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Varietal Screen for Susceptible stage of *Saccharum* hybrids for *Deltocephalus menoni* (Hemiptera: Cicadellidae), vector of sugarcane White Leaf Disease

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ABSTRACT

Deltocephalus menoni is a sap-sucking insect causing severe losses to the cane sugar industry by feeding on the crop and acting as the vector of Sugarcane white leaf disease (WLD) in Sri Lanka. This study was conducted to determine the most preferred age of the sugarcane plant for optimum feeding of *D. menoni*. Sugarcane plants of the varieties SL 92 5588, SL 97 1442, SLC 2009 01, and SL 96 128 at six age categories from 1-6 months, were selected for the study. Female *D. menoni* adults fed on to measure the amount of feeding. Variation of the feeding of *D. menoni* on each variety with the plant age was estimated separately. The feeding of the *D. menoni* significantly varied with the age of the crop, and the highest feeding rate of 6.8 mm^2, was recorded on four months old sugarcanes. The amount of secreted honeydew, measured as an indicator of the suitability of sugarcane for feeding of *D. menoni*, increased gradually from one month and reached the peak at four-month age. When the plant turned five-month-old, the amount of honeydew secretion reduced gradually, and the honeydew secretion significantly dropped at six months. A similar trend was observed in the amount of honeydew secreted by feeding on each variety, including the resistant check. The highest amount of feeding, 6.8, was recorded at four-month age, indicating four-month age of the sugarcane hybrids is the most vulnerable stage for *D. menoni* feeding, and the three to five-month period is the susceptible period for feeding.

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INTRODUCTION

About 103 insect species have been found in Sri Lankan Sugarcane plantations. Among them, eighteen species are homopterans (Kumarasinghe, 2003). Homopterans affect the crop in different growth stages; germination, tillering, grand growth, and ripening. *Deltocephalus menoni* (Hemiptera: Cicadellidae) belongs to Subfamily Deltocephalinae is one of the sap-sucking insects causing severe losses to the cane sugar industry, feeding on the crop and by acting as the vector of white leaf disease (WLD) in Sri Lanka (Senevirathne, 2008).

According to Chanchala *et al*. (2014), there is a strong relationship between the level of WLD symptoms that appeared in the field, and the population level of the vector found four weeks before the date of recording disease level. However, this type of vector and disease relationship was obvious only in the fields with a low level of apparent disease incidences. Further, a low population of *D. menoni* was found in fields at germination and ripening stages. Nevertheless, a higher population of *D. menoni* was reported in fields in the grand growth stage (SRI, 2014). The nutrition composition of plants varies with the variety and the growth stage (Bishop, 1965). Usually, leaf sap feeders select plants with high nutritional quality for feeding to have optimal growth. Female herbivore insects identify a suitable site for oviposition to maximize the development and survival of the offspring (Alyokhing *et al*., 2004; Weintraub, 2006 and Bonebrake *et al*., 2010).

It is vital to know the relationship of *D. menoni* with the sugarcane plant for its optimum feeding performance. The most preferred stage of the crop for feeding eventually becomes the most susceptible stage of the crop, and the most preferred cane sugar varieties eventually become the most susceptible varieties. It is a prerequisite to know the susceptible age of the crop for accurate varietal screening. The chance for the generation of false results could increase with the use of the non-susceptible stage of the crop.

Therefore, we conducted this study to determine the most preferred age of the sugarcane plant for optimum feeding of *D. menoni* and the varietal effect on the most preferred age of the crop.

METHODOLOGY

The study was conducted in the research farm and the entomology laboratory of the Sugarcane Research Institute, Uda Walawe, Sri Lanka (6^0 21' N; 80^0 48' E; Average temperature: 17-36 °C; Average rainfall: 1300-1600 mm; Elevation from mean sea level: 76 m).

As per the current information on sugarcane varieties, SL 92 5588 is considered a WLD resistant variety. While SL 97 1442 and SLC 2009 01 are considered susceptible varieties, SL 96 128 is considered as a less susceptible variety. Different resistance levels may cause differences in feeding preferences. Thereby, varieties with different levels of resistance may affect differently on the susceptible age of the crop. The above four varieties were selected as test varieties to determine the optimum feeding preference of the vector concerning the crop age.

D. menoni adults are typically observed in the sugarcane plantations of less than six months old, and the highest number of *D. menoni* has been recorded in the fields at four-month ages. Therefore, plants from six age categories were used in the study *viz*., one, two, three, four, five, and six-month-old sugarcane plants.

