Reduced fitness associated with spinosad resistance in *Helicoverpa armigera*

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Abstract The fitness cost of spinosad resistance was investigated in the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Laboratory experiments were conducted to compare relative fitness of *H. armigera* between the spinosad-susceptible and -resistant strains. During the experiments, the average development periods of the resistant strain were lengthened by 4–5 days, reflected in a prolongation of egg, larval and pupal periods. Furthermore, pupal survival, pupal weight, the mean life span of emerged adults, eggs laid and hatched decreased greatly in the resistant strain in comparison with the susceptible strain. Other life-cycle parameters such as larval survival, larval wet weights, prepupal periods, pupation ratio, and sex ratio did not change significantly. As a result, both net replacement rate (R_0) and intrinsic rate of increase (r_m) were reduced for the resistant strain. Our results clearly indicated that relative fitness of resistant individuals was reduced in the absence of spinosad. Rational measures including pesticide rotations should be expected to delay development of resistance to spinosad in *H. armigera* field populations from China.

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Introduction

The oriental cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is an economically important pest of cotton and vegetable crops throughout the world (Wu et al. 2002). In China, *H. armigera* has developed resistance to virtually all conventional insecticides that have been applied against it (Mu and Wang 1988; Mu et al. 1995; Rui et al. 1999; Sun et al. 1999). To control this pest effectively and sustain agricultural productivity, spinosad—a pesticide with a novel mode of action—has been introduced and registered on cabbage, eggplant and cotton in China (Gao et al. 2007). Currently, *H. armigera* populations from major cotton-growing regions in China can be controlled effectively by application of the naturally derived insecticide spinosad (Wang et al. 2009c). However, low levels of spinosad resistance have been

Fig. 1 Survival of different development stages of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains. Resistant strain data marked with an asterisk differ significantly ($P < 0.05$) from susceptible strain data for the same stage, on the basis of the *t*-test
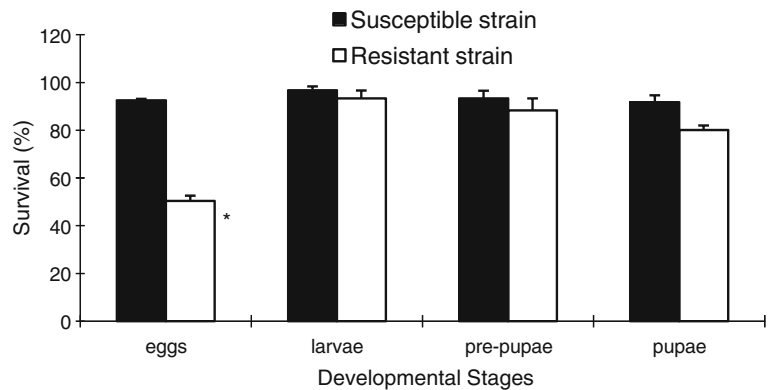
recorded in *H. armigera* in Pakistan (Ahmad et al. 2003), India (Kranthi et al. 2000) and Australia (Gunning and Balfe 2002). In addition, successful selections of spinosad-resistant strains under laboratory conditions in target pests have provided theoretical evidence that insects have the potential to evolve resistance to spinosad (Shono and Scott 2003; Wang et al. 2006; Wang et al. 2009b; Young et al. 2003). The probability of rapid resistance development will increase dramatically with increased persistence and coverage of spinosad under field conditions. Based on the facts, a potential threat exists of the failure of spinosad, similar to that of conventional insecticides.

Reduced relative fitness of resistant genotypes in insecticide-free environments is characteristic of many insect species (Sayyed et al. 2008a). Differences in the biological parameters affecting the net replacement rate (R_0) and the intrinsic rate of population increase (r_m) are of particular interest to insecticide resistance management (Haubruge and Arnaud 2001). Fitness costs associated with resistance genes expressed in the absence of insecticides can affect the evolution of insecticide resistance and the outcome of resistance

management programs. If resistance entails a fitness cost, an appropriate resistance management strategy will promote reversion of the resistant populations back to susceptibility.

