

# *Echinochloa crus-galli* seedlings surviving florpyrauxifen-benzyl applications have a greater potential to produce resistant seeds

## Research Article

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F<sub>1</sub> line; F<sub>2</sub> line; herbicide resistance; seed production; seed-setting course

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### Abstract

Florpyrauxifen-benzyl (FPB) is an important postemergence rice herbicide. This study tested the potential for seed production in an FPB-resistant barnyardgrass population. A barnyardgrass population (NL) collected from a rice field in eastern China was highly resistant to FPB with a GR<sub>50</sub> dose (the FPB dose causing a 50% reduction in fresh weight of aboveground parts) of 50.2 g ai ha<sup>-1</sup>. No significant differences in the percentages of surviving seedlings after treatment with different doses of the herbicide were found between F<sub>1</sub> lines collected from F<sub>0</sub> plants surviving a 36 g ai ha<sup>-1</sup> FPB treatment and those collected from nontreated control F<sub>0</sub> plants. Additionally, no significant differences were found in the rate of surviving seedlings after treatment with varying doses of FPB among the F<sub>2</sub> lines collected from F<sub>1</sub> plants that survived varying doses of FPB. At a constant temperature of 30 C, seeds from different F<sub>1</sub> and F<sub>2</sub> lines showed germination percentages of 85% to 92.0% and 68.3% to 89.0%, respectively. In the absence of competition, plants from the NL population surviving 0–144 g ai ha<sup>-1</sup> FPB showed no significant differences in plant height, dry weight of aboveground parts, effective accumulated temperature (EAT) from sowing to seed maturation, seed production per plant, or 1,000-seed weight. In the susceptible population (HJ), plants surviving 18 g ai ha<sup>-1</sup> FPB showed no significant differences compared to the nontreated control plants of the same population for the above variables. This is the first report of FPB-resistant barnyardgrass in China. Barnyardgrass seedlings that survived FPB application showed a higher potential for accumulating in the soil seedbank and negatively affecting rice.

### Introduction

Barnyardgrass is one of the most troublesome weeds in China rice production (Zhang et al. 2017). To date, many herbicide-resistant biotypes and populations have been reported, further intensifying challenges for weed management in rice fields (Heap 2023). In China, barnyardgrass has evolved resistance to various postemergence herbicides, including quinclorac, penoxsulam, bispyribac-sodium, and metamifop (Liu et al. 2019). This has resulted in limited postemergence herbicide options for effective weed control.

Florpyrauxifen-benzyl [FPB; IUPAC name benzyl-4-amino-3-chloro-6-(4-chloro-2-fluor-3-methoxyphenyl)-5-fluor-2-pyridincarboxylate], a synthetic auxin, WSSA Group 4 herbicide, is one of the most important postemergence herbicides widely used in China, particularly for

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controlling herbicide-resistant weeds in rice. It is highly effective against many weed species, including herbicide-resistant barnyardgrass (Beesinger et al. 2022; Haug et al. 2021; Mudge et al. 2021; Ortiz et al. 2022; Telo et al. 2019). Miller et al. (2018) reported that acetolactate synthase-, propanil-, and quinclorac-resistant barnyardgrass biotypes could be controlled with FPB at 30 g ai ha<sup>-1</sup>. FPB has strong tank-mix compatibility with various herbicides. No antagonistic interactions were found when FPB was mixed with other rice herbicides, such as acifluorfen, bentazon, carfentrazone, propanil, saflufenacil, 2,4-D, bispyribac, cyhalofop, fenoxaprop, halosulfuron, imazethapyr, penoxsulam, quinclorac, and triclopyr (Miller and Norsworthy 2018). FPB posed a low risk to subsequent crops after the rice-growing season (Zhou et al. 2022).

In China, FPB was registered for use in 2018 (CPIN 2023). In 2018, we collected a putative FPB-resistant barnyardgrass population (marked as NL) that infested a commercial rice field following application of FPB at 36 g ai ha<sup>-1</sup>. The spread of FPB-resistant barnyardgrass could become a major challenge for effective usage of this herbicide. Therefore, using the NL population as a case study, we aimed to test its potential to produce seeds upon treatment with different doses of FPB.

