Efficacy of *Bacillus thuringiensis* insecticidal proteins Cry1Ac and Cry2Ab2 on the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae)resistant to Pyrethroids in Burkina Faso.

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Abstract

Geographic variation in the sensitivity of cotton bollworm to the *B. thuringiensis* insecticidalproteins Cry1Ac and Cry2Ab2 and to the Pyrethroid, deltamethrin insecticides, was studied to establish a baseline for comparing the responses of future populations to the increased use of *Bt* cotton in Burkina Faso.Bollworm populations were collected from 3 ecological cotton growing areas in Burkina Faso and their susceptibilitiesto Cry1Ac and Cry2Ab2 proteins were evaluated by the diet contamination method; their susceptibility to deltamethrin was evaluated by topical application. The susceptible strain BK77 was used for resistance ratio calculation. The LC_{50}s (50% lethal concentration) resulting in larval mortality ranged from 0.003 to 0.109 µgml^{-1} of diet for Cry1Ac and from 0.341 to 4.222 µgml^{-1} Cry2Ab2, with resistance ratios respectively from 0.2 to 7 and from 1 to 7. The same field strains had resistance ratios to deltamethrin rangingfrom 11 to 69. These data will be useful as baseline susceptibility indexes for resistance monitoring of Cry toxins in *H. armigera* field strains in Burkina Faso.Theywill also help set up adequate strategies for Pyrethroid resistance management programs.

KEY WORDS: *Bacillus thuringiensis*, bollworm, cotton, Cry toxins,Pyrethroids, resistance.

Résumé

La variation géographique de la sensibilité de *Helicoverpa armigera* (Lepidoptera : Noctuidae) aux toxines *Bt* et à la deltaméthrine a été étudiée en vue d'établir la toxicité de base à ces toxines afin de suivre l'évolution du niveau de sensibilité des futures populations au cours de l'utilisation de la technologie *Bt* au Burkina Faso. Les souches de *H. armigera* ont été collectées dans trois zones écologiques du Burkina Faso et leur sensibilité aux toxines Cry1Ac et Cry2Ab2 a été évaluée par la méthode de la contamination de la diète ; leur niveau de sensibilité à la deltaméthrine a été évalué par la méthode de l'application topique. Le calcul du ratio de résistance a été fait grâce à la souche sensible de référence, la BK77.Les CL50 (Concentration Létale pour 50% de la population) se situaient entre 0.003 et 0.109 µgml^{-1} de diète et entre 0.341 to 4.222 µgml^{-1} de diète respectivement pour Cry1Ac et Cry2Ab2, avec des ratios de résistance compris entre 0.2 et 7 et entre 1 et 7 pour Cry1Ac et Cry2Ab2 respectivement. Les mêmes souches de terrain avaient des ratios de résistance de 11 à 69 vis-à-vis de la deltaméthrine. Ces données constituent une base pour le suivi de la résistance des souches de terrain de *H. armigera* aux toxines Cry et concourent à la mise en place des stratégies adéquates de gestion de la résistance de ces souches aux pyréthrinoïdes

MOTS-CLÉS : *Bacillus thuringiensis*, *Helicoverpa armigera*, coton, toxines Cry, pyréthrinoïdes, résistance.

