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Role of Leaf Fibro-vascular Bundle Configuration on Feeding of *Deltocephalus menoni* (Hemiptera: Cicadellidae) in the Transmission of White Leaf Disease in Sugarcane Varieties

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ABSTRACT

Purpose: *Deltocephalus menoni is the vector of Sugarcane White Leaf Disease (WLD) and the causative agent of White Leaf Disease Phytoplasma (WLDP). WLDP is restricted to the phloem tissues facilitating its dispersal via sap containing phytoplasma during the vector feeding. Here, the configuration of the vascular bundle would be an important characteristic in plant's resistance to vector feeding and thereby the disease spread. A study was conducted to determine the relationship between feeding of D. menoni and the configuration of leaf fibro-vascular bundles.*

Research Method: *Feeding amount of D. menoni on sugarcane varieties, the configuration of leaf fibro-vascular bundle of leaf blade in gross cross-section and the WLD infection in the natural environment were measured. Variations and correlations between configuration of leaf fibro-vascular bundles between varieties, vector feeding and WLD infection were studied. Major anatomical characteristics of vascular bundles that contribute to feeding were also determined.*

Findings: *The structure of the leaf fibro-vascular bundles varied with the sugarcane variety/ accession and the volume of feeding by D. menoni of sugarcane was correlated with the configuration of the leaf fibro vascular bundle. Distance from the epidermis to vascular parenchyma, distance from epidermis to vascular bundle and thickness of phloem fiber layer were negatively correlated with the feeding amount of D. menoni. The size of phloem tubes, the width of the vascular bundle and the total number of vascular bundles, were positively correlated. The level of WLD infection in natural environment was also correlated with the structure of leaf fibro vascular bundle.*

Originality/value: *Configuration of leaf fibro vascular bundles could be used to predict feeding of D. menoni, level of WLD infection, which could be incorporated into selection and directional breeding of sugarcane against the vector.*

Keywords: *Deltocephalus menoni, Feeding, Leaf vascular bundle, Sugarcane White Leaf Disease, Vector*

INTRODUCTION

Higher plants are characterized by vascular networks consisting of xylem and phloem distributes nutrients and water. As sugars, amino acids, other organic metabolites and water are translocated via phloem and xylem, they make the vascular system a target for insect pests (Brodbeck *et al.*, 1993; Gündüz and Douglas, 2009) especially, for phytophagous hemipterans.

There by the configuration of leaf vascular bundle of the plant plays an essential role in the

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interactions between plants and other organisms, both directly and obliquely.

Phytoplasma are insect-transmitted, phloem-limited, Gram-positive pathogens (Lee *et al.,* 2000) transmitted particularly by phloem-sap-feeding leafhoppers or psyllids among plants either in a persistent or non-persistent manner. In diseased plants, phytoplasma are located entirely in the phloem sieve tube elements, having been introduced by phloem-feeding homopteran insects, mainly leafhoppers (Cicadellidae), planthoppers (Fulgoromorpha), and less frequently by psyllids (Psyllidae) (Weintraub and Beanland, 2006). Once the phytoplasmas have entered the phloem sieve tube elements, they circulate systemically within the plant by transmitting through the phloem sieve plate pores.

Deltocephalus menoni (Hemiptera: Cicadellidae) is the solely identified vector of Sugarcane White Leaf Disease (WLD) in Sri Lanka (Senevirathna, 2008) which belongs to Subfamily Deltocephalinae. The feeding habits of the species within subfamily Deltocephalinae range from monophagous to polyphagous, and the species belong to this subfamily can transmit several taxa of phytoplasma to which, more than 75% of confirmed phytoplasma vector species have belong. Both adult and nymphs of *D. menoni* are sap-sucking from the lower surface of sugarcane leaf. The volumes of feeding on deferent sugarcane cultivars recorded to be variable.

Typically, to protect the configuration and the content of the sieve tubes, plants have varied chemical and physical defense mechanisms. Here, the configuration of the vascular bundle is considered an important characteristic. In sugarcane, anatomical characteristics transfer diverse levels of tolerance to stress conditions based on the genotypic characters, such as; the size of the midrib, the mesophyll, distance between vascular bundles, the leaf epidermis, thickness of the epidermis, cuticle thickness and the area of vascular bundles, *etc* (Akhtar *et al.,* 2001, Gardoni *et al.*, 2007, Castro *et al.,* 2009,

Ribeiro *et al.*, 2012, Pincelli and Silva, 2012).

Therefore, this study was conducted to determine whether the configuration of leaf fibro-vascular bundle affects the feeding of *D. menoni* in sugarcane varieties in order to design an enhanced management program for WLD by specific identification of resistant characteristics of varieties.

MATERIALS AND METHODS

This study was conducted at the Entomology Laboratory of the Sugarcane Research Institute (SRI), Uda Walawe in Sri Lanka. Sugarcane varieties: Co 775, SL 83 06, SL 92 5588, SL 96 128, SL 96 328, SL 97 1442, SLT 4921, SLC 2009 01, and the wild accessions SLC 92 95 *(Saccharum spontaneum)* and SLC 92 77 *(Erianthus arundinaceous)* were used in the study. Available information on WLD incidence in the natural environment was considered in selecting the test varieties/accessions for the study.

Configuration of leaf fibro-vascular bundle of sugarcane varieties that has with variable resistance to WLD

Sugarcane setts obtained from the mother plant nursery, established with hot water treated seed cane (54 ºC, 50 minutes), were used in the experiment. Single budded setts from each variety were obtained and potted in plastic pots (50 x 50 cm^2) using sterilized soil. 25 plants from each variety were kept in an insect proof screen house, maintained under recommended agronomic practices (SRI, 2004) for the experiments.

