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Sublethal effects of pyriproxyfen, a juvenile hormone analogue, on *Plutella xylostella* (Lepidoptera: Plutellidae): life table study

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The sublethal effects of pyriproxyfen (PYR, a juvenile hormone analogue) were investigated for two consecutive generations on life-history parameters, such as developmental time, pupal weight, fecundity, fertility and longevity of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). After topical application bioassay of third instar larvae, the LC_{25} and LC_{50} values (as sublethal doses) were determined to be 1.49 and 2.01 $\mu g/\mu l$, respectively. The results showed that the egg incubation period, larval and pupal duration times and oviposition period were increased in treated groups compared with the control (treated with distilled water). Pupal weight, adult longevity, fecundity and fertility were significantly reduced in the treatment groups compared with the control. Using the age-stage, two-sex life table analysis, we found that the intrinsic rate of increase (r), finite rate of increase (λ), gross reproduction rate (GRR) and net reproductive rate (R_0) significantly decreased while the mean generation time (T) and doubling time (DT) increased in two treatment groups compared with the control. In addition, administration of PYR induced morphogenetic abnormalities including untanned pupae, larval–pupal intermediates and various defective adults. The LC_{50} value was more effective on all of the above biological characteristics than the LC_{25} for both generations, although parents were more affected than the offspring. In general, our results revealed that PYR was highly effective against *P. xylostella* in the laboratory both directly (causing mortality) and indirectly (disruption of normal growth and development). We conclude that PYR is an excellent candidate for suppressing populations of *P. xylostella* through its sublethal effects.

Keywords: *Plutella xylostella*; pyriproxyfen; sublethal concentrations; age-stage; two-sex life tables; sublethal effects; life table

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive cosmopolitan insect pests of cruciferous plants in many parts of the world (Talekar and Shelton 1993). In many countries, *P. xylostella* has

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developed multiple- and cross-resistance to a wide range of conventional organic insecticides as well as *Bacillus thuringiensis* (Bt) products (Tabashnik et al. 1990; Tabashnik 1994; Zhao et al. 2002, 2006; Sarfraz and Keddie 2005; Raymond et al. 2007; Qian et al. 2008; Sayyed et al. 2008; Gassmann et al. 2009; Nehare et al. 2010; Santos et al. 2011). The development of insecticide resistance in field populations of DBM due to the high frequency of insecticides application has necessitated the use of alternative strategies and new management tactics. The use of very low doses of insecticides having strong sublethal effects represents an environmentally friendly option to improve existing integrated pest management strategies (Sial and Brunner 2010). The sublethal effects of pesticides influence the physiological or behavioural responses of individuals that survived prior exposure to pesticides (Desneux et al. 2004). The sublethal concentrations of insecticides may affect a number of physiological parameters including weight of larvae, pupae and adults (Yin et al. 2008; Nasr et al. 2010), fecundity, fertility (Perveen and Miyata 2000; Liu and Trumble 2005), egg size (Yin et al. 2008), adult longevity (Gerig 1975) and development speed (Cripe et al. 2003). They may also alter sex ratios (Shaalan et al. 2005) and affect behavioural parameters influencing feeding (Nasr et al. 2010), searching and oviposition (Dabrowski 1969; Fujiwara et al. 2002). The sublethal doses also affect reproduction parameters such as the intrinsic rate of increase (r), finite rate of increase (λ), gross reproduction rate (GRR), net reproductive rate (R_0), mean generation time (T) and doubling time (DT) (Zanuncio et al. 2005; Yin et al. 2008). A useful component of an integrated pest management is the use of insect growth regulators (IGRs) such as juvenile hormone analogues (JHAs) (Kostyukovsky et al. 2000; Sial and Brunner 2010). Pyriproxyfen (PYR), a JHA, mimics the action of juvenile hormones in a number of physiological processes and is a potent inhibitor of embryogenesis, metamorphosis and adult formation (Ishaaya and Horowitz 1992). In addition, it has been shown to have sterilising and toxic activities on many insects in a variety of orders, including Dictyoptera (Koehler and Patterson 1991), Hymenoptera (Reimer et al. 1991), Diptera (Langley et al. 1993), Orthoptera (Vennard et al. 1998), Homoptera (Liu and Chen 2001; Boina et al. 2009), Siphonoptera (Rajapakse et al. 2002), Coleoptera (Abo-Elghar et al. 2004) and Lepidoptera (Hatakoshi et al. 1991; Yokoyama and Miller 1991; Oouchi 2005; Kwon and Kim 2007; Sial and Brunner 2010). The objective of the present work was to evaluate the insecticidal activity and sublethal effects of PYR on life-history parameters of *P. xylostella*. In particular, the effects of PYR were observed on the mortality, developmental time of immature stages, pupal weight, adult fecundity, fertility, longevity and the incidences of various abnormalities in *P. xylostella* for two sequential generations.

Materials and methods

Insects culturing procedure

Plutella xylostella larvae and adults with no history of exposure to insecticides were originally collected from *Brassica* fields at the Horticultural Investigation Center of Tehran University in Karaj (Alborz province, Iran) during the 2009–2010 growth seasons. Chinese cabbage (*B. pekinensis*) cv. Spring Smile (Kumochon-Dong Co., Seoul, South Korea) was grown under greenhouse conditions (25 $\pm$ 5°C, 65 $\pm$ 10% RH and a photoperiod of 16L:8D). The stock culture of DBM was maintained on

8-week-old Chinese cabbage in screened hyaline cages (40 × 40 × 40 cm) under standard constant environment (25 ± 1°C, 65 ± 5% RH and a photoperiod of 16L:8D h).

Bioassay and determination of sublethal doses

The concentrations of 1.5, 1.75, 2, 2.4, 2.8 and 3 μg/μl of PYR (ADMIRAL® 10% EC; Sumitomo Chemical Co, Japan) were made using distilled water. To perform a bioassay, 0.5 μl of the chemical was topically applied onto the dorsum of the thorax of early third instar larva using a Hand micro-applicator (Burkard Manufacturing Co., Rickmansworth, UK). Batches of 15 treated larvae were transferred to fresh Chinese cabbage leaf discs (6 cm diameter) within individual Petri dishes (9 cm diameter, with a 20-mesh polyester net embedded in the lid for ventilation) and maintained in a growth chamber set at 25 ± 1°C, 65 ± 5% RH and a photoperiod of 16L:8D h. All treatments were replicated four times, and mortality was recorded after 96 h. Finally, the LC_{25} and LC_{50} values were selected as sublethal concentrations for the experiments.

