



Research Article

INTER AND INTRA PLANT MOVEMENT OF TOBACCO CATERPILLAR, *Spodoptera litura* (FAB.) IN BOLLGARD-II + NON BT COTTON FIELD

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Abstract-Larval migration of late second instar tobacco caterpillar *Spodoptera litura* (Fab.), was observed in pure and mixed stands of nontransgenic and BG-II cotton, *Gossypium* spp. L., expressing an insecticidal protein Cry 1 Ac + Cry 2 Ab from a bacterium, *Bacillus thuringiensis* Berliner subsp. kurstaki. Three plots i.e., two mixed stands (Mallika and Bunny BG-II along with non Bt) and one pure stand (BG-II cotton) were evaluated. In case of mixed planting non Bt plants were infested with late second instar larvae at 60, 75 and 90 days after sowing of the crop. In pure BG-II cotton only selected BG-II plants were infested with late second instar larvae at 75 days after sowing of the crop. These data provide evidence of larval movement between plants in seed mixtures. Migration of *S. litura* larvae from non Bt to BG-II cotton hybrid in mixed stand and BG-II cotton to BG-II cotton within pure stand was observed at 60, 75 and 90 DAS. The larval migration was more in pure stand (BG-II cotton field) compared with mixed stand cotton. More number of larvae was observed on leaves followed by flowers, squares and bolls irrespective of pure and mixed cotton.

Keywords- Dual toxin Bt cotton, Migration, *S. litura*.

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Introduction

Insecticidal proteins from *Bacillus thuringiensis* Berliner (Bt) are known insecticidal agents, both in spray formulations and transgenic crops [1]. The Cry 1 Ac protein produced by Bollgard cotton has insecticidal activity against the larvae of selected lepidopteran pests [2]. Whereas, beet armyworms, *Spodoptera exigua* (Hübner), fall armyworms, *S. frugiperda* (J. E. Smith), and soybean loopers, *Pseuoplusia includens* (Walker), are not readily controlled by this protein. Less than adequate control of the pest spectrum, coupled with concerns about resistance management, has prompted scientists with Monsanto Co. to develop other genetically engineered cottons that contain two separate crystalline proteins [3]. The second protein increases the insecticidal activity of Bollgard cotton against target pests and broadens the spectrum of total pests controlled.

The widespread planting of *B. thuringiensis* cotton increases the risk of resistance developing in all lepidopteran pests of cotton. The protein is produced throughout the plant during the entire season. Therefore, cotton insect pests are exposed to the protein for more than one generation each year. This constant selection pressure increases the frequency of resistance alleles in the population and could lead to high levels of tolerance in a relatively short amount of time [4]. Because of the threat of insect pests developing resistance to *B. thuringiensis* cotton, government agencies, industry, farmers, and academic researchers have adopted resistance management plans [5]. The seed mixture planting was proposed as a strategy to delay development of resistance during the early 1990s because of its potential ease in implementation. This strategy insured the presence of a refuge, but had potential limitations influenced by larval behavior [6]. Seed mixtures would hasten the development of resistance if insect movement was independent of the toxin inside plants [7]. These seed mixtures could delay resistance of insects to Bt toxins regardless of whether the movement of insect is independent of the toxin

inside the plant [8].

However, based on the field experiments conducted with diamondback moth, *Plutella xylostella* (L.) revealed that the seed mixtures did not delay the evolution of resistance to Bt toxins [9]. In addition to this that the movement of *P. xylostella* was independent of toxin between conventional and transgenic canola plants [10-11]. Furthermore, *P. xylostella* larvae have been shown to move to toxin plants from toxin-free plants if the population density on toxin-free plants was greater than that on toxin plants [12].

In greenhouse experiments, [13-15] observed increased movement of tobacco budworm larvae on Bollgard cotton plants compared with conventional plants. Findings such as these pose serious problems when using Bt for pest control. This study aimed to determine the movement of *S. litura* larvae in pure and mixed plantings of transgenic and nontransgenic cotton plants.

