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Effect of the Epicuticular Wax Level of Leaf Lamina on the Behaviour of Leaf Hopper *Deltocephalus menoni* (Hemiptera: Cicadellidae); A Vector of Sugarcane White Leaf Disease

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ABSTRACT

Sugarcane White Leaf Disease (WLD) is one of the major threats to the cane sugar industry in Sri Lanka and *Deltocephalus menoni* is the only recorded vector in local conditions. Epicuticular waxes (EW) play an important role in protecting plants against herbivore attacks. Therefore, this study was conducted with the objective of determining the effect of EW level on the behavioral characteristics of *D. menoni* in ten different sugarcane varieties. EW level of the selected varieties, behavioral characteristics of *D. menoni* and the level of WLD infection under natural conditions were studied. The effect of surface waxes on feeding behavior of *D. menoni* was also investigated under *in-vitro* conditions using agarose-sucrose. Pearson correlation coefficient test was performed to detect the associations between EW and insect behavioral characteristics. Varieties with higher level of EW showed a significantly lower *D. menoni* feeding and disease infection. There was a significantly positive correlation ($r = 0.78$, $P = <0.0001$) between the level of feeding and rate of disease infection in natural environment. Hence it is quite possible that wax may play a significant role in feeding of *D. menoni* on sugarcane. In vitro study also further confirmed the relationship of EW and feeding behaviour by recording high feeding preference in agarose-sucrose diet containers with less wax content. Therefore, sugarcane accessions having high level of EW could be incorporated into directional breeding of varieties to increase the resistance against WLD.

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INTRODUCTION

Sugarcane White Leaf Disease (WLD) is one of the major threats to the cane sugar industry in Sri Lanka and *Deltocephalus menoni* (Hemiptera: Cicadellidae, Deltocephalinae) is the only local vector, as identified to date, which is capable of transmitting this phytoplasma disease (Senevirathne, 2008). Under local conditions, hot water treatment of seed cane at 54ºC for 50 minutes (Chandrasena, 2001), rogueing out of infected sugarcane plants from the field and replanting or fallowing the fields where infection level is more than 20 percent (SRI, 2015) have been recommended to manage WLD. Although farmers have adoped these practices, the WLD has appeared and spread at an alarming rate; therefore, investigating of an effective strategy to manage WLD management is still a great need.

Management of WLD vector has been identified as a strategic and integrated approach to prevent the rapid spread of WLD disease in sugarcane plantations. In this context, the breeding of plants that are either resistant or deter the behavioural characteristics of vector would be a first line of defense to manage the WLD. In Sri Lanka, screening of sugarcane germplasm for WLD resistance is continuing using conventional screening methods under the natural infection of the WLD. In this breeding programme, 66 tolerant sugarcane varieties and 20 moderately tolerant sugarcane varieties for WLD have been identified.

Plant breeding is mostly directed towards the developing of varieties that are resistant to the disease rather than to the vectors. Breeding of plants that are resistant to vectors has been attempted as a strategy to control diseases and it has been successful in many crops viz., mite resistance to control virus disease of wheat (Martin, 1983), and planthopper resistance to control virus disease of rice (Heinrichs, 1986). However, there are many incidences that pest resistant varieties eventually became disease resistant varieties since particular insect pests of a crop is the vector of diseases.

Epicuticular waxes (EW) play an important role in protecting aerial parts against the damages caused by biotic and abiotic stresses (Wójcicka, 2013; Yin *et al.,* 2011; Rostàs *et al.,* 2008; Zhang *et al.,* 2007). These EWs are generally formed as a thin, continuous film but can also be decorated with protruding microscopic crystals occurring as filaments, rods, platelets, tubes or complex dendritic structures (Buschhaus and Jetter, 2011). Plant EWs are complex mixtures of long chain aliphatic and cyclic components. The morphology as well as the composition of EW vary widely among species or cultivars and are also affected by the plant age and certain environmental factors, such as heat, humidity and irradiance levels. However, the studies on the effect of EW on the plant resistance is poorly studied in local conditions, therefore, this study was conducted to determine the effect leaf lamina epicuticular wax on the behavioral characteristics of *D. menoni* on sugarcane varieties in order to design an effective management program for white leaf disease.

