agronomy

MDPI

Article

Resistance Realized Heritability and Fitness Cost of Cyproflanilide in Rice Stem Borer, *Chilo suppressalis* (Lepidoptera: Pyralidae)

Kexin Zhang [1], Enling Zhan [1], Xiaoli Chang [2], Eman Atef Fouad [3] and Chunqing Zhao [1,*]

[1] Key Laboratory of Integrated Pest Management on Crops in East China, Ministry of Agriculture, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China; 2020102107@njau.edu.cn (K.Z.); 2019202033@njau.edu.cn (E.Z.)
[2] Eco-Environmental Protection Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai Engineering Research Centre of Low-Carbon Agriculture, Shanghai 201403, China; xlchang981@126.com
[3] Department of Bioassay, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Giza 12618, Egypt; emansoliman28@hotmail.com
* Correspondence: zcq@njau.edu.cn; Tel.: +86-025-84399025; Fax: +86-025-84399063

Abstract: The rice stem borer (RSB) *Chilo suppressalis* is a devastating rice pest with resistance to a number of insecticides. Recently, the new meta-diamide insecticide cyproflanilide has been considered an effective insecticide to control RSB. However, its resistance risk has not been reported. In the present study, we aimed to assess the resistance risk and evaluate the fitness cost after the RSB was exposed to cyproflanilide. After five generations of selection, the resistance level of RSB increased by 1.5-fold. When h^2 was 0.125, a 10-fold resistance increase in the LD_{50} values was expected in fourteen and thirty-one generations at the selection intensity of 90% and 50%, respectively. The selected population (RSB-SEL) had significant differences in the developmental duration of eggs, 1st, 2nd, 3rd, and 6th instar larvae, and female pupae compared to the unselected population (RSB-UNSEL). Besides, the adult longevity was shortened, and the average pupal weight of males, the emergence rate, the sex ratio, the oviposition, the mean fecundity, and the full life cycle rate were decreased in RSB-SEL. The intrinsic rate of increase (r), the net reproductive rate (R_0), and the finite rate of increase (λ) of RSB-SEL were significantly lower than those of RSB-UNSEL, while the mean generation time (T) of RSB-SEL was significantly longer than that of RSB-UNSEL. Based on the results of the prediction of the generations required for a 10-fold resistance increase in the LD_{50}, a potential risk of resistance development exists in RSB after continuous and excessive use of cyproflanilide. These results will be useful in designing the dose of cyproflanilide to control *C. suppressalis* in field.

Keywords: GABA receptor; RDL; pest insect of rice; resistance risk; heritability; fitness cost

check for updates

Citation: Zhang, K.; Zhan, E.; Chang, X.; Fouad, E.A.; Zhao, C. Resistance Realized Heritability and Fitness Cost of Cyproflanilide in Rice Stem Borer, *Chilo suppressalis* (Lepidoptera: Pyralidae). *Agronomy* **2024**, *14*, 2249. https://doi.org/10.3390/agronomy14102249

Academic Editor: Christos Athanassiou

Received: 28 August 2024
Revised: 25 September 2024
Accepted: 25 September 2024
Published: 29 September 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The rice stem borer (RSB), *Chilo suppressalis* Walker, is one of the omnivorous pests of rice cultivation. Its larvae burrow into the stalks and lead to crop reductions and economic losses in China [1]. Among the five rice-producing regions of China, including Central China, South China, Southwest China, Northeast China, and North China, the average annual occurrence area of RSBs in the Central China region was the largest, reaching more than 9.0 million hm [2], and the yield loss reached 325,500 tons from 2010 to 2020 [1].

To date, usage of insecticides is still the main method for controlling RSB [1–3]. However, RSB has generated high resistance to the currently used insecticides, including flubendiamide, chlorantraniliprole, avermectin, triazophos, and monosultap [4,5]. Therefore, it is urgent to introduce efficient, novel, and green insecticides to solve its resistance problem. Cyproflanilide is a novel meta-diamide insecticide and has excellent insecticidal activity against Lepidoptera (e.g., RSB), Coleoptera, and Thysanoptera pests [6,7]. It is classified

into Group 30, in the γ-aminobutyric acid (GABA)-gated chloride channel category, by the Insecticide Resistance Action Committee (IRAC) [6], and its insecticidal mechanism was predicted to be broflanilide [6]. So far, CAC International (https://www.cacch.com/ (accessed on 12 July 2024)), the inventor of cyproflanilide, has registered it in China and is vigorously promoting cyproflanilide to be marketed for controlling RSB. However, the potential resistant risk of RSB to cyproflanilide has not been reported.

The estimation of realized heritability and its related fitness cost would be useful for understanding and managing the evolution of resistance [8,9]. In 1994, Tabashnik and Mcgaughey proposed the threshold trait analysis method to assess realized heritability (h^2) based on the short-term insecticide selection results in the laboratory population, to clarify the frequency distribution of resistance genes in the field population, and to predict the developmental trend of the corresponding insecticide resistance in the field population [10]. It was easier to detect resistance changes by short-term (four to six generations) selection of different field populations than by long-term (more than ten generations) selection of a single population [10]. To date, the threshold trait analysis method has been used in the assessment of the resistance of insects against a number of insecticides, for example, spotted-wing drosophila *Drosophila suzukii* (Matsumura) against spinosad and malathion [11], small brown planthopper *Laodelphax striatellus* (Fallén) against pymetrozine [12], tarnished plant bug *Lygus pratensis* (L.) against lambda-cyhalothrin [13], and RSB against fipronil, triazophos, and methoxyfenozide [14–16].

While realized heritability indicates the potential genetic variation of resistance in insects [8], the fitness cost has the potential to predict the development of insecticide resistance based on phenotypic variation [17]. In general, the insect development of resistance to insecticides is always accompanied by a fitness cost, e.g., the high energetic cost or significant disadvantage of population growth, the fecundity decrease, and the extension in the mean generation time, which diminishes its fitness compared with its susceptible counterparts in the population [9,18,19]. The fitness cost caused by resistance to insecticide has been reported on different pest species, e.g., cotton aphid *Aphis gossypii* Glover [20], brown planthopper *Nilaparvata lugens* (Stål) [21], diamondback moth *Plutella xylostella* L. [22], fall armyworm *Spodoptera frugiperda* (Smith, J.E.) [23,24], and house fly *Musca domestica* L. [17]. In Lepidoptera, resistance and the consequent detrimental impact on its biological fitness to *Bacillus thuringiensis* toxins and insecticides, e.g., benzoylureas and pyrethroids, have been reported [9]. Identifying the fitness cost as a result of resistance to any insecticide can be an advantage in designing an integrated pest management (IPM) program for limiting the spread of the resistant population [9]. When the fitness cost is higher, the time needed for resistant individuals to spread in the population is longer; thus, the fitness cost is an important factor in estimating insecticide use for resistance management [9].