Maintaining test plants

Sugarcane plants, obtained from the mother plant nursery (variety SL 96 128), which had been established using hot water treated seed cane (54 °C hot water for 50 minutes), were used in the experiment. Single budded setts from each variety were obtained and potted in plastic pots (20x20cm) using sterilized soil. Fifty plants from each variety were kept in insect-proof screen houses and maintained under the recommended agronomic practices (SRI 2004).

Maintaining of insect cultures

The adult insects of *D. menoni* were collected using a sweep net and a pooter from young sugarcane plants (less than 6 months) in the research farm, SRI, Uda Walawe. The insects were reared in insect-rearing cages according to the protocol developed by Senevirathne (2008). Nymphs were reared singly in glass vials to obtain two days old (2 days after final ecdysis) *D. menoni* adult female for the study.

Studying the relationship between feeding performance and plant age

Sugarcane plants of the varieties SL 92 5588, SL 97 1442, SLC 2009 01, and SL 96 128 at six age categories were used in the study viz., one, two, three, four, five, and six-month ages. Female *D. menoni* adults were collected singly with a pooter from the glass vials and starved them but provided water for three hours (3 h) on wetted tissue papers in glass containers. A selected portion of a leaf in each plant was encircled (4 cm) with a Parafilm ® sachet, and a water-starved young female vector was introduced to each sachet (Heinrichs et al., 1985). Each vector was left in a sachet for 24 hours for feeding. After 24 h period, the leaf was detached from the bottom margin of the Parafilm ® sachet.

Two techniques were used to measure the amount of feeding, weighing the honeydew and staining the honeydew using the bromocresole green treated filter paper. Erythrosine dye test was conducted to determine the number of salivary flanges on a leaf.

a. Weight of the honey dew

The weight of honeydew was measured by taking the deference of W1 (weight of sachet + leaf +honey dew) and W2 (weight of sachet + leaf). Honeydew on leaves was removed by staining them with bromocresol green-treated filter papers (Whatman No. 1) as in "b." Measurements were taken with electronic balance with ±0.0001 g accuracy (Axis, AGN220C).

b. Staining with the bromocresole green treated filter paper

Honeydew on the sugarcane leaf and the sachet were collected with the bromocresol green-treated filter papers (Whatman No. 1). Honeydew-stained filter papers were carefully wind dried, and stained areas on filter papers (blue) were measured using square millimetre grids. Fifty replicates from each age category were included from each variety.

Leaf portions where the insect had fed were collected and dipped in a staining solution of 0.1% erythrosine dye for 10-15 min. Then, the leaf portions were examined under a microscope. The salivary flanges on each plant sample stained in pink color were counted.

Statistical analysis

Data on salivary flanges were subjected to square root transformation and followed the analysis of variance (ANOVA procedure). In each considered variety, feeding data pooled under six age categories. Variation of the amount of feeding of *D.* menoni, on each variety with the increase of age and most preferred crop age of the vector for feeding, was estimated separately. Means were separated by Duncan's multiple range test at 0.05 probability level using the SAS software (9.0).

The feeding relationship was studied using the feeding measurement parameters viz., a) weight of honeydew b) area stained on bromocresole green treated filter papers and c) salivary flanges on the leaf.

