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Biology of Cotton Leafhopper *Amrasca devastans* (Ishida) on Transgenic Cotton and desi Cotton Hybrid

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Abstract
Biology of *Amrasca devastans* (Ishida) on transgenic cotton hybrid, RCH 650 BGII and *desi* cotton hybrid, AAH 1 cotton was studied in the laboratory of Department of Entomology, CCSHAU, Hisar. The average total nymphal period on RCH 650 BGII (transgenic cotton) lasted for 6.50 ± 0.55 days whereas, it lasted for 7.67 ± 0.52 days on AAH -1 (*desi* cotton hybrid). The nymphal survival on RCH 650 BGII ranged from 40 to 70 per cent with an average of 56.67 ± 10.33 per cent while it ranged from 30 to 50 per cent with an average of 43.33 ± 8.16 on AAH -1. Nymphal growth index was 8.72 for RCH 650 BGII and 5.65 for AAH 1. Sex ratio of male: female was 1: 0.84 and 1: 0.82 on RCH 650 BGII and AAH 1, respectively. Maximum number of eggs per leaf i.e. 35.20 ± 3.91and 29.60 ± 3.78 were found on August 7, 2016 (32nd SMW) on RCH 650 BGII and AAH 1, respectively. The maximum average number of eggs in lateral veins were 16.9 ± 3.51 and 12.5 ± 2.46 on RCH 650 BGII and AAH 1, respectively. On August 22, 2016, in lateral veins of RCH 650 BGII had 13.6 ± 2.37 eggs and AAH 1 had 7.5 ± 2.12 eggs which were maximum among other veins. On September 6, 2016 (34th SMW), maximum average numbers of eggs in sub veins had 6.1 ± 1.79 and 3.7 ± 1.34 number of eggs in RCH 650 BGII and AAH 1, respectively.

Keywords: *Amrasca devastans*, biology, transgenic cotton hybrid, *desi* cotton hybrid

Introduction
Cotton (*Gossypium* spp.) is one of the most important commercial crops playing a key role in economic, political and social affairs of the world. It is commonly known as "white gold" and unanimously designated as "King of Fibres". It has a global significance and grown for its lint and seed. In India, cotton is cultivated in an area of 12.65 m ha with a production of 37.39 million bales (170 kg/bale) of seed cotton during 2015-16. In Haryana, total area under cotton was 6.39 lakh ha and production was 22.00 lakh bales of 170 kg with productivity 665 kg per hectare [1]. Potential of leafhopper to cause damage depends on its oviposition preference and subsequent population on different cotton cultivars. In India and abroad, hundreds of genotypes of cotton have been screened for leafhopper to find out damage and population dynamics under similar environmental conditions [2].

More than 90% of the cotton cultivated in the country comprises transgenic or *Bt* cotton (Bollgard II) that expresses Cry1Ac and Cry2Ab toxins of *Bacillus thuringiensis* (*Bt*). Prior to the introduction of *Bt* cotton, whiteflies and bollworms were the major insect pests. After the introduction of *Bt* cotton, bollworm infestation became negligible, until 2010, during which pink bollworm developed resistance to the Cry1Ac toxin, but jassids, thrips, and whiteflies, however, continued to cause damage [3]. Among the sucking pests, the leafhopper (*Amrasca devastans* Ishida) became a major concern for the stakeholders. In addition to cotton, it is also found to cause serious damage to okra, cacao, potato, pepper etc. The nymphs and adults of *A. devastans* suck cell sap and cause 'hopperburn' symptoms. About 25% losses in yield are reported [4]. Before planning or executing any management tactic for any pest, the life cycle parameters of the pest must be well understood. To successfully manage any pest it is mandatory to detect or predict the fluctuations in the pest population and this in turn is extremely helpful in identifying the weak link in the life history of the pest.

More trichome density and length on mid rib adversely affect the oviposition. Female of cotton leafhopper lays eggs in the mid rib, lateral veins and sub veins of cotton leaves. To study the biology of the pest, it is essential to know the feeding habit, behaviour and duration of different developmental stages which in turn help in its management.

