agronomy

MDPI

Article

Detection of Resistance in *Echinochloa* spp. to Three Post-Emergence Herbicides (Penoxsulam, Metamifop, and Quinclorac) Used in China

Yangyang Zhang [1,2], Miao Wu [1,2], Shuijing Bao [1,2], Jiamin Li [1,2], Dingrong Liu [1,2], Liyao Dong [1,2] and Jun Li [1,2,*]

[1] College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
[2] Key Laboratory of Integrated Management of Crop Diseases and Pests, Nanjing Agricultural University, Ministry of Education, Nanjing 210095, China
* Correspondence: li_jun@njau.edu.cn

Abstract: In this study, rapid resistance in-season quick (RISQ) tests were developed for detecting the resistance in *Echinochloa* spp. to penoxsulam, metamifop, and quinclorac, which are widely used in rice fields to control *E.* spp. biotypes. Seedlings in 1–2 leaf stages from nine biotypes of *E. crusgalli*, *E. crusgalli var. zelayensis*, and *E. glabrescens*, with different susceptibility to the three herbicides tested, were transplanted to plates containing nutrient agar and different rates of herbicides. The survival rates were recorded at 8 days after treatment when no more new roots emerged for all the treatments. By comparing the results from RISQ tests and whole-plant pot bioassays statistically, discrimination rates could be determined to distinguish resistant plants from susceptible plants. For penoxsulam, metamifop, and quinclorac, the discrimination rates were 0.3, 0.6, and 2.4 μmol/L, respectively. Two additional biotypes of *E. crusgalli* collected in rice fields were used to confirm the validation of the RISQ test and the obtained results by the RISQ test were consistent with that of the whole-plant pot bioassay. Therefore, the developed RISQ test would be a possible alternative method to determine the susceptibility of *E.* spp. to certain herbicides.

Keywords: RISQ; herbicide resistance detection; bioassay; *Echinochloa* spp.

Citation: Zhang, Y.; Wu, M.; Bao, S.; Li, J.; Liu, D.; Dong, L.; Li, J. Detection of Resistance in *Echinochloa* spp. to Three Post-Emergence Herbicides (Penoxsulam, Metamifop, and Quinclorac) Used in China. *Agronomy* **2023**, *13*, 841. https://doi.org/10.3390/agronomy13030841

Academic Editors: Judith Wirth and Ilias Travlos

Received: 31 December 2022
Revised: 28 February 2023
Accepted: 9 March 2023
Published: 13 March 2023

1. Introduction

Echinochloa spp. are annual grass weeds with a wide range of distribution from latitudes 50″ N to 40″ S [1], among which *E. crusgalli* is one of the most troublesome weed species colonizing rice fields [2,3]. The various species of *Echinochloa* genus are well adapted to wettable soils and are commonly found growing among both temperate and tropical crops, including potatoes, snap beans, sugar beet, green peas, and melons, in over 60 countries. In many major rice-growing regions, intensive monocropping, typically with two or even three harvests each year of direct-seeded rice, has prompted farmers to use considerable amounts of herbicides to control these grass weeds [4]. It is generally considered as an effective, simple, and comparatively inexpensive approach for the control of noxious weeds [5]. Meanwhile, modern agricultural production has come to depend heavily on the use of herbicides to control crop weeds [6].

The increased use of pesticides has contributed immensely to enhancing agricultural productivity, decreased losses of stored grains, and generally improved people's living standard [7]. However, the application of large amounts of these chemicals would also bring negative repercussions for both human society and the environment, including economic loss to the user, inefficient control of pests, and potential environmental contamination [8]. Moreover, as a consequence of the large-scale use of herbicides, many weeds have evolved resistance to herbicides, which has accordingly become a threat to conventional agricultural practices, particularly as the number of reported incidences of

resistance are still rising. Since the discovery of resistance to triazine herbicides in common groundsel (*Senecio vulgaris*) in 1968, there has been a substantial increase in the incidence of herbicide resistance worldwide [9]. Over the past 50 years, herbicide-resistant weeds have infested both cultivated and non-cultivated areas of the world. To date, 515 unique cases of herbicide-resistant weeds (including 267 species) had been identified in 72 countries [10].

The first case of *E.* spp. resistance found was the resistance to triazine in US corn fields [11], and more were reported to be resistant to propanil [12], thiobencarb, butachlor, mefenacet [13], paraquat, quinclorac [14], and the acetolactate synthase (ALS) and acetyl CoA carboxylase (ACCase) inhibitors [15]. In China, the most widely used post-emergence herbicides to control *E.* spp. are ALS and ACCase inhibitors, and auxin-like herbicides, such as penoxsulam, metamifop, and quinclorac, respectively. However, the resistance to penoxsulam and quinclorac in *E.* spp. have evolved and resistance mechanisms have been reported [14,16,17].

Different resistance detecting methods have been developed for numerous herbicides and weed species [18], including the whole-plant pot bioassay, agar-based seedling assay, thermal imagery, spectral indices, molecular biology-based assay, as well as physiology and biochemistry-based assays [19]. However, the existing tests or technology have their disadvantages, such as cost inefficiency, the prerequisites for a good understanding of resistance mechanisms, the requirement for expensive instruments, not being in-season, and so on. The resistance in-season quick (RISQ) test was first developed to detect resistance to post-emergence ACCase and ALS inhibitor herbicides in *Lolium* spp. [20]. It is a more cost effective, simple, and early-season bioassay compared with the many existing ones. The RISQ test has also been used to detect glyphosate resistance in *Lolium*, *Eleusine*, *Conyza,* and *Amaranthus* species [21]; resistance to clodinafop-propargyl and pinoxaden in *L. rigidum* [22]; and ALS resistance in *Schoenoplectus juncoides* [23].

Given that most weed-management decisions must be made early in the growing season before plants mature and develop seeds, an ideal resistance test should be both effective and comparatively quick [24]. Therefore, the objective of the present work was to develop a reliable and quick assay for the detection of herbicide resistance in species of *Echinochloa* based on the RISQ test. Our specific objectives were as follows: (i) to determine the discriminating rates of different herbicides used against *E.* spp. in the RISQ test; and (ii) to verify the validity of the developed RISQ tests.

2. Materials & Methods

2.1. Plant Materials

In this study, eleven biotypes of *Echinochloa* species were used. Seeds of EC-2, EC-3, EC-4, EC-5, EZ-2, and EZ-3 were collected from rice fields with long histories of herbicide use in Anhui, Jiangsu provinces and Shanghai, whereas other seeds, EC-1, EZ-1, and EG-1, were collected from fallow fields in Jiangsu province that have not been sprayed with herbicides in recent years. The biotypes EC-6 and EC-7 were used in verifying this test. The resistant biotypes (EC-2 and EC-5) have been characterized as carrying mutations in ALS, responsible for the resistance to penoxsulam. The characteristics of the plant materials used in this study are shown in Table 1.

Table 1. Characteristics of the biotypes used to develop the RISQ test.