On the above mentioned background, we were interested in whether the lack of current spinosad resistance could be related to fitness cost associated with spinosad resistance in the field populations of *H. armigera* from China. However, there is no information regarding effects of spinosad resistance on fitness of *H. armigera*. In the current research, we investigated relative fitness of *H. armigera* resistant to spinosad and discussed the possible implications for resistance management in the field.

Materials and methods

Insects A laboratory susceptible strain (S) and a spinosad-resistant strain (R) of *H. armigera* were used in this study. The S strain was originally collected from Shandong Province, China, in 2007, and maintained in the laboratory under insecticide-

Fig. 2 Larval survival during the larval stages of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains
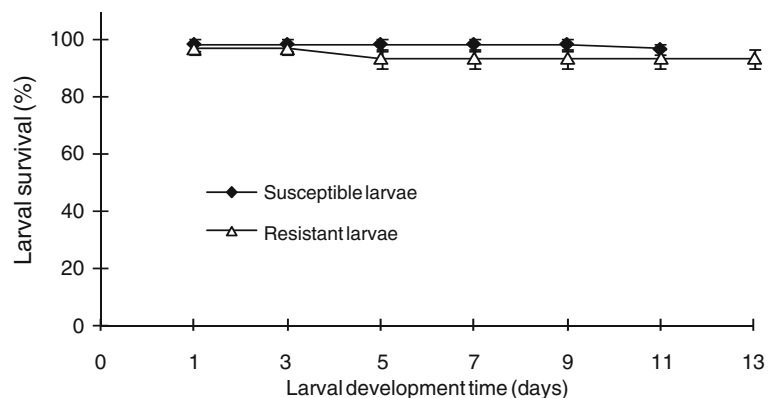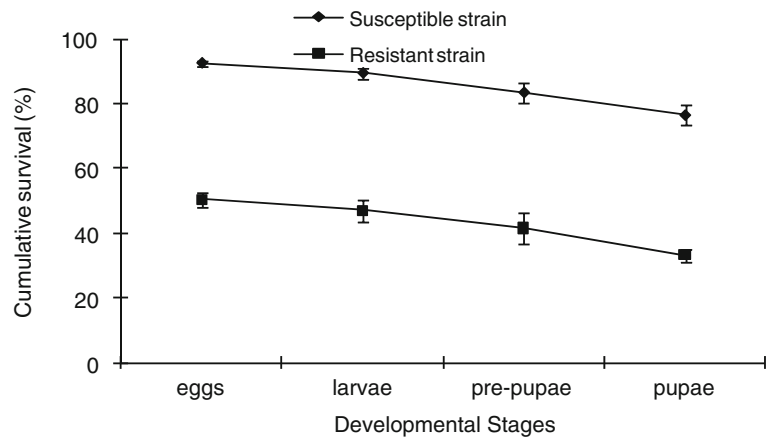
Fig. 3 Cumulative survival of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains during the immature stages

free conditions. For selecting the R strain of *H. armigera*, technical grade spinosad was dissolved in acetone and applied topically on the thoracic notum of 4th instar larvae with selection pressure to kill approximately 50% of the individuals of the population. After 15 generations of selection, the R strain was established until the resistance was >24-fold (Wang et al. 2009b). Larvae of both strains were maintained at 27±1°C with a photoperiod of 14L:10D and reared on the artificial diet described by Wang et al. (2009b).

Survival and development Eggs laid by the susceptible and resistant females within a 24-h period were incubated in an insectary maintained at 27±1°C with a 14L:10D photoperiod. The duration from eggs to neonates was recorded. Neonates was randomly selected and reared on artificial diet under the same conditions. Fresh artificial diet was provided every 2 days. The survival and growth of each individual

were checked twice daily until adult emergence. The duration from neonates to 3rd instar larvae, the duration of each larval instar (3rd to 6th instars), the first-day wet weights of larvae at each instar (3rd to 6th instars), the prepupal and pupal stages, and the ratio of pupation and adult emergence were recorded. Within 24–48 h of pupation, pupae were weighed and sorted by gender.

