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Variation in biological parameters of cypermethrin resistant and susceptible strains of *Helicoverpa armigera* from Benin Republic, West Africa

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ABSTRACT

The aim of this survey was to assess the costs of cypermethrin resistance in *Helicoverpa armigera* strains by using variation in their biological parameters such as fecundity, number of larval slough, development time, weight and survival at each stage of insect development in comparison with susceptible strains. AGB01 and AGB03 are the resistant strains compared respectively with BK77 and OKP01 as susceptible strains. Fecundity, fertility and survival from egg to adult emergence of AGB03 strain were significantly ($P < 0.001$) lower than those observed in OKP01 strain. No difference ($P > 0.05$) was observed with time of pre-adult development, survival at each stage of insect development, fecundity and number of larval slough in comparison with AGB01 and BK77 strains. Larvae of AGB01 strain took significantly ($P < 0.01$) longer time to develop and were significantly ($P < 0.01$) lighter than BK77 at the same age, but the slower development of larvae of AGB01 strain was counteracted by the shorter times of egg and pupae stages. The lower fecundity, fertility and survival from egg to adult emergence should represent the main costs for the AGB03 strain resistant to cypermethrin.
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Keywords: Cypermethrin resistance, resistance cost, fecundity, fertility, development time.

INTRODUCTION

From the 1980s, the entire planet has been spread with pyrethroids for agricultural pest management and environment is contaminated with these toxic molecules. Like all living organisms, the cotton bollworm *Helicoverpa armigera* (Hübner) can adapt to the new environment. This adaptation leads to *H. armigera* resistance to pyrethroids which can be defined as the ability of this insect to support pyrethroid doses which were originally lethal for it. At the beginning, *H. armigera* resistance to pyrethroids was reported in Australia in 1982 (Djihinto et al., 2009; Martin et al., 2005). After Australia, the widespread occurrence of this phenomenon of

resistance has been observed in several parts of world including West Africa at the end of the 1990s (Martin et al., 2000; Djihinto, 2004).

Genes responsible for an adaptation to a new environment are usually assumed to have a fitness cost or to be at a disadvantage in the previous environment (Berticat et al., 2008; Djihinto, 2004). It is not surprising that resistant and susceptible strains differ in properties other than their adaptation to insecticides, such as development time, fecundity and fertility. Since resistant individuals were not common prior to selection, it is generally assumed that resistant genotypes must have a reproductive disadvantage in the absence of pesticides. Thus, when selective pressure is relaxed, there may be intense selection against alleles conferring insecticide resistance, and these alleles will decline in frequency (Djihinto et al., 2009; Berticat et al., 2008).

The majority of biological parameters of resistant and susceptible strains studies show that there are costs associated with insecticide resistance (Martins et al., 2012; Fang et al., 2011; Paris et al., 2011; Roy et al., 2010; Djogbénou et al., 2010; Gassmann et al., 2009; Ellison, 2007; Xiaoxia et al., 2001). In some cases, there is no difference between resistant and susceptible strains, and the resistant strain sometimes is advantaged (Bielza et al., 2008; Arnaud et al., 2002).

Comparison of biological parameters such as fecundity, fertility, development time and insect survival of susceptible and resistant strains is one of the methods which can be used to assess the cost when resistance was costly (Martins et al., 2012; Fang et al., 2011; Paris et al., 2011; Roy et al., 2010; Djogbénou et al., 2010; Gassmann et al., 2009; Ellison, 2007; Bourguet et al., 2004; Xiaoxia et al., 2001; Boivin et al., 2001; Haubruge and Arnaud, 2001; Carrière et al., 2001; Gazave et al., 2001; Oppert et al., 2000). In the objective to assess the costs of cypermethrin resistance

of *Helicoverpa armigera* in Benin Republic, we compare the biological parameters of resistant and susceptible strains.