MATERIALS AND METHODS

This study was conducted at the Entomology Laboratory of the Sugarcane Research Institute (SRI), Uda Walawe in Sri Lanka. Sugarcane verities: 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. Level of leaf lamina epicuticle wax of each variety/ accession and performance of D. menoni on these varieties/accessions were studied. Available information on WLD incidences in the natural environment was

considered in selecting the test varieties/accessions for the study.

a. Measuring of the variation of leaf laminar wax among sugarcane varieties that has variable resistance to WLD

Sugarcane plants were obtained from mother plant nursery, which were established with hot water treated seed cane (54ºC, 50 minutes). Single budded setts from each variety were placed in plastic pots (50x50cm) using sterilized soil. 25 plants from each variety were kept in an insect-proof screen houses and maintained following recommended agronomic practices (SRI, 2004).

At the age of four months, 10 to 20 numbers of third leaves of the plants were detached and the middle portions of leaves were taken for wax extraction. The area (cm^2) of the selected leaf portions were measured using squire millimeter grid. The leaf portions were immersed in chloroform for about 10 seconds and the extracts were evaporated to separate wax. The amount of wax was weighed and calculated for unit area (cm^2).

b. Determining level of SWLD infection by *D. menoni* under natural environment

A land with infested sugarcanes with *D. menoni* was selected for the experiment. Three budded setts, obtained from mother plant nursery, were established in three rows (5 m) using randomized complete block design (RCBD) with three replicates. Trial was maintained under the recommended agronomic practices (SRI 2004) and the population of *D. menoni* monitored throughout crop growth. The clumps that were infested with WLD were detected using visual symptoms and recorded. The level of WLD infection by *D. menoni* was calculated as; Number of WLD symptomatic clumps/ Total number of clumps× 100.

c. Measuring the behavioural aspects of *D. menoni*

Rearing insects

The adult insects of *D. menoni* were collected using a sweep net and a pooter from young sugarcane plants (less than of 6 month) in the research farm, SRI, UdaWalawe. The collected insects were reared in insect-rearing cages according to the protocol developed by Senevirathne (2008). Aggregation, amount of feeding, fecundity, nymphal development and adult longevity were measured on each selected variety.

Aggregation

Three-month old ten sugarcane potted-plants representing one plant from each variety/accession were placed randomly but equidistantly apart in a circle with the radius of 15 cm inside the insect-proof laboratory cage. The arrangement was replicated nine times. Two-day old fifty adults of *D. menoni* were released from the center and choice test was conducted. Number of insects on each plant was recorded daily for ten days. Percentage of leafhoppers settled at each time on test plants was calculated.

Amount of feeding

Feeding of *D. menoni* was recorded by honey-dew production on each tested variety/accession, measured using Parafilm Sachet Technique (Heinrichs *et al.,* 1985). D. menoni adults were collected with a pooter from the insect cages and they were starved for 3hr on wetted tissue papers in glass containers. Female insect vectors, starved in similar manner, were inserted into the sachets individually where four-centimeter long leaf portion of third leaf of each plant 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 was 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 a grid (mm2).

Erythrosine dye test also was conducted *viz.,* leaf portions where insect fed 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. Then, the leaf portions were examined under a microscope. The salivary flangues on each plant species stained in pink colour were counted.

Fecundity

Plants grown in plastic pots were separately maintained in insect-proof cages up to the age of four months. The plants were thoroughly cleaned by removing ants, spiders and other insect predators before introducing vectors. Soil surface of each pot was covered with polythene as underpinnings to pave sterilized soil for oviposition. Five newly-emerged adult vectors (3 females and 2 males) were introduced to each plant enclosed in an insect proof cage and left for five days for oviposition. Then, substrates in each pot were observed using a light microscope (KYOWA TOKYO, 10x3) and the number of eggs in soil were counted.

Nymphal period

Four month old plants which were prepared as in fecundity study were used. Twenty-five neonatal nymphs were introduced to one plant from each of ten variety/ accession and this arrangement was replicated three times. Then, the infestation was allowed to proceed without interferences until final ecdysis. Nymphal period i.e. the number of days taken by them to reach adulthood was recorded on each variety/ accession.

Survival of nymphs

Four month old plants which were prepared as in fecundity study were used. Twenty-five neonate nymphs were introduced to one plant from each variety/ accession without interferences until nymphs develop into adults. This arrangement was replicated three times. The number of nymphs reached adulthood was recorded and survival percentages were calculated.

