRODUCTION

In the medical field, proliferative capacity is a key attribute responsible for persistent pathogenicity of a pathogenic microbe species. In the field of plant protection, proliferative capacity is also a key attribute responsible for persistent occurring of herbicide-resistant weeds. Many weed plants surviving from herbicide applications are able to regrow and reproduce before crop harvest; and thus, causing yield losses to the current crop and enriching seedbanks with herbicide-resistant individuals (Comont and Neve, 2021). Knowing the proliferative capacity of herbicide-resistant weeds is the basis for the integrated management.

*Echinochloa glabrescens* Munro ex Hook. f. is a serious rice (*Oryza sativa* L.) weed in Asia (Chauhan, 2013; Covshoff et al., 2016; Chen et al., 2019), which is very similar with *E. crus-galli* (L.) P. Beauv. in morphology and frequently co-occur with *E. crus-galli* in rice fields. These two *Echinochloa* species could be identified by their lemmas (Chen et al., 2019). Specifically, both upper and lower lemmas of *E. glabrescens* spikelet are coriaceous, hard and shining; while only upper lemmas of *E. crus-galli* spikelet is coriaceous ([www.efloras.org](http://www.efloras.org)). Metamifop, an acetyl-CoA carboxylase (ACCase) inhibiting herbicide was commonly used for *E. glabrescens* control in rice fields. A cyhalofop (another ACCase inhibiting herbicide)-

resistant *E. glabrescens* population has been collected in rice fields (Zhang et al., 2022). In eastern China, metamifop is one of the most frequently used herbicides in rice fields. Whereas, our fields survey suggested that *E. glabrescens* is the most serious *Echinochloa* weed species in rice fields in this area (Chen et al., 2019). How about capacities in regrowth and reproduction of *E. glabrescens* plants surviving metamifop applying practices? Illuminating the above question is of key importance to understand the distributing of metamifop-resistant *E. glabrescens* populations.

In October 2018, we collected two *E. glabrescens* populations from rice fields applied with metamifop in eastern China. Whole-plant bioassays suggested that one population showed resistance to metamifop and the other was susceptible. Furthermore, we tested the responses of seedlings grown from original seeds ( $F_0$ ) to metamifop, as well as those originating from seeds collected from individual plants that survived after being treated with metamifop at different doses ( $F_1$ ); we also conducted cultivating experiments to compare growing and reproducing abilities of plants treated with different metamifop doses. We hypothesize that *E. glabrescens* plants surviving metamifop applications with the label dose hold high potential to propagate offspring with metamifop resistance.

## MATERIALS AND METHODS

### Plant material

Seeds of two *Echinochloa glabrescens* Munro ex Hook. f. populations (WH and TS) were collected in October 2018 from commercial rice fields. The WH population was collected from a rice (*Oryza sativa* L.) field with a low infestation of *E. glabrescens* in Wuhu County (31°0'35" N, 118°29'48" E), Wuhu city, Anhui province; and the TS population originated from a rice field infested by *E. glabrescens* in Tongshan county (34°27'54" N, 117°11'34" E), Xuzhou city, Jiangsu Province. The TS plants were treated with pretilachlor, bensulfuron-methyl, bentazone, MCPA, metamifop, florpyrauxifen-benzyl and penoxsulam in the growing season when collected; the rice field where WH population was collected had been sprayed with pretilachlor, bensulfuron-methyl, bentazone, MCPA, metamifop and cyhalofop-butyl. Moreover, a metamifop-susceptible population of *E. glabrescens* (HJG) was collected from an experimental rice field located in the campus of Yangzhou University, Hanjiang county (32°23'37" N, 119°25'0" E), Yangzhou city, Jiangsu Province, China, in October 2018. On the experimental rice field where HJG population was collected, pre-emergence herbicides with low risks of rice seedling injury were applied, and post-emergence herbicides were not applied during rice cultivating experiments in the recent 5 yr. As well, the experimental rice field never accepted metamifop. Mature seeds were randomly collected from more than 100 individuals of each population by hands. Seeds were cleaned, dried and stored at room temperature for 1 yr and then stored in paper bags at 4 °C.