Biotypes *	Species	Origin	Susceptibility
EC-1 (JNBX-1)	*E. crusgalli*	Baixia, Jiangsu province in China (33°49′ N, 119°23′ E)	susceptible to penoxsulam, metamifop, and quinclorac

Table 1. *Cont.*

Biotypes *	Species	Origin	Susceptibility
EG-1 (JJXY-1)	*E. glabrescens*	Minhe, Xiannv, Jiangdu, Jiangsu province in China (32°42′ N, 119°63′ E)	susceptible to penoxsulam, metamifop, and quinclorac
EZ-1 (JNX-S)	*E. crusgalli* var. *zelayensis*	Academy of Agricultural Sciences, Jiangsu province in China (32°04′ N, 118°86′ E)	resistant to quinclorac
EC-2 (AXXZ-6)	*E. crusgalli*	Xuanzhou, Xuancheng, Anhui province in China (30°47′ N, 118°14′ E)	resistant to penoxsulam
EC-3 (AXXZ-8)	*E. crusgalli*	Xuanzhou, Xuancheng, Anhui province in China (30°47′ N, 118°14′ E)	resistant to penoxsulam
EC-4 (JHLS-1)	*E. crusgalli*	Lianshui, Huaian, Jiangsu province in China (33°25′ N, 119°30′ E)	resistant to penoxsulam
EC-5 (AXXZ-2)	*E. crusgalli*	Xuanzhou, Xuancheng, Anhui province in China (30°56′ N, 110°59′ E)	resistant to penoxsulam
EZ-2 (SSX-R)	*E. crusgalli* var. *zelayensis*	Xinbing, Songjiang, Shanghai in China (31°03′ N, 121°23′ E)	resistant to quinclorac
EZ-3 (JCW-R)	*E. crusgalli* var. *zelayensis*	Wujin, Changzhou, Jiangsu province in China (31°70′ N, 119°94′ E)	resistant to quinclorac
EC-6 (JNLS-1)	*E. crusgalli*	Lishui, Jiangsu province in China (31°32′ N,118°89′ E)	susceptible to penoxsulam, metamifop, and quinclorac
EC-7 (HYYJ-1)	*E. crusgalli*	Yuanjiang, Hunan province in China (28°84′ N,112°35′ E)	resistant to penoxsulam and quinclorac

*: To simplify the biotype coding, the biotypes have been re-coded and the original coding indicated in brackets, as most of the biotypes have been presented in previously published papers or dissertations.

2.2. Sensitivity of Different Biotypes to the Three Herbicides

Penoxsulam (25 g/L OD, Corteva Agriscience, Shanghai, China), metamifop (10% EC, FMC Cooperation, Philadelphia, PA, USA), and quinclorac (50% WP, Jiangsu Xinyizhongkai Agrochemicals, Xinyi, China) were used in the present study. The susceptibility of different biotypes to the three herbicides was determined by a whole-plant pot assay. Details of the doses of herbicides used for different biotypes are shown in Table 2. Plants were grown in $7 \times 7 \times 7$ cm plastic pots containing fertilizer: loam soil (1:3) under a day/night temperature regime of 30 °C/25 °C and 12-h photoperiod. For each herbicide treatment, 20 plants per biotype were sprayed at the early vegetative growth stages (2–3 leaves) using an automatic sprayer (3WP-2000), which was calibrated to deliver 600 L/ha solution at a speed of 291 mm s^{-1}, from a spray nozzle at 300 mm above the sprayed surface with a spraying pressure of 0.3 MPa. After being treated, all the plants were returned to the greenhouse and grown under the same conditions as described above. Each treatment had four replicates and each experiment was repeated twice at the same conditions. At 21 days after treatment (DAT), the above-ground parts of the seedlings were collected and fresh weights were recorded.

2.3. Development of the RISQ Test

Seedlings were prepared according to the procedure described by Kaundun et al. [20], with minor modifications. Square Petri dishes (13 × 13 cm) were used as alternatives and the incubated plates were put in the incubator vertically instead of horizontally, which would allow the roots of seedlings to grow downward due to geotropism, and make the visual observation of root emergence much easier than that described by Kaundun et al.

Table 2. Herbicide doses used in whole-plant pot bioassays for the *E.* spp. populations.

Herbicides	Herbicide Doses (g a.i./ha)	Biotypes
penoxsulam	0, 2.8125, 5.625, 11.25, 22.5, 45	EC-1, EZ-1, EG-1, EC-2, EC-4
	0, 5.625, 11.25, 22.5, 45, 90	EC-3
	0, 3.75, 7.5, 15, 30, 60	EC-6, EC-7
metamifop	0, 12.5, 25, 50, 100, 200	EC-1, EZ-1, EG-1, EC-2, EC-2
	0, 7.5, 15, 30, 60, 120, 240	EC-6, EC-7
quinclorac	0, 75, 150, 300, 600, 1200	EC-1, EZ-1 EG-1, EZ-3
	0, 150, 300, 600, 1200, 2400	EZ-2
	0, 46.88, 93.75, 187.5, 375, 750, 1500	EC-6, EC-7

2.3.1. Media Preparation

Agar powder (Beijing Solarbio Science & Technology Co., Ltd., Beijin, China) was added to ultrapure water at 1.2% *w*/*v* and placed in an autoclave (D-1-70, Beijing Faen Scientific Co., Ltd., Beijing, China) for sterilization. When the agar solution had cooled to approximately 50 °C, herbicides were added at the desired rates, and the resulting solutions were gently mixed at room temperature for 3 min. Thereafter, approximately 60 mL of each solution was poured into 13 × 13 cm square Petri dishes, which were placed horizontally in a laminar flow cabinet until the agar had cooled and solidified. Five square Petri dishes containing no herbicide were used as controls. All tests were performed immediately after the plates had been prepared.

2.3.2. Seedling Transplantation

The procedures used for seedling transplantation and subsequent growth followed the methods described by Kaundun et al. [20]. Initially, the seedlings from each biotype were cultured in the glasshouse under culture conditions used for whole-plant pot tests. When the seedlings had grown to the 2-leaf stage, they were carefully uprooted from the plastic pots. The roots were then washed in clean water to remove soil, and excess moisture was removed using filter papers. Seedlings were transplanted into square Petri dishes (13 × 13 cm) using a pair of forceps, placed horizontally with the roots below the growing point being gently pushed into the agar, and ensuring that the remaining roots were in contact with the agar. Ten seedlings were transplanted per Petri dish. A total of 50 plants per biotype were tested for each herbicide treatment. The Petri dishes were covered with lids and placed in an incubator with a day/night temperature regime of 30 °C/25 °C under a 12-h photoperiod. Plates containing no herbicides were used as controls.

2.3.3. Determination of Observation Time

The survival of the transplanted seedlings in each Petri dish under each treatment at different herbicide rates (0, 0.01, 0.1, 1, 10, and 100 µmol/L) was recorded daily until 11 DAT. When no more new roots emerged under the different treatments, the observation was stopped. The earliest day on which no more new roots emerged for all the treatments was taken as the optimal observation time.

2.3.4. Selection of Discriminating Rates of Herbicides

Serial rates for the selection of discriminating rates were set at the rate which could totally inhibit the root growth (Table 3), and the survived plants in each biotype were recorded.

Table 3. Herbicide rates for screening the discriminating rates of the three herbicides.

Herbicides	Herbicide Rates (μmol/L)	Tested Biotypes
penoxsulam	0, 0.3, 0.6, 0.9, 1.2, 1.5	EC-1, EZ-1, EG-1, EC-2, EC-3, EC-4
metamifop	0, 0.3, 0.6, 0.9, 1.2, 1.5	EC-1, EZ-1, EG-1, EC-2, EC-5
quinclorac	0, 0.6, 1.2, 2.4, 4.8, 9.6	EC-1, EZ-1, EG-1, EZ-2, EZ-3

2.3.5. The Whole-Plant Pot Assay

In this part, the whole-plant pot assay was performed as described in Section 2.2, except that 50 plants of each biotype and the recommended doses of penoxsulam (22.5 g a.i. ha^{-1}), metamifop (100 g a.i. ha^{-1}), and quinclorac (300 g a.i. ha^{-1}) were applied. Also, 50 plants without herbicide treatment for each biotype were taken as contrast. At 21 DAT, the number of surviving plants was recorded.