Adult longevity

Plants grown in plastic pots were separately maintained in insect-proof cages up to the age of four months. Just after final ecdysis, twenty-five adults were introduced to one plant from each variety/ accession and this arrangement was replicated three times. Then, the infestation was allowed to proceed without interferences until insects die. The number of days that adults survived on each genotype was recorded to calculate longevity of adults.

d. *In-vitro* Investigation of the effect of surface waxes on approaching and feeding behaviour of *D. menoni*

The effect of surface waxes on the feeding behaviour of *D. menoni* was investigated *in-vitro* using an agarose-sucrose diet. The diets were prepared by incorporating 0.8 % agarose (Sigma A-0169) into a 30% sucrose solution. The melted diet was poured into a watch glass with 1 cm radius. Waxes collected from a leaf which was of similar surface area to the watch glass was sprinkled evenly on the surface of the diet before it gets solidified and then it was covered evenly with stretched Parafilm M® membrane. Transparent gels formed after 1–2 minutes were offered to the insects for probing. Set up was replicated for 3 times for each variety

and insects were allowed to feed on membrane for 24-hour period. Fresh diets were used for recording performances of approaching and feeding of the insects.

Water-starved young female vectors were introduced to each container and following behaviour and the performance of the insect was recorded *viz.,* time taken up to first probing, duration of first successful probing and number of salivary flanges on the stretched parafilm membrane.

Data analysis

Analysis of variance was performed to test the effects leaf lamina epicuticle wax on the WLD infection and insect performance on tested varieties/accessions. Level of leaf lamina epicuticle wax was compared and used in correlation analysis.

The count data were transformed using square root transformation. Means were separated by using Duncan's Multiple Range Test (DMRT) at 0.05 probability level. Pearson correlation coefficient test was performed to detect the associations between level of leaf lamina epicuticle wax with level WLD infection and vector performance.

RESULTS AND DISCUSSION

a. Variation of leaf laminar wax among sugarcane varieties that has variable resistance to WLD

The amount of wax extracted from leaf lamina had a significant (P=0.05) variation among the tested sugarcane varieties. Higher level of leaf lamina epicuticle wax per unit area of leaves was extracted from the variety/ accession SL 83 06 and SL 92 5588. In contrast, low levels of leaf lamina epicuticle wax on unit area of sugarcane leaves were extracted from the SL 96 128 and Co 775 (Table 01).

b. Level of WLD infection by *D. menoni* under natural environment

Under the moderate pressure of *D. menoni* and WLD inoculum in the surrounding area, The WLD infection was significantly higher in varieties/accessions SL 97 1442, SL 96 128 and CO 775 and WLD infection was not observed in variety SL92 77 (Table 1).

c. Behavioral characteristics of *D. menoni* on varieties/accessions

Aggregation

The mean aggregation of *D. menoni* significantly varied among sugarcane varieties/accessions. The highest adult aggregation was observed on the varieties SL 97 1442 and the lowest aggregation was recorded on SLC 92 55 accession (Table 1).

Amount of feeding

The volume of honeydew production by *D. menoni* is directly proportional to the volume of feeding as shown in the previous studies (Heinrichs *et al,* 1985). Mean honeydew production of D. menoni was significantly higher when it fed on the varieties SL 96 128 and Co 775. Hence, these two varieties can be considered as the most preferred sugarcane varieties for feeding of WLD vector. The lowest honey dew production (1.34) was found when *D. menoni* fed on SLC 92 77 (Table 1).

Salivary flanges

Mean number of salivary flanges made by *D. menoni* was significantly different among the tested varieties (Table 1). It was higher when it fed on the varieties SL 92 77 and SL 92 5588. As number of feeding increase when there are high restrictions to access the phloem vessels and hence, these two varieties can be considered as the less preferred sugarcane varieties for feeding of vector of WLD. The lowest number of salivary flanges was found when *D. menoni* was feeding on SL 96 328 and SL 97 1442 (1.5) and hence these two varieties can be considered as the most preferred sugarcane varieties for feeding of WLD vector.

Survival of nymphs

The percentage of survival of nymphs of *D. menoni* was significantly varied with the sugarcane varieties that they fed on (P<0.05) (Table 1). The highest percentage of survival of nymphs (61.71%) was found when they were on the variety SL 96 128. In contrast, lower survival percentages were found when D. menoni was on SL 8306 followed by SLC 92 95 and Co 775. *D. menoni* was unable to survive on SLC 92 77.

