International Journal of Entomology Research
www.entomologyjournals.com
E-ISSN: 2455-4758
Received: 04-10-2026, Accepted: 03-11-2026, Published: 18-11-2026
Volume 11, Issue 4, 2026, Page No. 262-268

Evaluation of *BT* cotton hybrids for ovipositional response of Amrasca Biguttula Biguttula (Ishida)

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DOI: https://doi.org/10.66856/ijer.2026.11.4.11406

Abstract
The ovipositional response of the cotton leafhopper, *Amrasca biguttula biguttula* (Ishida), was evaluated on six *Bt* and non-*Bt* cotton hybrids to determine whether the incorporation of *Bt* genes influences egg-laying preference and distribution within different leaf-vein categories. The experiment was conducted on BIO 6488 and RCH 134 hybrids, comprising non-*Bt*, single-gene *Bt* (Cry1Ac) and double-gene *Bt* (Bollgard II; Cry1Ac + Cry2Ab) combinations, at the Research Farm, CCS Haryana Agricultural University, Hisar. Oviposition was studied on the main, lateral and sub-veins of fully expanded cotton leaves using a leaf-vein nomenclature system. Eggs deposited within the veins were made visible by processing the leaves in lactophenol solution and subsequently counted. The results revealed no significant differences in the total number of eggs laid per leaf among the six cotton hybrids, with the mean number ranging from 19.83 to 21.17 eggs per leaf. Similarly, the distribution of eggs among the main, lateral and sub-veins did not differ significantly among *Bt*, *BGII* and non-*Bt* hybrids. Across the hybrids, the respective mean numbers of eggs deposited in the main, lateral and sub-veins ranged from 2.17 to 3.00, 6.83 to 8.50 and 9.17 to 11.00. The findings indicate that the incorporation of Cry1Ac and Cry2Ab genes in cotton hybrids did not significantly influence the ovipositional behaviour or egg-laying preference of *A. biguttula biguttula*. The results further suggest that the leafhopper's selection of oviposition sites within leaves is largely independent of the *Bt* trait and may be governed by inherent leaf morphological characteristics.

Keywords: *Amrasca biguttula biguttula*, *Bt* cotton, Bollgard II, Cry1Ac, Cry2Ab, oviposition, leaf-veins, cotton leafhopper

Introduction

Bt cotton has been under commercial cultivation in India since 2002, primarily for the management of the American bollworm, Helicoverpa armigera (Hübner) (Arms *et al*., 1992; Likhitha *et al*., 2018) [3, 6]. Subsequently, certain other lepidopteran pests, particularly the tobacco caterpillar, Spodoptera litura (Fabricius), were also reported to cause considerable damage to transgenic cotton hybrids expressing the Cry1Ac protein. To improve the efficacy of *Bt* cotton against a broader range of lepidopteran pests, another insecticidal gene, Cry2Ab, was incorporated alongside Cry1Ac, resulting in the development of Bollgard II (BGII) technology (Stewart *et al*., 2001) [18]. Although *Bt* technology was primarily developed for managing lepidopteran pests, its influence on non-target sucking insect pests, particularly the cotton leafhopper, *Amrasca biguttula biguttula* (Ishida), has received considerable attention. However, reports concerning the relative abundance and performance of leafhoppers on *Bt* and non-*Bt* cotton hybrids have been inconsistent. Some studies recorded higher leafhopper populations on *Bt* cotton hybrids than on their non-*Bt* counterparts (Rao *et al*., 2002; Abro *et al*., 2003; Aggarwal *et al*., 2007) [10, 1, 2]. In contrast, Whitehouse *et al*. (2005) [19] reported comparatively lower leafhopper populations on *Bt* cotton. Other investigations found no significant differences in the abundance of sucking pests between *Bt* and non-*Bt* cotton hybrids (Kengegowda *et al*., 2003; Rajanikantha *et al*., 2004) [4, 9]. More recently, Kumar and Singh (2026) [5] reported an insignificant influence of transgenic *Bt* cotton hybrids on the survival, development and oviposition of the cotton leafhopper.