Originally, field collected seeds of the two populations were designated as  $F_0$ . Seedlings obtained from  $F_0$  seeds were treated with metamifop (2-[4-[(6-chloro-1,3-benzoxazol-2-yl)oxy]phenoxy]-*N*-(2-fluorophenyl)-*N*-methylpropanamide) at different doses. Plants that survived were allowed to mature and their seed was individually collected in 2019 and 2020 and grouped to  $F_1$  lines.

### Screening metamifop-resistant lines

In 2019 and 2020, seedlings grown from  $F_0$  seeds of TS and WH were cultivated under greenhouse conditions at 30/20 °C (day/night) and natural light. Each 7×7 cm plastic pot was sown with eight pre-germinated seeds, and sprayed with metamifop (10% emulsifiable concentrate, FMC Corporation, with a label dose of 120 g ai ha<sup>-1</sup>) at 0 (fresh tap water, the same as below), 60, 120, and 240 g ai ha<sup>-1</sup> at the three- to four-leaf stage using a laboratory sprayer equipped with an ex 80015 flat-fan nozzle delivering 450 L ha<sup>-1</sup> at 230 kPa (Chen et al., 2016). In China, farmers frequently take a spraying volume of 450 L ha<sup>-1</sup> at 230 kPa when apply herbicides with traditional manual and ground sprayers. Three weeks after treatment, surviving seedlings with green leaves were transplanted separately into larger (15×15 cm) plastic pots. Each pot was placed in a box (20×30×15cm) to facilitate watering. All transplanted seedlings were transferred into a greenhouse with windows totally opened at the campus of Yangzhou University, China. The soil used

in this study was obtained from Nanjing Duole Horticulture (Nanjing, Jiangsu, China) (with pH 6.8, total nutrient content 3.8% and total organic matter content > 40%). Plants were watered as needed. Seed was collected from each surviving individual and identified as F<sub>1</sub> lines. All seeds were cleaned, dried and stored at room temperature for 1 yr and then stored in paper bags at 4 °C.

### Whole-plant bioassay

In 2020, a set of bioassays was conducted to determine the response to metamifop of the F<sub>0</sub> populations. The TS seedlings were treated with metamifop at 0, 30, 60, 120, 240 and 480 g ai ha<sup>-1</sup>, and those of WH and HJG were treated with metamifop at 0, 7.5, 15, 30, 60 and 120 g ai ha<sup>-1</sup>. The studies were conducted under greenhouse conditions at 30/20 °C (day/night) and natural light, as described in our previous study (Chen et al., 2016). In all bioassays, each plastic pot (7×7 cm) was sown with 12 pre-germinated seeds. The seedlings were thinned to 8 plants per pot at the two-leaf stage. Herbicides were applied at the three- to four-leaf stage. Three weeks after treatment, the aboveground fresh weight per pot was recorded. Experiments were conducted as a completely randomized design with four replicates, and were conducted twice.

In 2021, bioassays were conducted to determine proportions of metamifop-resistant individuals among different F<sub>1</sub> lines. Seeds of different F<sub>1</sub> lines of TS and WH population were cultivated and treated with metamifop at a dose of 0, 120 and 240 g ai ha<sup>-1</sup>, under the same conditions previously described. Three weeks after treatment, the number of surviving seedlings was recorded. For each treatment (F<sub>1</sub> line × metamifop dose), a total of 24 seedlings in three pots were sprayed with the herbicide.

### Productivity tests

From June to October in 2020, TS and WH seedlings obtained from F<sub>0</sub> seeds were sprayed with metamifop at the three- to four-leaf stage with doses at 0 and 120 g ai ha<sup>-1</sup>, as before. Three weeks after treatment, surviving seedlings with green leaves were transplanted and grown in the same greenhouse. During this study, all windows of the greenhouse were kept fully open; the minimum temperature in the greenhouse was from 5 to 28 °C and the maximum temperature ranged from 16 to 38 °C, with an average of 28 °C. Mature seeds from each individual were collected separately. At 120 d after sowing (DAS), all plants were harvested. For each plant individual, dry weight of leaves and stems without panicles, number of reproductive tillers, number of mature seeds were determined, as well as 1000-seed weight (by weighing five replicates of 100 mature seeds) were determined.