Reproductive potential Virgin teneral moths were held in wooden frame cages (40×40×40 cm) to mate for about 2 days at 27±1°C with a 14L:10D photoperiod at 60% r.h., and supplied with a 10% honey solution. Then mating pairs were transferred to a smaller box covered with gauze and reared under the same conditions as those described above for egg laying. Non-egg-laying females were discarded and replaced until 30 egg-laying females had been procured from each strain. The number of eggs laid was counted daily until all females died. The

Fig. 4 Time of development from eggs to larvae for *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains
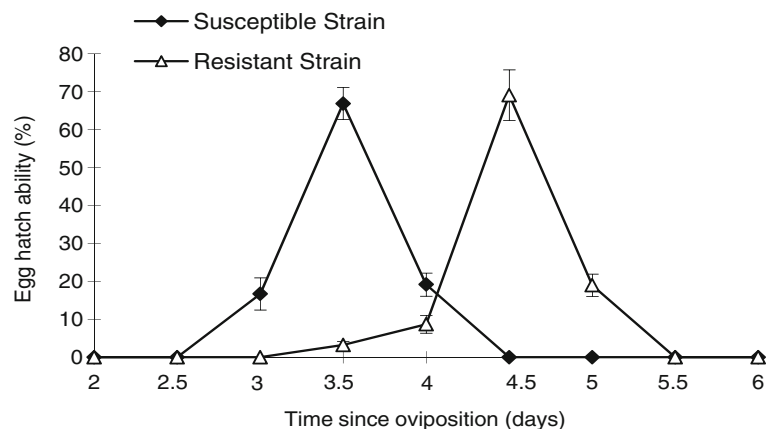
Table 1 Mean duration of different development stages of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains

Insect stage		Development period (days)	
		susceptible strain	resistant strain
Eggs		3.58±0.09 a [*]	4.52 ± 0.03 b
Larvae	1st–2nd instar	4.67±0.17 a	5.50±0.10 b
	3rd instar	1.67±0.22 a	2.10±0.06 b
	4th instar	1.87±0.16 a	2.13±0.06 b
	5th instar	1.61±0.07 a	1.96±0.36 b
	6th instar	2.09±0.06 a	2.67±0.05 b
Pre-pupae		2.53±0.17 a	2.49±0.69 a
Pupae	female	9.68±0.21 a	11.0±0.26 b
	male	10.4±0.15 a	12.0±0.24 b

[*] Within rows, means followed by the same letter do not differ significantly ($P<0.05$), based on the *t*-test using the SPSS program

longevities of adult males and females were recorded as well. Eggs that did not develop to the distinctive 'red ring' stage within 48 h were deemed to be infertile. Approximately 50 viable eggs were taken randomly from each pair of adult moths, and the number of hatching eggs was recorded.

Intrinsic rate of population increase The intrinsic rate of population increase (r_m) was calculated by the equation $r_m = (\ln R_0)/T$ (Birch 1948), where R_0 is the net replacement rate and T is the development time from eggs to adult eclosion. The R_0 was calculated by equation $R_0 = (n \times I_e \times I_a)/2$ (Birch 1948), where n is the mean number of eggs per female, I_e is the proportion of fertile eggs, I_a is the proportion of eclosing adults, and 2 is the sex ratio coefficient.

Statistical analysis For each parameter, the data were statistically analyzed by *t*-test ($P<0.05$) using the SPSS program. All percentage data were arcsine transformed before being subjected to analysis of variance.

