ความชอบสีและประสิทธิภาพในการบินของแมลงพาหะนำโรคใบขาวอ้อย *Matsumuratettix hiroglyphicus* (Matsumura) (Homoptera: Cicadellidae)

Color preference and flight ability of the sugarcane white leaf insect vector, *Matsumuratettix hiroglyphicus* (Matsumura) (Homoptera: Cicadellidae)

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บทคัดย่อ: การเปรียบเทียบกับดักสีต่างๆที่มีผลในการดึงดูดแมลงพาหะโรคใบขาวอ้อย *Matsumuratettix hiroglyphicus* โดยในพื้นปลูกอ้อย อำเภอโนนสะอาด จังหวัดอุดรธานี โดยใช้กับดักกาวเหนียวที่เป็นแผ่นพลาสติกสีต่างๆได้แก่ สีน้ำเงิน สีเหลือง สีเขียว สีขาว สีส้ม และใสไม่มีสีเป็นตัวควบคุม ผลการทดลอง พบว่า สีน้ำเงินและสีเหลืองมีประสิทธิภาพการดึงดูดแมลงพาหะ *M. hiroglyphicus* ได้ใกล้เคียงกัน รองลงมาคือ สีส้ม สีขาว และสีเขียว การทดลองเกี่ยวกับความสามารถในการบินของแมลงพาหะ *M. hiroglyphicus* ด้วยวิธี ทำเครื่องหมาย- ปลดปล่อย-สำรวจผล (mark-release-recapture) ในพื้นที่ปลูกอ้อยตำบลท่าพระ จังหวัดขอนแก่น โดยการจับตัวเต็มวัยแมลงพาหะมาทำเครื่องหมายด้วยผง fluorescent dye powder และนำไปปล่อยในกลางแปลงอ้อยที่มีกับดักกาวเหนียว ทั้งหมด 60 กับดัก ซึ่งวางระยะห่าง 5, 10, 20, 30 และ 50 เมตร ตามลำดับ ใน 6 ทิศทาง ภายในรัศมีวงกลม แล้วตรวจกับดัก ทุกๆ 2 วัน จนครบ 20 วัน หลังจากปล่อยซึ่งพบว่าในสภาพพื้นที่แปลงทดลอง แมลงพาหะ *M. hiroglyphicus* มีประสิทธิภาพในการบินได้ระยะทางไกล 4 เมตรต่อวัน และเมื่อนำมาคำนวณประสิทธิภาพการบินด้วย วิธี Yamamura method ประมาณการว่าแมลงพาหะ *M. hiroglyphicus* สามารถบินในสภาพธรรมชาติได้ระยะไกล 162.1 เมตร ภายในเวลา 20 วัน ข้อมูลพฤติกรรมความชอบชนิดสีและระยะทางการบินของแมลงพาหะนี้จะเป็นประโยชน์ในการนำมาใช้สำหรับการศึกษาการระบาดวิทยาและการพยากรณ์การระบาดของโรคใบขาวอ้อย
คำสำคัญ: โรคใบขาวอ้อย, *M. hiroglyphicus,* กับดักกาวเหนียวสี, ประสิทธิภาพการบิน

ABSTRACT: The experiment was conducted to evaluate the trapping efficiency of colored sticky traps to insect vectors, *Matsumuratettix hiroglyphicus* (Matsumura) in Kumpawapi, Udon Thani Province. In this experiment, sticky board traps with colors of blue, yellow, green, white, orange and colorless (control) were used. The result indicated that the blue and yellow color sticky trap showed the same efficiency in catching *M. hiroglyphicus* followed by the orange, white, green and colorless (control) sticky trap. The experiment on flight ability of *M. hiroglyphicus* was investigated by conducting the mark-release-recapture method in a sugarcane field in Tha Phra, Khon Kaen Province. Field collected adult vectors were marked with fluorescent dye powder, released and recaptured on the sticky traps in sugarcane filed. A total of 60 traps were placed at distances of 5, 10, 20, 30 and 50 m. in six directions radiating from the release point in the field. All traps were examined at two day intervals until 20 days after release. The actual field data of simple mean natural flight distance per day of *M. hiroglyphicus* was 4.0 m and the estimated mean natural flight distance using Yamamura method, within 20 days after released was 162.1 m. In conclusion, the results of color preference and flight distance of sugarcane white leaf insect vector would be useful for disease epidemiology study and for developing a spread-estimation insect and disease models for sugarcane white leaf disease control.
Keywords: sugarcane white leaf disease, *M. hiroglyphicus*, color sticky trap, flight ability

