Behaviour of laboratory-selected Cry1Ac-tolerant strain of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on *Bt*-cotton

Paramjit Kaur* and Vinod Kumar Dilawari

Department of Entomology, Punjab Agricultural University, Ludhiana 141 004, India

The effect of *Bacillus thuringiensis* toxin Cry1Ac on the behaviour of a laboratory-selected resistant population (72-fold) of *Helicoverpa armigera* on *Bt*-cotton was evaluated. Compared with non-*Bt*-cotton and Cry1Ac toxin incorporated in semi-synthetic diet, resistant larvae reared on *Bt*-cotton had only 0.13% survival and slower development. The results suggest that Cry1Ac from *Bt*-cotton exerts a greater toxic effect in terms of larval mortality coupled with decline in larval growth rate compared to semi-synthetic diet.

Keywords: *Bt*-cotton, Cry1Ac toxin, *Helicoverpa armigera*, resistant population.

Bt-cotton containing *Cry1Ac* gene holds great promise in controlling cotton bollworm *Helicoverpa armigera* (Hübner), which is one of the main target pests of transgenic cotton in India. The commercial cultivation of *Bt*-cotton was approved in India in 2002, but the North Indian states such as Punjab, Haryana and Rajasthan had to wait till 2005 to begin cultivation. The area under *Bt*-cotton reached 11.6 million hectares (m ha), equivalent to a high adoption rate of 95% of the total cotton area of 12.25 m ha in 2014, according to report from ISAAA[1]. India has already achieved a near phasing-out of the Bollgard™ 1 event, which has now been slowly replaced with the dual-gene Bollgard™ II (BG-II) cotton event. The widespread and large-scale application of *Bt* formulations and adoption of cultivation of *Bt* transgenic plants is suspected to expose the pest to a continuous selection pressure resulting in resistance development to *Bt*-cotton. In order to derive long-term benefits from this technology, regular resistance studies are necessary to develop management strategies. Laboratory studies have proved resistance development to *Bt* in several insect species upon continuous exposure. *Bt* resistance under laboratory has been reported in 13 insect species, 11 of which, i.e. *Ostrinia nubilalis* (Hübner); (European corn borer), *Heliothis virescens* (Fabricius); (tobacco budworm), *Pectinophora gossypiella* (Saunders); (pink bollworm), *Culex quinquefasciatus* Say (mosquito), *Caudra cautella* (Walker); (almond moth), *Chrysomela scripta* Fabricius (cottonwood leaf beetle), *Spodoptera exigua* (Hübner) (beet armyworm), *Spodoptera littoralis* (Boisduval); (Egyptian cotton leafworm), *Trichoplusia ni* (Hübner); (cabbage looper), *Aedes aegypti* (Linnaeus); (yellow fever mosquito) and *Leptinotarsa decemlineata* (Say); (Colorado potato beetle) have developed resistance to various strains of *Bt* in the laboratory but not in the field[2–8]. High survivorship of pink bollworm, *P. gossypiella* was found from Bollgard cotton fields in the adjoining states of Maharashtra and Madhya Pradesh in Central India[9]. These studies indicate the potential of these insects to develop resistance to *Bt*-toxins. Laboratory experiments for resistance study in India have shown tolerance in *H. armigera* under laboratory conditions[10–13]. In Mississippi and Arkansas, USA, *Bt*-resistance in bollworm, *Helicoverpa zea* on *Bt*-cotton has been reported[14]. So there is also a chance of resistance development in *H. armigera*. In 2010, field resistance in *H. armigera* was reported after a year of cultivation of *Bt*-cotton in China[15]. The positive results of resistance development to Cry1Ac toxin under laboratory conditions prompted us to find precautionary measures to delay resistance development in the field[16].