Third leaves of four-month-old plants were selected to study the configuration of the leaf fibro-vascular bundle. Tissue samples were collected from the leaf blades of collected leaves and tissue samples were excised from each side of the midrib, from the middle of the leaf along the length of the blade. Free-hand sections of leaves were obtained and the thin leaf sections

were observed under microscope at higher magnification (Olympus biological, model CX31 under 100X). Micro-photographs were taken and gross cross-sectional anatomy of the leaf blade was studied using micro-photographs. The thickness of the abaxial epidermis, and distance from the abaxial epidermis to vascular bundle, thickness of the phloem fiber layer, area of the phloem and width of the vascular bundle and number of vascular bundles available within 1.5mm length of the leaf lamina (Image analysis program, *CaptaVision* Imaging Software) were measured (figure 01)

Determining level of WLD infection by D. menoni under natural environment

Sugarcane setts obtained from a mother plant nursery established with hot water treated seed cane (54 ºC hot, 50 minutes) were used in the experiment. A *D. menoni* prevalent land was selected for the establishment of the trial. Three budded setts from each variety were obtained and three rows of 5 m each of sugarcane variety/ accessions were established according to the randomized complete block design (RCBD) in

three replicates. The trial was maintained under the recommended agronomic practices (SRI, 2004) and availability of *D. menoni* in the trial was confirmed by monitoring the population throughout plant crop stage using a sweep net. After rationing, WLD infected clumps were visually detected by considering the appearance of the test plants *viz.*, diffused, proliferated tillering, chlorotic leaves, yellow colour of fully-developed leaves of the mature canes and soft texture of symptomatic leaves (Chandrasena *et al.*, 2003; Senevirathne, 2008). Level of WLD infection by *D. menoni* was calculated as follows; (Number of WLD symptomatic clumps/ Total number of clumps) x 100.

Measuring the Amount of feeding of D. menoni

The adult insects of *D. menoni* were collected using a sweep net and a pooter from young sugarcane plants (< 6 months) in the research farm, Sugarcane Research Institute, Uda Walawe. The collected insects were reared in insect-rearing cages according to the protocol developed by Senevirathne (2008) to obtain one day old insects for the study.
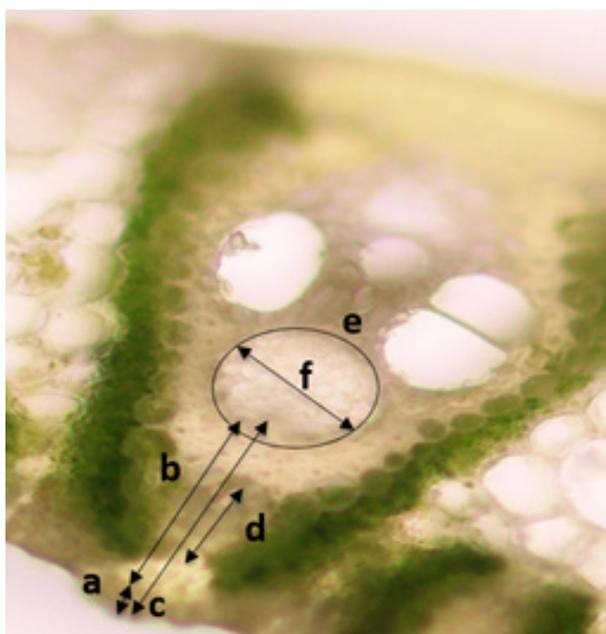
Figure 01: Measurements taken from cross section of a sugarcane leaf blade: **(a)** thickness of the abaxial epidermis, **(b)** distance from epidermis to vascular parenchyma **(c)** distance from abaxial epidermis to vascular bundle, **(d)** thickness of the phloem fiber layer, **(e)** area of the phloem and **(f)** width of the vascular bundle
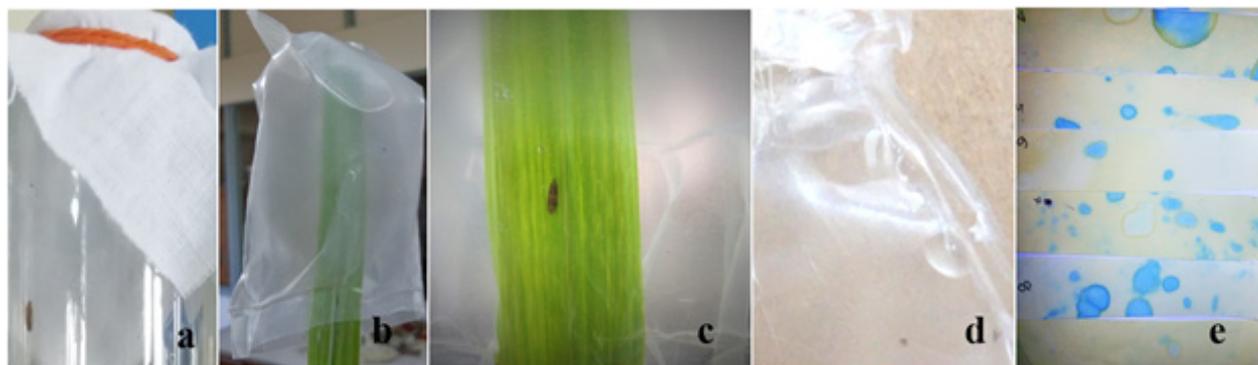
Figure 02: Experimental setup for measuring excreted honeydew amount of *D. menoni* (a).Water starving female *D. menoni* adult, (b).Encircling leaf portion of third leaf of each plant in Para Film ®sachet, (c). Female *D. menoni* adult in Parafilm ® sachet, (d). Honey- dew on the sachet and (e). Honeydew stained bromocresole green treated filter papers

Study on the amount of feeding

The feeding amount of *D. menoni* was recorded by honey-dew production on each test variety/accession, measured using the Parafilm sachet technique (Heinrichs *et al.*, 1985; Nugaliyadde, 1994).

