Gossypol-induced fitness gain and increased resistance to deltamethrin in beet armyworm, *Spodoptera exigua* (Hübner)

Muhammad Hafeez[1Θ], Liu Sisi[2Θ], Saad Jan[1], Bahar Ali[1], Muhammad Shahid[3], Muhammad Umer[2], G. Mandela Fernández-Grandon[4], Muhammad Nawaz1, Aqeel Ahmad[1], Mo Wang[1]*

[1]College of Plant Science and Technology, Huazhong Agricultural University Wuhan, Hubei 430070, People's Republic of China. [2]College of Science, Huazhong Agricultural University Wuhan, Hubei 430070, People's Republic of China. [3]Guandong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Key Laboratory of Animal Protection and Resource Utilization, Guangdong Institute of Applied Biological Resources, Guangzhou, 510260, China. [4]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent, UK.

Θ These authors contributed equally to this work.

*** Corresponding authors.**

E-mail address: wangmo@mail.hzau.edu.cn

Abstract

BACKGROUND: In plant-insect interactions, phytotoxins such as gossypol, exert a defensive role on behalf of the plant by interfering with the essential metabolic, biochemical and physiological pathways of herbivorous insects. The beet armyworm, *Spodoptera exigua* (Hübner), is a key pest for many important crops including a range of vegetables, ornamentals, and cotton. For this study we investigated how resistance to deltamethrin relates to enzyme activity in gossypol-pretreated larvae of *S. exigua*.

RESULTS: Through selection with deltamethrin insecticides on gossypol-pretreated larvae for 10 generations, the Gos-SEL population developed a 113.29-fold resistance. Under the same conditions the Delta-SEL selected population showed a 69.76-fold increase resistance along with corresponding levels of xenobiotic defense enzymes activity. Similarly, the fecundity of the Delta-SEL population along with male and female longevity were found to be significantly lower when compared to the Gos-SEL population and the lab susceptible-strain group (Lab-SS). In addition, the activities of cytochrome P450s in *S. exigua* were significantly enhanced when the insects were fed on a deltamethrin and gossypol-pretreated diet compared with being fed on deltamethrin alone.

CONCLUSION: The reproductive capacity of *S. exigua* is significantly reduced in the Delta-SEL and Gos-SEL populations compared to the control group (Lab-SS). Elevation of the major detoxification enzyme cytochrome P450 monooxygenase and esterase might play an important role to induce tolerance to the deltamethrin in gossypol-fed *S. exigua* populations. This study enhances our understanding of detoxification enzyme pathways for *S. exigua* gene expression and their role in responses to insecticides and plant secondary metabolites.

Keywords: *Spodoptera exigua*, insecticide resistance, reproductive parameters, midgut, detoxification enzyme, plant secondary metabolite

1 Introduction

Plant secondary metabolites exert important defensive roles by interfering with essential metabolic, biochemical, physiological functions and pathways of herbivorous insects[1]. Gossypol, and related phenolic sesquiterpenoid aldehydes, are the principal secondary metabolites produced by the subdermal glands of many cotton varieties which exhibit fungistatic and insecticidal activities[2,3]. Thus, success for phytophagous insects relies on them adapting to the changing biotic stress of different kinds of host plant secondary metabolites and modulate their defense states accordingly. To achieve this, insects have developed sophisticated defense systems to detoxify or eliminate various harmful compounds through the use of detoxifying enzymes. Of the detoxification enzymes commonly used, three are of primary importance for the detoxification of xenobiotics such as insecticides and phytotoxins; cytochrome P450 monooxygenases (P450s), esterases and glutathione S-transferases (GST)[4–7].

Various important plant secondary metabolites have been shown to provide plants with resistant against many herbivorous insects by acting as feeding deterrents, i.e., growth inhibitors or toxins for several insect orders: Lepidoptera, Coleoptera, Hymenoptera, and Hemiptera[8–11]. It is also reported that insecticide resistance mechanisms vary across insect pest populations feeding on different host plant species[12]. As an important plant secondary metabolite produced by some cotton varieties, gossypol, a phenolic sesquiterpenoid, may play a significant role in altering the defense mechanism of the beet armyworm.

The beet armyworm, *S. exigua*, is a pest of many important crops worldwide, including vegetables, ornamental plants, and cotton. The beet armyworm has developed a high level of resistance against a variety of different groups of insecticides due to its polyphagous behavior, overlapping development stages and long-term exposure to most insecticides[13,14]. To mitigate

the damage caused through their feeding, insecticides are used extensively as part of control programs. This exposure to insecticides has led to field-evolved resistance problems to conventional pesticides as reported in China[15,16] and other countries[17–19]. Like many other insect pests, *S. exigua* have developed resistance to the different group of insecticides, including chlorantraniliprole [20], spinosad [21] indoxacarb [22] tebufenozide [23] as well as diamide insecticides[24]. Insecticide resistance development among field populations has resulted in frequent failures to control the pest through conventional techniques[25].

Despite potential issues with resistance, the application of synthetic insecticides has continued to be the primary method for controlling *S. exigua*. Biological control approaches have often failed to combat the pest's high fertility and long-distance itinerant behavior, traits which also make it difficult to provide comprehensive monitoring[26,27]. Pyrethroid insecticides have contributed an essential role in decreasing crop damage by destructive insect herbivores, though, the extensive use of pesticides has led to increased environmental pollution and has decreased the insecticide's efficacy as a result of the selection for insect resistance[28,29]. The pyrethroid, deltamethrin, belongs to the α-cyano group of chemical pesticides that induce a long-lasting inhibition effect on sodium channel activation gates and produces successions of repetitive nerve signals in sensory organs of target organisms. The synthetic compound has been used to protect economically important crops, vegetables, and fruits from destructive insect pests[30,31]. Despite the economic importance of deltamethrin, and insecticide use generally, the effect of gossypol on the development of insecticides resistance and related important metabolic functions of insects has received little attention.