The United States Environmental Protection Agency has suggested the refuge strategy so that the farmer gets the prolonged benefits of transgenic cotton[17]. By adopting refuge strategy, the resistant insects from transgenic cotton will mate with the susceptible insects from the refuge cotton crop, which will produce progeny that can be killed by *Bt*-toxin. The benefits of refuges have been demonstrated with models and limited experimental evidence[18–20]. The best results of refuge strategy will be obtained when the mode of inheritance of resistance is recessive. In India, refuge strategy is 5% refuge, if no insecticides are used on it, or else 20% refuge. In order to determine the assumptions of refuge strategy and to manage resistance in the field, we have examined resistance to Cry1Ac in laboratory-selected strains of *H. armigera*. Based on previous studies, resistance was inferred to be polygenic, autosomal and inherited as a recessive trait in laboratory-raised resistant strains[11]. In the present study, we report behaviour, survival and corrected food intake of laboratory-selected Cry1Ac-resistant strain on artificial diet containing Cry1Ac toxin, *Bt*-cotton and non-*Bt*-cotton. The aim of this study is to evaluate the responses of the laboratory-derived resistant strain to *Bt*-cotton, because the results obtained from the study may be especially useful for understanding resistance in the field.

The larvae of *H. armigera* were collected from different districts of Punjab (Bathinda, Mansa, Muktsar, Abohar, Ludhiana, Faridkot and Hoshiarpur) from different host crops [barseem (*Trifolium alexandrium*), okra (*Abelmoschus esculentus*) and tomato (*Lycopersicon esculentum*) and cotton (*Gossypium hirsutum*)]. Larvae were reared at 27 ± 2°C and 75 ± 5% relative humidity on semi-synthetic diet. The ingredients and protocol of semi-synthetic diet are available in the literature[11]. The pupae

*For correspondence. (e-mail: paramjitkaur@pau.edu)

obtained from the collected larvae were kept singly in polycarbonate vials and allowed to become adults. Intercrosses were made using male and female of different populations, and a total of 104 crosses were made. The eight-day-old larvae were exposed to discriminating dose (1 μg/ml diet) incorporated in semi-synthetic diet. The larvae which survived were allowed to pupate and selected pupae of approximately the same weight were used for further studies. The progeny obtained by crossing females of Bathinda and males of Muktsar was considered as resistant strain because this cross showed highest survival (60.70%) after exposure to discriminating dose. So the selected resistant strain was subjected to bioassay at controlled temperature (27 ± 2°C) and relative humidity (75 ± 5%). In the first generation, the initial LC_{50} value was found to be 1.396 μg/ml. Continuous maintenance of resistant BM-R strain on Cry1Ac (2.0 μg/ml) for 19 generations resulted in LC_{50} value of 7.493 μg/ml. The effect in terms of survival and development of resistant strain (BM-R) of *H. armigera* was tested at 19th generations on *Bt*-cotton, Cry1Ac toxin incorporated in semi-synthetic diet and non-*Bt*-cotton.

We used MVP-II (19.7% Cry1Ac; Dow AgroSciences (NZ) Ltd, New Plymouth, New Zealand), a liquid formulation containing a hybrid protoxin similar to CryAc that is expressed in *Bt*-cotton and encapsulated by *Pseudomonas fluorescence*. Concentrations of Cry1Ac were calculated based on the amount of protoxin per millilitre of liquid formulation.

The relative survival of laboratory-developed Cry1Ac-resistant strain (BM-R) of *H. armigera* was studied on the same hybrid (RCH-134) of *Bt*-cotton (expression level ~5 μg/g at the age of 60–70 days) and non-*Bt*-cotton leaves and semi-synthetic diet incorporated with 5 μg Cry1Ac toxin/ml of diet. The eight-day-old weighed larvae were allowed to feed on weighed leaf-discs of equal size, which were replaced after every 24 h. Fresh weighed leaf-disc of the same size was kept in similar rearing tubes under the same conditions to estimate the natural loss of moisture, which was used to calculate the corrected weight of the consumed leaves. Fresh weight of leaf-discs, surviving larvae, food left and mortality was recorded daily. The mean amount of toxin consumed by different instars and total amount ingested during their life-span were computed using the calculated values of Cry1Ac toxin in *Bt*-cotton leaves[21]. One hundred larvae of the same population serving as control were fed with non-*Bt*-cotton leaves, while another 100 were exposed to field equivalent dose of MVP-II Cry1Ac (i.e. Cry1Ac expressed in *Bt*-cotton) by feeding them semi-synthetic diet incorporated with 5 μg Cry1Ac/ml of diet. The amount of MVP-II consumed was also calculated on the basis of semi-synthetic diet consumed by each instar. Besides, records were also made on the total developmental period of the larval populations under different treatments.