Generally, the fitness cost is determined by a life table [18]. The physiological and reproductive changes of pests to adapt and survive can be obtained by comparing life table parameters between susceptible and resistant populations [25]. In general, the selected life table parameters include the developmental duration of eggs, larvae, pupae, and adults; the adult lifespan; fecundity; and the reproductive potential [18]. For example, the resistant strains of *S. frugiperda* against chlorpyriphos have significantly changed life table parameters, e.g., prolonged developmental duration and decreased pupal weight, survival, and fecundity [26]. Based on the related life table parameters, we can get the relative fitness (R_f), which is a value indicating the differentiation of genotypes in resistant insects affected by insecticides [18].

Hence, the resistance risk of RSB to cyproflanilide will be predicted by the change of LD_{50} and resistance ratios after resistance selections and evaluated by the relative fitness with the fitness cost after selections.

2. Materials and Methods

2.1. Insects and Chemicals

The laboratory population (RSB-LAB) of RSB was collected from Ruichang County, Jiujiang City, Jiangxi Province, and continuously reared for over 150 generations without exposure to any insecticide in the lab provided by Professor Guanghua Luo (Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China). The RSB-LAB was regarded as the unselected population (RSB-UNSEL) in the present study. The RSBs were kept in the insect room at 27 ± 1 °C under a photoperiod of 16L/8D and with a relative humidity of 60–80%. Larvae were fed with an artificial diet (Table S1) [27]. Adults were fed 10% (*v*/*v*) honey water as a nutritional supplement, and fresh rice seedlings were provided for laying eggs. Cyproflanilide (CAS no. 2375110-88-4; technical grade with purity ≥ 99%) was provided by CAC Nantong Chemical Co., Ltd. (Nantong, Jiangsu, China). Acetone was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Bioassay

The insecticidal activity of cyproflanilide in RSB was measured by the topical application method [28,29]. The insecticide was first dissolved in acetone to create the stock solution (10,000 mg/L) and then diluted with acetone to give 7 serial concentration solutions in equal proportions (acetone was used as control). According to the Chinese Agricultural Standard (NYT2058-2011, [29]), the 4th instar larvae of RSB (6–9 mg/larva) were selected for topical application using an auto micro-applicator (PDE0006) (Burkard Manufacturing Co., Ltd., Rickmansworth, England, UK) and a micro-syringe (50 µL) (Shanghai Gaoge Industry and Trade Co., Ltd., Shanghai, China), and 0.04 µL of cyproflanilide solution was applied on the back of the anterior pectoralis of larvae. Ten 4th instar larvae as one replicate were treated with serial concentrations of cyproflanilide and then placed into a 9 cm diameter petri dish with an artificial diet. Three replicates were performed for each concentration. Mortality was recorded at 72 h after treatment.

If a larva did not respond after being gently pushed with a brush, was not able to recover within 1 min after inversion, or the body color became black and wrinkled and the body length was shorter than half of the control, the larva was considered dead.

2.3. Selection of RSB Population Using Cyproflanilide

The selected RSB population (RSB-SEL) was selected from RSB-UNSEL by the topical application method in the laboratory. The RSB in the F0 to F5 generations were discontinuously selected with cyproflanilide. For each generation, the 60% lethal dose (LD_{60}) of cyproflanilide to RSB was firstly determined by the topical application method mentioned in Section 2.2, and the remaining RSB larvae were treated with the LD_{60} cyproflanilide to select those resistant to cyproflanilide. The mortality was recorded at 72 h after treatment, and the survival larvae were reared to the next generation. If the survival RSBs were too few to generate sufficient next generation, the selection assay was skipped in the current generation and performed in the next generation. The h^2 of RSB to cyproflanilide was estimated using the threshold trait analysis method [10], the formulas are mentioned below:

(1) R = log (final LD_{50} − initial LD_{50})/n (n was the number of generations)

(2) $S = i\sigma_p$

(3) i = 1.583 − 0.0193336P + 0.000048P2 + 3.65194/P ($10 \leq P \leq 80$, i was intensity of selection)

(4) $\sigma_p = \left(\frac{1}{n}\sum_{i=1}^{n} b_i\right)$

The number of generations required for a 10-fold increase in LD_{50} at 50–90% selection intensity was calculated.

2.4. Evaluation of Fitness Cost

The growth and developmental parameters of the RSB population were observed and calculated according to Wu et al. (1980) [30] and Jiang (2011) [16]. Male and female pupae

(ratio 1.5:1, which was distinguished according to Figure S1) were randomly selected and placed in a breeding cage with fresh rice seedlings (10–18 d of rice growing period), which were used for mating and oviposition after eclosion. Two hundred eggs were randomly selected to determine the duration of the egg and the hatching rate. Subsequently, 150 larvae were randomly selected, transferred into a finger tube, and fed with an artificial diet. The artificial diet was changed every four days. The larval instar, the pupal stage, the pupation rate, the pupal weight, the male/female ratio, and the emergence rate were recorded every day. Then, three male and one female adults, which emerged on the same day, were mated and put into a disposable plastic cup (490 mL) with an absorbent cotton ball soaked in 10% (v/v) honey water for adult nutrition and a folded ridge of sulfate paper (length × width: 7.5 mm × 7.5 mm) for laying eggs. The survival, the oviposition period, and the fecundity of adults were recorded every day.

2.5. Statistical Analysis

The bioassay data were processed using IBM SPSS Statistics 22.0 software (IBM, Armonk, NY, USA) to calculate the regression equation, the median lethal dose (LD_{50}), the 95% confidence interval (95% CI), the Chi-square value (χ^2), and the correlation coefficient (R^2). The resistance ratio (RR) was determined by the ratio of LD_{50} between the RSB-SEL and the RSB-UNSEL. The resistance level was classified as follows [29]: sensitive level (RR < 3.0), decreased sensitivity (3.0 ≤ RR < 5.0), low-level resistance (5.0 ≤ RR < 10.0), moderate resistance (10.0 ≤ RR < 40.0), high-level resistance (40.0 ≤ RR < 160.0), and very-high-level resistance (RR ≥ 160.0).