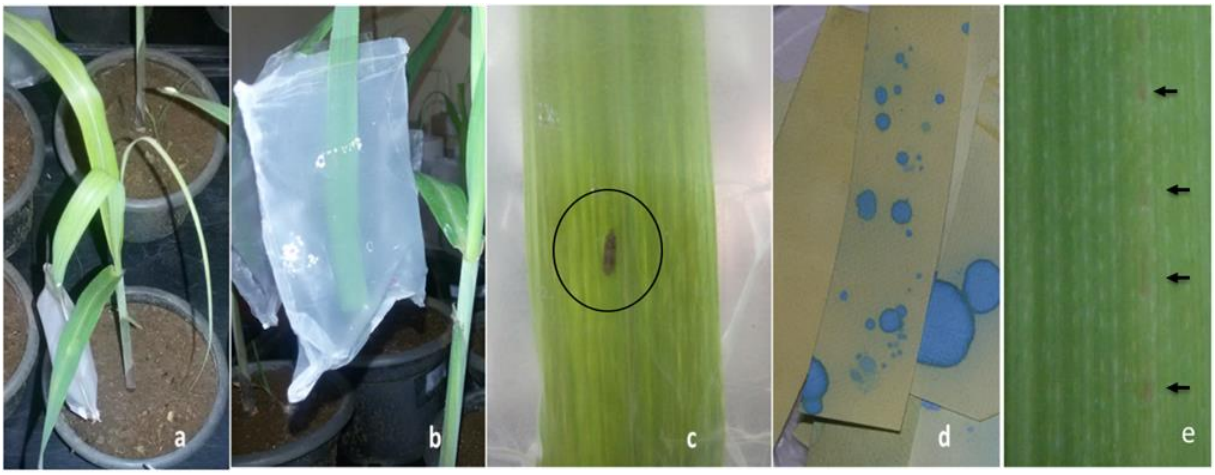

Figure 1. Experimental setup for measuring excreted honeydew amount of *D. menoni*. (a, b) Parafilm ® sachet attached to the plant, (c) Female *D. menoni* adult in Parafilm ® sachet, (d) Honeydew stained bromocresole green treated filter papers, (e) Stained salivary flange by erythrosine dye test

RESULTS AND DISCUSSION

a). Feeding of *D. menoni* on sugarcane as measured by the weight of honeydew

The amount of honeydew excreted by *D. menoni* significantly varied ($F_{5, 48}$ = 11694.1, $p<0.05$) with the age of the plant (Table 1). It increased gradually with the age of the crop, reaching the peak at four months (Table 1) as 3.2, 5.5,4 and 6.8 (µg) respectively in varieties SL 92 5588, SL 96 128, SL 97 1442, and SLC 2009 01. When the plant attained the age of five months, the amount of honeydew secretion reduced gradually, with the lowest honeydew secretion at six months. Amounts of secreted honeydew in three and five-month ages were more or less similar. The highest weight of honeydew was recorded at the four-month age, indicating a higher amount of feeding at the fourth month of the crop. The sugarcane variety significantly affects feeding, as measured by honeydew production ($F_{3, 48}$=9835.54, $p<0.05$). Of the four varieties tested, the highest weight of honeydew at four-month age was recorded on variety SLC 2009 01, followed by SL 96 128. Significantly less weight of honeydew was recorded on variety SL 92 5588 (Table 1) at four-month age. As such, a significant difference was found among varieties in terms of insect feeding, which is related to the disease resistance levels. Further, there was a significant interaction between crop age and variety on the amount of feeding ($F_{3, 48}$=547.57, $p<0.05$).

b). Feeding of *D. menoni* on sugarcane as measured by the area stained on bromocresole green treated filter papers

There is a significant variation of honeydew production as measured by stained area among varieties ($F_{3, 45}$= 1.88, $P<0.05$). Further, there was a significant correlation between the weight of honeydew and the area stained by the same honeydew amount ($P\leq0.01$, $y = 7116x + 4.859$, R^2=0.7302).

Considering the four varieties tested, the highest area stained by honeydew was recorded on variety SLC 2009 01 followed by SL 96 128, which are not significantly different. The significantly less honeydew stained area was recorded on variety SL 92 5588, followed by 97 1442, which are also not significantly different (Table 2). Honeydew-stained areas of different ages showed a similar pattern with the weight of honeydew excreted by *D. menoni*. In addition, there was a significant correlation between weight of honeydew and are stained by the same honeydew amount ($P\leq0.01$) for each variety but correlation coefficient and the equation were specific for sugarcane variety such as, $y = 3805.4x + 5.4968$, R^2=0.6996 for SL 92 5588 at $P\leq0.01$, $y = 6800.8x + 9.4476$, R^2=0.893 for SL 96 128 at $P\leq0.01$, $y = 12341x - 7.0004$, R^2=0.7732 for SL 97 1442 at $P\leq0.01$ and $y = 6850x + 8.8142$, R^2=0. 0.8771 for SLC 2009 01 at $P\leq0.01$.

c). Feeding of *D. menoni* on sugarcane as measured by the salivary flanges on leaf

Number of salivary flanges on leaf was in increasing pattern with the age ($P\leq0.01$, $y = 0.4883x + 1.5696$, $R^2 = 0.7085$). Considering the four varieties tested, the highest number of salivary flanges were recorded on variety SLC 2009 01 followed by SL 92 5588 at four-month age, but not significantly different to each other. A significantly lesser number of salivary flanges was recorded on variety SL 97 1442 and SL 96 128 (Fig 2). The four-month age crop was most preferred for feeding, and three and five-month age was also vulnerable for feeding compared with one, two, and six-month plants. Therefore, three, four, and five-month age crops were more susceptible for feeding of *D. menoni*, and eventually more vulnerable ages to acquire the disease inoculum during vector feeding. A higher population of *D. menoni* in the sugarcane fields at three to five-month ages has previously been recorded by Senevirathne (2008) and Chanchala *et al*. (2014), which support the above-identified ages of the sugarcane crop as the most susceptible age for feeding of *D. menoni*.