Materials and Method

Inter-plant (Mixed planting of BG-II and non Bt) and intra-plant (Pure stand of BG-II only) movement of larvae

To study the inter-plant movement of larvae two BG-II cotton hybrids namely Mallika and Bunny and their non Bt cotton plants were maintained at College Farm, College of Agriculture, Rajendranagar, Hyderabad during Kharif-2013. Mallika and Bunny Bollgard-II cotton was grown in the field with five per cent refuge [Plate-1] i.e., Out of 200 cotton plants, 190 were BG-II cotton plants and the remaining 10 plants were non-Bt cotton plants. These 10 non-Bt cotton plants and four BG-II cotton plants which were surrounded by each non-Bt cotton plants were tagged. To reduce the error in statistical analysis only the two hybrids were selected. For Bunny and Mallika BG-II cotton hybrids layout of field was similar. Standard agronomic practices maintained field plots, including herbicide

and fertilizer applications.

Rearing of *S. litura* culture was carried out at BT lab, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad. Initially the culture was collected from the field on castor plants. Later the larvae were reared on castor leaves in BT lab up to the first generation. Then the experiment was carried out with the late second instar larvae. Thirty late second instar *S. litura* larvae per plant were released at 60, 75 and 90 DAS on the tagged non-Bt plants only. The larvae were released at top, middle, and bottom strata of the plant.

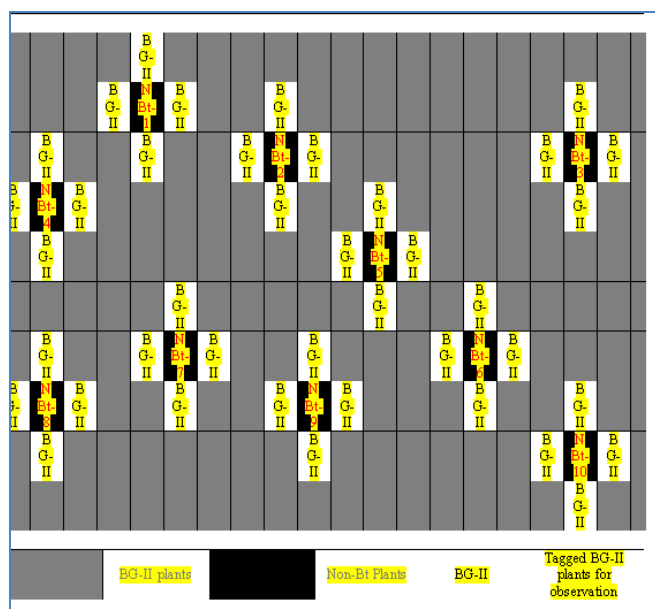


Plate-1 Field layout for inter-plant movement of late second instar larvae of *S. litura*.

For observing the intra-plant movement of late second instar larvae, eleven Bollgard-II cotton hybrids (namely Ankur-3034, Ajeet-155, Chetak, ATM, Bhakti, Brahma, Denim, Rasi-665, Rasi-668, Sudarshan, Yuva) were selected. Here, we assume that all eleven BG-II hybrids are similar i.e. they are only BG-II cotton plants having Cry 1 Ac and Cry 2 Ab protein.

Among these eleven BG-II hybrids ten BG-II cotton plants were selected and tagged along with four BG-II cotton plants that surrounded the selected BG-II hybrids [Plate-2]. On the center BG-II cotton plant, 30 late second instar *S. litura* larvae were released at 75 DAS and movement of the larvae was recorded.

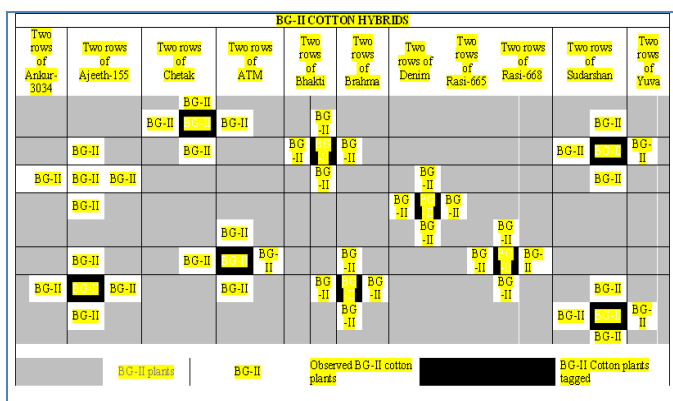


Plate-2 Field layout for intra-plant movement of late second instar larvae of *S. litura* (BG-II cotton hybrids).

