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Original Article

Effect of supply and demand of phloem sugar on the proportion of brachypterous forms of the rice pest *Nilaparvata lugens* Stål (Hemiptera: Delphacidae).

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Abstract

The sugar supplied by rice and the demand by *N. lugens* can affect the proportion of brachypterous forms in a population. Experiments were conducted to estimate the food ingestion by *N. lugens* per capita, to determine total food demand in a rice field, and to find the relation of food supply to the proportion of the brachypterous form. The results revealed that the sucrose content tended to decrease with the age of rice. However, the food demand by the *N. lugens* population dramatically increased in 40–89-day-old rice. The proportion of sugar supply to insect demand decreased as the insect population increased. The relative abundance of short-wing form insects increased when the ratio of sugar supply to insect demand was ≥0.02, but was decreased when the ratio was lower than 0.01. These results are useful for predicting *N. lugens* dispersal related to sugar supply in rice fields.

Keywords: brown planthopper, food demand, food ingested, macropterous, sucrose.

1. Introduction

Rice is one of the three most important grain crops in the world (Chauhan, Jabran, & Mahajan, 2017). It is a staple food for much of the world's population, with a production estimate of 519.5 million tons from 2022-2023 (Food and Agriculture Organization of the United Nations [FAO], 2023). Important factors that are contributing to the declining profitability in rice production are insect pests and diseases. *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) is an important pest of rice cultivation. It has serious effects on the growth of rice plants, resulting in very large yield losses in rice-growing areas (Jena et al., 2018). The damage is directly related to the desiccation of rice plants, which occurs as a result of insects consuming the plant fluids (referred to as 'hopper burn'), or is indirectly related to the transmission of viral diseases called 'rice grassy stunt' and 'ragged stunt virus' (Rivera, Ou, & Iida, 1966). Outbreaks of *N. lugens* often result in high economic losses. For example, in 2005, 1,880,000 tons of rice was lost due to *N. lugens* damage in China (Hu et al. 2014).

In the adult stage, there are two winged forms of adult *N. lugens*, a macropterous (long-winged) form and a brachypterous (short-winged) form (Figure 1). The brachypterous form has high fecundity and is flightless. This form is dominant if the insects have access to plenty of host plants. In contrast, the macropterous form has low fecundity (Xayyasin, Khlibsuwan, & Tangkawanit, 2014) and its population increases in older rice. The long wing of macropterous forms helps their dispersal. To predict *N. lugens* population dynamics, knowledge of the abiotic and biotic factors, such as accumulated degree days, fecundity, survivorship, and functional and numerical response of natural enemies and weather data, that influence their populations is important. Additionally, the changing balance of wing dimorphism types is important for estimating the timing of local insect dispersal in rice cultivation.

Wing dimorphism is considered to be influenced by environmental factors, population density, nutrition, juvenile hormones, interspecific interactions and abiotic factors such as photoperiod (Zhi-Fang, Ju-Long, Juan, Chao, & Xiang-Dong, 2014). The most important influence on the wing dimorphism of *N. lugens* is host plant quality (Kisimoto, 1956; Iwanaga, Tojo, & Nagata, 1985; Xu et al., 2015; Liu et al., 2020). Romadhon, Koesmaryono, and Hidayati (2017) found that the population of *N. lugens* becomes macropterous and emigrates during the 4–5 weeks after transplantation. Syobu, Mikuriya, Yamaguchi, Matsuzaki, and Matsumura (2002) reported that the incidence of brachypterous females dramatically decreased approximately 75–85 days after the rice was transplanted. A possible reason for this might be that the nutritional conditions tend to decline in older rice (Baqui & Kershaw, 1993; Wu, Yu, Tao, & Ren, 1994; Xayyasin et al., 2014).

Nilaparvata lugens feeds on rice phloem sap, which contains large amounts of sugars (Kikuta, Kikawada, Hagiwara-Komoda, Nakashima, & Noda, 2010). Okamura, Hashidaa, Hirosea, Ohsugia, and Aokia (2016) found that sucrose was the main soluble sugar in rice stems, but glucose and fructose were also present. Deepa, Pillai, and Murugesan (2016) revealed that the total sugar content was found to differ significantly in rice of different ages and varieties. Lin, Xu, Jiang, Lavine, and Lavine (2018) found that the glucose concentration in older rice plant is much greater than in younger ones. Knowing the quantity of food ingested by *N. lugens* per capita is important for estimating the food demand of the insects in a paddy field. We hypothesized that the supply of sugar from rice and the demand for it by insects affect the proportion of the brachypterous form of *N. lugens*. If the sugar supply is lower than the insect demand, the brachypterous form will decrease. The objectives of this research were: - (1) to estimate the food ingestion by *N. lugens* per capita for both nymphs and adults; (2) to estimate the insect food demand in rice field conditions; and (3) to determine the ratio of food supply to the proportion of the brachypterous form of *N. lugens*.