Oviposition

The mean number of eggs laid by *D. menoni* varied significantly with the sugarcane varieties/accessions (P<0.05) (Table 02). The highest oviposition (26.56 eggs / 5 days) was recorded when *D. menoni* was on the variety SL 8306 and the lowest oviposition (0.08) was recorded on SLC 92 77. Contradictorily, to the results in feeding, the highest oviposition was recorded on variety SL 8306.

Nymphal period

The nymphal period significantly varied with the varieties/accession (P<0.05). The lowest nymphal periods were recorded on varieties SL 96 128 and SL 96 328, which were the most favourable varieties for survival of *D. menoni* nymphs. A longer duration was taken to complete nymphal period when they were on SL 92 5588, SLC 92 95 and SL 8306. D. menoni nymphs did not survive on SLC 92 77. Time duration between moultings was also low in SL 96 128 and SL 96 328 and it was high on SL 92 5588, SL 83 06 and SLT 4921.

Adult longevity

Mean adult longevity significantly varied with the varieties/accessions (P<0.05). Adult longevity was significantly high when *D. menoni* was on Co 775, SL 96 328 and SLC 2009 01 (40.99). The lowest adult longevity was found when they were on SLC 92 95 and the variety SL 92 5588 (Table 1). *D. menoni* survived only for two days on SLC 92 77 and equal survival period (2dys) was recorded for *D. menoni* adults which subjected to starvation.

Correlations between Epicuticle wax and performance of D. menoni and Level of disease infection

The amount of feeding and level of disease infection was significantly negatively correlated. Correlation coefficient between level of leaf lamina epicuticle wax extracted from unit area and amount of feeding was - 0.62 (0.0003) and it was - 0.51 (0.0043) between the level of leaf lamina epicuticle wax extracted from unit area and level of disease infection. Amount of feeding by *D. menoni* and the level of disease infection was significantly positively correlated (r = 0.78, p<0.0001).

d. *In vitro* Investigation of the effect of surface waxes on approaching and feeding behaviour of *D. menoni*

Salivary flanges were of basically three different sizes viz., large, medium and small (Plate 01). The patterns of large, medium and small salivary flanges varied with the origin of wax sprinkled on agarose-sucrose diet plates.

The time taken for the first probe in agarose-sucrose diet containers which were sprinkled with wax was significantly higher in the varieties SL 83 06 (137.67 mins) and SL 92 5588 (76.33 mins). However, significantly lesser time was taken for the first probe in agarose-sucrose diet containers which were sprinkled with wax from variety SL 97 1442, SL 96 328, SL 96 128 and Co 775 (Table 02).

Table 1. Level of leaf lamina epicuticle wax extracted from unit area of leaf, Level of disease infection and Amount of feeding, rate of nymphs convert into adults, nymphal period, oviposition and adult longevity of *D. menoni* on sugarcane varieties/accessions tested

