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PUTATIVE VECTORS OF SUGARCANE WHITE LEAF DISEASE IN UDA WALAWE, SRI LANKA

Wijerathna W.G.C.J.N.[1], Ketipearachchi K.G.[1], Sumanarathna R.M.P.W.[1], Srimal R.A.S.[1], and Chanchala K.M.G.[2]*

[1]Department of Agro-Technology, Institute for Agro-Technology and Rural Sciences, Weligatta, Hambantota, Sri Lanka
[2]Sugarcane Research Institute, Uda Walawe, Sri Lanka

**Corresponding Author: g.chanchala@yahoo.com (https://orcid.org/0000-0002-8433-9372)*
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ABSTRACT

Sugarcane White Leaf Disease (WLD) is one of the major threats to the sugarcane industry in Sri Lanka. Deltocephalus menoni (Hemiptera: Cicadellidae, Deltocephalinae) is the only locally-identified vector of this phytoplasma disease. Understanding the vector and reservoir of the phytoplasma is essential for effective control of the disease. An experiment was conducted in the entomology laboratory and the Research farm of the Sugarcane Research Institute (SRI), Uda Walawe, Sri Lanka to identify the putative vectors of WLD of sugarcane. Insects available in the sugarcane ecosystem were identified by collecting them using a sweep net and pooter randomly from sugarcane fields, preserving samples and observing morphology. Among the collected insects, leaf hopper sp., Yamatotettix sp., Chlorita paolii, Cicadella viridis, Euscelis sp., Indiocerus lituratus, Kolla ceylonica and Tropidocephala signata were selected for the study as they belong to the family Cicadalidae, which belongs to 75% of phloem-feeding phytoplasma vectors. Deltocephalus menoni was considered as the reference sp. during the study. Honey dew test and erythrosine dye test were conducted to determine the feeding ability of selected insects on sugarcane. pH test and leaf cross-section study were conducted to study the feeding location of the insects on sugarcane leaves. Among test insects, only Yamatotettix sp. was identified as a phloem feeder exhibiting its potential to act as a vector of the sugarcane WLD. Further confirmation of the ability of Yamatotettix sp. to transmit the sugarcane WLD phytoplasma is crucial by conducting disease transmission tests in future.

Keywords: Deltocephalus menoni, Putative vectors, Phytoplasma, Sugarcane, White Leaf Disease

INTRODUCTION

Sugarcane is an important commercial crop and sugar is an important sub-sector in economy of Sri Lanka which offers great employment potential and income generation to the community in sugarcane cultivating areas, Sevanagala, Pelwatte, Monaragala, Siyambalanduwa, and Badulla (Keerthipala, 2007, 2016). At present, domestic sugar production has been reduced due to decrease in cane supply, labor unrest, cultivating inferior cane varieties, deficiencies in crop management and due to the pest and disease incidence.

The phytoplasma diseases that affect sugarcane include sugarcane white leaf disease (WLD), sugarcane grassy shoot disease, sugarcane yellow leaf syndrome, Ramu stunt disease of sugarcane, and sugarcane green grassy shoot disease (Marcone et al., 2001). Among them WLD is significant in Sri Lanka (Kumarasinghe & Jones, 2001). WLD is caused by phytoplasmas from group 16SrXI, including subgroup 16SrXI-B and a new subgroup, 16SrXI-D which includes other important phytoplasmas infecting plants of the Poaceae family, such as rice yellow dwarf (RYD), sugarcane grassy shoot (SCGS) and sorghum grassy shoot (SGS) phytoplasmas (Marcone, et al., 2001).

This disease spreads in sugarcane plantations, primarily, through infected seed cane (Jayaratne, 1996) and secondarily, by an insect vector, *Deltocephalus menoni* (Seneviratne, 2008) in Sri Lanka. Two other leafhopper species viz., *Matsumuratettix hiroglyphicus* Matsumura (Matsumuto et al., 1968) and *Yamatotettix flavovitatus* Matsumura (Hanboonsong et al., 2006) have been identified as the vectors of Sugarcane WLD Phytoplasma SWLDP in other countries, however, these species are not reported in Sri Lanka.

