



Fitness and inheritance of metaflumizone resistance in *Plutella xylostella*



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ABSTRACT

The diamondback moth, *Plutella xylostella* (L.) has developed resistance to many types of insecticides in the field. To study inheritance and fitness cost of metaflumizone resistance, a susceptible strain of diamondback moth was continuously selected with metaflumizone during 37 generations under laboratory conditions. The resistance to metaflumizone was at a high level (resistance ratios from 250.37 to 1450.47-fold). We investigated a metaflumizone resistance strain (G₂₇) and a susceptible strain of *P. xylostella*, using the age-stage, two-sex life table approach. Compared to the susceptible strain, egg duration, the developmental time of the first and second instar larvae, pupae duration, adult preoviposition period (APOP), total preoviposition period (TPOP), egg hatchability, the survival rate of second instar larva and the mean generation time (*T*) were significantly differences in the resistant strain. The resistant strain had a relative fitness of 0.78. The inheritance of metaflumizone resistance was also studied by crossing the metaflumizone resistant and susceptible populations. Results revealed an autosomal and incompletely recessive mode of inheritance for metaflumizone resistance in the resistant population of *P. xylostella*. The present study provided useful information for planning potential management strategies to delay development of metaflumizone resistance in *P. xylostella*.

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1. Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the major pest of *Brassica* vegetable and oilseed crops worldwide. Globally, direct losses and control costs are estimated to be US\$4–5 billion [1]. In China, losses is estimated to be approximately US\$0.77 billion annually [2]. To Date, the DBM has developed resistance to 95 active ingredients of insecticides including metaflumizone [3], which was introduced into the China market by BASF in 2009 and registered to control *P. xylostella* on vegetables [4,5].

Metaflumizone is a novel sodium channel blocker insecticide (SCBIs) in the semicarbazone class, which binds selectively to the slow-inactivated state of the sodium channel [6], leading to flaccid paralysis and, eventually, death of the affected insects [7]. Metaflumizone has been effectively used for control of a wide range of pests, including economically important lepidopterous pests and other pests in the orders Coleoptera, Hemiptera, Hymenoptera, Diptera, Isoptera, and Siphonaptera [8]. However, a high level resistance to metaflumizone has been reported in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in south China [9]. The resistance in the field populations of *P. xylostella* to metaflumizone was at medium levels (10–70-fold) to metaflumizone compared to the susceptible population [10].

Fitness costs can occur in resistant individuals and include reduced survival on nontreated plants and reduced fecundity [11]. Fitness cost associated with insecticide resistance is well documented in *P. xylostella* resistance to tebufenozide, fufenozide, abamectin and cyantraniliprole [12, 13,14,15]. Other examples of this phenomenon include *Musca domestica* L. (Diptera: Muscidae) to imidacloprid [16], *Spodoptera litura* (Fabricius) to imidacloprid and emamectin benzoate [17,18], *Nilaparvata lugens* (Hemiptera: Delphacidae) to imidacloprid [19], *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) to indoxacarb and deltamethrin [20] and *S. exigua* to tebufenozide [21]. Relative fitness is the ability of a resistant strain to survive and reproduce compared to the susceptible strain [18]. Studying the relative fitness of resistant strains is essential for understanding and managing resistance problems [22]. Fitness costs of metaflumizone resistance have not yet reported in *P. xylostella* anywhere in its worldwide distribution.

Selection for metaflumizone resistance in the laboratory and studies of its mode of inheritance and fitness costs is essential to the sustainable production of cruciferous vegetables and to establish management strategies to delay metaflumizone resistance development in the field. Therefore, the authors of this study used a laboratory-selected metaflumizone resistant and a susceptible strain to construct life tables and to investigate if there were fitness costs associated with metaflumizone resistance in *P. xylostella*. Studying the inheritance of metaflumizone resistance will enable researchers to develop programs to reduce resistance development to insecticides.

