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Baseline sensitivity of *Echinochloa glabrescens* to florpyrauxifen-benzyl on a regional scale: A case study of Jiangsu Province, China

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Abstract

Systematically monitoring the baseline sensitivity of troublesome weeds to herbicides is a crucial step in the early detection of their market lifespan. Florpyrauxifen-benzyl is one of the most important herbicides used in rice production throughout the world, and has been used for 5 yr in China. Barnyardgrass is one of the main targeted weed species of florpyrauxifen-benzyl. In total, 114 barnyardgrass populations were collected from rice fields in Jiangsu Province, China, and using whole-plant bioassays they were screened for susceptibility to florpyrauxifen-benzyl. The GR₅₀ values (representing the dose that causes a 50% reduction in fresh weight of aboveground parts) of florpyrauxifen-benzyl for all populations ranged from 1.0 to 34.5 g ai ha⁻¹, with an average of 6.8 g ai ha⁻¹, a baseline sensitivity dose of 3.3 g ai ha⁻¹, and a baseline sensitivity index of 34.5. Twenty-one days after treatment with florpyrauxifen-benzyl at the labeled dose (36 g ai ha^{-1}), 90% of the barnyardgrass populations exhibited >95% reductions in fresh weight of aboveground parts. Compared with the baseline sensitivity dose, 63, 44, and 7 populations had, respectively, no resistance (55%), low resistance (39%), and moderate resistance (6%) to florpyrauxifen-benzyl. Furthermore, the GR₅₀ distribution of barnyardgrass populations did not show a significant correlation with collection location, planting method (directseeding or transplanting), or rice species (Oryza sativa L. ssp. indica or ssp. japonica) at any of rice fields where seeds had been collected (P > 0.05). In conclusion, florpyrauxifenbenzyl remains effective for barnyardgrass control in rice fields despite serious resistance challenges.

Introduction

Barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], a C₄ plant, is one of the most troublesome weeds in agricultural systems (Alberto et al. 1996). In paddy fields, it is highly competitive with rice for space, sunlight, and soil nutrients. Morphological similarity between barnyardgrass and rice seedlings has made identification difficult (Chauhan and Johnson 2009). When barnyardgrass was easily distinguished by farmers, rice yield losses were inevitable: by 7% and 87% at weed infestation levels of 5% and 50% coverage, respectively (Diop and Moody 1984). Barnyardgrass has been reported in China, India, and Philippines (Chauhan and Abugho 2013; Donayre et al. 2014; Zhang et al. 2023). A survey of rice fields in China indicated that barnyardgrass is common found in East China but seldom found in southern and northwestern China (Chen et al. 2019).

Herbicides are the most effective weed control tools, providing >85% control of the targeted weeds (Chen et al. 2023). Nonchemical weeding may achieve similar efficacy but only by combining multiple methods, each of which is generally far more labor-intensive than applying herbicides (Delye et al. 2013). Long-term and excessive use of herbicides has led to the emergence and increasing trend of resistance to herbicides by weeds (Iwakami et al. 2012; Zhu et al. 2020), including barnyardgrass, which has been reported to be resistant to penoxsulam, a herbicide that inhibits acetolactate synthase (ALS); metamifop and cyhalofop-butyl, which inhibit acetyl-CoA carboxylase (ACCase); and quintrione, which inhibits 4-hydroxyphenylpyr-uvate dioxygenase (HPPD) (Li et al. 2023; Yan et al. 2019; Zhang et al. 2022). Developing a new herbicide is costly and usually takes more than 10 yr; accordingly, the scientific and reasonable use of herbicides in rice production to extend their effective life is the foundation for sustainable chemical control. Baseline sensitivity refers to a continuous distribution of weed isolates using a range of herbicides (Thomas et al. 2012; Wang et al. 2022). Knowing the baseline sensitivity of

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barnyardgrass to various herbicides can provide a basis for weed control by monitoring the evolution of weed resistance to herbicides (Moss 2001), and thereby supporting the promotion and application of newly developed herbicides.