Individual *D. menoni* adults were collected with a pooter from the insect cages and they were starved for 3 hrs on wet tissue papers in glass containers. Starved female vectors were inserted into the sachets individually where a four-centimeter- long leaf portion of the third leaf of each plant was encircled with Para Film ®. Vectors were left in the sachets for a period of 24 hours for feeding. Then the leaf was detached from the bottom margin of the Para film ® sachet to detect honey-dew production. Honey-dew on both sugarcane leaf and the sachet were stained with the bromocresol green-treated filter papers (Whatman No. 1) and they were carefully wind-dried. The stained areas on filter papers (blue) were measured using square millimeter grids.

Study of the salivary flanges on vascular area

Erythrosine dye test was conducted to study the salivary flanges on the vascular area. Leaf portions where insects fed (in Para-film sachet technique) were collected and dipped in staining solution of 0.1% erythrosine dye for 10-15 min and washed thoroughly for 5 mins with running water. The leaf portions were examined under a microscope. The salivary flanges on each plant species stained in pink colour were counted. Also free hand sections were taken from the leaf portions with salivary flanges to detect the stylet penetration path.

Data analysis

Analysis of variance was performed to test the configuration of leaf fibro-vascular bundle of the leaf blade, vector feeding and WLD infection on tested varieties/accessions (SAS 9.1.3). Square root transformed data were used in the analysis. Means were separated by using Duncan's Multiple Range Test (DMRT) at the 0.05 probability level.

Pearson correlation coefficient test was performed to detect the associations between level WLD infection and behavioral characteristics of the vector with the configuration of leaf fibro-vascular bundles of leaf blade. Principle Component Analysis (PCA) were performed using SAS software to identify major factors which contribute to the level of WLD infection and insect feeding on tested varieties/accessions.

RESULTS AND DISCUSSION

Structure of Leaf fibro-vascular bundles

In each sugarcane variety/accession, three types of vascular bundles were observed *viz.*, large (a), medium (b) and small (c) (Figure 03). The large

bundles were observed to be rhomboid or oval, the medium ones oval and the small ones were round. The small bundles were situated closer to the lower epidermis, while the large and medium ones slightly away from the epidermis. The large bundles were flanked by two small ones most of the times, the small and medium-sized bundles were observed between the large bundles alternatingly with each other (Figure 04).

In the leaf blade, fibro vascular bundles were surrounded by a ring of parenchyma cells, which comprised chlorophyll and they were termed chlorophyll-bearing bundle sheath. The bundle themselves consisted of xylem and phloem, while a cap of fibers each was found at the phloem side (Figure 04).

Colbert and Evert (1982), Ferreira (2007), Joarder *et al.,* (2010) and Van dillewijn (1952) described similar structures of sugarcane hybrids and the variation of fibro-vascular bundles in different sugarcane varieties. They have also described that there were no strict patterns of the structural arrangement of vascular bundles among varieties and in general all three types of vascular bundles may not be present in each variety/accession.

Configuration of leaf fibro-vascular bundle of the leaf lamina *viz.*, thickness of the abaxial epidermis, distance from abaxial epidermis to vascular bundle, the thickness of the phloem fiber layer, area of the phloem, width of the vascular bundle and the number of vascular bundles available within 1.5 mm length of the leaf lamina, cross-section were varied with different sugarcane accessions. Joarder *et al.,* (2010) also confirmed that quantitative expression of tissues varies between sugarcane cultivars.
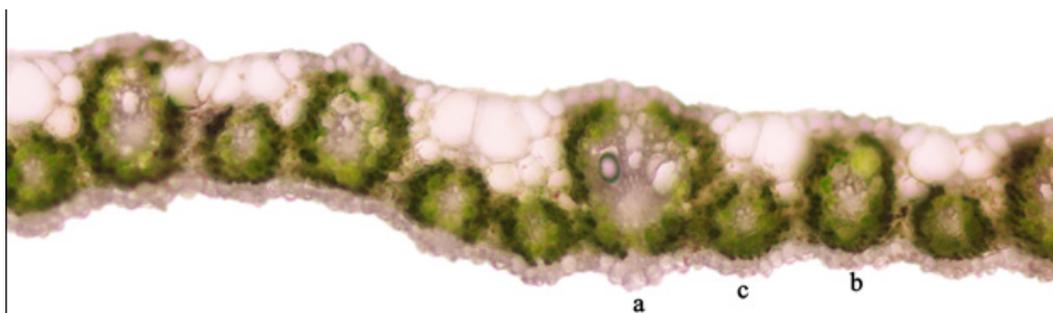
Figure 03: **Cross section of a sugarcane leaf blade showing large (a), medium (b) and small (c) types of fibro vascular bundles observed under 100X enlargement using free hand sections of the third leaf**
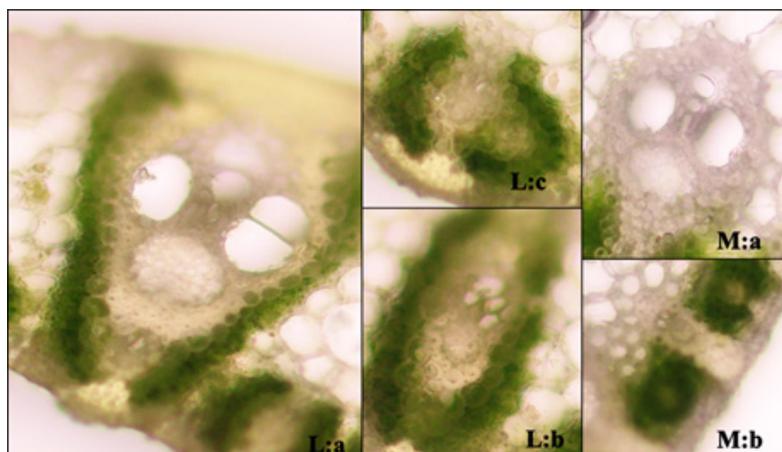
Figure 04: **Cross sections of sugarcane leaf blade fibro vascular bundles (large (L-a), medium (L-b) and small (L-c)) and vascular bundles in mid rib (large (M-a), small (M-b)) observed under 100X using free hand sections of the third leaf**

All the test varieties were associated with one large vascular bundle in a unit area (Table 01). The highest number of medium vascular bundles (4±0) were recorded in varieties SL 96 128 and SL 97 1442. The highest number of small vascular bundles was recorded in accession SLC 92 95 and the lowest in accession SLC 92 77.

