INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–113/WIB/2009/11–4–473–476
http://www.fspublishers.org

Full Length Article

Transgenic-Bt and Non-transgenic Cotton Effects on Survival and Growth of *Helicoverpa armigera*

MUHAMMAD ARSHAD[1], ANJUM SUHAIL, MUHAMMAD JALAL ARIF AND MUHAMMAD ASLAM KHAN†
Department of Agri. Entomology, University of Agriculture Faisalabad, Pakistan
†*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*
[1]Corresponding author's e-mail: arshaduaf@gmail.com; dranjumsuhailuaf@yahoo.com

ABSTRACT

The impact of Transgenic Bt cotton line (IR-FH-901) containing Cry1Ac toxin on Bt cotton leaves and flowers-bolls against *Helicoverpa armigera* Hubner with its parental non-Bt cotton variety (FH-901) was investigated. The parameters measured were larval mortality to pupation, larval period and pupal weight. Results indicated significant effects of Bt cotton on the percent cumulative mortalities of all instars compared with non-Bt cotton. However, a significant higher mortality (100%) was observed in neonates fed on Bt cotton leaves than those fed on Bt flower-bolls (93%). There was a marked difference in larval development period between Bt cotton (27.75 days) and on non-Bt cotton (16.68 days) flower-bolls. Pupae weight was significantly higher for larvae fed on non-Bt cotton compared with Bt cotton plant parts (leaves & flowers-bolls).

Key Words: Transgenic Bt cotton; *H. armigera*; Larval survival and growth

INTRODUCTION

Bt cotton was introduced in Punjab, Pakistan in 2001. The area under Bt cotton increased dramatically in 2005, when Pakistan Atomic Energy Commission (PAEC) provided 40,000.0 kg seed of the Bt cotton strains namely IR-FH-901, IR-NIBGE-2, IR-CIM-448 and IR-CIM-443, which were grown on over 3,238 ha (hectares) during the 2005-2006 cotton season (Rao, 2006; Arshad *et al*., 2007).

The use of transgenically modified cotton that expresses an insecticidal protein derived from *Bacillus thuringiensis* Berliner (Bt) is revolutionizing global agriculture (Head *et al*., 2005). Bt cotton expressing the Cry1Ac protein has been available commercially in the USA since 1996 and is also being grown in Mexico, Colombia, Australia, China, Argentina and South Africa (James, 2006). This insecticidal protein has the insecticidal activity against the larvae of specific lepidopterous pests (Kotchoni *et al*., 2005). Larvae stop feeding after Bt toxin ingestion, because of midgut paralysis, altered permeability and disintegration of the epithelium that render feeding impossible and finally death occurs within two to three days after exposure (Gill *et al*., 1992). The larval death may vary depending on insect species, larval age and the amount of toxin ingested (Halcomb *et al*., 1996). It has been noted somewhere, that there is a temporal and spatial variation in Cry1Ac protein levels in Bt cotton (Luttrell & Mink, 1999).

Although Bt toxin in transgenic cotton has adverse effects on larval survival and development of *H. armigera* (Bambawale *et al*., 2004), toxin level is different in various plant parts. This toxin level decreases as the crop matures and is very low or undetectable in square (Kranthi *et al*., 2005) and bolls (Greenplate *et al*., 2000). This variability in the expression of Cry1Ac toxin in different parts of Bt cotton plant can create the variability in the survival and development of target pests (Adamczyk & Gore, 2004).

The present study was carried out to determine the effects of Bt toxin on the larval survival, development time and pupal weight of *H. armigera* fed on leaves and flowers-bolls of transgenic Bt and non-Bt cotton.

MATERIALS AND METHODS

Cotton seed source. The transgenic Bt-cotton line, IR-FH-901, containing Cry1Ac provided by National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and non-transgenic parent cultivar, FH-901, obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan were used in this study. The cotton plants were grown in pots in a greenhouse at 25 ± 1°C, 40-70% RH and a photoperiod of 14:10 (L: D) h. Plants were kept free from any insecticide treatment.