Results

Survival of immature stages Survival of eggs, larval, prepupal and pupal stages was evaluated on the basis of hatch ratio, larval survival, pupation ratio and

emergence ratio (Fig. 1). In the present study, the ratio of hatching eggs from resistant adults was greatly decreased compared with that from the susceptible strain ($F=14.450$, df=28, $P<0.0001$). When larvae from both strains were supplied with an artificial diet, the survival of larvae was checked every 2 days until the prepupal stage (Fig. 2). We did not detect an obvious decrease in larval survival of the resistant strain during the whole larval stage ($F=3.177$, df=4, $P=0.421$). Similarly, pupation ratio did not differ significantly between the susceptible and resistant strains ($F=0.348$, df=4, $P=0.388$). However, more abnormal pupae were found for the resistant strain than the susceptible strain. In addition, the emergence ratio of resistant adults was 80.02%, which was significantly lower than that of the susceptible adults ($F=0.995$, df=4, $P=0.011$). Throughout the immature stages, the cumulative survival of susceptible and resistant *H. armigera* decreased to 76.47% and 33.24%, respectively (Fig. 3).

Development of immature stages The process of development from eggs to neonates took an average of 3.58 days for the susceptible strain and 4.52 days for the resistant strain, an obvious difference ($F=1.724$, df=4, $P<0.0001$). Susceptible eggs began to hatch at 3 days after oviposition and reached their maximum hatchability at 3.5 days, which was 1 day earlier than resistant eggs (Fig. 4). However, the emergence of resistant larvae was delayed, the first larva emerging at 3.5 days and with maximum hatch at 4.5 days. From days 1 to 4, almost all surviving eggs hatched for the susceptible strain while only

Table 2 Larval and pupal wet weights of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains

Insect stage		Wet weight (mg)	
		susceptible strain	resistant strain
Larvae	3rd instar	6.900±0.26 a [*]	5.990±0.44 a
	4th instar	16.32 ± 0.74 a	15.24±0.79 a
	5th instar	77.17±3.26 a	72.35 ± 2.15 a
	6th instar	130.9±3.44 a	123.2 ± 2.05 a
Pupae	female	218.4±6.43 a	195.9±7.40 b
	male	225.0±5.85 a	203.1 ± 3.20 b

[*] Within rows, means followed by the same letter do not differ significantly ($P<0.05$), based on the *t*-test using the SPSS program

Fig. 5 Time of development from eggs to adults for *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains
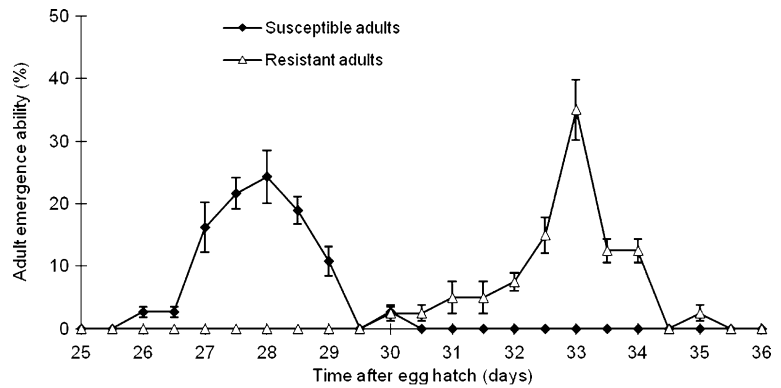
8.71% of surviving eggs reached the larval stage for the resistant strain.

The period larvae needed to complete each development stage differed significantly between the susceptible and resistant strains (Table 1) (1st–2nd instars: $F=16.00$, df=4, $P=0.007$; 3rd instar: $F=5.279$, df=4, $P=0.025$; 4th instar: $F=1.973$, df=4, $P=0.024$; 5th instar: $F=1.286$, df=4, $P=0.012$; 6th instar: $F=0.313$, df=4, $P=0.002$). For susceptible larvae, 11.91 days were needed to complete the larval stage. An extended development period was observed in the resistant strain, which was about 2 days longer than for the susceptible larvae.