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Introduction

Cotton is one of the main cash crops in Burkina Faso (Traoré*et al.*, 2008). In fact, cotton generates over 60% of Burkina Faso's export earnings and serves as a vital catalyst for the development of the country with over 2 million people earning all or part of their income from cotton (Vognan *et al.*, 2002). Unfortunately, the cotton plant is subject to many pest attacks (Ahmad *et al.*, 1997; Hoy, 1998; Kranthi*et al.*, 2001),the main one being Lepidopteran larvae. Since the 1960s,different types of insecticides were used to control these pests in cotton fields in Burkina Faso. During the 1990s, a number of pests (mainly the bollworm *Helicoverpa armigera*) developed resistance to Pyrethroids (Héma *et al.*, 2009a).Cotton growers had to spray eight to twelve times instead of four or five (Héma *et al.*, 2009b), with increasing production costs and decreasing yields. In west Africa, cotton entomologists have put in place since 1998 a project named Regional Project for Prevention and Management of Insecticide Resistance in Cotton Insect Pests, in order to monitor mainly the cotton bollworm's resistance to Pyrethroids which are being used since 1980s (Martin *etal.*, 2000). After five years of these resistance monitoring programs, resistance levels of*H. armigera* had not decreased. Therefore, scientists and politicians in Burkina Faso agreed in introducing *Bt* cotton as an alternative to Pyrethroids spraying. It is in these conditions that the transgenic cotton variety BOLLAGARD II® has been experimented in Burkina Faso since 2003 with the objective of its introduction in farm cultivation systems because of many advantages reported in other parts of the world. Since the commercialization of the first Cry1Ac *Bacillus thuringiensis* Berliner (*Bt*) cotton variety in 1996 (BOLLGARD®), there have been numerous advancements in insect control with transgenic technology (Adamczyk and Gore, 2004). Indeed, different advantages are known to be associated with the adoption of transgenic crops. These are higher yields, less labor intensive and a reduction in the use of insecticides that result in higher profits from the crop in

the USA, China, South Africa and Mexico (Fernandez-Cornejo & Klotz Ingram, 1998; Gianessi & Carpenter, 1999; Fernandez-Cornejo *et al*., 1999; Perlak *et al*., 2001; Ismael *et al*., 2001; Traxler *et al*., 2001; Huang *et al*., 2002; Pray *et al*., 2002; Bennett *et al*., 2004; Shankar & Thirtle, 2005).

BOLLAGARD II®is known to express Cry1Ac and Cry2Ab2 toxins used on Lepidopteran pests on cotton (Zhao *et al*., 2003; Russel *etal*., 2004, Luo et al., 2007, Carrière *et al*., 2010).These toxins have also been successfully used as bio insecticideson caterpillars, beetles, and flies, including mosquitoes and black flies (Palma *et al*., 2014). Because of their mode of action onthe intestinal villositiesof arthropods, these toxins have no cross-resistance with insecticides used in cotton cultivation and can be associated to optimize pest control (Luo et al., 2007; Pardo-López *et al*., 2013). In fact, Cry1Ac and Cry2Ab2 have no direct effecton sucking pests like aphids, jassids and aleurods (Hema *et al*., 2009b) and insecticides have to be sprayed to control these pests.

This paper reports for the first time in Burkina Faso, Cry1Ac and Cry2Ab2 toxicity on the cotton bollworm *H. armigera* strains collected from different geographic cotton growing areas. Deltamethrin, a largely used pyrethroids in Burkina Faso, was tested along with *Bt* toxins to monitor their field strain resistance levels compared with the susceptible *H. armigera* strain BK77. The main object of this work was to investigate the effectiveness of *Bt* toxins on cotton bollworm and to establish a baseline toxicity in order to managea possible resistance development.

Materials and methods

Insects

Three field populations of *H. armigera* were collected from conventional cotton varieties during four growing seasons, from 2007 to 2010, atone location from each of three cotton companies in Burkina Faso. The collection areas were Bittou (Bit) in the southeast, Datomo (Dat) in the northwest and Kompienga (Kom) in the east of the country (Fig. 1). Field insects were reared on artificial diet (Couilloud et Giret, 1980) in the laboratory at 25°C ± 2°C, 70 ± 5% RH and a photoperiod of 14:10 (L:D) h. Emerged pupae were disinfected, sexed and keptat 25°C for males and 20°C for females. Moths obtained were fed in 1/1 sex ratio (male/female) on 10% honey-water solution. Eggs laid on the packs were collected daily and disinfected with bleach and placed in pots until hatching. First generation larvae obtained in the laboratory were used for the bioassays. The BK77 *H. armigera* strain known to be susceptible to Pyrethroids was collected at Bouake in the Ivory Coast in 1977 (Martin *et al*., 2003). This stain was keptin the laboratory without any insecticidal treatment and was used as susceptible check.

