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Population fluctuation and Life history traits of tiny leafhopper *Singapora nigropunctata* Mahmood, 1967 (Hemiptera: Cicadellidae) in southwestern Taiwan

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Abstract

This study describes the life cycle of the tiny leafhopper *Singapora nigropunctata* Mahmood, 1967 (Cicadellidae), which lives on Burmese rosewoods (*Pterocarpus indicus* Willd, 1802) and Philippine padauks (*P. vidalianu*s Rolfe, 1884) in southwestern Taiwan. According to study observations, the average longevity of females was 11.9 ± 6.7 d, and the average number of eggs laid by one female was 91.5 ± 76.9 at 30.1 ± 2.1℃ (*n* = 10). The time for hatching of the tiny leafhopper eggs was 6.5 ± 2.4 d at 29.5 ± 2.4℃ (*n* = 65), and the time for development from hatching to maturity, including ten nymphal instars and the growth of mature wings, was 19.5 ± 4.7 d (*n* = 218). The duration of each nymphal stage was as follows: the first instar molted within hours; second to ninth instar lasted 1 to 4 d each; and the tenth instar lasted 3 d. The each shape-winged **instar nymph** was shown in the ninth to 26th photos. **In spring, the tiny leafhoppers began to hatch. At this time, the population density was low. On June 8, 2009, a temperature peak was observed at the first collection site KS, at which time synchronization was performed to obtain the peak number.**

Keywords: Agricultural insect pest, life history, nymphal development, instar, molt

Introduction

In population ecology and agricultural pest control, it is crucial to examine the manner in which small insect populations expand from few insects to huge populations within a short time. The leafhopper (Hemiptera: Cicadoidea: Cicadellidae) is an agricultural pest insect occurring extensively worldwide, including in Myanmar, Indonesia, Malaysia, the Philippines, Thailand, and Australia. Some species of the leafhopper are monophagous; others have migrated to different hosts over generations to obtain sufficient food (Nakasuji 1997; Sidumo et al. 2005; Al-Wahaibi & Morse 2009; Scott 2013, Cao et al. 2014). In normal environmental conditions and climates, leafhoppers can survive the winter in various stages of metamorphosis and nymphal instars. However, many species of the leafhopper undergo spring development in overwintering areas (Sidumo et al. 2005).

Mahmood first described the existence of the tiny leafhopper *Singapora nigropunctata* Mahmood, 1967, on pongam (*Pongamia pinnata* (L.) Pierre) in Taiwan (Mahmood 1967, Chiang and Knight 1990). Usually, the population density of tiny leafhoppers is higher on rosewood (*Pterocarpus* spp.) than on pongam. When the population density of tiny leafhoppers on rosewood increases to an extremely high level, the rosewood plant drops or dries its leaves as a form of defense. Tiny leafhoppers then die or move to other rosewood or pongam plants in the vicinity.

Leafhoppers lay their eggs on the leaves or branches of their hosts. Studies have reported that after hatching, several species of leafhopper have five nymphal instar stages (Raine 1960, Rose 1973, Marais 1989, Knight et al. 1991, Manurung et al. 2005). Other studies have reported the development of *Homalodisca coagulata*, *Megamelus scutellaris*, and *Empoasca fabae* from hatching to adulthood in five instar stages. Those insects are well-known pests of orange orchards in South America and North America (Castle et al. 2005, Sosa et al. 2005, Chasen et al. 2014); however, research on the tiny leafhopper (*S. nigropunctata*) remains limited. Therefore, this study aimed to elucidate its life history traits and population fluctuations through laboratory and field survey.

Materials and Methods

Host plants

Host plants of the tiny leafhopper in our surveyed sites comprised Burmese rosewoods (*P. indicus*), Philippine padauks (*P. vidalianu*s), and pongams. Because these host plants are distributed throughout Myanmar, Indonesia, Malaysia, Papua New Guinea, the Philippines, the Solomon Islands, Thailand, Australia, and Taiwan, the tiny leafhopper has become a widely distributed tropical pest.

The weather in southern Taiwan is humid in summer, with occasional typhoons and pouring rains, whereas it is arid in winter. In our research, the growth peak for the leaves of rosewoods occurred in May and June, and leaf changes occurred at least three times each year. Most rosewoods bloomed in April and May; however, some blossomed in June, September, and October, 2009.

This study consisted of two survey sites. The “KS” site was on the campus of Kun-Shan University (23°00′00″ N, 120°14′30″ E), where the numbers of host plants were one *P. vidalianu*s and two *P. indicus*. The distance between any two plants was at least 50 m. The “YS” site was at Yusin Road in Tainan City, where there were approximately 400 host plants in a row, most of which were *P. vidalianu*s and some were *P. indicus*.