The corrected weight of consumed leaves was calculated according to Waldbauer[22].

In order to analyse the response of Cry1Ac-resistant strain (BM-R) to *Bt*-cotton (expression level ~5 μg/g), eight-day-old larvae of the resistant BM-R strain after the 19th generation (LC_{50} = 7.493 μg/ml) was allowed to develop on *Bt*-cotton (RCH-134) and non-*Bt*-cotton leaves (60–70-days-old each), and semi-synthetic diet supplemented with 5 μg/ml MVP-II Cry1Ac toxin. The results indicate that in case of larval growth on non-*Bt*-cotton leaves, by 18 days, 91% of larvae survived and entered into pupation and subsequent development stages (Table 1) compared to *Bt*-cotton leaves, where the larval growth prolonged up to the 36th day; only 0.13% of larvae survived and entered into pupation. In the case of growth on semi-synthetic diet supplemented with MVP-II protein (that is equivalent to Cry1Ac concentration in *Bt*-cotton leaves), 60% of larvae survived by the 24th day before entering into pupation and subsequent development stages.

Out of the total of 744 second instars exposed to *Bt*-cotton, only 50, 17 and 4 could reach the third, fourth and fifth instars respectively. Of the 4 survived larvae on *Bt*-cotton, only 1 larva entered into pupation, but did not emerge into an adult. In the case of growth on semi-synthetic diet supplemented with MVP-II protein, the larvae survived by 24th day before entering into pupation and subsequent development stages. Figure 1 shows the weight of 187 individual larvae, which survived six days of exposure to *Bt*-cotton leaves. Each dot in the figure represents the maximum weight gained by a single larva. The total life cycle of the same population when fed with non-*Bt*-cotton leaves was completed in about 35 days, with all the larvae entering pupation at 20 days. On *Bt*-cotton leaves, only one surviving larva entered pupation after 38 days (Figure 2 which shows the mean weight of 100, 95, 73, 44, 27, 25, 18, 13, 9, 8, 5, 4, 2, 1, 1, larvae that survived after 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 34, 36 and 38 days respectively). The results suggest that Cry1Ac from *Bt*-cotton exerts a greater toxic effect in terms of larval mortality coupled with a decline in larval growth rate compared to semi-synthetic diet containing MVP-II Cry1Ac protein at levels equivalent to that expressed in *Bt*-cotton leaves. Presence of toxin either in semi-synthetic diet or in *Bt*-cotton exerted a high cost of fitness on the developmental parameters in the form of deformed and smaller sized larvae and pupae of *H. armigera.* In this context, the weight of fifth instar larvae was smaller on semi-synthetic diet containing toxin and *Bt*-cotton in comparison to larvae reared on non-*Bt*-cotton.

Table 2 provides data on the mean values of corrected food intake (CFI) by individual larvae from leaves of *Bt*-cotton and non-*Bt*-cotton as well as semi-synthetic diet along with the contribution of toxin protein in the ingested food per larva. The results suggest that the larvae express normal CFI on non-*Bt*-cotton leaves; the same in case of *Bt*-cotton leaves is reduced to around one-third.