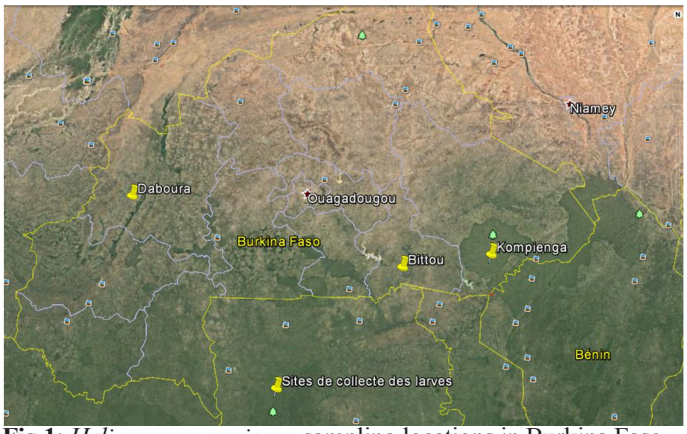

Fig 1: *Helicoverpa armigera* sampling locations in Burkina Faso.

Bioassays

*** Deltamethrin assay**

Technical deltamethrin containing 99.3% of a.i. was provided by Bayer CropScience. The assay conditions were the same as for thetoxins. The method used as described by Martin *et al*. (2000), was topical application with a micro applicator to apply a microliter on the thorax of the larva. Seven doses(0, 0.60, 0.95, 1.51, 2.39, 3.79 and 6.0 µg per g of insect) were tested on the three field strains. Six doses(0, 0.050, 0.0707, 0.0997, 0.141 and 0.2 µg per g of insect) were tested on the reference susceptible strain. Acetone was used to dissolve deltamethrin and also to treat the control batch. Third instar larvae were used for this assay. Each batch contained sixty larvae. Mortality was recorded daily for three days. The second day mortality record was used to calculate the median lethal dose (LD_{50}).

*** Cry1Ac and Cry2Ab2 toxins assay**

Toxins were provided by MONSANTO and contained 19.1% and 0.5727% respectively ofCry1Ac and Cry2Ab2. Assays were done in plastic boxes with wells containing artificial diet. The method used is diet surface treatment as described by Bird and Akhurst (2007). Serial dilutions of the toxins were done in phosphate buffer (10mM phosphate and 100mM NaCl). Seven concentrations, 0, 0.01µgml^{-1}, 0.022µgml^{-1}, 0.048µgml^{-1}, 0.096µgml^{-1}, 0.213µgml^{-1} and 1.032 µgml^{-1} were tested for Cry1Ac and eight concentrations, 0, 0.27µgml^{-1}, 0.41µgml^{-1}, 0.62µgml^{-1}, 0.93µgml^{-1}, 1.40µgml^{-1}, 2.10µgml^{-1} and 3.16 µgml^{-1} for Cry2Ab2. A 100 µl volume of toxin was associated with the diet in each well. One 1st-instar larva was placed in each well even if Kumar and Grewal (2015)showed that Bt toxins were efficient on second instar larvae of *S. litura* under laboratory conditions. We tested sixty larvae for each strain at each concentration (four replicates of fifteen). The buffer was used to treat control batch diet. Mortality was recorded daily until the seventh day. When mortality in the control batch reached ten percent, the assay was canceled. The seventh day mortality record was used to determine median lethal concentration (LC_{50}).

***Data analysis**

Probit analysis (Finney, 1971) was performed withWindl 2.0 software to estimate deltamethrin and Cry toxins

concentrations resulting in 10%, 50% and 90% mortality. Abbott natural mortality correction formula was automatically used by this software when the mortality was more than 5%: M = (M* – C) / (1 – C) where M = corrected mortality, M* = mortality observed at this dose of toxin and C = mortality observed in the control.

The data from all assays with field strains were pooled together and subjected to probit analysis to obtain a composite log dose response. For each toxin, the composite was compared to the laboratory susceptible strain BK77 by a non-parametric test that compares two independent populations, the Mann-Whitney test was performed using the XLSTAT software 2007 version.