Life tables were constructed based on different parameters, including the age-stage specific survival rate (s_{xj}), the age-specific fecundity of females ($f_{x,\,female}$), the age-specific survival rate (l_x), the age-specific fecundity (m_x), the age-specific maternity ($l_x m_x$), the age-stage specific life expectancy (e_{xj}), the intrinsic rate of increase (r), the finite rate (λ), the net reproductive rate (R_0), and the mean generation time (T) calculated using the TWOSEX-MS Chart program. The parameters were calculated by the formula below [31–33]:

(1) The age-stage specific survival rate (s_{xj}) is the probability that a newly-hatched individual will survive to age x and stage j.

(2) The l_x is the probability that an egg will survive to age x.

$$l_x = \sum_{j-1}^{m} s_{xj}$$

(3) $m_x = \frac{\sum_{j-1}^{m} s_{xj} f_{xj}}{\sum_{j=1}^{m} s_{xj}}$

(4) $\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$

(5) The age-stage specific life expectancy (e_{xj}) represents the survival probability of an individual of age x and stage j.

$$e_{xj} = \sum_{i=x}^{n} \sum_{j=y}^{m} s\prime_{xj}$$

(6) $\lambda = e^r$

(7) $R_0 = \sum_{x=0}^{\infty} l_x m_x$

(8) $T = \frac{\ln(R_0)}{r}$

The means and SEs for the life table parameters were calculated by paired bootstrap tests. The means and SE of pupa weight and pupation rate were calculated by IBM SPSS Statistics 22.0 software. The F value and the degree of freedom of the life table parameters were performed by independent sample t-test in IBM SPSS Statistics 22.0 software. Graphics were produced using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA).

According to the Chinese Agricultural Standard (NY/T1859.5-2014) [34], the resistance risk of a new insecticide is classified into the following three levels: if there is a usage history of similar insecticides and development of resistance, the new insecticide has a $h^2 \geq 0.2$, and after selection, the population fitness was at least not lower than that without selection and was not significantly reduced after the sublethal dose treatment, this new insecticide will be considered as a high-risk insecticide; if there is a history of similar insecticides, the new insecticide has a $0.1 \leq h^2 < 0.2$, and the fitness of the selected population is at least not lower than that without selection, and the fitness of the sublethal dose treatment is not significantly reduced, this new insecticide will be considered as a medium-risk insecticide; if there was no usage history of similar insecticides, the new insecticide has a $h^2 < 0.1$, the fitness of the population after selecting was significantly lower than that without selecting, and the fitness of the sublethal dose treatment was significantly reduced, this new insecticide will be considered as a low-risk insecticide.

3. Results

3.1. Selection of RSB with Cyproflanilide

According to the topical application method, the LD_{50} of cyproflanilide for the 4th RSB larvae in the F0 generation was 0.424 ng/larva, and increased to 0.636 ng/larva after selection for five generations (Table 1). The LD_{50} of F3, F4, and F5 increased, but their resistance ratios were less than 2 (Table 1), which indicated that no resistance of RSB against cyproflanilide happened after selection.

Table 1. Toxicity of cyproflanilide to 4th instar larvae of rice stem borer after 72 h treatment.

Generation	LD_{50} (95% CI) ng/Larva	R^2	χ^2	Regression Equation	Resistance Ratio
F0	0.424 (0.344–0.524)	0.926	4.786	Y = 2.56x + 0.94	-
F1 *	-	-	-	-	-
F2 *	-	-	-	-	-
F3	0.541 (0.373–0.726)	0.996	0.221	Y = 2.26x + 0.61	1.276
F4	0.552 (0.452–0.654)	0.930	1.719	Y = 3.42x + 0.97	1.302
F5	0.636 (0.485–0.802)	0.993	1.359	Y = 2.53x + 0.48	1.500

F1 was obtained by F0 treated with cyproflanilide. - F1 generation was not screened for resistance and not tested for bioassay, and F2 generation was screened for resistance but not tested for bioassay. * The LD_{50} of F1 and F2 was not examined, because the number of rice stem borer larvae in F1 and F2 after treatment with cyproflanilide was not enough.

3.2. Estimation of Realized Heritability (h^2)

The overall mean h^2 of resistance against cyproflanilide in RSB was 0.125, which was less than 0.2, meaning that the level of resistance development to cyproflanilide in RSB was not high (Table 2). The rate of resistance development to cyproflanilide in RSB was inversely proportional to h^2 and the selection intensity (Figure 1A). The mean slope (3.132) and h^2 (0.125) indicated that fourteen and thirty-one generations would be required for a 10-fold increase in LD_{50} at 90% and 50% selection intensity (Figure 1B).

Table 2. Estimation of realized heritability (h^2) of resistance to cyproflanilide in rice stem borer.

Mean Selection Response per Generation			Mean Selection Differential per Generation					
Initial LD_{50}	Final LD_{50}	Selection Response (*R*)	Mean Survival Percentage Generation (P%)	Intensity of Selection (i)	Mean Slope	Phenotypic Standard Deviation (σ_p)	Selection Differential (*S*)	h^2
0.424	0.636	0.0424	35.53	1.059	3.132	0.319	0.338	0.125

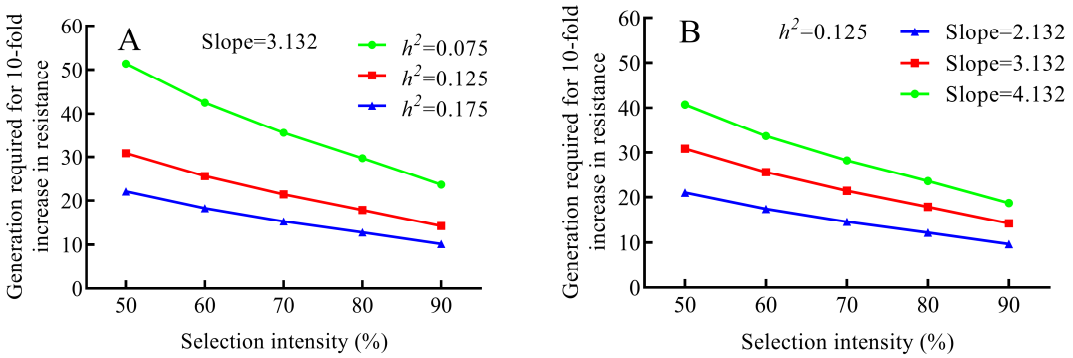

Figure 1. Effect of different realized heritability h^2 (**A**) and slope (**B**) values on the number of generations required for a 10-fold increase in LD_{50} of *C. suppressalis* to cyproflanilide at different selection intensities.

