

Technical constraints to the use of Bt technology in cotton farming. The case of toxin expression

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## 1. Introduction

Efforts to introduce Bt cotton in certain developing countries or countries with emerging economies over the past several years have met with varying degrees of success (Pray *et al.*, 2002; Bennett *et al.*, 2003; Hofs and Berti, 2006). However, we should point out that introgression of the first Bt gene, Cry1Ac, in cotton was originally designed to ensure the control of *Heliothis virescens* and *Pectinophora gossypiella* in the United States (Schell, 1997). The interest of the Cry1Ac gene in controlling *Helicoverpa armigera*, a predator found in most cotton-producing developing countries throughout Asia and Africa, became apparent very quickly after its launching in the United States. As a result, Bollgard I cotton was commercially introduced in China in 1996, in South Africa in 1997 and in India in 2002.

Right now, cotton-producing African countries have different attitudes towards the use of this technology in their agricultural systems. Four countries already have national regulations and have already conducted agronomic tests (South Africa, Burkina Faso, Sudan and Zimbabwe). Others are on the verge of enacting a regulatory framework and starting up tests or are still questioning the merit of embracing this new technology. The benefits of Bt cotton have been discussed at length over the past ten years in the scientific as well as the nonscientific literature and will not be dealt with in this article. On the other hand, the limitations of this technology are not often discussed objectively and, as such, warrant further clarification.

Genes determine the traits and functions of all living organisms. They are the basic units of diversity and heredity and are defined as a DNA segment containing a specific sequence of nucleotides. Genes express themselves through a protein or enzyme. Their expression varies according to their sequence of nucleotides, the nature of their promoter, their insertion site in the modified plant, the plant's internal environment and the different sources of modification of the external biotic (effects of the activity of living organisms) and abiotic (effects of a physical nature) environment. Thus, transgenes are liable to fully express themselves only in an optimal environment. High-input intensive farming is probably the farming system best able to meet or, at least, come close to meeting these conditions. In contrast, low-input family-owned farms in areas subject to numerous climatic hazards may well be the type of farming system least apt to meet the needs of genetically modified plant material.

This article recaps existing knowledge on the efficacy of the insecticide toxins produced by Bt cottons recommended for use in Africa and discusses opportunities for their introduction.

## 2. Different Bt genes in Africa and their efficacy in controlling *Helicoverpa armigera*

Depending on the country in question, there are two types of Bt cotton varieties commercially marketed or distributed in Africa. Bollgard® I cultivars containing the Cry1Ac gene were introduced in South Africa early on and are still being cultivated on a large scale. The Cry1Ac toxin provides good resistance to *Heliothis virescens*, *Earias sp.* and *Pectinophora gossypiella*. It is also recommended for controlling *Helicoverpa armigera* and *H. zea* in certain areas. However, numerous studies have shown that it is not quite as effective on

these caterpillars as it is against *H. virescens* (Luttrell *et al.*, 1999; Liao *et al.*, 2002; Kranthi, 2006; Russel & Deguine, 2006).

Bollgard® II cultivars contain two genes, namely the Cry1Ac and Cry2Ab genes. The Cry2Ab gene was introduced in an attempt to strengthen the cotton plant's defenses against bollworms and extend its range of resistance to other caterpillars such as *Spodoptera frugiperda* (Leonard *et al.*, 2006) and certain phyllophagous or leaf-eating caterpillars. It is for these reasons and due to the presence of higher concentrations of Cry toxins (Penn *et al.*, 2001) in flower organs (squares in particular) that the double gene has been used in Australia since 2002-2003, where it has produced excellent results in controlling *H. armigera* (Downes *et al.*, 2007). However, Liao *et al.* (2002) showed that the effect (lethal dose) of the Cry2Ab purified crystalline toxin on *H. armigera* was three times less than that of Cry1Ac. Accordingly, in the early stages of development, the extra measure of resistance provided by Cry2Ab in a plant already containing Cry1Ac may not be as important as one might think. The advantage of Cry2Ab lies mainly in its ability to extend protection beyond the peak flowering period. This trait has been established with regard to *H. virescens* (Greenplate *et al.*, 2000; Adamczyk *et al.*, 2001) but still needs to be confirmed with respect to *Helicoverpa armigera*.

