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Negative cross resistance of cry 1Ac toxin selected *Helicoverpa armigera* to chemical insecticides

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SUMMARY

Development of resistance to any xenobiotics imposed against cotton bollworm, *Helicoverpa armigera* (Hubner) is real and it has to be managed with a sound IRM strategy. Limited use of insecticide molecules in case of partial or complete failure of Cry toxin is well thought. As of now, ETL based application of chemical pesticides in Bt-cotton is recommended once after 90 DAS or 1-2 times based on ETL. Accurate prediction and management of resistance requires information on cross-resistance characteristics of the insecticide employed in BT - crops. Study on the pattern of cross-resistance of Cry 1Ac toxin selected (for seven generations) *H.armigera* to chemical insecticides (*viz.*, cypermethrin, fenvalerate, endosulfan, quinalphos, chlorpyriphos, methomyl and spinosad) conducted under laboratory conditions using discriminating doses of insecticides revealed negative cross resistance as Cry1Ac toxin selected *H. armigera* individuals were more susceptible to all the chemical insecticides tested irrespective of the group, compared to the unselected larvae from non-Bt cotton fields. The study strengthens the concept of "using chemical insecticides" as one of the tools in Bt resistance management strategy to increase the life of Bt technology.

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Key words : Cross resistance, Cry 1 Ac, Xenobiotics, *Helicoverpa armigera*, Bt cotton, Resistance management

The American bollworm, *Helicoverpa armigera* is a serious pest in many countries on various crops, including cotton, where it is the most serious. Different pesticide molecules have been employed for its control and that has resulted in development of resistance. Therefore, application of higher doses more frequently or discovery of new molecules for effective control has been practice. Since the commercial release of transgenic Bt- cotton, incorporating a gene for a highly specific insecticidal protein from *Bacillus thuringiensis* in 1996 in US, many countries including India have adopted the technology. Bt- cotton has found favour with farmers in many parts of the world and the area under Bt- cotton has been increasing year after year, currently at 0.5 m.ha. As a result there has been tremendous reduction in overall use of insecticides (Kranthi *et al*., 2004; Rajanikantha and Patil, 2004) and better environment in cotton growing regions.

It is expected that any competitive biological system would respond to high level of selection pressure by mechanisms that would either avoid or mitigate it. Random genetic changes that keep happening in a population of insects might include resistance alleles at very low frequency, which can rapidly increase when challenged. *H. armigera* has already developed resistance to many potent insecticides, especially to pyrethroids (McCaffery *et al*., 1989; Armes *et al*., 1996; Kranthi *et al*., 2001, Fakrudin *et al.,* 2004). There is also an indication that mechanisms of detoxification for different insecticides do overlap (Vijaykumar and Patil, 2005). In this context, wide spread use of Bt- cotton and other Bt- crops has to be considered. Like with chemical insecticides, *H.armigera* has a potential to develop resistance to Cry toxins under field conditions due to continued selection pressure, throughout the crop growth period, if proper resistance management tactics are not implemented. So far there is no field resistance observed for Bt Cotton. However, wide geographic variation in susceptibility of *H. armigera* to Cry1Ac toxin has already been reported in India (Gujar *et al.,* 2000; Kranthi *et al*., 2001; Fakrudin *et al.,* 2003; Jalali *et al*., 2004), China (Wu *et al*., 1999) and in Australia (Liao *et al.,* 2002). The ability of lepidopterans to develop

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resistance to Cry toxin under laboratory conditions was demonstrated well before the commercial release of Bt transgenics (Tabashnik *et al*., 1990, 1992, 1994; Gould *et al*., 1995; Kranthi *et al.,* 2000) and subsequent studies in laboratory and on field collected larvae do point to this fact (Vijaykumar and Patil, 2005).

Development of resistance to Cry protein under field conditions is a concern, which is being addressed by evolving different management strategies. Besides refuge crop, there are other ways of resistance management *viz*., gene pyramiding, application of insecticides and biorationals at a critical stage of the crop and other IPM strategies for delaying resistance build up in the insect population or to keep it at strategically low levels. Predictions based on a stochastic model with input parameters for Indian conditions, Kranthi and Kranthi (2004) have estimated that it required *H.armigera* 11 years to reach resistance allele frequency of 0.5. Semi-dominance for resistance to the toxin, 40% cotton area under Bt- cultivars, very low initial frequency of resistance allele were some of the assumptions and refuge crop at 20% would delay resistance development by two more years. In fact, 11-13 years is a good period for resistance to hold under modern agriculture. However, resistance development in insects is real and it has to be 1) managed with a sound IRM strategy, 2) Bt-technology used as a component of IPM, 3) limited use of insecticide molecules in case of partial or complete failure of Cry toxin and 4) gene pyramiding whenever necessary. As of now, ETL based application of chemical pesticides in Bt-cotton is recommended once after 90 DAS (Kranthi *et al*., 2004) or 1-2 times (Rajanikantha and Patil, 2004) based on ETL.