The mean data of ten BG-II cotton plants were pooled in a single table according to the days after release. Observations on movement of larvae were recorded at every 24 hours interval after releasing of larvae up to 10 days. The number of larvae retained on center and adjacent plant parts viz., leaves, squares, flowers and bolls were recorded. While taking the observations natural infestation also

observed in the field at 60, 75 and 90 DAS. But there was no natural infestation in the cotton field.

The data on the larval migration studies of second instar larvae of *S. litura* were subjected to student's t-test to determine the movement of larvae. By using the statistical program named Biostat-2009 student's t-test was analyzed. T-test was done separately for number of larvae retained on center plant and number of larvae retained on adjacent plant by taking the 'n' (total no. of larvae) as 30 for center and adjacent plant. Mean values which are obtained from the t-test are considered for comparing the movement of larvae in the field from the first day after release to 10 days after release.

Results and Discussion

Inter-plant Movement of *S. litura*

The movements of late second instar larvae varied in Bunny and Mallika BG-II cotton hybrids. In case of Bunny, larvae retained on centre plant (non-Bt Bunny) decreased from the first day after release (6.8) to fifth day (4.2) on leaves, as well as squares (3.4 to 1.5) at 60 DAS [Table-1]. Similar results were also obtained at 75 DAS (5 to 1 on leaves, 2.7 to 0.5 on squares, 1.4 to 0.3 on bolls and 1.3 to 0.2 on flowers) [Table-2] and 90 DAS (3.27 to 0.27, 1.18 to 0.45, 1.00 to 0.27 and 1.45 to 0.72 on leaves, squares, bolls and flowers, respectively) [Table-3]. Among leaves, squares, bolls and flowers more number of larvae were observed on leaves of non-Bt. Compared to non-Bt and BG-II cotton plants more number of larvae were observed on non-Bt. On non-Bt plant more larvae were observed on leaves. The number of *S. litura* larvae was decreasing day by day, that is after releasing of larvae, more number of larvae were found in the field and later the number of larvae were decreasing. The reason was they were not found in the field (missing larvae) and larvae died because of Cry protein. From the field dead larvae were recovered but very less in number. Different factors which effect on larval movement was growth of the crop and the Cry protein content and expression of Cry protein.

Intra-plant Movement of *S. litura*

The movement of *S. litura* was observed in between eleven BG-II cotton hybrids at 75 DAS. Larval movement was recorded with one day interval up to ten days after release. But the number of larvae was less after release of five days (i.e., sixth day). For statistical analysis the values up to 5 days after release only were considered. The mean number of larvae on centre BG-II cotton ranged between 3.27-0.27, 1.18-0.45, 1.0-0.27 and 1.45-0.72 on leaves, squares, bolls and flowers, respectively at 75 DAS. The mean number of larvae on adjacent BG-II cotton ranged in between 4.18-1.72, 2.09-1.00, 2.27-0.63 and 3.9- 1.18 on leaves, squares, bolls and flowers, respectively at 75 DAS [Table-4].

The number of larvae moved more rapidly in BG-II cotton field compared to field with five per cent refuge plant. The number of larvae on center plant was less, compared to adjacent plant in BG-II cotton field. But the results obtained with refuge cropping was reverse i.e., more number of larvae were present on center plant compared to adjacent plant. This indicates movement of second instar larvae was more in BG-II field than in refuge crop. The larval movement was significantly different on leaves and squares. In case of flowers and bolls the larval movement did not differ significantly.

These results are similar with the findings of [16], who reported that the larvae of *H. armigera* exposed to *B. thuringiensis* toxin moved more but fed less than larvae not exposed to the toxin. The results indicated that movement of *H. armigera* is not independent of plant genotypes, as assumed by [7]. Dispersal of early instar bollworm larvae may be different on Bollgard cotton plants compared to non-Bt cotton plants. In laboratory bioassays, bollworm larvae moved from cotton leaves treated with foliar *B. thuringiensis* formulations and were found at other locations in the test arena [17]. Bollworm larvae also avoided feeding on meridic diets containing purified *B. thuringiensis* proteins or lyophilized Bollgard plant tissues [18-19]. Tobacco budworm larval movement has been observed to be different on Bollgard cotton plants compared to non-Bt plants in field and greenhouse studies [6]. In both of these studies, tobacco budworm larvae moved from Bollgard plant terminals more rapidly than from non-Bt plant terminals. However, the fate of