Insect culture. *H. armigera* eggs laid on a cotton cloth were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan, where culture had been maintained for several generations. The cotton cloth deposited with eggs was placed in a small box (20 × 10 cm) and covered with a lid having small holes to ensure ventilation. The insect culture was maintained in a growth chamber at 27°C with a 70-75% RH and a photoperiod of 14:10 (L: D) h. After

To cite this paper: Arshad, M., A. Suhail, M.J. Arif and M.A. Khan, 2009. Transgenic-Bt and non-transgenic cotton effects on survival and growth of *Helicoverpa armigera*. *Int. J. Agric. Biol*., 11: 473–476

hatching 1st instar larvae were removed from cotton cloth using a camel hair brush under sterile conditions by washing rearing equipment in 1.8% sodium hypochlorite solution and then with distilled water.

Experimental details. The experiment was laid out in Randomized Complete Block Design with four replications, with 25 larvae in each replicate. The treatments included: (a) larvae fed on leaves of non-Bt cotton, (b) larvae fed on leaves of Bt cotton, (c) larvae fed on flowers of non-Bt cotton until the third instar, then fed with young bolls of non-Bt cotton and (d) larvae fed on flowers of Bt cotton until the third instar, then fed with young bolls of Bt cotton.

The cotton leaves were obtained from the third or fourth main stem node from terminal portion of the plants at 40 d after seedling emergence, while the reproductive parts (including fresh bloom & young bolls, <2.5 cm diameter) were obtained from the plants during the reproductive phase. Plant parts (leaves, flowers & bolls) of Bt and non-Bt cotton were brought into the laboratory and were dipped in 0.4% javelle water (sodium hypochlorite) for 10 min to kill the pathogens and then rinsed with water before the feeding bioassay (Men *et al*., 2005). One day old, first instar larvae of *H. armigera* were placed individually on each of the plant parts in an individual 8 cm × 2.5 cm glass tube. Plant parts were changed each day, until the larvae pupated. The numbers of live and dead larvae were checked daily. Larval mortality was recorded in all instars and cumulative mortalities were calculated. Pupae formed from larvae in different treatments were removed using feather-light forceps and weighed after one day of pupation, using a digital microbalance.

Statistical analysis. Data were analyzed using analysis of variance (ANOVA) and means were separated using Tukey's honestly significant difference (HSD) test using SPSS (SPSS Institute, Chicago, IL, USA).

RESULTS

The results showed significant differences ($P<0.05$) in mortalities of all larval instars (1-6th) fed continuously with leaves and flower-bolls of Bt and non-Bt cotton (Table I). Transgenic Bt cotton had significant ($P<0.01$) impact on the cumulative percent mortalities of 1st to 6th -instar larvae. Larval mortality was generally high among 1st and 2nd instar larvae on Bt cotton. The 2nd and third instar survival rate differed with different feeding structures of Bt cotton plant. Neonates were found more susceptible to leaves and flower-bolls of Bt cotton. In general, larval mortality was greater on leaves than those of flower-bolls of both Bt and non-Bt cotton. A significant higher mortality (100%) was observed in neonates fed with Bt cotton leaves than those fed with Bt flower-bolls (93%). All neonates were found to be dead up to the 5th instar fed continuously on Bt cotton leaves. Only 7.0% larvae were survived fed on Bt cotton flower-bolls and pupated successfully (Table I).