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Introduction

Sugarcane white leaf associated with phytoplasma is a serious disease of sugarcane in Thailand. The leafhopper, *Matsumuratettix hiroglyphicus* (Matsumura) and *Yamatotettix flavovittatus* Matsumura (Homoptera: Cicadellidae) are known vector of sugarcane white leaf (SCWL) phytoplasma (Matsumoto et al., 1968; Chen, 1974; Hanboonsong et al., 2002; 2006). It has been reported that the species of *M. hiroglyphicus* is not only the vector but also the pathogen reservoir (Hanboonsong et al., 2002). The transmission rate of 55% by *M. hiroglyphicus* is higher than that of 45% by Y. *flavovittatus* (Hanboonsong et al., 2006). Therefore, insect vector, *M. hiroglyphicus* is one of the main species of vectors contributing to outbreak of SCWL disease and decrease the sugarcane production in Thailand. Till now, there is no effective method or sugarcane resistant variety to control this disease, management of insect vector is one of the approaches to reduce the SCWL disease.

Information on flight ability is essential for the control of vector to transmit the pathogen of disease and to prevent the disease spread. Many experiments have been conducted to estimate the flight abilities of leafhoppers using mark-release-recapture (MRR) method; for example, the dispersal abilities were investigated for the different species of leafhopper vectors using yellow sticky traps (Larsen and Whalon, 1988; Whitney and Meyer, 1988; Blackmer et al., 2006). Moreover, it has been reported that the different color sticky traps are also used for trapping leafhoppers species on many crops (Chu et al., 2000; Lessio and Alma, 2004: Raja and Arivudainambi, 2004). Therefore, the study was carried out with two objectives; 1) to evaluate the trapping efficiency of different colored sticky traps to SCWL insect vector, *M. hiroglyphicus* and 2) to investigate the natural flight ability (distance) of this vector species in a sugarcane field using MRR method.

Materials and Methods

Evaluation of colored sticky traps for trapping vector, *M. hiroglyphicus*

Our flight ability study by mark-release-recapture method required the development of efficient trapping equipment for recapturing of released marked insects. Therefore, this experiment was carried out at farmer's field in Kumpawapi, Udon Thani Province in 2010. Sticky boards (30x20 cm) of yellow, green, white, orange, blue and colorless (control) traps were used. All treatment sticky boards were placed in the field from April to September, during the population abundance of vector in the fields. The transparent insect trapping adhesive (Beetle Glue, Greenplana Co. Ltd., Thailand) was applied on both surfaces of each colored board where the insect vector landed upon it. The colored sticky boards were fixed to bamboo sticks and placed in the field 10 cm above the sugarcane plant canopy. The traps were adjusted to canopy height later in the season. The experiment was carried out using completely randomized block design with six replications. The traps were collected and replaced every two weeks from April to September.

All different color sticky traps that caught insects were kept in plastic box and transferred to the laboratory for identification of leafhopper species *M. hiroglyphicus* and counting of individuals at each evaluation date. Data were subjected to analysis of variance (ANOVA) and the treatment means were separated by the least significant difference (LSD) at 5% probability level.

Mark-release-recapture experiment in a sugarcane field

This experiment was conducted to investigate the flight ability of leafhopper, *M. hiroglyphicus* in a sugarcane field, Tha Phra, Khon Kaen Province in 2011. For insect marking, the species of *M. hiroglyphicus* were collected from the fields in Non Sa-at, Udon Thani Province. The collection was done by light trap to attract the insects, and by aspirating leafhoppers from light traps. A groups of 50 leafhoppers were placed into transparent plastic tubes (12 cm in length, 3 cm in diameter) containing 20 mg of fluorescent dye powder for marking. Then, the tubes were shaken gently by hand for 20 sec to coat the leafhoppers with the powder. Three colors of micronized (3-4 µm particle size) fluorescent dye powder-purple pink, pinkish and green (Sinloihi Co. Ltd., Kamakura, Japan) were used for marking. After marking, the leafhoppers were immediately transferred from the plastic tubes to transparent plastic mass-rearing cages (80 cm in height, 25 cm in diameter) containing two-month-old healthy sugarcane. The marked leafhoppers, *M. hiroglyphicus* were released in three times with the number of 1,980, 1,200 and 800, respectively. The release dates were 28th July, 15th August and 31st August, respectively. Adult released in first time was marked purple pink, those released in second and third times were marked pinkish and green, respectively.