Florpyrauxifen-benzyl (benzyl-4-amino-3-chlor-6-[4-chlor-2fluor-3-methoxyphenyl]-5-fluor-2-pyridincarboxylate) is an important herbicide used worldwide in rice production to target the postemergence control of barnyardgrass. As a novel structural class of synthetic auxin herbicides, the herbicide has been used for managing weeds in rice fields since 2018, and has exhibited high efficacy in controlling susceptible and herbicide-resistant barnyardgrass biotypes. Nevertheless, barnyardgrass that is resistant to florpyrauxifen-benzyl has been found in the United States and China (Chen et al. 2024; Hwang et al. 2022b; Miller et al. 2018).

Farmers in Jiangsu Province, China, practice state-of-the-art rice and wheat planting, mainly by adopting the two-cropping pattern of rice-wheat rotation (Ren et al. 2023), with 2.22 million ha of paddy fields with $8,940 \text{ kg ha}^{-1}$. Due to the large rice market in Jiangsu Province, any new herbicide for use in rice production would be applied in Jiangsu Province once it is registered. Therefore, Jiangsu Province can serve as a typical region for studying the baseline sensitivity of regional weeds to important herbicides. Since 2017, florpyrauxifen-benzyl has been applied to control rice weeds in Jiangsu Province. In 2022, 114 barnyardgrass populations were collected from rice fields in Jiangsu Province. The objectives of this study were to 1) assess the sensitivity of barnyardgrass populations collected from paddy fields to florpyrauxifen-benzyl on a regional scale and 2) establish baseline sensitivity for monitoring the variation of resistance to florpyrauxifen-benzyl in the future.

Material and Method

Plant Materials

In October 2022, seeds were collected from paddy fields across 13 cities in Jiangsu Province, representing 114 populations. Sampling sites were not predetermined or selected based on the presence of barnyardgrass during collection. Therefore, herbicide application histories of the collecting fields were unknown. Seeds of each population were randomly collected from more than 100 mature plants, air-dried, and stored in a laboratory at room temperature before using. Barnyardgrass was identified based on its morphological traits (Chen et al. 2019).

Whole-Plant Dose-Response Bioassay

Seeds of each population were sown into a 9-cm-diam Petri dish containing two pieces of filter paper moistened with 6 mL distilled water and placed in a chamber at 30 /25 (light/dark) with a 12-h light/dark cycle. Twelve germinated seedlings were transplanted per plastic pot ($7 \times 7 \times 8$ cm) with a 2:1 mixture of natural soil and pH 5.6 nutritional soil (Nanjing Duole Horticulture Co., Ltd.), and grown in a greenhouse at Yangzhou University. Seedlings were thinned to nine uniform-sized plants per pot. At the 3- to 4-leaf stage, herbicides were applied using a laboratory sprayer (TBSHIELD) equipped with a flat-fan nozzle (1.1 mm diameter and jet angle 15 degrees), delivering 450 L ha⁻¹ at 230 kPa. Florpyrauxifen-benzyl 3% emulsifiable concentrate (36 g ai ha⁻¹; Corteva Agriscience, Shanghai, China) was sprayed at 0, 7.5, 15, 30, 60, 120, 240, or 480 g ai ha⁻¹. Aboveground fresh weight was measured 21 d after herbicide applications. Each treatment was

replicated four times, and the trial was repeated one additional time. Both trials were conducted between May and October 2023.

Data Analysis

Data from the two repeated trials were compared through ANOVA using SPSS software (v. 26.0). The data were pooled due to the nonsignificant interaction between the two repeated trials.