Oviposition is an important component of insect-host plant interactions and may be influenced by several morphological and anatomical characteristics of the host plant. Female *A. biguttula biguttula* insert their elongated, yellowish eggs within the leaf-veins, predominantly on the abaxial surface. The dimensions and structural characteristics of leaf-veins, together with leaf pubescence, have been reported to influence oviposition-site selection (Sharma and Singh, 2002; Lokesh and Singh, 2005) [12,7]. The distribution of eggs among different categories of leaf-veins provides an opportunity to understand the ovipositional preference of the leafhopper in relation to host plant characteristics. Earlier studies have described the pattern of egg deposition in the main, lateral and subsidiary veins of cotton leaves (Singh and Agarwal, 1988A; Singh and Agarwal, 1988B; Singh and Taneja, 1989) [14,15,16].

Considering the conflicting reports on the interaction between *Bt* cotton and sucking insect pests, the present investigation was undertaken to evaluate the ovipositional response of *A. biguttula biguttula* on *Bt*, BGII and non-*Bt* cotton hybrids. Particular emphasis was placed on determining the total number of eggs deposited per leaf and their distribution among the main, lateral and sub-veins, thereby assessing whether the incorporation of *Bt* genes influences the ovipositional behaviour of the cotton leafhopper.

Materials and Methods

Experimental material and field conditions: The experiment was conducted at the Research Farm of CCS Haryana Agricultural University, Hisar, using six cotton hybrids representing *Bt* and non-*Bt* combinations of two genetic backgrounds, namely BIO 6488 and RCH 134. The hybrids included were (i) BIO 6488 non-*Bt* (ii) BIO 6488 *Bt* (iii) BIO 6488 BGII (iv) RCH 134 non-*Bt* (v) RCH 134 *Bt* and (vi) RCH 134 BGII. The observations on leafhopper oviposition were recorded approximately 80 days after sowing. The experiment was conducted in three replications.

Classification and nomenclature of leaf-veins: The ovipositional behaviour of *A. biguttula biguttula* was studied by examining the distribution of eggs in different categories of leaf-veins, following the nomenclature proposed by Singh and Agarwal (1988A) [14]. The central midrib was designated as the main vein (M). The prominent veins originating from the base of the petiole on either side of the main vein were classified as lateral veins. Veins on the right-hand side were designated as R1, R2 and R3, beginning with the vein nearest the base, while those on the left-hand side were similarly designated as L1, L2 and L3. The smaller veins originating from the lateral veins were classified as sub-veins.This classification facilitated systematic examination and recording of egg deposition in the main, lateral and sub-veins of cotton leaves.

Assessment of oviposition and egg distribution: The oviposition experiment was conducted on the topmost third fully expanded leaves of selected cotton plants. Initially, all existing nymphs and adults of the leafhopper were removed from the selected leaves on two plants in each plot. The leaves were subsequently enclosed in nylon cages measuring 20 × 10 cm, following the method described by Sharma and Singh (2002)[12]. After 72 hours, any nymphs emerging within the cages were removed. Subsequently, 30 adult leafhoppers captured using an aspirator were released into each cage to facilitate egg laying. Following another 72-hour period, the leaves were detached and brought to the laboratory for examination.

Processing and examination of eggs: The collected leaves were processed in lactophenol solution for 10 minutes, following the procedure described by Moffit and Reynolds (1972) [8]. The solution was prepared by mixing one-part lactic acid, one part phenol, two parts glycerine and one-part distilled water. Treatment with lactophenol rendered the eggs inserted within the leaf-veins transparent and clearly visible, facilitating their identification and enumeration. The number of eggs deposited in the main vein, lateral veins and sub-veins was recorded separately for each leaf. The observations were subsequently compared among the six cotton hybrids to determine differences in total egg deposition and the distribution of eggs within different leaf-vein categories.

Data were subjected to analysis of variance (ANOVA) using a completely randomized design. Significant differences among treatments were determined at the 5% probability level (Snedecor and Snedecor 1996) [11].

Results and Discussion

Ovipositional response of *Amrasca biguttula biguttula* on different cotton hybrids: The ovipositional response of A. biguttula biguttula on the six *Bt*, BGII and non-*Bt* cotton hybrids revealed no significant differences in the total number of eggs deposited per leaf (Table 1). The mean number of eggs ranged from 19.83 eggs per leaf on BIO 6488 *Bt* to 21.17 eggs per leaf on RCH 134 non-*Bt*. The corresponding values for RCH 134 BGII, RCH 134 *Bt*, BIO 6488 BGII and BIO 6488 non-*Bt* were 20.17, 20.83, 20.50 and 20.33 eggs per leaf, respectively.