We repeated the experiment from June to October in 2022. The TS seedlings surviving metamifop treatments at doses of 0, 120, 240 g ai ha<sup>-1</sup> and the WH seedlings surviving metamifop at a dose of 0 and 120 g ai ha<sup>-1</sup> were transplanted. As well, the same variables were tested. During the study, all windows of the greenhouse were kept fully open; the minimum temperature in the greenhouse was from 6 to 32 °C and the maximum temperature ranged from 14 to 41 °C, with an average of 28 °C.

### Statistical analyses

The data presented are means ± SEs. The general linear model in SPSS16.0 software (IBM, Armonk, New York, USA) was used to test data of the two runs of whole-plant bioassays, fresh weight of seedling was set as dependent variable, experimental run, population and metamifop doses were set as fixed factors. The results of general linear model suggested that the experimental run showed nonsignificant influence on fresh weight of seedlings; thus, data of the two runs of whole-plant bioassays were pooled together. A four-parameter logistic function was fitted to test responses of fresh weight to herbicide treatments and responses of accumulated number of mature seeds collected to period after sowing, using the “drc” add-on package in R 3.1.3 (Ritz and Streibig, 2005):

$$Y = d + (a-d) / [1 + (x/GR_{50})^b]$$

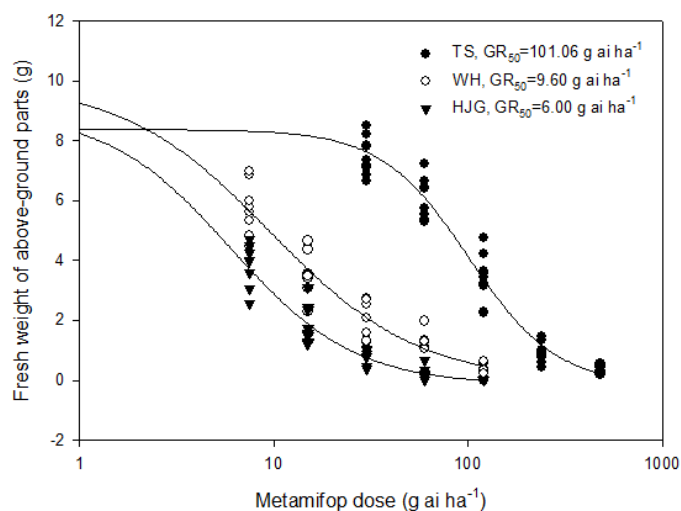
where Y denotes the fresh weight reduction response at dose x of the herbicide or day x after sowing; a is the upper limit; d is the lower limit; GR<sub>50</sub> is the dose of herbicide causing a 50% reduction in fresh weight, or the duration day after sowing for 50% mature seeds collected; and b is the slope. Resistance factors (RFs) were calculated by dividing the GR<sub>50</sub> of TS and WH by that of HJG. SigmaPlot 10.0 (Inpixon, Palo Alto,

California, USA) was used to show the dose-response curves. Populations were described as having no resistance ( $RF < 2$ ); low resistance (2-5); moderate resistance (6-10); and high resistance ( $> 10$ ), according to Beckie and Tardif (2012). To compare significant differences in proportions of seedlings surviving metamifop treatments with different treatments and life history traits of TS plants cultivated in 2022, least significant differences (LSD) were used when variances were homogeneous, and Dunnett's T3 were used, if variances were not homogeneous. Independent-sample t-tests were used to compare differences in life history traits between metamifop applied treatment and relative control treatment in TS plants cultivated in 2020 and WH plants cultivated in 2020 and 2022.

## RESULTS

### Metamifop resistance

The  $GR_{50}$  values of TS, WH and HJG were 101.06, 9.60 and 6.00 g ai ha<sup>-1</sup>, respectively (Figure 1), which suggesting resistance factors of 16.84 and 1.60 for TS and WH. Thus, TS showed high resistance to metamifop and WH was still susceptible.