MATERIALS AND METHODS
Strains

Four strains of *H. armigera* were used in this study (Table 1). The susceptible strain (BK77) was originally collected in Bouake (Côte d'Ivoire) in 1977 and was reared in the CIRAD Entomological Laboratory, Montpellier, France from 1977 to 2000 free of all insecticide treatment. Since 2001, this strain was introduced in laboratory of Cana in Benin and reared always in absence of insecticide.

BK77 and AGB01 strains were reared in this study at 25 °C, 75% HR and a photoperiod of 12:12 (L:D) h whereas OKP01 and AGB03 were reared at 20 °C, 75% HR and a photoperiod of 12:12 (L:D) h in the laboratory. Larvae were reared on an artificial diet and adults were fed on a 5% sugar solution as described by Djihinto (2004) and Djihinto et al. (2009).

Insecticide

The product used in this study was technical grade material of cypermethrin (two batches at 93.2 and 96.6%). The concentration of the *cis-form* was 48.4 and 50.7%. The cypermethrin came from FMC Corporation (Philadelphia, PA).

Cypermethrin susceptibility of strains

Standard third- and fourth-instar larval topical bioassays were used to determine insecticide toxicity (Martin et al., 2000; Djihinto, 2004; Djihinto et al., 2009). Technical grade product was weighted and dissolved in acetone. Calibrated doses of insecticide solutions were applied onto the insect thorax with an Arnold micro-applicator from Burkard Manufacturing (Rickmansworth, United Kingdom). Each test included acetone-treated control. After

Table 1: Strains of *H. armigera* and their origin**.**

Strain	Locality of collection		Date of collection mo/yr	Host plant
	Country	Village		
BK77	Côte d'Ivoire	Bouaké	- / 1977	*Gossypium hirsutum*
AGB01	Benin	Agblakindji	April 2001	*Cleome viscosa*
OKP01	Benin	Okpara	Sept. 2001	*Gossypium hirsutum*
AGB03	Benin	Agblakindji	May 2003	*Cleome viscosa*

treatment, the tested larvae were provided with adequate food and held individually at 25 °C, 75% RH, and at a photoperiod of 12:12 (L:D). Mortality was assessed 48 h after treatment. Larvae were considered dead if unable to move in a coordinated way when prodded with a needle. Five doses (30 insects per dose) of the insecticide were used, leading to between 0 and 100% mortality.

Biological parameters observation
Fecundity

Insect couples emerged the same day were directly introduced into the boxes and each female was followed for egg-laying. The boxes were ventilated and contained the gauze on which eggs can be laid. Adult were fed on sugar solution. Total number of eggs per female (for the duration of female life) was recorded for each strain.

Development times

- *Embryonic development time*. Eggs were collected into the box and followed for incubation. The time from egg laid to egg hatch was observed for each strain. The number of day taken by each egg to hatch was noted.

- *Larval development time*. Neonates of the same day and of the same strain were reared individually. The number of day taken by each larvae from neonate stage to pupation was recorded. During larval development, the number of the larval slough observed was noted.

- *Pupae development time*. Pupae of the same age for each strain were followed

individually for pupae duration. The number of day taken by each pupae was noted for males and for females.

Insect weight

Larvae were weighed daily until pupation. Pupae and adult males and females were also weighed separately for each strain.

Survival at each stage (egg, larvae and pupae) of insect development

Insect survival at each stage of insect development is the percentage of insect which was not dead at this stage. In the experiences used for development times, survival at each stage was recorded and fertility was determined by survival at egg stage.

Statistical analyses

LD_{50} (Lethal Dose 50%) of the insecticide was determined by using the Finney (1971) method. The data were subjected to probit analysis to generate dose mortality regression lines using WinLD software (CIRAD, Montpellier, France). Mortality in the control was always <10%. Data from all bioassays were corrected for control mortality using the formula of Abbott (1925). The resistance factor was determined dividing the LD_{50} of resistant strain by the LD_{50} for the susceptible strain. Differences among strains were considered significant when the 95% confidence intervals of the LD_{50} did not overlap.

The data of biological parameters were statistically analysed with STAT-ITCF software (ITCF, Boigneville, France). A completely randomized design with two

treatments (resistant and susceptible strains) was used and number of replications equal number of insects observed. The test of Newman-Keuls was the statistical test used to compare means of biological parameters.