Accurate prediction and management of resistance requires information on cross-resistance characteristics of the insecticide employed in BT - crops. Reports on the cross resistance between various categories of Cry toxins are available (Akhurst and liao, 1996; Zhao *et al*., 2000; Liao *et al*., 2002; Gould *et al*., 1992), but cross- resistance to chemical pesticides in insects resistant to Cry toxins is not available. It is desirable to have such information for resistance management in the insects and to have the option of using chemical pesticide to protect the crop, if needed. In this communication, we report the type of cross-resistance of Cry 1Ac toxin selected *H. armigera* to various chemical pesticides and its implications on insecticide resistance management.

MATERIALS AND METHODS

Seven insecticides representing five insecticide groups *viz*., carbaryl (Carbamates), monocrotophos, quinalphos, chlorpyriphos (Organophosphates) endosulfan (Cyclodine), cypermethrin and fenvalerate (Synthetic pyrethroids) and Spinosad (New molecule) were chosen for bioassay. Except spinosad, technical grade insecticides were used in all the cases. The Cry1Ac protein was produced according to the method in Albert *et al.* from *E. coli* strain containing hyperexpressivity recombinant plasmid vector pKK223 and purification and quantification of cry protein was done as per Krishnareddy and Kulkarni (2003).

Fourth to final instar larvae of *H. armigera,* which survived on Bt-cotton were collected from different fields of Dharwad district, Karnataka, India during 2004 season and were pooled to establish a bulk population, as very few larvae were available in any sampling area. They were maintained on Bt-cotton bolls and squares till pupation. In the next generation neonate larvae were reared in individual glass vials (5 x 2 cm) containing chickpea based semi-synthetic artificial diet as per NRI manual (Anonymous, 1995) incorporated with 0.3 ug of Cry 1Ac toxin/ml of diet and observed for mortality till the end of 6th day. The surviving larvae were reared on normal diet to obtain the next generation. In this manner, larvae were subjected to increamental concentrations of 0.5,1.0,2.0,3.0,4.0 and 5.0 ug/ml of the toxin in successive generations. By seventh generation, size of the colony reduced drastically due to increamental selection pressure. Third instar larvae hatched in eigth generation were subjected to insecticide bioassay using discriminating doses by topical application technique as in Armes *et al*. (1993). In each treatment, 20 larvae were used in five replications. Seven insecticides *viz*., cypermethrin, fenvalerate, endosulfan, quinalphos, chlorpyriphos, methomyl and spinosad were tested at discriminating doses and surviving larvae were counted 72 hours after treatment. Difference among the treatments was tested by 'z' test. Simultaneously, larvae collected from non Bt-cotton fields and susceptible individuals maintained in laboratory were also assayed with discriminating doses of the insecticides for comparison. During rearing, the larvae were kept in quarantine to maintain healthy culture by eliminating parasitization and removing unhealthy larvae. The environmental conditions of insectory were mentained as mentioned in the NRI manual (Anonymous, 1995). Cross-resistance index (CRI) for each insecticide was calculated as follows:

$$\text{CRI of insects selected on } X_1 \text{ with } X_2 = \text{Survival frequency of normal larvae following treatment with } X_2 - \text{Survival frequency of X1 tolerant insects following treatment with } X_2$$

where X_1 is one insecticide (Cry 1Ac toxin) and X_2

Table 1 : Response of Cry1Ac selected *H. armigera* larvae to different insecticides

Insecticides	Per cent survival of *H. armigera* #			Cross resistance index (CRI)	'z'-value between		
	Cry1Ac selected (1)	Field collected (Non-Bt) (2)	Lab maintained susceptible (3)		(1) & (2)	(1) & (3)	(2) & (3)
Cypermethrin (0.1µg / µl)	25	42	12	- 0.17	2.55*	2.37*	4.78*
Fenvalerate (0.2 µg / µl)	25	40	14	- 0.15	2.52*	2.45*	4.78*
Endosulfan(10µg / µl	18	40	10	- 0.22	3.43*	1.63	4.90*
Quinalphos (0.75 µg / µl)	20	36	8	- 0.16	2.05*	2.83*	4.11*
Chlorpyriphos (1.0 µg / µl)	16	28	4	- 0.12	2.45*	1.95	4.07*
Methomyl (1.2 µg / µl)	8	20	2	- 0.12	2.77*	1.42	3.57*
V. Spinosad (1.5µg / µl)	2	12	0	- 0.10	2.26*	1.96*	4.14*

100 larvae in each treatment * indicates significance of value at P=0.05

is the second insecticide

CRI close to '0' indicted complete cross resistance, '-ve' for negative cross-resistance (increased susceptibility) and '+ve' for positive cross-resistance (increased resistance).

