

# Influence of seed treatment and abiotic factors on damage to *Bt* and non-*Bt* cotton genotypes by the serpentine leaf miner *Liriomyza trifolii* (Diptera: Agromyzidae)

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**Abstract.** In recent years, the serpentine leaf miner *Liriomyza trifolii* (Burgess) has become a major pest of cotton (*Gossypium* spp.) in India, with high levels of incidence observed at the seedling stage, particularly in transgenic *Bt* cotton. We monitored the severity of infestation and damage by *L. trifolii* to transgenic and non-transgenic genotypes of *Gossypium arboreum* L. and *G. hirsutum* L. between 2002 and 2007. Overall, significantly lower *L. trifolii* damage occurred in *G. arboreum* varieties than in *G. hirsutum* varieties and hybrids. Among the *G. hirsutum* genotypes, damage was significantly lower in varieties than in hybrids. However, no significant differences in damage were observed between *Bt* and non-*Bt* cotton hybrids. Abiotic factors had a major influence on the severity of damage; there was a significant and negative association of *L. trifolii* damage with open pan evaporation (E), maximum temperature, solar radiation and sunshine, and a significant and positive association with relative humidity and rainfall (RF). Multiple stepwise regression analysis indicated that 99% of the variation in *L. trifolii* damage was accounted for by RF, open pan E and relative humidity.

**Key words:** serpentine leaf miner, *Liriomyza trifolii*, transgenic *Bt* cotton, *Gossypium hirsutum*, *Gossypium arboreum*, abiotic factors, India

## Introduction

The serpentine leaf miner *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) is a polyphagous pest that feeds on over 400 plant species, including cotton, cucurbits, solanaceous vegetables and brassicas (Parrella, 1987; Srinivasan *et al.*, 1995; Singh *et al.*, 1996; Murphy and La Salle, 1999; Lei *et al.*, 2007). Feeding and oviposition punctures made by females result in stippling of foliage, especially at the leaf tip and along the leaf margins. Larvae feed on the leaf mesophyll along the leaf veins, forming zigzag mines that become

noticeable a few days after oviposition and gradually widen and lengthen as the maggot matures. Both stippling and mining hamper photosynthesis, and severe infestation results in premature defoliation or complete desiccation of leaves (Parrella *et al.*, 1985). Wounding of the foliage also allows the entry of pathogenic bacteria and fungi into the plant (Broadbent and Matteoni, 1990).

*Liriomyza trifolii* is believed to be of Neotropical origin; until the 1970s, its damage occurred only in the Caribbean and in southern and eastern USA (Parrella, 1987). In India, *L. trifolii* damage was first reported from castor (*Ricinus communis* L.) in 1992 (Laxminarayana *et al.*, 1992) and was subsequently observed on cotton, cowpea, sunflower, cucumber,

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celery, chrysanthemum, chilli and rapeseed-mustard in 1993 (Singh *et al.*, 1996). Recently, *L. trifolii* has become a major pest of cotton in many countries, including India (Srinivasan *et al.*, 1995; Jeyakumar and Uthamasamy, 1996), Israel (Yathom, 1989), USA (Arizona; Palumbo, 1992) and Turkey (Gencsoylu, 2003). The pest causes severe damage during the seedling stage of the crop. The economic threshold of *L. trifolii* in beans (Cikman and Comlekcioglu, 2006) has been reported, but there is no information on economic thresholds for the pest on cotton. Such information is essential for timely and targeted pest management action.

Transgenic *Bt* cotton hybrids have been deregulated and adopted on a large scale by farmers in India (Mohan and Manjunath, 2002; James, 2008). Seed treatment with imidacloprid is a common practice for the management of sucking pests during the vegetative stage (up to 45 days after seedling emergence (DAE)). At the initiation of fruiting bodies, economically important insect pests such as whiteflies, leafhoppers, aphids and bollworms are managed predominantly with insecticides. Since the introduction of transgenic *Bt* cotton hybrids in the country, the serpentine leaf miner *L. trifolii* (at seedling stage) and the mealy bugs *Phenacoccus solenopsis* (Tinsley) and *Maconelliscoccus hirsutus* (Green) (throughout the crop season) are becoming major pests of cotton (Karihaloo and Kumar, 2009; Nagrare *et al.*, 2009).

We studied *L. trifolii* damage to different cotton genotypes, both non-*Bt* and *Bt*, with and without imidacloprid seed treatment. We also assessed the influence of abiotic factors, namely temperature, relative humidity, rainfall (RF), evaporation (E), solar radiation (SR) and sunshine (S), on the incidence of *L. trifolii*.

