

1659 Exploring the Bt Cotton Potential for the Management of Semilooper Complex, *Anomis flava* Fab. and *Tarache nitidula* Fab.

Dr. Umesh Chandra Shelker , Department of Entomology, Coimbatore, India
Prof. Ayyappan Regupathy , Department of Entomology, Coimbatore, India

Rationale: Semilooper complex consisting six species are known to cause damage occasionally. Among them, *Anomis flava* Fab., and *Tarache nitidula* Fab., occur in an epidemic form in years of heavy rainfall in Tamil Nadu, India.

Objectives: The performance of Bt cotton MECH 162 Bt and MECH 184 Bt against semiloopers was evaluated

Methods: Bioassay was conducted to investigate the Cry 1Ac toxin expression and its manifestation in *T. nitidula* and *A. flava* larval susceptibility, by excised leaf technique.

Results: There was cent per cent mortality of third instar, *T. nitidula* as well as *A. flava* larvae fed on MECH 184 Bt (60 DAS). Similarly, fifth instar larvae were also highly susceptible to Bt cotton cultivars (85.00-90.00 and 77.50-90.00 per cent). There was a gradual decrease in mortality 120 DAS and both instars, namely third and fifth, registered comparatively lower mortalities. Mortality of 67.50-77.50 per cent mortality in third instar and 62.50-72.50 per cent in fifth instar larvae were observed. Lower mortality of *A. flava* larva was observed in later stage of the crop (120 DAS); the extent of mortality being 72.50-82.50 and 55.00-72.50 per cent in third and fifth instars respectively.

Conclusions: Both *T. nitidula* and *A. flava* were highly sensitive to both Bt cotton cultivars viz., MECH 162 Bt and MECH 184 Bt. That both semiloopers were highly sensitive to Cry1Ac

Keywords: *emiloopers, transgenic, Anomis flava Fab., Tarache nitidula Fab*

Introduction

Cotton bollworm complex comprising *Helicoverpa armigera* Hub., *Earias vittella* F., *Earias insulana* Boisd., and *Pectinophora gossypiella* Saund., causes enormous damage to the crop. Chemical insecticides dominated the pest management strategy since 1960 (Regupathy and Ayyasami, 2003). Apart from bollworm complex six species of semiloopers viz. : ***Anomis flava* Fab.**, *Acontia graellsii*, *Tarache notabilis*, ***Tarache nitidula* Fab.**, *T. basifera* and *T. opalinoidea*. are known to infest cotton crop. They are all minor pests, doing damage occasionally. In India , *Anomis flava* occurs in an epidemic form in years of heavy rainfall. Its life and seasonal histories have been worked out in Mysore and Bombay . *T. notabilis* as an important pest in Amreli district of former Bombay state. However, in Surat district of Bombay State, it is known as a major pest and is serious during July to October – November, while in the Punjab, it is serious in June – July. . It usually attacks indigenous cotton by heavy defoliation (Deshpande and Nadkarny ,1936).). In Tamil Nadu, generally this is a minor pest of cotton and widely distributed in different cotton growing districts.

The introduction of genetic transformation techniques opened the new vistas for the management of pest . It is expected that transgenic crops will make pest management simpler to the farmer. The present day Bt cotton cultivars available in the market express modified crystal protein gene, *cry1Ac* derived from gram positive bacteria *Bacillus thuringiensis* (Bt, HD-73). The Cry1Ac toxin is reported to be very effective against an array of lepidopteran pests (Perlak *et al.*, 1990). Prior to field trials it was anticipated that expression of the Bt protein would be consistent throughout the growth of the cotton crop and expected to offer season long protection (Benedict, 1996). However, subsequent field trials revealed that the extent of Cry1Ac expression remained highly variable among the plant parts and some times the decline was noticed at the squaring stage itself (Daly and Fitt, 1998; Fitt *et al.*, 1998; Kranthi *et al.*, 2000).

Greenplate (1999) reported the decline in Cry1Ac levels in the terminal foliage as plant aged. Hence seasonal expression of Cry1Ac in Bt cotton and susceptibility of lepidopterous pest occurring on cotton are assessed. Recently while evaluating the Bt cultivars against resistant *H.armigera* (Shelkar, 2004), an outbreak of semiloopers, *T. nitidula*. and *A. flava* was observed. Availing the pest incidence, the performance of Bt cotton *in situ* against these species was evaluated .

Materials and Methods

Laboratory colony establishment of *T. nitidula*. and *A. flava*

Initial cultures both the semiloopers namely, *T. nitidula* (Noctuidae: Lepidoptera) and *A. flava* (Noctuidae: Lepidoptera) were collected from Agriculture Research Station (ARS), Bhavanisagar (Erode district) and ARS, Vaigaidam (Theni district) and were maintained on fresh cotton leaves of cv. MCU -5.