Variety	Level of leaf lamina epicuticle wax (µg)	Level of disease infection	Behavioral characteristics of *D. menoni*						
			Aggregation	Amount of feeding	Number of salivary flanges	Survival of nymphs (%)	Nymphal period (d)	Oviposition (no. of eggs)	Adult longevity (d)
Co 775	0.003320d (0.64)	4.80a (0.09)	14.27bcd (0.02)	32.45a (0.07)	1.79c (0.03)	35.28e (0.02)	17.33bc (0.03)	14.65d (0.04)	41.66a (0.49)
SL 83 06	0.024800a (0.07)	0.78d (1.73)	6.6ed (0.38)	8.38d (0.06)	2.66b (0.1)	30.33f (0.05)	21a (0.04)	26.56a (0.04)	15.16c (0.05)
SL 92 5588	0.020794a (0.26)	0.35de (1.73)	5.1ed (0.07)	8.09d (0.02)	6.23a (0.09)	44.60d (0.01)	22a (0.04)	7.79f (0.09)	11.7d (0.04)
SL 96 128	0.002300d (0.14)	4.86a (0.09)	17.17bc (0.01)	31.59a (0.04)	1.55c (0.02)	61.71a (0.01)	15.33d (0.03)	17.9b (0.03)	38b (0.02)
SL 96 328	0.004663d (0.42)	2.42c (0.03)	20.33b (0.07)	20.25b (0.08)	1.36d (0.03)	48.50c (0.03)	16cd (0.06)	7.54g (0.11)	40.33a (0.03)
SL 97 1442	0.008569cd (0.67)	5.26a (0.21)	30a (0.52)	18.81b (0.03)	1.55c (0.04)	43.33d (0.02)	18b (0.05)	6h (0.16)	37.66b (0.01)
SLC 2009 01	0.007765cd (0.96)	3.88ab (0.31)	9.93cde (0.44)	9.67d (0.05)	1.37d (0.04)	43.63d (0.03)	17.66b (0.03)	11.58e (0.03)	40.50a (0.01)
SLT 4921	0.013661bc (0.45)	0.93d (1.73)	10.17cde (0.07)	12.09c (0.12)	2.3b (0.05)	53.75d (0.02)	17bc (0.05)	15.75c (0.13)	41a (0.06)
SLC 92 95	0.002260d (0.1)	1.02cd (1.01)	3.27e (0.53)	8.82d (0.04)	4.78ab (0.09)	35.34e (0.06)	21a (0.04)	1.50i (0.01)	10.66d (0.03)
SLC 92 77	0.013803bc (0.18)	0.00e	6ed (0.86)	1.34e (1.1)	6.86a (0.18)	0.00g	0e (1.73)	0.08j	0e

Note: In a column, means followed by same letters are not significantly different in 5% probability level.

Table 2. Time taken by *D. menoni* for first probing, duration of first successful probing number of salivary flanges on the stretched parafilm membrane during the *In vitro* Investigation of the effect of surface waxes on approaching and feeding behaviour of *D. menoni*

Variety	Times to 1st Probe	Length of 1st probe	Number of Salivary flanges			
			Large	Medium	Small	Total
Co 775	23.50de (0.02)	86.33b (0.07)	1.33bc (0.43)	4.00c (0.34)	4.00c (0.25)	7.00e (0.29)
SL 83 06	137.67a (0.03)	3.33g (0.46)	5.00a (0.35)	0.33c (0.87)	0.33c (1.73)	8.67de (0.35)
SL 92 5588	76.33b (0.10)	20.17f (0.26)	0.33c (1.73)	52.33a (1.25)	52.33a (0.14)	54.33b (0.81)
SL 96 128	9.87e (0.46)	28.97f (0.09)	1.00c (0)	0.33c (1.73)	0.33c (1.73)	2.00e (0.5)
SL 96 328	7.29e (0.18)	43.54e (0.14)	1.33bc (0.43)	1.67c (0.69)	1.67c (1.73)	4.67e (0.81)
SL 97 1442	6.50e (0.23)	99.33a (0.18)	1.33bc (0.43)	3.33c (0.57)	1.67c (0.17)	8.33de (0.30)
SLC 2009 01	13.67de (0.15)	73.45c	1.33bc (0.43)	0.00c (1.73)	3.33c (0)	1.67e (0.35)
SLT 4921	39.70cd (0.11)	59.00d (0.12)	0.32c (1.73)	14.33b (1)	0.00c (0.26)	15.67d (0.21)
SLC 92 95	21.93de (0.2)	3.67g (0.15)	3.00b (0.68)	19.00b (0.94)	14.33b (0.24)	24.67c (0.24)
SLC 92 77	53.00bc (0.86)	0.67g (0.86)	0.00c	61.33a (1.73)	61.33a (0.88)	64.33a (0.88)

Note: In a column, means followed by same letters are not significantly different in 5% probability level.

The feeding of *D. menoni* found to be significantly varied with the level of leaf lamina epicuticle wax, however, no significant correlation was found in other behavioural characteristics of *D. menoni.* Our finding was also very much similar with the findings of many authors i.e., negative associations