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2. Materials and methods

2.1. Insect cultures

The susceptible strain (SS) of DBM was provided by the Department of Entomology, China Agricultural University, Beijing, China. The strain which was originally collected from a cabbage (*Brassica oleracea* var. *capitata*) field in Xuanhua, Hebei Province, China (40.37°N, 115.03°E) in 1996, was reared continuously in the laboratory without exposure to insecticides for over 10 years. The resistant strain (metaflu-SEL) was continuously selected with metaflumizone from this susceptible strain. The larvae were reared on vermiculite-grown radish (*Raphanus stivus* L.) seedlings, which were cultured in an aluminium container (20.5 × 12.5 × 5.5 cm) with vermiculite growing medium. The adults were provided with a 10% honey/water solution in the laboratory and allowed to oviposit on radish seedlings (*Raphanus sativus* L.). All populations were maintained at 25 ± 1 °C, 65 ± 5% RH and L: D = 16: 8 h in a separate greenhouse.

2.2. Insecticide

Bioassays on *P. xylostella* were performed with the insecticide metaflumizone (240 g/L SC; BASF Chemical Co., Ltd., Shanghai, China).

2.3. Bioassay

The leaf-dip bioassay method as described by IRAC method 18 [23] was used to determine the susceptibility of the third instar larvae of *P. xylostella* to metaflumizone. The insecticide was serially diluted to five to seven concentrations with water containing 0.1% Triton X-100 (a surfactant which facilitates uniform leaf disc coverage with its active ingredient). Cabbage (*Brassica oleracea*) leaf discs (7.0 cm diameter) were cut and dipped into those solutions for 10 s. Controls were treated with 0.1% Triton X-100 solution in water alone. The leaf discs were dried at room temperature for 1–2 h. Each treated leaf disc with 10 third instar larvae was placed in a separate plastic Petri dish (7.0 cm diameter) and kept at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16: 8 (L: D) h in a growth chamber. Three replicates of 10 third instar larvae were tested for each concentration. The mortality was assessed 72 h after exposure to metaflumizone. Larvae were considered to be dead if they did not respond to being touched with a probe. Control mortality was <5% in all bioassays.

2.4. Resistance selection

The resistant strain derived from the SS strain was continuously selected with metaflumizone during 37 generations under laboratory conditions since 2013. The concentrations of metaflumizone used for selection in the different generations were determined as LC₃₀–LC₅₀ of their parent's generation. Metaflumizone solution (25 mL) was sprayed onto the seedlings when they had reached 7–8 cm in height. Then the treated seedlings were moved into clear cages and reared the third instar larval to pupate. The number of larvae selected per generation ranged between 1000 and 2000. Viable pupae were collected and bred to the next generation, which were reared free from insecticides for resistance monitoring.

2.5. Genetics of resistance to metaflumizone

To determine the dominance of metaflumizone resistance, reciprocal crosses were performed between the metaflu-SEL (G₃₇) and SS of *P. xylostella* to produce two lines: F₁ (100 metaflu-SEL ♀ × 100 SS ♂) and F₁' (100 metaflu-SEL ♂ × 100 SS ♀).

The degree of dominance (D) was estimated on the basis of dose responses (LC₅₀) of F₁ or F₁' progeny from reciprocal crosses according to

Stone's method [24]:

$$D = \frac{2X_2 - X_1 - X_3}{X_1 - X_3}$$

where X₁, X₂ and X₃ are the logLC₅₀ values for metaflu-SEL, the reciprocal progeny (F₁ or F₁') and the susceptible strain, respectively; D = 1 indicates complete dominant, 0 < D < 1 indicates incomplete dominant, –1 < D < 0 indicates incomplete recessive and D = –1 indicates complete recessive. Maternal effects and sex linkage were estimated according to log dose-probit lines and the LC₅₀ values of reciprocal progeny (F₁ or F₁'). The inheritance of resistance to metaflumizone was autosomal if the log dose-probit lines of F₁ and F₁' progeny were mainly superposed and the LC₅₀ values did not differ between F₁ and F₁' progeny; otherwise, the inheritance of resistance was sex linked.

2.6. Fitness comparison

Life tables were separately constructed for SS and metaflu-SEL populations using the age-stage, two-sex life table approach [25]. 151 and 144 eggs, which laid on the same day by SS and metaflu-SEL (G₂₇) populations, respectively, were collected from at least ten pairs of adults to allow for adequate individuals. The eggs were individually transferred to the numbered plastic culture dish (7.0 cm diameter) containing a fresh cabbage leaf on the top of absorbent paper, and kept separately in the growth chamber at 25 ± 1 °C and 65 ± 5% RH, with a photoperiod of 16: 8 L: D. The incubation period was determined by observing each egg daily. After eggs hatched, the developmental time of individual larva was assessed and the leaves were replaced daily. Individual pupa was collected and placed individually in 1.5 mL centrifuge tubes. After emergence, its sex was determined, and the numbers of eggs laid by each female were recorded daily. Each adult was observed daily for survivorship.