Results

Deltamethrin LD_{50} for the field strains ranged from 0.965 to 5.995 μgg^{-1}oflarva. For the susceptible strain BK77, deltamethrin LD_{50} was 0.087μgg^{-1}oflarva. The susceptibility of *H. armigera* populations from Burkina Faso to deltamethrin is presented in Table I. Figure 2showsthe linear regression dose-mortality of *H. armigera* strains. It showed that the susceptible strain BK77 was more homogenous than the composite populationto deltamethrin. In comparison with the susceptible strain, the resistance factors ranged from 11 to 69. All fiducial limits for the field strains were higher than the BK77 ones. It means that the field populations were statistically different from the susceptible strain as far as resistance to deltamethrin is concerned.

Table I.LD_{50}, $LD_{90}$of deltamethrin andResistance Ratio of *Helicoverpa armigera* field strains.

Strain	n	LC_{50} (μgg^{-1})	95% FL (μgg^{-1})	LC_{90} (μgg^{-1})	SLOPE±SE	RR
Bit 07	60	2.844	0.889-5.278	33.515	1.196±0.003	33
Dat 07	60	3.81	0.901-9.051	83.479	0.956±0.003	44
Kom 07	60	2.637	0.726-5.436	44.813	1.042±0.003	30
Bit 08	60	0.965	0.164-2.218	32.638	0.838±0.004	11
Dat 08	60	3.5	0.932-7.443	60.173	1.038±0.003	40
Kom 08	60	1.08	0.315-2.097	17.307	1.064±0.003	12
Bit 09	60	1.134	0.262-2.407	26.615	0.935±0.003	13
Dat 09	60	2.301	0.779-4.170	25.284	1.231±0.003	26
Kom 09	60	1.416	0.449-2.684	19.932	1.116±0.003	16
Bit 10	60	2.979	0.860-5.932	43.403	1.102±0.003	34
Dat 10	60	5.995	1.418-15.786	115.091	0.999±0.003	69
Kom 10	60	1.446	0.475-2.687	18.275	1.163±0.003	17
Composite	720	1.961	1.508-2.457	34.975	1.024±0.001	23
BK 77	60	0.087	0.000-0.146	0.215	3.279±0.007	-

Susceptibility was estimated by deltamethrin concentration killing 50% larvae. *n* = total number of third instar larvae used in each dose of deltamethrin; LD_{50}= Medial Lethal Dose of deltamethrin expressed as μgg^{-1}; FL = Fiducial Limits; LD_{90} = Lethal Dose of deltamethrin killing 90% of 3rd-instar larvae expressed as μgg^{-1}; SE = Standard Error; RR = Resistance Ratio (deltamethrin LD_{50} for field strain divided by its LD_{50} for susceptible strain), Bit 07 = *H. armigera* strain collected at Bittou in 2007; Dat 08 = *H. armigera* strain collected at Datoma in 2008; Kom 09 = *H. armigera* strain collected at Kompienga in 2009.

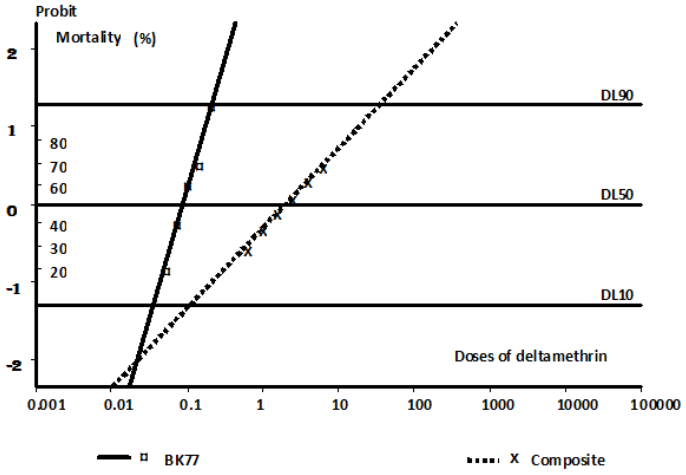

Fig. 2: **Susceptibility of *Helicoverpa armigera* to deltamethrin**. The graph shows in discontinuous line the dose-mortality of Composite population of field strains from 2007 to 2010; in continuous line is represented the dose-mortality of susceptible strain BK77.