Table-1 Migration of late second instar larvae of *S. litura* in five per cent refugia field at 60 DAS

Plant parts	DAR	Number of larvae retained on centre plant (non-Bt)						Number of larvae retained on adjacent plant (BG-II)					
		Bunny			Mallika			Bunny			Mallika		
		Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t
Leaf	First day	6.80±0.53	12.75	0	5.80±0.29	19.95	0	5.20±0.40	15.21	0	2.70±0.21	12.65	0
	Second day	5.30±0.30	17.67	0	3.60±0.33	10.59	0	5.60±0.42	13.11	0	2.10±0.23	9.00	0
	Third day	4.80±0.36	13.37	0	3.60±0.33	10.85	0	4.30±0.37	11.72	0	1.60±0.16	9.79	0
	Fourth day	4.30±0.30	14.33	0	1.60±0.22	9.79	0	3.70±0.30	12.33	0	1.30±0.15	8.51	0
	Fifth day	4.20±0.29	14.45	0	0.60±0.16	3.67	0.0005	3.10±0.23	13.28	0	0.30±0.15	1.96	0
Square	First day	3.40±0.22	15.37	0	3.20±0.16	16.00	0	2.60±0.40	6.50	0	2.00±0.21	9.48	0
	Second day	2.30±0.20	7.66	0	2.10±0.27	7.58	0	1.80±0.29	6.19	0	1.60±0.16	9.79	0
	Third day	1.80±0.20	9.00	0	0.60±0.16	3.60	0.005	1.00±0.21	4.74	0	1.30±0.21	6.09	0
	Fourth day	1.70±0.15	11.12	0	0.60±0.16	3.60	0.005	0.80±0.13	6.00	0	0.80±0.13	6.00	0
	Fifth day	1.50±0.17	9.00	0	0	N.A	N.A.	0.80±0.13	6.00	0	0.10±0.10	1.00	0

DAS - Days after sowing. DAR - Days after release.

Table-2 Migration of late second instar larvae of *S. litura* in five per cent refugia field at 75 DAS

Plant parts	DAR	Number of larvae retained on centre plant (non-Bt)						Number of larvae retained on adjacent plant (BG-II)					
		Bunny			Mallika			Bunny			Mallika		
		Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t
Leaf	First day	5.00±0.26	19.36	0	5.30±0.30	17.66	0	5.80±0.29	19.95	0	6.30±0.57	1.90	0
	Second day	3.00±0.15	20.12	0	4.60±0.22	20.80	0	4.20±0.20	21.00	0	4.70±0.21	10.89	0
	Third day	2.70±0.15	17.67	0	3.90±0.27	14.08	0	3.80±0.20	19.00	0	4.10±0.17	22.84	0
	Fourth day	1.50±0.22	6.71	0	2.90±0.27	10.47	0	2.10±0.10	21.00	0	3.70±0.15	24.22	0
	Fifth day	1.00±0.21	4.74	0.001	1.90±0.31	6.04	0	1.50±0.17	9.00	0	3.40±0.31	11.21	0
Square	First day	2.70±0.15	17.67	0	3.20±0.20	16.00	0	3.40±0.16	20.82	0	1.60±0.26	6.00	0
	Second day	1.40±0.16	8.57	0	2.00±0.21	9.48	0	1.90±0.18	10.58	0	1.30±0.21	6.09	0
	Third day	1.30±0.15	8.51	0	1.80±0.20	9.00	0	1.60±0.16	9.79	0	1.80±0.29	6.19	0
	Fourth day	0.60±0.16	3.67	0	0.90±0.17	5.01	0	0.70±0.15	4.58	0.001	1.40±0.26	5.25	0
	Fifth day	0.50±0.17	3.00	0.01	0.50±0.16	3.00	0.01	0.30±0.15	1.96	0.08	1.00±0.26	3.87	0.003
Boll	First day	1.40±0.16	8.57	0	0.50±0.16	3.00	0.01	0.90±0.18	5.03	0	1.30±0.30	4.33	0.001
	Second day	1.10±0.17	6.12	0	0.30±0.15	1.96	0.08	0.50±0.16	3.00	0.01	1.50±0.22	6.07	0
	Third day	0.90±0.10	9.00	0	0.30±0.15	1.96	0.08	0.30±0.15	1.96	0.08	1.30±0.15	8.51	0
	Fourth day	0.40±0.16	2.44	0.03	0.10±0.10	1.00	0.34	0.40±0.16	2.44	0.03	0.90±0.18	5.01	0
	Fifth day	0.30±0.15	1.96	0.08	0	N.A.	N.S.	0.30±0.15	1.96	0.08	0.60±0.16	3.67	0.005
flower	First day	1.30±0.15	8.50	0	1.90±0.17	1.90	0	0.60±0.16	3.67	0.005	1.60±0.16	9.79	0.001
	Second day	1.00±0.15	6.70	0	1.70±0.15	1.70	0	0.40±0.16	2.44	0.03	1.80±0.2	9.00	0
	Third day	0.60±0.16	3.67	0	1.40±0.22	1.40	0	0.20±0.13	1.50	0.16	1.70±0.21	7.96	0
	Fourth day	0.30±0.15	1.96	0.08	0.90±0.23	0.90	0.003	0.20±0.13	1.50	0.16	1.50±0.16	9.00	0
	Fifth day	0.20±0.13	1.50	0.17	0.60±0.16	0.60	0.005	0.20±0.13	1.50	0.16	1.00±0.21	4.74	0.005