The present study was undertaken to determine how the deltamethrin insecticide resistance and fitness traits of *S. exigua* were affected by feeding on the plant secondary metabolite, gossypol.

Through this we were able to assess the possible influence of gossypol and deltamethrin insecticide on the mechanism of three major detoxification enzymes, viz., Esterase, glutathione S-transferases, and cytochrome P450 monooxygenases. Availability of such information on secondary metabolites, utilization, and corresponding detoxifying enzyme profiles would help in determining the varying biology and virulence of the pest populations and potential for integrated control measures.

2 Materials and methods

2.1 Experimental insects rearing technique

Laboratory-reared susceptible strain (Lab-SS) of beet armyworm, (*Spodoptera exigua*) larvae were established with a field collection from Jingzhou, Hubei, China in 2003 and were maintained on artificial diet in the college of Plant Science and Technology, Huazhong Agriculture University Wuhan, China. Cultures were maintained in controlled environment laboratory conditions (25 ± 2°C, 65–75% RH) with a photoperiod of 14h:10h (L:D). A 10% honey solution was provided to adults as a food source. Populations reared continuously in the laboratory without exposure to any insecticides were considered a susceptible strain to deltamethrin.

2.2 Chemicals

The commercial insecticide products used in the bioassays were: deltamethrin (Decis 25EC Bayer crop sciences Company, Shanghai, China), gossypol 98% (Aldine chemical industry cooperation China). The L-glutathione reduced (GSH), bovine serum albumin (BSA), α-naphthol, and α-naphthyl acetate used in the experimental work were purchased from Sigma-Aldrich Chemical, Hong Kong. The 1,4-dithiothreitol (DTT), phenylmethylsulfonyl fluoride

(PMSF) 1,2-dichloro-4-nitrobenzene (DCNB), and phenylthiourea (PTU) were purchased from Cheng du Micxy Chemical Co Ltd, China.

2.3 Preparation of insecticide and gossypol-supplemented diets

To prepare the insecticide and gossypol-supplemented diets, gossypol was first dissolved in 1% dimethyl sulfoxide (DMSO). All diets contained a final concentration of 1% DMSO. The DMSO and gossypol were thoroughly mixed with the artificial diet and then transferred into small plastic cups before the solidification of agar (40–45℃). The control group diet was prepared using the same method but using an equal volume of DMSO without gossypol supplementation to the artificial diet.

2.4 Toxicity bioassays

Toxicity bioassays were conducted on newly molted second instar larvae of *S. exigua* under laboratory conditions using a diet incorporation method[32]. Five to six concentrations of insecticides diluted in distilled water were mixed into the semi-synthetic diet following a previously established methodology[33]. Each concentration was replicated three times. The early second instar larvae were first fed on artificial diet supplemented with gossypol 1 mg/g for a day, followed by a diet containing different concentrations of deltamethrin the following day. Distilled water in the semi-synthetic diet was used as a control. The range of concentrations of deltamethrin insecticide were 0.1875-180 μg ml^{-1} for both treatment populations. After preparation, the diet was cut into small cubes (3 cm^3) before being transferred into a 5 cm diameter Petri dish. Three Petri dishes were used for each concentration. A total of 630 newly molted second instar larvae were used for each bioassay with each selected group including control (90 larvae) and 90 newly molted second instar larvae for each concentration. Thirty individuals were tested per replicate with three replications for each concentration of toxicity

assessment bioassays. The toxicity bioassays were performed under the same environmental conditions as the insect rearing. Mortality was assessed after 72 h exposure to deltamethrin. Larvae were recognized as dead if they did not make any coordinated movement after being pushed with a probe.

2.5 Selection of gossypol & deltamethrin-resistant strains

For the *S. exigua* resistance selection experiments, the population was divided into two treatment groups after performing bioassays. For the gossypol with deltamethrin resistant strain, the early second instar larvae were first fed on artificial diet supplemented with gossypol 1 mg/g of artificial diet for a day, followed by a diet containing LC_{50} concentration of deltamethrin the following day. This group is identified as the Gos-SEL population. For the deltamethrin-resistant strain, the early second instar larvae were fed first on a standard artificial diet (i.e. without gossypol) for a day, followed by a diet containing LC_{50} concentration of deltamethrin the following day. This group is identified as the Delta-SEL population. This methodology was established after that used by Tao et al., 2012[34]. Following this method, 400–700 second-instar larvae of *S. exigua* for each group were selected and treated with artificial diet containing LC_{50} of deltmethrin inducing a mortality of 50–70%. The surviving larvae (after 72 h exposure) from the Gos-SEL population were reared to maturity on gossypol-treated artificial diet and Delta-SEL population on a standard artificial diet only. A separate control population considered as Lab-strain was reared without any pre or post treatment. This control population was reared in the same fashion, however, only artificial diet was provided for the next generation. The toxicity of deltamethrin to the Gos-SEL and Delta-SEL population was assayed at every generation for resistance selection. The survivors of every selection were reared to obtain the next generation. Insecticide resistance levels were determined by using the resistance ratio (RR)[17].