A four-parameter logistic function was fitted to test responses of fresh weight to herbicide treatments (Seefeldt et al. 1995), using the DRC add-on package in R 3.1.3 (Ritz and Streibig, 2005) as follows:

$$Y = d + (a - d) / [1 + (x/GR_50)^{b}]$$
(1)

where Y denotes the fresh weight reduction response at dose x of the herbicide, *a* is the upper limit, *d* is the lower limit, GR_{50} is the dose of herbicide causing a 50% reduction in fresh weight, and *b* is the slope. Fresh weight reduction was generated using the fresh weights and then ranked within the different responses according to the method reported by Chen et al. (2023) as follows: 100% = nosurvival individual of the target weed species observed; 95%-99% = very good control efficacy with sporadic individuals of the target weed species; 85%-94% = acceptable control efficacy with an obvious decrease in weed occurrence compared with the control; 70%-84% = general control efficacy and not enough to control the target weed species; 60%-69% = certain control efficacy without commercial value; and <60% = poor control efficacy and thus offering no commercial value. Resistance factors (RFs) were calculated by dividing the GR₅₀ value of each population by the most sensitive population to the herbicide. Using criteria reported by Beckie and Tardif (2012), populations were described as having no resistance (RF<2); low resistance (RF2–5); moderate resistance (RF6-10); and high resistance (RF>10).

The Shapiro-Wilk test was used to test for frequency distribution of GR_{50} values of florpyrauxifen-benzyl in Origin 2021 software. A Gaussian function was fitted to the baseline sensitivity to florpyrauxifen-benzyl of barnyardgrass as follows:

$$y = y_0 + \frac{A}{\left(\omega\sqrt{\frac{\pi}{2}}\right)} e^{-2\left(\frac{x-x_c}{\omega}\right)^2}$$
(2)

where y_0 is the offset, x_c is the center, ω is the width, and A is the area.

To compare significant differences in the average GR_{50} value for the 13 cities, least significant differences were used when variances were homogeneous, and Dunnett's T3 test was used if variances were not homogeneous. Correlation between the longitudes and latitudes of seed collection sites and GR_{50} values of barnyardgrass were determined with SPSS software using correlate analysis. To analyze the influence of environmental factors on the sensitivity of barnyardgrass, a general linear model was applied, where the GR_{50} value was the dependent variable, and independent variables including city, planting method, and subspecies of rice in locations where rice seeds were collected. The data presented are mean \pm SE.

Results and Discussion

Sensitivity to Florpyrauxifen-Benzyl

Fresh weight reduction with a half-labeled $(0.5\times)$ dose of florpyrauxifen-benzyl (18 g ai ha⁻¹) ranged from 19.2% to 100%

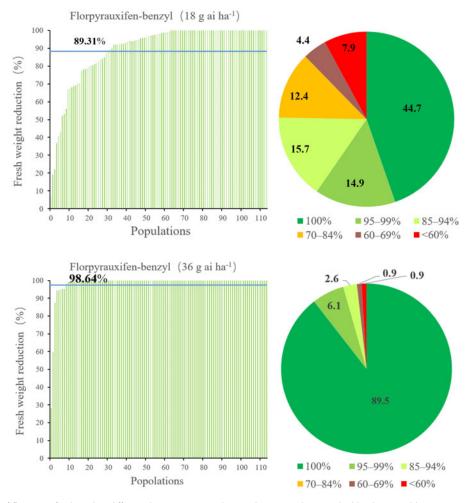


Figure 1. Control efficacy of florpyrauxifen-benzyl at different doses against 114 barnyardgrass populations. The blue horizontal lines represent the average fresh weight reduction of florpyrauxifen-benzyl to barnyardgrass populations. The pie chart represents six classes of control efficacy in 114 studied populations.

(89.3% on average) (Figure 1). Seedlings of 45% of the populations were completely killed, and 7.9% of the populations exhibited <60% fresh weight reduction. When the florpyrauxifen-benzyl dose increased to 36 g ai ha⁻¹ (1× dose), fresh weight reduction ranged from 28.0% to 100% (98.6% on average). Seedlings of 90% of the populations were completely killed, and 98.6% of the populations showed fresh weight reductions of >85%. Only 0.9% of the populations showed fresh weight reductions of <60%.

