

Ovipositional Preference of *Deltocephalus menoni* (Hemiptera: Cicadellidae), Vector of Sugarcane White Leaf Disease in Sri Lanka

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ABSTRACT

Information on the biology and ecology of *Deltocephalus menoni* (Homoptera: Cicadellidae), the only vector identified as responsible for spreading the White Leaf Disease (WLD)-causing phytoplasma in sugarcane in Sri Lanka, is essential to develop an effective disease management programme. Laboratory experiments involving choice tests and field studies were conducted at the Sugarcane Research Institute (SRI), Uda Walawe and sugarcane fields in Pelwatte from 2014 to 2017 to find out the ovipositional preference of the vector on six types of soil textures, with filter-mud and/or spent wash incorporation, with polythene mulches and plants mulched with sugarcane trash. The data on the number of eggs in different substrates were collected 14 days after the introduction of the vector. The differences in the number of eggs in different substrates were analyzed by the analysis of variance procedure and Duncan's multiple range test. The results revealed that the vector preferred sandy loam, fine sand soil, and filter-mud-incorporated soil for laying eggs. Mulching sugarcane trash did not show any significant effect on oviposition. Black and transparent polythene manage the oviposition significantly at the laboratory level but no significant effect of black polythene mulch was recorded under field conditions. Significantly higher rate of oviposition compared to the control recorded in filter-mud and spent wash incorporated fields into the soil. The findings confirmed that more attention should be given to sugarcane crops in the fields with sandy-loam and fine-sand soils in managing the disease. The incorporation of filter mud and spent wash into the soil should be minimised in the fields where WLD incidence is high. Rogueing out of the WLD-infected plants is essential to make the habitat unfavorable for laying eggs by the vector.

Keywords: *Deltocephalus menoni*, Ovipositional preference, Sri Lanka, Sugarcane White

INTRODUCTION

Until recently, the plant diseases caused by phytoplasma diseases have been managed by spraying insecticides, with or without managing the vector(s). Vector management by habitat manipulation has been identified as one of the effective strategies of the new integrated approach for managing phytoplasma diseases (Weintraub and Wilson, 2010). *Deltocephalus menoni* (Hemiptera: Cicadellidae, Subfamily: Deltocephalinae) is the only vector

identified so far transmitting the sugarcane White Leaf Disease (WLD) in Sri Lanka (Senevirathne *et al.*, 2008). Therefore, management of the vector *D. menoni* is a prerequisite to managing this devastating disease (Kumarasinghe and Jones 2001; Senevirathne *et al.*, 2006; Chanchala *et al.*, 2014; Chanchala *et al.*, 2015).

Vector-borne diseases of several crops have been managed successfully by

disrupting the lifecycle of the vectors by manipulating their egg-laying habitats (Howard and Oropeza, 1988; Summers and Stapleton, 2002; Mannini, 2007). An integrated approach developed to control the X-disease of stone fruits suggests that the management of ground cover in the orchards is an effective cultural measure for reducing leafhopper vector populations and feeding damage to peaches (Douglas and McClure, 1988). It has been identified that the insect vector of grapevine yellows lays eggs under the barks of grapevine. After identification, both phytoplasma and the eggs of the vector were simply eliminated by treatment with heat (Cauudwell, 1966). The current practice is treating grape vines with hot water (Mannini, 2007). The insect vector of bois noir disease of grapevine *Hyaletthes obsoletus* oviposits at or just below the soil surface, and vector movement into the soil has been physically prevented by using synthetic mulches such as plastic sheeting (Maixner, 2007). Summer and Stapleton (2002) reported that better control of maize leaf hopper *Dulbulus maidis*, a vector of maize stunt, leads to a higher maize yield. The control has been achieved by using plastic reflective mulch in which oviposition in the soil had been prevented. Similarly, the aster yellow phytoplasma vector on carrot, *Macrosteles quadrilineatus*, has been controlled by laying aluminum foil mulch to prevent oviposition by the vector (Stewan and Ragsdale, 1987).