Cry1Ac exhibited LC_{50} in the range 0.003 to 0.109 μgml^{-1} for the field strains (Table II) while Cry2Ab2LC_{50} ranged from 0.341 to 4.222 μgml^{-1} for the field strains (Table III). For the susceptible strain BK77, Cry1Ac exhibited an LC_{50} of 0.016 μgml^{-1} and Cry2Ab2LC_{50} was 0.575 μgml^{-1}. Figures 3 and 4showrespectively Cry1Ac and Cry2Ab2dose-mortalitylinear regression lines. Both showed homogeneity of BK77 as compared to the composite. The Resistance Ratios were very low for these toxins and ranged from 0.2 to 7 for Cry1Ac and from 1 to 7 for Cry2Ab2. The fiducial limits of all the field and laboratory strains overlapped. This means that the sensitivity of the different strains was not statistically different for the two Cry toxins. Thus, the comparison between the composite population and the susceptible strainhad shown no difference for the two Cry toxins while the comparison of susceptibility to deltamethrin showed that the composite was significantly more resistant than the susceptible strain BK77 (Table IV).

Susceptibility was estimated by the Cry1Ac concentration killing 50% of larvae. *n* = total number of 1st-instar larvae used for each Cry1Ac concentration used; LC_{50} = Medial Lethal Concentration of Cry1Ac expressed as μgml^{-1}; LC_{90} = Lethal Concentration of ninety percent of *H. armigera* population expressed as μgml^{-1}; FL = Fiducial limits; SE = Standard Error; RR = Resistance Ratio; Bit 07 = *H. armigera* strain collected at Bittou in 2007; Dat 08 = *H. armigera* strain collected at Datoma in 2008; Kom 09 = *H. armigera* strain collected at Kompienga in 2009.

Table III. LC_{50} andLC_{90} of Cry2Ab2 toxin and Resistance Ratio of *H. armigera* field strains.

Strain	n	LC_{50} (µgml^{-1})	95% FL (µgml^{-1})	LC_{90} (µgml^{-1})	SLOPE±SE	RR
Bit 07	60	4.201	0.789-15.318	151.44	0.823±0.004	7
Dat 07	60	4.222	0.866-14.368	132.729	0.856±0.004	7
Kom 07	60	3.936	0.511-26.390	374.643	0.648±0.005	7
Bit 08	60	1.715	0.011-270.128	50.25	0.874±0.019	3
Dat 08	60	3.177	0.11-925.939	165.977	0.746±0.021	6
Kom 08	60	3.165	0.263-38.136	79.479	0.916±0.009	6
Bit 09	60	0.564	0.256-0.91	4.08	1.490±0.002	1
Dat 09	60	0.536	0.273-0.802	2.698	1.826±0.018	1
Kom 09	60	0.492	0.285-0.699	1.944	2.146±0.002	1
Bit 10	60	0.508	0.286-0.752	2.236	1.992±0.002	1
Dat 10	60	0.346	0.229-0.451	2.714	1.433±0.001	1
Kom 10	60	0.341	0.114-0.581	2.9	1.379±0.003	1
Composite	720	1.013	0.793-1.250	15.657	1.078±0.001	2
BK77	60	0.575	0.257-0.932	4.173	1.488±0.002	-

Susceptibility was estimated by the Cry2Ab2 concentration killing 50% of larvae. *n* = total number of 1st-instar larvae used for each Cry2Ab2 concentration used; LC_{50} = Medial Lethal Concentration of Cry2Ab2 expressed as µgml^{-1}; LC_{90} = Lethal Concentration of ninety percent of *H. armigera* population expressed as µgml^{-1}; FL = Fiducial limits; SE = Standard Error; RR = Resistance Ratio; Bit 07 = *H. armigera* strain collected at Bittou in 2007; Dat 08 = *H. armigera* strain collected at Datoma in 2008; Kom 09 = *H. armigera* strain collected at Kompienga in 2009.

Table II.LC_{50} andLC_{90} of Cry1Ac and Resistance Ratio of *H. armigera* field strains.