Florpyrauxifen-benzyl provides excellent control of barnyardgrass in paddy fields despite being used for the past 5 yr. When using 1× florpyrauxifen-benzyl, a whole-plant bioassay indicated that the herbicide controlled 96% of the 114 populations with very good efficacy (fresh weight reduction >95%). Florpyrauxifenbenzyl was reported to be highly effective in controlling barnyardgrass that is demonstrating resistance to ACCase- or ALS-inhibiting herbicides or to quinclorac (Miller et al. 2018; Yang et al. 2022). Considering the 98.6% average fresh weight reduction among the total barnyardgrass populations evaluated, florpyrauxifen-benzyl was still an important rice herbicide in the area surveyed.

Baseline Sensitivity to Florpyrauxifen-Benzyl

The GR_{50} values of florpyrauxifen-benzyl ranged from 1.0 to 34.5 g ai ha⁻¹ through 114 barnyardgrass populations (6.8 average,

4.6 median), and the average R^2 value was 0.90, and a value of > 0.80 for 99 populations (Table S1). All values were lower with 1× florpyrauxifen-benzyl (36 g ai ha⁻¹; Figure 2), and 107 out of 114 GR₅₀ values of the populations were lower than those when the 0.5× dose of florpyrauxifen-benzyl was used. The GR₅₀ values obtained were grouped into class intervals of 2 g ai ha⁻¹ (Figure 3), with a baseline sensitivity dose of florpyrauxifen-benzyl of 3.3 g ai ha⁻¹, and a baseline sensitivity index of 34.5.Twenty-nine of the 114 populations exhibited GR₅₀ values that were less than the baseline sensitivity dose.

The baseline sensitivity test was used primarily for determining the variation in sensitivity, and the baseline sensitivity value was a more practical parameter for baseline sensitivity (Wang et al. 2022). This study of 114 barnyardgrass populations collected from overall rice planting counties could be important data for reflecting the full view of the sensitivities of barnyardgrass to florpyrauxifenbenzyl in rice fields in Jiangsu Province. The baseline sensitivity dose of florpyrauxifen-benzyl could provide a reference base for studies on barnyardgrass that is resistant to florpyrauxifen-benzyl and for determining time-dependent changes in florpyrauxifenbenzyl sensitivity of barnyardgrass in rice paddy fields (Escorial et al. 2019).

Compared with the baseline sensitivity dose (2.53 g ai ha^{-1}) to florpyrauxifen-benzyl in Korea in 2021 (Lim et al. 2021), the baseline sensitivity dose (3.34 g ai ha^{-1}) of barnyardgrass in China

40 Label dose of florpyrauxifen-benzyl (36 g ai ha-1) 3 35 30 GR50 (g ai ha-1) 25 20 Half of label dose of florpyrauxifen-benzyl (18 g ai ha-1) 15 10 5 0 **107**₁₁₀ 10 40 50 60 70 100 0 20 30 80 90 Poplations

Figure 2. GR₅₀ values of florpyrauxifen-benzyl against 114 barnyardgrass populations. The red horizontal lines represent the various doses of florpyrauxifen-benzyl. The red vertical line represents the number of populations.

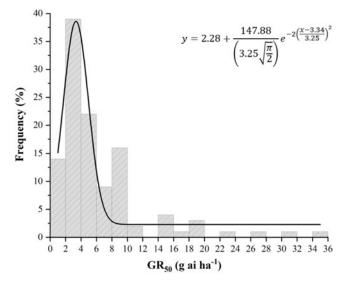


Figure 3. Frequency distribution of florpyrauxifen-benzyl doses that caused a 50% growth reduction (GR₅₀) in barnyardgrass populations. Individual isolates are grouped in class intervals of 2 g ai ha⁻¹.

in 2023 was slightly higher. In another *Echinochloa* rice weed species, Chen et al. (2024) and Gao et al. (2022) reported that the GR_{50} values of barnyardgrass that is susceptible to florpyrauxifenbenzyl were 4.2, and 4.1 g ai ha⁻¹, respectively. Therefore, the current baseline sensitivity dose of barnyardgrass could serve as a fixed sensitivity value in the study of barnyardgrass resistance to florpyrauxifenbenzyl.