D. menoni oviposits in the soil near the sugarcane plants, occasionally on the leaf sheaths at the base of the plants and on sugarcane trash (Senevirathne *et al.*, 2008). Information on its preference for laying eggs in different substrates has not been documented. Therefore, this study was conducted with the objective of determining the ovipositional preference of *D. menoni* in the different soil textures, amendments and mulches for formulating the management strategies of WLD in sugarcane in Sri Lanka.

MATERIALS AND METHODS

Laboratory and field studies were conducted to determine the ovipositional preference of the *Deltocephalus menoni* with different soil textures, amendments, and mulches.

- I. six different soil textures (gravel, coarse sand, fine sand, sandy loam, clay loam and clay)
- II. soils incorporated with filter mud and spent wash
- III. soil mulched with sugarcane trash and
- IV. soil mulched with polythene

Four laboratory experiments were conducted in insect-proof rearing cages in the entomology laboratory of the Sugarcane Research Institute (SRI), Uda Walawe, keeping the inside temperature at 25-30°C, relative humidity at 70 - 80% and under natural light to determine the ovipositional preference of *D. menoni*.

Field experiments were conducted in farmer fields in Sevanagala, Ginigalpelessa area (i), Pelwatte, Menik-Ganga nursery area (ii) and research farm (iii & iv) of the Sugarcane Research Institute (SRI), Uda Walawe.

The experiments on ovipositional preference of *D. menoni* in different soil textures were repeated three times covering both cropping seasons *Maha* 2014/15 (Mid-September to Mid-March) and *Yala* 2015 (Mid-March to Mid-September). The other three experiments on the ovipositional preference of *D. menoni* in filter mud and spent wash as soil amendments, sugarcane trash and polythene as soil mulch and infected sugarcane plants were conducted three times from February to April 2015. Field experiments were conducted from 2015 to 2017.

The following procedures were followed throughout the research period to maintain insect cultures and test plants

Maintaining insect cultures

The adult insects of *D. menoni* were collected, using a sweep net and a pooter, in sugarcane fields of less than six months old in the research farm, SRI, Uda Walawe. The insects collected were reared in insect-rearing cages according to the protocol developed by Senevirathne *et al.*, (2008).

Maintaining healthy plants

Healthy seed cane of the variety SL 96 128 was obtained from a well-maintained nursery raised using hot water-treated (dipping seed cane in 54°C hot water for 50 minutes) seed cane, by considering the visual symptoms of the plants (Chandrasena *et al.*, 2003; Senevirathne, 2008). Single-budded setts were again treated with hot water for further assurance of free of the phytoplasma, and the plants were maintained in insect-proof field cages under the recommended agronomic practices (SRI, 2004).

Preparation of experimental pots

Healthy and WLD symptomatic plants grown in plastic pots (14 x14cm) were separately maintained in insect-proof cages until three months of age. The plants were thoroughly cleaned by removing ants, spiders, and other insect predators before introducing treatments. The soil surface of each pot was covered with polythene as underpinnings to pave egg-laying substrates for the experiment.

I. Ovipositional preference of *D. menoni* in different soil types

a. Laboratory study

Six substrates representing different soil textures, viz., gravel, coarse sand, fine sand, sandy loam, clay loam and clay were used for the experiment. Sandy loam and clay-loam soils were selected based on laboratory analysis. The river sand sieved by 0.35 mm sieve was used as fine sand and the portion left above 0.35 mm sieve

and passed through 0.59 sieve was used as coarse sand. Gravel and clay substrates were selected by looking at the physical appearance and nature of roughness while touching soil samples. All substrate samples were sterilised to eliminate other entomophagous and entomopathogenic soil fauna and were paved on the polythene sheet to make a 2cm thick layer below each plant. Six pots with a single plant in each, and each containing one testing substrate were enclosed in insect-proof rearing cages (50 x30 x 20cm) and were arranged randomly in a cage for multiple-choice tests. Ten gravid females of *D. menoni* were introduced to the cage and maintained for two weeks for oviposition. After 2 weeks in the insect-proof rearing cages, the substrate in each pot was collected separately and examined through a light microscope (KYOWA TOKYO, 10x3). The number of eggs found in each substrate was counted and recorded. The experiment procedure was repeated six times; three times in the *Maha* 2014/15 season and three times in the *Yala* season 2015.