Table 1. Comparative mortality of laboratory-developed Cry1Ac-resistant strain of *Helicoverpa armigera* on *Bt*-cotton and non-*Bt*-cotton leaves of RCH-134, and semi-synthetic diet incorporated with Cry1Ac

	Bt-cotton		Non-*Bt*-cotton		Semi-synthetic diet	
Days	No. dead	Mortality (%)	No. dead	Mortality (%)	No. dead	Mortality (%)
10	65	8.74	3	3	8	8
12	207	27.82	2	2	11	11
14	286	38.44	1	1	12	12
16	84	11.29	1	1	2	2
18	20	2.69	2	2	1	1
20	17	2.28	0	0	2	2
22	14	1.88	0	0	0	0
24	10	1.34	0	0	1	1
26	11	1.52	0	0	–	–
28	9	1.21	0	0	–	–
30	6	0.76	0	0	–	–
32	5	0.67	0	0	–	–
34	5	0.67	0	0	–	–
36	4	0.54	0	0	–	–
38	–	0.00	0	0	–	–
Total	743 (out of 744)	99.85	9 (out of 100)	9	37 (out of 100)	37

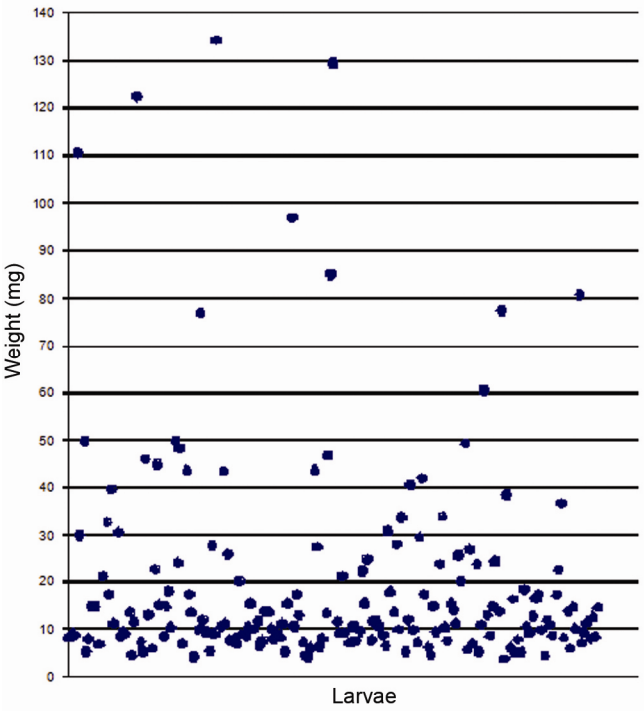

Figure 1. Weight gained by different individuals of *Helicoverpa armigera* (BM-R strain) reared on *Bt*-cotton leaves.

However, comparing the contribution of CFI towards consumed toxin, the ingested toxin from *Bt*-cotton is only one-twelfth of that ingested in semi-synthetic diet, though the latter shows lesser mortality and also lesser deteriorating influence on the growth of the larvae. Thus, Cry1Ac expressed in *Bt*-cotton possesses high toxicity than Cry1Ac toxin incorporated in semi-synthetic diet.

The results have been surprising in the sense that the resistant BM-R strain (LC_{50} = 7.493 µg Cry1Ac/ml semi-synthetic diet) showing 72-fold higher resistance level over the susceptible HP-S strain (LC_{50} = 0.104 µg Cry1Ac/ml semi-synthetic diet) failed to survive on *Bt*-cotton leaves (Cry1Ac ~ 5 µg/g). The larvae exposed to *Bt*-cotton leaves during their development from second to fifth instar showed total CFI of 155.69 mg compared to 440.60 mg from non-*Bt*-cotton suggesting that Cry1Ac protein besides slowing down larval development also exerts a negative effect on feed intake. Comparison of toxin intake from *Bt*-cotton leaves (0.761 µg) and semi-synthetic diet (9.375 µg) though was associated with similar negative effects on larval growth and development, higher mortality coupled with retardation of larval growth on *Bt*-cotton relative to semi-synthetic diet strongly suggest that Cry1Ac toxin expressed by *Bt*-cotton possesses high toxicity compared to MVP-II protein supplemented in semi-synthetic diet, both in terms of mortality and delayed growth, and development. Interpretation of these observations in Table 2 strongly suggests that Cry1Ac protein expressed in *Bt*-cotton leaves at a much lower concentration exerts a much higher level of depressive effect on food consumed and all developmental stages, including larval growth than even higher levels of MVP-II toxin.