2. Materials and Methods

2.1 Insect rearing

Nilaparvata lugens were collected from a paddy field and released in a cage (50×70×100 cm) made of a wooden frame with a wire mesh covering the top and sidewalls, maintained at the Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University. Twenty 40-day-old rice plants of the variety Taichung native 1 (TN1) were placed in the cage as the host plant for feeding and oviposition. Old rice plants were replaced with new plants after 10 days.

2.2 Food demand

2.2.1 Assimilation and ingestion of food

The experiment was conducted in a 7 cm diameter plastic pot containing a 45-day-old rice plant (TN1). Nymphs (third nymphal stage) and adults of *N. lugens* were starved for 2 hours before use. Each individual was weighed and released in a parafilm sachet (5x10 cm), which was attached to the base of the plant (3 cm above the soil surface). There were 20 replicates for both nymphs and adults. After 24 hours, the insect was removed from the sachet and weighed separately. The honeydew on the parafilm sachets was also weighed. The sachet and honeydew were weighed together, then the honeydew was removed and the sachet was reweighed. A control was conducted to assess the loss of body weight from catabolism, with moist cotton being provided instead of a rice plant.

Food assimilation was calculated by the method of Smith, Khan, and Pathak (1994).

Food assimilated = W1 x [(C1-C2)/C1] + (W2-W1), where

W1 = Initial weight of the insect,

W2 = Final weight of the insect,

C1 = Initial weight of the control insect,

C2 = Final weight of the control insect

Food ingested = Food assimilated + weight of the honeydew.

Food assimilated, honeydew excreted and food ingested by nymph and adult *N. lugens* on the Taichung native 1 (TN1) rice variety were compared by a paired t-test (p=0.05) using Statistix10.

2.2.2 Field experiment

An insect outbreak was simulated and studied in the field research area of the Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, using Jasmine rice (KDML 105 variety), a susceptible variety that is the preferred variety cultivated in Thailand and other Asian countries. Forty-day-old seedlings of KDML 105 were transplanted to a field with an area of 400 m^2. Four field cages made of iron frames (1.25x1.25x2 m), covered with a fine mesh on the tops and sidewalls with a door with a zipper-opening on one side, were positioned to enclose the rice plants. There were 25 rice hills per cage at a spacing of 25x25 cm. Three adults of *N. lugens* per rice hill were released (75 adults per cage with 2:1 female to male ratio). Nymphs and adults (brachypterous and macropterous) of *N. lugens* were recorded by direct counting on 13 hills per cage every week. One rice plant per hill was collected for further sugar analysis.

Food demand was estimated by multiplying the food ingested by the populations of nymphs and adults (total food demand = food ingested x insect population).

2.3 Sugar analysis

2.3.1 Rice plant material

Pieces of rice stem (5 cm long and cut 3 cm above the soil surface) were collected from the experimental cages. A total of 0.1 grams of each rice stem was cut into smaller pieces placed in a test tube, and 3 ml of 80% ethanol was added, held in a boiling water bath at 100 °C for 1 min and then transferred to a 65 °C water bath. After 1 hour, the supernatant was transferred to a new tube. Residual solid rice tissues were extracted with 3 ml of 80% ethanol and warmed in a 65 °C water bath for 1 hour two additional times. The supernatants of the three extractions were combined for the determination of sucrose content.

2.3.2 Sucrose content

Sucrose content was analyzed in supernatants from the plant extraction using the method described by Robbins and Pharr (1987). A total of 500 µl of the supernatant was transferred to a tube, and then 0.25 ml of 1% resorcinol in 95% ethanol and 0.75 ml of 30% HCL were added. The solution was incubated in an 80 °C water bath for 10 min. The tube was removed and cooled to room temperature. Absorbance at wavelength 520 nm was measured with a spectrophotometer. Sucrose content was quantitated by comparison to sucrose standards.