2.4. Verification

Susceptible and resistant biotypes of *E. crus-galli*, collected from Pailou, Jiangsu Province (EC-6), and Ruanjiang, Hunan Province (EC-7), respectively, were tested with single discriminating rates of penoxsulam, metamifop, and quinclorac to verify the accuracy of the developed RISQ test. The specific method was the same as above. For each biotype, 50 individual seedlings (five plates and 10 plants of each) were tested.

2.5. Statistical Analysis

2.5.1. The Sensitivity of Different Biotypes

Fresh weights were measured and percentage inhibitions were calculated using the following equation [25]:

$$percentage\ inhibition\ (\%) = \frac{control\ treatment}{control} \times 100 \quad (1)$$

A probit regression Equation (2) [14] was fitted by SPSS to calculated the ED_{50} value of different biotypes to penoxsulam, metamifop, and quinclorac,

$$Y = A + BX \quad (2)$$

where Y represents the probit value; A is the intercept; B is the regression coefficient; and X is the base 10 logarithm of herbicide doses.

Biotypes were classified as high, moderate, or low resistance, or susceptible, based on the ED_{50}. Herbicide resistance was classified into five groups: no resistance (S, RI < 2); low resistance (L, RI = 2–5); moderate resistance (M, RI = 6–10); high resistance (H, RI = 11–100); and very high resistance (VH, RI > 100) [26].

2.5.2. RISQ Test

The results of the RISQ test and whole-plant pot assay were analyzed with the Fisher exact test using GraphPad Prism 7.00 to determine correlations between the two tests. p-values greater than 0.05 indicated that there was no significant difference between the two tests and that the results were highly correlated.

3. Results

3.1. Susceptibility of Different Biotypes to the Three Herbicides

Based on the results from the whole-plant pot assay, for penoxsulam, EC-1, EG-1, EC-6, and EZ-1 were identified as susceptible (S) biotypes; EC-4 as low resistant (L); EC-7 as moderate resistant (M); and EC-2 and EC-3 as highly resistant (H) biotypes (Table 4).

Table 4. The susceptibility of different *E.* spp. to penoxsulam.

Population	ED_{50} ± SE (g a.i./ha)	RI	Susceptibility
EC-1	3.10 ± 0.32	1.00	S
EG-1	5.67 ± 1.57	1.83	S
EZ-1	6.10 ± 0.60	1.97	S
EC-4	10.40 ± 1.79	3.35	L
EC-3	58.29 ± 5.68	18.80	H
EC-2	40.45 ± 4.14	13.05	H
EC-6	<3.75	<2	S
EC-7	28.61 ± 3.53	9.23	M

Similarly, for metamifop, EC-1, EG-1, EZ-1, EC-6, and EC-7 were classified as S; EC-5 and EC-2 as L (Table 5), whereas for quinclorac, EC-1, EG-1, and EC-6 were classified as S; EZ-1 as L; EC-7 and EZ-3 as H; and EZ-2 as VH (Table 6). In addition, because the plants from EC-6 were all suppressed at the lowest doses of herbicides [penoxsulam (3.75 g a.i. ha^{-1}), metamifop (7.5 g a.i. ha^{-1}), and quinclorac (46.88 g a.i. ha^{-1})], it was identified as a susceptible (S) biotype (Tables 4–6).

Table 5. The susceptibility of different *E.* spp. to metamifop.

Population	ED_{50} ± SE (g a.i./ha)	RI	Susceptibility
EC-1	15.59 ± 3.81	1.00	S
EG-1	29.65 ± 3.76	1.90	S
EZ-1	29.96 ± 1.79	1.92	S
EC-2	63.09 ± 7.69	4.05	L
EC-5	51.85 ± 12.43	3.33	L
EC-6	<7.5	<2	S
EC-7	22.10 ± 3.41	1.42	S

Table 6. The susceptibility of different *E.* spp. to quinclorac.

Population	ED50 ± SE (g a.i./ha)	RI	Susceptibility
EC-1	23.89 ± 1.66	1.00	S
EG-1	42.26 ± 2.80	1.77	S
EZ-1	94.96 ± 1.29	3.97	L
EZ-2	2457.52 ± 149.69	102.87	VH
EZ-3	416.79 ± 3.05	17.45	H
EC-6	<46.88	<2	S
EC-7	354.36 ± 73.33	14.83	H

Meanwhile, all the three species of *Echinochloa* genus shared the same susceptibility to penoxsulam and metamifop. However, biotype EZ-1 of *E. crusgalli* var. *zelayensis* appearred to be less susceptible to quinclorac than the other two species, which would be due to the different susceptibility between species, as the EZ-1 biotype had been confirmed to be susceptible to quinclorac.

3.2. Development of the RISQ Test

3.2.1. Determination of Observation Time

The numbers of surviving plants in different treatments were counted daily until 11 DAT to determine the point at which there were no further changes in the number of survivors. Percentage survival was calculated and plotted against DAT. The earliest day on which no more new roots emerged, resulting in a constant percentage survival for all the

treatments, was selected as the optimal observation time. As shown in Figures 1–3, 8 DAT was selected as the optimal test time for all three herbicides.