Strain	n	LC_{50} (µgml^{-1})	95% FL (µgml^{-1})	LC_{90} (µgml^{-1})	SLOPE±SE	RR
Bit 07	60	0.064	0.039-0.101	4.42	0.697±0.002	4
Dat 07	60	0.072	0.000-1398.100	16.124	0.545±0.036	5
Kom 07	60	0.103	0.000-200253000	154.251	0.404±0.079	6
Bit 08	60	0.085	0.002-0.730	278.352	0.365±0.008	5
Dat 08	60	0.109	0.000-3572610	102.907	0.431±0.064	7
Kom 08	60	0.06	0.030-0.109	16.833	0.523±0.002	4
Bit 09	60	0.02	0.006-0.039	0.145	1.153±0.003	1
Dat 09	60	0.005	0.000-0.019	0.641	0.629±0.006	0,3
Kom 09	60	0.003	0.000-0.010	0.24	0.666±0.007	0,2
Bit 10	60	0.012	0.003-0.026	3.905	0.513±0.003	1
Dat 10	60	0.035	0.011-0.073	0.696	0.985±0.003	2
Kom 10	60	0.038	0.000-0.137	0.511	1.134±0.007	2
Composite	660	0.032	0.020-0.048	6.704	0.552±0.001	2
BK 77	60	0.016	0.004-0.039	0.517	0.857±0.004	-

Susceptibility was estimated by the Cry2Ab2 concentration killing 50% of larvae. *n* = total number of 1st-instar larvae used for each Cry2Ab2 concentration used; LC_{50} = Medial Lethal Concentration of Cry2Ab2 expressed as µgml^{-1}; LC_{90} = Lethal Concentration of ninety percent of *H. armigera* population expressed as µgml^{-1}; FL = Fiducial limits; SE = Standard Error; RR = Resistance Ratio; Bit 07 = *H. armigera* strain collected at Bittou in 2007; Dat 08 = *H. armigera* strain collected at Datoma in 2008; Kom 09 = *H. armigera* strain collected at Kompienga in 2009.

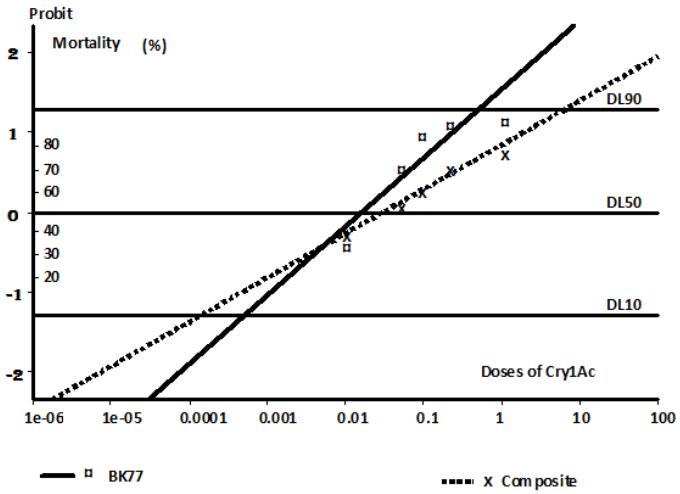

Fig. 3: **Susceptibility of *Helicoverpa armigera* to Cry1Ac toxin**. The graph shows in discontinuous line the dose-mortality of Composite population of field strains from 2007 to 2010; in continuous line is represented the dose-mortality of susceptible strain BK77.

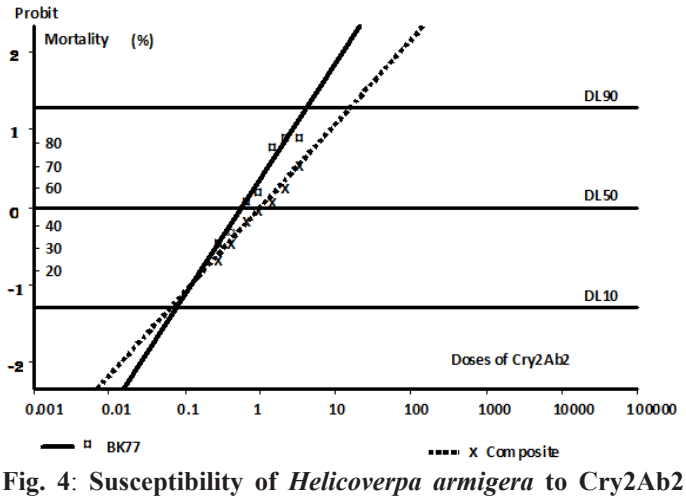

Fig. 4: **Susceptibility of *Helicoverpa armigera* to Cry2Ab2 toxin**. The graph shows in discontinuous line the dose-mortality of Composite population of field strains from 2007 to 2010; in continuous line is represented the dose-mortality of susceptible strain BK77.