Table 1: Ovipositonal preference of Amrasca *biguttula biguttula* for different leaf veins of *Bt* cotton hybrids

Hybrid	Average number of eggs laid/vein			Total number of eggs laid/ leaf
	Main vein	Lateral vein	Sub vein	
RCH 134 BGII	2.67	6.83	10.67	20.17
RCH 134 *Bt*	3.00	7.67	10.17	20.83
RCH 134 non *Bt*	2.83	7.67	10.67	21.17
BIO 6488 BGII	2.33	7.17	11.00	20.50
BIO 6488 *Bt*	2.17	8.50	9.17	19.83
BIO 6488 non *Bt*	2.67	7.00	10.67	20.33
SEm(±)	0.27	0.69	0.98	1.11
CD(p=0.05)	N S*	N S	N S	N S

*N S – Non significant

The relatively narrow range in egg deposition indicates that the presence of Cry1Ac alone or in combination with Cry2Ab did not significantly alter the overall ovipositional response of female leafhoppers. Furthermore, the absence of significant differences between the *Bt* and their corresponding non-*Bt* counterparts suggests that the genetic background and *Bt* status of the hybrids had no detectable influence on the number of eggs deposited under the experimental conditions.

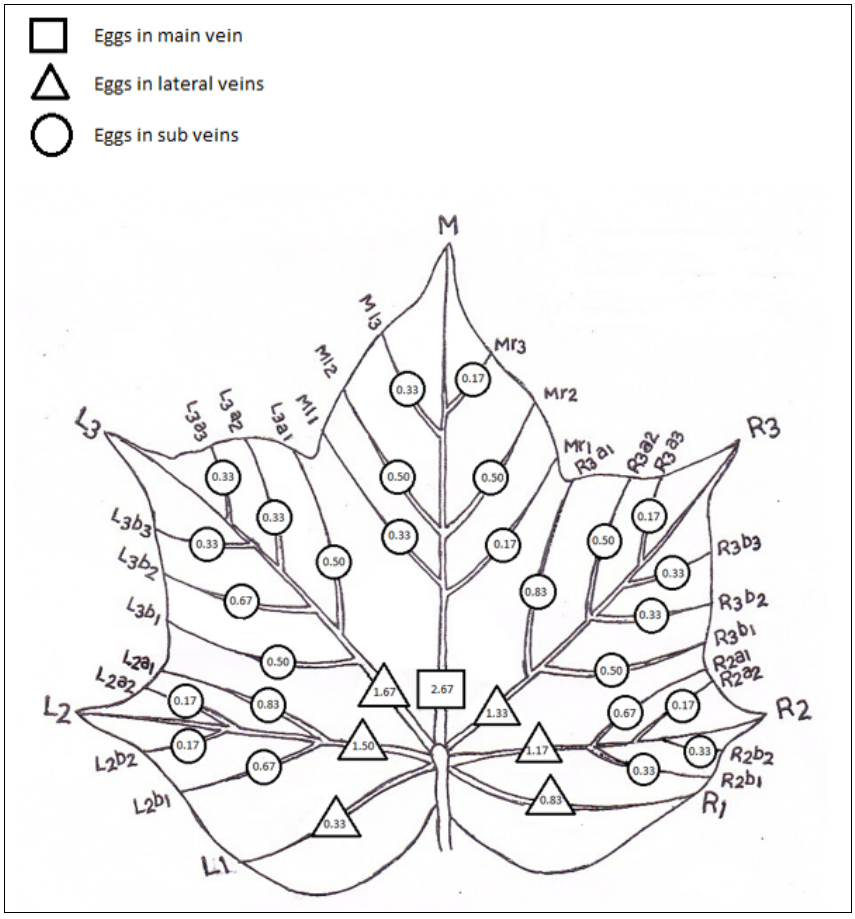


Fig 1: Distribution of *Amrasca biguttula biguttula* eggs in RCH 134 BG II leaf-veins