RESULTS AND DISCUSSION

The results of the bioassay using seven different insecticides representating five different chemical groups, are presented in Table 1. In all cases the cross-resistance index was –ve. It is clear that Cry1Ac toxin selected *H. armigera* individuals were more susceptible to all the commonly used chemical insecticides and some new molecules, irrespective of the group, compared to the unselected larvae from non-Bt cotton fields. Sensitive laboratory strain, as expected, was very susceptible to the chemicals and do not represent a real situation. Cry toxin resistant larvae were the most susceptible to endosulfon followed by cypermethrin and quinalphos. Positive changes in susceptibility of Cry1Ac selected larvae to different groups of chemical insecticides rules out the possibility of cross-resistance. Wu and Guo (2004) observed lack of cross-resistance between Cry1Ac toxin and synthetic insecticides in China and concluded that growers could confidently use such insecticides in cases where resistance for Cry1Ac protein is observed.

Cross-resistance is a function of the mode of action of the bioactive agents and the nature of the resistance mechanism developed in the insect to either avoid or detoxify the chemical. Toxic molecules similar in structure and function are likely to invoke the same or similar resistance mechanism and will have CRI values close to '0'. Cry toxins are a class apart, proteins that need a specific receptor site on the epithelial membrane of brush border cells of the insect gut for their effectiveness. Any resistance to these toxins should arise due to loss or modification of the receptor site or the modification of / degradation of the protein itself by enzymes in the insect gut. Different contact and systemic insecticides call for different detoxification mechanisms. Therefore, pattern of cross-resistance for Cry toxins and insecticide molecules is likely to be in a favorable direction for resistance management. While insects develop resistance to continued presence of toxic molecules, they also tend to loose it in the absence of selection pressure. The present ETL based application of insecticides in Bt- crops is sound both in theory and practice. Cross-resistance factor has to be considered in any prediction on resistance development to Cry proteins. In fact, the present practice should delay or even limit the development of resistance to Cry toxins without loosing the option of using insecticides as a last resort in controlling *H. armigera* and other insects.

REFERENCES

Akhurst, R. and Liao, C. (1996). Protecting an investment-managing resistance development to transgenic cotton by *Helicoverpa armigera*. In Proceedings of the Eighth Australian Cotton Conference, 1996, Broadbeach, queensland, Australian Cotton Growers Research Association, Brisbane, pp. 299-305.

Anonymous (1995). Monitoring and management of nsecticide resistance in *Helicoverpa armigera*. Training exercise on bioassay techniques for monitoring insecticide resistance. NRI/ICRISAT/ICAR Collaborative Project 1993-95.

Armes, N.J., Jadhav, D.R. and Desouza, K.R. (1996). A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bulletin Entomological Res.*, **86** : 499-514.

Armes, N.J., Lonergan, P.A. and Jadhav, D.R. (1993). Insecticide resistance in the pod borer, *Helicoverpa armigera* and prospects for its management in India. Paper presented at the National Symposium on Pesticides: Future scenario IARI, New Delhi, 15-17 April, 1993.

Fakrudin, B., Badariprasad, Prakash, S.H., Krishnareddy, K.B. and Patil, B.V. (2003). Baseline resistance to Cry 1Ac protein in geographic populations of *Helicoverpa armigera* in south Indian Cotton ecosystem. *Curr. Sci.*, **80**: 1304-1307.

Farkrudin, B., Vijaykumar, Krishnareddy, K.B., Patil, B.V. and Kuruvinashetty, M.S. (2004). Status of insecticide resistance in geographical populations of cotton bollworm, *Helicoverpa armigera* in South Indian Cotton Ecosystem During 2002-03. *Resistant Pest Management Newsletter*, **13**(2): 12-15

Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. and Moar, W. (1995). Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J. Econ. Entomol.,* **88**:1545–1559.

Gould, F., Martinez-Ramirez,A., Anderson, A., Ferre, J., Silva, F.J. and Moar, W.J. (1992). Broad-spectrum resistance to *Bacillus thuriengiensis* toxin in *Heliothis virescens*, *Proc. National Acad. Sci., USA*, **89**: 7986

Gujar, G.T., Kumari, Archana, Kalia, Vinay and Chandrashekar, K. (2000) Spatial and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hubner) to *Bacillus thuringiensis* var. Kurstaki in India. *Curr. Sci.,* **78**: 995 – 1001.

