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Sugarcane White Leaf Disease Incidences and Population Dynamic of Leafhopper Insect Vectors in Sugarcane Plantations in Northeast Thailand

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ABSTRACT
The work consisted of two experiments, i.e. Experiment 1 was conducted under controlled environments where sugarcane plants were used as hosts. This investigation aimed to monitor the occurrence of the Sugarcane White Leaf disease and the abundance of Leafhopper insect vectors and also the work aimed to provide useful information in understanding some aspects on epidemiology of the Sugarcane White Leaf disease. A Completely Randomized Design with three replications was used to justify growth and development of Leafhopper insects as affected by different temperatures: 20 (T1), 25 (T2), 30 (T3) and 35°C (T4). Experiment 2 was carried out to determine the numbers of Leafhopper insects with the use of light traps in the sugarcane Field 1 (ratoon plants), Field 2 (newly planted), Field 3 (newly planted) and Field 4 (ratoon plants). The results of Experiment 1 showed that growth and development of Leafhopper insects were highly affected by temperatures i.e. the higher the environmental temperature the faster the growth and development of the insects to reach its full adulthood. At 20°C, Leafhopper insects took 12 days to lay eggs whereas at 25°C the insects took only 6 days. Male reached its adulthood approximately 9 days earlier than female when cultured at 25°C and became approximately one week at 30°C or higher. The results of Experiment 2 showed that the majority of Leafhopper insects were found within the months of June and July for both newly planted and ratoon crops. A small amount was found in May and August with an exceptional case of Field 4 where the highest number of Leafhopper insects was found in April followed by June and July. For Sugarcane White Leaf disease, the disease was found in all months of the year except February for Fields 2 and 3. Newly planted sugarcane plants attained much smaller percentages of disease than those of the ratoon plants.

Key words: Leafhopper insect vector, newly planted plants, ratoon plants, Sugarcane White Leaf disease

INTRODUCTION

Sugarcane plant (*Saccharum officinarum* L.) is one of many important economic crops being cultivated annually in Thailand, particularly in many provinces in both the Central Plain and Northeastern regions of the country. Sugarcane plant has long been cultivated by a large number of growers of more than 90 countries in the tropical and subtropical regions. The crop plants provide a considerable amount of sugar in stems when it reaches a full stage of growth. In Northeast Thailand, sugarcane plantations have been established in many provinces such as Udon Thani, Burirum, Khon Kaen, Korat, Mukdahan, Chaiyaphume, Mahasarakham and other provinces where numbers of sugar mills are located. It has been advocated that the largest sugarcane exporter in the 2011 was Brazil followed by Thailand, Australia and India (FAO., 2011). The low cane contents and the poor sugar yields have been considered to be a significant problem caused by several contributing factors such as poor soil fertility, salinity, drought, pests and diseases. Of many important sugarcane diseases, Sugarcane White Leaf disease (SWL) collaborated with Phytoplasma has long been considered as one of the most significant threats to sugarcane production in the Asian countries (Kumarasinghe and Jones, 2001; Marcone, 2002; Burgess, 2010). The SWL disease can spread out largely among sugarcane plantations by a mean of Phytoplasma-infected cane stalks where two species of phloem feeding Leafhoppers have been naturally and commonly inhabited. They include *Matsumuratettixhiroglyphicus* (Matsumura) and *Yamatotettixflavovittatus* (Matsumura). These two species of Leafhoppers are important vectors and once the plantation is infested these vector species could easily multiply themselves and become vectors to carry out the disease of Sugarcane White Leaf more rapidly with time (Matsumoto *et al*., 1969; Chen, 1974; Maramorosch *et al*., 1975; Hanboonsong *et al*., 2002, 2006). As a part of sugarcane integrated management program to be applied for the development of a large scale plantation, an investigation on vector population levels may possibly provide some essential information for growers of the sugarcane plants in making their decision on any control measurements against the infestation of the disease. This investigation aimed to monitor the occurrence of the Sugarcane White Leaf disease and the abundance of Leafhopper insect vectors and also the work aimed to provide useful information in understanding some aspects on epidemiology of the Sugarcane White Leaf disease. Furthermore, there is a limited published work on Sugarcane White Leaf disease in Thailand, particularly in the northeastern region.