The results suggest that the increased resistance (72-fold compared to susceptible strain)[11] to Cry1Ac in laboratory-selected resistant strain (BM-R) differs when exposed to *Bt*-cotton leaves. Tests on cotton leaves using progeny of BM-R strain continuously selected for 19 generations on diet-incorporated Cry1Ac (MVP-II) showed only 0.15% survival. Difference in survival

Table 2. Comparative consumption of non-*Bt* cotton and *Bt*-cotton leaves, and semi-synthetic diet incorporated with Cry1Ac by laboratory-developed Cry1Ac-resistant strain of *H. armigera*

Instar	Corrected food intake			Cry1Ac toxin consumed (µg)	
	Non-*Bt*-cotton leaves (mg)	*Bt*-cotton leaves (mg)	Semi-synthetic diet (mg)	*Bt* leaves	Semi-synthetic diet
Second	43.84	33.72	0.176	0.165	0.88
Third	53.48	35.18	0.402	0.172	2.01
Fourth	162.59	40.05	0.499	0.196	2.49
Fifth	180.69	46.73	0.798	0.229	3.99
Total	440.60	155.69	1.875	0.761	9.375

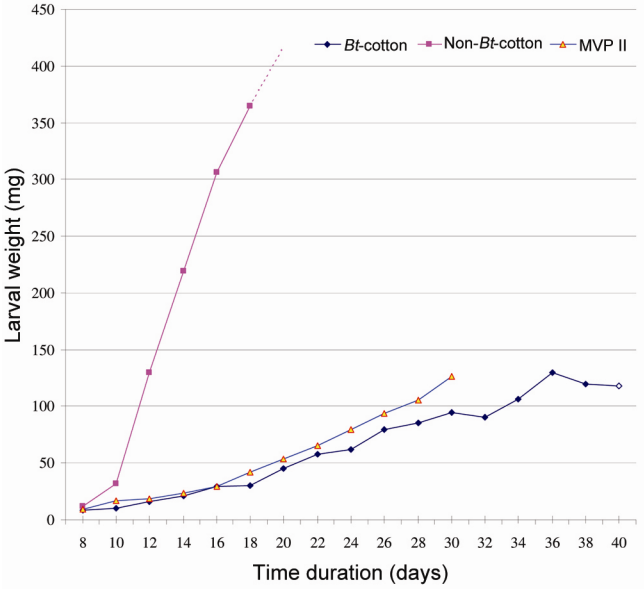

Figure 2. Mean weight gained by surviving larva of BM-R strain on leaves of *Bt*-cotton expressing ~5 µg/g of leaf, non-*Bt*-cotton and semi-synthetic diet incorporated with 5 µg/g MVP-II protein.

between laboratory diet bioassay and cotton leaf bioassay could be attributed to difference in toxin concentration in diet and cotton leaves, and also variation in toxicity in Cry1Ac from cotton leaves and MVP-II. There is 1% difference in the amino acid sequence of the active toxin between the Cry1Ac in *Bt*-cotton and Cry1Ac used in the diet (MVP-II), which is likely to cause a major difference in toxicity[23]. There are reports on resistance to *Bt* formulations or toxins in the laboratory, but only few may have survived on transgenic crops[24]. The results reported here confirm and support previously published evidence on the effects of Cry1Ac on survival and development of pink bollworm[4,25,26]. The susceptible and selected resistant strains of pink bollworm, *P. gossypiella* (Saunders) were tested for their survival and development on Cry1Ac toxin. The study concluded that increased Cry1Ac concentration in artificial diet reduced development rate and pupal weight, and resistant larvae reared on *Bt*-cotton showed lower survival, pupal weight and fecundity[27]. The slower development results in more mortality of resistant population due to exposure of natural enemies and abiotic factors[28,29]. A modelling study with tobacco bud worm showed that the chances of resistance development in the field can be decreased due to slower development of resistant larvae on *Bt*-cotton[30]. Our results corroborate those of Nadaf and Goud[31] in respect of loss of larval weight (6.1 mg) and longer time taken for pupation of pink bollworm (23.2 days) on *Bt*-cotton compared to non-*Bt*-cotton (larval weight:8.0 mg; time taken to pupation: 18.8 days).