Species in *Deltocephalinae* subfamily have feeding habits that can range from monophagous to polyphagous, and they are releasing honey dew on the leaf blades. Members of this group can transmit one or more different phytoplasma species where more than 75% of all confirmed phytoplasma vector species belong to a specific species (Chanchala et al., 2022). Weintraub & Beanland (2006) pointed out that this group collectively possesses several fundamental characteristics that make its members efficient vectors of phytoplasmas: i) Hemimetabolous, ii) Feed specifically and selectively on certain plant tissues, which makes them efficient vectors of pathogens residing in those tissues and iii) Feeding is non-destructive, promoting successful inoculation of the plant vascular system without damaging conductive tissues and eliciting defensive responses.

Recently, WLD incidences in all sugarcane cultivating areas of Sri Lanka are increasing rapidly creating doubts on presence of any other vectors in the country. Also, effective control of the disease requires a good understanding of the vector and reservoir of the phytoplasma. It is important to know the impact of currently undiscovered vectors on the spread of WLD. Hence, this study was started to identify the putative vectors except *D. menoni* (if present any) that transmit sugarcane WLD.

MATERIALS AND METHOD

The experiment was conducted at the research farm and entomology laboratory of the Sugarcane Research Institute (SRI), Uda Walawe, Sri Lanka (6.4185° N, 80.8239° E, Average Temperature: 32^0C, Average Rainfall: 1500 mm, Elevation from mean sea level: 109 m) from April to August 2024 (Wanasinghe et al., 2014).

Collecting test insects

Insects in the sugarcane eco system were collected from the research farm of the SRI Uda Walawe using sweep net and pooter in the morning between 7-8am and using light traps at night time between 7-8 pm (Figure 1). Leaf hopper spp trapped in the sweep nets were collected in the vial attached to the pooter. Immediately after removing the vial from the pooter, it was held upside down in order to allow the insects to move upward. The mouth of the vial was then covered with a piece of cloth and securely fastened with a rubber band. Thus, insects were collected in many vials changing the vial of the aspirator after enough insects had been collected in the vial. The insects in vials were then transported alive in an air-conditioned vehicle to the laboratory at SRI on the same day.

Figure 1: Instruments and methods used in insect collection; (a) sweep net, (b) pooter, (c) Insects collecting using sweep net and pooter and (d) Insects collecting using light trap at night

Sample preservation and identification

The collected insects were knocked down by keeping them in a deep freezer for 2 hours and dried in an oven for 5-to 6 hours under 45ºC temperature and stored in small paper packets. Specimen preparation for identification was done by placing dried insect samples on specific triangular shaped paper pieces attached to the top of the insect pins. Leaf hopper species were separated based on the morphological features. Identification was done based on the morphology of genital parts and other body parts (wings, legs, etc.) using available literature, expert guidance and identification keys.

Selection of insects for the feeding test

Insect selection was done considering previous studies conducted at SRI (Senevirathna, 2008).

Study the feeding potential on sugarcane

The feeding amount and ability of collected insects on sugarcane were studied using the honey dew test and the Erythrosine dye test. The feeding amount was measured by staining with the bromocresol green treated filter paper. Honeydew on the sugarcane leaf and the sachet were collected with the bromocresol green-treated filter papers. Honeydew-stained filter papers were carefully wind-dried, and stained areas on filter papers (blue) were measured using square millimeter grids. In order to study the ability to feed, leaf portions where the insect had fed were collected and dipped in a staining solution of 0.1% erythrosine dye for 10-15 min. Then, the leaf portions were examined under a microscope. The salivary flanges on each plant sample, which were stained in pink color, were counted based on size variation following the method described by Nugaliyadde (1994).