### Materials and methods

*Gossypium arboreum* varieties, *G. hirsutum* varieties and *G. hirsutum* hybrids (*Bt* hybrids expressing *cry1Ac* and *cry1Ac + cry2Ab* genes and non-transgenic ones) (Table 1) were grown under field conditions on deep black vertisols during the 2002–2007 crop growing seasons (July–December) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, Andhra Pradesh, India. Seeds of the test cultivars (Aravinda, MDL 2450, L 604, LK 861, Mech 12, Mech 162, Mech 184, RCH 2, NCS 207 and MRC 7201 BGII) were sown in four-row plots of 4-m length on ridges with 75-cm inter-ridge spacing and 50-cm inter-plant spacing, accommodating a total of 36 plants per plot. The experiment was planted in three replications in a split-plot design. Seed treatment was the main treatment, and genotype was the sub-treatment. Seeds of one set of genotypes were treated with

**Table 1.** Cotton genotypes evaluated for *Liriomyza trifolii* damage during the 2002–2007 cropping seasons

Genotypes	Cropping seasons (years)
<i>Gossypium arboreum</i> varieties	
Aravinda	2002, 2003, 2004
MDL 2450	2002, 2003, 2004, 2007
<i>Gossypium hirsutum</i> varieties	
LK 861	2002, 2003, 2004
L 604	2002, 2003, 2004, 2007
<i>Gossypium hirsutum</i> non- <i>Bt</i> hybrids	
Mech 162	2002, 2003, 2004, 2005, 2006, 2007
Mech 12	2003, 2004, 2005, 2006
Mech 184	2003, 2004, 2005, 2006, 2007
RCH 2	2005, 2006, 2007
NCS 207	2006, 2007
MRC 7201 BGII	2006, 2007
<i>Gossypium hirsutum</i> <i>Bt</i> hybrids	
Mech 162	2002, 2003, 2004, 2005, 2006, 2007
Mech 12	2003, 2004, 2005, 2006
Mech 184	2003, 2004, 2005, 2006, 2007
RCH 2	2005, 2006, 2007
NCS 207	2006, 2007
MRC 7201 BGII	2006, 2007

imidacloprid at 2 g/kg seed, while another set was left untreated. The crop was raised under rain-fed conditions following normal agronomic practices, including the application of a basal fertilizer (N:P:K = 100:40:60 kg/ha).

The total number of leaves and the number of leaves mined by *L. trifolii* at 30 DAE were counted on five randomly tagged plants in the middle two rows of each plot, and the data were used to calculate the percentage of leaves mined.

To determine whether there was any *Bt* toxin uptake by the leaf miners from *Bt* cotton, we collected *L. trifolii* pupae from transgenic *Bt* and non-transgenic cotton hybrids in eppendorf tubes, crushed them in phosphate-buffered saline buffer at the ratio of 1:10 (insect sample : buffer) and used a semi-quantitative ELISA (Agdia<sup>®</sup>, Elkhart, Indiana, USA) to detect *Bt* toxins present in the pupae (Sharma *et al.*, 2008).

Data on maximum (MaxT) and minimum (MinT) temperatures (°C), morning (RHM) and evening (RHE) relative humidity (%), rainfall (RF) (mm), sunshine (S) (h), open pan evaporation (E) (mm), wind velocity (WV) and solar radiation (SR) (mJ/m<sup>2</sup>) were obtained from the ICRISAT meteorological observatory. Means for each weather parameter (from crop germination to 30 DAE) were used for analysing the correlation of abiotic factors with *L. trifolii* incidence across seasons.

### Statistical analysis

The data were subjected to ANOVA using factorial analysis, with transgenic *Bt*/non-transgenic and

seed treatment as the main treatments and cotton cultivars as the sub-treatments (GenStat, 2008). The significance of differences between the treatments and their interactions were determined using an *F*-test. The interaction effects of genotypes (different varieties/hybrids of a specific cotton species)  $\times$  treatments (seed treatment/untreated)  $\times$  seasons and cotton types (*G. arboreum* varieties, *G. hirsutum* varieties, *G. hirsutum* *Bt* hybrids and *G. hirsutum* non-*Bt* hybrids) were also computed. Association between *L. trifolii* incidence and abiotic factors, and the influence of abiotic factors on *L. trifolii* incidence were analysed using Spearman's correlation analysis and multiple linear and stepwise regressions (Dhillon *et al.*, 2005).

## Results

### Effect of seed treatment and genotype

There was a significant influence of cotton types ( $F_{3,134} = 168.83$ ;  $P < 0.001$ ) and seed treatment ( $F_{1,134} = 9.32$ ;  $P = 0.003$ ) on the severity of damage by *L. trifolii*, which varied significantly across years ( $F_{5,134} = 78.59$ ;  $P < 0.001$ ). Leaf miner damage to different cotton genotypes, both with and without seed treatment, increased from 2002 to 2007, except during 2006 (Fig. 1). However, cotton type  $\times$  treatment  $\times$  season interaction effects were non-significant ( $F_{9,134} = 0.26$ ;  $P = 0.98$ ).

