*J. Cotton Res. Dev.* **36**(2), 231-236 (July, 2022) **ISSN No. 0972-8619**

# Function of plant volatile cues in leafhopper resistance and susceptibility in cotton

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**Abstract :** Cotton is one of the most significant fibre crops in India, and it is known as "**White Gold**". The leafhopper, *Amrasca devastans* (Distant), poses a real challenge to cotton production (Hemiptera: Cicadellidae). One possibility for controlling the leafhopper is to exploit volatile semio chemicals released by plants following insect herbivory. Here, we evaluated the repellent properties of volatile chemicals alone and in various combinations as tools for the management of this pest in cotton ecosystems. Volatiles obtained from young cotton seedlings of three kinds after air entrainment method with or without infestation of leafhoppers in 24, 48, 72, and 96 hr periods were pooled and later analysed by gas chromatography. Analysis of the volatile organic compounds (VOCs) collected from the three cotton varieties (CO17, KC3 and DCH32) reveals substantial interspecies variation. With the help of authentic standards and GC-MS and GC peak amplification, including chiral GC, these chemicals were identified as being benzaldehyde; caryophyllene; α-pinene, α-myrcene and trans-α-Ocimene, 9-Oximino-2,7-diethoxyfluorene with confirmation through Y tube olfactometer. Despite the fact that these plants were bred to be resistant to sucking pests like leafhoppers, the reported variability has important ecological implications for the use of plant volatiles as repellents for leafhoppers.

**Key words:** *Amrasca devastans*, HPR, leafhopper, plant volatile Cues, semio-chemicals, VOC

The cotton green leafhopper, *Amrasca devastans* (Distant) (Hemiptera: Cicadellidae, formerly *Amrasca biguttula biguttula*), is a sap-feeding insect pest of cotton plants, *Gossypium* sp., in India (PrasadaRao *et al.*, 2015). Other synonyms are *Amrasca biguttula, Chlorita biguttula, Chlorita biguttula biguttula, Empoasca biguttula, Empoasca devastans, Sundapteryx biguttula, Sundapteryx biguttula biguttula, Amrasca splendens, Jacobsiana distinguenda* and *Jacobsiana formosana* (Robert Mensah, 2021). Nymphs and adults of *Amrasca devastans* suck the sap from young and fresh leaves and female adults lay their eggs in leaf lamina. Early symptoms include yellowing and leaf curling followed by browning of the marginal leaf region and a burnt appearance. It's called "hopper burn symptoms". Generally, the presence of *A. devastans* throughout the season results in considerable reductions in all seasons' yields, with economic losses of up to 25 per cent (Nagrare *et al.*, 2012; Vennila *et al.*, 2016). Chemical control of *A. devastans* is common, although this leafhopper has evolved resistance to a number of insecticides. Excessive pesticide use has harmed other biological control agents and resulted in residual issues in cotton.

In this case, the only option for eco-friendly and farmer friendly management is to use a host plant resistant technique. Plant volatile chemicals play an important role in plant insect interactions, particularly for leaf feeding insects that rely on plant volatiles to find suitable hosts for feeding or ovipositing. Herbivores use their olfactory system to distinguish between host and non-host plants, coping with a complicated background of volatiles. The leafhoppers behavioural and electrophysiological responses were studied with the goal of finding the chemicals responsible for host location and expanding the possibilities for using this information in integrated pest management (IPM) techniques (Roleen *et al.*, 2017). Cotton VOCs involved in leafhopper attraction to the host plant, on the other hand, have yet to be definitively identified. The study's goal is to identify attracted plant volatile cues for leafhoppers. As a result of this circumstance, integrated pest management solutions based on host plant resistance have been developed, with repellent chemicals playing a key role.

## HOST PLANT VOLATILES

Some highly susceptible and resistant cotton varieties were investigated for their volatile composition and role of this volatile blend in insect's orientations to their host. Volatile compounds, which involve in insect attractions to their host plants for appropriate food, oviposition sites and shelter, are mainly monoterpenes (Metcalf, 1987). Plant volatiles are important cues for insects to find and locate their hosts (Zheng Hao *et al.*, 2002).