M. hiroglyphicus, a vector of sugarcane WLD, recorded a similar phenomenon (Rattanabunta, and Hanboonsong (2015). *M. hiroglyphicus,* multiply its populations more rapidly at the rapid growth of the sugarcane plants when the plant is nearly 2 m in height and age of four-month. Another study on the population dynamics of *M. hiroglyphicus,* by Hanboonsong *et al.* (2006), suggests that transmits of WLD phytoplasma happen increasingly when the plants are at the early elongation stage (90 to 150 days).

Insects have often shown a feeding preference for specific plant organs or tissues and foliage and tissues of certain physiological age. In addition, variations in physical growth conditions such as plant canopy and plant height can influence host selection and subsequent population development (War *et al.,* 2012).

Table 1: Feeding of *D. menoni* on four sugarcane varieties at different age in relation honeydew weight, honeydew-stained area and number of feeding punctures on a leaf

Age (Month)	Variety			
	SL 92 5588	SL 96 128	SL 97 1442	SLC 2009 01
Honeydew stained area (mm^2)				
1	09.32^{b}	24.14^{dc}	13.88^{bc}	24.54^{b}
2	12.85^{b}	26.41^{bc}	14.80^{bc}	24.44^{b}
3	13.58^{b}	37.90^{ab}	18.78^{b}	27.32^{b}
4	22.38^{a}	47.16^{a}	37.14^{a}	55.94^{a}
5	14.15^{b}	29.70^{bc}	21.02^{b}	26.58^{b}
6	04.88^{c}	14.10^{d}	08.72^{c}	09.18^{c}
Honeydew weight (µg)				
1	1.4^{b}	2.7^{b}	2.1^{b}	2.0b^{c}
2	1.5^{b}	3.1^{b}	1.5^{b}	2.8^{b}
3	1.6^{b}	3.3^{b}	2.0^{b}	2.3b^{c}
4	3.2^{a}	5.5^{a}	4.0^{a}	6.8^{a}
5	2.7^{ab}	2.9^{b}	2.3^{b}	1.3b^{c}
6	0.6^{c}	0.5^{c}	0.6 ^{c}	0.4^{c}
Feeding punctures (#)				
1	2.36^{d}	0.76^{c}	0.68^{b}	1.40^{e}
2	5.48^{c}	1.91^{b}	2.02^{a}	4.89^{b}
3	4.21^{bc}	1.55^{bc}	1.45^{a}	4.90^{dc}
4	5.73^{ab}	1.33^{d}	1.20^{a}	5.80^{d}
5	6.40^{ab}	1.70^{bc}	1.60^{a}	5.20^{c}
6	7.06^{a}	2.46^{a}	2.60^{a}	6.00^{a}

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

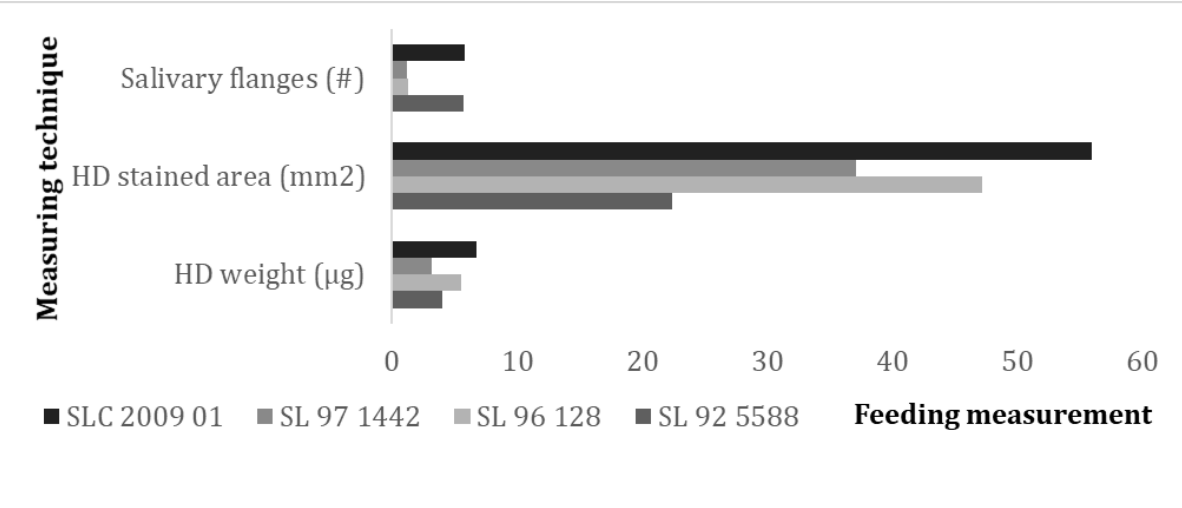

Figure 2: Varietal effect on the feeding of *D. menoni* at four-month age plants as measured by honeydew weight, honeydew stained area and number of salivary flanges on leaf