## Materials and Methods

### Plant Material

Seeds of a putative FPB-resistant population (NL) and susceptible barnyardgrass population (HJ) were collected in October 2018. Seeds of NL were collected from a commercial rice field in Nanling county (30.94°N, 118.24°E), Wuhu City, Anhui Province, China. This field was treated with pretilachlor, bensulfuron-methyl, bentazon, MCPA, metamifop, FPB, and cyhalofop during the growing season. The grower of this field did not keep records of earlier herbicide applications. Seeds of HJ (not treated with any herbicide) were collected from plants on roadsides surrounding the greenhouse located on the campus of Yangzhou University, Hanjiang county (32.39°N, 119.42°E), Yangzhou City, Jiangsu Province, China. Mature seeds were hand-collected randomly from more than 100 individuals in the NL population and five individuals with a large biomass in the HJ population. The seeds were cleaned, dried, stored at room temperature (15–25 C) for 1 yr, and then stored in paper bags at 4 C in a cold-storage room of at the Research Institute of Rice Industrial Engineering Technology of Yangzhou University.

The seeds collected from the two populations were designated as F<sub>0</sub>. Seedlings obtained from the F<sub>0</sub> plants were treated with different doses of FPB. The surviving plants were allowed to mature, and their seeds were individually collected in 2020 and grouped into F<sub>1</sub> lines. Seedlings obtained from F<sub>1</sub> plants were treated with different doses of FPB in 2021. Seeds from the F<sub>2</sub> lines were obtained using the same method.

### Whole-Plant Bioassay

In 2019, a series of bioassays were conducted to determine the susceptibility of NL and HJ to FPB [Rinskor-Dan<sup>®</sup>, 3 g ae L<sup>-1</sup> emulsifiable concentrate (EC); Corteva Agriscience, Chaoyang, Beijing, China]. The labeled dose of the FPB formulation was 36 g ai ha<sup>-1</sup>. Seedlings of NL were treated with FPB at 0 (nontreated control = not treated with any herbicide), 9, 18, 36, 72, and 144 g ai ha<sup>-1</sup>, and HJ seedlings were treated with 0, 2.25, 4.5, 9, 18, and 36 g ai ha<sup>-1</sup>. As described in our previous study, the experiments were conducted under greenhouse conditions at 30/20 C (day/night) and natural light (Chen et al. 2016). In all bioassays, each plastic

pot (7 by 7 cm) was sown with 12 pregerminated seeds. The seedlings were thinned to eight plants per pot during the two-leaf stage. FPB was applied at the three- to four-leaf stages, with an air-pressurized sprayer equipped with a Meiji W-71 spray gun (Meiji Machine Co., Ltd., Chiyoda Ward, Tokyo, Japan) delivering 400 L ha<sup>-1</sup> at 0.20 MPa; the caliber of the flat-fan spray nozzle on the gun was 1.0 mm. Seedlings treated with FPB were put on the ground out of the greenhouse for 6–8 h before moving back. The aboveground fresh weight per pot was collected at 30 d after treatment (DAT). The experiments were conducted twice using a completely randomized design with four replicates. The soil used in this study was produced by the same company (Haosheng<sup>®</sup>; Nanjing Duole Horticulture Co., Nanjing, Jiangsu, China) with a pH of 6.8, a total nutrient content of 3.8%, and a total organic matter content of about 40%. The plants were not fertilized during the bioassays, and were watered as needed to maintain ordinary growing conditions.

### Testing Proportions Of FPB-Resistant Individuals Among Different F<sub>1</sub> And F<sub>2</sub> Lines

In 2021 and 2022, bioassays were conducted to determine the proportion of FPB-resistant individuals in the different F<sub>1</sub> and F<sub>2</sub> lines. Seeds from the F<sub>1</sub> lines were terminated using tillage and treated with FPB at doses of 0, 36, and 72 g ai ha<sup>-1</sup> using the above-mentioned method. Seeds from the F<sub>2</sub> lines were grown and treated with FPB at doses of 0, 36, 72, and 144 g ai ha<sup>-1</sup>, as mentioned above. The number of seedlings surviving in each pot were recorded at 30 DAT. For each treatment (line by FPB dose), 18 seedlings in three pots were sprayed with the herbicide. The numbers of F<sub>1</sub> and F<sub>2</sub> lines used for each FPB treatment are shown in Tables 1 and 2.