In addition, significant ($P<0.01$) differences were also found in larval development time among treatments (Table II). Larvae fed on non-Bt cotton leaves and flower-bolls completed development faster than those fed on Bt cotton flower-bolls. No bollworm larvae survived when fed Bt cotton leaves, thus the effect of Bt cotton leaves on larval development and pupal weight could not be determined. A comparison of average larval period (days) showed that there was a marked difference in larval development time on non-Bt cotton flower-bolls (16.68 days) compared to Bt cotton flower-bolls (27.75 days). Pupae weights were significantly ($F = 510.83$; $df = 3$; $P = 0.00$) higher for larvae fed on non-Bt compared with Bt cotton (Table II). There was a little difference in average pupal weight produced by larvae fed on leaves and flower-bolls of non-Bt cotton, whereas a marked difference was found in average pupae weights between Bt and non-Bt cotton plant structures.

DISCUSSION

The early instar larvae of *H. armigera* prefer tender leaves, then the later instars, which feed on reproductive parts (flowers, square & bolls) and can infest several fruits and flowers over the course of larval development (Sarfraz *et al*., 2005). Bt cotton appears to be a new strategy for effective control of this pest. However, for sustainable Bt transgenic technology, it is important that the toxin expression level should be adequate on appropriate plant parts and at required time of the season to provide protection against major target insect pests (Kranthi *et al*., 2005). In this laboratory assay, wherein the neonates were fed on various plant parts indicated that transgenic Bt cotton line (IR-FH-901) proved to be more toxic to *H. armigera* than its parent conventional non-Bt cultivar (FH-901). In general, larval mortality was greater on the leaves of both cotton varieties.

It is noteworthy that *H. armigera* is primarily, a bollworm and prefers feeding on fruiting parts and seldom on foliage (Kranthi *et al*., 2005). Results indicated that larval survival rate in different instars was different on various parts of Bt cotton plant. Early instars were more susceptible and results indicated the maximum mortality in 1st and 2nd instars larvae on Bt cotton. Results showed that the efficacy of Bt cotton flowers was lower than that of Bt leaves against 1st, 2nd and 3rd instar larvae (Table I), which corroborated with the earlier reports (Gore *et al*., 2000; Zhao *et al*., 2000). Leonard *et al*. (1997) found a non-significant difference in the mortality of third instar bollworm larvae feeding on Bollgard squares as compared to conventional cotton. Because of, spatial-temporal variation in Cry1Ac protein levels in Bt cotton, Shanmugam *et al*. (2006) reported the susceptibility of *Helicoverpa armigera* larval instars in the order of neonates > second instar > third instar and the efficacy of plant parts in the order of top leaves > middle leaves > squares > bolls. Results showed that no larval survival on Bt cotton leaves, while only 7% larvae survived and pupated on Bt flower-bolls. Men *et al*. (2005) showed 100% mortality, while Xu

Table I. Percent cumulative mortality (Mean ± SE) of larval instars of *Helicoverpa armigera* fed with leaves and flowers-bolls of Bt and non-Bt cotton

	Leaves		Flowers-bolls	
Instar	Non-Bt cotton	Bt cotton	Non-Bt cotton	Bt cotton
I	1.00 ± 0.58a	16.50 ± 0.96c	0.50± 0.50a	8.00 ± 0.82b
II	4.00 ± 0.82a	79.50 ± 0.96c	1.50 ± 0.50a	54.00 ± 0.81b
III	8.50 ± 0.96b	91.00 ± 1.00d	4.00 ± 0.00a	63.50 ± 0.96c
IV	10.00 ± 0.82a	97.00 ± 0.58c	7.00 ± 0.58a	79.00 ± 1.29b
V	13.00 ± 0.58b	100.00 ± 0.00d	8.50 ± 0.50a	88.50 ± 0.96c
VI	15.00 ± 0.58b	100.00 ± 0.00d	9.00 ± 0.58a	93.00 ± 0.58c

In and Table II, neans ± SE followed by the same letters within the row are not significantly different (p>0.05; Tukey's HSD)

Table II. Larval development time and pupal weight of *Helicoverpa armigera* fed with leaves and flowers-bolls of Bt and non-Bt cotton