In the field condition there is a specific time period at which leafhopper population reach at its peak, so, to record and predict peak period of leafhopper incidence, biology of cotton leafhopper must be studied. Keeping the above facts in view, the present study was undertaken to study the comparative biology of cotton leafhopper on both the *Bt* and non-*Bt desi* cotton hybrids.

Material and Methods

The biology of *A. devastans* on transgenic cotton hybrid (Cv. RCH 650 BGII) and *desi* cotton hybrid (Cv. AAH 1) was studied during July to September, 2016 in the laboratory of Dept. of Entomology, CCSHAU, Hisar. Crop was sown at Experimental Area, Dept. of Entomology on 17th May, 2016.

Development and survival of cotton leafhopper under field conditions

The development and survivorship of nymphs were studied on transgenic cotton hybrid (RCH 650 BGII) and *desi* cotton hybrid (AAH 1) under field conditions according to the methodology [5]. For these studies, muslin cloth cages (20 cm × 10 cm) were fixed on each variety to enclose one expanding crown leaf per cage. A total of six crown leaves were caged on each variety on different plants (85 days old) selected at random. After three days the leaves were checked to remove the nymphs or any other stage of the insect. Then 10 first instar nymphs collected from field with camel hair brush from cotton leaves, were released on ventral surface of each caged leaf. Nymphs were examined each day to record number surviving and time taken to turn into adults.

$$\text{Nymphal growth Index} = \frac{\text{Nymphal survival (\%)}}{\text{Mean nymphal duration (days)}}$$

Sex ratio of cotton leafhopper, *A. devastans*

The cotton leafhopper adults were captured with the help of an aspirator separately from cotton fields. A total of 300 leafhopper adults were collected from each variety. Sex identification was done under compound microscope after killing them with ethyl acetate fumes in the laboratory. The males and females were identified based on abdominal appendages. Sex ratio was recorded as per methodology earlier derived [5].

Oviposition behaviour and peak period of cotton leafhopper, *A. devastans*

The ovipositional behaviour and peak period were analysed following the procedure [6]. The oviposition behaviour of cotton leafhopper was studied by recording the number of eggs laid, distribution of eggs and pattern of egg laying on the cotton leaves. For this, ten leaves were caged per variety on 85 days old plants for the oviposition studies. Before confining the cotton leafhopper on the cotton leaves, they were covered with muslin cloth cages for 72 hours, to allow the eggs to hatch out, if already laid on the test leaves. These newly emerged nymphs were removed from the leaves by a camel hair brush and the leaves were enclosed again with muslin cloth cage.

Thirty adult females were captured from the field with the help of an aspirator and released on third to fourth leaf from top enclosed in muslin cloth cage (20 cm × 10 cm) and confined for 72 hours in the field, to study the oviposition behaviour. The cages on the leaves were fixed with the help of wooden sticks in both hybrids (RCH 650 BG II and AAH 1). Ten leaves were caged for each variety. After the stipulated period, the leaves exposed to female leafhopper were cut by a pair of scissors and brought to laboratory. Then these leaves were processed in lactophenol solution for 5 to 10 minutes by standardized method [7]. After processing, the eggs laid by females inside the veins become clear and visible. Eggs laid in mid rib, lateral vein and sub veins were examined and counted under microscope and as such the oviposition behaviour, distribution and pattern of eggs were studied. A total of three observations on August 7 and 22 and September 6, 2016 were recorded for peak period of leafhopper activity based on earlier findings [7].

Statistical Analysis

The data recorded during the experiments was subjected to statistical analysis by proper methods using online statistical package OPSTAT [8].

Results and Discussion

Nymphal duration

Data presented in Table 1 revealed that the average nymphal period, under field conditions varied significantly on both hybrids of cotton, being significantly shorter on RCH 650 BGII than on AAH 1. It ranged from 6 to 7 days on RCH 650 BGII and 7 to 8 days on AAH 1. The average nymphal period on RCH 650 BGII was 6.50 ± 0.55 days whereas, the average nymphal duration on AAH 1 was 7.67 ± 0.52 days. The nymphal period indicated that the development of the leafhopper was faster on RCH 650 BGII than on AAH 1.