2.6 Life table construction

Beginning with the 11th generation, a total of 90 neonates were taken randomly from each population of Gos-SEL and Delta-SEL. Each was reared separately in the small transparent plastic cups containing 5-7 g of artificial diet. Larvae were checked every day for the occurrence of a molt and for survivorship from 1^{st} to 5^{th} instars prior to pupation. Individuals that survived to pupation were removed, separated by sex and weighed for comparison between strains. Male and females were identified at the third day of pupation. For this experiment, each larvae provided one replicate[35]. Overall survivorship, development time, and pupal weights were compared. The newly emerged male and female adults were separately paired into a family. We established 18 families, and these were divided into three groups. Each group consisted of six families, and each group served as a replicate for each strain. Plastic boxes (8 × 11 cm) were used and provided with nappy liner hung vertically to permit oviposition. Fecundity as eggs/female, and adult longevity were recorded. The diet was replaced once after three days to avoid any effects of spoilage throughout the experiment.

2.7 Enzyme assays

To assay the detoxification enzyme activities, beginning with the 11th generation, newly molted fourth instar individuals from the Gos-SEL population were fed first on artificial diet supplemented with 1 mg/g gossypol for one day before being transferred to a diet containing LC_{50} of deltamethrin for the second day. Preparation of the Delta-SEL strain was conducted in the same manner, however, the first day they fed only on artificial diet (no gossypol). On the third day individuals from both treatment groups were fed on a deltamethrin-treated artificial

diet. The control group, Lab-SS, were not exposed to any pre- or post- treatment. After 48 h the midgut of all the larvae were dissected to assess enzyme activity.

To prepare the total midgut proteins, four to six midguts from Gos-SEL and Delta-SEL strain larvae were transferred into separate 1.5-mL Eppendorf tubes and each sample was homogenized in 500 µl of ice-cold homogenizing buffer (0.1 M sodium phosphate buffer, pH 7.8, containing 1 mM EDTA, 1 mM DTT, 1 mM PMSF). The homogenates were centrifuged at 4 C, 2400 × g for 10 min. The supernatant from all replicates of each treatment was used immediately for enzyme activity. For each treatment group, three biological replicates were completed.

2.8 Assays of P450 PNOD activities

To complete assays of P450 and PNOD activities, 2 µl of 15 mg /mL p-nitroanisole (P-Na) was added to 200 µl of total proteins (200 µg). The reaction mixture containing substrate was incubated for 3 min at 30 ºC in a water bath with the reactions initiated by adding of 30 µl of 10 mM NADPH. After 30 min in water bath, ethanol (200 µl) was added to the reaction mixture to precipitate the protein and stop the reaction. As a blank control, proteins were added after ethanol to account for the absorbance value of each sample. The tubes were centrifuged at 2500 × g for 10 min, and the supernatant was used to read the absorbance in a spectrophotometer at 405 nm. The change in absorbance was calculated as the difference between the sample absorbance and the absorbance of each blank control. A p-nitrophenol standard curve was used to obtain the molar extinction coefficients to convert the absorbance into concentration. The activity was expressed as nmol p-nitrophenol per min per mg protein.

2.9 Assays of Glutathione S-transferase (GST) activity

A GSH-ST detection kit (Nanjing Jiancheng Bioengineering) was used, in which GST catalyzes the conjugation of L –glutathione (GSH) to 1, 2-dichloro-4-nitro-benzene (DCNB) through the thiol group of the glutathione. After allowing reaction at 37 ºC for 10 min, the remaining GSH were then detected by reaction with the general thiol reagent (5-5¢-dithiobis [2-nitrobenzoic acid], DTNB) to form the 412 nm chromophore, 5-thionitrobenzoic acid (TNB), as described in Teitze, 1969. The activity was expressed as the nmoles of GSH decreased per min per mg protein.

2.10 Esterase activity towards a-naphthyl acetate (a-NA)

The assay was performed following previously described methods[36,37]: twenty microliters of total proteins (80 µg) from each sample in three replicates were added to 300 µl of sodium phosphate buffer (0.1 M pH 7.6) in a 1.5-mL Eppendorf tube. Two hundred microlitres of substrate solution was added from solution containing 5 mL 0.1 M pH 7.6 phosphate buffer, 10 mg Fast Blue RR salt and 0.1 mL 100 mM a-NA and mixed gently. Absorbance values were immediately measured using a BIO-RAD xMark Microplate Spectrophotometer once every 15 s at 450 nm with recordings lasting for 2 min. The mixture without protein was used as a blank control. A naphthol standard curve was used to convert absorption into concentration. The activity was expressed as nmol naphthol per min per mg protein.

2.11 Statistical analysis

Concentration-mortality data was analyzed by probit analysis[38] with POLO software[39], to determine the LC_{50} values, their standard errors, slopes and 95% fiducial limits (FL). Mortality was corrected as necessary by application of the formula described by Abbot, 1925[40]. Resistance Ratio (RR) and its 95% FL were calculated by dividing the LC_{50} value and its 95% FL of Gos-SEL strain divided by the LC_{50} of the Lab-strain.

2.12 Age-stage, Two-sex Life Table Analysis

Different life stage developmental times, survival, adult longevity, and fecundity parameters were statistically analyzed using age-stage two-sex life table theory[41,42] and the TWOSEX-MSChart software[43]. Means and standard errors (SEs) of long-term table parameters were calculated via 100,000 bootstrap replicates to obtain stable SE estimates[44,45]. All treatments were compared using the paired bootstrap test; both bootstrap and paired bootstrap tests were computed in TWOSEX-MSChart[43], while the software Sigma Plot 12.5 was used to generate

curves for all population life table parameters, including survival rate, fecundity, reproductive values, and life expectancy. The age-specific survival rate (l_x) and age-specific fecundity (m_x) were calculated as:

$$l_x = \sum_{j=1}^{k} s_{xj} \quad (1)$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj}\, f_{xj}}{\sum_{j=1}^{k} s_{xj}} \quad (2)$$