The relative fitness of the resistant strain was calculated as [12,26]: $R_f = R_0$ of the resistant strain/ R_0 of the susceptible strain. $R_f > 1$ suggests that the fecundity of resistant strain is enhanced; $R_f < 1$ suggests that the resistant strain has a fitness defect.

2.7. Statistical analysis

The program *Probit-MS Chart* [27] was used for probit analysis of concentration-response data. Classification of the insecticide resistance level was according to Shao et al. [28]: Low resistance (RR ≤ 10), medium resistance (10 < RR < 100) and high resistance (RR ≥ 100). The raw data of the life cycle of each individual was analyzed using the age stage, two-sex life table theory [25,29]. The basic life-table parameters, such as age-stage survival rate (s_{xj}) (where x is the age and j is the stage), age-specific

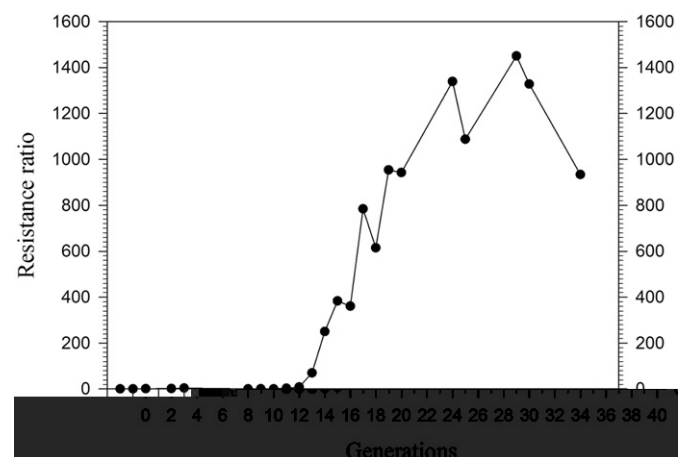


Fig. 1. Dynamics of metaflumizone resistance in *P. xylostella* during selection.