2.4 Sugar supply to insect demand

The sugar supply from rice to *N. lugens* demand (S2D) for each observation was determined as

$$S2D = CH2O/\text{Food demand}.$$

The data for food demand were calculated by the food ingestion of nymphs and adults multiplied by the number of insects examined in the cage for each observation. CH2O was the sugar content in the rice in each observation.

2.5 Relative abundance of the short-wing form

Brachypterous and macropterous forms of *N. lugens* in the insect cage were recorded every 7 days. The relative abundance of short-wing form was calculated as relative abundance = Nb/(Nb+Nm),

where Nb = number of brachypterous forms, and Nm = number of macropterous forms.

3. Results

3.1 Food demand

3.1.1 Assimilation and ingestion of food

The amount of food assimilated, honeydew excreted and food ingested by nymphs and adult *N. lugens* on 45-day-old TN1 rice after 24 hours are shown in Table 1. The quantities of those 3 parameters were significantly higher in the adult stage than in the nymphal stage. From this experiment, the food ingestion of *N. lugens* per capita for nymphs and adults was 1.21 and 10.60 mg/day, respectively.

3.1.2 Food demand in rice field conditions

The population of *N. lugens* was recorded every week. Food demand for each week was estimated as shown in Table 2. The results revealed that the number of nymphs slightly increased on rice that was 54–82 days old. The first released adult population slightly decreased during the first to third weeks. When the rice age was 61 days, the first released adult population died. The total number of adults was 0 in 61-day old rice. Then, the 2nd adult population increased and developed to adult in the rice field. The total food demand increased during vegetative growth until the rice age was 89 days old (1,033.97 mg/hill). After the rice was in reproductive growth, the insect population and food demand both dropped.

3.2 Rice sucrose content

The sucrose content of rice infested by *N. lugens* was significantly lower than that of non-infested rice at 47–103 days old. The sucrose content of infested rice and non-infested rice was dramatically decreased at 40–61 days. After 61 days, the sucrose content of the non-infested rice was stable, whereas the sucrose content of the infested rice had decreased slightly (Figure 2).

3.3 Sugar supply to insect demand and relative abundance

The proportions of sugar content and insect demand for each age of the rice plants are presented in Table 3. The results indicated that the ratio of sugar supply to insect demand tended to increase when the rice was 40–54 days old. Thereafter, the ratio decreased dramatically at 54–68 days old. Sugar supply to insect demand was lowest at 89 days old.

The relative abundance of the short-wing form in the first release was 0.5, and then the value subsequently increased until the rice was 54 days old. When the rice was 61 days old, the adults of the first released population had died and were no longer found in the experimental cage. For this rice age, the ratio of sugar supply to insect demand is not zero (0.09) because there are some nymphs of 2nd population from eggs laid by the first generation in the field. After that, the 2nd adult population of brachypterous adults, arising, began to increase until the rice was 75 days old. After the rice was 82 days old, the supply to demand ratio trended to lower than 0.01, and the relative abundance value was lower than 0.6. The results indicate that there were fewer brachypterous forms than macropterous forms. The relative abundance of short-wing form then slightly decreased to zero when the rice was 103 days old.

4. Discussion

The quantity of food ingested was higher in the adult stage than in the nymphal stage. This result is similar to that obtained for nymphs and adults of the grasshopper *Oxya hyla hyla*, which is a common rice pest (Ghosh, Haldar, & Mandal, 2014). This result may be related to the larger size of the adults, which requires them to ingest more food, nutrients and energy. In addition, adults expend most of the energy from their food in reproduction (Ghosh et al. 2014). The calculated values of food ingested found in this experiment differed from those from previous studies (Senthil-Nathan, Choi, Paik, Seo, & Kalavani, 2009; Latif et al., 2012; Mollah, Samad, Hossain, & Khatun, 2011). There are some ecological factors influencing the parameters used for the calculation of food ingestion, such as rice age and rice variety. Baqui and Kershaw (1993) found that the honeydew secreted by *N. lugens* was lower on 90 days old rice plants than on younger rice plants. Latif et al. (2012) and Mollah et al. (2011) reported that the amount of food ingested and assimilated was significantly decreased when *N. lugens* was reared on a resistant variety of rice compared to a susceptible variety of rice. In addition, Lu et al. (2007) and Wu et al. (2017) revealed that ingestion rates of the brown planthopper were increased when feeding on N-fertilized plants. Food ingested in this experiment was estimated in a rice variety (TN1) susceptible to *N. lugens*. Therefore, the results indicated the amount of food ingested when food is plentiful and favoured by insect pests. Forty-day-old rice was studied because this age of rice has often been detected in *N. lugens* outbreaks.