Where s_{xj} is the age-stage specific survival rate, i.e., the probability that an individual will survive to age *x* and in stage j. The intrinsic rate of increase (*r*) was then estimated iteratively from the Euler–Lotka equation with age indexed from 0[46]:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x\, m_x = 1 \quad (3)$$

The net reproductive rate R_0 is calculated as:

$$\sum_{x=0}^{\infty} l_x\, m_x = R_0 \quad (4)$$

The relationship between R_0 and mean female fecundity (*F*), of all population is as follows:

$$R_0 = F\, \frac{N_f}{N} \quad (5)$$

Where *N* is the total number of individuals used for the life table study, and N_f is the number of female adults[41]. The gross reproduction rate is defined as follows:

$$GRR = \sum_{x=0}^{\infty} m_x \quad (6)$$

The mean generation time is defined as the time duration that a population needs to increase to R_0-fold of its size (i.e. $e^{rT} = R_0$ or $\lambda^T = R_0$ at the stable age-stage distribution. The formula for *T* is:

$$T = \frac{\ln R_0}{r} \quad (7)$$

3 Results

3.1 Toxicity of Gos-SEL and Delta-SEL population

Resistance to deltamethrin pre-exposure on artificial diet containing 1 mg/g gossypol in *S. exigua*

Table 1. Shows the development of resistance in *S. exigua* larvae to Gos-SEL and Delta-SEL populations over ten generations. The LC_{50} values to deltamethrin from G_1 to G_5 were 0.52, 1.59, 3.82, 3.36, 11.51 μg ml^{-1} for Gos-SEL and 0.59, 1.2, 2.74, 2.99, 8.34 μg ml^{-1} for Delta-SEL. These values increased with successive generations from G_6 to G_{10} reaching 17.97, 27.65, 34.30, 42.20, 66.86 μg ml^{-1} for Gos-SEL and 14.72, 18.23, 26.22, 33.54, 41.16 μg ml^{-1} for Delta-SEL as a result of selection pressure by the insecticide and gossypol. After 10 generations of selection with deltamethrin, the Gos-SEL population developed a resistance ratio (RR) of 113.29-fold as compared to the Delta-SEL population which was 69.76-fold at G_{10}.

3.2 Pre-adult developmental time for Gos-SEL and Delta-SEL strains

Comparisons of Gos-SEL strains and Delta-SEL with Lab-strain showed fitness costs linked with Gos-SEL resistance (**Table 2)**. There were no significant differences in eggs incubation period between Gos-SEL and Delta-SEL strains as compared with the SS-Strain. While the mean duration of 1st, and 5th larval instar of Gos-SEL strains (3.21 and 3.1 days) and Delta-SEL (3.71 and 3.52 days) was significantly longer as compared to the mean duration of 1^{st}, and 5^{th} larval instar Lab-Strain (3.0 and 2.96 days) respectively. Moreover, larval duration of *S. exigua* in the Delta-SEL population was significantly extended, when compared with those larvae of the Gos-SEL population. No differences were observed between 3^{rd} and 4^{th} larval instar of Gos-SEL (2.93

and 2.93 days) and Delta-SEL strain (3.03 and 3.02) when compared to the Lab-strain (3.01 and 2.97). The mean total larval duration from egg to 5th instar in Delta-SEL populations (19.35 days) and Gos-SEL group (18.27 days) was significantly longer, when compared with the Lab-Strain (17.89 days). While, larval duration from egg to 5th instar in Delta-SEL populations (19.35 days) was also significantly extended as compared to the Gos-SEL group (18.27 days). Meanwhile, pupation period in Delta-SEL strain (8.82 days) and Gos-SEL strain (7.45 days) was significantly longer than the Lab-strain (7.25 days) respectively. Although, pupation period in Delta-SEL strain was significantly longer as compared to the Gos-SEL strain. The same trend in mean pupal weight was observed as the mean pupal weight in Delta-SEL strain (113.26 mg) was significantly lower when compared to the Lab-strain (126.27 mg), while no significant differences were observed in mean pupal weight between Gos-SEL strain and Lab-strain.

3.3 Adult longevity and growth metrics of *S. exigua* Gos-SEL and Delta-SEL

Resistance effects of Gos-SEL and Delta-SEL population on adult longevity, adult's pre-oviposion period (APOPs) and total pre-oviposition period (TPOPs), Oviposition-day, fecundity, and mean growth time (MGT) of Gos-SEL and Delta-SEL strain of *S. exigua* were evaluated with respect to Lab-strain (**Table 3).** Mean adult longevity in Delta-SEL strain (24.29 days) and the Gos-SEL strain (24.29 days) were significantly shorter compared to the mean adult longevity (30.63 days) of Lab-strain. Similarly, the mean longevity of male and female (9.22 and 13.61 days) in Gos-SEL strain and Delta-SEL strain (6.95 and 10.26 days) were significantly shorter as compared to the mean longevity of male and female (9.78 and 13.94 days) of Lab-strain. Mean longevity of males and females in Delta-SEL strain were significantly shorter when compared to the Gos-SEL strain. No significant differences were observed in adult TPOPs (APOPs) with Delta-SEL and Gos-SEL strains when compared with Lab-strain. While, the total

POPs (TPOPs) in the Delta-SEL strain (31.21 days) and Gos-SEL strain (27.78 days) were significantly increased when compared to the Lab-strain (26.67). Oviposition days in the Delta-SEL strain (4.42 days) were significantly shorter than the oviposition days of Lab-strain (6.56 days) (**Table 3).** No significant difference was observed between the Gos-SEL and Lab-strain for oviposition days. Delta-SEL and Gos-SEL female egg production was significantly lower (273.58 and 479.22 eggs/female, respectively) than the Lab-strain (532.67) eggs per female. Moreover, females in Gos-SEL strain significantly produced more eggs than those in the Delta-SEL strain.