Earlier workers reported the nymphal period of leafhopper on cotton ranging from 7 to 8.15 days. Time period of 7-8 days was reported as nymphal duration of leafhopper on non-*Bt* cotton [5]. The nymphal duration of leafhopper (7.56 days) was recorded on HS-6 (non-*Bt* cultivar) [6]. The nymphal period of leafhopper was found to be 7.41 days [10]. The nymphal period of 8.15 days was also reported on cultivar NCS-855 (BGII) [11]. These findings fall in the line with the present findings.

Nymphal survival

Observations made on the survival of nymphs of leafhopper reared on different cotton hybrid indicated that significant higher number of nymphs survived on RCH 650 BGII than on AAH 1 (Table 1). The nymphal survival on RCH 650 BGII ranged from 40 to 70 per cent with an average of 56.67 ± 10.33 while ranged from 30 to 50 per cent with an average of 43.33 ± 8.16 on AAH 1.

The nymphal survival of 30 to 60 per cent was reported on non-*Bt* cotton [5]. Nymphal survival was found to be 83.88 and 55.00 per cent on non-*Bt*[4] and *Bt* cultivar [11] (NCS-855 BGII) of cotton, respectively. The results of the present study are more or less in accordance with the result of these earlier workers. The variations between the present and earlier findings may be due to the cultivars used, handling and the climatic conditions.

Nymphal growth index

Data on nymphal growth index of leafhopper was higher (8.72) on RCH 650 BGII than AAH 1 (5.65) presented in the Table 1. Sharma and Sharma (1996)[5] observed nymphal growth index on non-*Bt* cotton which is not conformity with present study.

Table 1: Nymphal duration, survival and nymphal growth index of *A. devastans* on cotton hybrids

Hybrids	Nymphal period		Nymphal survival		Nymphal growth index
	Average (days)	Range (days)	Average (%)	Range (%)	
RCH 650 BGII	6.50 ± 0.55	6-7	56.67 ± 10.33	40-70	8.72
AAH 1	7.67 ± 0.52	7-8	43.33 ± 8.16	30-50	5.65
t value	2.48*		3.80*		

* Indicate significant at p = 0.05

Sex ratio

Data presented in Table 2 indicated that out of 300 adults collected from each hybrid 173 were males and 127 were females in RCH 650 BGII while 169 male and 131 females were found on AAH 1. Sex ratio of male: female was 1: 0.84 and 1: 0.82 on RCH 650 BGII and AAH 1, respectively. These results indicated that higher number of males were present in the field population.

Similar sex ratio (male: female) was found 1:0.73, 1:0.72 and 1:0.88 on non-*Bt* hybrids HHH 81, H 777 and H 974, respectively [5]. The sex ratio of 1:1.08 (male: female) was reported on *Bt*-cotton [12]. Similar sex ratio of 1:1.37 (male: female) under field conditions on *Bt* cultivar (NCS-855 BGII) was reported [11], which was not in the conformity with present study.

Table 2: Sex ratio of *A. devastans* on cotton hybrids

Hybrids	Male	Female	Sex ratio Male: female
RCH 650 BGII	5.77 ± 1.36	4.23 ± 1.36	1: 0.84
AAH 1	5.63 ± 1.19	4.37 ± 1.19	1: 0.82

Ovipositional behaviour of cotton leafhopper

The ovipositional behaviour of the pest was studied on two hybrids of cotton *viz*., RCH 650 BGII and AAH 1 in the field conditions. The freshly laid eggs were elongate, translucent having a yellowish tinge, slightly hooked towards the anterior end and the other end being broadly pointed. The curved, greenish-yellow eggs were embedded in the leaf veins singly. The ovipositional behaviour of cotton leafhopper was studied in terms of number of eggs laid/vein category and the pattern of egg laying on cotton hybrids.

The results are presented in Table 3. revealed that maximum number of eggs per leaf i.e. 35.20 ± 3.91and 29.60 ± 3.78 were found on August 7, 2016 on RCH 650 BGII and AAH 1, respectively. Minimum numbers of eggs were found on September 6, 2016 on both the hybrids i.e. 14.00 ± 4.08 eggs/leaf on RCH 650 BGII and 8.30 ± 1.34 eggs/leaf on AAH 1. Among all observations average number of eggs was significantly different on both hybrids *viz*., Bt and non-*Bt* cotton. The peak period of egg laying was found on August 7, 22 and September 6, 2016 in present study whereas Sharma and Sharma (1996) [5] found peak period on August 23.