Effects of sublethal doses on biological characteristics of parent generation

The sublethal doses of PYR were applied on 100 early third instar larva as explained in the previous section. Thereafter, larvae were individually placed on fresh Chinese cabbage leaf discs (3 cm diameter) in Petri dishes (5 cm diameter) and allowed to develop to pupation. To prevent starvation of larvae, the leaf discs were replaced every 12 h. The early pupae were preserved at 4°C until all the larvae had pupated and then the pupae were kept at room temperature until adult emergence. The experiments were conducted under controlled environment conditions (25 ± 1°C, 65 ± 5% RH and 16L:8D h). Life stage, mortality and deformities were recorded every 4 h until all the insects had either died or emerged as adults. The pupal weight was measured using an OHAUS Analytical Plus AP250D digital scale (OHAUS® Co. USA).

To measure fecundity and biological parameters of the DBM, a pair of moths (male and female) that emerged on the same day was placed into a Petri dish (5 cm diameter) containing a fresh Chinese cabbage leaf disc (3 cm diameter) to mate and lay eggs. The eggs laid on the Petri dishes wall and leaves were counted every 12 h and the Petri dish replaced with a fresh one until the females died. Twenty-five pairs of *P. xylostella* adults were used for each treatment. At the end of the experiment, adult sizes were measured using a stereoscopic microscope (Stemi V6, ZIESS Germany) fitted with a drawing tube.

Effects of sublethal doses on biological characteristics of offspring generation

One hundred eggs were obtained from parent adults of each treatment. Each egg was placed in a Petri dish similar to that described in the previous section. The egg incubation, larval and pupal periods, pupal weight, mortality and abnormalities were recorded as in the previous generation. Following the emergence of adults, we paired one male with one female as above. Thereafter, we supplied fresh Chinese cabbage leaf discs for oviposition and recorded the fecundity every 12 h until the death of all individuals.

Age-stage, two-sex life table analysis

All data obtained on the survival rates of adults and those individuals that died before maturity, and the female daily fecundity as described above, were analysed according to the age-stage, two-stage life table theory (Chi and Liu 1985; Chi 1988). The age-stage specific survival rate (s_{xj}) (where $x =$ age in days and $j =$ stage; the first, second, third, fourth and fifth stages are the egg, larval, pupal, adult including female and male, respectively), the age-stage specific fecundity (f_{xj}), the age-specific survival rate (lx), the age specific fecundity (m_x) and the population parameters (r, the intrinsic rate of increase; λ, the finite rate of increase, $\lambda = e^r$; R_0, the net reproductive rate; T, the mean generation time) of the offspring are calculated accordingly. The intrinsic rate of increase is calculated iteratively from the Euler–Lotka equation with age indexed from 0 (Goodman 1982):

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

In the age-stage, two-sex life table, according to Chi and Liu (1985), the l_x and m_x are estimated as:

$$l_x = \sum_{j=1}^{k} s_{xj} \tag{2}$$

and

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} \, f_{xj}}{\sum_{j=1}^{k} s_{xj}} \tag{3}$$

where k is the number of stages (Chi and Liu 1985). The GRR is defined as follows, where δ is the last age of the cohort.

$$\mathrm{GRR} = \sum_{x=o}^{\delta} m_x. \tag{4}$$

The net reproduction rate (R_0) is calculated as $R_0 = F(N_f/N)$, where F is the mean female fecundity, N is the total number of individuals used for the life table study and N_f the number of female adults (Chi 1988). The mean generation time (T) is the period of time that a population needs to increase to R_0-fold of its size at the stable age-stage distribution and is estimated by using the following formula: $T = [(ln \ R_0)/r]$. To simplify analysis of the raw data, the computer program, TWOSEX-MSChart (Chi 2008), was used for data analysis and the jackknife method (Sokal and Rohlf 1995) in Visual BASIC for the Windows operating system. [The computer program is available at http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey)].

Statistical analysis

The data on larval cumulative percentage mortality were corrected for control mortality by using the Schneider Orelli's formula (Püntener 1981). The LC_{25} and LC_{50} values were estimated using probit analysis, carried out by the statistical program POLO-PC (Leora Software 1987). The sublethal effects of PYR on the moth biological characteristics were analysed using the PROC General Linear Model [The same applies for following tests, as well]. Pairwise comparisons were performed using Fisher's Least Significant Difference (F-LSD) test. All statistical analyses (GLM and pairwise comparisons) were completed in SYSTAT 12.02 (SYSTAT Software 2007).

Results

Insecticidal activity of PYR and determination of sublethal concentration

The responses of third instar larvae of *P. xylostella* to PYR were dose-dependent (Figure 1). The LC_{25} and LC_{50} values measured 96 h after treatments were 1.49 and 2.01 $\mu g/\mu l$, respectively, and these concentrations were selected as sublethal doses for the experiments (Table 1).

Effect of sublethal doses on developmental period of P. xylostella

Developmental times of subimago stages including egg, larva and pupa were significantly increased by PYR treatment during both generations (Table 2). Due to direct application of sublethal doses on parents, PYR affected the developmental times of parents more than the offspring generation (see Table 2 for more details).

Percentage mortality, pupal weight, adult emergence, size and sex ratio

In both generations and especially in the parent, mortality of larva and pupa was remarkably increased by sublethal concentrations of PYR (see Tables 3 and 4 for

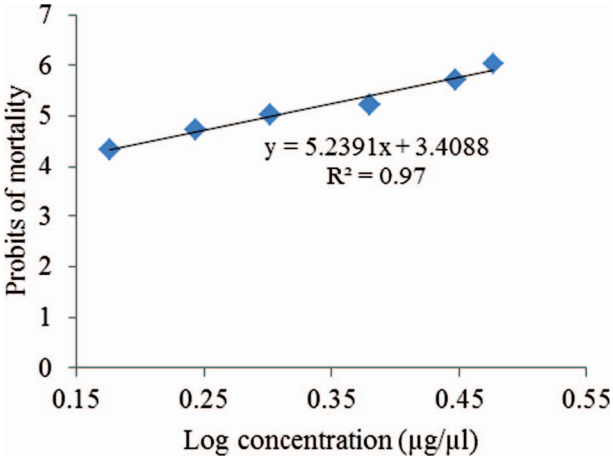

Figure 1. Corrected mortality percentage rate equivalent to probit unit of the third-instar larvae of *Plutella xylostella* caused by preparation of pyriproxyfen at different concentrations.