The food demand of *N. lugens* in the rice field depended on the size of the insect population. The highest population number of *N. lugens* was usually found when rice was in the late vegetative stage. Sawada, Subroto, Suwardiwijaya, Mustaghfirin, and Kusmayadi (1992) and Khlibsuwan, Hanboonsong, Pannangpetch and Sriratanasak (2014) showed that there were approximately 3 generations of *N. lugens* in rice fields, which corresponds to our results. In this experiment, the initial population died within 2–3 weeks after release. Then, a 2nd generation emerged from the eggs that had been oviposited by the 1st generation (Table 2). The population increased until the rice was

89 days old; then, a final generation of long-winged morphs became established in the rice field.

Sucrose is the major sugar product of photosynthesis in rice plants and is the main soluble sugar in the rice stem (Kikuta et al., 2010; Okamura et al., 2016). Sucrose is transported from source tissues to various sink tissues by the phloem to sustain plant development, such as pollen development and pollen tube growth before the heading stage. In developing seeds, the phloem releases sucrose into maternal tissues to produce the grains (Jung & Im, 2005). Therefore, the sucrose content in the rice tilling of older rice was lower. A decrease in the total sucrose content with increasing plant age was observed in this experiment. This result corresponds with the report of Deepa et al. (2016) that the total sugar content was decreased 20, 40, and 60 days after rice was transplanted. The trend of sucrose content in rice plants was opposite to that of the glucose content reported by Lin et al. (2018). Sucrose is a disaccharide consisting of one glucose and one fructose molecule; therefore, it may be that decreasing sucrose content may have resulted in increase of glucose content arising through hydrolysis of sucrose in older plants.

The main food source for *N. lugens* is sugar. It was observed that the sugar supply to insect demand decreased with an increasing insect population. The relative abundance of short wing form was highest when the rice age was 75 days. The ratio of the supply by plant and the demand by the insect at this age was 0.02. This amount of supply may be sufficient for the insects. However, after the rice was 82 days old, when the sugar supply to insect demand was lower than 0.01, the brachypterous form was less abundant than the macropterous adults. This is the critical value that indicates the imminent population migration of *N. lugens*. This result is similar to those in a report by

Syobu et al. (2002) that showed brachypterous females dramatically decrease approximately 75–85 days after rice is transplanted and that rice plant stage affects the female wing-form ratio. At 96 days into the experiment, the rice reached the booting stage of reproductive growth and developed a panicle primordium. The sugar may transfer to sink cells for panicle development. Therefore, the food supply was very low and not enough to support a high density of insects. Compared to non-infested plants, the sugar content in infested plants was very low, and most of the sugar was lost because of insect sucking. Some rice plants showed symptoms of hopper burn and turned from green to reddish-brown at 89 days (S2D=0) (Figure 3). Therefore, this rice symptom indicates a high density of the macropterous form and that the insect is ready for dispersal to the next locality. This is a critical point for vigilance in detecting outbreaks in neighboring areas. However, the amount of sugar content varied depending on the rice variety (Deepa et al. 2016) and macropterous forms may be present earlier in low-sugar varieties than in high-sugar varieties.

Iwanaga et al. (1985) and Ayoade, Sunao, and Sumio (1996) reported that high levels of juvenile hormones in the early nymphal stage induced the brachypterous form. However, juvenile hormones are affected by food quantity. Saxena, Okech, and Liquid (1981) suggested that inadequate food affects juvenile hormones released from the corpus allatum. It is possible that a starvation situation brought about by an older rice age may result in a change in the level of juvenile hormones, which affects the wing form of *N. lugens*. This report corresponded to the report of Simpson and Raubenheimer (1993), that metabolic activities during insect development are dependent on the quality of food. When foods are nutritionally imbalanced, insect herbivores have to adapt by evolving an appropriate behavioural and physiological mechanism (Behmer, 2009).