Table 3: Oviposition of *A. devastans* on different cotton hybrids

Hybrids	Average number of eggs per leaf in different Standard Meteorological Weeks		
	32	34	36
RCH 650 BGII	35.20 ± 3.91	28.60 ± 3.95	14.00 ± 4.08
AAH 1	29.60 ± 3.78	18.20 ± 3.05	8.30 ± 1.34
t value	3.26*	6.59*	4.20*

* Indicate significant at p=0.05

Oviposition of leafhopper in relation to leaf veins

Total numbers of eggs laid by individual female on two cotton hybrids were further distributed according to the locations of eggs in different category of veins *viz.* main vein (mid rib), lateral veins and sub veins. It is evident from Table 4 that lateral veins of RCH 650 BGII and AAH 1 leaves received more number of leafhopper eggs on August 7, 2016 and August 22, 2016 while on September 6, 2016 more numbers of eggs were found in sub veins of both hybrids.

On August 7, 2016, 7.0 ± 1.49 eggs were found on main vein of RCH 650 BGII while 6.6 ± 1.17 eggs on main vein of AAH 1. In lateral veins, average numbers of eggs were 16.9 ± 3.51 and 12.5 ± 2.46 on RCH 650 BGII and AAH 1, respectively and these were significantly different. Sub veins of RCH 650 BGII and AAH 1 received 11.3 ± 1.16 and 10.5 ± 1.58 number of eggs per leaf, respectively. On RCH 650 BGII and AAH 1, average numbers of eggs on main veins were 7.2 ± 2.39 and 3.6 ± 1.35, respectively, and found to be significantly different on August 22, 2016. In lateral veins of RCH 650 BGII had 13.6 ± 2.37 eggs and AAH 1 had 7.5 ± 2.12 eggs which were also significantly different while in sub veins, RCH 650 BGII had 7.8 ± 1.48 and AAH 1 had 7.1 ± 1.19 number of eggs.

On September 6, 2016, main vein of RCH 650 BGII and AAH 1 received 3.5 ± 1.43 and 2.1 ± 0.88 eggs, respectively. Numbers of eggs in lateral veins were 4.4 ± 1.9 on RCH 650 BGII and 2.5 ± 0.97 on AAH 1. Sub veins had 6.1 ± 1.79 and 3.7 ± 1.34 number of eggs in RCH 650 BGII and AAH 1, respectively. Numbers of eggs in all vein categories were significantly different on both the hybrids.

Present data showed that the maximum average number of eggs were found in lateral veins while minimum were found in main veins, in both the cultivars observed. These findings are in conformity with the earlier findings [6] where more eggs were reported in the lateral veins of cotton leaves of non-*Bt* cotton as compared to the main vein. In contrary to these findings, maximum number of eggs in midrib followed by lateral veins were reported in *Bt* cotton [12]. Also, more number of eggs in midrib than lateral veins were found on non-*Bt* cotton [5]. Therefore, it is concluded that the pattern of eggs laid by leafhopper in different leaf veins differ significantly between the *Bt* and non-*Bt* hybrids.

Table 4: Ovipositional preference of *A. devastans* in different veins of cotton leaf

Standard Meteorological Week	Hybrids	Average number of eggs/leaf vein category		
		Main vein	Lateral veins	Sub veins
32	RCH 650 BGII	7.0 ± 1.49	16.9 ± 3.51	11.3 ± 1.16
	AAH 1	6.6 ± 1.17	12.5 ± 2.46	10.5 ± 1.58
	t value	0.67	3.25*	1.29
34	RCH 650 BGII	7.2 ± 2.39	13.6 ± 2.37	7.8 ± 1.48
	AAH 1	3.6 ± 1.35	7.5 ± 2.12	7.1 ± 1.19
	t value	4.14*	6.07*	1.16
36	RCH 650 BGII	3.5 ± 1.43	4.4 ± 1.9	6.1 ± 1.79
	AAH 1	2.1 ± 0.88	2.5 ± 0.97	3.7 ± 1.34
	t value	2.64*	2.75*	3.39*

* Indicate significant at p=0.05

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