Table 1. Comparison of results of probit, Polo-PC analysis of dose response data for pyriproxyfen applied to early third instar larvae of *P. xylostella*.

IGR	N[1]	Slope ± SE	*df*	χ^2	LC_{50} ($\mu g/\mu l$) (95% CI)	LC_{25} ($\mu g/\mu l$) (95% CI)
Pyriproxyfen	360	5.164 ± 0.694	4	1.45	2.01 (1.868–2.15)	1.49 (1.2–1.6)

[1]Number of *P. xylostella* larvae assayed.

more details). In the parent generation, the pupal mortality of groups treated with the LC_{25} and LC_{50} doses was 15.73- and 20.60-fold more than those of the control treatment. The pupal weight was diminished by 1.11- and 1.22-fold, respectively. Adult emergence of those given the LC_{25} and LC_{50} treatments decreased to 0.75 and 0.64 of that in the control (Table 3). In the parent generation, adult abnormalities such as twisted wings increased from zero in the control to 13.75% and 25.12% in the LC_{25} and LC_{50} treatments, respectively (Table 3). The adult size of LC_{25} and LC_{50} groups were 1.13- and 1.19-fold (male wingspan), 1.16- and 1.20-fold (female wingspan), 1.08- and 1.16-fold (male length) and 1.09- and 1.14-fold (female length) smaller than the untreated group (Table 3). The indirect effects of the two sublethal doses of PYR on the next generation (offspring) obtained from treated parents included an increase in egg mortality (unhatched eggs). This was 5.5- and 9.01-times greater in LC_{25} and LC_{50} treatment groups than in the control (Table 4). The LC_{25} and LC_{50} values led to 4.13- and 6.81-fold enhancement in larval mortality compared with the control (Table 4). The offspring pupal mortality caused by LC_{25} and LC_{50} values was 7.39- and 9.38- times higher than in the untreated group. Moreover, in the offspring, normal adult emergence from pupae was significantly reduced in PYR-treated groups. Indirect effects of PYR led to other anomalous conditions in adults in the LC_{25}- and LC_{50}-treated groups about 3.44- and 5.96-times more than in the controls (Table 4). Furthermore, treatment with the LC_{25} and LC_{50} values of PYR significantly reduced the pupal weight to 1.07- and 1.16-times lower than those in the control group (Table 4). There was also an obvious decrease in the adult sizes of *P. xylostella* treated with the LC_{25} and LC_{50} dose of PYR. Wingspan decreased to between 0.89 and 0.93 times (males) and 0.88 and 0.92 times (females) that of untreated moths. Overall body length was 0.89–0.96 times (males), and 0.91 and 0.97 times (females) that of untreated moths (Table 4). The number of emerged females and males showed that the sex ratio [number of female/(female + male)] was close to 1:1 in all treatments, and there were no differences between treatments (see Table 4 for more details).

Effect of sublethal doses on adult performance

Treatment with the LC_{25} and LC_{50} values of PYR significantly influenced the duration of adult pre-oviposition, oviposition, post-oviposition periods, longevity and fecundity of *P. xylostella* in both generations, though this was especially evident in the parent generation (see Tables 5 and 6 for more details). Compared with the control, pre- and oviposition periods of PYR-treated females were prolonged, but there was no significant difference between the LC_{25} and LC_{50} treatments. The post-oviposition period was shortened in both generations and it was significantlty shorter

Table 2. The effects of pyriproxyfen on developmental period of immature stages of *P. xylostella* in two successive generations.

	Developmental time (days; mean ± SE; n[1] = 100)										
	Parent generation				Offspring (F1 generation)						
Treatments	L_3	L_4	L_3-Pupation	Pupa	Egg	L_1	L_2	L_3	L_4	Egg-Pupation	Pupa
Control	2.07 ± 0.02a[2]	2.56 ± 0.01a	4.63 ± 0.02a	4.30 ± 0.02a	2.39 ± 0.02a	2.18 ± 0.02a	2.05 ± 0.03a	2.14 ± 0.02a	2.54 ± 0.02a	11.30 ± 0.05a	4.25 ± 0.03a
LC_{25}	3.67 ± 0.07b	3.11 ± 0.05b	6.75 ± 0.09b	5.26 ± 0.06b	3.11 ± 0.02b	2.36 ± 0.04b	2.25 ± 0.04b	2.36 ± 0.03b	2.82 ± 0.03b	12.82 ± 0.09b	5.12 ± 0.04b
LC_{50}	4.02 ± 0.05c	3.79 ± 0.05c	7.86 ± 0.07c	5.81 ± 0.07c	3.83 ± 0.04c	2.93 ± 0.04c	2.86 ± 0.04c	2.99 ± 0.04c	3.87 ± 0.05c	16.45 ± 0.12c	5.25 ± 0.05b
df[3]	2,231	2,194	2,194	2,154	2,259	2,235	2,219	2,206	2,184	2,187	2,156
F	486.39	280.23	782.12	316.719	768.5	140.5	138.9	155.4	433.1	8351.4	239.4
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Total number of third instar larvae that assayed by estimated LC25 and LC50 of PYR., Control, distilled water. L_1, L_2, L_3 and L_4 denote the first, second, third and fourth instar moth larvae, respectively. [2]Means followed by the same letter within columns are not significantly different ($p < 0.05$; Fisher-LSD Test). [3]The numbers show the degrees of freedom of treatment and error, respectively.

Table 3. The effect of pyriproxyfen on larval mortality, pupation, adult emergence and size (mean ± SE) of *P. xylostella* (Parent).