b. Field study

A field survey was conducted in farmer fields in Sevanagala (Ginigalpelessa area). Thirteen (13) farmer fields with plant crop of variety SL 96 128 was used for the study and which was located among WLD-infected sugarcane fields where vectors were available naturally. 25x10m² size 6 plant plots were considered for the study. A random sampling technique was used to collect soil samples from 0-15 cm depth by using a soil augur. Five soil samples were collected from each plot and one composite sample was prepared for final analysis representing each farmer field. Collected samples from 13 farmer fields were analyzed for their textural classes followed by the hydrometer method. Vector populations in each plot were recorded in monthly intervals from 3 to 5 months of age as, the number of vectors captured for 500 sweeps within the plot during ratoon I and ratoon II crops.

II. Ovipositional preference of *D. menoni* in soils incorporated with filter-mud and spent wash

a. Laboratory study

Filter-mud

Fresh filter mud, 2 – 3 days after disposal from the factory, was used as the substrate. Ten pots with healthy plants were selected for the experiment. A 2cm thick, dried filter-mud layer was paved on the polythene sheet placed on the soil surfaces as the egg-laying substrate in five pots. The remaining five pots were used to pave with a 2cm thick, sterilised sandy loam soil layer (Steamed at 100°C for 3hrs) as the control. Both the filter-mud and soil-paved pots were enclosed in an insect-proof rearing cage for a choice test. The newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two weeks for oviposition. The oviposition was evaluated as explained in experiment 1. Treatments were replicated fifteen times.

Spent wash

Healthy sugarcane plants grown in plastic pots (14 x14cm) were maintained in insect-proof cages until three months of age. The plants were thoroughly cleaned before introducing treatments. The soil surface of each pot was covered with polythene as underpinnings to pave egg-laying substrates for the experiment. Spent wash obtained from the Sevanagala sugar mill was used for the experiment, and 5 ml of spent wash was mixed thoroughly with the same amount of sandy loam soil paved on each plant plot to make spent wash incorporated soil substrate. Ten pots (single plant in each) were used to pave with a 2cm thick layer of soil and similarly, ten plots with a single plant in each were used to pave each with a 2cm thick layer of sterilised sandy-loam soil as the control. One pot with spent wash incorporated soil and one pot with normal soil-paved pots were enclosed together in an insect-proof rearing cage for the choice test. The

newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two week for oviposition. After 2 weeks insects were removed from cages and the substrate in each pot was collected separately. Soil samples were separately examined through a light microscope (KYOWA TOKYO, 10x3). The number of eggs found in each substrate was counted and recorded.

b. Field study

The study was conducted in the Menik-Ganga nursery in Lanka Sugar Pelwatta Pvt (Ltd). Sugarcane fields at 4 month age and variety SL 96 128 were selected with the following treatments *ie.* filter mud incorporated fields (20 tons/ha), spent wash incorporated fields (40 L³), filter mud (20 tons/ha), and spent wash incorporated fields (40 L³) and fields without any incorporations. Three plots were considered for each treatment. Vector populations in each plot were recorded in weekly intervals during the four-month age as, the number of vectors captured for 500 sweeps within the plot during the plant crop stage.