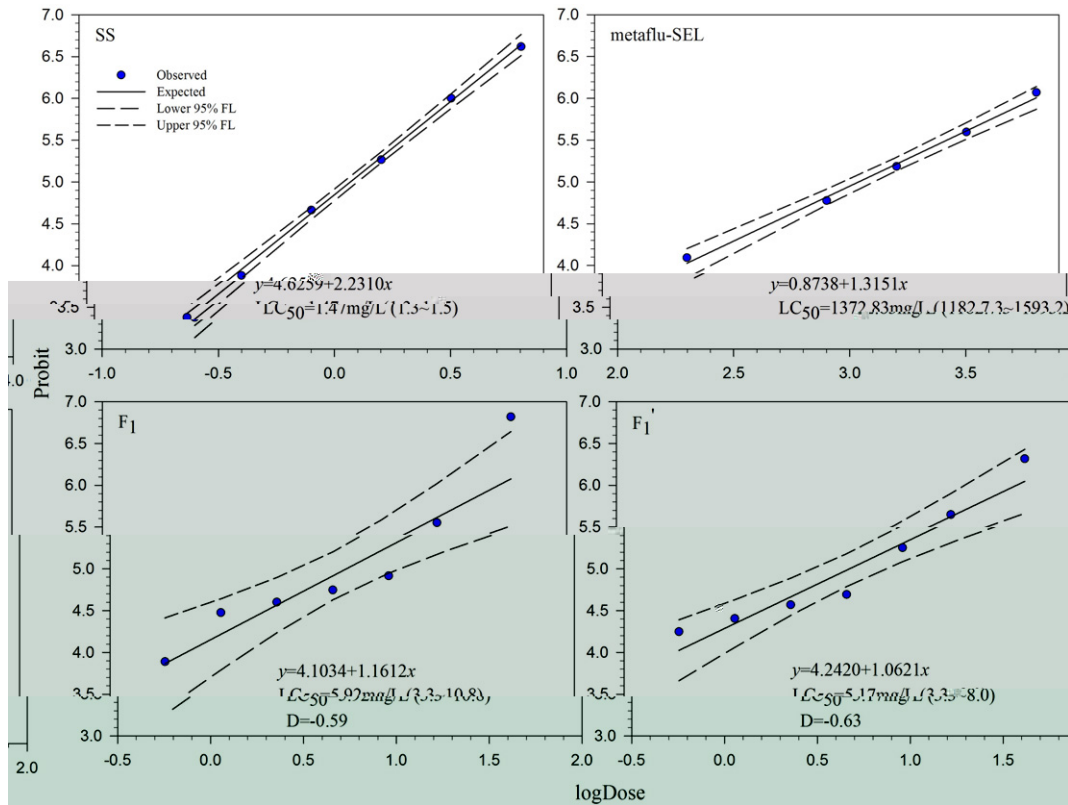


Fig. 2. Response to metaflumizone of SS and metaflu-SEL parental strains of *P. xylostella* and reciprocal crosses F₁ and F₁'.

Table 1

Duration of the development, reproduction, survival rate and life table parameters for the susceptible and metaflu-SEL strains of *P. xylostella*. Standard errors (SE) were estimated with bootstrapping (200,000 re-samplings).

Stage	Strain		n	metaflu-SEL	P	95% CI ^a
	n	SS				
Egg	127	3.07 ± 0.03	134	2.81 ± 0.04	0.000	0.158–0.372
L1	123	2.55 ± 0.06	127	3.29 ± 0.07	0.000	0.555–0.922
L2	121	2.31 ± 0.05	119	2.76 ± 0.07	0.000	0.273–0.628
L3	120	2.47 ± 0.07	116	2.59 ± 0.06	0.165	–0.050–0.289
L4	113	3.43 ± 0.11	109	3.45 ± 0.12	0.920	–0.300–0.333
Pupa	97	4.54 ± 0.06	91	4.77 ± 0.06	0.006	0.068–0.398
Female adult	45	9.69 ± 0.61	44	10.66 ± 0.52	0.225	–0.600–2.545
Male adult	52	9.88 ± 0.62	47	9.94 ± 0.54	0.949	–1.550–1.656
APOP (day)	43	0.72 ± 0.23	43	1.67 ± 0.31	0.014	0.198–1.708
TPOP (day)	43	18.42 ± 0.36	43	20.35 ± 0.49	0.002	0.736–3.123
Oviposition days	45	5.74 ± 0.41	44	5.70 ± 0.42	0.939	–1.106–1.197
Fecundity (eggs)	45	126.42 ± 13.71	44	95.74 ± 10.94	0.080	–3.649–65.012
Survival rate (%)						
Egg	127	84.10 ± 0.03	134	93.05 ± 0.02	0.013	0.018–0.161
L1	123	97.35 ± 0.01	127	95.14 ± 0.02	0.278	–0.021–0.066
L2	121	98.67 ± 0.01	119	94.45 ± 0.02	0.035	0.001–0.084
L3	120	99.34 ± 0.01	116	97.92 ± 0.01	0.216	–0.012–0.041
L4	113	95.37 ± 0.02	109	95.14 ± 0.02	0.890	–0.046–0.051
Pupa	97	89.42 ± 0.03	91	87.51 ± 0.03	0.638	–0.054–0.092
Female adult	45	70.20 ± 0.04	44	69.44 ± 0.04	0.876	–0.097–0.112
Male adult	52	65.56 ± 0.04	47	67.36 ± 0.04	0.756	–0.090–0.126
Parameters						
r (d ⁻¹)		0.1820 ± 0.01		0.1603 ± 0.01	0.099	–0.004–0.048
λ (d ⁻¹)		1.1996 ± 0.01		1.1738 ± 0.01	0.099	–0.005–5.632
R ₀ (offspring individual ⁻¹)		37.67 ± 6.21		29.26 ± 4.95	0.287	–7.142–23.969
T (d)		19.96 ± 0.29		21.09 ± 0.26	0.005	0.364–1.893
R _f				0.78		

^a The difference between two treatments was evaluated by using paired bootstrap test. If the CI includes 0, there is no difference.

fecundity of female (f_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), age-specific maternity ($l_x m_x$), age-stage specific life expectancy (e_{xj}), reproductive value (v_{xj}), intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T), were calculated using the computer program *TWOSEX-MS Chart* [30]. The variances and standard errors of the population parameters were estimated using the bootstrap procedure include in the *TWOSEX-MS Chart* with 200,000 random resampling. The developmental time, adult longevity, APOP, TPOP, oviposition days, fecundity and the population parameters (r , λ , R_0 , and T) were compared by using the paired bootstrap test based on the confidence interval of differences [31,32].