No significant differences were recorded in prepupal periods between the two strains (Table 1) ($F=2.463$, df=4, $P=0.605$). Until day 10 after pupation, all female pupae and approximately 30% male pupae from the susceptible strain reached the adult stage. However, just 10% female adults and no male adults were found until day 10 after formation of resistant

pupae. The development period from pupae to adults was significantly longer in the resistant strain than in the susceptible strain, which was delayed about 1.5 days for both female and male pupae (Table 1) (♀: $F=0.365$, df=4, $P=0.016$; ♂: $F=0.439$, df=4, $P=0.004$).

Weights of larvae and pupae Compared with the susceptible strain, the resistant larvae had smaller wet weights at every instar (Table 2). However, no statistical differences were found in the wet weights of susceptible and resistant larvae at each time point measured (3rd instar: $F=0.864$, df=4, $P=0.148$; 4th instar: $F=3.444$, df=4, $P=0.264$; 5th instar: $F=2.853$, df=4, $P=0.113$; 6th instar: $F=1.707$, df=4, $P=0.127$). Interestingly, statistically significant decreases in the pupal weights were observed in the resistant strain (♀: $F=6.187$, df=4, $P=0.026$; ♂: $F=1.588$, df=4, $P=0.030$).

Adult emergence and longevity There were no obvious differences in sex ratio, which was approximately 1:1
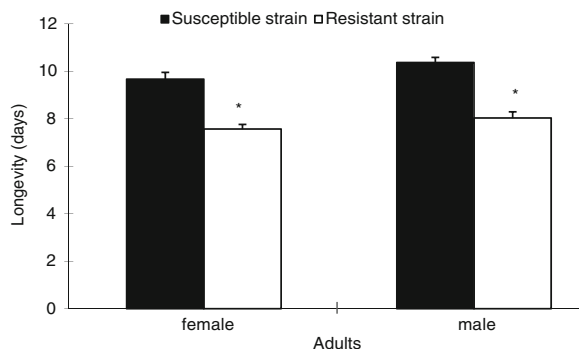

Fig. 6 The longevity of emerged adults of *Helicoverpa armigera* from the spinosad-susceptible and -resistant strains. Resistant strain data marked with an asterisk differ significantly ($P<0.05$) from susceptible strain data for the same sex, on the basis of the *t*-test

Table 3 Fitness parameters for the spinosad-susceptible and -resistant strains of *Helicoverpa armigera*

Life history trait	Susceptible strain	Resistant strain
Emergence ratio (%)	91.67±0.03 a [*]	80.02±0.02 b
Number of eggs laid per female	707.2±34.20 a	359.4±24.62 b
Net replacement rate (R_0)	226.9	57.52
Intrinsic rate of population increase (r_m)	0.20	0.13

[*] Within rows, means followed by the same letter do not differ significantly ($P<0.05$), based on the *t*-test using the SPSS program

($\female$: $\male$) in both strains (F=2.255, df=4, P=0.911). The first susceptible adults emerged at 26 days after egg hatch. About 2 days later, maximum emergence could be observed (Fig. 5). Compared with the susceptible strain, the emergence of adults was obviously delayed for the resistant strain, the first imago emerging at 30 days and with maximum emergence at 33 days (Fig. 5).

The mean life span of emerged adults of both sexes was significantly shortened in the resistant strain, and was 2 days shorter than that of susceptible adults (Fig. 6) ($\female$: F=0.163, df=4, P=0.004; $\male$: F=0.145, df=4, P=0.002).

Reproductive potential and intrinsic rate of population increase Compared with susceptible females, lower fecundity was found in the resistant females, with the difference being significant (F=1.038, df=28, P<0.0001). The number of eggs laid per susceptible female was 707, which was double that of the resistant female. Net replacement rate (R_0) was 226.90 for susceptible females, and 57.52 for resistant females (Table 3). Based on the R_0 value and the development periods, the intrinsic rate of population increase (r_m) was 0.20 for the susceptible strain (Table 3). Compared with the susceptible strain, development to eclosion in the resistant strain was delayed by about 4 days. This delay, combined with low emergence ratio and decrease in egg production, resulted in a r_m value of 0.13 for the resistant strain (Table 3).