MATERIALS AND METHODS

For this study, two experiments were carried out, i.e. Experiment 1 was carried out from May-September 2012 at Khon Kaen University, Khon Kaen, Thailand under controlled environments where sugarcane plants were used as Leafhoppers hosts. A Completely Randomized Design (CRD) with three replications was used. Four treatments were carried out with the use of a day old Leafhopper insects (*M. hiroglyphicus*). The insects were cultured at 20°C (T1), 25°C (T2), 30°C (T3, control) and 35°C (T4). Six Leafhopper insects were used for each replication. This experiment was carried out to provide some initial information on growth behavior of the insects as affected by different environmental temperatures. With the first experiment the following parameters were used, i.e., numbers of days to lay egg, numbers of days to reach molting stages of its growth (larvae stages of growth from stages 1-5) and number of days to reach a full adulthood stage as influenced by the temperatures. For Experiment 2, the experiment was carried out under field conditions during February-November 2012. Four field areas of the sugarcane plantation were used. This experiment was carried out to determine numbers of Leafhopper insects available in different areas of the four sites of the sugar plantations (Fields 1 up to 4), particularly in different months of the year. The four sites are located within the area of the Non sa-art District, Udon Thani Province, Northeast Thailand. This area was considered as the most infested area of Sugarcane White Leaf disease of the northeastern region of the country (Wongkaew *et al*., 1997; Marcone, 2002). The four sites of the sugarcane plantations include: Field 1 for ratoon plants, Field 2 for newly planted plants, Field 3 for newly planted plants and Field 4 for ratoon plants. The four sites are located nearby each other of the same district of the province. The KK88-92 sugarcane variety was used in all experimental fields. An area of 1600 m^2 of each site occupied with sugarcane plants was chosen for experimental observations. The sugarcane plants were sown at distances of a 50×80 cm within rows and between rows, respectively. The experimental observations commenced when the sugarcane plants reached an age of four months old for both newly planted and the first year ratoon plants. Thirty rows of seventy planted holes of sugarcane plants of each field were randomly marked for both Leafhopper insects and Sugarcane White Leaf disease determinations. The percentages of the Sugarcane White Leaf disease were monthly determined and calculated throughout the study period. The meteorological data on radiant energy, minimum and maximum temperatures, relative humidity, wind speed and rainfall were recorded throughout the experimental period (Table 1). The soil samples were taken at random from each field to the depth of approximately 30 cm at the beginning of the experimental period and they were used for the determinations of soil nitrogen (N), soil phosphorous (P), soil potassium (K), soil Organic Matter (OM) and soil pH. Soil analysis was carried out in the laboratory of the Department of Plant Science and Agricultural Resources, Khon Kaen University (Table 2).

Of the four chosen sugarcane fields of the experimental sites, the populations of the Leafhopper insects (*M. hiroglyphicus*) were observed and collected twice a month with the use of four light traps where each light trap was combined with the use of a sticky plastic sheet. Each sticky plastic sheet was fixed with a PVC plastic pipe frame (50×50 cm in width and length), white cloth, transparent plastic sheet and a light source (black light-blue 20 W). The insect sticky glue obtained from the Beetle Glue, Green Plana Co., Ltd., Thailand was applied as a thin layer on both sides of

Table 1: Mean values of meteorological data measured at the sugarcane experimental fields (Fields 1-4) from February to November 2012 at Udon Thani Province, Northeast Thailand

Experimental sites	Radiant energy (MJ/m^2 day^{-1})	Mini. temp. (°C)	Max. temp. (°C)	Relative humidity (%)	Wind speed (m sec^{-1})	Rainfall (mm day^{-1})
Field 1	15.30	21.74	31.32	71.32	1.29	3.65
Field 2	13.59	21.67	31.47	71.84	0.80	3.74
Field 3	15.02	21.90	31.60	71.72	1.18	4.04
Field 4	15.10	21.69	31.79	72.28	1.03	5.44

Table 2: Mean values of soil analysis data on nitrogen, phosphorus, potassium, organic matter and pH of the Oxic Paleustults great soil group at sugarcane experimental field (Fields 1-4) at Udon Thani Province, Northeast Thailand

Field number	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Organic matter (%)	pH
Field 1	0.08^{a}	63.60^{c}	47.00^{a}	1.56^{a}	4.60^{c}
Field 2	0.06^{b}	104.60^{a}	25.00^{d}	1.23^{d}	5.50^{a}
Field 3	0.06^{b}	101.80^{b}	44.00^{b}	1.27^{c}	4.90^{b}
Field 4	0.07^{a}	47.70^{d}	36.00^{c}	1.29^{b}	4.40^{d}
F-test	*	**	**	**	**
C.V. (%)	22.08	0.19	3.29	1.12	3.07

Letters in each column indicated least significant differences of probability (p) of **: 0.01 and *: 0.05 of Duncan's Multiple Range Test. C.V. %: Covariant percentages

the plastic sheet of each frame. The light traps were placed (one trap for each field) at 1 m away from the border rows of the sugarcane plants at a height of 1 m from 18:00-21:00 PM for insect pest attraction. All of the trapped insects in each field were identified for Leafhopper (*M. hiroglyphicus*) populations and individual Leafhoppers were used for each counting period under an illuminated microscope unit in the laboratory. The number of Leafhopper insect vectors was calculated monthly. The obtained data were calculated where appropriate with the use of a computer program (SAS., 1998).