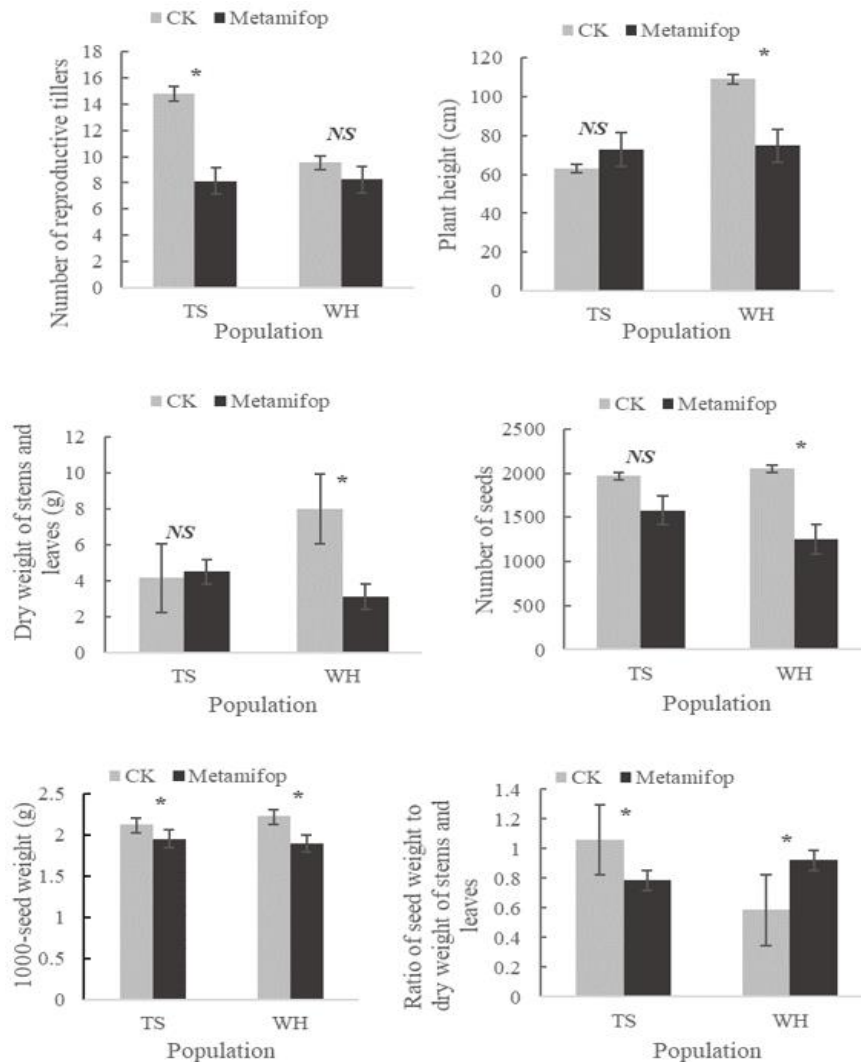


**Figure 1.** Responses of three *Echinochloa glabrescens* populations to metamifop at increasing doses. TS: *Echinochloa glabrescens* population collected from Tongshan county; WH: *E. glabrescens* population collected from Wuhu County; HJG: metamifop-susceptible population collected from Hanjiang county;  $GR_{50}$ : effective dose of herbicide causing 50% growth reduction.

### Productivity of plants surviving treatment with metamifop

In 2020, totally 50 TS seedlings surviving metamifop treatments at the label dose were transplanted and harvested at 120 DAS. Compared with the control treatment, the number of reproductive tillers decreased ( $P < 0.05$ ) by 45% (Figure 2), 1000-seed weight decreased by 8% and ratio of total seed weight to dry weight of stems and leaves decreased by 26%. Plant height, dry weight of stems and leaves, and seed production showed nonsignificant difference.