RESULTS

Cypermethrin susceptibility of the BK77, AGB01, OKP01, and AGB03 strains

Table 2 presents the cypermethrin LD_{50} values for BK77, OKP01, AGB01 and AGB03 strains. The BK77 strain cypermethrin LD_{50} value was significantly ($P < 0.05$) different from those of the AGB01, and AGB03 strains. The AGB01 and AGB03 strains were resistant to cypermethrin. However, cypermethrin LD_{50} value of AGB03 strain was significantly ($P < 0.05$) higher than cypermethrin LD_{50} value of AGB01 strain. On the other hand, no significant ($P > 0.05$) difference was observed between the LD_{50} values of BK77 and OKP01 strains. The BK77 and OKP01 strains were susceptible to cypermethrin.

Comparison of biological parameters of BK77 and AGB01 strains

In a first experiment, two strains were compared, a laboratory susceptible strain (BK77), and a resistant field strain (AGB01). Times of pre-adult development (from egg to adult emergence) were not significantly ($P > 0.05$) different for the two strains. Nevertheless, comparison of development times of each stage showed that times for egg stage and male pupae stage were significantly ($P < 0.01$) shorter for resistant strain than susceptible whereas resistant larvae took longer time to develop (Table3). Fecundity and number of larval slough were not significantly ($P > 0.05$) different for the BK77 and AGB01 strains.

Larvae weights of the same age were significantly ($P < 0.001$) different in the two strains; larvae from the resistant strain were lighter than larvae from the susceptible strain. However, at the end of larval development (just before pupation), resistant larvae weight was similar to susceptible (Table 4).

Survival at each stage of insect development showed that fertility or percentage egg hatch (18% for AGB01 strain and 23% for BK77 strain), larval survival (90% for both strains) and pupae survival (100% for both strains) were not significantly ($P > 0.05$) different from the BK77 and AGB01 strains.

Comparison of biological parameters of OKP01 and AGB03 strains

In a second experiment, two strains were compared, a susceptible strain (OKP01), and a resistant strain (AGB03). Times of pre-adult development of susceptible strain were significantly ($P < 0.001$) longer than those of the resistant strain (Table 5). However, resistant larvae took longer time to develop. At the beginning, resistant larvae were lighter than susceptible larvae whereas at the end of larval development, larvae from the susceptible strain were lighter than larvae from the resistant strain (Table 6). Thus, pupae of OKP01 strain were lighter than pupae of AGB03 strain.

Fecundity and fertility (Table 7) were significantly ($P < 0.001$) increased in susceptible strain (OKP01) in comparison with the resistant strain (AGB03). Survival from egg to adult emergence (pre-adult survival) of OKP01 strain was significantly ($P < 0.0001$) higher than survival from egg to adult emergence of AGB03 strain. However, pupae survival was significantly ($P < 0.0001$) lower for OKP01 strain than AGB03 strain.

These results of biological parameters variation of cypermethrin resistant and susceptible strains of *H. armigera* can allow assessing the costs of the resistance.

Table 2: Cypermethrin susceptibility of the BK77, AGB01, OKP01, and AGB03 strains.

Strain	Date of collection M/Y	LD_{50} (µg/g)	Confidence interval (95 %)	χ^2	Df [a]	Slope ± SE	RF[b]
BK77	-	0.44	0.38-0.58	11.4	7	2.98 ± 0.42	-
OKP01	9/01	0.5	0.2-1	10.4	7	0.78 ± 0.11	1
AGB01	04/01	15.00	9-27	7.4	4	0.96 ± 0.16	34
AGB03	5/03	40.2	29.9-54.7	7.6	8	1.64 ± 0.18	91.4

[a] Degree of freedom; [b] Resistance factor

Table 3: Mean (± SE)[1] fecundity, larval slough and insect development times (day) of BK77 and AGB01 strains at 25 °C, 75% HR and a photoperiod of 12:12 (L:D) h.