A gene's efficacy with respect to a particular insect species varies in both time and space. It depends on the intrinsic sensitivity of the insect population (and lineage) in a given area (or country) and, in addition, will vary according to the speed with which the individuals making up such population build up a resistance to the toxins. A strain of Cry2Ab-resistant *H. armigera* was recently discovered in Australian fields (Mahon *et al.*, 2007) despite stringent resistance management programs (Downes *et al.*, 2007).

Aside from the aforementioned two widely commercially marketed genes, there are many other such genes, including Cry2Aa, which has proved to be even more effective than Cry2Ab against *H. armigera* (Liao *et al.*, 2002; Avilla *et al.*, 2005).

### 3. Genetic stock

The same transgene will not express itself exactly the same way in all cultivars. As a result, the efficacy of toxins varies widely from one variety to another. This proven inconsistency in the control of *Helicoverpa armigera* (Wan *et al.*, 2005; Olsen *et al.*, 2005) as well as other species (Adamczyk and Gore, 2004) is found in *Gossypium hirsutum* cultivars as well as in hybrid cultivars (Kranthi *et al.*, 2005; Dong *et al.*, 2006). This variability is illustrated in Figures 1 and 2, which recap the results obtained with genetically distant Chinese, Australian and U. S. materials. In the case of the Cry1AC gene, Adamczyk *et al.* (2000, 2001) showed how, among U. S. cultivars, season-long concentrations can vary from simple to double.

### 4. Parts of the plant

Concentrations of Bt protein (and, thus, its efficacy) vary according to the organ in question (Figures 3 and 4). In general, petals, leaves and squares have higher concentrations of Bt toxins than anthers and ovules (Adamczyk *et al.*, 2000; Wan *et al.*, 2002).

Concentrations of Bt toxins in cotton plants vary according to the position of the boll (Akin *et al.*, 2003). Bolls in position 1 (close to the main stem) have higher concentrations than those in positions farther down the same fruiting branch. Concentrations of Bt protein steadily decline between nodes 9 and 17. Compare this tendency with the effect of the plant's age, which is discussed in section 5 below.

## 5. Age of the plant

The degree of protection afforded by the Bt gene varies over time, decreasing according to the age of the plant (Daly and Fitt, 1998; Greenplate *et al.*, 2000; Gore *et al.*, 2003). Bt protein concentrations generally decline after the peak flowering period (Adamczyk *et al.*, 2000; Kranthi *et al.*, 2005; Olsen *et al.*, 2005) to values under the efficacy threshold. This tendency is especially apparent in leaves and squares (Figure 4).

## 6. Effect of chloroplast concentrations in tissue

Those parts of the plant which are not exposed to light have less protection from Bt toxins. Abel and Adamczyk (2004) showed how, in Bt cultivars, bolls with remaining corollas on top had some discoloration in their apex area (the mucron) and how this part of the plant was discovered to be more sensitive to infestations of *Spodoptera frugiperda*. A study of the bolls revealed a smaller Bt protein content in those parts with a smaller chloroplast content. This same phenomenon was also observed in corn.

## 7. Effect of temperature

High temperatures cause physiological imbalances in the plant (Burke *et al.*, 1985) and can trigger the degradation of soluble proteins, reducing the concentration of Bt toxins. Chen *et al.* (2003, 2005) showed how exposure to temperatures of over 37 degrees Centigrade for a 24-hour period reduces concentrations of Cry1A proteins by more than 50%. Olsen *et al.* (2005) report that the survival of *H. armigera* on Bt cotton is influenced by short-term or protracted exposure to high and low temperatures. They point out that low temperatures of around 14 to 20 degrees Centigrade are a real handicap to toxin expression.

## 8. Effect of drought

The effect of drought is oftentimes confused with the effects of high temperatures. However, Martins *et al.* (submitted in 2007) show that water stress plays only a small part in reducing concentrations of Bt toxins. They also demonstrate the variability of the effect of drought within a plant population.

## 9. Effect of salinity and waterlogging

Salinity is a serious problem for many crops. In the case of cotton, which is considered moderately resistant (Leidi and Saiz, 1997), salinity alone reduces Bt toxin rates to what are still acceptable levels for the control of *H. armigera* (Jiang *et al.*, 2006). Moreover, field observations in South Africa (Hofs, 2001) and recent greenhouse studies in China suggest that waterlogging may have a much more damaging effect on the expression of Bt toxins.