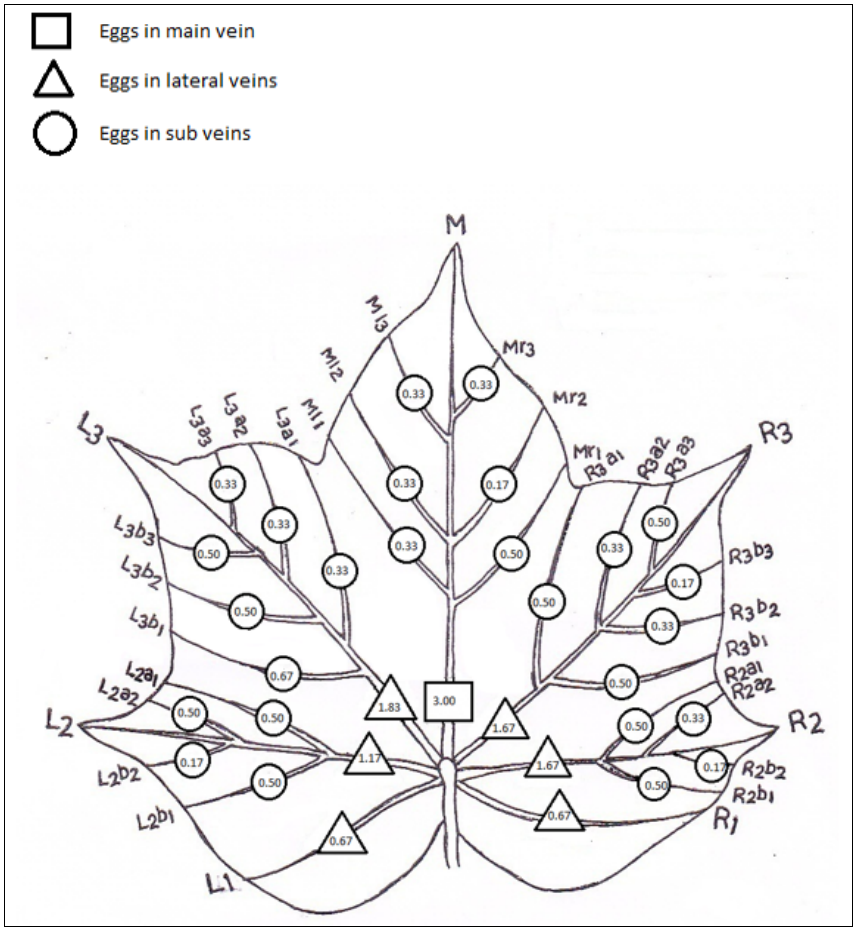


Fig 2: Distribution of *Amrasca biguttula biguttula* eggs in RCH 134 *Bt* leaf-veins