	Larvae ($n^1 = 100$)	Pupa		Adult					
Treatment	Mortality% (mean ± SE)	Mortality% (mean ± SE)	PW ($\times 10^{-5}$ g)	Emergence%	Abnormality%	MW	FW	ML	FL
Control	6.00 ± 1.02a[3]	2.12 ± 0.03a	628.53 ± 13.15c	96.78 ± 1.64c	0.0 ± 0.0a	13.67 ± 0.09a	15.03 ± 0.05a	5.83 ± 0.09a	5.76 ± 0.06a
LC_{25}	36.25 ± 1.69b	34.52 ± 4.02b	520.05 ± 17.41b	72.33 ± 5.59b	13.75 ± 0.07b	12.05 ± 0.08b	12.94 ± 0.06b	5.38 ± 0.07b	5.30 ± 0.05ab
LC_{50}	61.00 ± 2.80c	46.15 ± 3.69c	408.94 ± 16.95a	61.67 ± 4.05a	25.12 ± 0.10c	11.50 ± 0.06c	12.45 ± 0.08c	5.01 ± 0.04b	5.05 ± 0.03bc
df[2]	2,297	2,194	2,91	2,140	2,140	2,72	2,72	2,72	2,72
F	1980.8	391.97	42.08	147.4	22.4	77.78	105.28	7.64	3.21
p	<0.001	<0.001	<0.01	<0.001	<0.001	<0.01	<0.001	0.001	0.046

[1]Total number of third instar larvae that assayed by estimated LC_{25} and LC_{50} of PYR. [2]The numbers given show the degrees of freedom of treatment and error, respectively. PW, pupal weight (mean ± SE), weight that was recorded within 24 h after pupation; MW, male wingspan; FW, female wingspan; ML, male length; FL, female length. [3]Means followed by the same letter within columns are not significantly different ($p < 0.05$; Fisher-LSD Test).

Table 4. The effect of pyriproxyfen on larval mortality, pupation, adult emergence, sex ratios and size (mean ± SE) of *P. xylostella* (offspring).

	Egg (n = 100)	Larvae	Pupa		Adult						
Treatment	Mortality % (mean ± SE)	Mortality % (mean ± SE)	Mortality% (mean ± SE)	PW ($\times 10^{-5}$ g)	Emergence %	Abnormality %	Sex ratio	MW	FW	ML	FL
Control	2.48 ± 1.02a[1]	7.14 ± 0.04a	3.29 ± 1.03a	608.7 ± 12.45a	97.05 ± 1.57a	1.42 ± 1.38a	50.50 ± 3.41	13.72 ± 0.11a	14.95 ± 0.10a	5.93 ± 0.08a	5.81 ± 0.08a
LC_{25}	13.64 ± 1.05b	28.73 ± 2.02b	22.58 ± 2.37b	558.33 ± 12.91b	84.83 ± 5.32b	9.5 ± 3.08bb	48.42 ± 4.15	12.07 ± 0.06b	13.68 ± 0.07b	5.68 ± 0.06b	5.62 ± 0.05ab
LC_{50}	22.35 ± 2.18c	48.05 ± 3.05c	30.16 ± 2.04c	454.7 ± 11.4b	75.92 ± 6.14c	14.81 ± 0.11b	45.38 ± 3.18	12.19 ± 0.08b	13.17 ± 0.04c	5.26 ± 0.07c	5.28 ± 0.05bc
df[2]	2,297	2,259	2,190	2,123	2,159	2,159	–	2,72	2,72	2,72	2,72
F	410.5	815.7	336.15	33.14	43.22	16.47	–	124.78	127.36	26.59	18.07
p	< 0.001	< 0.001	0.001	0.01	0.002	0.007	–	< 0.01	< 0.01	0.027	< 0.01

PW, pupal weight (mean ± SE), weight that was recorded within 24 h after pupation; MW, male wingspan; FW, female wingspan; ML, male length; FL, female length. [1]Means followed by the same letter within columns are not significantly different ($p < 0.05$; Fisher-LSD Test). [2]The numbers given show the degrees of freedom of treatment and error, respectively.

Table 5. The effect of pyriproxyfen on adult fitness and performance of *P. xylostella* in parent.

	Parent (n = 100[1], sex ratio:1:1)						
	Total oviposition period (mean ± SE; days)			Longevity (mean ± SE; days)		Reproduction potential (mean ± SE; number)	
Treatments	Pre-ovi	Ovi.	Post-ovi.	Female	Male	Fecundity[2]	% Egg viability[3]
Control	0.42 ± 0.01a[4]	7.84 ± 0.09a	19.19 ± 0.09c	27.45 ± 0.62a	31.10 ± 0.55c	300.2 ± 4.39c	97.12 ± 0.15c
LC_{25}	1.75 ± 0.55b	11.13 ± 0.84b	4.51 ± 0.67b	17.39 ± 0.76b	17.08 ± 0.82b	131.2 ± 10.90b	77.02 ± 6.00b
LC_{50}	2.84 ± 1.14b	9.55 ± 2.29b	0.63 ± 0.15a	13.02 ± 1.57c	13.98 ± 1.37a	89.00 ± 17.08a	60.83 ± 7.45a
df[5]	2,64	2,64	2,64	2,70	2,71	2,64	2,62
F	368.65	141.74	1042.9	82.50	156.3	282.66	511.15
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Total number of third instar larvae that assayed by estimated LC_{25} and LC_{50} of PYR. [2]Total number of eggs laid by each female. [3]Percentage of hatched eggs per adult females. [4]Means sharing same letters in a column are not significantly different from each other ($p < 0.05$; Fisher-LSD Test). [5]The numbers given show the degrees of freedom of treatment and error, respectively.

Table 6. The effect of pyriproxyfen on adult fitness and performance of *P. xylostella* in offspring (F1 generation).

	Offspring (n = 100[1], sex ratio:1:1)						
	Total oviposition period (mean ± SE; days)			Longevity (mean ± SE; days)		Reproduction potential (mean ± SE; number)	
Treatments	Pre-ovi	Ovi.	Post-ovi.	Female	Male	Fecundity[2]	% Egg viability[3]
Control	0.36 ± 0.01a[4]	7.36 ± 0.12a	20.55 ± 0.48a	28.26 ± 0.50a	31.11 ± 0.61a	322.85 ± 4.62a	97.38 ± 0.18 a
LC_{25}	1.41 ± 0.16 b	9.85 ± 0.90b	7.20 ± 1.23d	18.46 ± 1.47b	23.8 ± 2.07c	209.79 ± 11.16b	88.54 ± 4.94b
LC_{50}	1.90 ± 0.24c	9.11 ± 1.04b	2.94 ± 0.47c	13.94 ± 1.3c	15.39 ± 1.26b	168.78 ± 14.73c	77.87 ± 3.32c
df[5]	2,66	2,66	2,66	2,70	2,74	2,66	2,64
F	96.2	22.2	242.5	69.48	36.91	95.2	122.85
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Total number of third instar larvae that assayed by estimated LC_{25} and LC_{50} of PYR. [2]Total number of eggs laid by each female. [3]Percentage of hatched eggs per adult females. [4]Means sharing same letters in a column are not significantly different from each other ($p < 0.05$; Fisher-LSD Test). [5]The numbers given show the degrees of freedom of treatment and error, respectively.