RESULTS

Experiment 1: The results showed that number of days to reach the first day in laying eggs of T1 up to T4 ranged from 5.76-12.14 days for T4 and T1, respectively. The differences were large and highly significant (Table 3). A decrease in temperature highly increased number of days to reach a stage of laying eggs. At larvae stage 1, the results showed that number of days to reach stage 1 of molting ranged from 2.48-5.36 days for T4 and T1, respectively. The differences were large and highly significant. A similar trend was found with stage 2 up to stage 5 where number of days in molting highly increased with a decrease in environmental temperature. This trend was found from stage 1 up to the last stage (stage 5). The differences were large and highly significant. The number of days to reach a full growth for both male and female highly increased with a decrease in environmental temperature where male Leafhoppers attained number of days ranged from 18.32-21.78 days for T4 and T1, respectively and female ranged from 25.11-30.70 days for T4 and T1, respectively. A decrease in temperature highly increased number of days to reach a full growth of adulthood stage. High environmental temperature tremendously hastened life of the Leafhoppers where the female Leafhoppers possessed much higher number of days to reach a full adulthood stage of growth than the male Leafhoppers. Male required a shorter period of time to reach its full adulthood stage than female insects.

Experiment 2: With meteorological data, the results showed that mean values of radiant energy from the sun of Field 1 up to Filed 4 ranged from 15.02-15.59 $MJ/m^2/day$ for Field 3 and Field 2, respectively (Table 1). Mean values of minimum temperature ranged from 21.67-21.90°C for Field 2 and Field 3, respectively whilst mean values of maximum temperature ranged from 31.32-31.79°C for Field 1 and Field 4, respectively. Mean values of relative humidity ranged from 71.32-72.28% for Field 1 and Field 4, respectively. Mean values of wind speed ranged from 0.80-1.29 m sec^{-1} for Field 2 and Field 1, respectively. Mean values of rainfall ranged from 3.65-5.44 mm day^{-1} for Field 1 and Field 4, respectively.

For soil analysis data, the results showed that mean values of soil nitrogen (N) ranged from 0.06-0.08 for Field 3 and Field 1, respectively (Table 2). Mean values of soil phosphorus (P) ranged from 47.7-104.6 ppm for Filed 4 and Field 2, respectively. Mean values of soil potassium (K) ranged from 25.0-47.0 ppm for Field 2 and Field 1, respectively. Mean values of soil sodium (Na) ranged from 1.3-1.4 ppm for Field 1 and Field 4, respectively. Mean values of soil Organic Matter (OM) ranged from 1.23-1.56% for Field 2 and Field 1, respectively. Mean values of soil pH ranged from 4.4-5.5 for Field 4 and Field 2, respectively. Mean values of electrical conductivity ranged from 0.02-0.03 dS m^{-1} for Field 1 and Field 4, respectively.

With the results on Leafhopper insect vectors and Sugarcane White Leaf disease of Field 1 up to Field 4, the results showed that there were no Leafhoppers found from

Table 3: Growth stages and development of the *Matsumuratettix hiroglyphicus* insects cultured under controlled environments at different treatments and temperature at the Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

		Days							
Treatments	Temperature	Egg laid	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Male	Female
T1	20°C	12.14[a]	5.36[a]	5.14[a]	5.42[a]	5.66[a]	5.32[a]	21.78[a]	30.70[a]
T2	25°C	6.72[b]	3.10[b]	3.46[b]	3.64[b]	4.46[b]	4.24[b]	20.96[a]	28.40[ab]
T3	30°C	6.26[c]	2.36[c]	2.74[c]	3.46[b]	3.92[c]	4.06[b]	20.02[a]	26.04[b]
T4	35°C	5.76[c]	2.48[c]	2.50[c]	2.58[c]	3.22[d]	3.70[c]	18.32[b]	25.11[b]
F-test		**	**	**	**	**	**	**	**
C.V. (%)		18.65	20.61	23.95	19.14	16.55	18.76	9.46	6.37