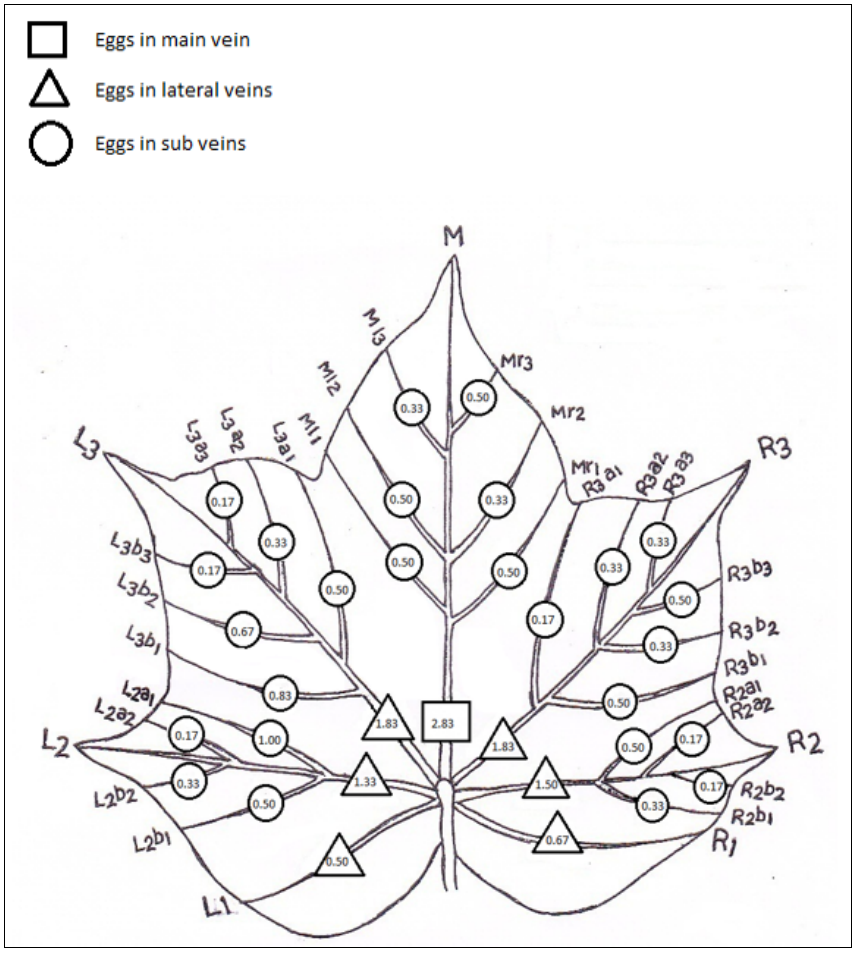


Fig 3: Distribution of *Amrasca biguttula biguttula* eggs in RCH 134 non *Bt* leaf-veins

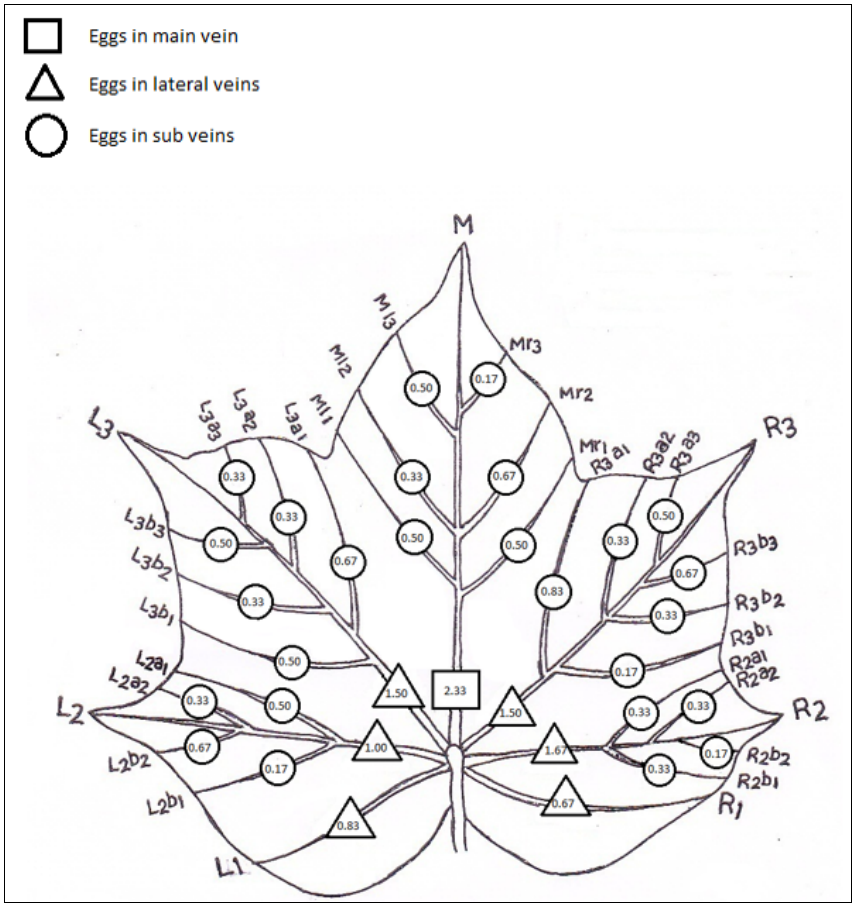


Fig 4: Distribution of *Amrasca biguttula biguttula* eggs in BIO 6488 BG II leaf-veins