with the larger treatment dose (see Tables 5 and 6 for more details). PYR-treatment caused a significant reduction in the adult longevity of both sexes in each of the two consecutive generations (Tables 5 and 6). Treatment with the LC_{50} dose was more effective than the LC_{25} dose in reducing adult longevity. There was not much difference in adult longevity between the two generation except that the offspring males of the LC_{25} treatment had a higher longevity than males of the parent generation (Tables 5 and 6). The experimental doses of PYR did, however, significantly reduce female fecundity and the percentage of viable eggs in each generation, especially in the parent generation. In the parent generation, the female fecundity values in the LC_{25}- and LC_{50}-treated groups were 0.43 and 0.29, respectively, compared to the control number of offspring. In the offspring, these values increased to 0.64 and 0.52 of those found for the control. The percentage of egg viability affected by the LC_{25} and LC_{50} treatments was 0.79 and 0.63 of the control in parent and 0.91 and 0.80 of the control in offspring (Tables 5 and 6).

Age-stage, two-sex life table of P. xylostella

In Figure 2, age-stage survival rates (s_{xj}) of *P. xylostella* in PYR-treated (LC_{25} and LC_{50} values) and untreated groups (control) are shown. s_{xj} indicates the probability that a newborn will survive to age x and develop to stage j. According to Chi and Yang (2003), since the variable developmental rates that occur among individuals of a population are consolidated into the age-stage, two-sex life table, the stage survival rate curves of a cohort show stage overlapping. Equations (2) and (3) were used to calculate the age-specific survival rates (l_x) and fecundity rates (m_x) (Figure 3). A single age-specific survival rate (l_x) gives the probability that an egg will survive to age x (Figure 3). The age-stage specific fecundity rate (f_{xj}) of female adult *P. xylostella* gives the number of eggs produced by adult females (the fourth stage, $j = 4$) of age x, where the age x is counted from the egg stage. Age specific fecundity (m_x) curves show that reproduction began at age 37 and 49 days in LC_{25} and LC_{50} treatments, respectively (Figure 3). However, the first reproduction in the untreated group (control) was at age 30 days. The ovipositional period of *P. xylostella* lasted about 23, 52 and 33 days for the control, LC_{25} and LC_{50} treatments respectively, but the number of eggs produced by adult females (f_{x4}) in PYR-treated were much lower than by the control (Figure 3).

Sublethal effects of PYR on biological parameters of the offspring

In the two treatment groups (LC_{25} and LC_{50}), the biological parameters of *P. xylostella* including net reproduction rate (R_0), GRR, intrinsic rate of increase (r), mean generation time (T), finite rate of increase (λ) and DT were significantlty affected by two the tested sublethal concentrations compared to the control (Table 7). In addition, there were significant differences in all mentioned parameters between the LC_{25} and LC_{50} treatments except GRR. Treatment with the LC_{25} and LC_{50} values significantly decreased the GRR to about 40% and 50%, and R_0 to approximately 70% and 90%, respectively, in comparision to the control. The intrinsic rate of increase (r) in the LC_{25} and LC_{50} groups declined to 0.63 and 0.39 of that in the control, respectively ($F_{2,297} = 61.17$, $p < 0.001$, $df = 2$, 297). The mean generation time (T) tended to be longer (7.36 and 17.25 days in the LC_{25} and LC_{50} treated *P. xylostella*) than that of the control ($F_{2,297} = 144.65$, $p < 0.001$,

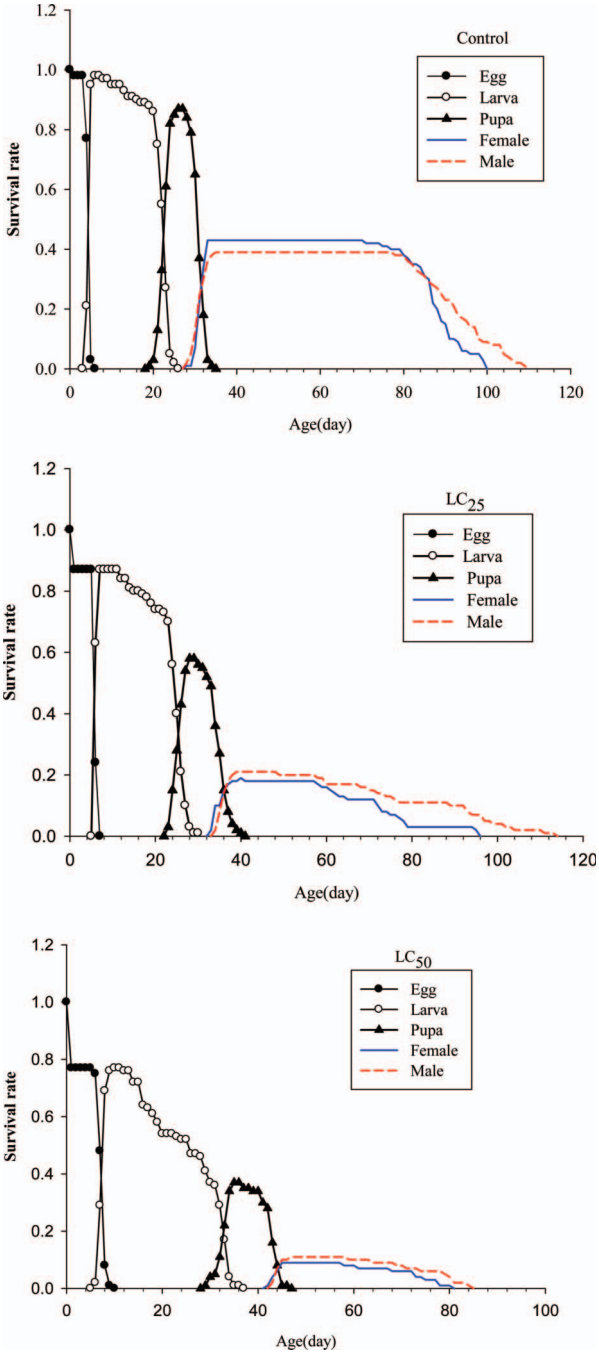

Figure 2. Age-stage survival rates (s_{xj}) of *P. xylostella* treated with sublethal concentrations of pyriproxyfen (LC_{25} and LC_{50}) and control (as untreated group).

df = 2, 297). The DT was affected by the LC_{25} and LC_{50} treatments and increased to 1.86- and 3.71-fold, respectively, of that in the control ($F_{2,297} = 429.25$, $p < 0.01$, df = 2, 297).