Jalali, S.K., Mohan, K.S., Singh, S.P., Manjunath, T.M. and Laitha, Y. (2004). Baseline susceptibility of the old-world bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) population from India to *Bacillus thurinngiensis* Cry1Ac insecticidal protein. *Crop Prot.*, **23**: 53-59.

Kranthi, K..R., Jadhav, D., Wanjari, L.R., Kranthi, S. and Russell, D. (2001). Pyrethroid resistance and mechanisms of resistance in field strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.,* **94**: 1-6.

Kranthi, K.R. and Kranthi, N.R. (2004). Modeling adaptability of the cotton bollworm, *Helicoverpa armigera* (Hubner) to Bt-cotton in India. *Curr. Sci.*, **87**:1096-1107.

Kranthi, K.R., Kranthi, S. and Wanjari, R.R. (2001). Baseline toxicity of Cry 1Ac toxin to *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in India. *Internat. J. Pest Management*, **47**: 141-145.

Kranthi, K.R., Kranthi, S., Naidu, S., Dhawad, C.S., Mate, K., Wadaskar, R., Chaudhary, A., Bharose, P., Siddhabhatti and Patil, E. (2004). IRM and Bt-cotton. International Symposium on "Strategies for Sustainable Cotton Production – A Global Vision" 3. Crop Protection, 23-25 November 2004, University of Agricultural Sciences, Dharwad, Karnataka (INDIA).

Kranthi, K.R., Kranthi, S., Ali, S. and Banerjee, S.K. (2000). Resistance to Cry1Ac delta endotoxin of *Bacillus thurinngiensis* in a laboratory selected strain of *Helicoverpa armigera* (Hubner). *Curr. Sci.*, **78**: 1001-1004.

Kranthi, K.R., Kranthi, S., Behre, G.T., Dhawad, C.S., Wadaskar, R.M., Benergee, S.K., Sheoraj, and Mayee, C.D. (2004). Recent advances in IRM strategies for sustainable cotton pest management in India. pp. 221-231. Chauhan M.S. and Saini, R.K. (Eds.). National symposium on changing world orders-Cotton research, development and policy in context, 10-12th August 2004. Acharya N.G. Ranga Agricultural University, Hyderabad.

Krishnareddy, K.B. and Kulkarni, K.A. (2003). Baseline susceptibility to Cry1Ac protein and molecular diversity analysis of geographical populations cotton bollworm *Helicoverpa armigera* (Hubner). M.Sc. (Ag.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka (India).

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

McCaffery, A.R., King, A.B.S., Walker, A.J. and El-Nayir, H. (1989). Resistance to synthetic pyrethroids in the bollworm, *Heliothis armigera* from Andhra Pradesh, India. *Pesticide Sci.,* **27** : 65–76.

Rajanikantha, R. and Patil, B.V. (2004). Performance of Bt cotton against major insect pests and their natural enemies under irrigated ecosystem. M.Sc. (Ag.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka (India).

Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K. and Adang, M.J. (1994). Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proc Natl Acad Sci USA* 91:4120–4124.

Tabashnik, B.E., Cushing, N.L., Finson, N. and Johnson, M.W. (1990). Field development of resistance to *Bacillus thurinngiensis* in diamondback moth (Lepidoptera : Plutellidae). *J. Econ. Entomol.*, **83**: 1671-1676.

Tabashnik, B.E., Schwartz, J.M., Finson, N. and Johnson, M.W. (1992). Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera : Plutellidae). *J. Econ. Entomol.*, **85**: 1046-1055.

Vijaykumar and Patil, B.V. (2005). Geographic variation in morphometry, genetics and insecticide resistance in cotton bollworm, *Helicoverpa armigera* (Hubner) occurring in south indian cotton ecosystem and validation of IPM and IRM modules. Ph.D Thesis, University of Agricultural Sciences, Dharwad, Karnataka (India).

Wu, K. and Guo, Y. (2004). Changes in susceptibility to conventional insecticides of a Cry1Ac-selected population of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Pest Management Sci.*, **60** : 680–684

Wu, K., Guo, Y. and Lv, N. (1999). Geographical variation of susceptibility of *Helicoverpa armigera* to *Bacillus thuriengiensis* insecticidal protein in China. *J. Econ. Entomol.*, **92**: 273-278.

Zhao, J.Z., Rui, C., Lu, M.G., Fan, X.L., Ru, L. and Meng, X.Q. (2000). Monitoring and management of *Helicoverpa armigera* resistance to transgenic Bt cotton in Northern China. *Resistant Pest Management*, **11**(1): 28.

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