### Productivity Tests

From April to October 2021, the NL seedlings were cultivated from F<sub>0</sub> seeds, and were sprayed with FPB at the three- to the four-leaf stage at doses of 0, 36, and 72 g ai ha<sup>-1</sup>, using the above-mentioned methods. Subsequently, at 30 DAT, the seedlings that survived and had green leaves were transplanted into cylindrical plastic pots (15 by 15 cm), with one seedling per pot, and grown in the same greenhouse. Each pot was placed in a box (20 by 30 by 15 cm) to facilitate watering. The soil used in this study was the same as mentioned above. The plants were watered as required to maintain healthy growing conditions. Nitrogen (Granular urea, 46.4%; Yangmei fengxi fertilizer Group Co., Ltd., Yuncheng, Shanxi, China) was applied to the surface of each pot (15 by 15 cm) for 7 d (40 kg ha<sup>-1</sup>) and 45 d after transplantation (50 kg ha<sup>-1</sup>). During this study, all the greenhouse windows were kept open. The minimum and maximum temperatures in the greenhouse ranged from 14 to 30 C and 22 to 37 C, respectively, with an average of 26.7 C.

The initiation date of seed maturation was recorded for each plant. A thermometer was fixed in the greenhouse center at a height of 2 m above the ground. The effective accumulated temperatures (EAT) from sowing to seed maturation were calculated according to the daily temperatures (Chen et al. 2022). Mature seeds of each plant were collected separately. For each plant, plant height, dry weight of aboveground parts, and the number of mature seeds were determined 180 d after sowing. The 1,000-seed weight was determined by weighing five replicates of 100 mature seeds.

Experiments were repeated from April to October 2022 with the HJ and NL populations using the above-mentioned methods. The HJ seedlings surviving the 18 g ai ha<sup>-1</sup> FPB and control treatments that did not receive any herbicide were terminated using tillage.

**Table 1.** Percentage of germination under a constant temperature of 30 C and surviving seedlings among different F<sub>1</sub> lines of the floryprauxifen-benzyl (FPB)-resistant barnyardgrass population (NL) 30 d after treatment with different dosages of FPB.<sup>a</sup>

Dose treated for F <sub>0</sub> g ai ha <sup>-1</sup>	No. of lines tested	Germination of F <sub>1</sub> seeds* %	Surviving seedlings		
			Dose treated for F <sub>1</sub>		
			g ai ha <sup>-1</sup>		
			0 <sup>NS</sup>	36 <sup>NS</sup>	72 <sup>NS</sup>
0	26	92.0 ± 2.0*	100 ± 0	26.7 ± 4.6	14.3 ± 3.0
36	21	85.1 ± 2.6*	100 ± 0	24.5 ± 15.9	17.7 ± 4.5

<sup>a</sup>F<sub>1</sub> lines were collected from F<sub>0</sub> lines surviving 36 g ai ha<sup>-1</sup> FPB treatment or control treatment (not treated with any herbicide). Seedlings were treated with FPB at the three- to four-leaf stage. \*Significant difference between the two values in the same column at P < 0.05. NS = nonsignificant.

**Table 2.** Percentage of surviving seedlings among different F<sub>2</sub> lines of the floryprauxifen-benzyl (FPB)-resistant barnyardgrass population (NL) 30 d after treatment with different dosages of FPB and germination percentage of F<sub>2</sub> seeds under a constant temperature of 30 C.<sup>a</sup>

Treated dose F <sub>0</sub>	F <sub>1</sub>	No. of tested lines	Germination of F <sub>2</sub> seeds %	Surviving seedlings			
				Dose treated for F <sub>2</sub>			
				g ai ha <sup>-1</sup>			
				0 <sup>NS</sup>	36 <sup>NS</sup>	72 <sup>NS</sup>	144 <sup>NS</sup>
0	0	6	86.0 ± 1.4 ab	100 ± 0	43.7 ± 7.9	34.3 ± 14.6	22.2 ± 12.3
0	36	11	80.2 ± 3.2 ab	100 ± 0	50.4 ± 6.1	24.1 ± 5.5	6.1 ± 2.0
0	72	5	89.0 ± 1.3 a	100 ± 0	65.2 ± 15.9	31.8 ± 11.3	13.8 ± 6.0
36	0	10	73.8 ± 3.5 bc	100 ± 0	60.6 ± 9.6	30.4 ± 12.2	16.0 ± 7.9
36	36	9	80.0 ± 2.6 b	100 ± 0	43.9 ± 6.3	15.7 ± 6.0	10.4 ± 4.6
36	72	7	68.3 ± 4.2 c	100 ± 0	56.1 ± 9.5	23.3 ± 5.4	21.4 ± 7.5

<sup>a</sup>F<sub>2</sub> lines tested were collected from F<sub>1</sub> lines surviving FPB treatments with different doses or control treatment, and F<sub>1</sub> lines were collected from F<sub>0</sub> lines surviving 36 g ai ha<sup>-1</sup> FPB treatment or control treatment. Seedlings were treated with FPB at the three- to four-leaf stage. Different letters suggest significant difference among values in the same column at P < 0.05. NS = nonsignificant.