Letters in each column indicated least significant differences at probability (p) of 0.05 of Duncan's Multiple Range Test. C.V. %: Covariant percentages

Table 4: Monthly numbers of Leafhopper insects (*Matsumuratettix hiroglyphicus*) and infected percentages of Sugarcane White Leaf disease (SWLD) of sugarcane experimental fields (Fields 1-4) recorded from February to November 2012 at Udon Thaini Province, Northeast Thailand

	Field 1		Field 2		Field 3		Field 4	
Months	Insect number	SWLD (%)	Insect number	SWLD (%)	Insect number	SWLD (%)	Insect number	SWLD (%)
February	0	0.19	0	0	0	0	0	1.12
March	0	0.31	0	0.03	0	0.05	0	2.10
April	0	5.19	0	0.14	0	0.29	208.50	11.24
May	12.00	23.16	24.50	0.34	11.50	0.41	12.00	54.36
June	174.00	29.04	251.50	1.08	308.50	0.46	190.00	72.55
July	218.00	29.90	530.40	1.74	446.00	0.48	151.50	86.00
August	3.00	30.24	8.50	1.84	9.00	0.75	2.00	86.94
September	0	36.38	0	1.95	0	0.94	0	89.38
October	0	34.91	0	1.33	0	0.79	0	85.20
November	0	32.83	0	1.12	0	0.54	0	80.19

February to April for Fields 1, 2 and 3 but with a large number of Leafhopper insects (208.5 individuals) was found in April for Field 4 (ratoon plants). Mean values of Leafhopper insects found in May ranged from 11.50-24.50 individuals for Field 3 and Field 2, respectively (Table 4). Mean values of Leafhopper insects found in June ranged from 174.00-308.50 individuals for Field 1 and Field 3, respectively. Mean values of Leafhopper insects found in July ranged from 151.50-530.00 individuals for Field 4 and Field 2, respectively. Mean values of Leafhopper insects found in August ranged from 2.00-9.00 individuals for Field 4 and Field 3, respectively. There were no Leafhopper insects found in September up to November, 2011 in Field 1 up to Field 4 of the province of Udon Thani.

For the distribution of the Sugarcane White Leaf disease, the results showed that White Leaf disease infested from February until November where Field 1attained percentages ranged from 0.19-36.38 for February and November, respectively (Table 4). Field 2 attained infested percentages ranged from 8.5-530 for August and July, respectively. Field 3 possessed infestation % ranged from 9.00-446 for August and July, respectively. Field 4 attained infestation % ranged from 2.00-208 for August and April, respectively.

DISCUSSION

With the results carried out under controlled environments on the effect due to different levels of temperature of the Experiment 1, the results showed that Leafhopper insects (*M. hiroglyphicus*) tremendously responded to low and high environmental temperatures, i.e. the results on growth revealed that the higher the temperature the shorter the number of days to reach its adulthood stage. The differences were large and highly significant. The results agree with the work reported by Hanboonsong *et al.* (2006). The results evidently showed that under controlled conditions at 20°C, Leafhopper insects reached its maturity stage at day 12 and started to lay eggs whereas at 25°C the Leafhopper insects could be able to use number of days to lay eggs twice earlier than those of the growth at 20°C (day 6 only) hence a warm environmental temperature hastened maturity age of the insects tremendously. The results indicated that at a high environmental temperature of 25°C or higher, the Leafhopper insects could be able to reach its maturity stage much earlier and able to multiply its populations more rapidly with time. Thus with the environmental temperature in the tropical areas like in Thailand, Leafhopper insects could be able to multiply its populations more rapidly with time throughout the year provided that its hosts are available. It was found that male Leafhopper insects reached its adulthood stage earlier than female insects about one week earlier when the environmental temperature was higher than 25°C. The results suggested that Leafhopper insects could easily remain in any worm climate like in any other Asian countries and they could be ready to act as vectors for the spread out of diseases at any time of the year. The spread out of the disease must be attributable to the suitable climatic conditions where radiant energy from the sun attained a mean value greater than 13.5 MJ m^{-1} day^{-1} and mean values of rainfall ranged from 3.6-5.4 mm day^{-1} with a mean value of minimum temperature higher than 21°C. Thus this tropical climate favors the rapid multiplication of Leafhopper insects. That is why Sugarcane White Leaf disease could remain in the sugarcane plantations when growers always preserve their sugarcane plants for any new coming planting season or even in the ratoon plants for the second crop.