3. Results

3.1. Metaflumizone resistance selection

A susceptible population of DBM was continuously selected with metaflumizone for 37 generations in the laboratory (Fig. 1). The results showed that the resistance increased slightly (RR increased from 0.52 to 8.96 -fold) from the G_1 to the G_{15} generation. While from the G_{16} to the G_{27} generation, the resistance developed quickly (RR increased from 70.24 to 1338.99-fold). However, the resistance appeared to remain at a constant high level, with the last eleven selections showing no obvious resistance increase.

3.2. Genetics of resistance to metaflumizone

Reciprocal crosses were made between the metaflu-SEL (G_{37} , RR = 933.90 -fold) and SS strains. The LC_{50} values did not differ significantly between the F_1 and F_1' progeny of the reciprocal crosses (Fig. 2). Similarly, the mean slope of the concentration-mortality did not differ significantly ($P > 0.05$) between the reciprocal crosses. These indicate that the inheritance of resistance to metaflumizone was autosomal. The degree of dominance (D) was -0.59 and -0.63 for F_1 and F_1' strains, respectively, suggesting that the resistance to metaflumizone was incompletely recessive.

3.3. Fitness comparison

The biotic fitness of metaflu-SEL and SS strains of *P. xylostella* were compared when the resistant ratio was 1338.99-fold (G_{27}). Compared to the SS, egg duration of the metaflu-SEL strain was significantly shortened (Table 1). While the developmental time of first and second-instar larvae, pupae duration, APOP and TPOP were significantly longer in the metaflu-SEL strain than in the SS strain, which was delayed about 0.74, 0.45, 0.23, 0.85 and 1.83 days, respectively (Table 1). The egg hatchability of metaflu-SEL strain ($93.05 \pm 0.02\%$) was greatly increased compared with that of the susceptible strain ($84.10 \pm 0.03\%$) (Table 1). The survival rate of second instar larva was significantly lower in the metaflu-SEL strain ($94.45 \pm 0.02\%$) than in the SS strain ($98.67 \pm 0.01\%$) (Table 1). There were no significant differences in the developmental time of the third and fourth instar larvae, female and male adult lifespans between the metaflu-SEL and SS strains (Table 1), whereas female and male adults of SS strain emerged at the 15th day and reached their maximal mean longevity at the 37th day, which were 1 day earlier than metaflu-SEL strain (Figs. 3 and 4). Similarly, The oviposition period and total fecundity of the two strains were also not significantly different (Table 1), but the highest age-stage specific fecundity (f_{x7}) peaks occurred on the 18th day with 30.8 offspring in the metaflu-SEL strain, and on the 15th day with 68.0 offspring in the SS strain, showing that the oviposition peak was delayed in the metaflu-SEL strain (Fig. 4). No significant differences were found in r , λ and R_0 values between the metaflu-SEL and SS strains (Table 1). However, the mean generation time (T) was significantly prolonged in the metaflu-SEL strain (21.09 day) compared to the SS strain (19.96 day) (Table 1). Compared with the SS strain, the relative fitness value of metaflu-SEL was calculated to be 0.78, indicating that