Thus, slower development of larvae on *Bt*-cotton could increase their mortality in field conditions. On the other hand, the negative effects of *Bt*-cotton on laboratory-raised resistant *H. armigera* bollworm may help to delay resistance. Based on differences in life-history traits of resistant insect reared on *Bt*-cotton versus non-*Bt*-cotton, *Bt*-cotton could greatly reduce the growth.

We observed negative effects of *Bt*-cotton on the survival and development of resistant larvae which could delay resistance development. The results of this study suggest that Cry1Ac protein expressed in *Bt*-cotton exerts a higher level of depressive effect on food consumed, larval survival and its subsequent growth/development than MVP-II toxin. Thus, MVP-II toxin is not an ideal substitute for Cry1Ac toxin protein in *Bt*-cotton for use in resistance studies in *H. armigera* due to difference in toxicity.

1. Choudhary, B. and Gaur, K., Biotech cotton in India, 2002 to 2014. ISAAA Series of Biotech Crop Profiles, ISAAA, Ithaca, NY, 2015.
2. Huang, F., Buschman, L. L., Higgins, R. A. and McGaughey, W. H., Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science*, 1999, **284**, 965–967.
3. Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. and Moar, W., Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera : Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxin. *J. Econ. Entomol.*, 1995, **88**, 1545–1559.
4. Liu, Y. B., Tabashnik, B. E., Dennehy, T. J., Patin, A. L. and Bartlett, A. C., Development time and resistance to *Bt* crops. *Nature*, 1999, **400**, 519.
5. Tabashnik, B. E., Evolution of resistance to *Bacillius thuringiensis*. *Annu. Rev. Entomol.*, 1994, **39**, 47–79.