III. Ovipositional preference of *D. menoni* in soil mulched with sugarcane trash

a. Laboratory study

Sterilised soil mulched with trash and without trash mulch as control was used as the oviposition substrates. The sugarcane trash composited with dried sugarcane leaves and leaf sheaths collected from a newly-ratooned field of the variety SL 96 128 was used for the study. A 2cm height layer of the substrates was paved on a polythene sheet laid on each pot, A pair of pots with mulched soil and un-mulched soil was kept enclosed in an insect-proof rearing cage (45 x 30 x 30cm). The newly-emerged five adult vectors (3 females: 2 males) were introduced to each cage and left for two weeks for oviposition. The same procedure was repeated fifteen times. The oviposition was evaluated as explained in experiment 1.

b. Field study

The field survey was conducted in Research Farm (SRI), Uda Walawe. A ratoon crop of variety SL 96 128 was used for the study and was located among WLD-infected sugarcane fields where vectors were available naturally. 25x10m² size 6 plant plots were considered for the study. Sugarcane trash mulch was maintained in 3 plots and trash mulch was removed in 3 plots (un-mulched). Un-mulched three plots were maintained as control treatment. Vector populations in each plot were recorded in weekly intervals up to six months of age as, the number of vectors captured for 500 sweeps within the plot during ratoon I and ratoon II crops.

IV. Ovipositional preference of *D. menoni* in soil mulched with polythene

a. Laboratory study

Five mulches were used for the study. Polythene in four colours (Black, yellow, blue, transparent) and aluminum foil were used as mulches. Un mulched soil is considered as control. Three-month-old healthy sugarcane plants grown in plastic pots (14 x14cm) were obtained for the study. The plants were thoroughly cleaned by removing ants, spiders, and other insect predators before introducing treatments.

Ten plant pots from each mulch type were arranged by covering the soil with a layer of mulching material (polythene/aluminum foil). Six pots from black, yellow, blue, transparent polythene, and aluminum foil enclosed in a single insect cage with un mulched plant pot for multiple choice test. Ten gravid females were introduced into the cage. Experimental setup was kept undisturbed for two week period for oviposition. The test setup was replicated ten times. After 2 weeks, polythene/aluminum foil was removed

and the top soil layer in each pot was collected separately and examined through a light microscope (KYOWA TOKYO, 10x3) for the presence of eggs.

a. Field study

A field trial was established in Research Farm (SRI), Uda Walawe. The trial was located among WLD-infected sugarcane fields where vectors were available naturally. According to the results of the laboratory study on the effect of polythene mulch on the egg laying of vector, black colour polythene mulch was selected for the field test. Normal, un-mulched soil is considered as a control. 25x10m² size 6 plant plots were established with hot water-treated seed cane (SL 96 128) during the Maha season. After the germination of plants, the base of the plants in randomly selected three plots (both sides) were covered with 1.5' black polythene. Polythene mulch was fixed into the soil with wooden pegs to cover the soil properly at the base of the plants. The remaining three plots were maintained as control treatment. Vector populations in each plot were recorded in weekly intervals up to six month age as, the number of vectors captured for 500 sweeps within the plot.

Statistical Analysis

Data on egg counts were subjected to square root transformation, and the analysis of variance was conducted for the transformed data. Means were separated by Duncan's multiple range test at 0.05 probability level using the SAS software.

RESULTS AND DISCUSSION

I. Ovipositional preference of *D. menoni* in different soil types

a. Laboratory study

The highest number of eggs of *D. Menoni* was found in sandy loam soil followed by fine-sand soil. Similar observations of more eggs in sandy loam soil were found in all three experiments conducted in two seasons (*Maha* and *Yala*). There was no significant difference in number of eggs recorded between the two cropping seasons (Table 1).