3.4 Pre-adults Pupal, Adult's survival rate and Hatchability of the Gos-SEL and Delta-SEL population of *Spodoptera exigua*

Adult and pre-pupal survival (%) in Gos-SEL and Delta-SEL resistance strains were compared (**Table 4).** The mean survival rate from 1^{st} instar to 2^{nd} instar (67.65%) was significantly lower in Delta-SEL compared to the mean survival rate from 1^{st} instar to 2^{nd} instar (83.34%) of the Gos-SEL population strain. A similar trend was observed in mean survival rate from 3^{rd} to 5^{th} instar with 61.23% survival in the Delta-SEL population being significantly lower than the 77.05% survival observed for the Gos-SEL strain. Similarly, the mean pupal survival rate in Delta-SEL strain (77.50%) was significantly lower than the mean pupal survival rate in Gos-SEL population (89.367%). No significant differences were observed in emergence rate of the healthy adults between the Delta-SEL and Gos-SEL populations. In addition, mean hatchability percentage of *S. exigua* in the Delta-SEL strain (63.97%) was significantly lower than that of the Gos-SEL and Lab-strain (84.51 and 93.16%, respectively) (**Table 4).**

3.5 Fitness comparison

The intrinsic rate of population increase (r_m) was significantly lower for the Delta-SEL strain (0.115) than that of the Gos-SEL (0.143) and Lab-strain (0.149) populations (**Table 5)**. Also, these results show that the finite rate of increase (ƛ) (1.12) markedly decreased in the Delta-SEL strain as compared to the finite rate of increase (ƛ) of the Gos-SEL and SS-strain (1.154 and 1.161); while no significant differences were observed between Gos-SEL and Lab-strain. Similarly, mean generation time (T) in Delta-SEL strain (34.97) was significantly extended compared to the mean generation time of Gos-SEL (31.67) and Lab-strain (31.005). No significant differences were observed in net reproductive rate (R_0) between Delta-SEL and Gos-SEL strains compared to the Lab-strain (**Table 5).**

3.6 Survival rate, life expectancy, reproductive value and fecundity of the Gos-SEL and Delta-SEL populations of *Spodoptera exigua*

The age-stage survival rate (s_{xj}) shows that the expected survival of newly laid eggs is to age *x* and stage *j* **(Fig. 1).** The results in this study revealed that a change occurs in the developmental rate between individuals. It was also shown that there is a significant difference in overlapping projected curves between the different developmental stages for Delta-SEL and Gos-SEL strain compared with the Lab-strain. The peak lines in the plotted curves showed a different pattern for every developmental stage of both populations when compared to the Lab-strain. Thus, s_{xj} values for male and female adults were negatively affected in the Delta-SEL strain. It is shown that s_{xj} reached a maximum in the Gos-SEL strain (0.42 for males and 0.22 for females), whereas this value constantly decreased in the Delta-SEL strain (0.23 for males and 0.24 for females) compared to the Lab-strain control group (0.58 for males and 0.21 for females). The curves indicated that emergence occurred after 23 days for males and 26 days for female in Delta-SEL

strain, and in Gos-SEL strain emergence occurred after 24 days for males and 23 days for males, while in Lab-strain emergence occurred after 22 days for females and 24 days for males (**Fig. 1).** Age-stage-specific survival rate (l_x), female age-stage-specific(f_x), age-stage-specific fecundity of the total population (m_x) and age-stage-specific maternity ($l_x m_x$) of *S. exigua* for Delta-SEL and Gos-SEL populations were compared to the Lab-strain **(Fig. 2).** These results show that the fecundity level in Delta-SEL and Gos-SEL strain was lower than Gos-SEL and population. Indeed, the highest recorded (f_{xj}) peak in a Gos-SEL strain was 82.00 eggs female^{-1}day^{-1} laid over 29.0 days, while in the Delta-SEL populations this was 60.00 eggs female^{-1} day^{-1} laid over 35.00 days compared to the Gos-SEL and Lab-strain which produced 94.00 eggs female-1 day-1 laid over 30.00 days . Interestingly, the highest recorded m_{ax} values for Gos-SEL strain were 22.8 eggs individual^{-1} day^{-1} which happened on the day 29, noticeably lower than the value for the Delta-SEL strain of 40.2 eggs individual^{-1} day^{-1}, which occurred after 35 days **(Fig. 2).** Correspondingly, the age-stage reproductive values (v_{xj}) of *S. exigua* were recorded **(Fig. 3).** At the pupal stage, the reproductive value Delta-SEL populations were lower compared to the Delta-SEL and Lab-strain. The result also showed that when females emerged there is a lower plotted curve Delta-SEL strain compared to the Gos-SEL group (Fig. 3). The life expectancy (e_{xj}), which is the amount of the total time that individuals of age *x* and stage *j* would be expected to live, is different between the Delta-SEL strain, Gos-SEL when compared to the SS-strain population **(Fig. 4).** The life expectancy of newly laid eggs by the female in the Delta-SEL group was (24.0 d) which was much shorter than that of eggs from the Gos-SEL group (29.0 d) and SS-strain (30.0 d). The peak life expectancy of newly hatched first instar larvae was 21.6.4 d and 24.0 d in the Delta-SEL and Gos-SEL strains respectively which was comparably lower than that seen in the Lab-strain (27.0 d) **(Fig. 4).**

3.7 Detoxification enzymes activity for Gos-SEL and Delta-SEL strains

Spodoptera. exigua fitness traits are interpreted in light of differential activity of three major xenobiotic detoxifying enzymes mechanism, the esterases (ESTs), cytochrome P450 monooxygenases (CYPs) and glutathione S-transferases (GSTs) in Gos-SEL and Delta-SEL resistance population. The activities of esterase (EST) (163.17 nanomole per min per mg pro) and cytochrome P450 monooxygenase (P450) (0.8437 nanomol /min / mg pro) were significantly increased in the Gos-SEL strain compared to those of the Delta-SEL population (133.39 nanomole per min per mg pro for EST and 0.5281 nmole per min per mg pro for P450) (**Table 6)**.