Treatment	Mean development time (days) ± SE	Mean pupal weight (mg) ± SE
Non-Bt cotton leaves	19.35 ± 0.10c	0.232 ± 0.005c
Bt cotton leaves	0.00 ± 0.00a	0.000 ± 0.00a
Non-Bt cotton flowers-bolls	16.68 ± 0.37b	0.254 ± 0.004d
Bt cotton flowers-bolls	27.75 ± 0.20d	0.166 ± 0.008b

et al. (2008) reported 2.6% larval survival of *H. armigera* fed on Bt cotton leave. 1st-6th instar larvae fed on Bt cotton leaves, however none of those pupated successfully.

The differences in larval mortality of *H. armigera* among different plant parts of Bt cotton line in the current study could be attributed to variation in toxin expression among these structures. The current and previous studies (Adamczyk *et al*., 2000) assigned it to the lower expression of Cry1Ac toxin in the reproductive parts (flower & bolls) than critical level required for the effective control of bollworm. Many previous studies witness that Bt cotton leaves were more effective against cotton bollworm and showed the highest level of Cry1Ac expression than flowers, squares and bolls (Greenplate *et al*., 2000; Kranthi *et al*., 2005).

Other fitness indicators (larval development & pupal weight) were also significantly different among treatments. The results on larval developmental period showed that Bt cotton significant delayed the larval development (Khalique & Ahmed 2003). The survived 1st-instar larvae continued to be in first instar even after four days, while those on non-Bt cotton moulted to 2nd-instar within 2-3 days of the experiment. The Bt toxic proteins in transgenic cotton suppress the feeding, because larvae take longer time than those of control to recover the Bt toxicity (Bambawale *et al*., 2004). Pupal weight is an easily measurable biological parameter of lepidopteran insect fitness (Polanczyk & Alves, 2005). The current study showed significant decrease in pupal weight for pupae developed by larvae fed on Bt than pupae produced by larvae fed on non-Bt cotton.

CONCLUSION

The study highlight the importance of sustainable temporal and intra-plant expression of Cry1Ac in Bt cotton varieties for effective control of lepidopterous pests especially *H. armigera*. The biotechnological efforts, in developing the transgenic Bt cotton varieties, in countries, where *H. armigera* is a major pest of cotton, should focus on the higher level of toxic genes expression in fruiting parts.

Acknowledgement. We thank Dr. Feng Ge, Institute of Zoology, Chinese Academy of Sciences, Beijing, China for helping in developing method for the experiments, Dr. Denis J. Wright, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, for help in rearing insects and bioassays. The research was supported by Higher Education Commission, Pakistan.

REFERENCES

Adamczyk, J.J. Jr., L.C. Adams and D.D. Hardee, 2000. Quantification of CrylAc delta-endotoxin in transgenic Bt cotton: correlating insect survival to different protein levels among plant parts and varieties. In: *Proc. Beltwide Cotton Conf., San Antonio, USA, 4-8 January 2000,* Vol. 2, pp: 929–932

Adamczyk, J.J. and J. Gore, 2004. Development of bollworms, *Helicoverpa zea,* on two commercial Bollgard (R) cultivars that differ in overall Cry1Ac levels. *J. Insect Sci.*, 4: 32

Arshad, M., A. Suhail, M. Asghar, M. Tayyib and F. Hafeez, 2007. Factors influencing the adoption of Bt cotton in the Punjab, Pakistan. *J. Agric. Soc. Sci.*, 3: 121–124

Bambawale, O.M., A. Singh, O.P. Sharma, B.B. Bhosle, R.C. Lavekar, A. Dhandapani, V. Kanwar, R.K. Tanwar, K.S. Rathod, N.R. Patange and V.M. Pawar, 2004. Performance of Bt cotton (MECH-162) under Integrated Pest Management in farmers' participatory field trial in Nanded district, Central India. *Curr. Sci.*, 86: 1628–1633