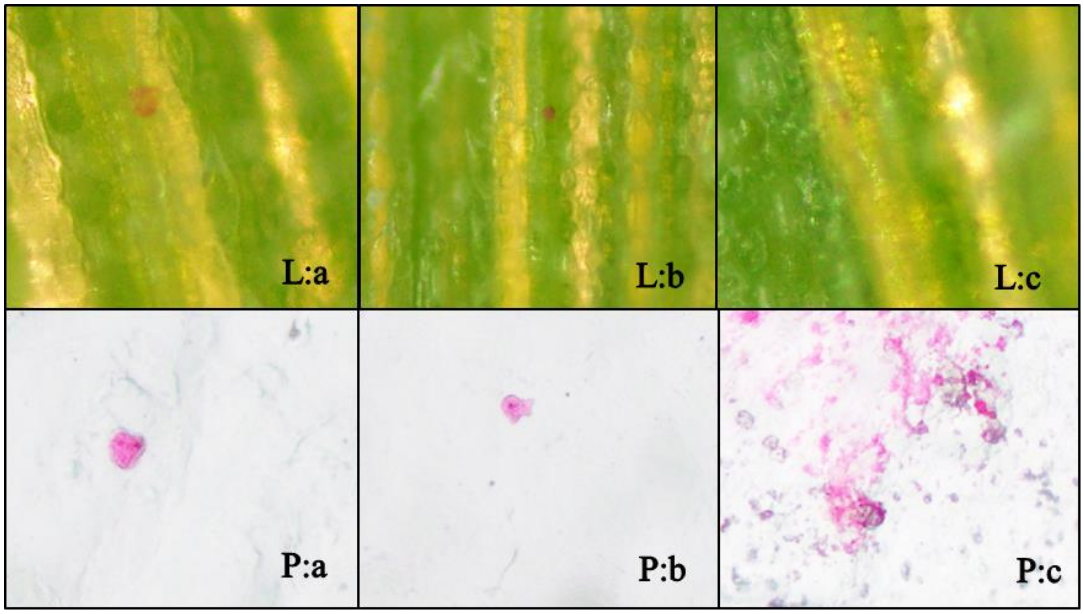

Figure 01: Salivary flanges; L- leaf (a) Large, (b) Medium and (c) Small. P- Para film M® membrane; (a) Large, (b) Medium and (c) Small

The longest time period after the first probing was recorded on SL 97 1442 expressing the higher preference to feed on particular variety and length of probing period was significantly less on SLC 92 77 and SLC 92 95.
Nearly one salivary flange was recorded on varieties SL 97 1442, SL 96 328, SL 96 128 and Co 775 showing their low resistance and the lower requirement of building higher number of salivary flanges and more
The distribution pattern of salivary flanges was similar on the leaf and parafilm membranes. Higher numbers of salivary flanges were recorded on leaves than the number of flanges on para film covered the agarose sucrose diet with EW of particular variety. It may be due to effect of some other factor/s associated with leaf morphology or composition other than the wax.

The feeding of *D. menoni* found to be significantly varied with the level of leaf lamina epicuticle wax, however, no significant preference by *D. menoni.* The varieties SLC 92 77 and SL 92 5588 showed significantly higher number of small salivary flanges (Fig 01- P:c) confirming the difficulty to pierce the surface for feeding. Similarly, the highest number of total flanges were recorded on variety SLC 92 77 and SL 92 5588 followed by SLC 92 95, SLT 4921 and SL 8306 which showed the higher resistance to penetrate and feed.
correlation was found in other behavioural characteristics of *D. menoni.* Our finding was also very much similar with the findings of many authors i.e., negative associations between surface waxes and insects performances. Effect of epicuticular waxes on insects performances have been well studied in many crops. For example, increased levels of surface wax have been correlated with resistance in cabbage (*Brassica oleracea L.*) for the aphid *Bravicoryne brassicae L.*, sorghum (*Sorghum bicolor L.*) for the green bug *Schizaphis graminum*, of the winter wheat

(*Triticum aestivum L.*), on English grain aphid *Sitobiona venae* (Shepherd et al., 1999, Ni et al., 1998, Eigenbrode and Espelie, 1995; Eigenbrode *et al.,* 2000) and the evidences have shown that wax blooms on leaf surfaces reduce adult and larval feeding by herbivores. Glossy genotypes of Brassica are more susceptible to the green peach aphid when compared to waxy Brassica genotypes (Thompson, 1963; Way and Murdie, 1965; Stoner, 1990). Epicuticular waxes negatively affected both the neonate larval movement and development of the fall armyworm *Spodoptera frugiperda* on corn, Zea maize (Östrandet al., 2008). Moreover, examples of epicuticular wax herbivore interaction have been identified and characterized in Eucalyptus globulus (Brennan and Weinbaum, 2001), Hordeumvulgare L. (Tsumuki *et al.,* 1989), S. bicolor (Nwanze *et al.,* 1992) and *Triticum aestivum* (Lowe *et al.,* 1985). Frazier and Chyb (1995) suggested that insect feeding can be inhibited by three kinds of effects that occur at different stages of the insect-plant interactions: pre-ingestional, ingestional and post-ingestional effects. In many cases as mentioned above, waxes deter aphid probing and feeding and waxy surface acts as an anti-feedant.