DAS - Days after sowing. DAR - Days after release

Table-3 Migration of late second instar larvae of *S. litura* in five per cent refugia field at 90 DAS.

Plant parts	DAR	Number of larvae retained on centre plant (non-Bt)						Number of larvae retained on adjacent plant (BG-II)					
		Bunny			Mallika			Bunny			Mallika		
		Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t	Mean	t-test	p>t
Leaf	First day	5.20±0.25	20.84	0	6.80±0.46	14.57	0	5.50±0.28	21.3	0	5.90±0.40	13.7	0
	Second day	3.10±0.18	17.26	0	6.30±0.30	21.00	0	3.80±0.20	19.00	0	5.10±0.23	21.85	0
	Third day	2.30±0.26	15.05	0	4.00±0.29	15.49	0	3.10±0.10	31.00	0	3.60±0.26	13.50	0
	Fourth day	1.80±0.13	13.50	0	2.50±0.30	8.13	0	2.90±0.28	10.47	0.009	3.10±0.27	11.19	0
	Fifth day	1.70±0.15	11.12	0	2.30±0.33	6.86	0	1.80±0.13	13.5	0.01	3.20±0.29	11.01	0
Square	First day	3.30±0.21	15.46	0	1.90±0.27	6.86	0	1.30±0.15	8.51	0	2.20±0.32	6.73	0
	Second day	1.40±0.16	8.57	0	1.80±0.13	13.5	0	1.30±0.15	8.51	0	1.60±0.22	7.23	0
	Third day	0.80±0.13	6.00	0	1.70±0.15	11.12	0	1.00±0.21	4.74	0	1.50±0.27	5.58	0
	Fourth day	0.60±0.16	3.67	0.005	1.10±0.28	3.97	0	0.70±0.21	3.27	0.009	1.30±0.26	4.99	0
	Fifth day	0.40±0.16	2.44	0.03	1.00±0.30	3.35	0	0.50±0.17	3.00	0.01	1.30±0.26	4.99	0
Boll	First day	0.80±0.13	6.00	0	1.10±0.34	3.16	0.01	1.30±0.15	8.51	0	0.80±0.20	4.00	0.003
	Second day	0.80±0.13	6.00	0	0.70±0.21	3.27	0.009	0.80±0.13	6.00	0	0.50±0.17	3.00	0.01
	Third day	0.40±0.16	2.44	0.03	0.30±0.15	1.96	0.08	0.60±0.16	3.67	0.005	0.40±0.16	2.44	0.03
	Fourth day	0.30±0.15	1.96	0.08	0.10±0.10	1.00	0	0.50±0.17	3.00	0.01	0.20±0.13	1.50	0.16
	Fifth day	0.30±0.15	1.96	0.08	0.10±0.10	1.00	0	0.30±0.15	1.96	0.08	0.30±0.15	1.96	0.08
Flower	First day	1.1±0.1	11.00	0	1.3±0.26	4.99	0	1.3±0.15	8.51	0	1.2±0.24	4.8	0
	Second day	0.8±0.13	6.00	0	1.3±0.15	8.51	0	1.3±0.15	8.51	0	1.2±0.24	4.8	0
	Third day	0.6±0.16	3.67	0.005	1±0.14	6.07	0	1±0.21	4.74	0.001	0.8±0.25	3.2	0.01
	Fourth day	0.4±0.16	2.44	0.03	0.9±0.18	5.01	0	0.7±0.21	3.28	0.009	1.0±0.26	3.8	0.003
	Fifth day	0.3±0.15	1.96	0.08	0.9±0.18	5.01	0	0.5±0.17	3.00	0.01	1.0±0.26	3.8	0.003

DAS - Days after sowing. DAR - Days after release.