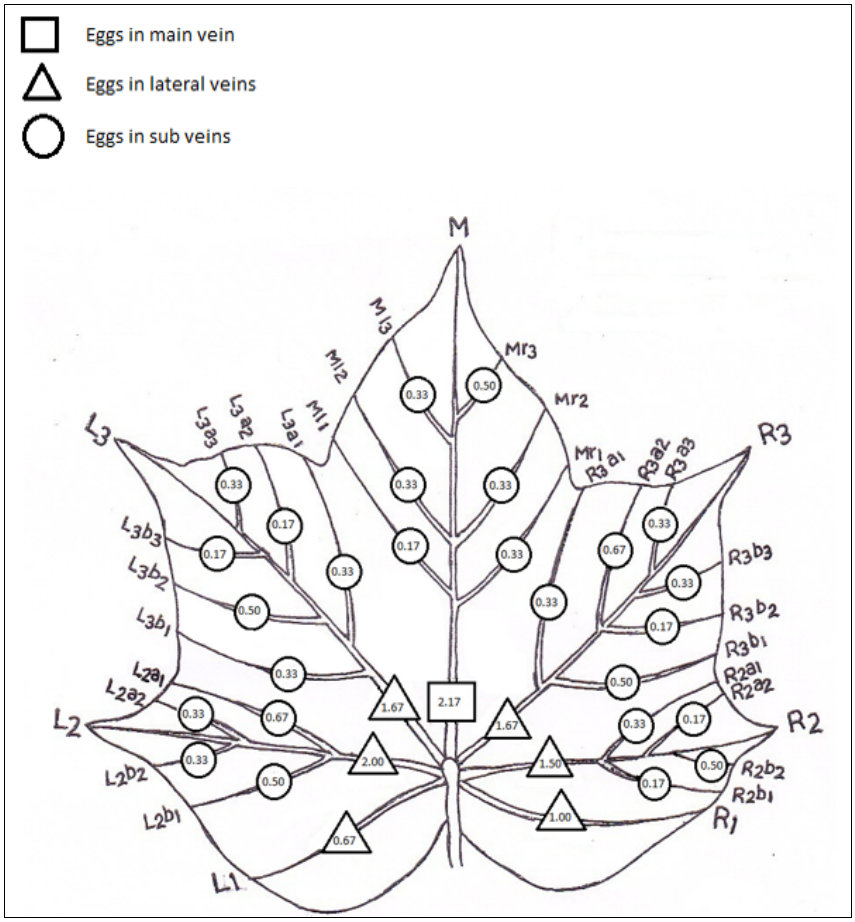


Fig 5: Distribution of *Amrasca biguttula biguttula* eggs in BIO 6488 *Bt* leaf-veins

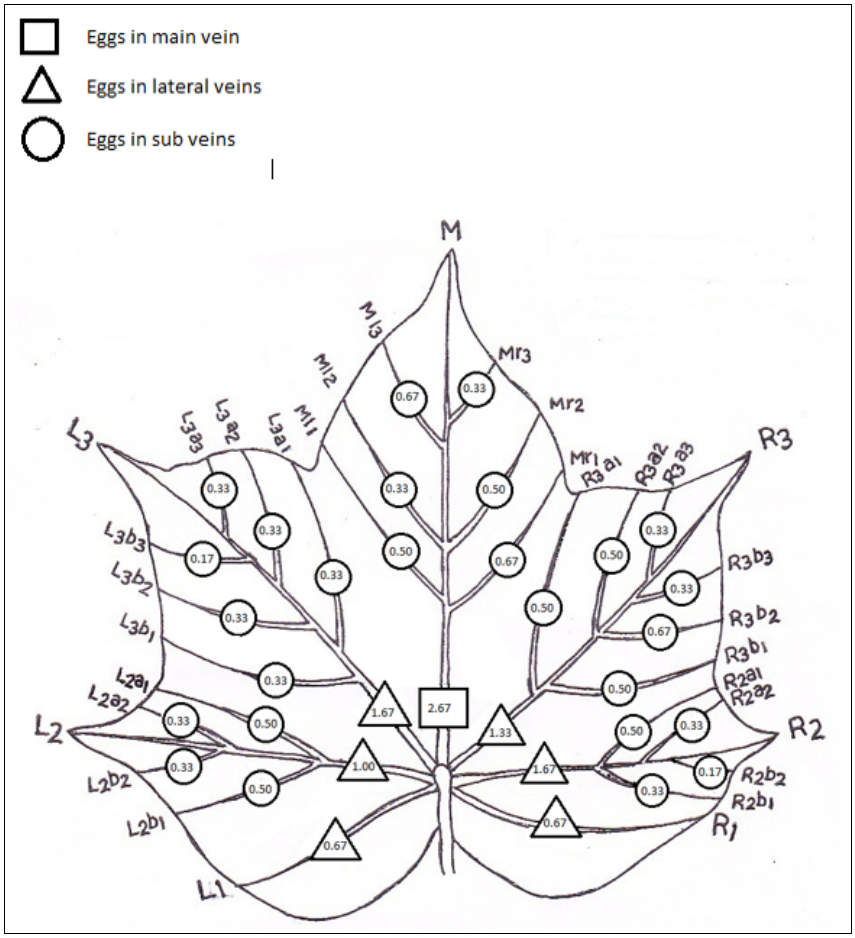


Fig 6: Distribution of *Amrasca biguttula biguttula* eggs in BIO 6488 non *Bt* leaf-veins