For the recapturing marked *M. hiroglyphicus*, yellow and blue colored sticky traps with the size of (25 cm x 40 cm) were fixed on bamboo sticks and placed in the field 1.5 m above the ground level at distances of 5, 10, 20, 30 and 50 m, in six directions (north (0^{o})), northeast (60^{o}), southeast (120^{o}), south (180^{o}), southwest (240^{o}) and northwest(300^{o}) from a release point. A total of 60 traps were placed in the field. All sticky traps were collected and replaced at two-day intervals until 20 days after each release. Trapped leafhoppers were viewed under a microscope to examine their marking. Weather data were obtained from the weather station at Tha Phra, Khon Kaen Province.

Estimated natural flight distance

The dispersal distance estimation method of Yamamura et al., (2003) has been used for analyzing SCWL vectors dispersal study by Thein et al., (2012). The present study, estimated flight distance of *M. hiroglyphicus* was analyzed using Yamamura method and modified from Thein et al., (2012).

Firstly, we calculated the expected number of settled individuals at a distance f(r), assuming that the traveling individuals are settled in a position at the rate of λ:

$$f(r)=\frac{n_0}{2\pi}\lambda_D K_0\left(r\sqrt{\lambda_D}\right)$$

where n_0 is the number of released insects; $K_0(\cdot)$ is a modified Bessel function of the second kind, of order zero; and λ_D is λ/D, where D is the diffusion coefficient. λ_D is the rate of settlement scaled by the diffusion coefficient.

Then we calculated the expected number of individuals captured by the *i*-th trap placed at a distance r_i as:

$$g(r_i) = \frac{cn_0}{2\pi}\lambda_D K_0\left(r_i\sqrt{\lambda_D}\right)$$

where c is constant.

Then, we calculated the estimate mean dispersal distance of individuals captured by traps as:

$$E(r) = \frac{\pi}{2\sqrt{\hat{\lambda}_D}}$$

where a hat (^) indicates the corresponding estimate.

The standard error SE is estimated as: $\mathrm{SE} = \mathrm{SE}\left(\hat{\lambda}_D\right) \times \frac{\pi}{\left(4\hat{\lambda}_D^{\ 1.5}\right)}$ where SE(λ_D) indicates the SE of λ_D (Seber, 1982).

The Microsoft Excel spreadsheet for this estimation is available at http://cse.niaes.affrc.go.jp/yamamura/Yamamura_et_al_2003_estimation.xls.

Results and Discussion

Trapping efficiency of colored sticky traps to *M. hiroglyphicus*

SCWL leafhopper-transmitted phytoplasma disease is causing increasing damage and yield losses, both to farmers and the broader sugarcane industry. The study was conducted to evaluate the attractiveness of SCWL insect vector to different color sticky traps in the sugarcane fields. The result indicated that the blue and yellow color sticky traps caught the highest number in species of M. hiroglyphicus ($F = 7.05$, $df = 5, 25$, $P < 0.05$). The orange, white and green color sticky traps were found to be caught in descending order, and less attractive than blue and yellow. A colorless (control) sticky trap was the least attractive for this vector species (**Table 1**). The present study of trapping efficiency using colored sticky traps revealed that the blue color sticky had the best efficiency in catching leafhopper, M. hiroglyphicus than yellow color sticky trap, but it did not show difference significantly (**Table 1**). Mostly, insects are attracted to special reflectance ranging from 350-650 nm, where as different color varies in wave length band. The blue and yellow colors have the wave length band of 450-500 nm and 570-600 nm, respectively (Tanton, 1977). This result indicated that insect vector, M. hiroglyphicus was attracted by the wave length range of 450-600 nm. It have been reported that yellow and orange color sticky traps were significantly attractive for leafhopper, Empoasca decipiens in cotton, and the red color sticky trap caught more Scaphoideus titanus, vector of grapevine flavescence doree phytoplasma than white, yellow or blue (Demirel and Yildirim, 2008; Lessio and Alma, 2004).