The highest width of the epidermis was recorded in varieties SLC 92 77 (11.85±0.35) and SL 83 06 (11.68±0.01). The width of the epidermis was less in variety SLC 2009 01 (06.94±0.01) and accession SLC 92 95 (06.95±0.08).

The highest thickness of the leaf blades was recorded on varieties SL 96 128 (328.63±0.74) and SL 96 328 (331.00±1.24) while the lowest in variety SLT 4921 (110.85±0.15).

Distance from the epidermis to vascular parenchyma, distance from epidermis to vascular bundle, thickness of phloem fiber layer, area of phloem and width of the vascular bundle on the third leaf of test sugarcane variety/accession varied in the test varieties (Tables 01 and 02). The highest distance from epidermis to vascular parenchyma in the leaf-large vascular bundle was recorded in variety SL 92 5588 (40.98±0.48) and accession SLC 92 95 (40.26±0.17), and the lowest distance in variety SL 96 128 (17.83±0.02). In leaf-medium vascular bundle, the highest distance from epidermis to vascular parenchyma was recorded in variety SL 83 06 (42.42±0.27) and the lowest distance in variety SL 97 1442 (13.17±0.03). In leaf-small vascular bundle, the highest distance from epidermis to vascular parenchyma was recorded in variety SL 92 5588 (40.11±0.48) and the lowest in variety SL 97 1442 (11.22±0.01).

The highest distance from epidermis to vascular bundle in leaf-large vascular bundle was found in variety SL 83 06 (112.49±0.29), and the lowest distance in varieties SL 96 128 (57.63±0.10) and SLC 2009 01 (57.05±0.03). In leaf-medium vascular bundle, the highest distance from epidermis to vascular bundle was recorded in variety SL 83 06 (90.23±0.39) and the lowest distance in variety SL 97 1442 (31.36±0.03). In leaf-small vascular bundle, the highest distance from epidermis to vascular bundle was recorded in variety SL 96 328 (63.14±0.09) and accession SLC 92 77 (61.49±0.01), and the lowest distance in variety SL 97 1442 (31.37±0.02).

Table 01: **Variation of number of vascular bundles, width of epidermis and thickness of leaf blade of tested sugarcane varieties/accessions**

Variety	Number of vascular bundles (#)			Width of Epidermis (μm)	Thickness of Leaf blade (μm)
	Large	Medium	Small		
Co 775	1±0[a]	2±0[c]	6±0[c]	7.62±0.003[e]	293.56±0.22[b]
SL 8306	1±0[a]	3±0[b]	5±0[d]	11.68±0.01[a]	207.40±5.97[c]
SL 92 5588	1±0[a]	2±0[c]	4±0[e]	10.80±0.06[b]	223.16±0.12[c]
SL 96 128	1±0[a]	2±0[c]	7±0[b]	08.66±0.33[d]	328.63±0.74[a]
SL 96 328	1±0[a]	4±0[a]	4±0[e]	09.76±0.03[c]	331.00±1.24[a]
SL 97 1442	1±0[a]	4±0[a]	5±2[d]	08.38±0.02[d]	219.70±1.22[cd]
SLT 4921	1±0[a]	2±0[c]	5±0[d]	07.49±0.01[e]	110.85±0.15[f]
SLC 2009 01	1±0[a]	3±0[b]	4±0[e]	06.94±0.01[f]	211.58±4.24[cd]
SLC 92 95	1±0[a]	3±0[b]	8±0[a]	06.95±0.08[f]	179.93±12.1[e]
SLC 92 77	1±0[a]	3±0[b]	3±0[f]	11.85±0.35[a]	173.60±4.62[e]

Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.

Table 02: **Variation in fibro vascular bundle on third leaf of tested sugarcane varieties/accessions**