3.3. Effects of Cyproflanilide Selection on the RSB Life Cycle

Cyproflanilide had different effects on the development stages of RSBs. The 1st instar larvae, 6th instar larvae, and female pupae of RSB-SEL significantly prolonged (Table 3). In contrast, the developmental duration of the eggs, 2nd instar larvae, and 3rd instar larvae of RSB-SEL were significantly shorter than the respective stages of RSB-UNSEL (Table 3). No significant difference was found between RSB-UNSEL and RSB-SEL in the 4th instar larvae, male pupae, and total larval developmental duration (Table 3).

Table 3. Developmental duration of egg, larvae, and pupae in RSB-SEL and RSB-UNSEL population.

Developmental Stages	Duration (Day)		*F*	*df*	*P*
	RSB-UNSEL (Mean ± SE)	RSB-SEL (Mean ± SE)			
Egg	5.00 ± 0.022 a	4.18 ± 0.327 b	214.77	1 (298)	0.012
1st instar	3.66 ± 0.066 a	3.97 ± 0.317 b	15.225	1 (298)	0.008
2nd instar	3.14 ± 0.070 a	2.78 ± 0.229 b	0.879	1 (255)	0.012
3rd instar	3.12 ± 0.072 a	2.82 ± 0.235 b	8.854	1 (244)	0.017
4th instar	3.57 ± 0.086 a	3.58 ± 0.289 a	1.907	1 (237)	0.906
5th instar	5.63 ± 0.116 a	4.92 ± 0.389 b	37.640	1 (233)	0.012
6th instar	13.49 ± 0.569 a	15.61 ± 1.317 b	11.604	1 (231)	0.013
Total larva	32.29 ± 0.598 a	33.57 ± 0.618 a	13.101	1 (298)	0.139
Female pupa	6.24 ± 0.136 a	6.96 ± 0.556 b	2.313	1 (58)	0.006
Male pupa	6.69 ± 0.116 a	6.97 ± 0.558 a	10.295	1 (73)	0.173

Values are mean ± SE, different lower-case letters in the same column indicate significant differences ($P < 0.05$).

Compared with RSB-UNSEL, the average weight of male pupae of RSB-SEL significantly decreased from 43.43 mg to 37.28 mg (Table 4). The parameters of pupation rate, pupal weight of female, emergence rate, sex ratio, oviposition, mean fecundity, and complete full life cycle rate in RSB-SEL were decreased compared to RSB-UNSEL, and the adult longevity in RSB-SEL was shorter RSB-UNSEL (Table 4).

Table 4. The survival of pupa and adult, female oviposition, and complete life cycle rate of RSB-UNSEL population and RSB-SEL population.

Parameters	RSB-UNSEL (Mean ± SE)	RSB-SEL (Mean ± SE)	*F*	*df*	*P*
Pupation rate (%)	48.67 ± 4.667 a	46.00 ± 7.856 a	5.783	1 (28)	0.773
Pupal weight of female (mg)	50.06 ± 2.010 a	45.77 ± 2.112 a	2.026	1 (58)	0.155
Pupal weight of male (mg)	43.43 ± 1.435 a	37.28 ± 1.014 b	0.962	1 (73)	0.001
Emergence rate (%)	42.00 ± 4.598 a	36.67 ± 7.082 a	7.408	1 (28)	0.534

Table 4. *Cont.*

Parameters	RSB-UNSEL (Mean ± SE)	RSB-SEL (Mean ± SE)	*F*	*df*	*P*
Sex ratio (%)	57.40 ± 7.541 a	48.90 ± 9.674 a	1.720	1 (28)	0.060
Female longevity (day)	3.47 ± 0.287 a	2.96 ± 0.353 a	1.256	1 (54)	0.188
Male longevity (day)	3.79 ± 0.319 a	3.06 ± 0.345 a	3.304	1 (59)	0.076
Oviposition (day)	2.00 ± 4.152 a	1.00 ± 5.024 a	2.291	1 (28)	0.680
Mean fecundity (egg female^{-1})	30.94 ± 9.671 a	11.74 ± 5.356 a	6.384	1(28)	0.084
Complete full life cycle rate (%)	21.33 ± 5.243 a	18.00 ± 4.899 a	0.099	1(28)	0.646

Values are mean ± SE, different lower-case letters in the same column indicate significant differences ($P < 0.05$).

3.4. Effects of Cyproflanilide Selection on the RSB Life Table and Life Expectancy

As shown in Table 5, the r, R_0, and λ of RSB-SEL were 0.013, 1.80, and 1.01, respectively, significantly lower than those of RSB-UNSEL (0.045, 7.01, and 1.04, respectively). The T of RSB-SEL (46.60 d) was significantly longer than that of RSB-UNSEL (42.96 d). The R_f, calculated from R_0 of RSB-SEL relative to the RSB-UNSEL was 0.257 (Table 5).

Table 5. Life table parameters of RSB-UNSEL population and RSB-SEL population.

Parameters	RSB-UNSEL (Mean ± SE)	RSB-SEL (Mean ± SE)	*P*
λ (day^{-1})	1.04 ± 0.009 a	1.01 ± 0.012 b	0.039
R_0 (offspring/individual)	7.01 ± 2.414 a	1.80 ± 0.887 b	0.042
r (day^{-1})	0.045 ± 0.009 a	0.013 ± 0.013 b	0.035
T (day)	42.96 ± 0.943 a	46.60 ± 0.720 b	0.006
R_f	-	0.257	-

Values are mean ± SE, different lowercase letters in the same column indicate significant differences ($P < 0.05$).

The s_{xj} curves had clear overlapping regions in the developmental duration of RSB-UNSEL and RSB-SEL (Figure 2). However, RSB-UNSEL and RSB-SEL curves were different between the larval stage and the adult stage. Specifically, the survival rates in the larval stage and the adult stage of RSB-SEL, especially in 2nd, 3rd, and 4th instar larvae, which were 72.00%, 60.00%, and 56.67%, respectively, were significantly lower than in RSB-UNSEL (87.33%, 78.67% and 75.33%, respectively) (Figure 2).