Olfactory cues may enhance the attractiveness of host plants to leafhoppers. For example, the maize leafhopper *Dalbulus maidis*, found that a combination of green light and maize volatiles was about twice as appealing as green light plus hexane (Todd, *et al.*, 1990). In the absence of other stimuli, certain leafhoppers respond to odour cues. In olfactory bioassays, nymphs of the American grapevine leafhopper, *Scaphoideus titanus* Ball, preferred grapevine leaves or apical shoots of the grapevine rootstock *Vitis riparia x rupestris* over purified air, and in electroantennogram (EAG) tests, higher responses to a plant extract were recorded compared to purified air (Mazzoni *et al.*, 2009). *Amrasca biguttula* an Indian cotton leafhopper employed olfactory cues to distinguish between cotton a host plant and castor, a non-host species (Saxena and Saxena, 1974). When compared to resistant lucerne, the potato leafhopper, *Empoasca fabae* (Harris) was more attracted to leaf volatiles produced by susceptible glandular haired lucerne. Leafhoppers were attracted to a 0.0001 per cent solution of 1-octen-3-ol, a significant volatile component of both genotypes, which produced differing ratios of the same volatiles (Ranger *et al.*, 2005). Arthropods are repelled by plants in the Alliaceae family. Cotton bollworms, aphids, whiteflies, cabbage root flies, citrus psyllids, and mosquitoes have all been proven to be repelled by volatiles from *Allium sativum* (L.) or *Allium tuberosum.* Oluwafemi *et al.*, (2011) found that sulphur compounds play a crucial role in the repelling impact of volatiles from members of the Alliaceae family. Tea leafhopper management could benefit from volatiles from the Alliaceae family or fragrant plants. The repellent activity of five semiochemicals against *A. devastans* was studied in this study.

Adult leafhopper *Amrasca devastans* were collected from the cotton experimental trial of the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. Six week-old plants were taken for this experiment. Leafhoppers were maintained on young plants in pots (10 x 20 cm). The age of *A. devastans* adults was estimated from their body colour based on our experience. Newly emerged "pale green" and too old "dark green" adults of *A. devastans* were not used in the laboratory assays. The proportions of tested male and female leafhoppers were random. Each insect was used only once.

**Air entrainment method**

Air entrainment is a method in which a stream of purified air stream is passed over a substance which emits volatiles and the emitted volatiles are trapped onto a glass rod containing an adsorption media Porapak Q. The trapped volatiles are then desorbed by elution with a solvent such as diethyl ether. Volatiles were desorbed from the Porapak by eluting with purified solvent and the extracts of volatiles were concentrated to low volume of 10-20 l and further analysed by Gas Chromatography coupled with Mass

Spectrometry (GC-MS) (Oluwafemi *et al.*, 2011).

Cotton variety Co17 was grown in pots in the greenhouse and compared to DHC32 and KC3, which were susceptible and resistant, respectively. Air entrainment was carried out following standard procedures using 6 week old cotton plants. Cotton volatiles were collected over a period of 24, 48, 72, and 96 hours before and after the release of leafhoppers. Plant volatiles were collected from three cotton plant varieties. Samples were stored in vials at -20°C until used in chemical analysis.

**a) Gas Chromatography - Mass Spectrometry (GC-MS)**

Eluted volatile compounds from the air entrainment in plants infested with leafhopper were further investigated by analytical techniques such as Gas Chromatography (GC) and Mass Spectrometry (GC-MS). GC analyses were done on a methyl silicon column with nitrogen as carrier gas in the Flame Ionization Detector (FID). By linking chromatography directly to Mass Spectrometry (MS), it was possible to obtain spectra in sub-nanogram ($10^{-9}$) quantities (Oluwafemi *et al.*, 2011).

**Olfactometer y tube bioassay**

The first step in studying signals in any insect is to demonstrate unequivocally a behavioural response, which can be done using an olfactometer or a wind tunnel in which an insect is exposed to a volatile source and a behavioural response can be monitored numerically and statistically by removing other cues such as vision and contact effects (Oluwafemi *et al.*, 2011). Response of *A. devastans* to volatile blend of susceptible cotton variety DCH32 and resistant variety KC3 were also observed in a Y tube alfactometer. One compartment was treated with 0.1 ml of cotton extract and others remained untreated. Cotton leafhoppers were collected randomly from the mother culture and released through the hole in the entrance of the lid. The hole was then closed with cotton plug. Number of stem weevil in each chamber was counted after 1 hour.

Each of the five compounds was first dissolved in liquid paraffin (2% v/v) and tested for repellency using a Y tube olfactorometer. Then, two 2.5-cm diameter filter papers were filled with 20 µL of volatile chemical complex solution and 20 µL of liquid paraffin. To keep leafhoppers from contacting the filter papers, they were placed individually in the two lateral stoppers and covered with muslin cloth netting on the exterior. The stopper was replaced after twenty jassid adults were inserted into the tube through the middle hole. Leafhoppers in the two reaction zones were counted at 0, 15, 30, 45, and 60 minutes following release. Each chemical was put through its paces two times. Each drug was tested in two tubes on each experimental day, with at least four trials done. There was a minimum of 0.5 m between tubes. The treated filter paper was placed in the tubes at random. Bioassays were carried out between 9:00 and 10:00 a.m. in a temperature controlled chamber (24 2°C in complete darkness). Between trials, each bioassay tube was washed with water and 90 per cent ethanol and oven-dried at 80°C. In Y tube olfactometer trials, this apparatus was employed. Between the muslin cloth netting and the glass stopper, a volatile chemical (repellent) was put to the filter paper before the assay. On the other side, liquid paraffin (control) was added to the filter paper.