Parameters observed	BK77(susceptible)		AGB01 (resistant)	
	Mean ± SE	n	Mean ± SE	n
Fecundity	365.9 ± 68.7	21	339.2 ± 56.3	21
Number of larval slough	4.6 ± 0.1	100	4.7 ± 0.1	100
Embryonic development time	3.0 ± 0.0	100	2.1 ± 0.1*	100
Larval development time	14.3 ± 0.1	100	16.3 ± 0.1*	100
Pupae development time: Male	16.4 ± 0.2	10	14.9 ± 0.2*	10
Female	15.0 ± 0.6	10	14.2 ± 0.2	10
Pre-adult development time: Male	33.4 ± 0.2	10	32.9 ± 0.2	10
Female	32.0 ± 0.6	10	32.2 ± 0.2	10

[1] SE = Standard Errors, * Significant difference at the 5% level between two strains. n = individual number observed

Table 4: Mean (± SE)[1] larval and pupae weight (mg) of BK77 and AGB01 strains and larval growth rate (mg/day) at 25 °C, 75% HR and a photoperiod of 12:12 (L:D) h.

Parameters observed	BK77(susceptible)		AGB01 (resistant)	
	Mean ± SE	n	Mean ± SE	n
Larval weight 10 days after egg hatching	112.2 ± 4.1	100	58.3 ± 2.1*	100
Larval weight 11 days after egg hatching	198.6 ± 9.0	100	101.3 ± 3.5*	100
Larval weight 12 days after egg hatching	334.6 ± 12.3	100	151.4 ± 4.3*	100
Larval weight 13 days after egg hatching	402.9 ± 10.2	100	242.5 ± 9.1*	100
Larval weight 14 days after egg hatching	pupation	100	360.0 ± 11.9	100
Larval weight 15 days after egg hatching	pupation	100	452.5 ± 10.9	100
Pupae weight: Male	387.7 ± 6.4	10	361.3 ± 10.3*	10
Female	347.7 ± 11.3	10	366.1 ± 9.9	10

[1] SE = Standard Errors; * Significant difference at the 5% level between two strains. n = individual number observed

Table 5: Mean (± SE)[1] larval slough and insect development times of OKP01 and AGB03 strains at 20 °C, 75% HR and a photoperiod of 12:12 (L:D) h.

Parameters observed	OKP01(susceptible)		AGB03 (resistant)	
	Mean ± SE	n	Mean ± SE	n
Number of larval slough	5.2 ± 0.1	300	4.0 ± 0.1	300
Embryonic development time (hour)	78.3 ± 0.5	300	60.8 ± 0.4*	300
Larval development time (day)	26.4 ± 0.1	162	27.1 ± 0.2*	162
Pupae development time: Male (day)	16.9 ± 0.2	86	15.0 ± 0.1*	86
Female (day)	15.7 ± 0.2	70	14.0 ± 0.2*	70
Pre-adult development time: Male (day)	47.1 ± 0.2	86	44.8 ± 0.3*	86
Female (day)	46.1 ± 0.2	70	43.8 ± 0.4*	70

[1] SE = Standard Errors , * Significant difference at the 5% level between two strains. n = individual number observed

Table 6: Mean (± SE)[1] larval, pupae and adult weight (mg) of OKP01 and AGB03 strains at 20 °C, 75% HR and a photoperiod of 12:12 (L:D) h.

Parameters observed	OKP01(susceptible)		AGB03 (resistant)	
	Mean ± SE	n	Mean ± SE	n
Larval weight 10 days after egg hatching	15.1 ± 0.4	300	10.8 ± 0.3*	300
Larval weight 13 days after egg hatching	57.4 ± 1.4	288	59.5 ± 2.5	288
Larval weight 16 days after egg hatching	184 ± 4.9	251	157.4 ± 4.7*	251
Larval weight 19 days after egg hatching	345.5 ± 5.3	220	312.6 ± 5.3*	220
Larval weight 22 days after egg hatching	351.1 ± 3.7	176	411.8 ± 4.9*	176
Pupae weight: Male	289.8 ± 4.2	89	304.3 ± 4.6*	89
Female	274.6 ± 3.7	73	291.0 ± 5.2*	73
Adult weight: Male	131.3 ± 2.0	86	120.9 ± 2.1*	86
Female	122.0 ± 1.7	70	118.5 ± 2.5	70