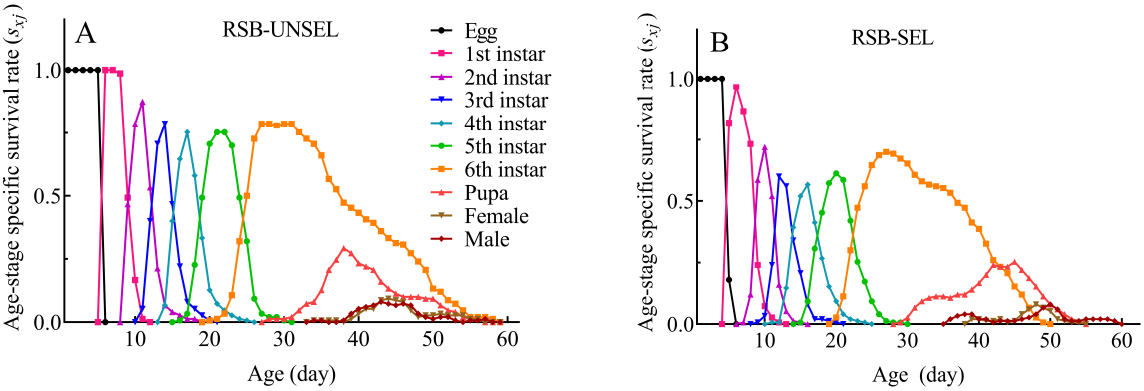

Figure 2. Age-stage specific survival rate (s_{xj}) of RSB-UNSEL (**A**) and RSB-SEL (**B**).

The l_x values of RSB-SEL were lower than those of RSB-UNSEL (Figure 3). However, three peaks on the 38th, 42nd, and 53rd day were displayed in the $f_{x,\,female}$ curves of RSB-UNSEL, while only one peak on the 45th day was found in RSB-SEL, but the highest fecundity peak values of $f_{x,\,female}$ in RSB-SEL appeared in the 44th day, later than in RSB-UNSEL, in which they appeared in the 39th day. Additionally, the values of the age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) also showed similar trends to $f_{x,\,female}$. Of

note, the values of $f_{x,\ female}$, m_x, and $l_x m_x$ in RSB-SEL appeared during 4 days, shorter than RSB-UNSEL (Figure 3).

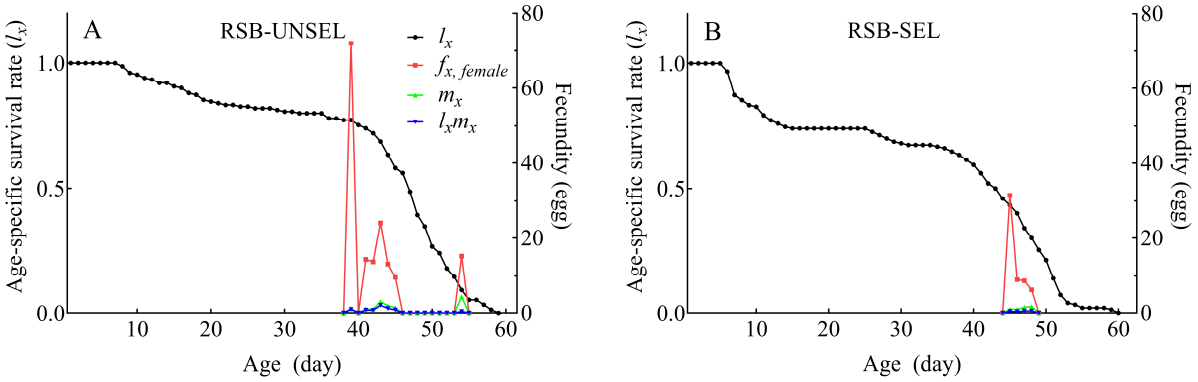

Figure 3. Age-specific survival rate (l_x), age-specific fecundity of female ($f_{x,\ female}$), age-specific fecundity of total population (m_x), and age-specific maternity ($l_x m_x$) of RSB-UNSEL (**A**) and RSB-SEL (**B**).

As shown in Figure 4, the value of e_{xj} in RSB-SEL was lower than in RSB-UNSEL, and the day on which the e_{xj} values appeared in every development stage of RSB-SEL was earlier than RSB-UNSEL. The maximum values of e_{xj} appeared in the egg stage (41.21) of RSB-UNSEL, whereas they appeared in the 2nd instar larvae (36.58) of RSB-SEL. Intriguingly, the e_{xj} value of the male in RSB-SEL first appeared on the 35th day, 3 days earlier than the female value, even though the first e_{xj} value of the male and female in RSB-UNSEL appeared on the same day (the 33rd day), earlier than in RSB-SEL (Figure 4).

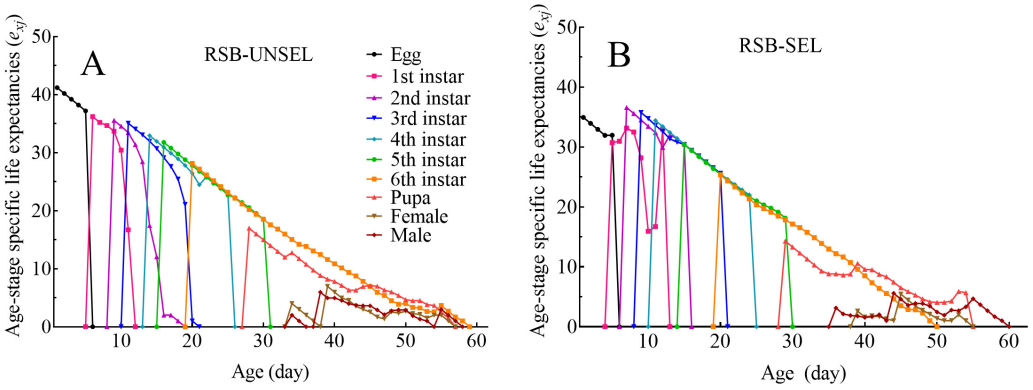

Figure 4. Age-stage specific life expectancies (e_{xj}) of RSB-UNSEL (**A**) and RSB-SEL (**B**).

4. Discussion

To date, the usage of chemical insecticides is still the most effective method for managing agricultural pests; thus, understanding the resistance development to new chemical insecticides in target pests is important to determine its reasonable application guidance. Cyproflanilide has high insecticidal activity against Lepidoptera, with a LC_{50} to *S. frugiperda* and *P. xylostella* of 1.61 mg/L and 0.056 mg/L, respectively [35,36]. Liu et al. (2020) reported that the cyproflanilide has excellent insecticidal activity against RSB, with a LC_{50} of 0.453 mg/L [7], a result that is equal to that of this study (Table 1). Therefore, cyproflanilide would be one potential choice for controlling RSB, but its resistance risk cannot be ignored.

After RSB was discontinuously exposed to cyproflanilide for five generations, the RR of RSB was only 1.5-fold, indicating that the resistance development was not rapid in five generations. Similarly, the RR of lepidopteron to meta-diamide insecticides is low after selection for ten generations. The RR of *P. xylostella* and *S. frugiperda* was 1.4- and 1.71-fold,

respectively, after being selected with broflanilide, another novel meta-diamide insecticide, for ten generations [37,38]. This may be caused by the short usage history of meta-diamide insecticides, which was consistent with the results of the h^2 in this study.

