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...
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 °C, 50 minutes), were used in the experiment. Single budded setts from each variety were obtained and potted in plastic pots (50 x 50 cm²) 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 °C 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](image.png)

**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
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.

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 (40.11±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 92 5588 (40.11±0.48) 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±0a  | 2±0b   | 6±0c  | 7.62±0.003e          | 293.56±0.22b             |
| SL 8306 | 1±0a  | 3±0b   | 5±0d  | 11.68±0.01a          | 207.40±5.97c             |
| SL 92 5588 | 1±0a   | 2±0c   | 4±0e  | 10.80±0.06b          | 223.16±0.12c             |
| SL 96 128 | 1±0a  | 2±0c   | 7±0b  | 08.66±0.33d          | 328.63±0.74c             |
| SL 96 328 | 1±0a  | 4±0b   | 4±0e  | 09.76±0.03c          | 331.00±1.24a             |
| SL 97 1442 | 1±0a   | 4±0b   | 5±2d  | 08.38±0.02d          | 219.70±1.22ad            |
| SLT 4921 | 1±0a  | 2±0c   | 5±0d  | 07.49±0.01e          | 110.85±0.15f             |
| SLC 2009 01 | 1±0a   | 3±0b   | 4±0e  | 06.94±0.01f          | 211.58±4.24ad            |
| SLC 92 95 | 1±0a  | 3±0b   | 8±0e  | 06.95±0.08f          | 179.93±12.1c             |
| SLC 92 77 | 1±0a  | 3±0b   | 3±0f  | 11.85±0.35e          | 173.60±4.62c             |

Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.
| Variety | Distance from epidermis to vascular parenchyma (µm) | Distance from epidermis to vascular bundle (µm) | Thickness of phloem fiber layer (µm) | Area of phloem (µm²) | Width of vascular bundle (µm) |
|---------|---------------------------------------------------|---------------------------------------------|----------------------------------|-------------------|---------------------------|
| **Leaf-Large vascular bundles** |
| Co 775   | 25.88±0.19f                                      | 60.65±0.67f                                  | 14.09±0.02f                                    | 2658.18±19.44f                             | 227.74±1.16b                        |
| SL 8306  | 30.67±0.27d                                      | 112.49±0.29g                                 | 40.68±0.29a                                   | 1264.72±02.62b                             | 271.62±0.31b                        |
| SL 92 5588 | 40.98±0.48c                                     | 79.86±0.44c                                  | 38.25±0.17b                                   | 1976.42±03.21d                             | 218.67±5.10c                        |
| SL 96 128 | 17.83±0.02b                                     | 57.63±0.10g                                  | 12.83±0.04g                                   | 2056.11±02.86c                             | 350.09±0.63a                        |
| SL 96 328 | 38.12±0.15h                                     | 67.46±0.26d                                  | 13.30±0.25g                                   | 2059.14±27.92c                             | 236.22±2.40c                        |
| SL 97 1442 | 14.35±0.09g                                     | 62.91±0.01f                                  | 13.38±0.01g                                   | 1296.96±01.98f                             | 184.26±0.37g                        |
| SLT 4921 | 31.60±0.25c                                     | 63.00±0.06f                                  | 26.51±0.29d                                   | 1403.47±02.62f                             | 169.55±0.04h                        |
| SLC 2009 01 | 22.64±0.23g                                    | 57.05±0.03g                                  | 11.88±0.01h                                   | 2088.01±00.05b                             | 181.47±0.29g                        |
| SLC 92 95   | 40.26±0.17f                                     | 83.58±0.49h                                  | 19.38±0.23e                                   | 1289.67±00.42e                             | 170.89±0.06b                        |
| SLC 92 77  | 26.66±0.18e                                     | 66.91±0.04a                                  | 31.34±0.08e                                   | 1563.15±00.58e                             | 206.00±0.57f                        |
| **Leaf-Medium vascular bundles** |
| Co 775   | 21.71±0.15f                                      | 60.11±0.06e                                  | 12.37±0.02f                                   | 1095.40±00.31e                             | 99.35±0.28b                         |
| SL 8306  | 30.66±0.17d                                      | 90.23±0.39a                                  | 30.25±0.15b                                   | 1020.76±00.63f                             | 142.47±9.74f                        |
| SL 92 5588 | 42.42±0.27d                                     | 66.15±0.05g                                  | 37.57±0.05a                                   | 1269.25±76.24a                             | 170.53±0.36c                        |
| SL 96 128 | 25.54±8.44a                                     | 62.97±1.60f                                  | 20.56±8.50g                                   | 1421.74±01.19f                             | 183.81±6.65a                        |
| SL 96 328 | 31.41±0.22c                                     | 66.73±0.38g                                  | 11.52±0.01h                                   | 1238.58±02.26d                             | 180.72±6.65b                        |
| SL 97 1442 | 13.17±0.03c                                     | 31.36±0.03b                                  | 19.66±0.04d                                   | 916.13±00.03b                              | 162.41±0.30c                        |
| SLT 4921 | 29.54±0.04e                                     | 64.94±0.03e                                  | 27.54±0.01c                                   | 0977.41±00.89f                             | 150.77±0.67f                        |
| SLC 2009 01 | 21.77±0.09f                                    | 56.07±0.07b                                  | 10.07±0.03f                                   | 1206.42±00.30b                             | 157.80±0.15d                        |
| SLC 92 95   | 32.96±0.14h                                     | 68.63±0.07b                                  | 13.82±0.18e                                   | 564.56±01.77f                              | 144.70±0.25f                        |
| SLC 92 77  | 20.30±0.01f                                     | 67.80±0.20b                                  | 37.67±0.09a                                   | 902.36±00.33f                              | 131.92±0.04f                        |
| **Leaf-Small vascular bundles** |
| Co 775   | 19.79±0.01f                                      | 25.99±0.03f                                  | 7.85±0.04f                                    | 976.74±01.12f                              | 99.63±0.02b                         |
| SL 8306  | 33.50±0.09h                                      | 52.49±0.07g                                  | 27.57±0.16b                                   | 840.00±00.01f                              | 90.54±0.03d                         |
| SL 92 5588 | 40.11±0.03f                                     | 49.05±0.01f                                  | 22.63±0.02d                                   | 451.13±00.07f                              | 89.78±0.01c                         |
| SL 96 128 | 24.67±7.71l                                     | 50.43±0.70c                                  | 15.63±3.50f                                   | 534.37±41.62f                              | 83.89±2.94b                         |
| SL 96 328 | 28.54±0.03g                                     | 63.14±0.09e                                  | 4.94±0.02f                                    | 916.49±00.01f                              | 97.45±0.03c                         |
| SL 97 1442 | 11.22±0.01l                                     | 31.37±0.02f                                  | 15.87±0.09e                                   | 55.37±00.10f                               | 75.45±0.04f                         |
| SLT 4921 | 29.52±0.02d                                     | 54.46±0.02f                                  | 26.84±0.01c                                   | 810.98±00.52f                              | 83.7±0.006f                         |
| SLC 2009 01 | 17.73±0.01b                                    | 49.35±0.18f                                  | 8.08±0.01c                                    | 469.11±00.06f                              | 105.33±0.03a                        |
| SLC 92 95   | 32.80±0.01c                                     | 48.06±0.03b                                  | 8.99±0.01c                                    | 390.69±00.15f                              | 57.09±0.01l                         |
| SLC 92 77  | 28.90±0.01c                                     | 61.49±0.01b                                  | 29.22±0.00a                                   | 666.35±00.32c                              | 87.76±0.00f                         |