4 Discussion

Plants have groups of biochemical pathways responsible for synthesizing various kind of phytotoxins for defense against herbivores and pathogens[47,48]. To cope with this, generalist insect herbivores have evolved a range of strategies and mechanisms to cope with a large diversity of toxic secondary metabolites produced by their host plants. Such strategies include: behavioral avoidance, rapid excretion, target site mutation and induction of detoxification including cytochrome P450 monooxygenase (P450), glutathione S-transferase (GST), and carboxyl esterase (COE)[6,49,50]. Gossypol, a phenolic sesquiterpenoid, is an allelochemical produced by sub-epidermal glands of some cotton cultivars which exhibits antibiosis to many cotton pests and contributes to their pest resistence[51–53].

Our results demonstrate that *S. exigua* larvae which fed on the artificial diet containing 1 mg/g gossypol induced greater tolerance to deltamethrin than those larvae that fed on the diet without gossypol after 10 generations of selection. One possible explanation for the synergistic effects of gossypol and Cry1Ac on AR-larvae is that gossypol is degraded and induced greater tolerance to deltamethrin by upregulation of a cytochrome P450 as seen in *Helicoverpa armigera* [34,54]. This finding provides an important contribution to the growing body of literature showing that the insecticide's toxicity to herbivorous insects might be affected by exposure to plant secondary metabolites in the host plant. For example, it has previously been shown that *Helicoverpa zea* larvae after being fed to xanthotoxin show induced tolerance to alpha-cypermethrin[55]. The present finding indicates that, under constant selection pressure of deltamethrin and gossypol, the costs associated with larval growth and pupal weight were different for Gos-SEL and Delta-SEL populations. Knowledge of the resistance to deltamethrin induced by gossypol may help to elucidate the mechanisms leading to plasticity in fitness costs.

Previous studies show that the P450 enzyme CYP6AE14 to be a gossypol-induced gene. The enzyme is also related to deltamethrin tolerance and appears to play a key role in development as its expression correlates with the larval growth of bollworm[34,54,56]. Gossypol ingested by larvae may potentially increase the detoxification enzyme's activity to induce greater resistance against deltamethrin and boost the larval development. Thus, our results indicate that gossypol increased the magnitude of fitness costs associated with the deltamethrin in *S. exigua*. Gossypol-induced resistance to deltamethrin may possibly link with multiple P450s and esterase genes in *S. exigua*. Our results support previous studies[34] which have reported that gossypol-induced p450s are highly divergent in cotton bollworm, with at least CYP321A1, Cyp9a12, Cyp9a14, Cyp6ae11 and Cyp6b7 contributing towards tolerance to deltamethrin. The acquisition of resistance to synthetic insecticides often carries an associated fitness cost when resources generally directed toward fitness-enhancing traits are redirected instead toward production and maintenance of resistance[57]. Adult male and female longevity, the total developmental time from egg to adults and fertility of female adults were significantly decreased in Delta-SEL and Gos-SEL strains compared to Lab-strain of *S. exigua*. Fitness costs associated with insecticide resistance, such as increased development time of larval and pupal stages, show a trade-off in the sharing of various resources between resistant populations. These fitness costs have been observed in many other lepidopteran pests, including the tobacco budworm (*Heliothis virescens*)[58], cotton bollworm (*Helicoverpa armigera*)[59], pink bollworm (*Pectinophora gossypiella)*[60], tobacco cutworm (*Spodoptera litura)*[61], and diamondback moth (*Plutella Xylostella)*[62,63].

The intrinsic rate of natural increase (r_m) provides an estimate of the growth potential of insect populations[64] which, along with other life history parameters, can provide great insight

into the population growth of species. While it is also possible to calculate the net reproductive rate (R_0) this, again, is not the only component needed to evaluate the potential growth of the population as the intrinsic rate of natural increase depends on productiveness, % hatching, growth and adult emergence[65,66]. For that reason, distinctions in the above life history abilities could simulate the rate of *S. exigua* population increase. The intrinsic rate of natural increase (r_m) of males and females in Delta-SEL Population was significantly decreased compared with that in the Gos-SEL population **(Fig. 5).** Similarly, the average generation time (34.97 days) was considerably extended in the Delta-SEL population compared to the mean generation time (31.67 days) of the Gos-SEL population **(Fig. 5)**. Previously, the effects of imidacloprid-resistance on the intrinsic rate of natural increase in the populations of *S. litura*[61], deltamethrin and indoxacarb-resistant *H. virescens*[67], spinosad-resistant in *P. xylostella*[68] have been documented. However, the results of this experiment provide a more realistic understanding of resistance mechanisms by taking into account the effects of the plant secondary metabolic gossypol as an induced resistance to deltamethrin in *S. exigua.*

Our results demonstrate that life table parameters of *S. exigua* were found to be adversely affected by the Delta-SEL and Gos-SEL treatments which reflect the adverse effects on population growth traits. Specifically, S_{xj} significantly decreased in the Delta-SEL population as well as f_{xj} and m_x which showed a marked reduction in the Delta-SEL population compared to the Gos-SEL population, and V_{xj} was significantly different for the Delta-SEL population. Also, $e_{xj,}$ a measure of the contribution of new individuals in population growth, declined sharply in second stage larval instars in the Delta-SEL population and showed marked increased for subsequent instars. These results, therefore, show that after ingestion of gossypol the *S. exigua* population may have developed a higher resistance to deltamethrin.