The development rate of resistance to cyproflanilide was slow in RSB (Tables 1 and 2, Figure 1). The additive genetic variation in h^2 of resistance is a function of the corresponding allele frequency in the population [10]. When the allele frequency tends to zero, h^2 will tend to zero, indicating that the resistance genes in the population tend to be sensitive [15]. When the allele frequency tends to 0.5, the additive genetic variation ratio is higher, so h^2 is also larger [39]. In this study, the h^2 of RSB selected after five generations with cyproflanilide (h^2 = 0.125) was lower than that of RSB selected after six generations with triazophos (h^2 = 0.4835) [15] and after seven generations with fipronil (h^2 = 0.3388) [15], indicating that there was a low allele frequency of resistance genes in RSB-UNSEL.

Under selection with cyproflanilide, a significant fitness cost appeared in the development of RSB. After exposure to insecticide, insects sometimes reduce toxicity through detoxification enzymes and, at the same time, change in development and reproduction to adapt to the environment [18]. It has been reported that the relative fitness of resistant insects can be reflected more comprehensively and scientifically by considering the parameters of population growth ability and developmental duration [18,40]. Therefore, the fitness cost of RSB after selection with cyproflanilide was evaluated, and the results showed that the duration of 1st instar larvae, 6th instar larvae, and pupae of RSB-SEL were significantly prolonged. Similarly, the duration of 6th instar larvae and female pupae of *S. frugiperda* was significantly prolonged after selection of ten generations with broflanilide [38], which has a similar structure to cyproflanilide [7]. Furthermore, lowered appetite, feeding disturbance, aberrant metabolism, stress of starving, or an imbalance between physiological development and metabolic detoxification could be reasons for the prolonged larval duration in insecticide exposure [41,42]. Meanwhile, prolonged larval duration may be helpful in the field management of RSB by raising the likelihood of natural predation and forcing neonate larvae to feed on foliage with poor nutritional value to complete their life cycle, which reduces fecundity and survival [41]. Conversely, the sulfoxaflor and clothianidin lead *Aphis gossypii* Glover to a shorter larval development period, which may be caused by the different expression levels of detoxification metabolism enzyme genes and their combined effect [43,44]. Similarly, the development stages of the 2nd and 3rd instar larvae RSB-SEL were significantly decreased in the present study (Table 3). Additionally, the pupal weight of males of RSB-SEL was significantly decreased after being selected with cyproflanilide. Pupation rate, pupal weight, and adult longevity are all important factors affecting the reproductive capacity of the population [18]. Thus, cyproflanilide could affect the development and reproductive capacity of RSB after selection.

In addition, the selection with cyproflanilide also changed the reproductive capacity of RSB. Compared with RSB-UNSEL, the λ, $R_{0,}$ and r of RSB-SEL were significantly lower, and the T of RSB-SEL was significantly extended, which was similar to the previous reports in *S. frugiperda* treated with fluxametamide [41] and broflanilide [38]. The decreased survival rate, the decreased R_0, r, and λ, and the prolonged T could result in a slowdown in the population dynamics of RSB after cyproflanilide selection. Moreover, the significantly reduced R_0 of RSB-SEL caused the relative fitness to be 0.257. Similarly, the values of relative fitness in *S. frugiperda* treated with fluxametamide or broflanilide, two insecticides targeting GABA receptors, were 0.353 and 0.35, respectively [38,41]. It has been reported that significant variation in most of the biological parameters of the insecticide-selected population is due to the trade-off between resistance development and fitness cost [45]. Such developmental asynchrony in insect populations could probably affect the evolution of resistance [46].

5. Conclusions

In summary, as a novel meta-diamide insecticide, cyproflanilide has shown excellent activity against larvae of RSB. After selected RSB with cyproflanilide during five

generations, the results of the estimated h^2 which was less than 0.2 and the prediction of generations required for a 10-fold increase in LD_{50} suggested that the development of resistance in RSB to cyproflanilide was slow during fourteen generations under the selection intensity of 90%. However, RSB displayed a fitness cost under cyproflanilide selection pressure. Our study indicated that cyproflanilide could be used as a new fast-acting insecticide to control RSB. Based on the prediction of generations required for a 10-fold increase in LD_{50}, continuous and excessive use of cyproflanilide will accelerate the development of resistance. Thus, it is necessary to design appropriate dosages and rotations with different insecticides to slow down the development of potential resistance, and to prolong the usage of cyproflanilide in the control of RSB. In this study, the continuous three generations used for building up resistance were not enough to make a judgment on resistance, however, able to predict the development of resistance in RSB to cyproflanilide, which will be useful to guide the usage of cyproflanilide and to design scientific resistance management strategy in the field.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14102249/s1, Table S1: Composition of the artificial food; Figure S1: The female and male pupae of *Chilo suppressalis*.

Author Contributions: Conceptualization, K.Z. and C.Z.; methodology, K.Z.; formal analysis, investigation, data curation, K.Z.; writing—original draft preparation, K.Z. and E.Z.; writing—review and editing, and visualization, K.Z., E.Z., X.C., E.A.F. and C.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the National Key R&D Program of China [grant number 2022YFD1400900] and Jiangsu Agricultural Science and Technology Independent Innovation Fund Project (grant number CX(24)3010).

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Acknowledgments: We would like to thank Guanghua Luo from Jiangsu Academy of Agricultural Sciences for providing insects and help. We would also like to thank Hsin Chi for providing the free software TWOSEX-MS Chart program, and Jueping Ni from CAC Nantong Chemical Co., Ltd. for providing the cyproflanilide sample.

Conflicts of Interest: The authors declare no competing financial interests.

Abbreviations

95% CI, 95% confidence interval; χ^2, Chi-square value; λ, finite rate; d, day; e_{xj}, age-stage specific life expectancy; $f_{x,\,female}$, age-specific fecundity of females; GABA: γ-aminobutyric acid; h^2, realized heritability; RSB, rice stem borer; RSB-LAB, the laboratory population of *Chilo suppressalis*; RSB-UNSEL, the unselected population of *Chilo suppressalis*; RSB-SEL, the selected population of *Chilo suppressalis*; LD_{50}, lethal median dose; LD_{60}, the 60% lethal dose; l_x, age-specific survival rate; $l_x m_x$, age-specific maternity; m_x, age-specific fecundity; r, intrinsic rate of increase; R, the mean response; R_0, net reproductive rate; R^2, correlation coefficient; R_f, relative fitness; RR, resistance ratio; S, the overall mean; SE, slope ± standard error; s_{xj}, age-stage survival rate; T, mean generation time.