Table IV: Mann-Whitney statistical susceptibility comparison between composite and BK77

Toxic	observed z	critical z	Probability	Significance
Cry1Ac	1.143	1.960	0.253	NS
Cry2Ab2	1.156	1.960	0.248	NS
Deltamethrin	**-2.739**	1.960	**0.006**	**HS**

Mann-Whitney non-parametric test was used to compare composite to susceptible strain BK77. The z test uses observed values that must be compared to the critical value of 1.960. There is significant difference if observed z is lower than critical z. The test is rejected if observed z is higher than critical z. NS = Non-Significant; HS = Highly Significant.

The composite population dose-mortality line indicated that Cry1Ac was 32 times more toxic than Cry2Ab2to*H. armigera* strains.

Discussion

The introduction of *Bt* cotton was expected to keep Lepidopteran pest populations under economic thresholds. Before the introduction of any new component of pest management, especially toxic compounds, it is appropriate to examinethe baseline toxicity data to enable detection of changes in susceptibility in field populations as influenced by the compound after prolonged exposure (Kranthi *et al.*, 1999; Siegfried *et al.*, 2007; Tabashnik *et al.*, 2008b; Brévault *et al*., 2009). Since cotton pest management in Burkina Faso used no *Bt* toxins, it was presumed that the cotton bollworm had not been subjected to these compounds (Greenplate, 1999).

Susceptibility to Cry1Ac and Cry2Ab2 toxins was measured in fifteen *H. armigera* populations inBurkina Faso, from 2007 whenno *Bt* cotton was cultivated by farmers up to the 2010 growing season whenfarmers had planted more than 200,000 ha of *Bt* Cotton. Two populations are said to be significantly different as far as their susceptibility to a chemical is concerned if it is demonstrated that there is no overlap in the 95% fiducial limits of their LC_{50} values (Tabashnik *et al*., 1987). Our results showed the same level of susceptibility between field strains and the susceptible one reared in the laboratory free of all chemicals since 1977,whereas deltamethrin LD_{50}s showed differences between BK77 and field strains during the four years' tests. These results suggest that there was no cross resistance between the two categories of insecticide in *H. armigera*. Deltamethrin is a neurotoxic insecticide (Ahmad, 2004) whereas the *Bt*delta-endotoxins act on the intestinal micro villosities (Olsen and Daly, 2000; Uraichuen, 2002; Pardo-López*et al*., 2013). Cry1Ac and Cry2Ab2 expressed by the transgenic cotton plant would be effective in the management of *H. armigera* resistance to Pyrethroids.

Bt toxin effectiveness was shown by several authors: Kranthi and Russel (2004) reported that Cry1Ac LC_{50} on *H. armigera* varied from 0.01 to 0.67 μgml^{-1} of diet on field strain in China and from 0.091 to 9.093 μgml^{-1} of diet in India from 2001 to 2003. These results are similar to ours which showed the three field collected strains' susceptibility to the two toxins from 2007 to 2009 was not significantly different. These results are in opposition with that fund found by Brévault *et al.* (2009) who revealed that the susceptibility to *Bt* toxins of *H. armigera* field-collected strains in four non-growing *Bt* cotton West African countries (Benin, Cameroon, Nigeria, and Chad) was significantly different from 2006 to 2008. In fact, the LC50 data of Cry2Ab2 and Cry1A shown by the authors, ranging respectively from 5.12 to 50.71 µgml-1 of diet and from 0.38 to 16.71 µgml-1 of diet, proved to be higher than our results obtained with the same toxin though the Fiducial Limits are not statistically different. Furthermore, if Wu *et al.* (1999) found differences of Cry1Ac toxicity among field strains collected from five areas in China, our study did not show any difference in the toxicity of the two toxins to the three field collected strains in Burkina Faso.