Fresh cotton leaves were obtained from the cotton plants maintained in the Insectary, Department of Entomology, TNAU, Coimbatore. Leaves were washed with 0.1 per cent sodium hypochlorite and subsequently washed with distilled water repeatedly and shade dried. Fresh stalks of cotton with leaves were immersed in conical flask (Borosil, 250 ml capacity) filled with water. The whole set up was transferred to the larval rearing cages and larvae were introduced inside. Larvae were allowed to feed *ad libitum* and complete the life cycle on same host.

The emerging adults were transferred to the adult emergence cages stocked with 10 percent sucrose solution fortified with ABDEC multivitamin solution. Fresh stalks with leaves were provided as oviposition substrate for the adults inside the oviposition cage. The eggs were collected, surface sterilized with sodium hypochlorite and introduced on fresh tender cotton leaves for hatching and subsequent feeding. Emerging larvae were again fed with cotton leaves as explained earlier.

Measurement of Susceptibility of cotton semilooper complex to Bt cotton

Cotton semiloopers namely *T. nitidula* and *A. flava* of third and fifth instars were separated from the nucleus culture and bioassayed on Bt cotton cultivars aged 60 and 120 DAS. The fresh leaves of Bt cotton cultivar namely MECH 162 Bt and MECH 184 Bt along with their NBt counterparts and checks were collected and sterilized as discussed earlier. Leaf petiole was tied with the help of moistened cotton wool so as to maintain the freshness of the leaves. Known numbers of larvae were introduced in a plastic container along with the leaves. Container lids were perforated for providing ventilation and avoid condensation of

vapor and an absorbent filter paper disc was placed. Three replications were maintained for each treatment. The observations on larval mortality were recorded after every 24 h and continued up to 72 h.

Results and Discussion

Both semiloopers were highly susceptible to Bt cotton cultivars (Tables 1 and 2). There was cent per cent mortality of third instar, *T. nitidula* as well as *A. flava* larvae fed on MECH 184 Bt (60 DAS). Similarly, fifth instar larvae were also found to be highly susceptible to Bt cotton cultivars (85.00-90.00 and 77.50-90.00 per cent). There was a gradual decrease in mortality 120 DAS and both instars, namely third and fifth, registered comparatively lower mortalities thus, indicating reduction in Bt protein. Mortality of 67.50-77.50 per cent mortality in third instar and 62.50-72.50 per cent in fifth instar larvae were observed. Lower mortality of *A. flava* larva was observed in later stage of the crop (120 DAS); the extent of mortality being 72.50-82.50 and 55.00-72.50 per cent in third and fifth instars respectively. The mortality of *T. nitidula* and *A. flava* on non Bt cotton did not exceed 17.50 per cent. The possible reason of higher mortality of semiloopers can be explained from the fact that semiloopers solely feed on fresh leaves which contain the highest protein concentration among the various plant parts. It also indicates the presence of binding sight of Bt protein in both semiloopers midgut. Secondly, it has to be remembered that *T. nitidula* and *A. flava* are the sporadic pests and do not appear regularly. Only during heavy rainfall periods, they emerge mainly as weed feeders and subsequently get transferred to cotton causing severe defoliation. They are least exposed to insecticide selection pressure and hence respond positively to any compound directed to control them.

Both semiloopers were highly sensitive to Cry1Ac toxins even at lower concentrations as compared to other defoliator, *S. litura* which is least susceptible to Bt cotton expressing Cry1Ac (Kranthi *et al.*, 2004). It could be possibly due to absence of protein binding sight in *S. litura* midgut. There is a wide variation in lepidopteron larval response to Bt protein. The variation in susceptibility may arise from innate ability to tolerate the Cry toxins (Henneberry *et al.*, 2003; MacIntosh *et al.*, 1990). Bt cotton feeding caused 90-100 per cent mortality in *P. gossypiella* (Wilson *et al.*, 1994), *E. insulana* (Horowitz *et al.*, 2004), *H. virescens* and *H. zea* (Halcomb *et al.*, 1996). The per cent mortality in each instar of the *H. armigera* in the decreasing order of susceptibility was 75.00-100.00 > 72.93-96.67 > 47.90-91.68 > 31.25- 87.50 per cent in neonates, first, second and third instar, respectively (Shelker, 2004).

This experiment proves beyond doubt that *A. flava* and *T. nitidula* are highly susceptible to Bt cotton (MECH 162 Bt and MECH 184 Bt) in different crop growth phases under study. It can be safely assumed that Bt cotton can effectively check the semiloopers complex in the event of unexpected outbreak

References

- Benedict, J.H., E.S. Sachs, D.W. Altman, W.R. Deaton, R.J. Kohel, D.R. Ring and S.A. Berberich. 1996. Field performance of cotton expressing transgenic Cry1A insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). **J. Econ. Entomol.**, **89**: 230-238
- Daly, J.C. and G.P. Fitt. 1998. Efficacy of Bt cotton plants in Australia what is going on? **Proc. World Cotton Res. Conf.**, Athens, Greece, 6-12 September pp. 675-678.

Deshpande, B. P. and N. T. Nadkarny. 1936. The spotted bollworm of cotton, *Earias fabia* Stoll and *Earias insulana* Boisd., in south Gujarat, Bombay Presidency. Indian Coun. Agr. Res. Sci. Monogr. **10**: 1-208.