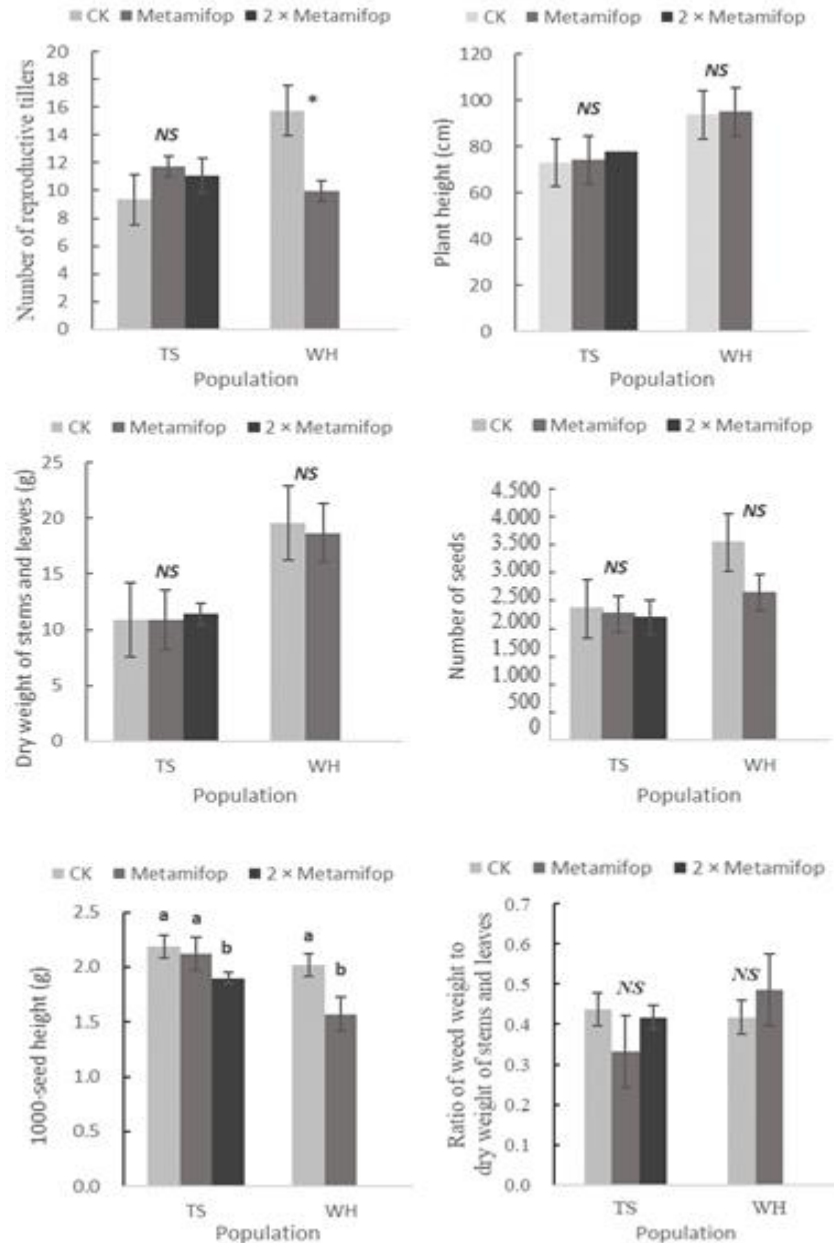
For WH population in 2020, 15 seedlings that survived from metamifop at the label dose were transplanted and harvest at 120 DAS. Compared with the controls, there were significant differences in five indices; plant height, dry weight of stems and leaves, 1000-seed weight and seed production decreased by 31%, 61%, 15% and 39%, respectively, while ratio of total seed weight to dry weight of stems and leaves increased 50% (Figure 2).



**Figure 2.** Productivity traits of *Echinochloa glabrescens* seedlings of TS and WH populations surviving metamifop treatments at different doses. Seedlings were treated with metamifop at the three- to four-leaf stage and surviving individuals were transplanted, allowed to grow, and harvested at 120 d after sowing in 2020. \*Suggested significant differences between the two adjacent columns. TS: *Echinochloa glabrescens* population collected from Tongshan county; WH: *E. glabrescens* population collected from Wuhu County.

In 2022, 14 TS seedlings surviving metamifop treatment at the label dose and 15 seedlings surviving 2 × label dose of metamifop treatment were transplanted and harvested at 120 DAS, respectively. Compared with the control treatment, only 1000-seed weight of seeds collected from individuals surviving metamifop treatments at 2 × label dose decreased significantly (by 13%, Figure 3).

For WH population in 2022, 30 seedlings surviving metamifop treatment at the label dose were transplanted and harvest at 120 DAS. Compared with the control treatment, the number of reproductive tillers and 1000-seed weight of seeds collected from individuals surviving metamifop treatments at the label dose decreased by 37% and 22%, respectively (Figure 3).



**Figure 3.** Productivity traits of *Echinochloa glabrescens* seedlings of TS and WH populations surviving metamifop treatments at different doses. Seedlings were treated with metamifop at the three- to four-leaf stage and surviving individuals were transplanted, allowed to grow, and harvested at 120 days after sowing in 2022. Different letters suggested significant differences among adjacent columns. TS: *Echinochloa glabrescens* population collected from Tongshan county; WH: *E. glabrescens* population collected from Wuhu County.