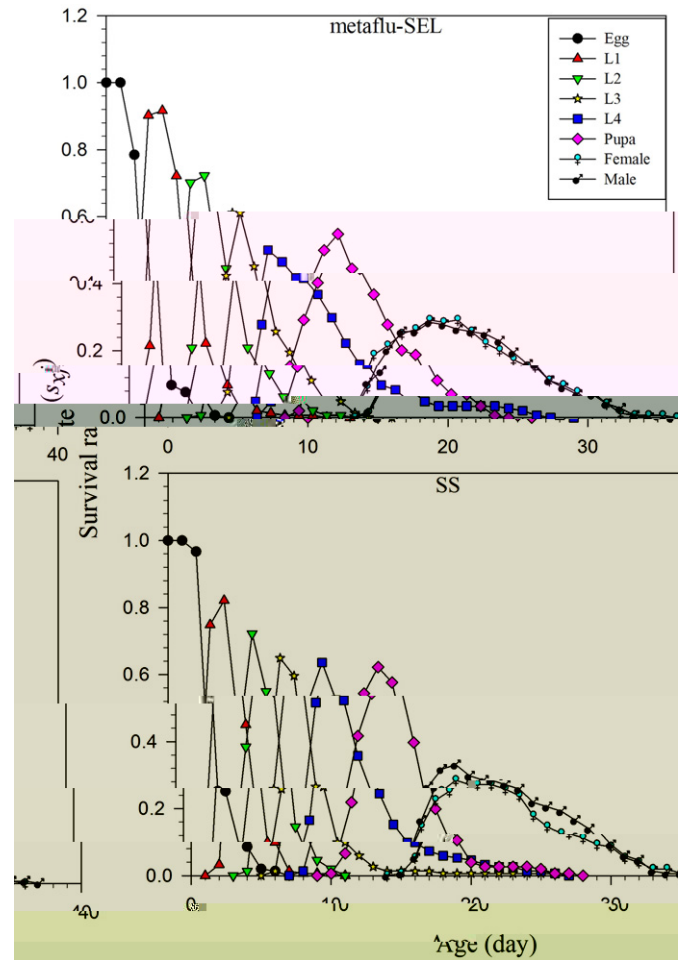


Fig. 3. Age-stage specific survival rates (s_{xj}) of *P. xylostella* in the metaflu-SEL and SS strains.

fitness costs were associated with metaflumizone resistance in the metaflu-SEL strain of *P. xylostella*.

The age-stage survival rate (s_{xj}) represents the probability that a newly laid egg will survive to age x and stage j (Fig. 3). Owing to the variable developmental rates among individuals, significant overlaps between stages were clearly observed between the metaflu-SEL and SS strains. The projected curves for each developmental stage of the metaflu-SEL and SS, showed a similar pattern. The life expectancy (e_{xj}) is the length of time that an individual of age x and stage j is expected to live after age x . In most instances, the e_{xj} values were higher in the metaflu-SEL strain than in the susceptible strain. For example, the longevity of DBM at age zero (e_{01}) was higher in the metaflu-SEL strain (24.13 days) than in the SS group (22.17 days) (Fig. 5). The age-stage-specific reproductive values (v_{xj}) of *P. xylostella* represented the contribution of an individual at age x and stage j to the next generation. The reproductive value increased significantly when *P. xylostella* began to lay eggs. In the SS strain, the increase in reproductive value occurred at age 15 days and reached a peak of 180. In the metaflu-SEL strain, the reproductive value increased at age 16 days and remained at high reproduction for a few days (Fig. 6).

4. Discussion

Metaflumizone, which belongs to the voltage-dependent sodium channel blockers class of insecticides, was registered to control *P. xylostella* on cabbage in China in 2009 [4,5]. In this study, after 37 generations of selection with metaflumizone under laboratory conditions, the resistance to metaflumizone was >1000-fold compared with the