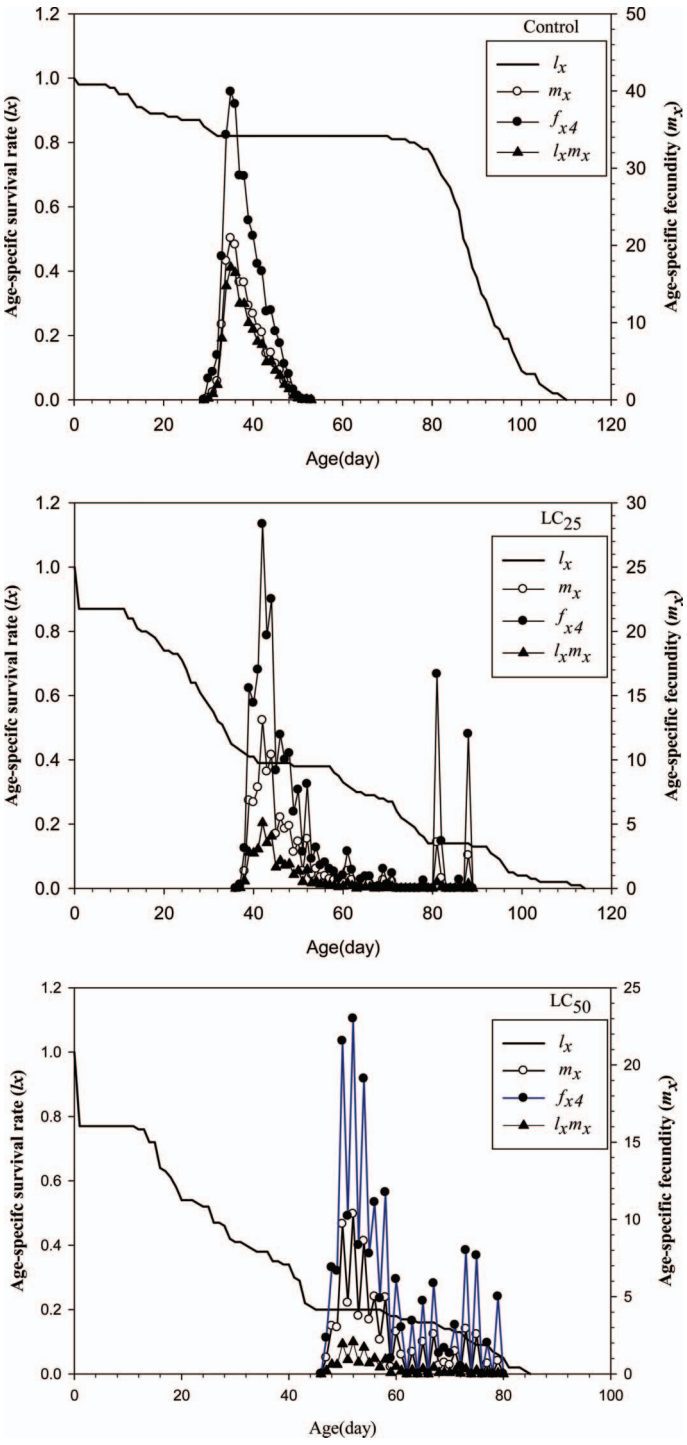

Figure 3. Age-specifc survival rate (l_x) and age-stage fecundity of female (f_{x4}) (eggs/female), and age-specific fecundity (m_x) of *P. xylostella* treated with sublethal concentrations of pyriproxyfen (LC_{25} and LC_{50}) and control (as untreated group) by using the age-stage, two-sex life table.

Table 7. Population parameters (mean ± SE) of *P. xylostella* treated with sublethal concentrations of pyriproxyfen and control (as untreated treatment), calculated by using the age-stage, two-sex life table.

Treatments	R_0[1]	GRR[2] (offspring)	$r(d^{-1})$[3]	T(d)[4]	$\lambda(d^{-1})$[5]	DT[6]
Control	139.11 ± 16.21c[7]	169.63 ± 18.12b	0.129 ± 0.003c[4]	38.22 ± 0.19a	1.138 ± 0.003c	5.67 ± 0.12a
LC_{25}	38.6 ± 8.38b	104.46 ± 20.12a	0.081 ± 0.005b	45.58 ± 0.76b	1.084 ± 0.006b	10.52 ± 0.31b
LC_{50}	14.81 ± 4.94a	80.71 ± 22.88a	0.050 ± 0.007a	55.47 ± 0.97c	1.05 ± 0.006a	21.06 ± 0.56c
df[8]	2,297	2,297	2,297	2,297	2,297	2,297
F	36.54	5.061	61.17	144.65	63.908	429.25
p	<0.01	0.007	<0.001	<0.001	<0.001	<0.01

[1]Net reproductive rate, [2]Gross reproductive rate, [3]Intrinsic rate of increase, [4]Mean generation time, [5]Finite rate of increase, [6]Doubling time, [7]Means sharing same letters in a column are not significantly different from each other ($p < 0.05$; Fisher-LSD Test). [8]The numbers given show the degrees of freedom of treatment and error, respectively.

Abnormalities caused by PYR treatment

PYR treatment caused a number of abnormalities in DBM larvae. These include failure of some larvae to completely shed their old cuticles (e.g. inability to withdraw from the head capsule – usually caused by entrapment of their mouth parts); larvae with disordered setae; arrested larval growth due to cessation of feeding; overlapping of the larval anal legs following ecdysis and subsequent death due a combination of factors including the cessation of feeding, failure of the larvae to pupate and the formation of larval–pupal intermediates; untanned pupae or pupae with imperfect sclerotisation; adults that failed to emerge and remain trapped in the cocoon, eventually dying; adults with twisted wings; females with reduced reproduction capacity due to either being sterile or having reduced fecundity and/or fertility and laying unviable eggs (Figures 4 and 5).