### Proportions of metamifop-resistant offspring among F<sub>1</sub> seedlings

Totally 33 696 seedlings belonging to 117 F<sub>1</sub> lines of the TS population (117 lines × 4 F<sub>0</sub> treatments × 3 metamifop doses × 24 seedlings) were tested. After treated with metamifop at the label dose, 33%-84% (average 56%) F<sub>1</sub> lines contained surviving seedlings (Table 1); and among total seedlings



of the same treatment, 12%-47% survived, with an average of 24% (Table 2). After treatment with metamifop at 2 × label dose, 20%-53% (average 32%) F<sub>1</sub> lines contained surviving seedlings; and among total seedlings of the same treatment, 4%-13% survived, with an average of 9%.

**Table 1.** Proportion of lines containing seedlings surviving from metamifop treatments at different doses of two *Echinochloa glabrescens* populations. <sup>1</sup>Seeds collected from a rice field were marked as an F<sub>0</sub> population; seeds of the F<sub>0</sub> population were used to cultivate plants and the 2<sup>nd</sup> generation seeds collected from one F<sub>0</sub> plant individual were marked as an F<sub>1</sub> line. Data in parentheses are Nr of F<sub>1</sub> lines with surviving seedlings/Nr of total F<sub>1</sub> lines.

Population	Treated dose g ai ha <sup>-1</sup>	F <sub>0</sub> plants escaped from metamifop dose (g ai ha <sup>-1</sup> ) <sup>1</sup>				Average %
		0	60	120	240	
TS	120	43.33 (13/30)	33.33 (10/30)	68.42 (26/38)	84.21 (16/19)	55.56 (65/117)
	240	33.33 (10/30)	20.00 (6/30)	31.58 (12/38)	52.63 (10/19)	32.48 (38/117)
WH	120	20.00 (6/30)	0.00 (0/30)	20.00 (2/10)	38.46 (5/13)	15.66 (13/83)
	240	0.00 (0/30)	0.00 (0/30)	0.00 (0/30)	15.38 (2/13)	2.41 (2/83)

**Table 2.** Proportions (as percent) of seedlings surviving metamifop treatments with different doses of two *Echinochloa glabrescens* populations. Different letters in the same row suggested significant differences.

Population	Treated dose g ai ha <sup>-1</sup>	Sum of square	F <sub>0</sub> plants escaped from metamifop dose (g ai ha <sup>-1</sup> )				Average %
			0	60	120	240	
TS	120	800.25	13.99 ± 4.64 <sup>b</sup>	12.36 ± 3.90 <sup>b</sup>	30.53 ± 5.03 <sup>a</sup>	47.20 ± 6.67 <sup>a</sup>	24.01 ± 2.73
	240	53.33	3.72 ± 1.56 <sup>a</sup>	7.57 ± 3.74 <sup>a</sup>	12.50 ± 3.71 <sup>a</sup>	12.34 ± 4.75 <sup>a</sup>	8.96 ± 1.78
WH	120	3.19	2.19 ± 1.12 <sup>a</sup>	0 <sup>a</sup>	0.83 ± 0.56 <sup>a</sup>	2.00 ± 0.80 <sup>a</sup>	1.33 ± 0.48
	240	1.39	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	1.36 ± 0.92 <sup>a</sup>	0.21 ± 0.15

Moreover, F<sub>0</sub> plants of TS population escaped from metamifop treatments with higher doses reproduced higher proportions of metamifop-resistant F<sub>1</sub> progenies. For example, 53% TS F<sub>0</sub> plants surviving metamifop treatment at 240 g ai ha<sup>-1</sup> generated F<sub>1</sub> progenies with resistance to the same dosage of metamifop, with a proportion of 12% among total F<sub>1</sub> seedlings tested; while 20% F<sub>0</sub> plants surviving metamifop treatment at 60 g ai ha<sup>-1</sup> generated seeds with resistance to metamifop treatment at 240 g ai ha<sup>-1</sup>, with a proportion of 8% among total F<sub>1</sub> seedlings tested.