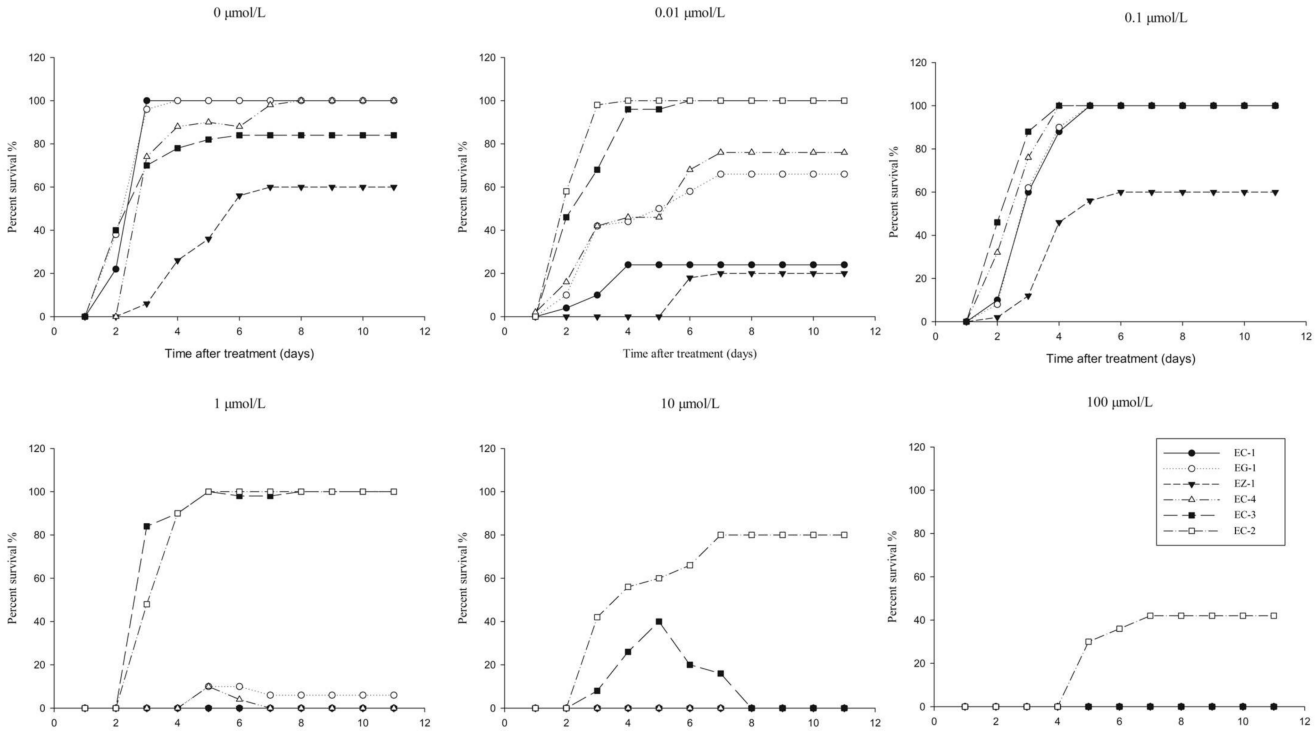

Figure 1. Percentage of daily survival of various biotypes at different penoxsulam concentrations (%).

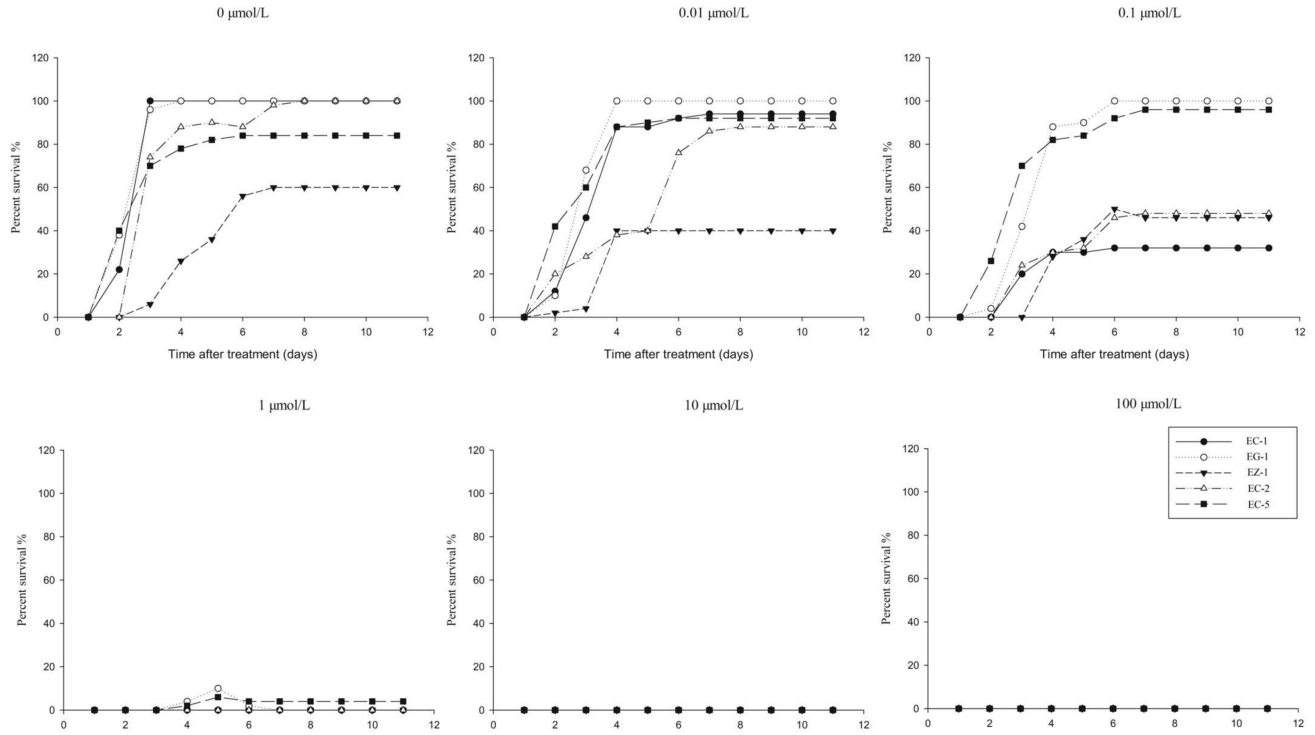

Figure 2. Percentage of daily survival of various groups at different metamifop concentrations (%).

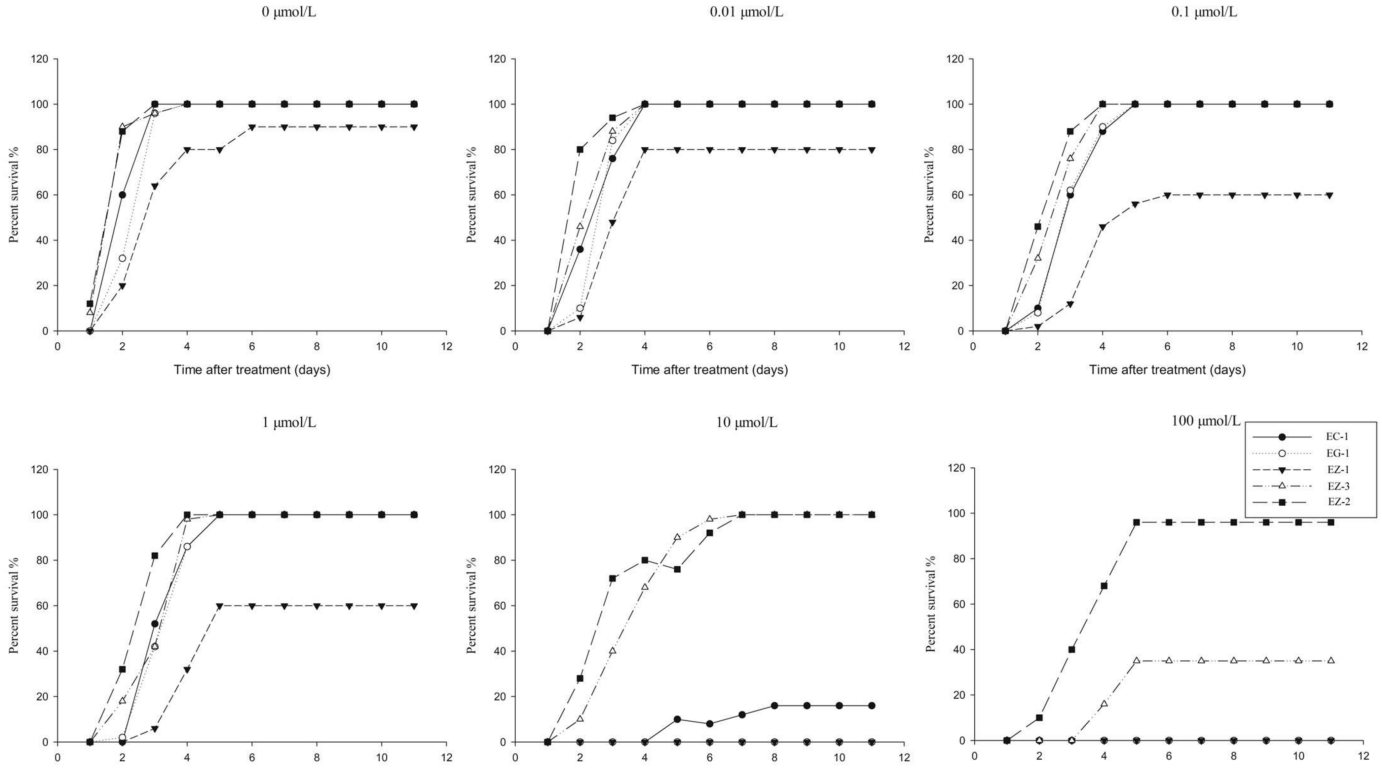

Figure 3. Percentage of daily survival of various biotypes at different quinclorac concentrations (%).