## 10. Effect of nitrogen deficiency

High doses of nitrogen increased concentrations of Bt toxins by 14%, compared with a minimal fertilization program (Pettigrew and Adamczyk, 2006). The same study suggests that, with inadequate fertilization, any nitrogen contained in the plant in the form of protein, including CryA toxins, is remobilized for the development of fruiting organs. Reassignment of the plant's nitrogen for another use would translate into a sharp drop in its concentrations of Bt toxin.

## 11. Other abiotic factors

High levels of CO<sub>2</sub> reduce the rate of Bt toxins in a given plant (Coviella *et al.*, 2000, 2002). However, there are numerous interactions with the CO<sub>2</sub> and, thus, we need to use caution in interpreting correlations between CO<sub>2</sub> rates in the atmosphere and toxin rates. The C/N factor may offer a more satisfactory explanation of the variation in concentrations of Bt toxins: an increase in this ratio would cause the quantity of toxins to decline. Conversely, an increase in the level of CO<sub>2</sub> may have a positive effect on the toxin rate by promoting photosynthesis and improving the plant's water efficiency (Samarakoon and Gifford, 2004).

## 12. Effect of concentrations of secondary compounds

Plants contain many secondary compounds such as phenols, orthoquinones, terpenoids and tannins. Studies have shown that the quantities of these compounds vary according to the age of the plant and exposure to external factors. Some of these compounds create synergies with Bt toxins (gossypol), while others (tannins) create negative interference (Dally and Fitt, 1998). Kranthi *et al.* (2005) suggested that, in times of stress, the relative increase in the concentration of gossypol makes up for the reduction in the concentration of Bt toxin. These findings show that changes in efficacy do not only depend on the level of Bt toxin in the plant, but also on the plant's physiological condition.

## 13. Conclusions and outlook for Africa

Obviously, a reduction in the efficacy of Bt toxins exposes the plant to predators and reduces expected yields. It also allows generations of caterpillars exposed to sublethal doses to build up a resistance to Bt toxins.

The success of efforts to control cotton bollworms depends on two factors: the population's sensitivity to a specific toxin and the toxin's level of expression in the part of the plant attacked by this pest.

Establishing a gene's specificity (and efficacy) with regard to a given insect species or population is the first step in any program for the introduction of pest-resistant cultivars. Right now, there are few first-generation genes recommended for use in African cotton industries and they are all « ready-made » genes whose efficacy against targeted insects, while relatively good, is still inferior to their results against *Heliothis virescens*. As part of a better targeted control program, following the example of Brazil (Silva *et al.*, 2004), African research could select the most effective Bt toxins against local populations of different species of lepidopteran pests.

Until we see results and pending the development of new Bt strains, the use of Cry1Ac, Cry2Ab or even Cry1F genes must categorically be accompanied by strict agricultural monitoring to maintain an optimal level of toxin expression and prevent any further losses of efficacy. In other words, it is a question of introducing genetically modified cottons in tandem with the use of more intensive farming practices. Unfortunately, the cost of this type of intensive farming system will be unaffordable for certain small farmers.

We cannot lose sight of the fact that the transgene interacts with highly complex physiological mechanisms in the plant and its environment.

It is important that special attention be paid to the plant's nitrogen nutrition, which affects protein metabolism and, as a result, Bt toxin production. Maintaining good soil fertility is a real challenge in many African countries suffering from losses of fertility, shortages of

inputs and disorganized agricultural training and extension systems resulting in noncompliance with research recommendations.

Finally, abiotic stress factors such as high temperatures and periods of drought during the plant's growing cycle (dry spells without rain) are all limiting factors if not serious risks which need to be taken into account in rainfed farming systems.

Given the complex workings and demands of Bt technology, most likely, efforts to introduce genetically modified cotton in Africa will meet with varying degrees of success, depending on the pest situation prior to its introduction, the degree of intensification of the local farming system and the financial situation of local farmers, as well as the level of organization of the cotton sector.

## Bibliography

Abel C., Adamczyk J.J. (2004). Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of Fall armyworm (Lepidoptera:Noctuidae) and Southwestern Corn Borer (Lepidoptera:Crambidae) on maize whorl leaf profiles. *J. Econ. Entomol.* 97(5), 1737-1744.