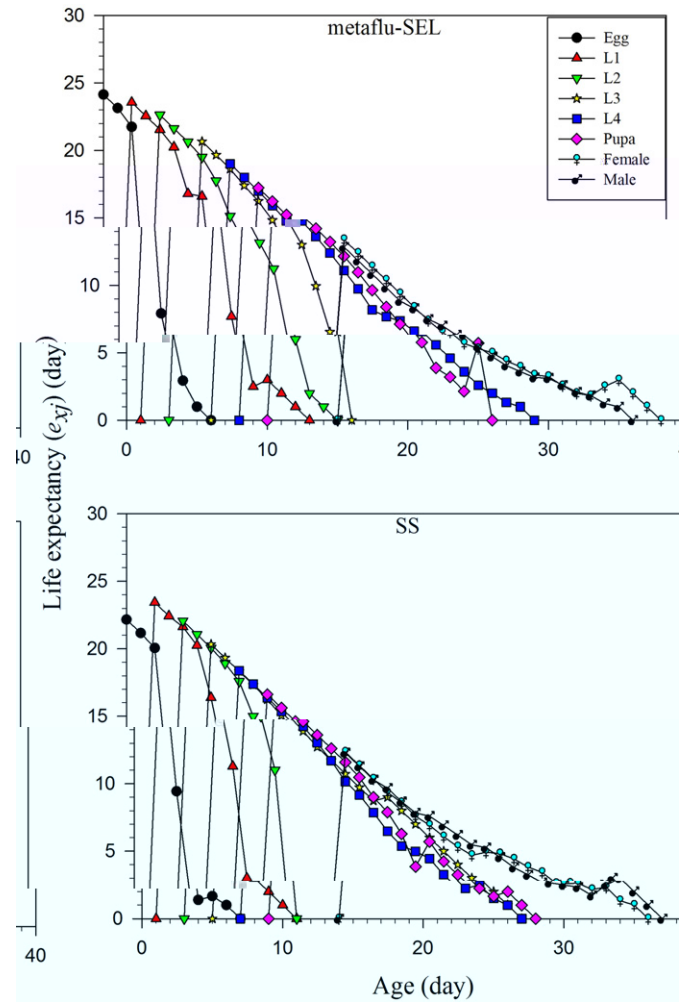


Fig. 5. Age-stage life expectancy (e_{xj}) of *P*

susceptible strain (SS) of *P. xylostella*, indicating that the DBM has the capability of developing a high resistance level to metaflumizone. Development of metaflumizone resistance has previously been reported only in field populations of *S. exigua* and *P. xylostella* [5,9,10]. However, in another SCBI, indoxacarb [33], highly resistant strains were obtained quite readily from field populations after consecutive selection for only three and seven generations in *M. domestica* and *P. xylostella*, respectively [34,35]. Thus, insects have the potential to develop high resistance to sodium channel blockers insecticides, and it should be used with a protective resistance management procedures.

In the present study, logistic regression analysis of F_1 and F_1' reciprocal crosses between metaflu-SEL and SS indicated that resistance to metaflumizone is inherited as an autosomal, incompletely recessive trait. Likewise, Sayyed and Wright [34] reported that in indoxacarb resistant *P. xylostella*, the resistance was an incompletely recessive trait. Nehare et al. [36] also observed similar results in indoxacarb resistant *P. xylostella* showing autosomal and an incompletely recessive trait. This information should be useful to delay the development of metaflumizone resistance in pest insects. More specifically, based on the assumption that indoxacarb resistance is an incompletely recessive trait, a high-dose/refuge strategy was used to delay the development of indoxacarb resistance [35,36]. Due to the genetic basis of metaflumizone resistance, it is possible that the development of metaflumizone resistance in *P. xylostella* strains can be delayed by the use of a high-dose/refuge strategy [37,38].

Fitness cost determination is very important in homozygous-resistant individuals [21]. The genetic background of populations can affect the fitness associated with resistance [39]. Having a similar genetic background, the resistant and susceptible populations differ only in small regions of the genome, which facilitates the assessment of fitness costs. We

therefore selected a population of *P. xylostella* with a high-level of resistance to metaflumizone under laboratory conditions and a susceptible to evaluate the fitness associated with metaflumizone resistance.

Fitness costs of insecticide resistance are considered to be a major factor affecting the evolution of resistance, and a better understanding of the costs may be invaluable in devising effective resistance management strategies [14]. Fitness costs related to insecticide resistance occur when the development of insecticide resistance is accompanied by a reduction in fitness. This may involve a longer development time, lower survival rate, or reduced reproductive performance, in the absence of insecticide [37,40]. Generally, biological characteristics such as increases in development duration, reduced fecundity and reproductive rate, affect the relative fitness [16]. Decreases in fitness due to the development of various insecticide resistances have been observed in *P. xylostella* [11–15]. Factors selected for resistance may present direct pleiotropic effects in one or more life-trait aspects, and alterations in different traits can be considered manifestations of the insect physiological commitment to face the challenge represented by insecticide exposure [41]. For instance, developmental time is a primary aspect of fitness in dispersing mosquito populations [42]. A delay in developmental dynamics was observed in a *Spodoptera exigua* strain selected with fenvalerate [43], in two *Cydia pomonella* strains selected in the laboratory with deltamethrin and with diflubenzuron [44], and in a *Aedes aegypti* population selected in the laboratory with deltamethrin [41]. The current study also demonstrates that metaflumizone resistance could decrease fitness in *P. xylostella*. The developmental time of first and second instar larval, pupal duration, APOP and TPOP were significantly prolonged in