Variety	Distance from epidermis to vascular parenchyma (µm)	Distance from epidermis to vascular bundle (µm)	Thickness of phloem fiber layer (µm)	Area of phloem (µm^2)	Width of vascular bundle (µm)
Leaf-Large vascular bundles					
Co 775	25.88±0.19[f]	60.65±0.67[f]	14.09±0.02[f]	2658.18±19.44[a]	227.74±1.16[b]
SL 8306	30.67±0.27[d]	112.49±0.29[a]	40.68±0.29[a]	1264.72±02.62[h]	271.62±0.31[b]
SL 92 5588	40.98±0.48[a]	79.86±0.44[c]	38.25±0.17[b]	1976.42±03.21[d]	218.67±5.10[e]
SL 96 128	17.83±0.02[h]	57.63±0.10[g]	12.83±0.04[g]	2056.11±02.86[c]	350.09±0.63[a]
SL 96 328	38.11±0.15[b]	67.46±0.26[d]	13.30±0.25[g]	2059.14±27.92b[c]	236.22±2.40[c]
SL 97 1442	14.35±0.09[i]	62.91±0.01[e]	13.38±0.01[g]	1296.96±01.98[g]	184.26±0.37[g]
SLT 4921	31.60±0.25[c]	63.00±0.06[e]	26.51±0.29[d]	1403.47±02.62[f]	169.55±0.04[h]
SLC 2009 01	22.64±0.23[g]	57.05±0.03[g]	11.88±0.01[h]	2088.01±00.05[b]	181.47±0.29[g]
SLC 92 95	40.26±0.17[a]	83.58±0.49[b]	19.38±0.23[e]	1289.67±00.42[gh]	170.89±0.06[h]
SLC 92 77	26.66±0.18[e]	66.91±0.04[d]	31.34±0.08[c]	1563.15±00.58[e]	206.00±0.57[f]
Leaf-Medium vascular bundles					
Co 775	21.71±0.15[f]	60.11±0.06[g]	12.37±0.02[f]	1095.40±00.31[e]	99.35±0.28[h]
SL 8306	30.66±0.17[d]	90.23±0.39[a]	30.25±0.15[b]	1020.76±00.63[f]	142.47±9.74[f]
SL 92 5588	42.42±0.27[a]	66.15±0.05[d]	37.57±0.05[a]	1269.25±76.24[a]	170.53±0.36[c]
SL 96 128	25.54±8.44[h]	62.97±1.60[f]	20.56±8.50[g]	1421.74±01.19[d]	183.81±6.65[a]
SL 96 328	31.41±0.22[c]	66.73±0.38[d]	11.52±0.01[h]	1238.58±02.26[b]	180.72±0.65[b]
SL 97 1442	13.17±0.03[i]	31.36±0.03[i]	19.66±0.04[d]	916.13±00.03[h]	162.41±0.30[dc]
SLT 4921	29.54±0.04[e]	64.94±0.03[e]	27.54±0.01[c]	0977.41±00.89[g]	150.77±0.67[ef]
SLC 2009 01	21.77±0.09[f]	56.07±0.07[h]	10.07±0.03[i]	1206.42±00.30[c]	157.80±0.15d[e]
SLC 92 95	32.96±0.14[b]	68.63±0.07[b]	13.82±0.18[e]	564.56±01.77[j]	144.70±0.25[f]
SLC 92 77	20.30±0.01[g]	67.80±0.20[c]	37.67±0.09[a]	902.36±00.33[i]	131.92±0.04[g]
Leaf-Small vascular bundles					
Co 775	19.79±0.01[f]	25.99±0.03[j]	7.85±0.04[i]	976.74±01.12[a]	99.63±0.02[b]
SL 8306	33.50±0.09[b]	52.49±0.07[d]	27.57±0.16[b]	840.00±00.01[c]	90.54±0.03[d]
SL 92 5588	40.11±0.03[a]	49.05±0.01[g]	22.63±0.02[d]	451.13±00.07[h]	89.78±0.01[e]
SL 96 128	24.67±7.71[i]	50.43±0.70[e]	15.63±3.50[f]	534.37±41.62[f]	83.89±2.94[h]
SL 96 328	28.54±0.03[g]	63.14±0.09[a]	4.94±0.02[j]	916.49±00.01[b]	97.45±0.03[c]
SL 97 1442	11.22±0.01[j]	31.37±0.02[i]	15.87±0.09[e]	55.37±00.10[j]	75.45±0.04[i]
SLT 4921	29.52±0.02[d]	54.46±0.02[c]	26.84±0.01[c]	810.98±00.52[d]	83.7±0.006[g]
SLC 2009 01	17.73±0.01[h]	49.35±0.18[f]	8.08±0.01[h]	469.11±00.06[g]	105.33±0.03[a]
SLC 92 95	32.80±0.01[c]	48.06±0.03[h]	8.99±0.01[g]	390.69±00.15[i]	57.09±0.01[j]
SLC 92 77	28.90±0.01[e]	61.49±0.01[b]	29.22±0.00[a]	666.35±00.32[e]	87.76±0.00[f]

Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.

Thickness of phloem fiber layer in leaf-large vascular bundle was high in varieties SL 83 06 (40.68±0.29) and SL 92 5588 (38.25±0.17), and low in variety SLC 2009 01 (11.88±0.01). Thickness of phloem fiber layer in leaf-medium vascular bundle was high in varieties SLC 92 77 (37.67±0.09) and SL 92 5588 (37.57±0.05), and low in variety SLC 2009 01 (10.07±0.03). The thickness of phloem fiber layer in leaf-small vascular bundle was high in variety SL 83 06 (27.57±0.16) and accession SL 92 77 (29.22±00), and low in variety SL 96 328 (4.94±0.02).

Area of phloem in leaf-large vascular bundle was high in variety Co 775 (2658.18±19.44) and it was lower in variety SL 83 06 (1264.72±2.62). Area of phloem in leaf-middle vascular bundle was high in variety SL 96 128 (1421.74±01.19) and it was low in variety SLC 92 95 (0564.56±01.77). Area of phloem in leaf-small vascular bundle was high in variety Co 775 (976.74±1.12) and it was low in variety SL 92 5588 (451.13±0.07). (Table 03)

Level of WLD infection by D. menoni under the natural environment

Under moderate pressure of *D. menoni* and WLD inoculum in the surrounding area, WLD infection was significantly higher ($F_{8, 18} = 4.75$, $p<0.05$) in varieties/accessions SL 97 1442, SL 96 128 and Co 775. There was no WLD infection observed in variety SL 92 77 (Table 04).

Table 03: **Variation in fibro vascular bundle on midrib of the third leaf of test sugarcane varieties/ accessions**