Similarly, the NL seedlings surviving the 36, 72, and 144 g ai ha<sup>-1</sup> FPB and control treatments were terminated using tillage. The variables were tested using the above-mentioned methods. During the study, all the greenhouse windows were kept open. The minimum and maximum temperatures in the greenhouse ranged from 6 to 32 C and 14 to 41 C, respectively, with an average of 25.5 C.

### Seed Germination

The germination percentages for the different F<sub>1</sub> and F<sub>2</sub> lines surviving various doses of FPB treatments were determined in a growth chamber (constant 30 C, 12 h dark/12 h light). A total of 100 seeds from each line were sown in Petri dishes containing two pieces of filter paper moistened with 6 ml of distilled water (pH 6.8). Exactly 25 seeds were sown per dish and incubated under white fluorescent lamps (photosynthetic photon flux density of 140 μmol m<sup>-2</sup> s<sup>-1</sup>) with a photoperiod of 12 h dark/12 h light at a constant temperature of 28 C. The dishes were checked for germination 14 d after sowing.

### Statistical Analysis

Effective accumulated temperatures (EAT) were calculated using the equation:

$$EAT = \sum (T - T_0) \times GD \quad [1]$$

where *T* is the daily mean temperature, *T*<sub>0</sub> is the biological zero temperature of barnyardgrass at 10 C, and *GD* is the duration of the growth stage (d).

A general linear model (GLM) in SPSS (version 16; SPSS Inc., Chicago, IL, USA) statistical software was used to analyze the data from the two runs of whole-plant bioassays. Fresh weights of seedlings were set as dependent variables, and the experimental run, population, and doses were set as fixed factors. The GLM results suggested that the experimental run did not significantly influence the fresh weight of the seedlings. Therefore, data from the two whole-plant bioassays were pooled. The following three-parameter logistic function was fitted to test the responses of fresh weight to herbicide treatments and the accumulated number of mature seeds collected after sowing using SigmaPlot software (version 12.0; Systat Software Inc., San Jose, CA, USA):

$$Y = a/[1 + (x/GR_{50})^b] \quad [2]$$

where *Y* denotes the fresh-weight reduction response at FPB dose or day after sowing, *a* is the upper limit, *GR*<sub>50</sub> is the FPB dose causing a 50% reduction in fresh weight or EAT of the period after sowing to 50% mature seeds collected, and *b* is the slope. Accordingly, *GR*<sub>90</sub> (FPB dose causing a 90% reduction in fresh weight) was calculated for the two populations. The NL population's resistance factor (RF) to FPB was calculated by dividing the *GR*<sub>50</sub> of NL by that of HJ. Logistic response curves were generated using SigmaPlot. Populations were described as having no resistance (RF < 2), low resistance (2–5), moderate resistance (6–10), or high resistance (>10), according to Beckie and Tardif (2012). To compare the significant differences in the proportion of seedlings surviving different FPB treatments,

germination percentages of different treatments, and life history traits of NL plants from the various treatments were recorded. Data were subjected to analysis of variance in SPSS using the ANOVA procedure. Data were checked for normality and constant variance prior to analysis, and were log-transformed before analysis. Nontransformed means are reported with statistical interpretation based on transformed data. Treatment means were separated using LSD test at  $P = 0.05$ . Independent-sample  $t$ -tests were used to compare the differences in variables between the two treatments with SPSS. Additionally, GLMs were used to analyze the influence of the source of the mother plants ( $F_0$  and  $F_1$ ) on seed germination and the percentage of  $F_2$  seedlings surviving different FPB treatments with SPSS. Data are presented as the mean  $\pm$  SEs.