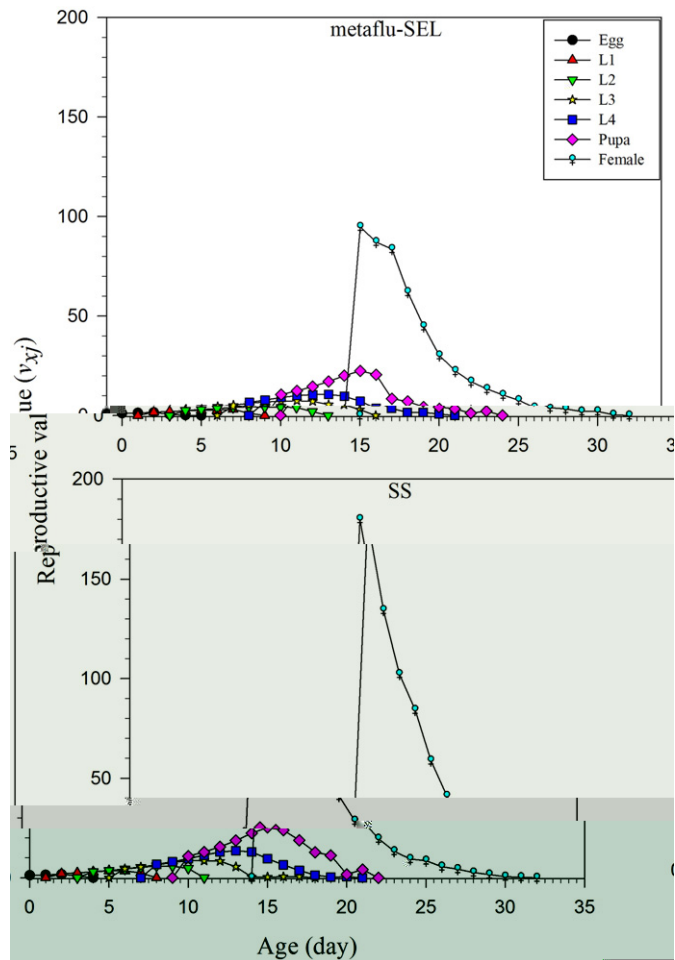


Fig. 6. Age-stage reproductive value (v_{xy}) of *P. xylostella* in the metaflu-SEL and SS strains.

metaflu-SEL strain compared to the susceptible population. Therefore, metaflumizone resistance in *P. xylostella* corresponds with a significant disadvantage in developmental duration. The mean generation time (T) is an important indicator of population dynamics, which prolonged in resistant strain would lead to apparent fitness defects [17,45]. Our results showed that the metaflu-SEL strain had a significantly longer the mean generation time (T), with a relative fitness of 0.78. Of the life history traits examined in our study, the major differences affecting fitness costs are the significant differences found in the developmental duration of the resistant and susceptible strains. Based on the developmental data, it can be concluded that the metaflu-SEL strain would not be able to increase as rapidly as the susceptible strain if metaflumizone selection was discontinued.

In summary, this study demonstrates that *P. xylostella* have the potential to develop resistance to metaflumizone. The resistance was shown to have an autosomal and incompletely recessive mode of inheritance. Using the age-stage-specific life table, a population projection can reveal the change of stage structure during growth of a given population. Understanding stage structure is important to pest management because the dispersal and damage capability of insects varies with stage. This study demonstrates that the life table is capable of providing a comprehensive description of the fitness of an insect population. The data obtained from the two life tables calculated for the metaflu-SEL and SS strains of *P. xylostella* provide a wealth of interesting and useful information that will ultimately be invaluable in IPM programs and/or other studies involving *P. xylostella* biology. Our study reveals that selection pressure can have a disadvantageous effect on the population fitness-related traits of *P. xylostella*. Specifically, we found that population traits such as first and second larval duration, pupal duration, APOP and TPOP

as well as on the population demographic parameters (T) were significantly prolonged, and the survival rate of second larval was significantly decreased in the metaflu-SEL strain, suggesting the rotational use of metaflumizone with other types of insecticides may delay the development of resistance.

Author Contributions

J. S., X.Z., H.W., and J.H.L designed the experiment; J. S., S.Z.Z., and D.Y.L collected data; J.S. and J. L analyzed the data and wrote the manuscript, J. S., S.Z.Z., D.Y.L., X.Z., H.W. and J.H.L read, corrected and approved the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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