Feeding location

The feeding location of the leaf was determined by conducting a leaf cross-section study and pH test. The pH test was done by using litmus paper. If the insect was fed the phloem, the pH of honeydew is acidic, and if the insect was fed the xylem, the honeydew is bases.

In order to confirm the feeding location, Erythrosine dye-stained cross sections were studied to confirm whether the phloem has been fed precisely by the test insect spp. (Heinrichs et al., 1985).

RESULTS AND DISCUSSION

Ten insect species primarily belonging to order Hemiptera, were recorded during the study period (Table 1). Recorded insects were belonging to three families Cicadellidae, Lophopidae, and Delphacidae families

Table 1: Insect species belong to order Hemiptera recorded during the study period

	Insect collected	Order	Family
01	*Chlorita paolii*	Hemiptera	Cicadellidae
02	*Cicadella viridis*	Hemiptera	Cicadellidae
03	*Yamatotettix spp.*	Hemiptera	Cicadellidae
04	*Kolla ceylonica*	Hemiptera	Cicadellidae
05	*Tropidocephala signata*	Hemiptera	Cicadellidae
06	*Idiocerus lituratus*	Hemiptera	Cicadellidae
07	*Euscelis sp.*	Hemiptera	Cicadellidae
08	*Pyrilla perpusilla*	Hemiptera	Lophopidae
09	*Perkinsiella saccharicida*	Hemiptera	Delphacidae
10	*Deltocephalus menoni*	Hemiptera	Cicadellidae

Eight hopper species belonging to the family Cicadellidae were recorded *i.e.*, *Chlorita paolii*, *Cicadella viridis, Yamatotettix* spp., *Kolla ceylonica, Tropidocephala signata, Idiocerus lituratus*, *Euscelis sp* and *Deltocephalus menoni*. The family consists of various leafhopper species, known for their plant-feeding habits and sometimes their role as disease vectors (Weintraub & Beanland 2006).

One hopper species from the family Lophopidae, named *Pyrilla perpusilla* and one species from the family Delphacidae named *Perkinsiella saccharicida* were also recorded during the study.

Pyrilla perpusilla, also known as the sap-feeding planthopper leading to direct damages to the sugarcane. *Perkinsiella saccharicida* is also a major pest in sugarcane. It also acts as the vector of Fiji disease of sugarcane. Since *Perkinsiella saccharicida* and *Pyrilla perpusilla* do not belong to the family Cicadellidae, they were not considered for further studies.

Identifications of the leafhopper spp feed on Sugarcane

Yamatotettix spp., *Chlorita paolii*, *Cicadella viridis*, *Euscelis sp.*, *Idiocerus lituratus*, *Kolla ceylonica* were considered for the study, including *Deltocephalus menoni*, which is the already known vector of sugarcane WLD as a reference and has been demonstrated to carry and transmit the WLD phytoplasma in previous studies (Senevirathne, 2008; Chanchala et al., 2014; 2019; 2020(a,b); 2021; 2022).