Adamczyk J.J., Adams L.C., Hardee D.D. (2000). Quantification of Cry1Ac  $\delta$ -endotoxin in transgenic Bt cotton: correlating insect survival to different protein levels among plant parts and varieties. Proc. Beltwide Conf. 929-933.

Adamczyk JJ, Bew K., Adams LC., Hardee DD. (2001) Evaluation of Bollgard® (cv.DP50BII) in the mississippi delta: field efficacy against various Lepidoptera while profiling season-long expression of Cry1Ac and Cry2Ab. Proc. Beltwide Conf. 835-836.

Adamczyk Jr.J.J., Gore J. (2004). Development of bollworms, *Helicoverpa zea*, on two commercial Bollgard ® cultivars that differ in overall Cry1Ac levels. [En ligne] *J. Insect Sci.*, 4:32, accessible à l'adresse: <http://www.insectscience.org/4.32> , 5 p., consulté le 26 octobre 2006.

Akin D.S., Layton M.B., Stewart S.D., Adamczyk J.J. (2003). Profiling seasonal expression of Cry2Ab in bolls of dual-toxin Bt cotton. Beltwide Conf. 1157-1160.

Avilla C., Vargas-Osuna E., Gonzalez-Cabrera J., Ferré J., Gonzalez-Zamora J.E. (2005). Toxicity of several  $\delta$ -endotoxins of *Bacillus thuringiensis* against *Helicoverpa armigera* (Lepidoptera :Noctuidae) from Spain. *Journal of Invertebrate Pathology* 90, 51-54.

Bennett R., Buthelezi T.J., Ismaël Y., Morse S. (2003). Bt cotton, pesticides, labour and health: a case study of smallholder farmers in the Makhathini Flats, Republic of South Africa. *Outlook on Agriculture* 32 (2), p. 123-128.

Burke J.J., Hatfield J.L., Klein R.R., Mullet J.E. (1985). Accumulation of heat shock proteins in field-grown cotton. *Plant Physiol.* 78, 394-398.

Chen D., Yan C., Chan Y., Nie A., Wu Y. (2003). Effect of high temperature stress on the leaf Bt protein content and nitrogen metabolism of Bt cotton. *Cotton Science* 15 (5), p. 288-292.

Chen D., Ye G., Yang C., Chen Y., Wu Y. (2005). The effect of high temperature on the insecticidal properties of Bt Cotton. *Environmental and Experimental Botany* 53 (3), p. 333-342.

Coviella C.E., Morgan D.W., Trumble J.T.(2000a) Interactions of elevated CO<sub>2</sub> and Nitrogen fertilization: effects on production of *Bacillus thuringiensis* toxin in transgenic plants. *Environ. Entomol* 29(4), 781-787.

Coviella C.E., Stipanovic R.D., Trumble J.T.(2002). Plant allocation to defensive compounds: interactions between elevated CO<sub>2</sub> and Nitrogen in transgenic cotton plants. *J. Exp. Bot.* 53, 323-331.

Daly J.C., Fitt G.P. (1998). Efficacy of Bt cotton Plants in Australia: What is going on ? In: P. Petridis (éd.). *Proceedings of the World Cotton Research Conference-2*. Thessaloniki: (Greece), 6-12 September 1998, p. 675-678.

Dong H.Z., Li W.J., Tang W., Li Z.H., Zhang D.M. (2006). Effect of genotypes and plant density on yield, yield components and photosynthesis in Bt transgenic cotton. *J. Agronomy & Crop Science* 192, 132-139.

Downes S., Mahon R., Olsen K. (2007). Monitoring and adaptive resistance management in Australia for Bt-cotton: current status and future challenges. *Journal of Invertebrate Pathology* 95, 208-213.

Gore J., Leonard B.R., Adamczyk J.J. (2001). Bollworm (Lepidoptera:Noctuidae) survival on Bollgard and Bollgard II cotton flower bud and flower components. *J.Econ. Entomol.* 94 (6), 1445-1451.

Gore J., Leonard B.R., Gable R.H. (2003). Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. kurstaki Berliner cotton. *J. Econ. Entomol.* 96, 699-705.