[1] SE = Standard Errors; * Significant difference at the 5% level between two strains. n = individual number observed

Table 7: Mean (± SE)[1] Fecundity and survival during each stage of OKP01 and AGB03 strains at 20 °C, 75% HR and a photoperiod of 12:12 (L:D) h.

Parameters observed	OKP01(susceptible)		AGB03 (resistant)	
	Mean ± SE	n	Mean ± SE	n
Fecundity	1218 ± 129.7	16	814 ± 191.8*	16
Fertility	55.6 ± 0.01	300	26.4 ± 0.01*	300
Larvae survival (%)	39.5 ± 0.01	300	40.3 ± 0.02	300
Pupae survival (%)	86.1 ± 0.02	162	96.3 ± 0.01*	162
Pre-adult survival (%)	18.9 ± 0.01	300	10.3 ± 0.01*	300

[1] SE = Standard Errors; * Significant difference at the 5% level between two strains. n = individual number observed

DISCUSSION

Insecticide resistance, in several cases, has induced resistance fitness costs to most of the resistant individuals (Martins et al., 2012; Fang et al., 2011; Paris et al., 2011; Roy et al., 2010; Rivero et al., 2010; Djogbénou et al., 2010; Miller et al., 2009; Gassmann et al., 2009; Araujo et al., 2008; Ellison, 2007; Bourguet et al., 2004; Guillemaud et al., 2003; Foster et al., 2003; Berticat et al., 2002; Xiaoxia et al., 2001; Boivin et al., 2001; Haubruge and Arnaud, 2001; Carrière et al., 2001; Gazave et al., 2001; Oppert et al., 2000). In Benin republic, our previous studies suggested that *H. armigera* resistance to pyrethroids was associated with fitness cost (Djihinto et al., 2009). The aim of this study was to find out the costs of the resistance to cypermethrin in AGB03 and AGB01 strains of *H. armigera* based on variation in their biological parameters in comparison with susceptible strains.

The comparison of the resistant (AGB03) and the susceptible (OKP01) strains revealed that AGB03 strain was less fecund. A lot of cases of resistance were correlated with decrease production of eggs, and then, the resistance was costly (Martins et al., 2012; Fang et al., 2011; Paris et al., 2011; Roy et al., 2010; Xiaoxia et al., 2001). Xiaoxia et al. (2001) have evaluated the effects of monocrotophos resistance on fitness of cotton bollworm, *H. armigera* in terms of fecundity or number of eggs laid per female of resistant and susceptible strains. Result indicated that fecundity of resistant strain was lower than susceptible. Fang et al. (2011) studied the effects of insecticide resistance on fecundity of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) in Taiwan. Result showed that resistant strain had the lowest fecundity. Another example was pyrethroids and organophosphates resistant strain of the dengue vector *Aedes aegypti* from South America which was less fecund (Martins et al., 2012). A bacterio-insecticide *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) resistant strain of *Aedes aegypti* had sometimes very

significantly reduced fecundity compared to a susceptible strain (Paris et al., 2011).

The resistant strain (AGB03) compared with the susceptible strain (OKP01) revealed also that AGB03 strain was less fertile. Insecticide resistance has induced the decrease of fertility in several resistant insects. Ellison (2007) has compared fitness of spinosad resistant and susceptible strains of *Plutella xylostella* from Hawaii in terms of fertility or percentage of eggs hatch. Result showed that fertility of resistant strain was significantly lower. A deltamethrin resistance of another strain of *Aedes aegypti* from Brazil had sometimes very significantly reduced fertility compared to a susceptible strain (Martins et al., 2012). In *H. armigera* resistance to monocrotophos (Xiaoxia et al., 2001), hatchability of eggs was also lower for resistant strain than susceptible.