Zhang, Mao, & Liu (2023) revealed that 4 developmental regulated genes of wing, NlInR1, NlInR2, NlAkt, and NlFoxo were expressed in the short-winged adults, but silenced in the long winged adults. Lin et al. (2018) showed that two insulin receptors (NlInR1 and NlInR2) regulated wing type of *N. lugens*. Recently, Liu et al. (2020) revealed that ultrabithorax (Ubx) is a key regulator for promoting short wing form in *N. lugens*. They suggested that a high quality of plant nutrition at a later stage of nymph increased NlInR2 expression. NlInR2 induced high level of Ubx and consequently suppress the NlInR1 resulting in short wing form in *N. lugens.* Based on the present results and earlier researcher, food is identified as the important factor for wing development in *N. lugens*. However, some abiotic factors such as temperature and photoperiod may influence wing dimorphism. The results of this study should prove useful for predicting *N. lugens* dispersal in rice fields.

5. Conclusions

Nilaparvata lugens Stal's primary food source is sugar from rice phloem sap. Adults consumed 10.6 mg of food each day, while nymphs only consumed 1.21 mg. As rice grew older, the sugar concentration tended to diminish. The number of brachypterous forms in a population can vary depending on the amount of sugar that is produced by rice and the demand from *N. lugens*. The proportion of sugar supply to insect demand decreased with an increasing insect population. The relative abundance of short-wing form insects increased when the sugar supply to insect demand was $\geq$0.02, and became decreased when the sugar supply to insect demand was lower than 0.01. This information is useful for *N. lugens* population prediction in the rice production.

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Figure 1. ***Nilaparvata lugens*** **life stages: (A) eggs; (B) nymph; (C) brachypterous form; (D) macropterous form.**

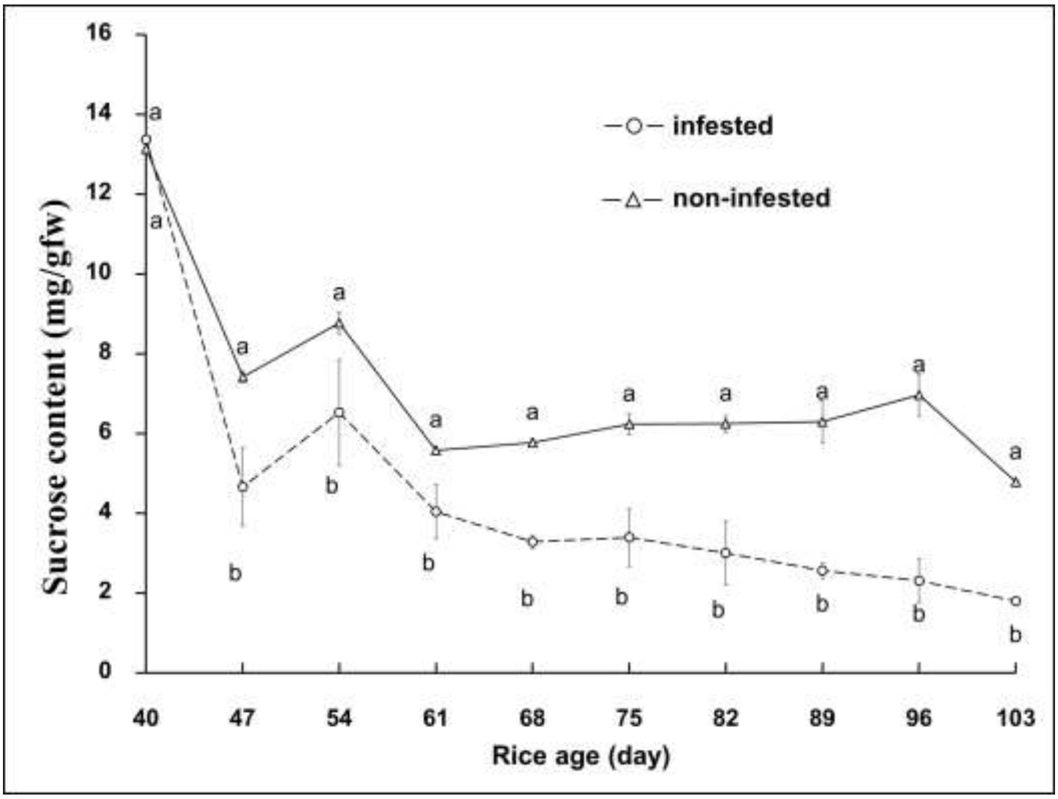

Figure 2. Sucrose content (mg/gfw) for rice infested with 3 adults of *Nilaparvata lugens* per clump of rice and for non-infested rice.