The establishment of baseline sensitivity data was critical in managing fungicide resistance because the results can be used to illustrate shifts in pathogenic sensitivity and provide evidence that control failures occurring in the future can be correlated with the appearance of resistance in the population (Russel 2004; Veloukas and Karaoglanidis 2012). Baseline sensitivity is also applicable to weed resistance management. Subsequent surveys of weed sensitivity to herbicides on a regional scale will be necessary to prevent resistance evolution to the level at which control fails (Avenot and Michailides 2010). Systematically monitoring the baseline sensitivity of troublesome weeds to important herbicides

is the basis for developing an early warning system for the market lifespan of herbicides.

Florpyrauxifen-Benzyl Resistance

Compared with the baseline sensitivity dose (3.3 g ai ha⁻¹), 63, 44, and 7 barnyardgrass populations had, respectively, no resistance (55%), low resistance (39%), and moderate resistance (6%) to florpyrauxifen-benzyl (Figure 4). Barnyardgrass populations in the southwest Jiangsu Province exhibited high resistance, especially populations with the highest proportion of low resistance collected from Suzhou (Table 1). However, the most resistant population (YC5, $GR_{50} = 34.5$ g ai ha⁻¹) was found in Yancheng.

Herbicide resistance is an inevitable problem in agricultural intensification due to its excessive use (Hulme, 2023; Owen, 2016). Developing an effective and feasible herbicide is a difficult process that takes a great deal of time and money (Rueegg et al. 2007). Additionally, herbicide resistance evolution is accelerating with their repeated use on cereal crops. Some populations of troublesome weeds might even show resistance to new herbicides that have never been used against them (Baucom 2019; Takano et al. 2023). Before the commercialization of florpyrauxifen-benzyl, barnyardgrass had evolved resistance to the herbicides quinclorac, penoxsulam, metamifop, and bispyribac-sodium, all of which are used to control weeds in rice production (Choudhary et al. 2023; Damalas and Koutroubas 2023; Xia et al. 2016). Thus, florpyrauxifen-benzyl resistance by barnyardgrass might be a result of multiple resistance being caused by applications of other rice herbicides (Takano et al. 2023).

The average GR₅₀ values for florpyrauxifen-benzyl recorded from 13 cities ranged from 3.4 to 11.7 g ai ha⁻¹. Neither city, rice planting method (direct seeding or transplanting), or rice subspecies (*Oryza sativa* subsp. *japonica* or *O. sativa* subsp. *indica*) at the seed-collecting site showed any significant influences on the distributions of GR₅₀ values (Table S2). The median GR₅₀ values were consistent with the average value. Moreover, neither longitude nor latitude of the seed-collecting sites had any significant (P > 0.05) correlations with the GR₅₀ value of tested populations (Figure S1), with low R^2 values of 0.026 and 0.006, respectively.

Barnyardgrass escaping chemical control in rice fields may be attributable to inappropriate agronomic practices, such as uneven

Table 1. ${\rm GR}_{50}$ values and resistance factors of florpyrauxifen-benzyl for barnyardgrass collected from 13 cities.^=-c

| | | Florpyrauxifen-benzyl | | |
|-------------|-----------------------|-----------------------|-------------------------------|--|
| City | No. of populations | GR ₅₀ | Median of GR ₅₀ | Proportion of resistance factors |
| | | g ai ha ⁻¹ | g ai ha ⁻¹ | NR:LR:MR:HR |
| Huaian | 8 | 3.4 ± 0.4 c | 3.5 | 8:0:0:0 |
| Lianyungang | 9 | 3.9 ± 0.8 c | 3.4 | 8:1:0:0 |
| Changzhou | 8 | 5.0 ± 0.7 bc | 5.0 | 4:4:0:0 |
| Xuzhou | 8 | 5.4 ± 1.1 abc | 5.4 | 4:4:0:0 |
| Nanjing | 6 | 5.7 ± 1.3 abc | 4.3 | 4:2:0:0 |
| Yangzhou | 12 | 6.7 ± 2.5 abc | 3.1 | 8:3:1:0 |
| Suqian | 14 | 6.0 ± 1.3 abc | 4.3 | 10:3:1:0 |
| Nantong | 8 | 6.1 ± 0.9 abc | 6.1 | 3:5:0:0 |
| Taizhou | 8 | 6.3 ± 1.8 abc | 5.0 | 4:4:0:0 |
| Wuxi | 6 | 7.5 ± 2.5 abc | 6.0 | 3:2:1:0 |
| Zhenjiang | 6 | 9.4 ± 3.5 abc | 4.9 | 3:2:1:0 |
| Suzhou | 14 | 10.6 ± 1.8 ab | 9.4 | 3:9:2:0 |
| Yancheng | 7 | 11.7 ± 3.9 a | 8.8 | 7:3:1:0 |