References

1. Che, L.; Jiang, Q.H.; Wang, Y.; Li, C.G.; Yan, S. Comparative analysis of occurrence and control of pests in five rice producing regions in China. *Plant Prot.* **2022**, *48*, 233–241.
2. Wang, Q.W.; Ding, C.H.; Mo, C.A. Cause analysis and control measures of the outbreak of rice stem borer in Taojiang, Hunan Province in recent years. *China Plant Prot.* **2020**, *40*, 59–61.
3. Li, W.B.; Chen, Q.F.; Xie, W.; He, X.X.; Chen, J.Z.; Zheng, L.J.; He, H.; Duan, B.F.; Lu, X.L.; Zhang, D.Y.; et al. Analysis on the occurrence characteristics and control measures of rice stem borer in Hengyang, Hunan Province in recent years. *China Plant Prot.* **2018**, *38*, 48–52+77.
4. Sun, Y.; Hu, Y.Y.; Qin, W.J.; Wang, P.; Zhou, F.; Huang, J.S. Resistance of Asiatic rice borer *Chilo suppressalis* (Lepidoptera: Crambidae) population to four insecticides in Jiangxi Province from 2017 to 2019. *J. Plant Prot.* **2021**, *48*, 396–406.

5. Zhao, D.D.; Zhou, L.Q.; Zhang, S.; Yao, R.; Qiu, Y.X.; Gao, C.F. Resistance monitoring and cross-resistance to the diamides in the rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae). *Chin. J. Rice Sci.* **2017**, *31*, 307–314.
6. Tan, J.H. Gama-aminobutyric acid-gated chloride channel receptor and its target insecticides. *World Pestic.* **2022**, *44*, 38–53.
7. Liu, J.Y.; Zhou, L.Q.; Xiang, J.C.; Ni, J.P.; Li, Z.C.; Pang, H.L.; Lu, L. The R&D of cyproflanilide. *World Pestic.* **2020**, *43*, 34–38.
8. Tabashnik, B.E. Resistance risk assessment: Realized heritability of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae), tobacco budwornl (Lepidoptera: Noctuidae), and Colorado potato beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1992**, *85*, 1551–1559. [CrossRef]
9. Hina, G.; Gowda, G.B.; Ali, G.; Saniya, T.; Farman, U.; Nicolas, D.; Liu, X.X. Fitness costs of resistance to insecticides in insects. *Front. Physiol.* **2023**, *14*, 123811.
10. Tabashnik, B.E.; Mcgaughey, W.H. Resistance risk assessment for single and multiple insecticides: Responses of indianmeal moth (Lepidoptera: Pyralidae) to *Bacillus thuringiensis*. *J. Econ. Entomol.* **1994**, *87*, 834–841. [CrossRef]
11. Disi, J.O.; Sial, A.A. Laboratory selection and assessment of resistance risk in *Drosophila suzukii* (Diptera: Drosophilidae) to spinosad and malathion. *Insects* **2021**, *12*, 794. [CrossRef] [PubMed]
12. Ban, L.F.; Zhang, S.; Huang, Z.Y.; He, Y.P.; Peng, Y.Q.; Gao, C.F. Resistance monitoring and assessment of resistance risk to pymetrozine in *Laodelphax striatellus* (Hemiptera: Delphacidae). *J. Econ. Entomol.* **2012**, *105*, 2129–2135. [CrossRef] [PubMed]
13. Tan, Y.; Ma, Y.; Jia, B.; Rafael, A.H.; Martin, S.W.; Gao, S.J.; Han, H.B.; Xiang, K.F.; Sun, X.H.; Gao, X.; et al. Laboratory selection, cross-resistance, risk assessment to lambda-cyhalothrin resistance, and monitoring of insecticide resistance for plant bug *Lygus pratensis* (Hemiptera: Miridae) in farming-pastoral ecotones of northern China. *J. Econ. Entomol.* **2021**, *114*, 891–902. [CrossRef]
14. Liu, Y.; He, L.F.; Wang, S.C.; Yang, F.X.; Gao, C.F.; Wu, S.F. Resistance risk, cross resistance and sublethal effects of methoxyfenozide on rice stem borers (*Chilo suppressalis*). *Chin. J. Rice Sci.* **2023**, *37*, 417–435.
15. Cao, M.Z. Monitoring of Insecticide Resistance, Genetic Analysis of Triazophos and Monosultap Resistance and Resistance Risk Assessment for Fipronil in *Chilo suppressalis* Walker. Ph.D. Thesis, Nanjing Agricultural University, Nanjing, China, 2004.
16. Jiang, W.H. Study on Insecticide Resistance and Integrated Control of Rice Stem Borer, *Chilo suppressalis*. Ph.D. Thesis, Nanjing Agricultural University, Nanjing, China, 2011.
17. Alam, M.; Shah, R.M.; Shad, S.A.; Binyameen, M. Fitness cost, realized heritability and stability of resistance to spiromesifen in house fly, *Musca domestica* L. (Diptera: Muscidae). *Pestic. Biochem. Physiol.* **2020**, *168*, 104648. [CrossRef]
18. Wang, Z.Y.; Wang, W.F.; Miao, S.J.; Lu, Y.J. Progress in research on the relative fitness of insecticide-resistance insects. *Chin. J. Appl. Entomol.* **2021**, *58*, 487–496.
19. Guo, Y.J.; Hu, K.; Zhou, J.N.; Xie, Z.S.; Zhao, Y.J.; Zhao, S.Y.; Gu, J.B.; Zhou, X.H.; Yan, G.Y.; James, A.A.; et al. The dynamics of deltamethrin resistance evolution in *Aedes albopictus* has an impact on fitness and dengue virus type-2 vectorial capacity. *BMC Biol.* **2023**, *21*, 194. [CrossRef]
20. Ullah, F.; Gul, H.; Tariq, K.; Desneux, N.; Gao, X.; Song, D.L. Acetamiprid resistance and fitness costs of melon aphid, *Aphis gossypii*: An age-stage, two-sex life table study. *Pestic. Biochem. Physiol.* **2021**, *171*, 104729. [CrossRef]
21. Zhang, X.; Mao, K.; Liao, X.; He, B.; Jin, R.; Tang, T. Fitness cost of nitenpyram resistance in the brown planthopper *Nilaparvata lugens*. *J. Pest Sci.* **2018**, *91*, 1145–1151. [CrossRef]
22. Banazeer, A.; Afzal, M.B.; Hassan, S.; Ijaz, M.; Shad, S.A.; Serrão, J.E. Status of insecticide resistance in *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) from 1997 to 2019: Cross-resistance, genetics, biological costs, underlying mechanisms, and implications for management. *Phytoparasitica* **2021**, *50*, 465–485. [CrossRef]
23. Barbosa, M.G.; Andre, T.P.; Pontes, A.D.; Souza, S.A.; Oliveira, N.R.; Pastori, P.L. Insecticide rotation and adaptive fitness cost underlying insecticide resistance management for *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Neotrop. Entomol.* **2020**, *49*, 882–892. [CrossRef] [PubMed]
24. Elias, O.P.F.; Hideo, K.R.; Omoto, C.; Sartori, G.A. Fitness costs associated with chlorantraniliprole resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains with different genetic backgrounds. *Pest Manag. Sci.* **2022**, *78*, 1279–1286. [CrossRef] [PubMed]
25. Kliot, A.; Ghanim, M. Fitness costs associated with insecticide resistance. *Pest Manag. Sci.* **2012**, *68*, 1431–1437. [CrossRef] [PubMed]
26. Garlet, C.G.; Moreira, R.P.; Gubiani, P.D.; Palharini, R.B.; Farias, J.R.; Bernardi, O. Fitness cost of chlorpyrifos resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on different host plants. *Environ. Entomol.* **2021**, *50*, 898–908. [CrossRef]
27. Li, B.; Han, L.Z.; Peng, Y.F. Development of a standardized artificial diet and rearing technique for the striped stem borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae). *Chin. J. Appl. Entomol.* **2015**, *52*, 498–503.
28. Chen, C.K.; Li, X.F.; Han, Z.J.; Li, G.Q.; Wang, M.Z. Method for monitoring insecticide resistance in rice stem borer (*Chilo suppressalis* Walker) and relative susceptible baeslines data. *J. Nanjing Agric. Univ.* **2000**, *23*, 25–28.
29. *NYT2058-2011*; Technological Rules for Monitoring Insecticide Resistance in Striped Stem Borer, Chilo suppressalis Walker, Using Topical Application Method. The Ministry of Agriculture of China: Beijing, China, 2011.
30. Wu, K.J.; Chen, Y.P.; Li, M.H. Effect of temperature on experimental population growth of cotton bollworm. *Acta Entomol. Sin.* **1980**, *23*, 358–368.
31. Huang, Y.B.; Chi, H. Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): With an invalidation of the jackknife technique. *J. Appl. Entomol.* **2013**, *137*, 327–339. [CrossRef]

