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

1°Omer Sacamba Aimé Héma, 2°Isoufou Ouédraogo, 3°Oumar Traoré, 1°Blaise K. Zagré.

**Abstract**

Geographic variation in the sensitivity of cotton bollworm to the *B. thuringiensis* insecticidal proteins 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 susceptibility to 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_{90}^(-1) (50% lethal concentration) resulting in larval mortality ranged from 0.003 to 0.109 µg/ml of diet for Cry1Ac and from 0.341 to 4.222 µg/ml of Cry2Ab2, with resistance ratios respectively from 0.2 to 7 and from 1 to 7. The same field strains had resistance ratios to deltamethrin ranging from 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. They will 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 µg/ml de diète et entre 0.341 et 4.222 µg/ml 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.

**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 (Vogman 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 et al., 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 BOLLGARD 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 & Thirile, 2005).

BOLLAGARD II® is known to express Cry1Ac and Cry2Ab2 toxins used on Lepidopteran pests on cotton (Zhao et al., 2003; Russel et al., 2004, Luo et al., 2007, Carrière et al., 2010). These toxins have also been successfully used as bio insecticides on caterpillars, beetles, and flies, including mosquitoes and black flies (Palma et al., 2014). Because of their mode of action on the intestinal villosity of 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 effect on sucking pests like aphids, jassids and aleurodes (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 manage 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, alone 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 kept at 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 strain was kept in the laboratory without any insecticidal treatment and was used as susceptible check.

**Bioassays**

**Deltamethrin assay**

Technical deltamethrin containing 99.3% of a.i. was provided by Bayer CropScience. The assay conditions were the same as for the toxins. 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, 0.05, 0.07, 0.141, 0.2 µg per g of insect) were tested on the three field strains. Six doses(0, 0.050, 0.0707, 0.213 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 (LD₅₀).

**Cry1Ac and Cry2Ab2 toxins assay**

Toxins were provided by MONSANTO and contained 19.1% and 0.5727% respectively of Cry1Ac 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, 0.050, 0.0707, 0.141, 0.2 µg per g of insect) were tested on the three field strains. Six doses(0, 0.050, 0.0707, 0.213 µg per g of insect) were tested on the reference susceptible strain. Acetone was used to dissolve the toxins 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 (LD₅₀).

**Data analysis**

Probit analysis (Finney, 1971) was performed with Windl 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%: 

\[ M = \frac{M^* - C}{1 - C} \]

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 \( \mu g^{-1} \) larva. For the susceptible strain BK77, deltamethrin \( LD_{50} \) was 0.087 \( \mu g^{-1} \) larva. The susceptibility of \( H. armigera \) populations from Burkina Faso to deltamethrin is presented in Table I. Figure 2 shows the linear regression dose-mortality of \( H. armigera \) strains. It showed that the susceptible strain BK77 was more homogenous than the composite population to 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.

Susceptibility was estimated by deltamethrin concentration killing 50% larvae. In each dose of deltamethrin; \( LD_{50} = MEDIAL \) Lethal Dose of deltamethrin expressed as \( \mu g^{-1} \); \( FL = Fiducial Limits; LD_{90} = Lehalt \) Dose of deltamethrin killing 90% of 3rd-instar larvae expressed as \( \mu g^{-1} \); \( 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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( LD_{50} ) (µg/g)</th>
<th>( LC_{90} ) (µg/g)</th>
<th>( 95% FL )</th>
<th>( SLOPE )</th>
<th>( SE )</th>
<th>( RR )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bit 07</td>
<td>0.284</td>
<td>0.089</td>
<td>0.825</td>
<td>0.507</td>
<td>0.024</td>
<td>1.00</td>
</tr>
<tr>
<td>Bit 08</td>
<td>2.841</td>
<td>0.934</td>
<td>0.837</td>
<td>0.510</td>
<td>0.037</td>
<td>0.96</td>
</tr>
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<td>Kom 07</td>
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<td>0.037</td>
<td>0.96</td>
</tr>
<tr>
<td>Kom 08</td>
<td>4.921</td>
<td>1.146</td>
<td>1.301</td>
<td>0.520</td>
<td>0.040</td>
<td>0.93</td>
</tr>
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<td>4.921</td>
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<td>1.301</td>
<td>0.520</td>
<td>0.040</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Susceptibility of \( H. armigera \) 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.
Susceptibility was estimated by the Cry2Ab2 concentration killing 50% of larvae. \( n \) = total number of 1\(^{st}\)-instar larvae used for each Cry2Ab2 concentration used; \( LC_{50} \) = Lethal Concentration of Cry2Ab2 expressed as µg ml\(^{-1}\); \( LC_{90} \) = Lethal Concentration of ninety percent of \( H. \) armigera population expressed as µg ml\(^{-1}\); 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\(_{90}\) and LC\(_{90}\) of Cry1Ac and Resistance Ratio of \( H. \) armigera field strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( n )</th>
<th>( LC_{90} ) (µg ml(^{-1}))</th>
<th>95% FL (µg ml(^{-1}))</th>
<th>LC(_{90}) SLOPE ± SE</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK 77</td>
<td>60</td>
<td>0.19</td>
<td>0.009-0.11</td>
<td>0.161 ± 0.007</td>
<td>1.01</td>
</tr>
<tr>
<td>Kom 07</td>
<td>60</td>
<td>0.93</td>
<td>0.69-1.16</td>
<td>0.32 ± 0.015</td>
<td>1.00</td>
</tr>
<tr>
<td>Dat 07</td>
<td>60</td>
<td>1.43</td>
<td>1.11-1.75</td>
<td>0.38 ± 0.025</td>
<td>1.00</td>
</tr>
<tr>
<td>Bit 07</td>
<td>60</td>
<td>1.99</td>
<td>1.81-2.17</td>
<td>0.58 ± 0.022</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Fig. 3: Susceptibility of \( H. \) 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 \( H. \) 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.
The composite population dose-mortality line indicated that Cry1Ac was 32 times more toxic than Cry2Ab2 to 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 examine the 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 in Burkina Faso, from 2007 when no Bt cotton was cultivated by farmers up to the 2010 growing season when farmers 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 µg/ml of diet on field strain in China and from 0.091 to 9.093 µg/ml 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 LC$_{50}$ data of Cry2Ab2 and Cry1A shown by the authors, ranging respectively from 5.12 to 50.71 µg/ml of diet and from 0.38 to 16.71 µg/ml 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 Cry1A 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 Hawai and the Virgin Islands; they ranged from 8 fold for H. virescens to 16-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 a. zea strains obtained from 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 Cry1A protein was close to its maximum at protein concentrations above 3 µg/g Cry1Ac while mortality response to Cry2Ab2 increased steadily as concentration increased to 1200 µg/g Cry2Ab (Knight et al., 2016). Moreover, Sun et al. (2002) had found that the mortality of H. armigera first instar larvae on different Bt cotton lines from China was high reaching 100% 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 Arojan, 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).

Conclusion

This study has allowed to establish the baseline susceptibility of Helicoverpa armigerazfield 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.

Acknowledgements

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