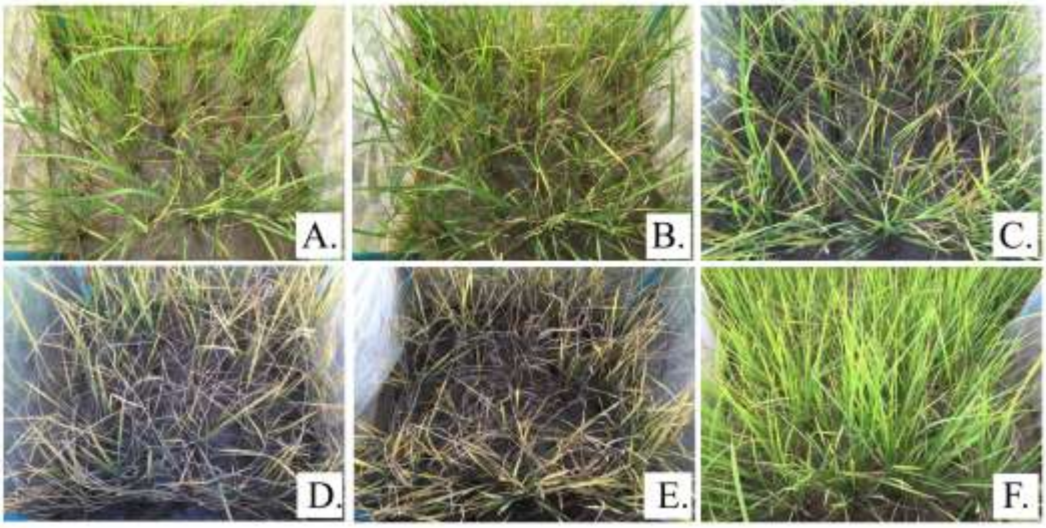

Figure 3. Rice infestation symptoms at different ages after *Nilaparvata lugens* were released when rice was 40 days old: (A) 68 days old; (B) 75 days old; (C) brachypterous 82 days old; (D) 89 days old; (E) 96 days old; and (F) 96 days old (control rice).

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Table 1 Food assimilated, honeydew excreted and food ingested by nymph and adult *Nilaparvata lugens* on the Taichung native 1 (TN1) rice variety after 24 hours.

Nilaparvata lugens (n=30)	Food assimilated (mg) (Mean±SD)	Honeydew excreted (mg) (Mean±SD)	Food ingested (mg/day) (Mean±SD)
Nymph	0.72±1.33b*	0.49±0.26b	1.21±1.38b
Adult	5.75±0.72a	4.85±1.90a	10.60±2.14a

*Means followed by a lowercase letter within the columns are not significantly different at the 5% level according to a t-test.

Table 2 *Nilaparvata lugens* populations and food demands in the rice field experiment for 40- to 103-day-old rice.

Rice age (Days)	Rice stages*	Population (insects per hill)		Food demand (mg/day)		
		Nymph	Adult	Nymph	Adult	Total
40	V	0.00	3.00	0.00	31.80	31.80
47	V	0.00	1.73	0.00	18.35	18.35
54	V	9.69	0.38	11.73	4.08	15.80
61	V	38.46	0.00	46.54	0.00	46.54
68	V	11.77	14.71	14.24	155.94	170.18
75	V	47.81	13.12	57.85	139.02	196.87
82	V	197.02	11.92	238.39	126.38	364.78
89	V	9.54	96.46	11.54	1022.42	1033.97
96	R	2.58	40.95	3.12	434.06	437.17
103	R	0.00	4.10	0.00	43.42	43.42

* V = vegetative growth, R= reproductive growth.

Table 3 Sugar supply to insect demand ratio and the relative abundance of short-wing form for 40- to 103-day-old rice.

Rice age (day)	Supply to demand (gfw*/insect/hill)	Adult (insect/hill)	Relative abundance	Insect generation
40	0.42	3.00	0.50	
47	0.25	1.73	0.51	
54	0.41	0.38	0.90	1st
61	0.09	0.00**	0.00	
68	0.02	14.71	0.83	
75	0.02	13.12	0.95	2nd
82	0.01	11.92	0.60	
89	0.00	96.46	0.03	
96	0.01	40.95	0.02	3rd
103	0.04	4.10	0.00	

***gfw = gram fresh weight**

** number of adults in 1st generation died