Variety	Distance from epidermis to vascular parenchyma (μm)	Distance from epidermis to vascular bundle (μm)	Thickness of phloem fiber layer (μm)	Area of phloem /A (μm²)	Width of vascular bundle (μm)
Co 775	35.64±0.08[e]	82.43±0.008[b]	14.05±0.03[g]	162.50±0.24[j]	155.69±0.10[a]
SL 8306	50.25±0.07[a]	74.22±0.10[d]	28.95±0.30[a]	1033.16±0.09[d]	82.23±0.07[i]
SL 92 5588	44.01±0.02[c]	75.93±0.02[c]	22.47±0.01[d]	843.66±0.23[g]	89.62±0.12[h]
SL 96 128	29.48±7.31[i]	66.03±4.94[g]	15.17±3.65[h]	1657.70±1.28[a]	139.18±0.09[c]
SL 96 328	47.3±0.035[b]	84.56±0.01[a]	18.06±0.06[f]	1652.44±0.27[b]	140.2±0.008[b]
SL 97 1442	20.76±0.13[j]	47.47±0.28[i]	20.59±0.13[e]	1633.09±0.12[c]	155.87±0.01[a]
SLT 4921	26.82±0.06[h]	63.34±0.02[f]	25.04±0.02[c]	1007.97±0.02[e]	109.56±0.30[e]
SLC 2009 01	35.22±0.07[f]	41.99±0.04[j]	10.27±0.02[j]	610.16±0.03[i]	112.09±0.10[d]
SLC 92 95	33.12±0.10[g]	57.45±0.22[h]	10.33±0.03[i]	829.37±0.33[h]	103.02±0.04[g]
SLC 92 77	38.92±0.03[d]	68.79±0.14[e]	26.73±0.02[b]	1.02±0.59[f]	106.19±0.10[f]

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Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.

Table 04: **Level of disease infection and amount of feeding of *D. menoni* in sugarcane varieties/ accessions tested**

Variety/Accession	Level of disease infection	Amount of feeding
Co 775	4.80 ±0.09[a]	32.45 ±0.07[a]
SL 83 06	0.78 ±1.73[d]	08.38±0.06[d]
SL 92 5588	0.35 ±1.73[de]	08.09 ±0.02[d]
SL 96 128	4.86 ±0.09[a]	31.59 ±0.04[a]
SL 96 328	2.42 ±0.03[c]	20.25 ±0.08[b]
SL 97 1442	5.26 ±0.21[a]	18.81 ±0.03[b]
SLC 2009 01	3.88 ±0.31[ab]	09.67 ±0.05[d]
SLT 4921	0.93 ±1.73[d]	12.09 ±0.12[c]
SLC 92 95	1.02 ±1.01[cd]	08.82 ±0.04[d]
SLC 92 77	0.00[e]	01.34 ±1.10[e]

Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.

Amount of feeding by D. menoni

Mean honeydew production of *D. menoni* was significantly higher ($F_{8, 34}$ =12.6, $p<0.05$) when it fed on the varieties SL 96 128 (31.59 ± 0.07 mm^2) and Co 775 (32.45 ± 0.07 mm^2). Hence, these two sugarcane varieties could be considered as the most preferred for feeding by WLD vector. The lowest honeydew production (1.34 ± 1.1 mm^2) was found when *D. menoni* fed on SLC 92 77 (Table 04).

Salivary flanges on vascular area

Less number of salivary flanges were recorded on varieties SL 96 328, SLC 2009 01, SL 97 1442, SL 96 128, and Co 775, which can be identified as preferred for feeding by *D. menoni*. The high number of salivary flanges recorded on SLC 92 77 (*E. arundinaceous*), SL 92 5588 and SLC 92 55 (*S. spontaneum*), varieties considered to be less preferred by WLD vector.

According to many authors, leafhoppers apparently form a stylet sheath and puncture usually to reach the phloem tissues (Fife and Frampton, 1936). They continue feeding via previously prepared stylet sheaths several times. When leaf surface is less preferred or resistant for feeding, insects search for a suiTable 0position to insert the proboscis (test bites). When the leaf is more resistant for feeding, the number of test bites on a leaf increases. When the leaf is more preferable/vulnerable for feeding either one or a very few number of punctures are made on the leaf. Our results are in agreement with the above statement that in the varieties associated with higher honeydew production numbers of feeding punctures were less and, in the varieties associated with less honeydew production number of feeding punctures recorded to be high.

A significantly higher number of the salivary flangues of *D. menoni* stained with Erythrosine dye were detected on leaf lamina which followed stylet pathways up to large vascular bundles. Comparatively, a less number of salivary flanges were associated with stylet pathways that reached medium and small vascular bundles. No salivary flanges of *D. menoni* were observed to be in association with stylet pathways that reach phloem of central or smaller vascular bundles of the midrib.
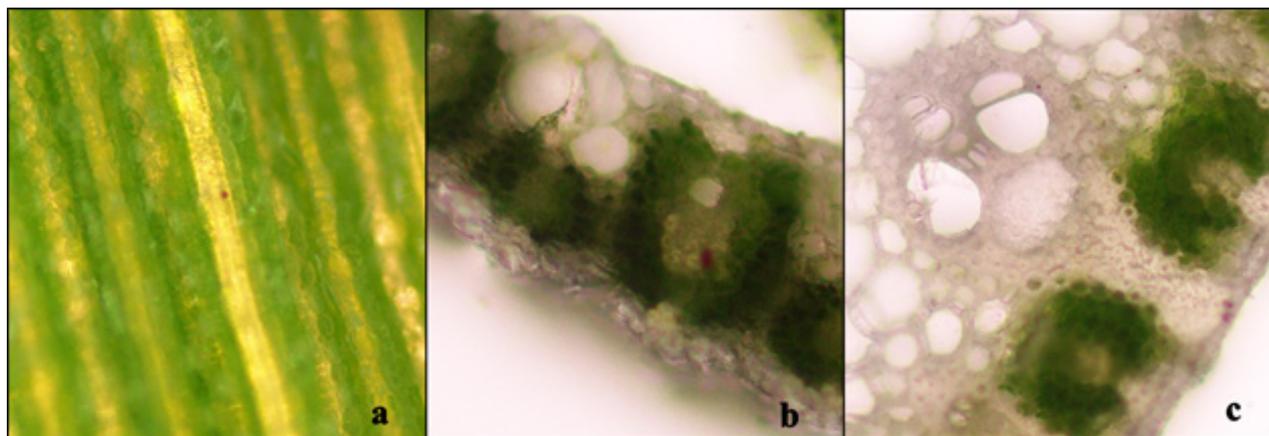
Figure 05a: A salivary flanges of *D. menoni* stained with Erythrosine dye on leaf lamina; b: Stylet pathway of *D. menoni* that reached phloem of a large vascular bundle; c: A salivary flange of *D. menoni* on central vascular bundle of midrib which did not form stylet pathway up to phloem, observed under 100X enlargement using free hand sections.