Discussion

Other insect species have also been shown to have dose-dependent responses to PYR, including *Choristoneura rosaceana* (Sial and Brunner 2010) and *Plodia interpunctella* (Ghasemi et al. 2010). On the other hand, the results obtained by Oochi (2005) on larvae of *P. xylostella* treated with PYR were the opposite of those obtained in the present study, as were those of Hatakoshi et al. (1987) studying *Manduca sexta*. Oochi (2005) suggested that this inverse relationship may be due to either a more rapid excretion or more readily breaking down of higher concentrations in the alkaline environment of the larval intestine. Moreover, in our study, the negative effects of the LC_{25} treatment on biological characteristics of *P. xylostella* were lower than the higher sublethal dose (LC_{50}). There are two JH sensitive periods in the last instar larvae of holometabola: in the first, JH must be absent in the hemolymph for the development of pupal characters to begin, and in the next, JH must be present to stabilise the pupal determined state of imaginal disk structures and prohibits their commitment to adult development (Nijhout 1994).

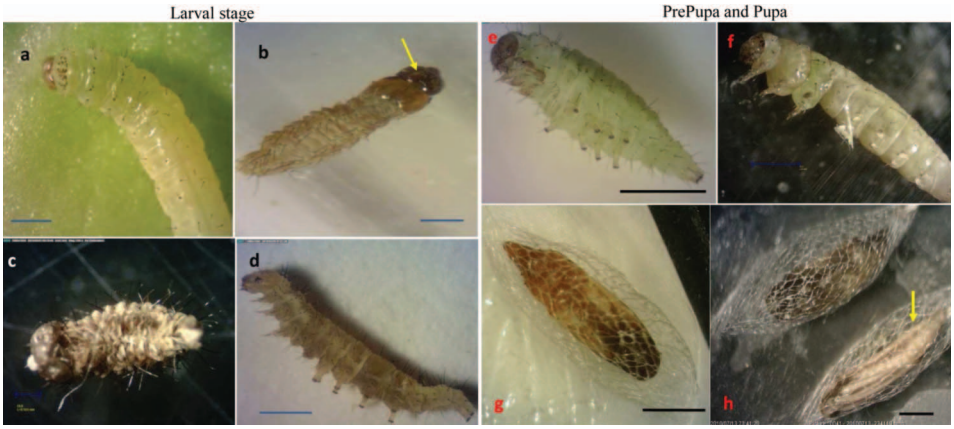

Figure 4. Various morphological abnormalities in treated *P. xylostella* with sublethal concentrations of pyriproxyfen at larval (a–d), prepupal and pupal (e–h) stages: (a) larva with disordered hairs; (b) inability to shed head capsule, old head capsule (arrow); (c and d) arrested larval growth; (e and f) larval–pupal intermediates; (g and h) untanned pupae, normal pupa (arrow); scale bar: 1 mm.

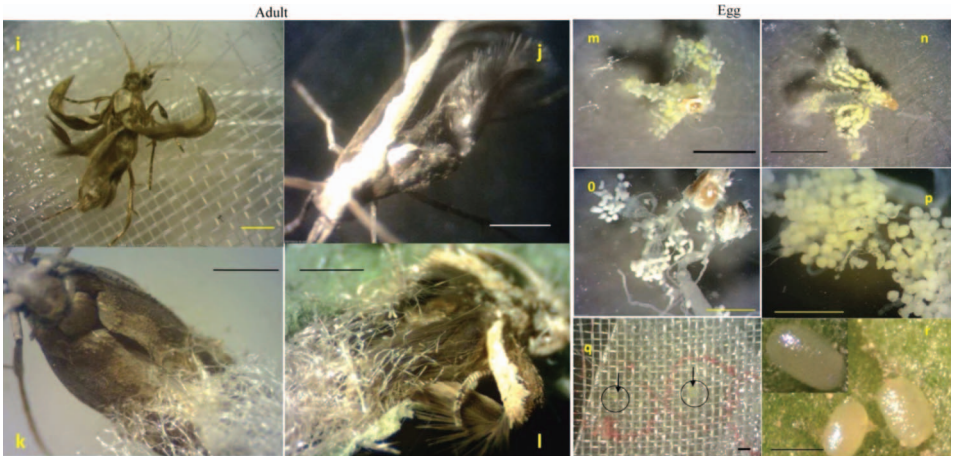

Figure 5. Various morphological abnormalities in treated *P. xylostella* with sublethal concentrations of pyriproxyfen at eggs and adults: (i and j) twisted wings in adults; (k and l) failure in adult emergence; (m and o) abnormal ovaries; (n) normal ovary; (p) deformed oocytes; (q) eggs without yolk; (r) normal eggs; scale bar: 1 mm.

Therefore, the prolonged duration of larval and pupal periods noticed in DBM, the production of larval–pupal intermediates, failure to shed the old larval cuticule, other morphological abnormalities and mortality may be a result of the existence of PYR during the critical time of the DBM life cycle. The production of larval–pupal intermediates as a result of exposure to PYR in *C. rosaceana* (Sial and Brunner 2010) and *Spodoptera littoralis* to JHI (Khafagi and Hegazi 1999) have been reported. Similarly, the increase in time to pupation, without creation of supernumerary larvae, has been shown in *P. interpunctella* treated with PYR (Ghasemi et al. 2010) and delay in pupation due to larval–larval intermediates followed by treatment with JH's or JHA's have been observed previously by Khafagi and Hegazi (1999), Oochi (2005), Nomura and Miyata (2000) and Kostyukovsky et al. (2000). In this study, despite of increase in larval period, the weight of pupae declined. Leonardi et al. (2001) demonstrated that oral and topical applications of fenoxycarb on *Bombyx mori* decreased larval frass production because of the toxic effect of fenoxycarb on the brush border membrane (BBM) of midgut columnar cells (especially in its anterior–middle regions) and disordered leucine uptake in these areas. It also modified the lipid composition of BBM by changing the balance between saturated and unsaturated fatty acids that both affect amino acid absorption in the larval midgut (Leonardi et al. 2001). There is a K^+/amino acid cotransporter for Lucine uptake in the BBM of midgut columnar cells (Giordana et al. 1994) that it is in charge of the absorption of many other essential amino acids (Parenti et al. 2000). Moreover, Fenoxycarb severely disturbed lipid synthesis and catabolism in the fat body of *Choristoneura fumiferana* (Mulye and Gordon 1993) and reduced intake of food and growth rate in silkworms (Leonardi et al. 1996). Hence, the reduced pupal weight of DBM may be attributed to the disturbed uptake of amino acids, lipid synthesis and catabolism. In our study, arrested larval growth due to feeding interruption was similar to the results found by Arthur (2001) in *Tribolium castaneum*. At the beginning of the pupal stage of holometabola, there is an additional JH-sensitive period for pupal versus adult determination that JH must be absent in