^aAbbreviations: NR, no resistance; LR, low resistance; MR, moderate resistance; HR, high resistance.

 b Different letters in the same column indicate significant differences among 13 cities at P < 0.05, the maximum value noted letter a, compared in turn.

^cGR₅₀ represents the dose of herbicide that causes a 50% reduction in fresh weight.

land, failure to maintain flood depth, and inappropriate application of herbicides. Late-emerging weed seeds may avoid chemical control. Among 13 cities, barnyardgrass populations from Suzhou and Yancheng clearly exhibited higher median resistance; however, some other cities also contained high resistant populations. These results provide insight for producers to focus specific ways they can mitigate herbicide resistance.

Practical Implications

After 5 yr of florpyrauxifen-benzyl being used in Jiangsu province, China, the herbicide is still highly effective at controlling a majority of barnyardgrass populations. To date, florpyrauxifen-benzyl is an outstandingly useful herbicide in many rice planting areas worldwide. On the other hand, reports are beginning to reveal that barnyardgrass is beginning to show resistance to florpyrauxifen-benzyl (Chen et al. 2024; Hwang et al. 2022a; Yang et al. 2022). Additionally, 45% of the barnyardgrass populations showed resistance to this herbicide. Florpyrauxifen-benzyl had low efficacy against a few populations of barnyardgrass when it was applied at the $1 \times$ dose (36 g ai ha⁻¹). Hence, resistance to florpyrauxifenbenzyl could be a challenge if this herbicide continues to be used. Considering the serious resistance to ACCase- and ALS-inhibiting herbicides in Echinochloa weeds, the effectiveness of florpyrauxifen-benzyl against Echinochloa species is important for weed management in rice. Therefore, an integrative management approach to control weeds that are resistant to florpyrauxifenbenzyl should be implemented (Lim et al. 2021; Miller and Norsworthy 2018; Wang et al. 2021). For example, preemergence chemical control; agronomic measures that target the suppression of weed emergence such as deep tillage, crushing, and deep burying of previous crop straw; leveling planting areas; and removing panicles could also be useful (Chen et al. 2022).

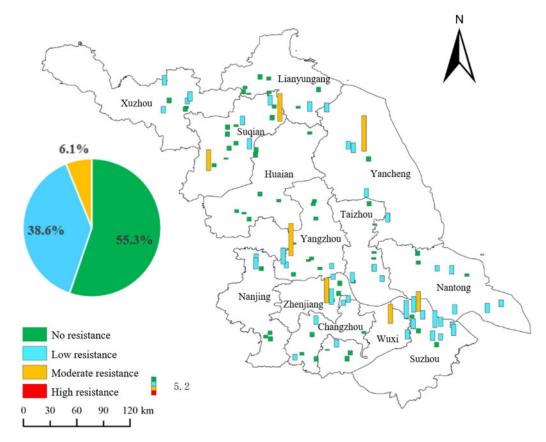


Figure 4. Distribution of resistance factors and resistance levels to florpyrauxifen-benzyl among 114 barnyardgrass populations. The pie chart represents the proportions of the four resistance levels.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2025.3

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Competing Interests. The authors declare they have no conflicts of interest.

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