Colbert and Evert (1982) reviewed that, larger veins are accounTable 0for the bulk of longitudinal translocation, while the miner bundles were more responsible in the collection of photosinthates and their movement over shorter distances. Significantly, a higher number of the salivary flanges of *D. menoni* stains on leaf lamina were detected with stylet pathways up to large vascular bundles and may due to higher concentrations of photosinthates translocate within the larger vascular bundles.

Relationship of configuration of leaf fibro vascular bundle with behavioural characteristics of D. menoni and the level of disease infection in natural environment

The aggregation, amount of feeding, rate of nymphs convert into adults, oviposition and adult longevity of *D. menoni* on sugarcane were correlated with the configuration of leaf fibro vascular bundles. Distance from the epidermis to vascular parenchyma, distance from epidermis to vascular bundle and thickness of phloem fiber layer were negatively correlated with the behavioural characteristics of *D. menoni*. The size (A) of phloem tubes and the width of vascular bundles were positively correlated with the behavioural characteristics of *D. menoni*

(Table 04). The total number of vascular bundles in the sheath and the thickness of leaf blade were positively correlated with the behavioural characteristics of *D. menoni*. The area of the phloem was positively correlated with the thickness of leaf blade suggesting that thickness of leaf blade can predict the size of phloem.

Level of WLD infection in the natural environment was also correlated with the configuration of fibro vascular bundles. Distance from epidermis to vascular parenchyma, distance from epidermis to vascular bundle and thickness of phloem fiber layer were negatively correlated to the level of disease infection and width of vascular bundle was positively correlated with the behavioural characteristics of *D. menoni* (Table 05). No significant correlation was observed between the total number of vascular bundles in sheath, thickness of leaf blade and the level of disease infection.

Varieties/accessions with higher leaf width *i.e.* SL 96 128, SL 96 328 and Co 775 were mostly associated with thinner phloem fiber layers and the least sucking distances which facilitate higher amounts of feeding by *D. menoni*. Conversely, Varieties/accessions with the least leaf width were associated with thicker phloem fiber layers and widest sucking distances, which reduced the amounts of the feeding by *D. menoni*.

Table 05: Correlations (R) of configuration of leaf fibro vascular bundles with feeding amount of *D. menoni*

Relationship for feeding	R	P
Thickness of Leaf blade	0.58	0.0008
Total number of vascular bundles	0.52	0.003
Fiber layer: Large vascular bundle	-0.49	0.005
Width of vascular bundle: Large vascular bundle	0.81	<0.0001
Fiber layer: Medium vascular bundle	-0.65	0.0001
Epidermis to vascular parenchyma: Small vascular bundle	-0.58	0.0007
Epidermis to vascular bundle : Small vascular bundle	-0.51	0.004
Fiber layer: Small vascular bundle	-0.58	0.0007
Fiber layer: Vascular bundle of Mid-rib	-0.49	0.0052
Width of vascular bundle: Vascular bundle of Mid-rib	0.81	<0.0001

Table 06: Correlations of configuration of leaf fibro vascular bundles with the level of WLD infection in natural environment

Relationship with the level of WLD	R	P
Leaf-Large Vascular Bundles		
Epidermis to vascular parenchyma	-0.685	0.0001
Epidermis to vascular bundle	-0.533	0.0024
Fiber layer	-0.760	<0.0001
Phloem area	0.448	0.0118
Leaf-Medium Vascular Bundles		
Epidermis to vascular parenchyma	-0.667	0.0001
Epidermis to vascular bundle	-0.635	0.0002
Fiber layer	-0.685	<0.0001
Leaf-Small Vascular Bundles		
Epidermis to vascular parenchyma	-0.849	<0.0001
Epidermis to vascular bundle	-0.602	0.0004
Fiber layer	-0.598	0.0005
Mid Rib-Vascular Bundles		
Epidermis to vascular parenchyma	-0.535	0.0023
Fiber layer	-0.559	0.0013

In line with our findings Kumarasinghe *et al,* (2001) confirmed that, thickness of the phloem fiber layer in the miner vascular bundle of sugarcane affects the relative growth rate of the first nymphal stage of *Pyrilla perpusilla* (Homoptera: Lophophidae). Khanna *et al.* (1950) described that it is difficult for the insect to suck sap from plants where phloem bundles are protected by a concealment formed by the merging of vascular sheaths.

Agrawal (1969) recorded that, the thickness of leaf sheath and the distance between the inner epidermis and the vascular bundles affect the resistance of sugarcane to sugarcane mite (*Eriophyid* sp.). Further, resistance of crop species (yard long bean) to insect (*Aphis craccivora*) attack due to thicker epidermis have been reported (Benchasri *et al.*, 2012).

Peeters (2002a) confirmed that, lamina thickness, distance to phloem, lower and upper depths

between phloem of primary and secondary veins and cuticle thickness are negatively correlated with feeding and population density of phloem feeders on their host plants. Also, Peeters (2002a) reviewed that, the internal anatomy of leaves influences the herbivores' feeding and distribution. Feeding preference of the sucking insects, this feed on vascular tissues links to vein size, depth vain density, and distance from the surface and vein sclerification (Sosnovsky, 2016).

And also, Cohen *et al.* (1996), Peeters (2002b) and Sosnovsky (2016) confirmed that, a comparatively higher distance between the veins and sucking distance negatively affects the host acceptance of herbivores directing vascular tissues in the thick-leaved species of Ficus.