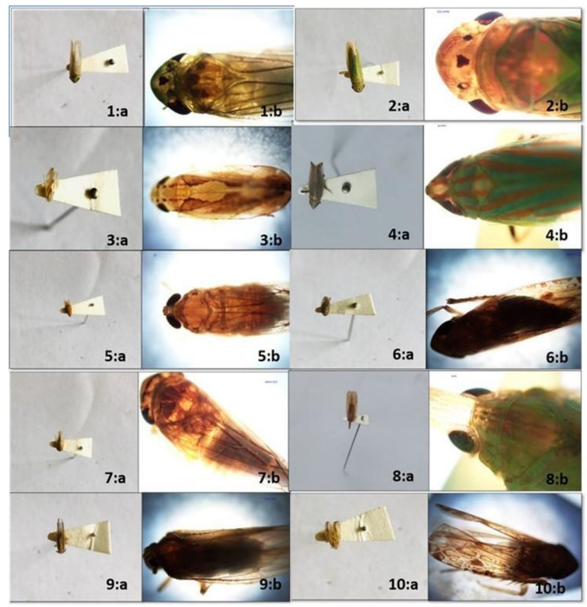

Figure 2: Collected leaf hopper specimens; (1) *Chlorita paolii,* (2) *Cicadella viridis,* (3) *Yamatotettix spp.* (4) *Kolla ceylonica,* (5) *Tropidocephala signata,* (6) *Idiocerus lituratus* (7) *Euscelis sp.* (8) *Pyrilla perpusilla,* (9) *Perkinsiella saccharicida, and* (10) *Deltocephalus menoni. (a)* Photograph and *(b)* microscope capture of each specimen.

Feeding potential of test insect spp. on sugarcane plant

The feeding potential of test insect species was measured using honeydew production and production of salivary flanges on sugarcane leaves. All the insect species produced honeydew and salivary flanges, which confirm all the spp feed on sugarcane (Table 2). All species produce honeydew, which confirms the insect’s ability to feed on a particular plant.

Deltocephalus menoni, *Kolla ceylonica,* and *Yamatotettix spp.* have produced large salivary flanges. Among them, *D. menoni* shows the highest mean honeydew production (24.1 ± 0.02 mm²), followed by *Yamatotettix* spp. (13.7 ± 0.13 mm²), confirming their intensive phloem-feeding behavior. Production of large salivary flanges by *Deltocephalus menoni* and *Yamatotettix spp*. Exhibits more intensive feeding activity of these two-insect spp.

Table 2: Status of honeydew production, feeding flanges, feeding location and response to pH tests in selected hopper species.

Test insect *spp*	Production of Honey dew (mm^2)	Presence of feeding flanges	Feeding location	Response to pH test
Deltocephalus menoni	24.1^a (±0.02)	1 (large)	Phloem	Red color convert blue
Kolla ceylonica	4.3^c (±0.15)	2 (large)	Epidermal cells	No reaction
Tropidocephala signata	3.2^c (±0.18)	3	Bulliform cells	No reaction
Idiocerus lituratus	3.1^c (±0.16)	4	Epidermal /Mesophile	No reaction
Euscelis sp	2.9^c (±0.2)	4	Epidermal /Mesophile	No reaction
Cicadella viridis	4.1^c (±0.05)	4	Epidermal /Mesophile	No reaction
Chlorita paolii	3.9^c (±0.21)	4	Bulliform cells	No reaction
Yamatotettix spp.	13.7^b (±0.13)	2 (large)	Phloem	Red color convert Blue

This heightened activity could correlate with increased risk for pathogen acquisition and transmission.

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Although *Kolla ceylonica* has produced large flangers they were located in mesophyll cells rather than in phloem tissues. Honey dew production was low (4.3 ± 0.15 mm²) and absence of any pH color change further confirmed non-phloem feeding behavior of the insect showing its restricted capacity to act as a vector for WLD despite the visible damage caused.

Tropidocephala signata, *Idiocerus lituratus*, *Euscelis* sp., *Cicadella viridis*, and *Chlorita paolii*, exhibited minimal honeydew production (2.9–4.1 mm²) and no pH reaction, reflecting their limited feeding depth and negligible vector potential.

Salivary-sheath feeding appears to be particularly common among the phloem- feeding hemipterans. Thus, the number of salivary sheaths, or flanges, has been considered as a good predictor of crop damage from hemipteran feeding. Leafhoppers expend a considerable energy cost when making salivary flanges to access plant tissues. If a leafhopper successfully creates a feeding well and feeds, they often do not need to make additional feeding attempts on the same plant. However, if the first attempt fails or the plant defenses are too strong, they may create additional feeding wells in an attempt to establish a successful feeding site (Nugaliyadde et al., *1994).*

Accordingly, *Deltocephalus menoni*, *Kolla ceylonica*, and *Yamatotettix spp*. were capable of penetrating the leaf tissues efficiently, indicating that their penetration and feeding mechanisms are adapted to establish feeding sites and feed with minimum energy expenditure.