Almost all phytophagous insects utilize diverse mechanisms of metabolic detoxification to evade the toxicity of plant secondary metabolites and synthetic insecticides. Overexpression of these detoxifying enzymes, capable of metabolizing insecticides and phytotoxins, can result in higher levels of metabolic tolerance/resistance to synthetic insecticides and plant secondary metabolites[34,69]. Our results show that the activities of esterase (EST) (163.17 nmole/min/mg pro) and cytochrome P450 monooxygenase (P450) (1.467 nmole/min/mg pro) might play a key role in resistance development to deltamethrin after ingestion of gossypol in *S. exigua*. The activities of these enzymes significantly increased in Gos-SEL population compared to the activities of esterase (EST) (133.39 nmole/min/mg pro) and cytochrome P450 monooxygenase (P450) (1.194 nmole/min/mg pro) of the Delta-SEL population. Our results are in agreement with those previous studies[70] which have suggested that the resistance to deltamethrin may be associated with the increase of esterase activity. It has also been indicated that the esterase's inhibitor dramatically potentiates the toxicity of metaflumizone against the field-evolved resistant populations of *S. exigua*[71]. Additionally, elevated CarE enzyme activity in quercetin-fed larvae of *H. armigera* has been shown to contribute to the induced tolerance to lambda-cyhalothrin insecticide[72]. Elevated P450 enzyme activities in the greater midgut have also been linked to induced tolerance to deltamethrin insecticides in gossypol-fed cotton bollworm larvae[34]. As a result, the occurrence of existing plant secondary metabolites provides a selective pressure for the herbivorous insects to develop a rich pool of defense genes, which is likely to be a contributing factor in the rapid acquisition of pyrethroid resistance in beet armyworm. To the author's knowledge, this is the first study of resistance and fitness cost of deltamethrin after ingestion of gossypol in *S. exigua*.

We also investigated the biochemical mechanisms toward deltamethrin resistance in laboratory-reared populations of *S. exigua*. These data are useful for the application of

deltamethrin resistance management in the field. Indeed, quick shifts in the susceptibility of *S. exigua* to deltamethrin have started to occur. An effective resistance management strategy when using conventional pesticides may be the rotation or mixture with another insecticide in the field, applied in succession to avoid or retard the further development of insect pest resistance to deltamethrin. Future studies may benefit from examining the role of different pathways in detoxification enzymes of *S. exigua* gene expression responses to insecticides and phytotoxins.

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Table 1 Toxicity of Delta-SEL, and Goss-SEL strains of *Spodoptera exigua* to deltamethrin insecticides

Generations	**Insecticides**	**LC_{50} (mg l^{-1}) (95% FL)**	**Slope ± S.E.**	**X^2**	**df**	**RR[a]**
UNSEL-(G1)	Deltamethrin	0.59(0.48-0. 69)	1.9±0.17	0.13	-------	-------
Gos-SEL (G1)		0.52(0.41-0.63	1.5+0.15	0.23		
Delta-SEL-(G2)	Deltamethrin	1.2(0.99- 1.44)	1.8±0.18	0.43	4	2.03
Gos-SEL (G2		1.59(1.32-1.88)	1.7±0.15	0.40	4	2.69
Delta-SEL (G3)	Deltamethrin	2.74(2.14- 3.41)	1.4±0.15	0.56	4	4.64
Gos-SEL (G3)		3.82(3.03-4.73)	1.5±0.16	1.10	4	6.47
Delta-SEL (G4)	Deltamethrin	2.99(2.35- 3.687)	1.5±0.16	0.69	4	5.07
Gos-SEL (G4)		3.36(2.66-4.15)	1.56±0.16	0.53	4	5.69
Delta-SEL (G5)	Deltamethrin	8.34(6.97-9.75)	2.07±0.19	3.35	4	14.14
Gos-SEL (G5)		11.51(9.75-13.41)	1.97±0.17	1.61	4	19.51
Delta-SEL (G6)	Deltamethrin	14.42(11.48-17.75)	1. 6±0.17	0.97	4	24.44
Gos-SEL (G6		17.97(14.68-22.05)	1.3±0.14	0.73	4	30.46
Delta-SEL (G7)	Deltamethrin	18.23(14.48-22.44)	1.4±0.15	0.98	4	31.02
Gos-SEL (G7)		27.65(21.19-36.63)	0.98±0.13	1.72	4	46.86
UNSEL-(G8)	Deltamethrin	26.22(21.58-31.79)	1.4±0.13	1.44	4	44.44
Gos-SEL (G8)		34.30(29.57-39.26)	2.30±0.24	1.28	4	58.14
Delta-SEL (G9)	Deltamethrin	33.54(27.59-40.60)	1.5±0.14	1.71	4	56.86
Gos-SEL (G9)		42.20(35.15-49.95)	1.6±0.16	1.91	4	71.53
Delta-SEL (G10)	Deltamethrin	41.16(34.11-49.72)	1.6±0.17	2.00	4	69.76
Gos-SEL (G10)		66.84(57.66-76.55)	2.2 ±0.26	3.38	4	113.29

Numbers of larvae exposed in bioassay, including control were 270.

a RR = Resistance ratio, calculated as (LC50 of Goss-SEL and Delta-SEL) / (LC50 of UNSEL strain)

Table 2 Pre-Adults developmental time and pupal wet (mg) (Mean ± SE) of the Gos-SEL and Delta-SEL strains of *Spodoptera exigua*