There are rear cases that record epicuticular waxes are attractive to the herbivores too viz., epicuticular waxes of the wheat are attractive to adult oviposition of the Hessian fly Mayetiola destructor (Foster and Harris, 1992).

Scientists have confirmed that removal of epicutical waxes have reduced the level of resistance for herbivores viz., removal of the surface waxes with chloroform from seedlings of *Sorghum bicolor* (L.) caused their acceptance by nymphs of *Locusta migratoria L.* (Woodhead, 1983). Hexane extracts of surface lipids from resistant rice cultivars enhanced feeding of the brown planthopper, *Nilaparvata lungens* (Woodhead and Padgham, 1988). Brennan and Weinbaum (2001) showed that the epicuticular wax on juvenile leaves reduced stylet probing by *Ctenaryta inaspatulata* and *C. brimblecombei.* Moreover, epicuticular wax on juvenile leaves of Eucalyptus globulus plays a primary role in resistance to *C. spatulata* and *C. brimblecombei*, because these species survived longer and settled more often on 'de-waxed' than on 'waxy' juvenile leaves.

It was reported that, herbivore attack in a specific stage of the crop also occurs with the variation of epicuticle wax content at particular crop stages. According to the Shepherd et al. (1999), preference of raspberry aphids (*Amphorophora idaei*) to older leaves of raspberry is due to lower wax coverage on these leaves relative to the younger emerging leaves. These phenomena were also reported in spotted alfalfa aphids, *Therioaphism aculata*, in the foliar canopy of alfalfa.

Results of the in-vitro investigation conducted using an agarose-sucrose diet are in agreement with previous findings. Lei *et al.* (2001) describe that, host acceptance by sucking insects starts with the first labial contact with the plant surface, followed by stylet penetration through successive tissue layers between the epidermis and the vascular tissues and final feeding. The 'time to the first probe' can be considered as the insects' evaluation of sub epidermal tissues (Troncoso *et al.,* 2005). This is important, as epicuticular wax composition and thickness of the epidermal cuticle can influence the host plant's acceptance (Lei *et al.,* 2001).

A longer period of time spent on a plant before probing suggests adverse effects of the plant exterior on the insect (Sandanayaka *et al.,* 2013). Hence, the deterrent effects of the epicuticular waxes are reflected by longer

non-probing periods. Troncoso *et al.,* (2005) recorded that the acceptance or rejection of plant for insect feeding is one assessment of host resistance. Prado and Tjallingii (1993) noted that stylet penetration is a key factor to evaluate feeding of sap-sucking insects. The parameters describing aphid behaviour during probing and feeding, such as total time of probing, number of probes, average time of probing and duration of the first probing are good indicators of plant suitability or interference of probing by chemical or physical factors in a particular plant surface.

Herbivores use tactile and gustatory cues to determine the value of a plant as a feeding and oviposition host and during initial encounters with a plant, these insects often use their stylets to tap on and make shallow probes of the leaf surface. It is combined with secretion of small amounts of watery saliva to dissolve surface chemicals and imbibition of the liquids at the surface (Powell *et al.,* 2006, Miles, 1999). Mu¨ller and Riederer (2005) confirmed that these behaviours detect differences in the carbohydrate content of cell walls, epicuticular waxes, and presence or absence of secondary metabolites to determine non-host or host status and avoid unacceptable plants.

CONCLUSIONS

Varieties/ accessions with a higher level of leaf lamina epicuticle wax of sugarcane leaves caused a significant reduction of the *D. menoni* feeding on leaves and level of disease infection. Wax may play a significant role in feeding of *D. menoni* on sugarcane and transmission of WLD phytoplasma. In-vitro Investigation confirmed the effect of surface waxes on approaching and feeding behaviour of *D. menoni*. Therefore, sugarcane varieties and wild relatives having leaf lamina with higher compositions level of leaf lamina EW 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 and the level of disease infection in natural environment.

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