Greenplate J.T., Penn S.R., Shappley Z., Oppenhuizen M., Mann J., Reich B. Osborn J. (2000). Bollgard II efficacy: quantification of total lepidopteran activity in a 2-gene product. In: Dugger C.P. et Richter D.A. (éds), *Proceedings Beltwide Cotton Conferences-2*, 4-8 janvier 2000, San Antonio, TX., p. 1041-1043.

Hofs J.L. (2001). Rapport d'activité du Projet CGM. Cirad-Université de Pretoria.

Hofs J.L. et Berti F. (2006). Les cotonniers (*Gossypium hirsutum* L.) génétiquement modifiés, Bt : quel avenir pour la petite agriculture familiale en Afrique francophone ? *Biotechnol. Agron. Soc. Environ.* 10 (4), 335-343.

Jiang L., Duan L., Tian X., Wang B., Zhang H., Zhang M., Li Z. (2006). NaCl salinity stress decreased *Bacillus thuringiensis* (Bt) protein content of transgenic Bt cotton (*Gossypium hirsutum* L.) seedlings. *Environmental and Experimental Botany* 55, 315-320.

Kranthi K.R., Naidu S., Dhawad C.S., Tatwawadi A., Mate K., Patil E., Bharose A.A., Behere G.T., Wadaskar R.M., Kranthi S. (2005). Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae:Lepidoptera). *Current Science* 89 (2), p. 291-298.

Kranthi K.R. (2006). Bt-cotton: high toxin level in fruiting parts is most critical for bollworm control. *Current Science* 90 (3), p. 279.

Leidi E.O., Saiz J.F. (1997). Is salinity tolerance related to Na accumulation in Upland cotton under salt stress? *Plant Soil* 190, 67-75.

Leonard B.R., Tindall K.V., Emfinger K.D. (2006). Fall armyworm survivorship and damage in Bollgard and Bollgard 2 cotton. Beltwide cotton Conf. San Antonio, Texas –January 3-6,2006, 1080-1084.

Liao C., Heckel D.G., Akhurst R. (2002). Toxicity of *Bacillus thuringiensis* insecticidal proteins for *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera:Noctuidae), major pests of cotton. *Journal of Invertebrate Pathology* 80, 55-63.

Luttrell R.G., Ali I., Allen K.C., Young S.Y., Szalanski A., Williams K., Lorenz G., Parker C.D., Blanco C. (2004). Resistance of Bt in Arkansas populations of cotton bollworm. In: Dugger C.P. et Richter D.A. (éds), *Proceedings Beltwide Cotton Conferences 2004*, San Antonio, TX., p. 1373-1383.

Mahon R.J., Olsen K.M., Garsia K.A., Young S.R. (2007). Resistance to *Bacillus thuringiensis* toxin Cry2Ab in a strain of *Helicoverpa armigera* (Lepidoptera:Noctuidae) in Australia. *J. Econ. Entomol.* 100(3), 894-902.

Martins C.M., Beyene G., Hofs J-L., Krüger K., Van der Vyver C., Schlüter U., Kunert K.J. (2007). Effect of water deficit stress on cotton plants expressing the Bt-toxin. *Annals of Applied Biology* (submitted).

Olsen K.M., Daly J.C., Finnegan E.J., Mahon R.J. (2005). Changes in Cry1Ac Bt transgenic cotton in response to two environmental factors: temperature and insect damage. *J. Econ. Entomol.* 98 (4), 1382-1390.

Penn S.R., Reich B., Osborn J., Embry K., Greenplate J. (2001). Quantification of lepidodteran activity in a 2-gene product: a 2-year summary of Bollgard II®. Beltwide Conf. 830-832.

Pettigrew W.T., Adamczyk J.J. (2006). Nitrogen fertility and planting date effects on lint yield and Cry1Ac (Bt) endotoxin production. *Agron. J.* 98, 691-697.

Pray C., Huang J., Hu R., Rozelle S. (2002). Five years of Bt cotton in China –the benefits continue. *The Plant Journal*, **31** (4), p. 423-430.

Russel D., Deguine J.-P. (2006). Durabilité de la culture des cotonniers en Chine et en Inde. *Cahiers Agricultures* **15** (3), p. 54-59.

Samarakoon AB., Gifford RM. (2004). Water use and growth of cotton in response to elevated CO<sub>2</sub> in wet and drying soil. *Aust. J. Plant Physiol.* 23, 63-74.