Sugarcane crop at three to five-month age seems to be associated with the most favorable characters for feeding by *D. menoni. A*ll available literature on plant vector relationships suggests that the nutritional and morphological status of the crop make it vulnerable for vector by providing required nutrients and making leaves more succulent and less resistant for piercing at feeding.

Plant nutrition has a substantial impact on the predisposition of plants to insect pests. Hemipteran herbivores are also sensitive to alterations in host plant nutrition. The chemical composition of plants changes with plant age, and it affects herbivore feeding. Bishop (1965) has recorded the influence of age on N, P, K, and Ca percentage levels in the third leaf of the sugarcane plant on susceptibility. According to Dillewijn (1952), concentrations of P and N decrease as the leaf gets older, due to the migration of these elements into the stalk. In addition, a significant accumulation of Si, Ca and Mg is found in leaves as it gets older. These changes in the chemical composition of the canopy with age are compounded by the change of chemical composition in individual leaves (Klun and Robinson, 1969; Rossi *et al.,* 1966; Suksri, 1999; Connick, 2011 and Singh and Sood, 2017).

The phloem sap consists of a wide range of organic and inorganic substances, with a high concentration of sugar. The composition of phloem sap, however, varies depending on several factors including plant age (Ziegler, 1975; Douglas, 1993; Geiger and Servaites, 1994; Kehr *et al.,* 1998; Ponder *et al.,* 2000; *Corbesier et al.,* 2001; Karley *et al.,* 2002 and Douglas, 2006).

Salivary-sheath forming at feeding appears to be particularly common among the phloem-feeding hemipterans (Hollay *et al.,* 1987; Medrano *et al.* 2007, 2009; Depieri and Panizzi, 2011; Cao *et al.,* 2013; Morgan *et al.*, 2013 and Peiffer and Felton, 2014). Thus, the number of salivary sheaths or flanges has been considered as a good predictor of crop damage from hemipteran feeding. Many authors have used these criteria to infer food consumption and feeding preference previously (Bowling, 1980; Kester *et al.,* 1984, Lye and Story, 1988; Simmons and Yeargan, 1988).

The relationship between feeding flanges and feeding volume is not simple. Pentatomid- and aphid-resistant crop varieties reduce herbivore development but do not affect the number of salivary flanges produced (Kester *et al.,* 1984; Ni and Quisenberry, 1997). When the food source is not well suited for the hemipteran feeding, increased probing behavior of the insect has been observed (Zeilinger *et al.* (2015), which is similar to the current study. Zeilinger *et al.,* (2015) suggested that excreta may be an effective measure of consumption rate than salivary flanges, where growth rates are low (*i.e.*, adult insects) and directly measuring food consumption is difficult (*i.e.,* piercing-sucking insects).

However, as in many other crops, the relationship between the amount of feeding and salivary flanges of *D. menoni* with sugarcane varieties was unpredictable. Nevertheless, the number of salivary flanges on a particular variety increases with age.

CONCLUSIONS

The amount of secreted honeydew, an indicator of plant suitability for feeding by *D. menoni,* increases gradually from one month's age and, reached a peak at the four-month age of the plant. When the plant attained five-month age, the amount of honeydew secretion reduced gradually, and a significant drop of the amount of secreted honeydew was recorded at six-month-old plants. The highest amount of honeydew was recorded at the four-month age indicating a higher feeding at the four-month age crop. There was an increasing number of salivary flanges with the increase of plant age.

The amount of secreted honeydew was found different from the variety. The highest amount of honeydew was secreted by *D. menoni,* feeding on variety SLC 2009 01 followed by variety SL 96 128 and SL 97 1442. The lowest amount of honeydew was secreted by *D. menoni,* feeding on variety SL 92 5588 confirming the resistance level of the variety. Instead of the level of resistance of the test variety, the four-month age of the sugarcane hybrids is the most vulnerable stage for *D. menoni* feeding, and the three to five-month period is susceptible for feeding and disease transmission.

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