Table 1 Mean number of *M. hiroglyphicus* caught on sticky traps of different colors in sugarcane field from April to September, 2010

Sticky trap color	*M. hiroglyphicus*(±SE)/trap/day
Blue	3.02±0.38a
Yellow	2.57±0.21ab
Orange	1.37±0.20bc
White	1.17±0.16c
Green	0.83±0.14c
Colorless (control)	0.17±0.08d

Means in a column followed by the different letter are significantly different (P <0.05) by LSD

Mark-release-recapture experiments

A total of 3,980 adults *M. hiroglyphicus* were marked and released, in which a total of 402 were recaptured and the overall release-recapture rate (%) was found to be 10.1%. This was low in comparison with some other leafhoppers flight ability studies using the mark-release-recapture method (for example, 17.3-43.7% in a report on sharpnosed leafhopper on blueberry plants (Whitney and Meyer, 1988) but it was higher than the 1.8-3.7% rate reported by Larsen and Whalon (1988) in studying the vector leafhopper of X-disease in peach and cherry orchards (some of which were regularly treated with insecticide). The lower rate in our study might be the fact that vector flight movement in a sugarcane field is comparatively difficult in comparison to that among orchards or vegetable crops, owing to the obstruction caused by the broad leaf types found in sugarcane plantations.

The result of total number of recaptured leafhoppers also revealed that higher number of marked leafhopper *M. hiroglyphicus* was caught to the southwest (240^0 from north), followed by south (180^0 from north) and southeast (120^0 from north), and lowest to the northeast (60^0 from north) in all three releases (**Table 2**). The wind direction throughout the experimental period of our study was predominantly towards the south (S), southeast (SE) and southwest (SW). The total number of recaptured leafhopper individuals of *M. hiroglyphicus* was caught at higher in southern part (**Table 2**); which appears to have been influenced by the prevailing wind direction. Wind appears to be the main factor influencing the leafhopper dispersal direction and distance. Kobori et al. (2011) reported that wind was one of the major factors influencing the flight distance of citrus greening disease (Asian citrus psyllid, *Diaphorina citri* Kuwayama).

Table 2 Total number of recaptured marked leafhoppers during twenty days in each direction

Direction of flight	Number of recaptured marked leafhopper, *M. hiroglyphicus*			
	1st release	2nd release	3rd release	Combine all releases
North (0^{o})	18	17	6	41
Northeast (60^{o})	20	14	3	37
Southeast (120^{o})	39	29	8	76
South (180^{o})	47	33	9	89
Southwest (240^{o})	66	35	17	118
Northwest (300^{o})	17	21	3	41
Total	207	149	46	402

(modified from Thein *et al*., 2012)

The number of marked *M. hiroglyphicus* recaptured over the 20 days after release decreased exponentially following their release (**Fig. 1a, b, c**). The number of *M. hiroglyphicus* recaptured from seven times at two-day intervals (2, 4, 6, 8, 10, 12 and 14 days), in the 1st release was 57, 40, 34, 35, 28, 10, 3; in the 2nd release was 60, 42, 22, 11, 9, 4, 1 and in the 3rd release was 28, 8, 5, 3, 2, 0, 0, respectively. The number of recaptured vector decreased with the increasing number of observation days. Our flight ability study of mark-release-recapture experiments in the field, marked insects of *M. hiroglyphicus* were recaptured over 14 days in the first and second release, and over only 10 days in third release (**Figure 1**). The recaptured marked leafhopper was also less in 3rd release compare to 1st and 2nd release. A high mortality would appear to have occurred in the third release experiment, resulting in the low number of recaptured leafhopper (**Table 2**). This could be due to rainfall or unsuitable habitat. Normally, this vector species come to the fields during the raining season of May to August but the greatest abundance was found to be in June-July (Phisitkul et al., 1989; Hanboonsong et al., 2006). The present experiment, the third release of the *M. hiroglyphicus* vector was done at the end of August, which may be a period of unstable habitat in the field, leading to effect on fitness for this species.

