Synergistic Effect of Piperonyl Butoxide on the Toxicity of Delyamethrin Against Two Resistant Strains of the Bollworm *Helicoverpa armigera* in Côte d'Ivoire

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ABSTRACT

Helicoverpa armigera is a key-pest of cotton in West Africa, controlled by pyrethroids for more than twenty years. In the Côte d'Ivoire, susceptibility to pyrethroids is monitored every year on populations collected in Bouaké at the end of the season (BK strains). A loss of effectiveness to cypermethrin and deltamethrin has been observed for the last three years. Compared to the mean of 1985-94 LD_{50} values of the BK strains and to the LD_{50} of a susceptible laboratory strain (BK77), the H. armigera population collected in 1997 (BK97) expresses factors of resistance varying from 9 to 27 for deltamethrin. Using PBO, a threefold reduction in the LD_{50} is obtained in the resistant strain (BK97) while DEF has no effect. Observing that the LD_{50} obtained with PBO on the resistant strain remains tenfold above the susceptible one, we conclude that a) MFO are involved in the pyrethroid resistance mechanism and b) another one, excluding esterases, is probably occurring in this field population. The synergistic effect of PBO has been confirmed on another H. armigera population (NBG97) collected on the Burkina border.

Introduction

The bollworm *Helicoverpa armigera* (Hübner) is one of the main pests observed on cotton, in the old world and Australia. In west Africa, specially in the Côte d'Ivoire, *H. armigera* is active in raingrown crops area during the rainy season from June to November and attacks mainly maize and cotton (Nibouche, 1994).

In the Côte d'Ivoire, since 1996, an increase of LD_{50} was recorded for both cypermethrin and deltamethrin in field populations of *H. armigera*. The 1997 recorded LD_{50} values were twenty and ten times higher than the means of 1985-1992 LD_{50} respectively for cypermethrin and deltamethrin (Vassal *et al*, 1997).

In the countries where bollworms have developed insecticide resistance, it is most of the time multiple resistance to most of major chemical groups of insecticides. There are several mechanisms, from penetration resistance, nerve insensitivity to the metabolization of the insecticide under the effect of monooxygenases or esterases (Gunning *et al.,* 1993 ; McCaffery, 1994 ; Ottea *et al.*, 1995).

With the knowledge of the importance of metabolic resistance in *H. armigera*, we decided first to determine if metabolic detoxification could be involved in *H. armigera* pyrethroid resistance in Côte d'Ivoire.

Materials and methods

Third and fourth instar larvae (40 to 80 mg weight) in the F1 generation of field collected *H. armigera* are used for the tests. For each instar, larvae are selected immediately after moulting, weighed, and classified in 10 mg weight groups. The impact of pretreatment with piperonyl butoxide (Pbo) and s,s,s-tributyl-phosphorotrithioate (DEF) was tested on larvae of both laboratory susceptible (BK77) and field collected strains (BK97, NB97).

Insecticidal activities were assessed by topical application of measured droplets of insecticide solutions with an Arnold microapplicator (Burkard Manufacturing). One microliter of solution per 100 mg of larvae is laid on the neck of each larva. One hour before the insecticide treatment, the maximum sub lethal dose of each synergist was applied topically, according the same way, in 1µl of acetone per 100 mg of larva (0.02 µg of synergist per 100 mg of larvae).

Mortality data, collected 48 h after application were subjected to probit analysis according to Finney (Finney, 1971) to obtain LD_{50} values of each insecticide by using a PC-software, "WINDL" developed at CIRAD (Giner *et al.*, 1998). Resistance factors (RF) were expressed as LD_{50} resistant strain divided by LD_{50} susceptible strain. Synergism factors (SF) were expressed as LD_{50} without synergist divided by LD_{50} with synergist.

Results

Deltamethrin susceptibility

Results obtain with deltamethrin in 1997 on the different strains of *H. armigera* (Table 1) show that LD_{50} of both two field strains (BK97 and NB97) are higher than the reference one (0.049 µg a.i./g larva). On the other hand, a noticeable decrease of the

regression lines slopes is observed. The resistance factors of 27 and 18 show the development of a resistance mechanism in these two field populations of *H. armigera*.