In addition, Stone and Sims (1993) had studied the baselines of Cry1Ac on *Heliothis virescens* and *H. armigera* from several developing countries, plus Hawaï and the Virgin Islands; they ranged from8 fold for *H. virescens*to16-fold for *H. zea*. These results suggest that the susceptibility of the different field strains of *H. armigera*, *H. virescens* and *H. zea* to *Bt* toxins depends on their age and the length of exposure to these toxins. For *H. zea* strains obtainedfrom the field in 1992-1993, before Cry1Ac cotton was commercialized, the maximum resistance ratio was 1.2 and the maximum LC_{50} value among field-derived strains was 5.97 µg Cry1Ac/ml diet (Luttrell *et al*. 1999, Tabashnik *et al.* 2008a). The procedures, the choice of insect, its development stage, and the methods used for toxin production are factors of significant variation LC_{50}(Karim et *al*., 1999; Olsen and Daly 2000).

This difference in baseline susceptibility may be explained by the frequency of resistance alleles in these populations (Downes *et al*., 2010).

Several studies had reported the efficacy of transgenic cotton on Lepidopteran pests. However, the expression of Cry toxin in transgenic plants is known to decline steadily in fruit and terminal parts as the growing season progresses, sometimes reaching undetectable levels (Greenplate *et al.*, 2003). For *H.armigera*, the fitted mortality response to the Cry1Ac protein was close to its maximum at protein concentrations above 3 µg/g Cry1Ac while mortality response to Cry2Ab2 increased steadily asconcentration increasedto 1200 µg/g Cry2Ab (Knight *et al.*, 2016). Moreover, Sun *etal.* (2002) had found that the mortality of *H. armigera*first instar larvae on different *Bt* cotton lines from China was high reaching100 % before flowering but decreased to 50-70% by peak flowering and boll set.

As the primary threat to the continued success of *Bt* crops is the evolution of resistance by pests (Tabashnik, 1994, Gould, 1998, Tabashnik *et al*., 2003; Griffits and Aroian, 2005; Bravo and Soberon, 2008; Onstad, 2008), resistance monitoring and management systems must be implemented in order to avoid an eventual apparition of resistance to *Bt* toxins. The adoption of second generation biotech cotton containing two toxins is a first tool in the prevention of resistance apparition because several studies demonstrated that transgenic plants expressing two *Bt* toxins can delay insect resistance evolution

(Tabashnik *et al*., 2002; Zhao *et al*., 2003; Bird & Akhurst, 2004). Pyramiding different *Bt* genes in cotton is valuable for managing resistance evolution. Bollgard II® (MON 15985) cotton expressing both Cry1Ac and Cry2Ab proteins has been developed to delay resistance development (Zhao *et al*., 2003). Indeed, a study conducted by Luo *et al.* (2007) indicated no cross-resistance of Cry1Ac resistant *H. armigera* larvae towards Cry2Ab. They concluded that transgenic cotton expressing Cry1Ac and Cry2Ab genes may be deployed for management of Cry1Ac resistant *H. armigera* in China.
Natural or constituted refuges may be implemented to dilute the resistance genes in insect populations (Onstad *et al*., 2011); because resistance ratios >10 are more likely to reflect genetically based decreases in susceptibility (Tabashnik, 1994).

Monitoring method must be improved by using tests estimating alleles frequency that confer toxin resistance on insect pests (Gould *et al*., 1997; Blanco *et al*., 2009; Brévault *et al*., 2009; Tabashnik *et al*, 2009).

Conclusion

This study has allowed to establish the baseline susceptibility of *Helicoverpa armigera*field strains to Cry toxins contained in BOLLGARD II. It has also allowed to show the resistance level of these field strains to Pyrethroids, the most used insecticide family in Burkina Faso. The susceptibility of these deltamethrin resistant strains to Cry toxins is an indication of the efficacy of these toxins in the management of cotton lepidopteran pests. In order to develop an effective pest management program and a rational strategy to prevent resistance, there is a need to carry on monitoring over certain period of time and space, eventual changes in the susceptibility of *H. armigera* field strains to Cry toxins.

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