3.2.2. Selection of Discriminating Rates of Herbicides in the RISQ Test

When plants were exposed to the recommended field doses of herbicides [penoxsulam (22.5 g a.i. ha^{-1}), metamifop (100 g a.i. ha^{-1}), and quinclorac (300 g a.i. ha^{-1})], it was found that almost all plants of the susceptible biotypes were totally killed, whereas resistant biotypes showed varied inhibiting effects (Table 7a–c).

In subsequent tests, the lowest rate of penoxsulam that killed all the plants from all three susceptible biotypes was 0.3 µmol/L. At that rate, 100% of the individuals from EC-2 and EC-3, and 22% of EC-4 survived, of which EC-2, EC-3, and EC-4 were identified as highly resistant, highly resistant, and lowly resistant to penoxsulam, respectively. For the whole-plant pot assay at the recommend dose of penoxsulam, all the plants from the three sensitive biotypes were killed at 22.5 g a.i. ha^{-1}, except for EG-1 (6% of the tested plants survived), while for the resistant biotypes, EC-2, EC-3, and EC-4, the survival rates were 100%, 100%, and 30%, respectively. These results were well correlated as no p values were less than 0.05 (Table 7a). Therefore, 0.3 µmol/L was taken as the discriminating rate for *E.* spp. to penoxsulam.

For metamifop, the lowest rate that killed all individuals from the three susceptible biotypes was 0.6 µmol/L, at which the survival rate for EC-2 (L) and EC-5 (L) were 28% and 6%, respectively (Table 7b). Therefore, 0.6 µmol/L was taken as the discriminating rate for *E.* spp. to metamifop.

For quinclorac, the lowest rate that killed all the susceptible biotypes was 2.4 µmol/L. At this rate, the percentage surviving of EZ-2 (VH) and EZ-3 (H) were 100% and 52%, respectively, which were not significantly different with the results from the whole-plant pot assay. Although EZ-1 was an L biotype, it was completely killed at 2.4 µmol/L (Table 7c). However, in the whole-plant pot assay, all the plants could be killed by the recommended dose of quinclorac, and between these two results, no significant difference were found. Therefore, such a false negative result would not affect the choice of quinclorac. As a result, 2.4 µmol/L was taken as the discriminating rate for *E.* spp. to quinclorac, although this rate could not be used to identify R biotypes.

Table 7. The percentage surviving in the RISQ test compared with whole-plant pot tests for different susceptible and resistant *Echinochloa* spp. biotypes treated with (a) penoxsulam, (b) metamifop, and (c) quinclorac.

(a) penoxsulam								
Biotypes	**Whole-Plant Pot Test (g a.i./ha)**	**Herbicide Rate in RISQ Test (μmol/L)**						***p*-Value ***
	22.5	**0**	**0.3**	**0.6**	**0.9**	**1.2**	**1.5**	
EC-1	0 ± 0.00	100 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EG-1	6 ± 2.45	100 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.2424
EZ-1	0 ± 0.00	100 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EC-2	100 ± 0.00	100 ± 0.00	100 ± 0.00	72 ± 5.83	54 ± 5.10	0 ± 0.00	0 ± 0.00	>0.9999
EC-3	100 ± 0.00	100 ± 0.00	100 ± 0.00	52 ± 3.74	44 ± 5.10	2 ± 2.00	0 ± 0.00	>0.9999
EC-4	30 ± 3.16	100 ± 0.00	22 ± 3.74	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.1609
(b) metamifop								
Biotypes	**Whole-Plant Pot Test (g a.i./ha)**	**Herbicide Rate in RISQ Test (μmol/L)**						***p*-Value ****
	100 ± 0.00	**0**	**0.3**	**0.6**	**0.9**	**1.2**	**1.5**	
EC-1	0 ± 0.00	100 ± 0.00	34 ± 5.19	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EG-1	0 ± 0.00	100 ± 0.00	14 ± 2.45	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EZ-1	0 ± 0.00	100 ± 0.00	30 ± 3.16	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EC-2	32 ± 3.74	100 ± 0.00	66 ± 5.10	28 ± 3.74	2 ± 2.00	0 ± 0.00	0 ± 0.00	0.6753
EC-5	16 ± 2.45	100 ± 0.00	70 ± 3.16	6 ± 2.45	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.1997
(c) quinclorac								
Biotypes	**Whole-Plant Pot Test (g a.i./ha)**	**Herbicide Rate in RISQ Test (μmol/L)**						***p*-Value *****
	300	**0**	**0.6**	**1.2**	**2.4**	**4.8**	**9.6**	
EC-1	0 ± 0.00	100 ± 0.00	78 ± 3.74	26 ± 2.45	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EG-1	0 ± 0.00	100 ± 0.00	92 ± 3.74	26 ± 4.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EZ-1	0 ± 0.00	100 ± 0.00	72 ± 3.74	22 ± 2.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	>0.9999
EZ-2	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	>0.9999
EZ-3	50 ± 0.32	100 ± 0.00	100 ± 0.00	88 ± 3.74	52 ± 3.74	2 ± 2.45	0 ± 0.00	>0.9999

* Fisher's exact test to evaluate the correlation between the whole-plant pot test at the recommended field rate (22.5 g a.i./ha) and best discriminating rate of penoxsulam in the RISQ test (0.3 μmol/L) method. ** Fisher's exact test to evaluate the correlation between the whole-plant pot test at the recommended field rate (100 g a.i./ha) and best discriminating rate of metamifop in the RISQ test (0.6 μmol/L) method. *** Fisher's exact test to evaluate the correlation between the whole-plant pot test at the recommended field rate (300 g a.i./ha) and best discriminating rate of quinclorac in the RISQ test (2.4 μmol/L) method.

3.3. Verification

Two *E. crusgalli* biotypes, which were identified by whole-plant bioassays as susceptible to all three herbicides (EC-6) and resistant to penoxsulam and quinclorac (EC-7), were used in the RISQ verification. When plants from EC-6 were subjected to the discriminating rates of penoxsulam, metamifop, and quinclorac, almost all of the tested seedlings from EC-6 were killed, from which it could be identified as being susceptible to these three herbicides, except for quinclorac. At 2.4 μmol/L quinclorac, 18% of the plants from EC-6 survived, which is a very high value. For EC-7, more than 50% of the tested seedlings survived from the treatment of penoxsulam and quinclorac at the discriminating rates, while nearly all the tested seedlings were killed by metamifop, which were consistent with the results from Section 3.1 (Table 8). From the results, it is verified that the developed RISQ test

could be used to identify the resistance to penoxsulam and metamifop in *E*. spp., although for the field test, it still requires more research. However, for quinclorac, 2.4 µmol/L could only discriminate the *E*. spp. with high resistance to quinclorac, but not the susceptible biotypes, which is a problem to be solved in the future.