Discussion

This study investigated the changes in fitness of *H. armigera* after 15 generations of selection with spinosad. Fitness was evaluated in terms of survival, development time and fecundity. Comparisons between unselected and spinosad-resistant *H. armigera* on untreated diets showed a fitness cost associated with spinosad resistance (Table 3).

No significantly lower survival was found for the resistant strain in comparison with the susceptible strain, throughout the larval stage (Fig. 2). However, the selected *H. armigera* had a significantly longer development period to reach the adult stage by the prolongation of egg periods, larval periods and pupal periods (Fig. 4, Table 1). Both strains tested in this

study had the same genetic background because they were from the same field. Therefore, the observed longer development periods in the resistant strain might be caused by related resistant alleles emerged during the selection process. The period from egg production to adult emergence was lengthened by 4–5 days, which indicated that relative fitness of resistant individuals was reduced in the spinosad-free conditions. Similarly, the spinosad resistance allele significantly delayed the development time for *Plutella xylostella* (Linnaeus) populations (Sayyed et al. 2008b). In the current study, although longer development periods were found in the various larval instars, the wet weights of resistant larvae were not increased accordingly (Table 2). On the contrary, the resistant larvae had slightly lower wet weights, which translated into lower final pupal weight compared with the susceptible larvae. Similarly, the pupal weights of the spinosad-resistant strain of the tobacco budworm were ~30 mg lower than that of the susceptible pupae (Wyss et al. 2003). Our results showed that there were no statistical differences in the larval wet weight in the resistant strain, whereas the pupal weights were significantly decreased by 20 mg compared with the susceptible strain.

For the susceptible and resistant larvae, the same proportion of pupation was found in the present study. However, more abnormal pupae existed in the resistant strain, resulting in a lower emergence ratio. After emergence, obviously shorter adult longevity was observed for both resistant males and females. Moreover, resistant females had a lower reproductive capacity and egg hatchability compared with susceptible females. Egg production was reduced by 49.2% after 15 generations of selection. Of the eggs laid by the susceptible females, 92.5% hatched *vs* only 50.9% in the resistant strain. Data from the current study were consistent with previous work that demonstrated the population density was reduced by the decrease in ratio of adult eclosion and hatching eggs (Wang et al. 2009a). Therefore, spinosad resistance in *H. armigera* was associated with a fitness cost, suggesting insecticide dose should not be increased blindly for controlling the resistant population density. As with *H. armigera* in the current study, fitness cost of *Heliothis virescens* (Fabricius) was found in the spinosad-resistant strain, which was supported by observations of a lower biotic potential in comparison with a susceptible population (Wyss et al. 2003). Li et

al. (2007) found that fitness costs of resistance to spinosad in the diamondback moth were temperature-dependent, increasing in scale at unfavorably low and high temperatures. However, no changes in relative fitness were found in the spinosad-resistant strain of *Frankliniella occidentalis* (Pergande) (Bielza et al. 2008). These results imply that fitness costs associated with spinosad resistance are probably related to the pest species.

Based on the laboratory results, the lower rate of *H. armigera* population increase seemed to be due largely to a decrease in number of eggs laid and hatched, and adult eclosion. Although we have not measured the fitness of F_1 progeny, we believe that our data provide some insight into possible development under field conditions before resistance to spinosad has become a problem for controlling pests. Data from the current study showed that relative fitness of resistant individuals was significantly reduced in the absence of spinosad, suggesting that relaxation of selection pressure was likely to favor reversion to susceptibility for the *H. armigera* population. Combined with our previous work (Wang et al. 2009c), the development of resistance to spinosad in *H. armigera* should be delayed by rational resistance management measures such as applications of synergists or pesticide rotations.

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