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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 (G_{27}) 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*.

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 LC_{30}-LC_{50} 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 dominancy of metaflumizone resistance, reciprocal crosses were performed between the metaflu-SEL (G_{37}) and SS of *P. xylostella* to produce two lines: F_1 (100 metaflu-SEL ♀ × 100 SS ♂) and F_1' (100 metaflu-SEL ♂ × 100 SS ♀).

The degree of dominance (D) was estimated on the basis of dose responses (LC_{50}) of F_1 or F_1' progeny from reciprocal crosses according to Stone's method [24]:

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

where X_1, X_2 and X_3 are the $\log LC_{50}$ values for metaflu-SEL, the reciprocal progeny (F_1 or F_1') 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 LC_{50} values of reciprocal progeny (F_1 or F_1'). The inheritance of resistance to metaflumizone was autosomal if the log dose-probit lines of F_1 and F_1' progeny were mainly superposed and the LC_{50} values did not differ between F_1 and F_1' 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 (G_{27}) 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

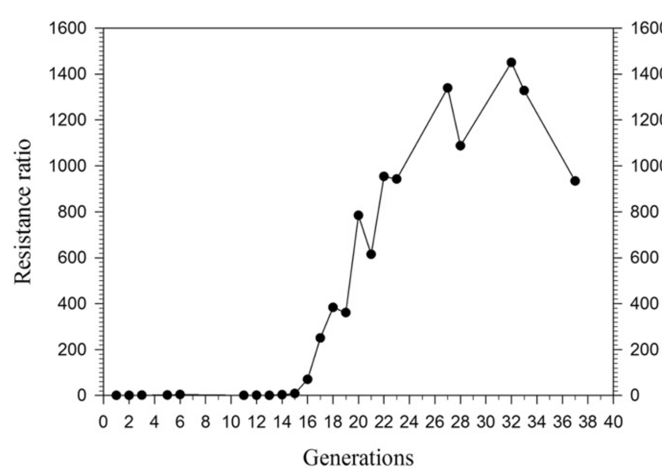

Fig. 1. Dynamics of metaflumizone resistance in *P. xylostella* during selection.