A total 23 904 seedlings belonging to 83 F<sub>1</sub> lines of the WH population (83 lines × 4 F<sub>0</sub> treatments × 3 metamifop doses × 24 seedlings) were tested. After treated with metamifop at the label dose, 0%-38% (average 16%) F<sub>1</sub> lines contained surviving seedlings (Table 1); and among total seedlings of the same treatment, 0%-2% survived, with an average of 1% (Table 2). After treated with metamifop at 2× label dose, only two F<sub>1</sub> lines contained surviving seedlings, with an average of 0.21% among total seedlings tested.

Moreover, WH F<sub>0</sub> plants escaped from metamifop treatments with higher doses also reproduced higher proportions of metamifop-resistant F<sub>1</sub> progenies (Tables 1 and 2). For example, 15% WH F<sub>0</sub> plants surviving metamifop treatment at 240 g ai ha<sup>-1</sup> generated F<sub>1</sub> progenies with resistance to metamifop treatment at the same dose, with a proportion of 1% among total F<sub>1</sub> seedlings tested; while none of F<sub>0</sub> plants escaped from metamifop treatment at a dose of 60 g ai ha<sup>-1</sup> generated F<sub>1</sub> progenies with resistance to metamifop treatment at 240 g ai ha<sup>-1</sup>.

## DISCUSSION

*Echinochloa glabrescens* surviving from metamifop treatments hold high ability to reproduce metamifop-resistant offspring, especially for seedlings surviving higher metamifop treated doses. Metamifop holds high efficacy to *Echinochloa* weed species at the 3- to 5-leaf stage (Wang et al., 2015; Xia et al., 2016; Yan et al., 2019). To date, no study reported the metamifop resistance in *E. glabrescens*. Zhang et al. (2022) reported a cyhalofop (ACCase inhibiting herbicide)-resistant *E. glabrescens* population. Wang et al. (2015) reported that an *E. glabrescens* population collected from a rice field showed a reduction of 92% in the fresh weight of aboveground parts 21 d after treated with 45 g ai ha<sup>-1</sup> metamifop at the 3-leaf stage. The GR<sub>50</sub> values of metamifop to TS population were 101.06 g ai ha<sup>-1</sup>, which was closed to its label dose (120 g ai ha<sup>-1</sup>), and was 17 times of the value of the sensitive population. Seeds of the sensitive population (HJG) were collected from an experimental rice field, which never accepted metamifop. Therefore, compared with HJG, TS was highly resistant to metamifop. As a metamifop-resistant population, a TS plant individual surviving metamifop applying at the label dose was able to reproduce > 1500 mature seeds; and 68% plant individuals (Table 1) cultivated from these seeds may reproduce progenies holding metamifop resistance, with a proportion of 31% among the total progenies (Table 2). As a metamifop-susceptible population, a WH plant individual surviving metamifop at the label dose was able to reproduce > 1200 mature seeds; and 20% plant individuals cultivated from these seeds may reproduce progenies holding metamifop resistance, with a proportion of 0.8% among the total progenies (Table 2). In other words, one WH seedling surviving metamifop treatment at the label dose may generate 10-21 progenies resistant to the label dose of metamifop; and one TS seedling surviving metamifop treatment at the label dose may generate 490-701 progenies resistant to the label dose of metamifop. Therefore, persistent application of metamifop may select *E. glabrescens* populations with increased proportions of metamifop-resistant individuals. Continuous selection pressure driving quick evolution of ACCase-inhibitor resistance has been reported in different weed species (Comont and Neve, 2021; Yuan et al., 2021). Rotating application of different herbicides is important (Vasilakoglou et al., 2018; Vila-Aiub et al., 2019).

*Echinochloa glabrescens* seedlings surviving metamifop application hold high ability to grow and reproduce; and thus, cause damages to rice growing. In the resistant population, TS individuals surviving 2 × label dose of metamifop showed nonsignificant difference in plant heights, biomass and seed production at 120 DAS (Figures 2 and 3); and in the susceptible population, WH individuals surviving the label dose of metamifop also showed nonsignificant difference in plant height, and decreases in seed production by 25%-39%. Ordinarily, there is only one opportunity for applying metamifop to control *Echinochloa* seedlings in rice fields, during the period of three-leaf to two-tiller stage (about 12-20 d after rice planting). Determining the efficacy of metamifop application in rice fields needs > 20 d (Chen et al., 2016), after which surviving *E. glabrescens* seedlings should have been grown enough tolerant to various rice herbicides. Meanwhile, the space, moisture and nutrients in rice fields are rich enough for surviving *E. glabrescens* seedlings growth during the above period. Therefore, *E. glabrescens* surviving metamifop applications may still cause serious yield losses of rice.