Table 01: The mean number of eggs laid by ten *D. Menoni* (mean) in six soil types at fourteen days after introducing gravid females into rearing (laboratory) cage in the *Maha* 2014 and *Yala* 2015 seasons

Soil type	Average number of Eggs	
	<i>Yala</i> season	<i>Maha</i> season
Fine sand	5.66 ^{ab}	7 ^{ab}
Coarse sand	0 ^b	0 ^b
Sandy loam	20 ^a	23.25 ^a
Clay loam	0 ^b	0 ^b
Gravel	0 ^b	0 ^b
Clay	0 ^b	0 ^b

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

D. menoni preferred sandy loam soil for laying eggs, as it had a significantly higher number of eggs. This could be attributed to the porous nature of the soil (Foth, 1978). Loose, porous soil facilitates the penetration of ovipositor into the soil for egg laying. The compacted soils restrict penetration. Also, poor ventilation in compact soil disturbs laying eggs and hatching (Simelane, 2007; Chaudarie, 1985). Yang and Pang (1979) have reported that, *Epitettix hiroglyphicus*; the vector of WLD in Taiwan, prefers fine sand, coarse sand and sandy loam soils for oviposition, and 10 % (w/w) was the most preferred soil moisture level for its oviposition.

b. Field study

According to a field survey conducted in farmers' fields in Sevanagala rain-fed sector, all highly WLD-infected fields were associated with sandy loam soils and 10% (w/w) moisture content (Table 02). The population levels of the vector were also comparatively high in those fields.

Table 02: Sand, clay, silt composition, soil textural class and vector population captured during 500 sweeps in each test field

Field No	Sand%	Clay%	Silt%	Texture class	Vectors
1	76.72	15.28	8.00	Sandy Lome	7
2	72.72	18.00	9.28	Sandy Lome	5
3	70.72	20.00	9.24	Sandy Lome	5
4	66.72	16.00	22.08	Sandy Lome	4
5	74.72	11.20	9.28	Sandy Lome	7
6	70.72	20.00	9.28	Sandy Lome	4
7	68.72	20.00	11.28	Sandy Lome	2
8	68.72	20.00	11.28	Sandy Lome	2
9	80.72	14.00	5.28	Sandy Lome	8
10	61.03	28.24	10.72	Sandy Clay Lome	1
11	70.72	22.24	7.04	Sandy Clay Lome	0
12	78.72	18.24	3.04	Sandy Lome	8
13	72.72	16.24	11.04	Sandy Lome	12

This confirms the finding of the laboratory study, the high preference of the vector for sandy loam soil for oviposition under favorable moisture levels. Hence, more attention should be given to managing WLD in the fields with sandy loam and fine sand soils in areas with high incidence of this disease.

ii. Ovipositional preference of *D. menoni* for soils incorporated with filter-mud and spent wash

a. Laboratory study

The number of eggs in filter-mud incorporated soil was 67.5% more than that in the unincorporated soils. The number of eggs laid in spent wash incorporated soil is significantly high compared to the spent wash unincorporated soil (Table 03).

Table 03: The mean number of eggs laid by three female adults of *D. menoni* fourteen days after introducing into the rearing cage

Treatment	Number of eggs (mean)
<i>Filter mud</i>	
Incorporated soil	8 ^a
Un-incorporated soil	2.6 ^b
<i>Spent wash</i>	
Incorporated soil	8 ^a
Un-incorporated soil	3 ^b

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

a. Field study

High incidences of WLD vector have been recorded in the fields incorporated with filter-mud, spent wash, and both filter mud and spent wash incorporated fields than the filter-mud unincorporated fields (Table 04). Even though filter mud enhances soil quality by adding organic matter into the soil, this practice enhances the soil porosity facilitating penetration of ovipositor 1-2cm deep into the soil for laying eggs.

Table 04: Mean number of *D. menoni* adults captured during 500 sweeps in each plot with each treatment

Treatment	Number of insects (mean)
Filter mud-incorporated soil	8 ^a
Spent wash incorporated soil	7.5 ^a
Filter mud and spent wash incorporated soil	9 ^a
Control/Un-incorporated soil	3.5 ^b

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

iii. Ovipositional preference of *D. menoni* for the soil mulched with sugarcane trash

In the laboratory study, the mean number of eggs in the soil mulched with sugarcane trash was slightly higher (5.4^a) than that in un-mulched soil (5.2^a), but the difference was not statistically significant. Also, according to the data collected from the field study, the number of vectors in the fields with sugarcane trash was slightly higher (8.9^a) than that in the fields without sugarcane trash (8.4^a), but the differences were statistically not significant. However, *D. menoni* occasionally lays eggs on sugarcane trash (Senevirathne, 2008).