Although the pupae survival was significantly lower for OKP01 strain than AGB03 strain, survival from egg to adult emergence of AGB03 strain was significantly lower than survival from egg to adult emergence of OKP01 strain. These results, in addition with the lower fecundity and fertility, represented the substantial costs for resistant strain (AGB03) as they directly decreased the proportion of individuals able to contribute to the next generation. In another insect, recently, Djogbénou et al., (2010) found that resistance decreased pupae survival of *Anopheles gambiae* and this result constituted the main cost of the resistance. Survival from egg to adult emergence of AGB03 strain was not in contradiction with this finding and was also in accord with Gassmann et al. (2009) and Gazave et al. (2001).

Furthermore, resistant larvae of AGB03 strain took longer time to develop, even if the times of pre-adult development of susceptible strain (OKP01) were significantly longer than those of the resistant strain (AGB03). In natural populations, shorter development times of susceptible larvae reduced risk of larval mortality due to predation or their environmental conditions, whereas longer

development times of resistant larvae disadvantaged them. Resistance increases development times in several insects (Roy et al., 2010; Gassmann et al., 2009). Measurement of larval development times indicated that a fitness cost was associated with resistance to *Bacillus thuringiensis* Berliner in *Bt*-resistant colonies of *Plodia interpunctella* (Hübner). In many cases, the development of *Bt*-resistant moths on *Bt*-treated diet was slower than the unselected moths on untreated diet (Oppert et al., 2000). Resistance to organophosphate of *C. pipiens pallens* (Bourguet et al., 2004), to monocrotophos of *H. armigera* (Xiaoxia et al., 2001), and to malathion of *Tribolium castaneum* (Herbst) (Haubruge and Arnaud, 2001) involved significant slower development of resistant strains than susceptible. Our results were also in accord with the deltamethrin and diflubenzuron resistance in another insect, the codling moth, *Cydia pomonella* (L), where resistance has decreased fecundity and fertility of this insect and resistant individuals developed more slowly and were lighter than susceptible individuals (Boivin et al., 2001).

In Benin Republic, our previous survey showed that *H. armigera* resistance to pyrethroids was unstable in field and in laboratory. Several resistant field strains monitoring indicated that resistance level increased in insecticide selection period and decreased when treatments were stopped. In laboratory, LD_{50} of several resistant strains reared without insecticide decreased significantly from the first generation through the following generations. These previous results evidenced that resistance was associated with fitness cost (Djihinto et al., 2009). In accord with our previous results, the main costs of resistance found for AGB03 strain in Benin involved lower fecundity, lower fertility and lower survival from egg to adult emergence.

No difference was observed about time from egg laying to adult emergence, larval weight at the end of larval development and survival during egg, larval and pupae stages in comparison of the resistant strain (AGB01) and the susceptible strain (BK77). These results were not in contradiction with those of Bielza et al. (2008) and Arnaud et al. (2002) in other insects. However, larvae of AGB01 strain took significantly longer time to develop and were significantly lighter than BK77 at the same age. The slower development of larvae of AGB01 strain was counteracted by the shorter times of egg and pupae stages. It appears that, if it exists a cost associated to AGB01 strain resistance to cypermethrin, the lower resistance level of this strain would not allow to assess clearly resistance cost.

Conclusion

Results of cypermethrin toxicity to AGB01 and AGB03 strains showed that both strains were resistant to cypermethrin. This resistance was associated with fitness cost and resistant strains could be disadvantaged when biological parameters were compared with susceptible strains. The study showed that larvae of AGB01 strain took significantly longer time to develop and were significantly lighter than BK77 at the same age. However, the slower development of larvae of AGB01 strain was contrasted with the shorter times of egg and pupae stages. Fecundity, fertility and survival from egg to adult emergence of AGB03 strain were negatively affected and were significantly lower than those observed in OKP01 strain. It appears that the lower fecundity, fertility and survival from egg to adult emergence should constitute the main costs for the AGB03 strain resistant to cypermethrin.

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