Table-4 Migration of late second instar larvae of *S. litura* in BG-II field at 75 DAS

Plant parts	DAR	Number of larvae retained on centre plant (BG-II)			Number of larvae retained on adjacent plant (BG-II)		
		Mean	t-test	p>t	Mean	t-test	p>t
Leaf	First day	3.27±0.19	16.78	0	4.18±0.35	11.87	0
	Second day	2.18±0.22	9.63	0	3.45±0.24	13.96	0
	Third day	1.36±0.20	6.70	0	2.54±0.24	10.29	0
	Fourth day	0.45±0.16	2.88	0.02	2.18±0.22	9.63	0
	Fifth day	0.27±0.14	1.93	0.08	1.72±0.19	8.85	0
Square	First day	1.18±0.32	3.63	0.004	2.09±0.28	7.34	0
	Second day	1.18±0.18	6.50	0	1.72±0.14	12.26	0
	Third day	1.00±0.13	7.41	0	1.36±0.15	8.96	0
	Fourth day	0.54±0.16	3.46	0.006	1.09±0.16	6.70	0
	Fifth day	0.45±0.16	2.88	0.02	1±0.19	5.24	0.0004
Boll	First day	1.00±0.26	3.70	0.004	2.27±0.30	7.47	0
	Second day	0.72±0.19	3.73	0.003	1.36±0.27	4.89	0.001
	Third day	0.90±0.16	5.59	0	1.27±0.23	5.36	0.0003
	Fourth day	0.45±0.15	2.88	0.16	0.90±0.21	4.30	0.002
	Fifth day	0.27±0.14	1.93	0.08	0.63±0.20	3.13	0.01
Flower	First day	1.45±0.15	9.23	0	3.9±0.21	18.50	0
	Second day	1.18±0.18	6.50	0	3.09±0.16	19.00	0
	Third day	0.90±0.25	3.62	0.004	1.90±0.21	9.03	0
	Fourth day	0.81±0.18	4.50	0.001	1.45±0.16	9.23	0
	Fifth day	0.72±0.14	5.16	0	1.18±0.18	6.50	0

DAS - Days after sowing. DAR - Days after release.

larvae after leaving the terminals was not reported.

In a laboratory bioassay, [20] found that tobacco budworm larvae could avoid *B. thuringiensis* proteins. Terminal foliage expresses higher levels of Cry 1 Ac than other plant parts [3]. Levels of Cry 1 Ac expression in terminal foliage and fruiting forms on node nine of cotton plant averaged 68.1 and 26.5 µg/g dry weight, respectively [21]. In a similar study, Cry 1 Ac expression was higher in white flowers compared with squares and bolls [22]. Although protein expression was not measured in foliage, Cry 1 Ac expression was higher in bracts compared to flowers, squares, and bolls [22]. Variation in protein expression among different plant parts combined with bollworm detection and avoidance of the protein could result in bollworm populations becoming established on those structures with low protein expression. Small bollworm larvae remain near the terminals of non-Bt cotton plants feeding on small squares [23]. It is found that 78 to 100 per cent of damaged fruiting forms could be found in the top 0.6 m of plants at any given time [24]. As larvae develop, they typically move down the plants feeding on larger squares and bolls [25]. Data in the present study indicate that bollworm larvae disperse more rapidly on Bollgard cotton compared to non-Bt cotton. Larvae remained near the top of non-Bt cotton plants feeding on terminal foliage and small squares. In contrast, larvae were observed lower in the plant canopy on Bollgard cotton feeding on white flowers and bolls.

Conclusion

More number of larvae were observed on leaves of non Bt cotton. Hence, the movement of *S. litura* larvae was more in pure BG-II cotton field when compared with the mixed (BG-II and non Bt) cotton field.

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Conflict of Interest: None declared

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