Distribution of eggs in different leaf-veins: The distribution of eggs among the main, lateral and sub-veins was also statistically comparable across all six cotton hybrids (Table 1; Figs. 1–6). The mean number of eggs deposited in the main vein ranged from 2.17 to 3.00 per leaf, whereas the corresponding numbers in the lateral and sub-veins ranged from 6.83 to 8.50 and 9.17 to 11.00, respectively. Although numerical variations were evident among the hybrids, none of these differences was statistically significant.

The main vein received a comparatively smaller proportion of eggs than the lateral and sub-veins when their respective aggregate numbers were considered. Among the individual vein categories, the sub-veins generally received the highest number of eggs, followed by the lateral veins and the main vein. The maximum number of eggs in the main vein (3.00) was recorded on RCH 134 *Bt*, while the maximum number in the lateral veins (8.50) occurred on BIO 6488 *Bt*. The highest number of eggs in the sub-veins (11.00) was observed on BIO 6488 BGII. Nevertheless, these numerical differences were not statistically significant, indicating that the distribution of eggs within different leaf-vein categories remained broadly similar irrespective of the *Bt* status of the hybrids.

The graphical presentation of egg distribution in individual hybrids (Figs. 1–6) further illustrates the similarity in ovipositional patterns across the three categories of cotton hybrids. The observations indicate that the incorporation of Cry1Ac and Cry2Ab genes did not bring about any detectable change in the preference of female leafhoppers for particular leaf-vein categories.

The present findings indicate that *Bt* cotton technology, although developed primarily for the management of lepidopteran pests, did not significantly influence the ovipositional response of *A. biguttula biguttula*. Both the total number of eggs deposited and their distribution among different leaf-vein categories remained statistically comparable in *Bt*, BGII and non-*Bt* hybrids. The results are consistent with the observations of Singh (1988) [17], Shivanna *et al*. (2009) [13], Sharma and Singh (2002) [12], Singh and Agarwal (1988A) [14] and Lokesh and Singh (2005) [7], who documented the ovipositional preferences of cotton leafhoppers in relation to different leaf-vein characteristics. The preferential utilization of particular leaf-vein categories for egg deposition may be associated with their structural and anatomical suitability for egg insertion. Since leafhoppers insert their eggs within the leaf tissues, vein dimensions and associated morphological features may play a more important role in determining oviposition sites than the presence or absence of *Bt* genes. The lack of significant differences between the corresponding *Bt* and non-*Bt* hybrids also suggests that the expression of Cry1Ac and Cry2Ab proteins did not produce a detectable effect on the egg-laying behaviour of the leafhopper. However, the present investigation was restricted to ovipositional response and does not establish the absence of possible *Bt*-related effects on other biological parameters or field population dynamics (Kumar and Singh, 2026) [5].

Conclusion

The present investigation demonstrated that the incorporation of *Bt* genes (Cry1Ac) and their combination with Cry2Ab in Bollgard II cotton hybrids did not significantly influence the ovipositional response of *Amrasca biguttula biguttula*. Neither the total number of eggs deposited per leaf nor their distribution among the main, lateral and sub-veins differed significantly between *Bt*, BGII and non-*Bt* cotton hybrids. The average egg deposition remained within a narrow range of 19.83–21.17 eggs per leaf across all six hybrids. Similarly, the distribution of eggs among the three leaf-vein categories exhibited no significant variation. These findings suggest that *Bt* technology, designed primarily for managing lepidopteran pests, has no detectable influence on the ovipositional preference of the cotton leafhopper under the experimental conditions. The selection of egg-laying sites appears to be governed more by the inherent morphological and anatomical characteristics of cotton leaf-veins than by the presence of *Bt* genes. Thus, the deployment of *Bt* and BGII cotton hybrids does not appear to alter the ovipositional behaviour of *A. biguttula biguttula*.

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