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±0.00), 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±1.19) 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 (F8, 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±0.08a                                        | 82.43±0.008b                                  | 14.05±0.03g                         | 162.50±0.24j           | 155.69±0.10a                 |
| SL 8306    | 50.25±0.07a                                        | 74.22±0.10d                                   | 28.95±0.30a                         | 1033.16±0.09d           | 82.23±0.07i                  |
| SL 92 5588 | 44.01±0.02c                                        | 75.93±0.02c                                   | 22.47±0.01d                         | 843.66±0.23g            | 89.62±0.12h                  |
| SL 96 128  | 29.48±7.31i                                        | 66.03±4.94g                                   | 15.17±3.65h                         | 1657.70±1.28g           | 139.18±0.09c                 |
| SL 96 328  | 47.3±0.035b                                        | 84.56±0.01a                                   | 18.06±0.06f                         | 1652.44±0.27b           | 140.2±0.008h                 |
| SL 97 1442 | 20.76±0.13j                                        | 47.47±0.28i                                   | 20.59±0.13c                         | 1633.09±0.12c           | 155.87±0.01a                 |
| SLT 4921   | 26.82±0.06h                                        | 63.34±0.02f                                   | 25.04±0.02c                         | 1007.97±0.02e           | 109.56±0.30e                 |
| SLC 2009 01| 35.22±0.07f                                        | 41.99±0.04i                                   | 10.27±0.02e                         | 610.16±0.03i            | 112.09±0.10d                 |
| SLC 92 95  | 33.12±0.10g                                        | 57.45±0.22b                                   | 10.33±0.03f                         | 829.37±0.33b            | 103.02±0.04g                 |
| SLC 92 77  | 38.92±0.03d                                        | 68.79±0.14e                                   | 26.73±0.02b                         | 1.02±0.59f              | 106.19±0.10f                 |

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 (F8, 34 =12.6, p<0.05) when it fed on the varieties SL 96 128 (31.59 ± 0.07 mm²) and Co 775 (32.45 ± 0.07 mm²). Hence, these two sugarcane varieties could be considered as the most preferred for feeding by WLD vector. The lowest honeydew production (1.34±1.1mm²) 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 position 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 flanges 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.

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**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<sup>a</sup>     | 32.45 ±0.07<sup>b</sup> |
| SL 83 06          | 0.78 ±1.73<sup>d</sup>     | 08.38±0.06<sup>d</sup>  |
| SL 92 5588        | 0.35 ±1.73<sup>de</sup>    | 08.09 ±0.02<sup>d</sup> |
| SL 96 128         | 4.86 ±0.09<sup>a</sup>     | 31.59 ±0.04<sup>a</sup> |
| SL 96 328         | 2.42 ±0.03<sup>c</sup>     | 20.25 ±0.08<sup>b</sup> |
| SL 97 1442        | 5.26 ±0.21<sup>a</sup>     | 18.81 ±0.03<sup>b</sup> |
| SLC 2009 01       | 3.88 ±0.31<sup>bc</sup>    | 09.67 ±0.05<sup>d</sup> |
| SLT 4921          | 0.93 ±1.73<sup>d</sup>     | 12.09 ±0.12<sup>c</sup> |
| SLC 92 95         | 1.02 ±1.01<sup>cd</sup>    | 08.82 ±0.04<sup>d</sup> |
| SLC 92 77         | 0.00<sup>e</sup>           | 01.34 ±1.10<sup>e</sup> |

Note: In a column, means (±SE) followed by the same letter are not significantly different at 5% probability level.
Colbert and Evert (1982) reviewed that, larger veins are accountable for the bulk of longitudinal translocation, while the minor bundles were more responsible in the collection of photosynthates 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 photosynthates translocate within the larger vascular bundles.

**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.

**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*. 
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 vein 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.,

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