It has been widely advocated that the majority of soils in Northeast Thailand are those of the Oxic Paleustults great soil group with a relatively poor fertility (Trelo-ges *et al*., 2002). With the soil analysis data, in most cases, the soil indicated unfavorable conditions for growth of the sugarcane plants, e.g., mean values of soil phosphorus (P) ranged from 47.7-104.6 ppm for Fields 4 and 2, respectively. These extremely high mean values of P could have been attributed to the previous history of chemical fertilizers added to the soil. Amount and type of fertilizer application could have been carried out without any soil analysis information hence it is a waste of capital investment. Soil available P should not be exceeded a mean value of 50 ppm since higher P could provide toxic effect on growth of the crop plants and soil pH should be at a range of 6-6.5 (1:2.5 soil: water by volume). This range of soil pH could facilitate a better release of soil nutrients around the roots zone of the crop plants. Furthermore, the attained mean values of potassium (36-47 ppm for Fields 4 and 1, respectively) were inadequately available for growth of the crop plants. Mean value of the potassium in inorganic soils should be at least 80 ppm (Mengel and Kirkby, 1987; Miller and Donahue, 1990; Suksri, 1998a, b; Kasikranan, 2011). The high mean values of organic matter (1.23-1.56% for Fields 2 and 1, respectively) must be attributable to the previous history of the added amount of crop residues to the soil hence soil nitrogen levels were moderately supplied.

With number of Leafhopper insects (*M. hiroglyphicus*) in Field 1 up to Field 3, the results showed that the collected numbers of Leafhopper insects, in most cases, were highest in the month of July followed by June and May whereas in Field 4 the numbers of insects were highest in April followed by June and least with August. The results indicated that Leafhopper insects were able to multiply its populations more rapidly with time in the rainy season, particularly in the months of May up to August. This must be attributable to the rapid growth of the sugarcane plants since the plant stand was nearly 2 m in height. However, with Field 4 (ratoon crop), it was found that the highest numbers of Leafhopper insects were found in the month of April followed by the months of June and July. The highest amount of the insects found in April must be attributable to the high amount of available sugarcane plants of the ratoon crop where the Leafhopper insects used them for sources of food and hosts. The rapid increases in the amount of insects may be due to the low value of soil Ca^{2+} due to the low value of soil pH resulted in a low amount of Ca^{2+} in leaves and stems of the sugarcane plants where the juices available in sugarcane leaves and stems could be of a high palatability for the insects (Suksri, 1999). Therefore, growers should add more of dolomite and organic residues to their lands to attain a suitable mean value of soil pH within a range from 6-6.5 as described earlier.

The severely high percentages of Sugarcane White Leaf disease were found with Field 2 and Field 4. This must be attributable to the rapid growth of the existing ratoon plants continued to supply food for the insects without any vacancy period whilst the percentages of those newly sown plants of Fields 2 and 3 (lesser than 2%) were tremendously lower than those of the ratoon plants. The results suggested that the plants of the ratoon crop may not be of advantages when it comes to the high percentages of the diseases those remain in the plots for the coming rainy season. The disease could spread out easily when Leafhopper insects multiply its populations, particularly in the rainy season (April-August). However, the first ratoon crop should remain in the plots or not it depended on economic returns where growers must evaluate the margin of the profit. The use of some chemical supplies such as insecticides, herbicides and others chemical substances may not be of significant advantages since some hazardous effects may be degrading the quality of the products derived from the sugarcane plants.

To sum up, the results of Experiment 1 carried out under controlled environmental temperatures revealed that growth and development of Leafhopper insects were highly affected by temperature levels, i.e. the higher the environmental temperature the faster the growth and development of the Leafhopper insects to reach its full adulthood stage hence tropical environmental temperature from 25-35°C suited most for a rapid multiplication of this insect vector. The results on the distribution of Leafhopper insects found among different months of the year in the sugarcane plantation of Field 1 up to Field 4 of the Experiment 2 indicated that the majority of Leafhopper insects were found within the months of June up to July for both newly planted and ratoon plants. A small number was found in May and August with exceptional case of Field 4, ratoon plants, i.e. high numbers of Leafhopper insects were found in April. With the Sugarcane White Leaf disease, in most cases, were severely found with the ratoon plants of Fields 1 and 4. The left out of ratoon plants for second crop may not be of a high advantage due to a certain amount of Leafhopper insects and disease remained in the sugarcane fields of the sugarcane plantation.

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