**Statistical analysis**

All statistical tests were carried out using SPSS v11. For laboratory bioassays, the repellency index (RI) was calculated using the following formula: RI = $[(C-T)/(C+T)]\times 100\%$, where $T$ and $C$ are the numbers of leafhoppers in the treated and control response zones, respectively. A higher value indicates a stronger repellent effect. In Y tube olfactometer trials, the leafhopper numbers in two response zones were square root transformed, and two sample paired *t*-tests were used to compare the transformed values between two response zones on each sampling time point.

VOC samples collected from 6 week old cotton plants, according to chemical analysis, were quantitaively and qualitatively varied in intensity. However, the total amount of VOCs produced by leafhopper damaged cotton plants at 6 weeks old differed significantly from the volatile chemicals emitted by leafhopper damaged plants during 24 hours. Using authentic standards, GC-MS and GC peak enhancement, including enantioselective (chiral) GC, showed the identities of these chemicals as benzaldehyde; caryophyllene; α-pinene, α-myrcene and trans-α-Ocimene, 9-Oximino-2,7-diethoxyfluorene (Table 1).

Repellent chemicals can be useful in the management of a wide range of agricultural pests as part of an integrated pest management strategy. Cotton plant semio-chemicals have been demonstrated to repel sucking bugs. In this study, we found that semio-chemicals from cotton plants were highly repellent to adult leafhoppers in the lab. The repellent effect was powerful enough to diminish the number of nymphs in the field.

Only five organic compounds were discovered to be cotton leafhopper repellant among the chosen organic compounds. Previous research has yielded conflicting outcomes. Cotton leafhoppers were repelled by benzaldehyde; caryophyllene; α-pinene, α-myrcene and trans-α-Ocimene, 9-Oximino-2,7-diethoxyfluorene. The uniqueness of interactions between insects and specific substances may account for the disparities in repellency. However, the findings are similar to those of prior studies on other pest species (Bruce *et al.*, 2005; Mann *et al.*, 2011; Diaz-montano and Trumble, 2013; Pan and Wyckhuys, 2013; Ferry *et al.*, 2009; Diaz-montano and Trumble, 2013; Pan and Wyckhuys, 2013; Ferry *et al.*, 2009).

Zhang *et al.*, 2014 reported that two selected terpenoids were shown to repel tea leafhopper in a Y-tube olfactometer assay method. Only terpenoids were shown to be repellant against tea leafhoppers in this investigation. Because the test concentration (2% in liquid paraffin, v/v) was sufficient for screening repellents, the various results are most likely due

**Table 1.** List of volatile organic compounds emitted by three varieties of cotton

| VOC | CO17 | | DCH32 | | KC3 | |
|---|---|---|---|---|---|---|
| | A | B | A | B | A | B |
| benzaldehyde | + | + | + | + | ++ | ++ |
| caryophyllene | | + | + | | ++ | ++ |
| a-pinene | + | | | + | ++ | ++ |
| a-myrcene | | + | | + | ++ | ++ |
| trans-a-Ocimene, 9-Oximino-2,7-diethoxyfluorene | + | | + | + | ++ | ++ |
| Heptane, 2,4-dimethyl | | + | + | + | ++ | |
| o-Cymene | | + | | + | | + |
| Benzene, 1-methyl-3-(1-methylethyl) | | + | + | | | + |
| Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) | | + | + | | + | |
| Benzothiazole | + | | + | + | | |
| 1,2-Benzisothiazole | | + | + | | | + |
| 3-Allylbenzothiazolium bromide | + | | + | | + | |
| Aromandendrene | + | | | + | | + |
| Isocaryophillene | | + | | + | + | |
| Benzothiazole | | + | + | | | |
| 2-Propanone, 1-(acetyloxy) | + | | | + | | + |

VOC – Volatile Organic Compounds, A – After leafhopper feeding, *B* – Before leafhopper feeding
++ - > 50 % area in GC peak; + - < 50 % of area in GC peak

to the different Y tube bioassay techniques used. According to Du *et al.*, 2016, the ideal way for volatile cues testing against insects is to use Y-tube olfactometer tests to investigate entirely different selection processes by insects. In conclusion, our study demonstrates the effectiveness of a five semiochemicals was repellent for cotton leafhopper adults and contributes to develop resistant cotton variety in future.

## ACKNOWLEDGEMENTS

The authors are grateful for the facilities provided by the Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, as well as financial support from Rainbow Agrosciences Private Limited, Ahmedabad, Gujarat.

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**Received for publication : February, 2022**
**Accepted for publication : April 16, 2022**