Table 8. The field samples and percentage surviving under discriminative rates of penoxsulam, metamifop, and quinclorac in the RISQ test.

Population	Characteristics	Origin	RISQ Test			
			Untreated	Penoxsulam 0.3 µmol/L	Metamifop 0.6 µmol/L	Quinclorac 2.4 µmol/L
EC-6	suspected resistant field	Lishui, Jiangsu province in China	100 ± 0.00	2 ± 0.20	3 ± 0.20	18 ± 0.66
EC-7	suspected resistant field	Yuanjiang, Hunan province in China	100 ± 0.00	64 ± 0.60	6 ± 0.40	62 ± 0.58

4. Discussion and Conclusions

Herbicides make an extremely valuable contribution to securing crop yields and are indispensable in traditional, mechanized arable farming [27]. However, intensive use of these herbicides worldwide has accelerated the selection of resistance genes in a number of weed grass species [28], and the number of reported cases of herbicide-resistant weeds continue to increase [10]. These resistant weeds endanger the productivity of modern cropping systems in many regions of the world [29], and represent a constraint on weed management in many cropping regions [30].

In China, *E*. spp. are the most noxious weeds in both direct-seeding and transplanting paddy fields. The most widely-used post-emergence herbicides to control *E*. spp. are penoxsulam, metamifop, and quinclorac, which belong to ALS inhibitor, ACCase inhibitor, and auxin-like herbicides, respectively. However, resistance to penoxsulam, metamifop, and/or quinclorac in *E*. spp. have evolved [14,16], although metamifop-resistance in *E. crusgalli* has not yet been reported (data in the present paper). Therefore, quick resistance detecting technologies have become urgent requirements for the management of herbicide resistance.

In order to effectively tackle the problem of herbicide resistance, it would therefore be beneficial to have a simple means of determining which weeds are resistant to a particular herbicide [31]. In this regard, an increasing number of methods have been developed for detecting resistant weeds. Whole-plant tests performed under glasshouse conditions could most closely simulate conditions experienced in the field, which is currently one of the most commonly used methods for screening herbicide resistance. However, a major limitation of the whole-plant test is that several months are required to obtain the final results. Pollen germination has been applied to detect target site-based resistance to ALS inhibitors and ACCase inhibitors in *Lolium rigidum* [32] and *A. myosuroides* [33], which was performed on specific agar mediums and the results could be observed in 2 h. Other seed-basedtests have also been developed, such as the tribenuron-methyl resistance in *Papaver rhoeas* [34,35]; quiclorac resistance in *E*. spp. [36]; ALS inhibitor resistance in *Avena sterilis* [37]; and dicamba resistance in *Chenopodium album*. However, these tests would take 10 to 20 d before the results were obtained and were labor intensive as the root and/or shoot lengths should be measured in general. All these mentioned tests could not be performed at the early vegetative growth stage of weeds in season and subsequently give directions for the choice of herbicides.

Some in-season detection methods have also been reported. Methods based on leaf chlorophyll fluorescence have been developed to detect ALS and ACCase inhibitor resistance in *E*. spp. [38,39], *Apera spica-venti* [40], and *A. myosroiudes* [41,42], and glyphosate resistance in *E. indica* [43], in 2 to 10 days, respectively. Although it could detect resistance in season, it requires special equipment and detecting sensors, which would be very expensive and professional grade. Leaf disc flotation was developed to detect glyphosate-resistant plants in 48 h [44], but complex procedures were needed. With further exploration on herbicide resistance mechanisms, molecular biology-based detections were developed for rapid and in-season resistance detection. Tests based on PCR (qPCR and/or droplet digital PCR etc), CAPS/dCAPS, and LAMP have been developed to detect target-site based resistance to ACCase and ALS inhibitors in *A. myosuroides* [27], *Beckmannia syzigachne* [45,46]; and *L*. spp. [47], glyphosate resistance in *L. perenne* [48] and *Amaranthus palmeri* [49]. Meanwhile, SNaPshot, genome-wide signatures and next-generation sequencing have also been used to detect herbicide resistance. A SNaPshot assay had been developed to detect the ACCase inhibitor resistance in *L*. spp. [50]. Genome-wide signatures had been used to detect 2,4-D resistance in *Trifolium pratense* [51], and the next-generation sequencing had been reported to detect ALS inhibitor resistance in *E. crusgalli* [52]. However, these methods are mutation-specific and limited to known mutations. Also, as various mutations in ACCase and/or ALS could confer resistance, it would be very costly and time consuming to develop methods and to identify each and every one of these mutations [20]. Furthermore, it could not be used to detect non-target site-based resistance, which had appeared to be a more dominant mechanism for resistance to ACCase and ALS inhibitors in grass weeds [53]. Novel techniques have emerged in herbicide resistance detection. Monoclonal antibody for CP4 EPSPS had been tested to detect glyphosate resistance in weeds, although it is still in development [54]. Image-based spectral reflectance has been conceptually proved to have the possibility in discriminating the glufosinate-resistant and -susceptible varieties of *Zea mays*, *Gossypium hirsutum,* and *Glycine max*, which would also assist in the detection of herbicide resistance in weeds [55].

Compared with the existing methods, the RISQ test is a reliable, quick, cost effective, and an in-season test to detect target-site based and non-target-site based resistance in *Conyza canadensis*, *L. multiflorum*, *L. rigidum*, *E. indica,* and *A. rudis* [20,21] to certain ACCase and/or ALS inhibitors, as well as glyphosate. In the present study, our RISQ tests could discriminate between the resistant and susceptible biotypes of *E*. spp. to penoxsulam, metamifop, and quinclorac, in 8 days, which is a much shorter observation time than those previously reported, and would allow the famers to choose proper post-emergence herbicides to control *E*. spp. in time. Also, the development of RISQ tests will also facilitate in monitoring and finding resistance, and assist weed scientists in subsequent researches. However, further research will be required to include more species of *E*. spp. and herbicides, to widen the scope of application and to shorten the observation time to meet the optional application stage of post-emergence herbicides.