*Liriomyza trifolii* incidence in *G. arboreum* varieties varied from 12.8 to 45.1% and was significantly greater in plots with insecticide-treated seed than in untreated control plots (Table 2), probably because of the abundance of healthy leaves in treated plots and the ineffectiveness of imidacloprid in the control of *L. trifolii*. Damage was significantly lower in *G. arboreum* than in *G. hirsutum* varieties (Table 2), and seed treatment with imidacloprid was ineffective in controlling leaf miner damage to *G. hirsutum* varieties and hybrids (Tables 2 and 3).

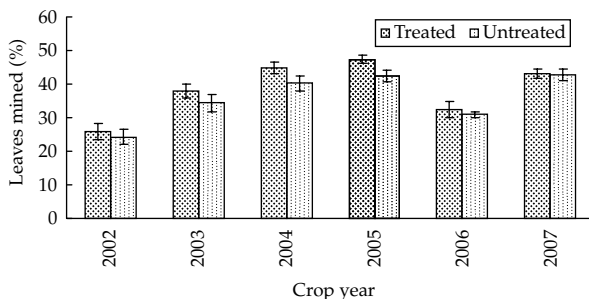


Fig. 1. *Liriomyza trifolii* damage to cotton under seed-treated and untreated conditions during the 2002–2007 cropping seasons

*Liriomyza trifolii* incidence in *G. hirsutum* *Bt* and non-transgenic cotton hybrids varied from 32.6 to 47.9% and from 31.2 to 52.2%, respectively, across cropping seasons (Table 3). Among the cotton hybrids, *L. trifolii* incidence was significantly lower in MRC 7201 BGII and NCS 207 than in the other test hybrids (Table 3). There were significant differences in damage among the genotypes ( $F_{5,174} = 13.74$ ;  $P < 0.001$ ) and across seasons ( $F_{5,174} = 67.91$ ;  $P < 0.001$ ). However, no significant differences were noted among the seed-treated genotypes ( $F_{3,174} = 2.43$ ;  $P = 0.07$ ). Genotype  $\times$  treatment  $\times$  season interaction effects for leaf miner incidence were non-significant ( $F_{33,174} = 1.18$ ;  $P = 0.24$ ).

The *L. trifolii* pupae collected from the leaves of *Bt* cotton hybrids contained up to 1.35 ppb *Bt* toxin, indicating uptake of the toxin by the leaf miner larvae; however, *Bt* uptake had no effect on damage by *L. trifolii* larvae to the *Bt* and non-*Bt* cotton hybrids.

### Influence of abiotic factors

Open pan evaporation ( $r = -0.53^{**}$ ), maximum temperature ( $r = -0.50^{**}$ ), solar radiation ( $r = -0.50^{**}$ ) and sunshine ( $r = -0.49^{**}$ ) had a negative influence on *L. trifolii* incidence, while relative humidity ( $r = 0.46^{**}$ ) and rainfall ( $r = 0.35^*$ ) had a positive influence (\*, \*\* =  $P$  0.05, and 0.01, respectively). Multiple linear regression indicated that abiotic factors, namely sunshine, open pan evaporation, maximum and minimum temperatures, morning and evening relative humidity, rainfall, solar radiation and wind velocity explained 73% of the variation in *L. trifolii* damage (leaf miner damage (%) =  $43377 + 8.0S + 244.0E + 103.9\text{MaxT} + 23.0\text{MinT} - 418.0\text{RHM} + 24.0\text{RHE} + 36.2\text{RF} - 893.0\text{SR} - 65.0\text{WV}$  ( $R^2 = 0.73$ )). However, stepwise regression (leaf miner damage (%) =  $74.74 - 1.36\text{RF} - 7.77E + 0.28\text{RHE}$  ( $R^2 = 0.99$ )) suggested that 99% of the variation in *L. trifolii* damage was associated with rainfall, open pan evaporation and relative humidity.

## Discussion

*Liriomyza trifolii* damages cotton during the early stages of crop growth (Srinivasan *et al.*, 1995; Jeyakumar and Uthamasamy, 1996), preferring younger leaves for feeding and oviposition (Lei *et al.*, 2009). The production of *Bt* proteins in the transgenic hybrids (expressing *cry1Ac* and *cry1Ac + cry2Ab* genes) occurs mostly during the vegetative stage of the crop, and predominantly in leaves (Kranthi *et al.*, 2005). Although the *L. trifolii* pupae collected from the leaves of *Bt* cotton hybrids imbibed a trace amount of *Bt* toxin, there were no

**Table 2.** Efficacy of imidacloprid seed treatment on reduction of serpentine leaf miner *Liriomyza trifolii* damage to *Gossypium arboreum* and *G. hirsutum* varieties