Schell J. (1997). Cotton carrying the recombinant insect poison Bt toxin : no case to doubt the benefits of plant biotechnology. *Current Opinion in Biotechnology* 8, 235-236.

Da Silva S.M.B., Silva-Werneck J.O., Falcao R., Gomes A.C., Fragoso R.R., Quezado M.T., Neto O.B., Aguiar J.B., De Sa M.F.G., Bravo A., Monnerat R.G. (2004). Characterization of novel Brazilian *Bacillus thuringiensis* strains active against *Spodoptera frugiperda* and other insect pests. *Journal of Applied Entomology.* 128 (2), 102-107.

Wan P., Zhang Y., Wu K., Huang M. (2005). Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for Bt cotton in the Yangtze River Valley of China. *J. Econ. Entomol.* 98(1), 195-201.

## Figures

Figure 1: Variation in bollworm (*H. armigera*) densities on 2 genetically modified Chinese cultivars throughout two consecutive seasons (from Wan *et al.*, 2005)

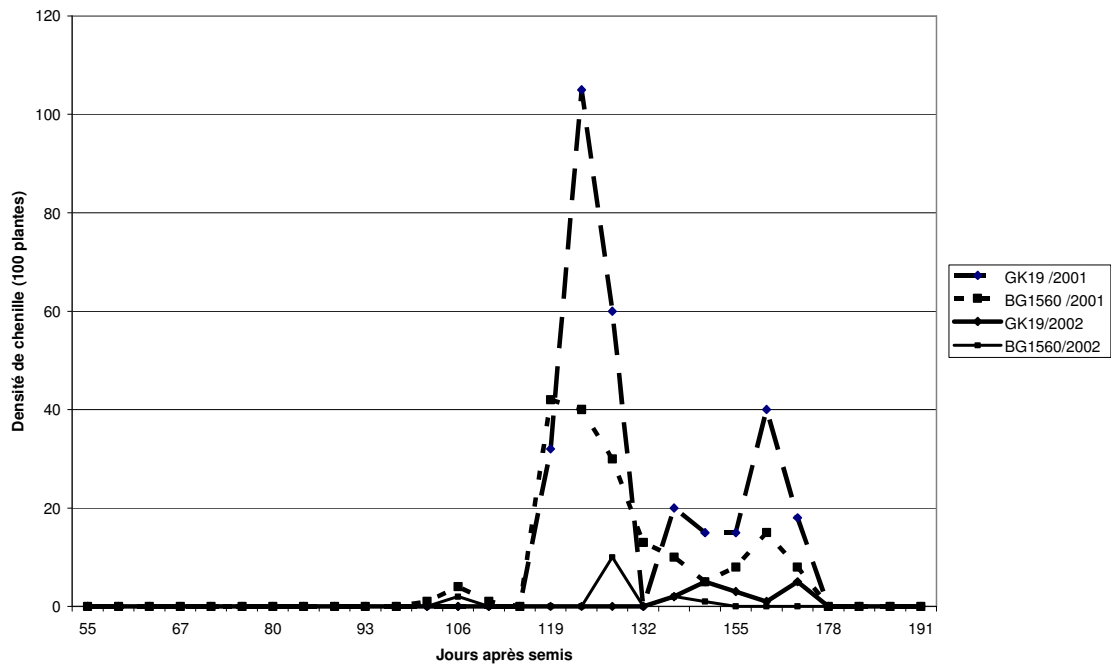


Figure 2: Variation in *H. armigera* mortality rates as a function of temperature and the cultivar (from Olsen *et al.*, 2005)