Author Contributions: Conceptualization, J.L. (Jun Li); methodology, J.L. (Jun Li); software, Y.Z. and D.L.; validation, Y.Z., M.W., S.B. and J.L. (Jiamin Li); formal analysis, J.L. (Jun Li); investigation, Y.Z. and D.L.; resources, J.L. (Jun Li) and L.D.; data curation, Y.Z. and D.L.; writing—original draft preparation, Y.Z.; writing—review and editing, J.L. (Jun Li); visualization, M.W.; supervision, J.L. (Jun Li); project administration, J.L. (Jun Li); funding acquisition, J.L. (Jun Li). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program (2021YFD1700100).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thanks to the Minstry of Science and Technology of China for the funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maun, M.A.; Barrett, S.C.H. The biology of Canada weeds.: 77. *Echinochloa-crussgalli* (L.) Beauv. . *Can. J. Plant Sci.* **1986**, *66*, 739–759. [CrossRef]
2. Lim, S.H.; Kim, H.; Noh, T.K.; Lim, J.S.; Yook, M.J.; Kim, J.W.; Yi, J.H.; Kim, D. Baseline Sensitivity of *Echinochloa crus-gall* and *E. oryzicola* to Florpyrauxifen-Benzyl, a New Synthetic Auxin Herbicide, in Korea. *Front. Plant Sci.* **2021**, *12*, 656642. [CrossRef]
3. Holm, L.G.; Pucknett, D.L.; Pancho, J.V.; Heberger, J.P. *The World's Worst Weeds. Distribution and Biology*; University Press of Hawaii: Honolulu, HI, USA, 1977.
4. Juliano, L.M.; Casimero, M.C.; Llewellyn, R. Multiple herbicide resistance in barnyardgrass (*Echinochloa crus-galli*) in direct-seeded rice in the Philippines. *Int. J. Pest Manag.* **2010**, *56*, 299–307. [CrossRef]
5. Zhang, N.Y.; Linscombe, S.; Oard, J. Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. *Euphytica* **2003**, *130*, 35–45. [CrossRef]
6. Tadeo, J.L.; Sanchez-Brunete, C.; Perez, R.A.; Fernandez, M.D. Analysis of herbicide residues in cereals, fruits and vegetables. *J. Chromatogr. A* **2000**, *882*, 175–191. [CrossRef] [PubMed]
7. Tiryaki, O.; Temur, C. The Fate of Pesticide in the Environment. *J. Biol. Environ. Sci.* **2010**, *4*, 29–38.
8. Quan, L.; Li, S.F.; Tian, S.J.; Xu, H.; Lin, A.Q.; Gu, L. Determination of organochlorine pesticides residue in ginseng root by orthogonal array design soxhlet extraction and gas chromatography. *Chromatographia* **2004**, *59*, 89–93.
9. Beckie, H.J.; Ashworth, M.B.; Flower, K.C. Herbicide Resistance Management: Recent Developments and Trends. *Plants* **2019**, *8*, 161. [CrossRef]
10. Heap, I. The International Herbicide-Resistant Weed Database. Available online: http://www.weedscience.org/Home.aspx (accessed on 13 February 2023).
11. Ryan, G.F. Resistance of common groundsel to simazine and atrazine. *Weed Sci.* **1970**, *18*, 614–616. [CrossRef]
12. Carey, V.F.; Hoagland, R.E.; Talbert, R.E. Resistance mechanism of propanil-resistant barnyardgrass: 2. In-vivo metabolism of the propanil molecule. *Pestic. Sci.* **1997**, *49*, 333–338. [CrossRef]
13. Cai, X.; Chen, J.; Wang, X.; Gao, H.; Xiang, B.; Dong, L. Mefenacet resistance in multiple herbicide-*resistant Echinochloa crus-galli* L. populations. *Pestic. Biochem. Physiol.* **2022**, *182*, 105038. [CrossRef] [PubMed]
14. Xu, J.; Lv, B.; Wang, Q.; Li, J.; Dong, L. A resistance mechanism dependent upon the inhibition of ethylene biosynthesis. *Pest Manag. Sci.* **2013**, *69*, 1407–1414. [CrossRef] [PubMed]
15. Bagavathiannan, M.V.; Norsworthy, J.K.; Smith, K.L.; Neve, P. Modeling the Simultaneous Evolution of Resistance to ALS- and ACCase-Inhibiting Herbicides in Barnyardgrass (*Echinochloa crusgalli*) in Clearfield (R) Rice. *Weed Technol.* **2014**, *28*, 89–103. [CrossRef]
16. Fang, J.; Zhang, Y.; Liu, T.; Yan, B.; Li, J.; Dong, L. Target-Site and Metabolic Resistance Mechanisms to Penoxsulam in Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv). *J. Agric. Food Chem.* **2019**, *67*, 8085–8095. [CrossRef]
17. Zhao, Z.; Liu, X.; Pan, X.; Napier, R.; Dong, L.; Li, J. Different regulation of auxin homeostasis would be a possible mechanism conferring quinclorac resistance in *Echinochloa crusgalli* var. zelayensis. *Weed Res.* **2022**, *62*, 318–327. [CrossRef]
18. Burgos, N.R.; Tranel, P.J.; Streibig, J.C.; Davis, V.M.; Shaner, D.; Norsworthy, J.K.; Ritz, C. Review: Confirmation of Resistance to Herbicides and Evaluation of Resistance Levels. *Weed Sci.* **2013**, *61*, 4–20. [CrossRef]
19. Tataridas, A.; Jabran, K.; Kanatas, P.; Oliveira, R.S.; Freitas, H.; Travlos, I. Early detection, herbicide resistance screening, and integrated management of invasive plant species: A review. *Pest Manag. Sci.* **2022**, *78*, 3957–3972. [CrossRef] [PubMed]
20. Kaundun, S.S.; Hutchings, S.J.; Dale, R.P.; Bailly, G.C.; Glanfield, P. Syngenta 'RISQ' test: A novel in-season method for detecting resistance to post-emergence ACCase and ALS inhibitor herbicides in grass weeds. *Weed Res.* **2011**, *51*, 284–293. [CrossRef]
21. Kaundun, S.S.; Hutchings, S.-J.; Harris, S.C.; Jackson, L.V.; Shashi-Kiran, R.; Dale, R.P.; McIndoe, E. A Simple In-Season Bioassay for Detecting Glyphosate Resistance in Grass and Broadleaf Weeds Prior to Herbicide Application in the Field. *Weed Sci.* **2014**, *62*, 597–607. [CrossRef]
22. Khammassi, M.; Hajri, H.; Menchari, Y.; Chaabane, H.; Souissi, T. Current status in resistance to ACCase and ALS-inhibiting herbicides in rigid ryegrass populations from cereal crops in North of Tunisia. *J. Agric. Sci.* **2019**, *157*, 676–683. [CrossRef]
23. Weiqiang, J.; Roh, S.-W.; Song, J.E.; Bo, A.B.; Cho, K.M.; Park, K.W.; Joo, L. Evaluation of Three in-season Tests for Rapidly Detecting Resistance to Acetolactate Synthase Inhibitors in Schoenoplectus juncoides. *Weed Turfgrass Sci.* **2021**, *10*, 13–23.
24. Koger, C.H.; Shaner, D.L.; Henry, W.B.; Nadler-Hassar, T.; Thomas, W.E.; Wilcut, J.W. Assessment of two nondestructive assays for detecting glyphosate resistance in horseweed (*Conyza canadensis*). *Weed Sci.* **2005**, *53*, 438–445. [CrossRef]
25. Chung, I.M.; Ahn, J.K.; Yun, S.J. Assessment of allelopathic potential of barnyard grass (*Echinochloa crus-galli*) on rice (*Oryza sativa* L.) cultivars. *Crop Prot.* **2001**, *20*, 921–928. [CrossRef]
26. Beckie, H.J.; Tardif, F.J. Herbicide cross resistance in weeds. *Crop Prot.* **2012**, *35*, 15–28. [CrossRef]