### Management implications

Chemical control is still the dominant weed control method in rice growing. Metamifop is one of the most important rice herbicides, which holds high efficacy to various grassy weed species in rice field including *Echinochloa* spp., *Digitaria sanguinalis* (L.) Scop., *Leptochloa chinensis* (L.) Nees, *Eleusine indica* (L.) Gaertn., and *Eragrostis japonica* (Thunb.) Trin. (McCullough et al., 2016; Xia et al., 2016). The proliferation and following distribution of metamifop-resistant *E. glabrescens* populations may cause the exit of metamifop in the market of rice herbicides. Moreover, considering the high frequency of cross-resistance and multiple resistance in *Echinochloa* species (Chen et al.,



2016; Zabala et al., 2019; Zhang et al., 2022), control the proliferation of metamifop-resistant *E. glabrescens* is also important for developing new ACCase-inhibitors (Antony and Karuppasamy, 2022). To control *E. glabrescens* populations with resistance to metamifop in rice fields, farmers frequently increase metamifop dosage and/or add other post-emergence herbicides such as florpyrauxifen-benzyl, propanil and quinclorac. Whereas, continuously applying metamifop may facilitate the infestation of metamifop-resistant *E. glabrescens* plants. Together our results highlighted the control of metamifop-resistant *E. glabrescens* plant individuals in rice fields.

Firstly, in rice fields with lower occurrence of *E. glabrescens* surviving metamifop application, manually removal is necessary. The WH population was susceptible to metamifop, while seedlings surviving label dose of this herbicide was still able to generate 10-21 times progenies with resistance to it. Large soil seedbanks with metamifop resistance might quickly accumulate in a few of years.

Secondly, panicle-cutting practice (Chen et al., 2022) should be taken to prevent accumulating metamifop-resistant *E. glabrescens* seedbanks in rice fields with obvious occurrence of this weed species after chemical control practices. As well, preventing the distributing of metamifop-resistant *E. glabrescens* seedbanks should also be highlighted. As a metamifop-resistant population, TS seedlings surviving metamifop treatment at the label dose may proliferate > 490 times of progenies.

Thirdly, in rice fields with occurrence of metamifop-resistant *E. glabrescens*, metamifop should be rotated with other post-emergence herbicides before well control of this weed species. Pre-emergence control against *E. glabrescens* should be highlight, for example, twice applications of pre-emergence herbicides with different active ingredients could be employed. Additional pre-emergence herbicides could also be tank-mixed with post-emergence herbicides and applied after rice planting.

## CONCLUSIONS

After treated with metamifop at the label dose (120 g ai ha<sup>-1</sup>), averagely 56% and 16% of TS and WH F<sub>1</sub> *Echinochloa glabrescens* lines contained surviving seedlings, and averagely 24% and 1% F<sub>1</sub> seedlings survived among total seedlings of the same population, respectively. After treated with metamifop at a dose of 0, 60, 120 or 240 g ai ha<sup>-1</sup>, surviving F<sub>0</sub> plants escaped from higher doses reproduced higher proportions of metamifop-resistant F<sub>1</sub> progenies in the both populations. One WH and TS plant individual surviving metamifop treatment at the label dose may generate 10-21 and 490-701 progenies resistant to the label dose of metamifop, respectively. *Echinochloa glabrescens* surviving from metamifop treatments hold high ability to reproduce metamifop-resistant offspring, especially for seedlings surviving higher metamifop treated doses. To avoid the loss of control of weeds in the field, farmers need to remove plants, cut panicle and use other post-emergence herbicides before well control of this weed species.

### Author contribution

Conceptualization: G.Q-C. Methodology: Y.C. Software: Y.C. Validation: Y.C. Formal analysis: Y.C. Investigation: C.L. Resources: F.Z., T.J-G. Data curation: Y.C. Writing-original draft: Y.C. Writing-review & editing: G.Q-C., C.L., G.Q-C. Project administration: G.Q-C. Funding acquisition: G.Q-C. All co-authors reviewed the final version and approved the manuscript before submission.

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