epidermal cell obligated to adult development (Nijhout 1994). Hence, the presence of JH (PYR in this study) at this critical time, resulted in the production of deformed pupae and adults (e.g. twisted wings) and failure in adult eclosion in *P. xylostella*. These data confirmed prevention of imaginal disk cell growth in *P. interpunctella* treated with JHI, JHIII, methoprene, fenoxycarb and farnesol (Oberlander et al. 2000), prohibition of silk gland growth in *B. mori* treated with JH's and JHA's (Kurata 1981; Garel 1983; Aribi et al. 2006), malformed wings in *L. migratoria* (De Kort and Koopmanschap 1991), *Blattella germanica* (Lim and Yap 1996), *T. castaneum* (Arthur 2003) treated with fenoxycarb, PYR and Hydroprene, respectively, and increased larval and pupal mortality and reduced adult emergence as a result of JH's or JHA's treatment (Khafagi and Hegazi 1999; Arthur 2001, 2003; Oochi 2005; Sial and Brunner 2010). In the present work, reduction in fecundity, egg viability percentage and adult longevity were observed in both sublethal treated groups at successive generations. Dissection of these females indicated that they had malformed ovaries. Yin et al. (2008) observed the reduction of fecundity in *P. xylostella* treated with sublethal doses of spinosad not in the offspring but in the parent. It may be due to the presence of PYR at the early pupal stage preventing the secretion of sufficient ecdyson. Shaaya et al. (1993) suggested that ovaries in *P. interpunctella* require high level of ecdysteroid in the hemolymph of the early pupal stage to mature. In other studies, topical application of Chlorfluzuron on *Spodoptera litura* (Perveen and Miyata 2000), PYR on *P. interpunctella* (Ghasemi et al. 2010) and Hexaflumuron on *Callosobruchus maculatus* (Kellouche and Soltani 2006) reduced lipid and protein concentration in ovaries. Kanost et al. (1990) reported that reduction of compounds such as lipids, proteins and carbohydrates may result in abnormal oogenesis. Pyriproxyfen application to young workers of *Aphis mellifera* damaged vitellogenin synthesis in the hemolymph (Pinto et al. 2000). Feeding of *A. mellifera* workers with Kinoprene contaminated pollen reduced their longevity (Gerig 1975). In the beginning of the pupal stage of *S. litura*, treatment with PYR resulted in adults with low ovarian weight and reduction in number of eggs oviposited because of losing an oviposition stimulator factor (a protein with molecular weight more than 14 KDa) in the treated females' hemolymph (Hatakoshi 1992). The reduced reproductive capacity of untreated *C. rosaceana* (Sial and Brunner 2010) and *M. domestica* (Chang and Borkovec 1990) via mating with PYR and diflubenzuron (A chitin synthesis inhibitor) treated male, respectively, proved the transfer of IGRs to the eggs via the sperm. This may be justification for continuing to study effects of PYR on subsequent generations in this study. Pyriproxyfen also influenced the biological parameters of the offspring in the present study. As was previously mentioned in the results, the net reproductive rate (R_0) of DBM was affected by both of the PYR sublethal doses. Reduced female fecundity and longevity led to a reduction in R_0 in the current study. This was the reverse of the sublethal effect of bifenthrin on enhancement of the net reproductive rate of cotton aphids (Kerns and Stewart 2000). On the other hand, some insects and mites show increased vigour (e.g. increase fecundity and the reproductive parameters) when exposed to sublethal doses of pesticide - a phenomenon known as hormoligosis. Sota et al. (1998) and Fujiwara et al. (2002) reported the phenomenon of hormoligosis in *P. xylostella* affected by treatment with different sublethal doses of fanvalerate. But as previously noted, IGR's reduced the fecundity of insects and Acari duo to physiological and morphological changes in both sexes (Hatakoshi 1992; Kellouche and Soltani 2006). In addition to R_0, other biological parameters of *P. xylostella*

such as GRR, intrinsic rate of increase (r) and finite rate of increase (λ) tended to be lower in both of the sublethal treatment groups than in the untreated group. This was consistent with the results reported by Yin et al. (2008) on *P. xylostella* treated with spinosad and Lashkari et al. (2007) on *Brevicoryne brassicae* treated by imidacloprid and pymetrozine. Unlike other population parameters, the mean generation time of the two sublethal treatments actually increased compared to the control in this investigation. It was due to longer immature stages and adult preoviposition periods compared with the control. In the present study, the negative effects of both tested sublethal concentrations of PYR influenced the biological characteristics of *P. xylostella* not only in the parent but also in the next generation, although the mentioned effects were less noticable in the offspring. Yin et al. (2008) reported on the sublethal effects of spinosad on consecutive generations of *P. xylostella*. The effects on the second generation, however, were much less apparent than those on the parent generation. In some cases, sublethal concentrations do not have an effect on the offspring. For example, in *Daphnia carinata*, the negative sublethal effect of chlorpyrifos was absent on the next generation (Zalizniak and Nugegoda 2006). The reduction or loss of effects of insecticides on the next generations of treated parents can be a result of the insect's growth and dilution of residues; in other words, an insect could be restored piecemeal to a normal metabolism, generation by generation (Yin et al. 2008). The sublethal effects of PYR on vital performance of two successive generation of DBM may generate more benefits to an integrated pest management program for this major pest than conventional insecticides that have a high acute toxicity (Michaud and Grant 2003). In conclusion, our findings indicated that PYR was highly effective in controlling *P. xylostella* in the laboratory. This IGR addition to direct mortality has indirect effects such as disrupting growth and development, reducing reproductive capacity, and is also capable of transferring abnormalities to the next generation. Further studies, however, are needed to investigate the sublethal effects of PYR on later generations of DBM and to display the mechanisms by which PYR exerts its sublethal effects in the laboratory and to understand its aggregate effects on population dynamics of *P. xylostella* and its interaction with natural enemies in the field.

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