32. Chi, H. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* **1988**, *17*, 26–34. [CrossRef]
33. Chi, H.; Liu, H. Two new methods for study of insect population ecology. *Bull. Inst. Zool. Acad. Sin.* **1985**, *24*, 225–240.
34. *NY/T 1859.5-2014*; Guidelines on the Risk Assessment for Pesticide Resistance—Part 5: The Risk Assessment of *Plutella xylostella* Resistance to Insecticides. The Ministry of Agriculture of China: Beijing, China, 2014.
35. Shi, Y.; He, L.; Ding, W.B.; Huang, H.; He, H.L.; Xue, J.; Gao, Q.; Zhang, Z.X.; Li, Y.Z.; Qiu, L. Function analysis of CYP321A9 from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) associated with emamectin benzoate, and a novel insecticide, cyproflanilide detoxification. *J. Econ. Entomol.* **2023**, *116*, 1812–1819. [CrossRef] [PubMed]
36. Ding, F.D.; Zhao, L.; Xiang, J.C. Bioactivity and field efficacy of cyproflanilide against *Plutella xylostella* (L.). *Agrochemicals* **2023**, *62*, 852–855.
37. Sun, X.; Wei, R.; Li, L.H.; Zhu, B.; Liang, P.; Gao, X.W. Resistance and fitness costs in diamondback moths after selection using broflanilide, a novel meta-diamide insecticide. *Insect Sci.* **2022**, *29*, 188–198. [CrossRef] [PubMed]
38. Ji, X.J.; Cui, L.; Wang, W.J.; Yang, Q.J.; Zhai, Y.H.; Rui, C.H. Effects of broflanilide on the fitness and detoxification enzyme activities of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Acta Entomol. Sin.* **2023**, *66*, 1569–1578.
39. Falconer, D.S. *Introduction to Quantitative Genetics*; Longman: New York, NY, USA, 1960.
40. Liu, H.; Lu, Y.Y. Improvement on the index of relative fitness in resistance population of insects. *J. Environ. Entomol.* **2016**, *38*, 393–400.
41. Roy, D.; Biswas, S.; Sarkar, S.; Adhikary, S.; Chakraborty, G.; Sarkar, P.K.; Al-Shuraym, L.A.; Sayed, S.; Gaber, A.; Hossain, A. Risk assessment of fluxametamide resistance and fitness costs in fall armyworm (*Spodoptera frugiperda*). *Toxics* **2023**, *11*, 307. [CrossRef] [PubMed]
42. Liu, D.; Jia, Z.Q.; Peng, Y.C.; Sheng, C.W.; Tang, T.; Xu, L.; Han, Z.J.; Zhao, C.Q. Toxicity and sublethal effects of fluralaner on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* **2018**, *152*, 8–16. [CrossRef]
43. Ullah, F.; Gul, H.; Tariq, K.; Desneux, N.; Gao, X.; Song, D.L. Fitness costs in clothianidin-resistant population of the melon aphid, *Aphis gossypii*. *PLoS ONE* **2020**, *15*, e0238707. [CrossRef]
44. Ma, K.S.; Tang, Q.L.; Xia, J.; Lv, N.N.; Gao, X.W. Fitness costs of sulfoxaflor resistance in the cotton aphid, *Aphis gossypii* Glover. *Pestic. Biochem. Physiol.* **2019**, *158*, 40–46. [CrossRef] [PubMed]
45. Shah, R.M.; Shad, S.A.; Abbas, N. Mechanism, stability and fitness cost of resistance to pyriproxyfen in the house fly, *Musca domestica* L. (Diptera: Muscidae). *Pestic. Biochem. Physiol.* **2015**, *119*, 67–73. [CrossRef]
46. Peck, S.; Gould, F.; Ellner, S.P. Spread of resistance in spatially extended regions of transgenic cotton: Implications for management of *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **1999**, *92*, 1–16. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.