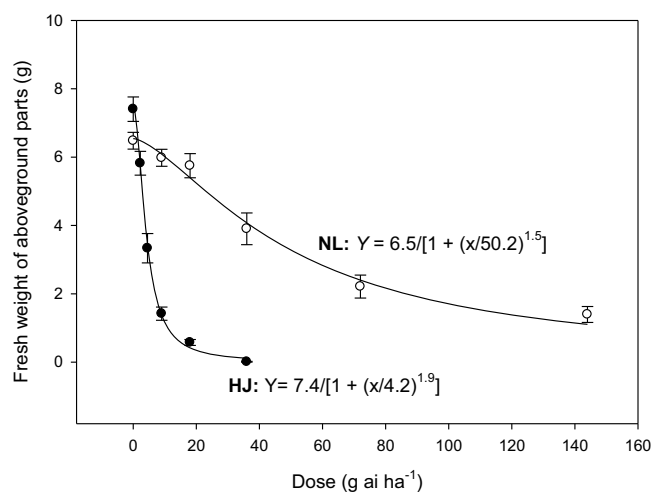
## Results and Discussion

### Florpyrauxifen-Benzyl (FPB) Resistance

The  $GR_{50}$  values for NL and HJ were  $50.2 \pm 5.5$  and  $4.2 \pm 0.2$  g ai ha<sup>-1</sup>, respectively, suggesting a resistance factor of 12 for NL (Figure 1). The  $GR_{90}$  values for NL and HJ were  $214.9 \pm 34.9$  and  $13.0 \pm 1.5$  g ai ha<sup>-1</sup>, respectively, indicating a 17 $\times$  reduction in the susceptibility to FPB in NL. The NL population escaped the application of metamifop, cyhalofop, and FPB in 2018 during the rice-growing season. In another set of experiments, NL showed a 6 $\times$  reduction in the susceptibility to metamifop, compared with HJ and based on  $GR_{50}$  values (data not shown). Prior to 2018, the herbicide-use history of the rice field-collected NL population was unclear. However, FPB was introduced in China in 2018. The resistance of NL to FPB may not be due to the continuous application of this herbicide. Similar results were also reported in South Korea. FPB was introduced into Korea in 2018, and Lim et al. (2021) reported that the  $GR_{50}$  of FPB to 70 barnyardgrass populations ranged from 6.2 to 16.1 g ai ha<sup>-1</sup>; moreover, these populations were collected from paddy fields of different provinces between 2006 and 2016. Gao et al. (2022) reported that the  $GR_{50}$  of FPB to a barnyardgrass population was 4.1 g ai ha<sup>-1</sup>. In the United States, FPB-resistant barnyardgrass populations collected in 2018 have also been reported (Hwang et al. 2022a, 2022b). Therefore, FPB resistance in NL might be a side effect of multiple resistance to other herbicides. More FPB-resistant barnyardgrass populations may exist in rice fields in China.

No significant differences in the percent seedling survival following FPB treatment at different rates were found between  $F_1$  lines collected from the  $F_0$  plants that survived 36 g ai ha<sup>-1</sup> FPB treatment and those collected from the control  $F_0$  plants with no herbicide treatment (Table 1). The  $F_2$  lines from the various  $F_1$  plants also showed no significant differences in percent seedling survival following FPB treatment at different rates (Table 2). The GLMs suggested that the source of the mother plants ( $F_0$  and  $F_1$ ) did not influence  $F_2$  seedlings survival rate following FPB at 36 to 144 g ai ha<sup>-1</sup>. These results suggest that FPB selection pressure may not be responsible for the reduced sensitivity in the NL population. Several other factors could reduce the sensitivity, such as an existing situation of metabolic/non-target site resistance to some other herbicide in this population. Hwang et al. (2022a, 2022b) reported non-target site resistance mechanisms of barnyardgrass to FPB in a mid-southern United States population, which showed multiple resistance to FPB and cyhalofop.

Under a constant temperature of 30 C,  $F_1$  seeds collected from the  $F_0$  plants that survived 36 g ai ha<sup>-1</sup> FPB treatment had an average germination rate of 85.1% (Table 1), and lower ( $P < 0.05$ )



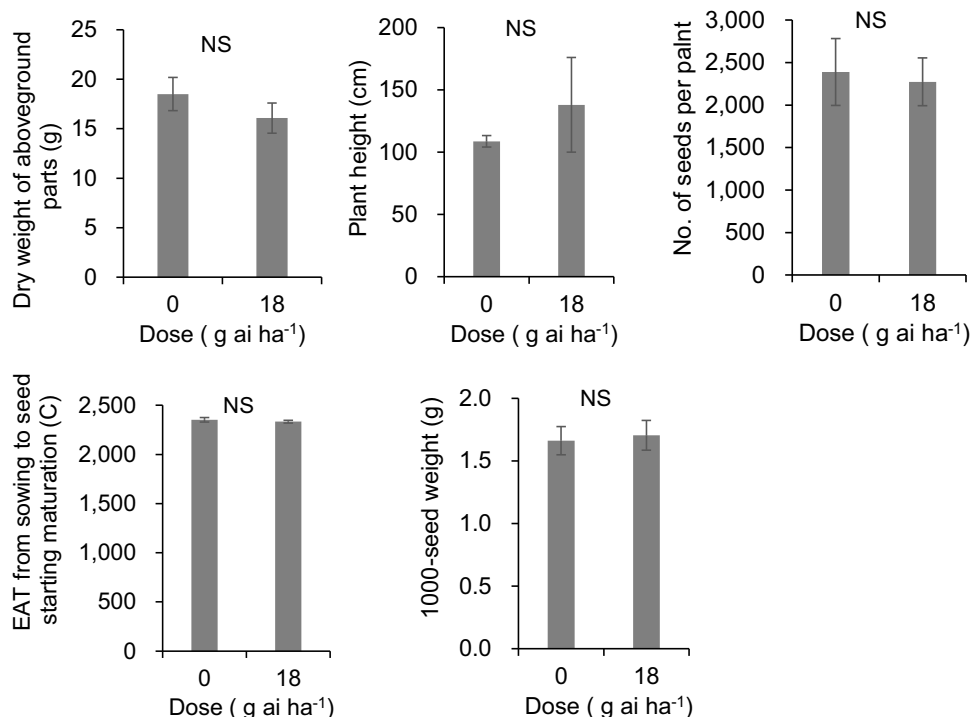
**Figure 1.** Florpyrauxifen-benzyl (FPB) dose–response tests for the two barnyardgrass populations. Seedlings were treated with FPB at the three- to four-leaf stage, and fresh weight of aboveground parts of surviving seedlings were determined for each treatment at 30 d after treatments.