Gill, S.S., E.A. Cowles and P.V. Pietrantonio, 1992. The mode of action of *Bacillus thuringiensis* endotoxins. *Annl. Rev. Entomol*., 37: 615–636

Gore, J., B.R. Leonard, C.E. Church and D.R. Cook, 2000. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. *J. Econ. Entomol.*, 95: 763–769

Greenplate, J., S.R. Penn, J.W. Mullins and M. Oppenhuizen, 2000. Seasonal Cry1Ac levels in DP50B: the "Bollgard basis" for Bollgard II. *Proc. Beltwide Cotton Conf., San Antonio, USA, 4-8 January, 2000*, Vol. 2, pp: 1039–1040

Halcomb, J.L., J.H. Benedict, B. Cook and D.R. Ring, 1996. Survival and growth of bollworm and tobacco budworm on non-transgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). *Environ. Entomol.,* 25: 250–255

Head, G., M. Moar, M. Eubanks, B. Freeman, J. Ruberson, A. Hagerty and S. Turnipseed, 2005. A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environ. Entomol.,* 34: 1257–1266

James, Clive. 2006. Global Status of Commercialized Biotech/GM Crops: 2006. *International Service for the Acquisition of Agri-Biotech Applications* (*ISAAA*) Brief No. 35, Ithaca, New York

Khalique, F. and K. Ahmed, 2003. Impact of *Bacillus thuringiensis* Subsp. *Kurstaki* on biology of *Helicoverpa armigera* (Hubn). *Pakistan J. Biol. Sci.*, 6: 615–621

Kotchoni, O.S., E.W. Gachomo and M. Mwangi, 2005. Commercial production of genetically modified crops: A prognosis towards global acceptance. *Int. J. Agric. Biol.*, 7: 681–688

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

Leonard, B.R., H. Fife, K. Torrey, J.B. Graves and J. Holloway, 1997. *Helicoverpa/ Heliothis* management in NuCOTN and conventional cotton cultivars in Louisiana. *Proc. Beltwide Cotton Conf., New Orleans, LA, USA, January 6-10, 1997,* Vol. 2, pp: 863–867

Luttrell, R.G. and J.S. Mink, 1999. Damage to cotton fruiting structures by the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Cott. Sci.*, 3: 35–44

Men, X., F. Ge, C.A. Edwards and E.N. Yardim, 2005. The influence of pesticide applications on *Helicoverpa armigera* Hubner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. *Crop Prot.*, 24: 319–324

Polanczyk, R.A. and S.B. Alves, 2005. Biological parameters of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) assayed with *Bacillus thuringiensis* Berliner. *Sci. Agric.*, 62: 464–468

Rao, I.A., 2006. *Pakistan-GM Cotton Grown*. Available at: http://www.afaa.com.au/news/n_news-1758.asp

Sarfraz, M., M.J. Arif and G. Ahmad, 2005. Comparative resistance of transgenic and conventional cotton against bollworms complex. *Int. J. Agric. Biol.*, 7: 308–310

Shanmugam, P.S., R. Ballagurunathan and N. Sathiah, 2006. Susceptibility of *Helicoverpa armigera* Hubner instars to *Bacillus thuringiensis* insecticidal crystal proteins. *Pestic. Res. J.,* 18: 186–189

Xu, Y., R.F. Ding, H.B. Li, J. Liu, K.M. Wu and X.X. Chen, 2008. Effect of transgenic Bt cotton GK62 on the development of cotton bollworm and its resistant efficiency in fields. *Acta Entomol. Sin.*, 51: 222–226

Zhao, J., K. Zhao, X. Fan, M. Lu, C. Rui, H. Zhang and S. Guo, 2000. Comparison of insecticidal activity of Bt cotton lines both developed in China and USA against *Helicoverpa armigera*. *Sci. Agric. Sin.*, 33: 100–102

(Received 09 April 2009; Accepted 20 April 2009)