Parameters	**Lab-strain**	**Gos-SEL Strain**	**Delta-SEL Strain**
Egg period (d)	3.01 ± 0.01 a	3.01 ± 0.01 a	3.02 ± 0.02 a
1st Instar (d)	3.00 ± 0.22 c	3.21 ± 0.05 b	3.71 ± 0.08 a
2nd Instar (d)	3.01 ± 0.11 b	3.07 ± 0.04 b	3.41 ± 0.08 a
3rd Instar (d)	2.97 ± 0.16 a	2.93 ± 0.03 a	3.03 ± 0.05 a
4th Instar (d)	2.95 ± 0.22 a	2.93 ± 0.03 a	3.02 ± 0.07 a
5th Instar (d)	2.96 ± 0.2 c	3.1 ± 0.05 a	3.52 ± 0.11 b
larval (d)	17.89 ± 0.43 c	18.27 ± 0.79 b	19.35 ± 0.17 a
Pupal (d)	7.25 ± 0.55 c	7.45 ± 0.09 b	8.82 ± 0.15 a
Pupal wet (mg)	126.27 ± 0.91a	123.98 ±1.835 a	113.26 ± 0.841 b

Means followed by the same letters in the same rows are not significantly different based on the paired bootstrap test at the 5% significance level. 90 insects were used for each treatment

Table 3 Adult longevity (d), APOP, TPOP, Ovi-day, Fecundity, and MGT (Mean ± SE) of the Gos-SEL and Delta-SEL strains of *Spodoptera exigua*

Parameters	**Lab-strain**	**Gos-SEL Strain**	**Delta-SEL Strain**
Adult longevity (d)	30.63 ± 1.14 c	27.68 ± 1.29 a	24.29 ± 1.33 b
Female longevity (d)	13.94 ± 0.36 b	13.61 ± 0.5 a	10.26 ± 0.54 b
Male longevity (d)	9.78 ± 0.5 b	9.22 ± 0.5 a	6.95 ± 0.42 b
APOP (d)	1.44 ± 0.12 a	1.50 ± 0.15 a	1.89 ± 0.17 a
TPOP (d)	26.67 ± 0.84 c	27.78 ± 0.24 b	31.21 ± 0.37 a
Ovi-day	6.56 ± 0.17 a	6.06 ± 0.29 a	4.42 ± 0.36 b
Fecundity (eggs/female)	532.67 ± 4.17 a	479.22 ± 17.18 b	273.58 ± 23.78 c
MGT[a]	36.62 ± 0.45 a	36.40 ± 0.51 a	34.15 ± 0.54 b

Means followed by the same letters in the same rows are not significantly different based on the paired bootstrap test at the 5% significance level. 90 insects were used for each treatment

Table 4 Pre-adults Survival %, pupal survival (%) Pupa, Adult's survival (%) and Hatchability (%) (Mean ± SE) of the Gos-SEL and Delta-SEL strains of *Spodoptera exigua*

Parameters	**Lab-strain**	**Gos-SEL Strain**	**Delta-SEL Strain**
1^{ST}-2^{ND} instar survival (%)	88.62 ± 1.64 a	83.337 ± 1.0912 a	67.650 ± 1.848 b
3^{RD} – 5^{TH} instar survival (%)	91.20 ± 1.12 a	77.053 ± 0.563 b	61.230 ± 1.088 c
Pupal survival (%)	94.43 ± 0.89 a	89.367 ± 1.4471 a	77.500 ± 1.905 b
Emergence rate of healthy Adult (%)	94.96 ± 1.16 a	90.163 ± 2.283 ab	81.630 ± 2.933 b
Hatchability (%)	93.16 ± 0.94 a	84.517 ± 1.128 b	63.966 ± 1.96 c

All means ± S.E. are based on three replicates within rows, means followed by the same letter did not differ significantly

Table 5 Mean generation time, Net reproductive rate, intrinsic rate of increase, and Finite rate of increase of the Gos-SEL and Delta-SEL strains of *Spodoptera exigua*

Parameters	**Lab-strain**	**Gos -SEL Strain**	**Delta-SEL Strain**
Intrinsic rate of increase (r) day^{-1}	0.149 ± 0.007 a	0.143 ± 0.007 b	0.115 ± 0.007 c
Net reproductive rate (R_0)	106.56 ± 22.44 a	95.89 ± 20.51 a	57.68 ± 12.72 a
Mean generation time (T) (days)	31.005 ± 0.28 b	31.67 ± 0.25 b	34.97 ± 0.36 a
Finite rate of increase (λ)(day^{-1})	1.161 ± 0.008 a	1.154 ± 0.008 a	1.12 ± 0.007 b

Means followed by the same letters in the same rows are not significantly different based on the paired bootstrap test at the 5% significance level. 90 insects were used for each treatment

Table 6 Esterase-αNA, P450 PNOD, and GST-DCNB (glutathione S-transferase) activity (Mean ± SE) against Gos-SEL and Delta-SEL Strain in *Spodoptera exigua*

Enzyme	**Lab-strain**	**Gos-SEL Strain**	**Delta-SEL Strain**
Esterase-αNA (nmole per min per mg pro)	119.93 ± 2.97 c	163.17 ± 3.049a	133.39 ± 2.043b
P450 PNOD (nmole per min per mg pro)	0.525 ± 0.026 c	1.467 ± 0.0578a	1.194 ± 0.0433b
GST-DCNB (nmole per min per mg pro)	40.52 ±1.42 a	44.933 ± 1.411a	40.240 ± 1.211a

All means ± S.E. are based on three replicates within rows, means followed by the same letter are not significantly different