than those collected from the nontreated control  $F_0$  plants (92%). The GLM suggested that FPB at 36 g ai ha<sup>-1</sup> applied to  $F_0$  plants influenced germination rate of the  $F_2$  seeds (Table 2). Germination rates of  $F_2$  seeds were 80.2% to 89% for lines sourced from  $F_0$  plants not treated with any herbicides, and germination rates were 68.3% to 80.0% for  $F_2$  seeds sourced from  $F_0$  plants that survived 36 g ai ha<sup>-1</sup> FPB. These results suggest that although FPB did not influence the survival rate, the seed quality was affected, as shown by reduced germination rates compared to a nontreated control. In a study with 43 naturally occurring populations of *Ipomoea purpurea* that vary in their resistance to glyphosate, Van Etten et al. (2016) found that highly resistant populations had lower germination. In contrast, in studies with *Lolium multiflorum* (Gundel et al. 2008) and *Beckmannia syzigachne* (Du et al. 2017), herbicide-resistant lines did not show lower seed germination.

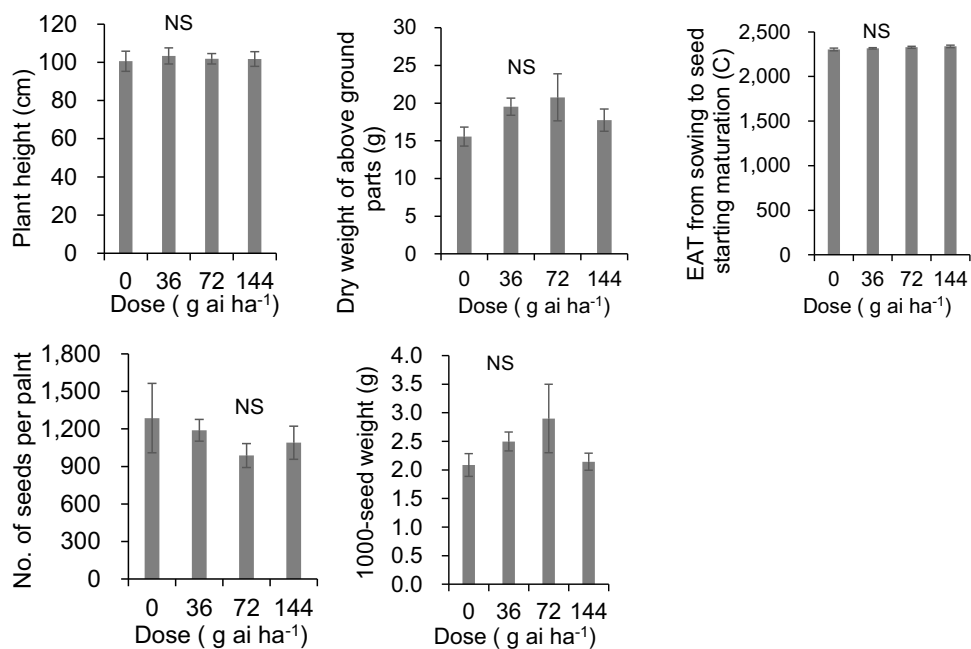
The spread of FPB-resistant barnyardgrass could become a major challenge for weed management in rice. Chemical control is the most important weed management method in rice fields in China (Liu et al. 2019). Time windows suitable for applying postemergence herbicides to control grassy weeds during the rice-growing season are frequently 15–20 d after planting (CPIN 2023). Various factors, such as the lack of homogeneity in seedling emergence, the rapid growth of grassy weeds, a rapid decrease in susceptibility to multiple herbicides with increasing tillers in grassy weeds, and rainy weather often cause the failure of postemergence chemical control (Chen et al. 2022). Furthermore, postemergence herbicides targeting barnyardgrass must be highly efficient against seedlings with growth stages from the three-leaf stage to the two-tiller stage, and meanwhile be safe for rice seedlings with the same growth stages. Among the registered rice herbicides in China, only metamifop and FPB meet these requirements when applied at label doses for postemergence barnyardgrass control (CPIN 2023). Developing new herbicides is difficult and expensive (Powles and Yu 2010; Yan et al. 2018), and thus FPB resistance in barnyardgrass causes a serious challenge to postemergence chemical control of this troublesome weed.