Three components of plant characters (PCs) of the selected sugarcane varieties/accessions were retained 100% (Table 07). The identified PCs for dependent variables are illustrated below *viz.,*

Table 07: **Component loadings from the Principle Component Analysis of configuration of leaf fibro vascular bundles of the selected sugarcane varieties/accessions with amount of vector feeding**

Plant Trait	Components		
	PC1	PC 2	PC3
Large vascular bundle			
Distance from epidermis to vascular parenchyma	0.71229	0.06840	0.69855
Distance from epidermis to vascular bundle	0.24513	-0.25518	0.93531
Thickness of phloem fiber layer	0.17753	-0.79663	0.57781
Area of phloem	0.73467	0.29524	0.61082
Width of vascular bundle	0.80435	0.23710	0.54481
Medium vascular bundle			
Distance from epidermis to vascular parenchyma	-0.01886	-0.94633	0.32265
Distance from epidermis to vascular bundle	0.32049	0.25171	0.91319
Thickness of phloem fiber layer	-0.00394	0.84981	0.52707
Area of phloem	0.62510	-0.33317	0.70586
Width of vascular bundle	0.98977	-0.12858	-0.06184
Small vascular bundle			
Distance from epidermis to vascular parenchyma	-0.13158	-0.99098	0.02544
Distance from epidermis to vascular bundle	0.99314	0.11347	-0.02816
Thickness of phloem fiber layer	0.93665	0.19553	0.29062
Area of phloem	0.89908	0.00894	0.43769
Width of vascular bundle	0.90554	0.14455	0.39887
Vascular bundle - mid rib			
Distance from epidermis to vascular parenchyma	0.07350	-0.98542	-0.15345
Distance from epidermis to vascular bundle	-0.19843	0.88471	-0.42179
Thickness of phloem fiber layer	-0.35944	0.72137	-0.59197
Area of phloem	0.00000	0.00000	0.00000
Width of vascular bundle	-0.09932	0.67649	-0.72972
Number of available vascular bundles			
Large vascular bundle	-0.75824	0.64194	0.11398
Medium vascular bundle	0.43707	0.86041	0.26202
Small vascular bundle	0.44345	0.88998	0.10620
Width of Epidermis	-0.21891	-0.41533	0.88294
Thickness of Leaf blade	0.46073	0.39997	0.79231

PC1 = (Distance from epidermis to vascular parenchyma: Large vascular bundle) 0.71 + (Area of phloem: Large vascular bundle) 0.73 + (Width of vascular bundle: Large vascular bundle) 0.81 + (Width of vascular bundle: Medium vascular bundle) 0.99 + (Distance from epidermis to vascular bundle: Small vascular bundle) 0.99 + (Thickness of phloem fiber layer: Small vascular bundle) 0.94+ (Area of phloem: Small vascular bundle) 0.89 + (Width of vascular bundle: Small vascular bundle) 0.91 + (Number of available vascular bundles: Large) -0.76

PC2 = (Thickness of phloem fiber layer: Large vascular bundle) -0.79 + (Distance from epidermis to vascular parenchyma: MVB) -0.94+ (Thickness of phloem fiber layer: Medium vascular bundle) 0.84+ (Distance from epidermis to vascular parenchyma: Medium vascular bundle) 0.99+ (Distance from epidermis to vascular parenchyma: Mid rib) 0.98+ (Distance from epidermis to vascular bundle: Mid rib) 0.88+ (Thickness of phloem fiber layer: Mid rib) 0.87+ (Number of available vascular bundles: Medium) 0.86+ (Number of available vascular bundles: Small) 0.88

PC3 = (Distance from epidermis to vascular bundle: Large vascular bundle) 0.93 + (Distance from epidermis to vascular bundle: Medium vascular bundle) 0.91 + (Area of phloem: Medium vascular bundle) 0.71 + (Width of vascular bundle: Vascular bundle - mid rib) − 0.73 + (Width of Epidermis) 0.88 + (Thickness of Leaf blade) 0.79

The findings of this study could facilitate the plant breeder to assess the plants resistant to WLD vector by enhancing those particular plant characteristics. PCA on plant characters and behavioral characteristics of *D. menoni* can be used in more precise identification of the resistant varieties in the variety evaluation process against WLD vector.

Sosnovsky (2016) reviewed that, as mobile phloem feeders are having active life habits, these herbivores are capable of choosing the most suiTable 0feeding sites on a plant. Arthropod herbivores are efficient in detecting the plants that provide food resources of higher quantity and quality. Leaf nutrient quality and the nonexistence of self-protective structures are the most considered factors in the host selection of herbivores. They prefer hosts where they could spend less costs on energy for feeding. Accordingly, *D. menoni* could be showing a higher rate of feeding on varieties with less fiber layer thickness, lower distance from the epidermis to vascular parenchyma and vascular bundle as they are supposed to penetrate a shorter distance to access phloem tissues and therefore, the least barrier to penetrate through.

CONCLUSIONS

The structure of leaf fibro-vascular bundle varied with the sugarcane variety/accession and the amount of feeding of *D. menoni* on sugarcane correlated with the configuration of leaf-fibro-vascular-bundle. Distance from epidermis to vascular parenchyma, distance from epidermis to vascular bundle and thickness of phloem fiber layer were negatively correlated to the amount of feeding of *D. menoni*. The size of phloem tubes, the width of vascular bundle and total number of the vascular bundles, were positively correlated with the amount of feeding of *D. menoni.* Level of WLD infection in the natural environment was also correlated with the structure of leaf-fibro-vascular-bundles. Thus, the configuration of leaf-fibro-vascular bundles could be used to predict behavioural characteristics of *D. menoni* and level of WLD infection in the natural environment. Therefore, sugarcane varieties/accessions having higher distances from epidermis to vascular parenchyma, from epidermis to vascular bundle and thickness of phloem fiber layer and smaller vascular bundle size, reduced phloem fiber layer could be incorporated into directional breeding of sugarcane varieties with resistance to the vector of WLD. Secondary transmission of WLD in commercial sugarcane plantations can be reduced by growing such varieties with a potential to reduce vector feeding thus reducing the level of disease in the natural environment.

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