27. Delye, C. Weed resistance to acetyl coenzyme A carboxylase inhibitors: An update. *Weed Sci.* **2005**, *53*, 728–746. [CrossRef]
28. Beckie, H.J.; Reboud, X. Selecting for Weed Resistance: Herbicide Rotation and Mixture. *Weed Technol.* **2009**, *23*, 363–370. [CrossRef]
29. Rosenhauer, M. Distribution, Detection and Genetic Background of Herbicide-Resistant Alopecurus myosuroides (Huds.) in Germany. Ph.D. Dissertation, Universität Hohenheim, Stuttgart, Germany, 2015.
30. Preston, C. Herbicide resistance in weeds endowed by enhanced detoxification: Complications for management. *Weed Sci.* **2004**, *52*, 448–453. [CrossRef]
31. Ghanizadeh, H.; Harrington, K.C.; James, T.K.; Woolley, D.J. A quick test using seeds for detecting dicamba resistance in fathen (*Chenopodium album*). *Aust. J. Crop Sci.* **2015**, *9*, 337–343.
32. Richter, J.; Powles, S.B. Pollen Expression of Herbicide Target Site Resistance Genes in Annual Ryegrass (*Lolium rigidum*). *Plant Physiol.* **1993**, *102*, 1037–1041. [CrossRef]
33. Letouze, A.; Gasquez, J. A pollen test to detect ACCase target-site resistance within Alopecurus myosuroides populations. *Weed Res.* **2000**, *40*, 151–162. [CrossRef]
34. Cirujeda, A.; Recasens, J.; Taberner, A. A qualitative quick-test for detection of herbicide resistance to tribenuron-methyl in Papaver rhoeas. *Weed Res.* **2001**, *41*, 523–534. [CrossRef]
35. Cirujeda, A.; Taberner, A. A qualitative quick-test for the detection of herbicide resistance towards tribenuron-methyl in *Papaver rhoeas* L. on agar medium. In Proceedings of the Actas Congreso 2001 Sociedad Española de Malherbología, León, Spain, 20–22 November 2001.
36. Concenco, G.; Melo, P.T.B.S.; Andres, A.; Ferreira, E.A.; Galon, L.; Ferreira, F.A.; Silva, A.A. A quick method for detecting resistance of *Echinochloa* spp. to quinclorac. *Planta Daninha* **2008**, *26*, 429–437.
37. Abdurruhman, A.M.; Uygur, S.; Mathiassen, S.K.; Uygur, N. Identification of sterile wild oat (*Avena sterilis* L.) resistance to acetolactate synthase (ALS)-inhibiting herbicides using different assay techniques. *J. Plant Prot. Res.* **2020**, *60*, 244–252.
38. Zhang, C.J.; Lim, S.H.; Kim, J.W.; Nah, G.; Fischer, A.; Kim, D.S. Leaf chlorophyll fluorescence discriminates herbicide resistance in Echinochloa species. *Weed Res.* **2016**, *56*, 424–433. [CrossRef]
39. Zhang, C.-J.; Kim, D.-S. Using Leaf Chlorophyll Fluorescence for In-Season Diagnosing Herbicide Resistance in Echinochloa Species at Reproductive Growth Stage. *Plant Prot. Sci.* **2018**, *54*, 194–202. [CrossRef]
40. Linn, A.I.; Kosnarova, P.; Soukup, J.; Gerhards, R. Detecting herbicide-resistant Apera spica-venti with a chlorophyll fluorescence agar test. *Plant Soil Environ.* **2018**, *64*, 386–392. [CrossRef]
41. Wang, P.; Peteinatos, G.; Li, H.; Gerhards, R. Rapid in-season detection of herbicide resistant Alopecurus myosuroides using a mobile fluorescence imaging sensor. *Crop Prot.* **2016**, *89*, 170–177. [CrossRef]
42. Kaiser, Y.I.; Menegat, A.; Gerhards, R. Chlorophyll fluorescence imaging: A new method for rapid detection of herbicide resistance in Alopecurus myosuroides. *Weed Res.* **2013**, *53*, 399–406. [CrossRef]
43. Zhang, T.-J.; Feng, L.; Tian, X.-S.; Yang, C.-H.; Gao, J.-D. Use of chlorophyll fluorescence and P700 absorbance to rapidly detect glyphosate resistance in goosegrass (*Eleusine indica*). *J. Integr. Agric.* **2015**, *14*, 714–723. [CrossRef]
44. Shaner, D.L.; Nadler-Hassar, T.; Henry, W.B.; Koger, C.H. A rapid in vivo shikimate accumulation assay with excised leaf discs. *Weed Sci.* **2005**, *53*, 769–774. [CrossRef]
45. Pan, L.; Li, J.; Xia, W.; Zhang, D.; Dong, L. An effective method, composed of LAMP and dCAPS, to detect different mutations in fenoxaprop-P-ethyl-resistant American sloughgrass (*Beckmannia syzigachne* Steud.) populations. *Pestic. Biochem. Physiol.* **2015**, *117*, 1–8. [CrossRef] [PubMed]
46. Pan, L.; Li, J.; Zhang, W.-N.; Dong, L. Detection of the I1781L mutation in fenoxaprop-p-ethyl-resistant American sloughgrass (*Beckmannia syzigachne* Steud.), based on the loop-mediated isothermal amplification method. *Pest Manag. Sci.* **2015**, *71*, 123–130. [CrossRef]
47. Panozzo, S.; Farinati, S.; Sattin, M.; Scarabel, L. Can allele-specific loop-mediated isothermal amplification be used for rapid detection of target-site herbicide resistance in *Lolium* spp.? *Plant Methods* **2023**, *19*, 14. [CrossRef] [PubMed]
48. Ghanizadeh, H.; Griffiths, A.G.; Buddenhagen, C.E.; Anderson, C.B.; Harrington, K.C. A PCR plus restriction enzyme-based technique for detecting target-enzyme mutations at position Pro-106 in glyphosate-resistant *Lolium perenne* . *PLoS ONE* **2021**, *16*, e0246028. [CrossRef] [PubMed]
49. Cao, J.; Wu, Q.; Wan, F.; Guo, J.; Wang, R. Reliable and rapid identification of glyphosate-resistance in the invasive weed Amaranthus palmeri in China. *Pest Manag. Sci.* **2022**, *78*, 2173–2182. [CrossRef]
50. Alarcon-Reverte, R.; Hanley, S.; Kaundun, S.S.; Karp, A.; Moss, S.R. A SNaPshot assay for the rapid and simple detection of known point mutations conferring resistance to ACCase-inhibiting herbicides in *Lolium* spp. *Weed Res.* **2013**, *53*, 12–20. [CrossRef]
51. Benevenuto, J.; Bhakta, M.; Lohr, D.A.; Ferrao, L.F.V.; Resende, M.F.R., Jr.; Kirst, M.; Quesenberry, K.; Munoz, P. Cost-effective detection of genome-wide signatures for 2,4-D herbicide resistance adaptation in red clover. *Sci. Rep.* **2019**, *9*, 20037. [CrossRef]
52. Delye, C.; Causse, R.; Gautier, V.; Poncet, C.; Michel, S. Using next-generation sequencing to detect mutations endowing resistance to pesticides: Application to acetolactate-synthase (ALS)-based resistance in barnyard grass, a polyploid grass weed. *Pest Manag. Sci.* **2015**, *71*, 675–685. [CrossRef]

53. Petit, C.; Bay, G.; Pernin, F.; Delye, C. Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. *Pest Manag. Sci.* **2010**, *66*, 168–177. [CrossRef]
54. Yoon, A.M.; Kim, I.R.; Choi, W. Monoclonal antibody production for CP4 EPSPS detection assays. *Korean J. Environ. Biol.* **2021**, *39*, 445–451. [CrossRef]
55. Jones, E.A.L.; Austin, R.; Dunne, J.C.; Cahoon, C.W.; Jennings, K.M.; Leon, R.G.; Everman, W.J. Utilization of image-based spectral reflectance to detect herbicide resistance in glufosinate-resistant and glufosinate-susceptible plants: A proof of concept. *Weed Sci.* **2022**, 1–11. [CrossRef]