Synergists evaluation

In Table 2, the addition of Pbo to deltamethrin significantly improves its toxicity in NB97 *H. armigera* strain (SF=19). The LD_{50} is reduced to that of the control. In BK97 strain, the increasing of deltamethrin toxicity by Pbo is less important, but significantly different from BK77 one. In this case, LD_{50} is still 10 times as high as that of the control. An other mechanism is involved in the deltamethrin resistance of the BK97 strain.

The use of DEF before the application of deltamethrin shows that this synergist has a minimal effect on the LD_{50} (Table 3). The LD_{50} of the BK97 strain remains 20 times as high as that of the susceptible strain one.

Discussion

Although BK97 and NB97 have the same resistance level, the synergism factors show that Pbo is more effective on the NB97 strain than on the BK97 strain collected in the experimental research station of IDESSA in Bouake. An other mechanism is involved in BK97 deltamethrin resistance. Synergist factors obtained with DEF on NB97 strain suggest that the predominant mechanism involved is mixed function oxydases (MFOs) not esterases.

These results suggest that pyrethroid resistance in the field is closely connected to the oxidative metabolic mechanism with perhaps a minor contribution of some other mechanisms like reduced penetration or nerve insensitivity in some special areas where selection pressure has been different.

Pbo could be used in resistance management strategies, but as mentioned by Forester *et al.* (1993), the use of synergists against insecticide resistant pests in the field has been not very effective, principally due to the development of new resistance to the synergistic insecticide. The recommendation to use Pbo in the field must be very careful. It will be not possible to mixe Pbo with all the treatment and it will be indispensable to alternate it with other synergists. On an other hand, it is necessary to develop our knowledge of the resistance extent in West Africa and on the different mechanisms involved in *H. armigera* pyrethroid resistance in each part of the cotton production area.

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Table 1. Deltamethrin toxicity and Resistance Factors (RF) on fourth instar larvae (µg a.i./ g larvae) of different susceptible and resistant strains of *Helicoverpa armigera* (topical application-probit analysis), with 95% confidence intervals and slopes of the mortality regression line with standard error.

Strain	LD_{50}	(95% C.i.'s)	slope ± SE	RF
BK77 (susceptible lab. strain)	0.049	(0.041, 0.058)	2.15 ± 0.27	
BK97 (field strain)	1.33	(0.89, 1.99)	1.10 ± 0.17	27
NB97 (field strain)	0.89	(0.53, 1.49)	1.14 ± 0.31	18

Table 2. Impact of the Pbo on the deltamethrin toxicity (Synergism Factor) on fourth instar larvae of different susceptible and resistant strains of *Helicoverpa armigera* (topical application-probit analysis), with 95% confidence intervals.

Strain	Deltamethrin alone		Deltamethrin + Pbo		SF
	LD_{50}	(95% C.i.'s)	LD_{50}	(95% C.i.'s)	
BK77 (susceptible lab. strain)	0.049	(0.041, 0.058)	0.046	(0.035, 0.060)	1.1
BK97 (field strain)	1.33	(0.89, 1.99)	0.43	(0.25, 0.76)	3
NB97 (field strain)	0.89	(0.53, 1.49)	0.047	(0.021, 0.106)	19

Table 3. Impact of the DEF on the deltamethrin toxicity (Synergism Factor) on fourth instar larvae of different susceptible and resistant strains of *Helicoverpa armigera* (topical application-probit analysis), with 95% confidence intervals.

Strain	Deltamethrin alone		Deltamethrin + DEF		SF
	LD_{50}	(95% C.i.'s)	LD_{50}	(95% C.i.'s)	
BK77 (susceptible lab. strain)	0.049	(0.041, 0.058)	0.045	(0.033, 0.062)	1.1
BK97 (field strain)	1.66	(0.89, 1.99)	0.860	(0.34, 2.17)	1.5