### Plant Productivity Following FPB Applications at Different Doses

In 2021, plants from the NL population surviving 36 or 72 g ai ha<sup>-1</sup> FPB did not differ from the nontreated control plants, in plant



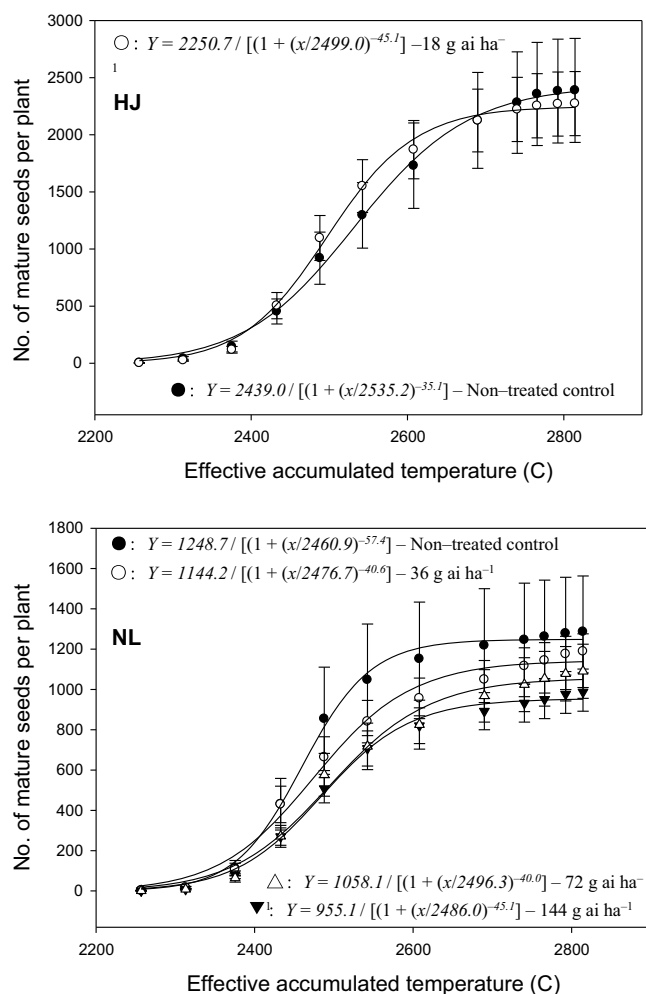
**Figure 2.** Productivity traits of *Echinochloa crus-galli* seedlings of the floryprauxifen-benzyl (FPB)-susceptible barnyardgrass population (HJ) surviving 18 g ai ha<sup>-1</sup> FPB treatment and the nontreated control. Seedlings were treated with FPB at the three- to four-leaf stage and surviving individuals were transplanted, allowed to grow, and harvested at 180 d after sowing in 2022. EAT, Effective accumulated temperature from sowing to seeds starting maturation. NS, No significant differences between the nontreated control and treatment at  $P < 0.05$ .