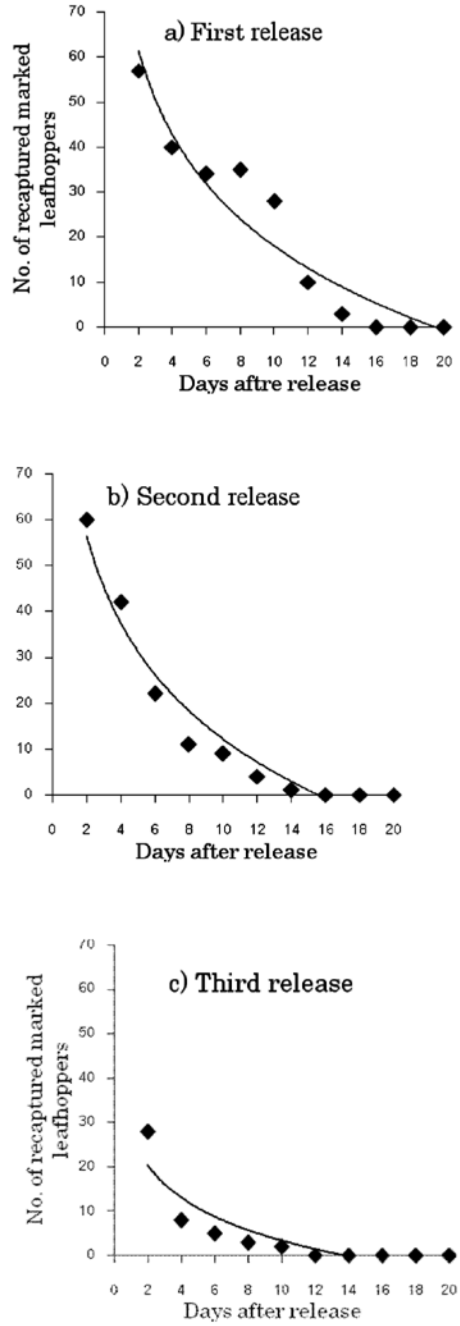

Figure 1 Number of marked leafhopper *M. hiroglyphicus* recaptured after each two-day interval, for 20 days after release: (a) 1st release, (b) 2nd release, (c) 3rd release (The curves were generated by the least squares method, assuming log approximation). (modified from Thein *et al*., 2012)

Moreover, the simple mean flight ability (distance) of *M. hiroglyphicus* was determined by dividing the distance recaptured from release point (m) by the days of observation. The species of *M. hiroglyphicus* had flown 3.56 m, 4.44 m and 4.77 m per day at first, second and third release, and the mean distance for three releases combined was 4.02 m per day, respectively. It seems that the vector, *M. hiroglyphicus* is not capable of moving further than the speed of 4.0 m per day. But in our experiment in the field, some individuals of vector leafhopper species once they arrived on the sticky traps, they could not move again, while some might move over 50 m outside the trapped area. Therefore, the result of simple mean flight distance per day may be underestimated.

Estimated natural flight distance

A total number of recaptured *M. hiroglyphicus* individuals from all traps in all directions over 20 days, was 402 (**Table 2**). We used this cumulative number of recaptured leafhoppers to predict the cumulative flight distance. The maximum likelihood estimate (± SE) of the rate of settlement of leafhopper *M. hiroglyphicus*, scaled by the diffusion coefficient, was $\hat{\lambda}_D = 9.4 \times 10^{-5} (\pm 5.5 \times 10^{-5})$. The estimated mean flight ability (distance) (± SE) was 162.1 (± 47.2) m. Our results showed that the estimated mean natural flight distance (± SE) of *M. hiroglyphicus* was 162.1 (± 47.2) m, using the Yamamura method (Yamamura et al., 2003). It also indicated that the estimated mean natural flight distance per day by Yamamura method was about 8.0 m because our mark-release-recapture experiment was carried out within 20 days for each release. Our estimation assumed that the vector' movement was isotropic, which meant that variations in wind speed and wind direction in the actual field were not factored into the analysis.

Conclusions

From this study, our findings would be useful not only in considerations of prevention techniques for SCWL disease transmitted by *M. hiroglyphicus*, but as a first step in developing spread-estimation models for SCWL disease. Based on our results, we may speculate that the disease risk decreases with increasing distance from infected fields; and that the SCWL infection risk is higher on the leeward side of an infected field than on the windward side. Further studies, concerning the vectors' transmission ability, intrinsic rate of natural increase, and movement patterns, are also needed in order to enhance SCWL disease management.

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