Feeding locations of test insect spp. on sugarcane plant

Feeding locations of test insect spp were measured by studying cross sections of insect fed- locations and conducting pH (litmus) tests for honeydew excreted by test insect spp. In considering the feeding locations of *Deltocephalus menoni* and *Yamatotettix spp*. have reached the phloem in leaf cross section, confirming their feeding on phloem sap and therefore the ability to acquire WLD phytoplasma, as it presents in phloem sap.

Phloem feeders are the primary vectors of phytoplasma, the causal agent of WLD, as they can access and transmit the pathogen directly from infected plants to healthy ones. This phloem access is crucial, as only phloem feeders have the capability to transmit such systemic pathogens effectively (Jarausch, 2010).

While *Kolla ceylonica*, *Tropidocephala signata*, *Idiocerus lituratus*, *Euscelis sp.*, *Cicadella viridis*, and *Chlorita paolii* feed on epidermal, mesophyll, or parenchyma cells, they are unlikely to act as vectors for WLD due to their inability to access the phloem, where phytoplasmas reside. Thus, these species, although damaging, do not pose the same disease threat as *Deltocephalus menoni* and *Yamatotettix spp*. Phloem sap in the plants generally has a slightly alkaline pH, often around 7.5 to 8.0, due to the presence of amino acids, sugars, and other nutrients that plants transport to different tissues via phloem tubes. Thereby, when an insects feed on phloem sap, their excreta; honeydew also shows an alkaline nature, which converts the red color of litmus paper into blue in pH test (Heinrichs et al., 1985).

Honeydew produced by both *Deltocephalus menoni* and *Yamatotettix spp.* convert red- colored litmus paper into blue-colored in pH test confirming their feeding from phloem tubes of the plant. All other test insect spp did not respond to the litmus paper test, showing they are not feeding from phloem tissues of the plant.

Potential vectors of the sugarcane WLD

Deltocephalus menoni and *Yamatotettix spp*. exhibit similar feeding ability and feeding location on sugarcane as *Deltocephalus menoni* and *Yamatotettix spp*. stand out due to their feeding on the phloem, response to the pH test, and large salivary flange production, making them significant potential vectors of WLD in sugarcane. Among two cicadalids, *Deltocephalus menoni* is already identified as a vector of the sugarcane white leaf disease phytoplasma (Senevirathne, 2008; Chanchala et al., 2019).

The other species contribute to physical damage and honeydew-related issues but lack the direct disease transmission capacity associated with phloem feeders. Literature confirmed that, *Kolla ceylonica* and *Tropidocephala signata* are not capable of acting as a vector to the WLD in Sri Lanka (Senevirathne, 2008 and SRI, 2017). Accordingly, *Yamatotettix spp*. exhibits its potential to act as a vector for sugarcane WLD which will require further confirmation by conducting disease transmission tests.

Yamatotettix spp., particularly *Yamatotettix flavovittatus*, is a leafhopper that feeds on sugarcane and poses a significant threat due to its role as a vector for the sugarcane white leaf disease phytoplasma (Hanboonsong et al., 2006; Roddee et al., 2018). Studies have shown that when these leafhoppers feed on infected plants, they can acquire the pathogen and transmit it to healthy plants, thus perpetuating the disease within sugarcane fields. This transmission ability has established *Yamatotettix flavovittatus* as a prominent vector, necessitating close monitoring and effective management strategies to control both the vector and the spread of WLD in sugarcane-producing regions (Thein, 2012; Wangkeeree et al., 2021).

CONCLUSIONS

Yamatotettix spp. exhibits its potential to act as a vector for sugarcane WLD in Sri Lanka. Further confirmation of the ability of *Yamatotettix spp.* to transmit the sugarcane WLD phytoplasma is crucial by conducting disease transmission test in the future. Leaf hopper spp. available in all growing areas are also important in the identification of putative vectors available in particular sugarcane growing areas of the country.

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