**Figure 3.** Productivity traits of barnyardgrass seedlings of the floryprauxifen-benzyl (FPB)-resistant population (NL) surviving FPB treatments at different doses. Seedlings were treated with FPB at the three- to four-leaf stage and surviving individuals were transplanted, allowed to grow, and harvested at 180 d after sowing in 2022. EAT, Effective accumulated temperatures. NS, No significant differences among different treatments at  $P < 0.05$ .

height, dry weight of aboveground parts, EAT from sowing to seed maturation, seed production per plant, and 1,000-seed weight. Similarly, in 2022, for the susceptible population (HJ), plants surviving 18 g ai ha<sup>-1</sup> FPB did not differ from the nontreated

control plants in the above-mentioned traits (Figure 2). For the NL population, plants treated with FPB at different doses did not differ significantly for any of the five variables tested (Figure 3). Moreover, plants treated with FPB at 72 g ai ha<sup>-1</sup> showed the



**Figure 4.** Logistics regressions showing response of number of mature seeds collected and effective accumulated temperature (EAT) after sowing in plant individuals surviving different doses of florpyrauxifen-benzyl treatment at the three- to four-leaf stage of barnyardgrass populations (HJ and NL).

highest biomass and 1,000-seed weight, and the lowest seed production (Figure 3). The responses of NL plants to FPB treated with different doses suggest potential hormetic effects (Belz 2020; Pincelli-Souza et al. 2020), and more studies are needed to illuminate FPB hormetic effects.

Logistic regressions for the two HJ treatments and four NL treatments showed significant relationships between the accumulated number of mature seeds and the EAT after sowing (Figure 4). Effective accumulated temperature (EAT) is a basic index to predict plant growing and development (Mei et al. 2015; Zmudzka 2013). In the HJ population, EATs needed for 50% of seeds to mature were 2,535.2 and 2,499.0 C for the control plants and plants surviving 18 g ai ha<sup>-1</sup> FPB treatment at the three- to four-leaf stage, respectively. Furthermore, HJ plants surviving 18 g ai ha<sup>-1</sup> FPB treatment showed seed-setting trends in line with the control plants. For the NL population, EATs needed for 50% of seeds to mature were 2,460.9, 2,476.7, 2,496.3, and 2,486.0 C for the control plants and plants surviving 36, 72, and 144 g ai ha<sup>-1</sup> FPB treatment at the three- to four-leaf stage, respectively (Figure 4). Plants subjected to the three FPB treatments exhibited seed-setting trends similar to those subjected to the nontreated control. Together the

above results did not show significant difference between seedlings surviving FPB treatments and nontreated control seedlings, in EATs needed for 50% of seeds to mature for both populations.

Barnyardgrass seedlings that survive FPB applications pose a high risk to rice production and accumulating seedbanks. Normally, there is only one opportunity to apply FPB to control *Echinochloa* seedlings in rice fields from the three-leaf stage to the two-tiller stage. Determining the efficacy of this herbicide in rice fields requires more than 20 d, after which the surviving barnyardgrass seedlings will likely become sufficiently tolerant to various rice herbicides. The space, moisture, and nutrients in the rice fields are sufficient to promote barnyardgrass seedling growth during this period (Chen et al. 2022).

Barnyardgrass surviving FPB treatments could produce seeds that would accumulate as a large seed bank. Seeds collected from barnyardgrass plants that escaped FPB application had relatively high germination rates. The NL plant surviving FPB application at the label dose (36 g ai ha<sup>-1</sup>) was able to produce >1,100 mature seeds on average, and >50% of the seedlings of this population survived this dosage. Therefore, the continuous application of FPB may lead to selection of barnyardgrass populations with increasing proportions of resistant plants. Palmer amaranth (*Amaranthus palmeri* S. Wats.) seedlings that escape FPB application have been reported to show significant reproduction ability (Beesinger et al. 2022). Herbicide-resistant barnyardgrass biotypes frequently show no substantial differences in growth or competitiveness (Bagavathiannan et al. 2011a). Given that some populations are tolerant to FPB, which practically limits the use of this herbicide in these regions, use of FPB would select for the already existing resistant populations and favor their dominance and possibly dissemination as well. Further complicating the situation, the already resistant and susceptible populations might be coexisting in the same region. Mapping all the fields for the existence of FPB-sensitive populations would be practically impossible and economically inviable, or both. Monitoring and controlling FPB resistance in barnyardgrass should be highlighted along with its market introduction.

### Practical Implications

Testing the sensitivity of representative barnyardgrass populations to FPB could be helpful before its widespread application in a certain area. Because they can grow and proliferate aggressively, FPB-resistant barnyardgrass plants should be removed from the field. Manual control should begin as early as possible to prevent numerous barnyardgrass plants from escaping FPB application. When many barnyardgrass plants elude FPB application, panicle-removing control should be initiated during the heading stage (Chen et al. 2022). Effective seed bank management is important for controlling FPB-resistant barnyardgrass populations (Bagavathiannan et al. 2011b; Chen et al. 2017; Norsworthy et al. 2018). Rotating the application of different herbicides is also important for barnyardgrass control in rice planting areas.

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