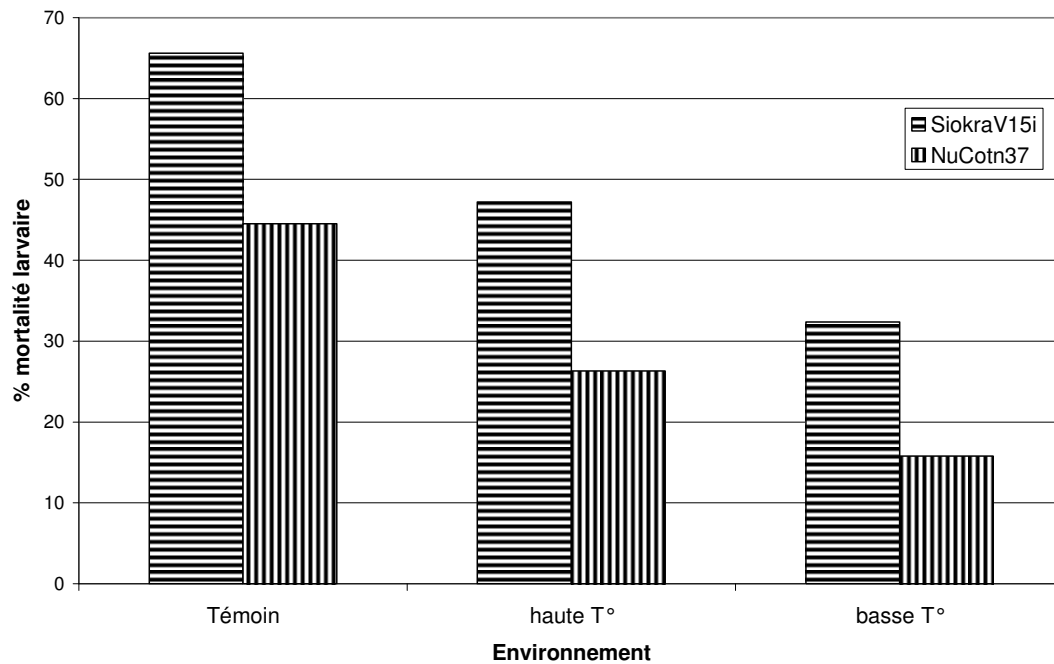


Figure 3: Percentage of surviving bollworms on Bt cotton (NuCOTN33B cultivar) 72 hours after infestation (from Gore *et al.*, 2001)

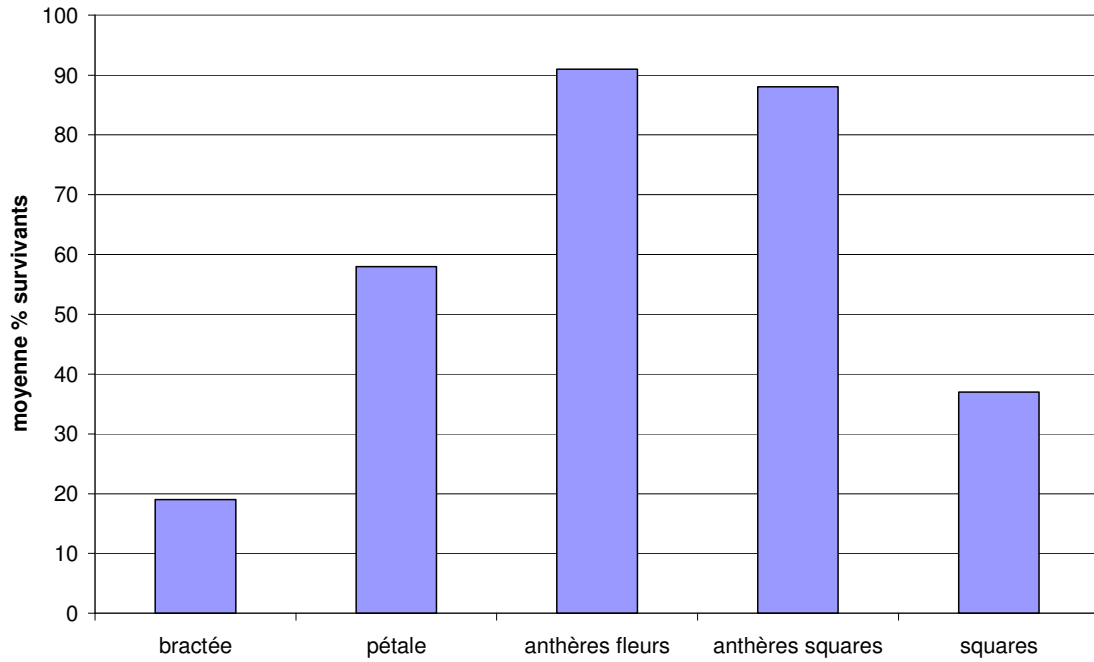


Figure 4: Cry1Ac protein concentrations over time and as a function of the plant organ (from Wan *et al.*, 2002)