Howard and Oropeza (1998) have discovered that different types of mulching materials have influenced on the abundance of the vector (*Myndus crudus*) of coconut lethal yellow disease and very few nymphs have been present near the coconut trees that have been mulched with coarse materials. Douglas and McClure (1988) have designed an approach to overcome the “X-disease of stone fruit” by manipulating the vector population by maintaining bare ground under the fruit crop. But this study reveals that there are no significant effects of trash on the vector population. It proves that removing or burning trash does not reduce the population level of WLD vectors in sugarcane fields.

iv. Ovipositional preference of *D. menoni* in soil mulched with polythene

a. Laboratory study

The lowest number of eggs was recorded in Black and transparent polythene mulched plant pots. A significantly high amount of eggs was recorded on pots with un-mulched soil (Table 05). There is no significant difference between black colour polythene mulch, transparent polythene mulch and aluminum foil mulch. Since aluminium foil is costly and

transparent polythene degrade easily, black colour polythene was selected for the field study.

Table 05: The mean number of eggs laid by three *D. menoni* females on six soil mulches at fourteen days after introducing gravid females into rearing (laboratory) cage

Mulch type	The average number of Eggs
Soil (control)	7 ^a
Blue colour polythene	3 ^{ab}
Yellow colour polythene	2.33 ^{ab}
Black colour polythene	0.33 ^b
Transparent polythene	0.33 ^b
Aluminium foil	0.66 ^b

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

a. Field study

Table 06: Mean number of *D. menoni* adults captured during 500 sweeps in each age of the black colour polythene mulched and un-mulched plots

Crop age	The mean number of vectors / 500 sweeps	
	Polythene mulched	Un mulched soil
1 st Month	0	0
2 nd Month	2 ^a	2.2 ^a
3 rd Month	3.33 ^a	3.5 ^a
4 th Month	7 ^a	7 ^a
5 th Month	3.5 ^a	3.33 ^a
6 th Month	0.75 ^a	0.8 ^a

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

As the number of eggs in the soil is not detectable, the number of insect vectors caught in the insect net is considered a relative measurement of eggs. There was no significant difference recorded in the mean number of *D. menoni* adults captured in polythene mulched and un-mulched plant plots (Table 06). Accordingly, no significant effect of black polythene mulch was recorded under field conditions.

Since it is not possible to cover the soil completely under field conditions, using polythene mulch to deduct the oviposition rate of the vector is not an effective vector management strategy. The ridge and furrow land preparation system for sugarcane is also may be a reason for the ineffectiveness of the mulch. Because due to irregular land proper mulching/covering soil is difficult. Tillering nature of the plant also may cause a reason for the incompatibility of mulch to the soil. However, the effect of the polythene mulch in ratoon one crop will be tested with the ratoon crop of the same plant crop used in the study.

CONCLUSIONS

There is a significant difference in ovipositional preference of the WLD vector *D. Menoni* between different soil groups and between different soil amendments. There is a high preference of *D. menoni* for oviposition in sandy loam and fine-sand soils. Therefore, more attention should be given to managing WLD in the fields with sandy loam and fine-sand soils in the areas with a high incidence of this disease. This could be accomplished by adopting recommendations given by SRI on the use of healthy seed cane, regular monitoring of the disease incidence in the field, and regular roguing out of the infected plants at the early stage. Filter mud was found to be a more preferred substrate for oviposition by *D. menoni*. This has to be considered in using filter mud to enhance the soil quality, particularly in WLD susceptible areas having a high vector population. The above investigations were conducted only at Uda Walawe under controlled conditions and a limited number of seasons, sites, and locations. The results reported are very useful as baseline information for developing a WLD management program, and further studies are required to confirm the applicability of findings under different field conditions.

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