Fitt, G. P., J.C. Daly, C.L. Mares and K. Olsen. 1998. Changing the efficacy Transgenic Bt cotton—Patterns and Consequences. **In: Pest Management—Future Challenges**. Zalucki, M.P., R.A.I. Drew, G.G. Whites (Eds.) University of Qld Press, Brisbane, pp. 189-196.

Greenplate, J.T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. **J. Econ. Entomol.**, **92**(6): 1377-1383.
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.

Henneberry, T.J., L.F. Hech and T. de la Torre. 2003. Cabbage looper, tobacco budworm and beet army worm larval mortalities, development and foliage consumption on Bt and non Bt cottons. **Arizona Cotton Report**, <http://cals.arizona.edu/pubs/crops/az1312>.

Horowitz, A. R. A. Navon, S. Levski, S. Yablonski and A. Niv. 2004. Contributions of Bt transgenic cotton to the Israeli Insecticide Resistance Management (IRM) strategy. **In: World Cotton Res. Conf.**, 9-13, March, RSA. Pp 1250-1252.

Kranthi, K.R., S. Kranthi, A.B. Dongre and M.S. Kairon. 2000. A brief review on transgenic cotton. **J. Indian Soc. Cotton Improv.**, **25**(1): 1-16.

Kranthi, K.R., S. Kranthi, S.K. Banerjee and C.D. Mayee. 2004. Perspective on resistance management strategies for Bt cotton in India. **In: World Cotton Res. Conf.**, 9-13, March. Cape Town, RSA. pp.1254-1260

MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, T.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff and R.L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. **J. Invertebr. Pathol.**, **39**: 323-328.

Perlak, F.J., R.W. Deaton, T.A. Armstrong, R.L. Fuchs, S.R. Sims, J.T. Greenplate and D.A. Fischhoff. 1990. Insect resistant cotton plants. **Bio/Technol.**, **8**: 939-943.

Reguapthy, A. and R. Ayyasami. 2003. Insecticide resistance in *Heliothis* species: An update. **Resist. Pest Mgmt**, **13** (1):19-25.

Shelkar U.R. 2004. Exploring the bt cotton potential in management of *Helicoverpa rmigera* hubner. Ph.d. thesis. Tamil Nadu agricultural University, Coimbatore, India.

Wilson, F.D., H.M. Frint, W.R. Dexton, D.A. Fischhoff, F.J. Perlak, T.A. Armstrong, R.L. Fuchs, S.A. Berberich, N.J. Parks and B.R. Stapp. 1994. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. **J. Econ. Entomol.**, **85**: 1516-1521.

Table 1 Effect of Bt cotton cultivars on cotton semi looper *A. flava* –Per cent mortality

Treatments	III Instar		V Instar	
	Days after sowing (DAS)			
	60	120	60	120
MECH 162 Bt	92.50 (76.17) ^b	72.50 (58.60) ^a	77.50 (62.14) ^a	55.00 (47.88) ^a
MECH 162 NBt	5.00 (9.47) ^d	10.00 (13.53) ^b	7.50 (11.50) ^b	5.00 (9.47) ^b
MECH 184 Bt	100.00 (89.86) ^a	82.50 (65.47) ^a	90.00 (74.14) ^a	72.50 (58.45) ^a
MECH 184 NBt	17.50 (24.53) ^c	5.00 (9.47) ^b	7.50 (13.95) ^b	10.00 (15.98) ^b
MCU 5	7.50 (13.95) ^{cd}	7.50 (11.50) ^b	5.00 (9.47) ^b	5.00 (9.47) ^b

Mortality expressed in per cent

Values in parenthesis are arcsine (sqr [$p/100$]) transformed values

Means followed by a common letter in a column are not significantly different at the 5% level by DMRT

Mean of four replications

** - Significant at 1% level

Table 2 Effect of Bt cotton cultivars on brown semilooper *T. nitidula*-Per cent Mortality

Treatments	III Instar		V Instar	
	Days after sowing (DAS)			
	60	120	60	120
MECH 162 Bt	92.50 (76.17) ^b	67.50 (54.44) ^a	85.00 (67.50) ^a	62.50 (52.27) ^a
MECH 162 NBt	7.50 (13.95) ^{cd}	10.00 (15.98) ^b	5.00 (9.47) ^b	2.50 (4.98) ^c
MECH 184 Bt	100.00 (89.86) ^a	77.50 (61.77) ^a	90.00 (77.09) ^a	72.50 (58.45) ^a
MECH 184 NBt	15.00 (22.50) ^c	17.50 (24.53) ^b	12.50 (20.47) ^b	12.50 (20.47) ^b
MCU 5	2.50 (4.98) ^d	7.50 (11.50) ^b	2.50 (4.98) ^b	7.50 (13.95) ^{bc}

Mortality expressed in per cent

Values in parenthesis are arcsine (sqr [$p/100$]) transformed values

Means followed by a common letter in a column are not significantly different at the 5% level by DMRT

Mean of four replications

** - Significant at 1% level