Genotypes	Leaves mined (%) during different crop years <sup>1</sup>							
	2002		2003		2004		2007	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
<i>G. arboreum</i>								
Aravinda	30.1 ± 5.0	14.8 ± 1.5	29.3 ± 4.9	17.6 ± 1.6	28.5 ± 4.9	20.3 ± 2.2	— <sup>2</sup>	— <sup>2</sup>
MDL 2450	12.8 ± 1.9	12.3 ± 3.7	21.2 ± 2.6	14.8 ± 1.8	29.6 ± 5.9	17.2 ± 1.7	23.3 ± 0.8	17.1 ± 2.4
<i>G. hirsutum</i>								
LK 861	37.0 ± 0.9	37.5 ± 3.7	40.5 ± 2.0	41.3 ± 2.2	44.1 ± 3.4	45.1 ± 0.8	— <sup>2</sup>	— <sup>2</sup>
L 604	25.2 ± 3.6	24.9 ± 2.3	34.1 ± 1.8	29.4 ± 1.1	43.0 ± 1.6	34.0 ± 1.1	40.2 ± 2.1	41.7 ± 1.5

<sup>1</sup> Values presented are means of three replications ± standard error; <sup>2</sup>—, data not available.

significant differences in leaf damage by the leaf miner larvae in the *Bt* and non-*Bt* cotton hybrids.

*Liriomyza trifolii* damage was significantly lower in *G. arboreum* cotton varieties than in the *G. hirsutum* varieties and hybrids across treatments and cropping seasons. No significant differences, however, were observed between *Bt* and non-*Bt* cotton hybrids.

Another plausible reason for the increased severity of *L. trifolii* damage to *Bt* cotton over the previously grown conventional cotton might be that broad spectrum insecticides for the control of lepidopteran pests are not as frequently used on *Bt* cotton as they are on conventional cotton. In addition, inherent differences in susceptibility to leaf damage among the different cotton genotypes, as previously observed by Sharma and Pampapathy (2006), may play a role.

Seed treatment with imidacloprid was found to be ineffective for the control of leaf miner damage, indicating the need to identify alternative strategies for the management of this and similar sucking pests.

Although *L. trifolii* pupae collected from the leaves of *Bt* cotton hybrids had ingested trace

amounts of *Bt* toxin, no significant differences in leaf damage by *L. trifolii* to *Bt* and non-*Bt* cotton hybrids were recorded in our study. Lei *et al.* (2009), however, observed a reduction in *L. trifolii* damage on *Bt* cotton leaves. In addition, *Bt*-sprayed chickpea and bean crops showed lower damage by *L. cicerina* (Rondani) (Cikman *et al.*, 2008) and *L. trifolii* (Cikman and Comlekcioglu, 2006), respectively.

Abiotic factors had a large and significant influence on *L. trifolii* damage, similar to previous studies. For instance, abiotic factors account for over 90% of *L. trifolii* incidence in castor (Singh *et al.*, 1996). The present study suggested that 99% of the variation in *L. trifolii* damage was due to rainfall, open pan evaporation and relative humidity. Evidently, the serpentine leaf miner is highly sensitive to changes in environmental conditions. As such, climate change and widespread cultivation of leaf miner susceptible cotton genotypes, including transgenic *Bt* cottons, might lead to *L. trifolii* becoming a major pest in the future. There is an urgent need to devise better control strategies for the pest, including alternative seed treatments and leaf miner-resistant genotypes.

**Table 3.** Effect of imidacloprid seed treatment on damage by the serpentine leaf miner *Liriomyza trifolii* to *Gossypium hirsutum* *Bt* and non-*Bt* cotton hybrids

Genotypes <sup>1</sup>	Leaves mined (%) in different treatments <sup>2</sup>			
	<i>Bt</i> + T	<i>Bt</i> + UT	Non- <i>Bt</i> + T	Non- <i>Bt</i> + UT
Mech 12 (4)	44.5 ± 2.5	38.9 ± 2.8	52.2 ± 5.5	44.3 ± 1.5
Mech 162 (6)	40.8 ± 2.9	41.0 ± 2.3	42.3 ± 2.5	41.6 ± 2.6
Mech 184 (5)	47.9 ± 1.7	43.8 ± 2.0	44.8 ± 2.1	44.1 ± 2.1
RCH 2 (3)	42.0 ± 3.9	41.6 ± 3.6	41.3 ± 3.1	36.5 ± 2.7
NCS 207 (2)	32.6 ± 4.0	35.2 ± 4.6	36.2 ± 5.2	31.2 ± 2.4
MRC 7201 BGII (2)	33.6 ± 2.8	36.2 ± 3.3	34.0 ± 3.2	35.6 ± 3.5

T, seed treated with imidacloprid; UT, no seed treatment; <sup>1</sup> figures in parentheses are numbers of years tested; <sup>2</sup> values presented are means across seasons ± standard error.

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