6. Wirth, M. C., Georghiou, G. P. and Federici, B. A., CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels of CryIV resistance in the mosquito, *Culex quinquefasciatus*. *Proc. Natl. Acad. Sci. USA,* 1997, **94**, 10536–10540.
7. Frutos, R., Rang, C. and Royer, M., Managing insect resistance to plants producing *Bacillus thuringiensis* toxins. *Crit. Rev. Biotech*., 1999, **19**, 227–276.
8. Whalon, M. E. and McGaughey, W. H., *Bacillus thuringiensis*: use and resistance management. In *Insecticides with Novel Modes of Action: Mechanism and Application* (eds Ishaaya, I. and Degheele, D.), Springer, Berlin, 1998, pp. 106–137.
9. Ojha, A. K. *et al.*, Analysis of resistance to Cry1Ac in field-collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera:Gelechiidae), populations. *GM Crops Food*, 2014, **5(**4), 280–286.
10. Kranthi, K. R., Ali, S. and Banerjee, S. K., Resistance to Cry 1AC δ-endotoxin of *Bacillus thuringiensis* in a laboratory selected strain of *Helicoverpa armigera* (Hübner). *Curr. Sci*., 2000, **78**, 1001–1004.
11. Kaur, P. and Dilawari, V. K., Inheritance of resistance to *Bacillus thuringiensis* Cry1Ac toxin in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from India. *Pest. Manage. Sci*., 2011, **67**, 1294–1302.
12. Gujar, G. T., Kumari, A., Kalia, V. and Chandrashekar, K., Spatial and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hübner) to *Bacillus thuringiensis* var. *kurstaki* in India. *Curr. Sci.*, 2000, **78**, 995–1001.
13. Nair, R., Kalia, V., Aggarwal, K. K. and Gujar, G. T., Variation in the cadherin gene sequence of Cry1Ac susceptible and resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae) and the identification of mutant alleles in resistant strains. *Curr. Sci.*, 2013, **104**, 215–223.
14. Tabashnik, B. E., Gassmann, A. J., Crowder, D. W. and Carriere, Y., Insect resistance to *Bt* crops: evidence versus theory. *Nature Biotechnol*., 2008 **26**, 199–202.
15. Liu, F. *et al.*, Evidence of field-evolved resistance to Cry1Ac-expressing *Bt* cotton in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. *Pest Manage. Sci*., 2010, **66**, 155–161.
16. Li, G. P., Wu, K. M., Gould, F., Wang, J. K., Miao, J., Gao, X. W. and Guo, Y. Y., Increasing tolerance to Cry1Ac cotton in cotton bollworm, *Helicoverpa armigera*, was conformed in *Bt* cotton farming area of China. *Ecol. Entomol*., 2007, **32**, 366–375.
17. Gould, F. and Tabashnik, B. E., *Bt*-cotton resistance management. In *Now or Never, Serious New Plans to Save a Natural Pest Control* (eds Mellon, M. and Rissler, J.), Union of Concerned Scientists, Cambridge, MA, 1998, pp. 67–105.
18. Gould, F., Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu. Rev. Entomol.*, 1998, **43**, 701–726.
19. Liu, Y. B. and Tabashnik, B. E., Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*. In *Proc. R. Soc. London, Ser. B*, 1997, **264**, 605–610.
20. Shelton, A. M., Tang, J. D., Roush, R. T., Metz, T. D. and Earle, E. D., Field tests on managing resistance to *Bt*-engineered plants. *Nature Biotechnol.*, 2000, **18**, 339–342.
21. Kumar, R., Effect of *Bt*-cotton genotypes and *Bt cry* gene interaction on the performance of *Helicoverpa armigera*. M Sc thesis, Punjab Agricultural University, Ludhiana, Punjab, India, 2005.
22. Waldbauer, G. P., The consumption and utilization of food by insects. *Adv. Insect Physiol*., 1968, **5**, 229–282.
23. Tabashnik, B. E., Liu, Y. B., Dennehy, T. J., Sims, M. A., Sisterson, M. S., Biggs, R. W. and Carrière, Y., Inheritance of resistance to *Bt* toxin Cry1 Ac in a field-derived strain of pink bollworm (Lepidoptera : Gelechiidae). *J. Econ. Entomol*., 2002, **95**, 1018–1026.
24. Ferre, J. and Van Rie, Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol*., 2002, **47**, 501–533.
25. Liu, Y. B., Tabashnik, B. E., Meyer, S. K., Carriere, Y. and Bartlett, A. C., Genetics of pink bollworm resistance to *Bacillus thuringiensis* toxin Cry1Ac. *J. Econ. Entomol*., 2001, **94**, 248–252.
26. Tabashnik, B. E., Patin, A. L., Dennehy, T. J., Liu, Y. B., Carriere, Y., Sims, M. A. and Antilla, L., Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. In *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 12980–12984.
27. Liu, Y. B., Tabashnik, B. E., Dennehy, T. J., Patin, A. L., Sims, M. A., Meyer, S. K. and Carriere, Y., Effects of *Bt* cotton and Cry1Ac toxin on survival and development of pink bollworm (Lepidoptera : Gelechiidae). *J. Econ. Entomol*., 2001, **94**, 1237–1242.
28. Gould, F., Kennedy, G. G. and Johnson, M. T., Effects of natural enemies on the rate of herbivore adaptation to resistant host plants. *Entomol. Exp. Appl*., 1991, **58**, 1–14.
29. Benry, B. and Denno, R. F., The slow growth–high mortality hypothesis: a test using the cabbage butterfly. *Ecology*, 1997, **78**, 987–999.
30. Peck, S. L., Gould, F. and Ellner, S. P., Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Heliothis virescens* (Lepidoptera : Noctuidae). *J. Econ. Entomol*., 1999, **92**, 1–16.
31. Nadaf, A. R. M. and Goud, K. B., Effect of *Bt* cotton on growth and development of pink bollworm, *Pectinophora gossypiella*. *Indian J. Plant Prot*., 2007, **35**, 57–59.

Received 4 January 2016; revised accepted 3 October 2016

doi: 10.18520/cs/v112/i07/1579-1583