Population fluctuation of the tiny leafhopper

The fluctuation of the population densities of the tiny leafhopper *S. nigropunctata* was evaluated from April 12, 2009, to April 17, 2010. A sweep net (diameter: 38 cm) was used to catch tiny leafhoppers (Manurung et al. 2005). Sweep net samples were used to estimate population densities at each site weekly.

The trees at KS were more than 6 m in height. They were sampled within 2 m around and under the tree. Each collection was performed 20 times (sampling) at three trees, totaling 60 collections, with a sweep net. At the YS site, ten trees were selected, and each side of the tree was sampled once, for a total of 20 times with a sweep net. After counting, the collected insects were released back into their original habitats.

During the survey period, there were two typhoons, Typhoon Lotus (June 21, 2009) and Typhoon Morakot (August 8, 2009) and some cold air masses from September to November 2009. The leaf changes of rosewoods occurred during three periods: from July to August 2009, from November to December 2009, and from February to March 2010.

Once a week, the average temperature of leaf surfaces was estimated using an infrared thermometer, type TES-1326S, manufactured in Taiwan, in the main time of insect collection at the sampling site KS.

Morphological studies

The body length (BL) and head width (HW) of each specimen was determined using collected individuals from the same habitats. Color images of specimens and measurements of body size were determined using a stereo-zoom microscope (SZ-60, Olympus, Japan). The BL and HW of nymphs was measured (precision = 0.01 mm) using a microscope (Eclipse 50i, Nikon, Japan).

Life history traits in the laboratory

For rearing the leafhoppers, the branches with leaves of Burmese rosewoods were cut at the sampling site YS. One end of a branch (5–7 cm in length) was put into a test tube filled with water (Fig. 1). Each cleaned branch was then placed into a rearing box. The nymph introduced into the box would feed on the leaves. Using this method, the leaves would last for 3 to 30 days before drying.

Figure 1: Laboratory setting for observation and recording of spawning of adult tiny leafhoppers (*S. nigropunctata* Mahmood, 1967) and the growth of nymphs

To evaluate the effects of seasonal temperatures on the life history traits of tiny leafhoppers, three experimental environments were examined. The 30℃ experiment was performed in a thermostatic container maintained at a constant temperature of 30℃ and a relative humidity of 78% in 12-h light and 12-h dark. In the laboratory, the winter experiment was conducted in winter (the measured temperature was from 16℃ to 29℃), and the summer experiment was conducted in summer (the measured temperature was from 27℃ to 31℃). The lower temperature in the winter- experiment resulted in a smaller sample size (n = 25) compared with those in the summer experiment (n = 55) and 30℃ experiment (n = 69).

Before commencing the raising process in the laboratory, it was ensured that there were no eggs in each feeding box by viewing under a microscope. The feeding boxes had a diameter of 10 cm and a height of 14 cm. The internal capacity was 870 mL. Two adults were moved into the feeding box to lay eggs, and the number of eggs laid each day and when they hatched were recorded. After the eggs hatched, each single nymph was moved into a new feeding box by self-crawling onto a new branch. Subsequently, the molting processes were observed daily.

When the tiny leafhoppers grew to the adult stage, they were paired by contacting the exposed genitals (Chiang and Knight 1990); then, each pair was reared in one feeding box (n = 10). The laboratory temperature was 30 ± 2℃. Each of the single females and her mating male were moved to a new feeding box daily until the females' death, and the average number of eggs laid by one female was counted.

Following the laying of eggs, their hatching time was evaluated. In the thermostatic container maintained at a constant temperature of 30 ± 2℃ and a humidity of 78% in 12-h light and 12-h dark, the number of evaluated eggs was 65.

From April to July of 2017 and 2018, at an average spring and summer room temperature of 27.7℃ (17.9–30.8℃), we collected eggs from the KS site and returned to the laboratory to wait for the eggs to hatch to repeat the experiment measuring the age of the larvae. To determine the age of the larvae and the appearance characteristics at each age, the breeding number was 135. Feeding with old ripe leaves extended the time of each nymph instar to facilitate observation.

Data analysis

We analyzed our results using the subroutine of analysis of similarity of the statistical package PRIMER v.5 (PRIMER-E, Plymouth, UK; Krebs, 1999). Results were considered significant when $P < 0.05$. Data are presented as the mean ± standard deviation.

Results

Population dynamics

The field temperatures near the Burmese rosewoods ranged from 12.3 to 35.9℃ during our survey period. In March and April, when the weather was warm, the tiny leafhoppers began to hatch, and the population density was less than 10 insects/m^3. Gradually, some of the nymphs appeared on the branches and leaves of the rosewoods and absorbed the sap from leaves. Throughout May and June, the population densities increased rapidly until the Burmese rosewoods and Philippine padauks were crowded with the insects. Before Typhoon Lotus, the population density at the KS site peaked to 155.0 ± 173.9 insects/m^3 on June 8, 2009. However, the summer heavy rain and typhoons from

June to October always led to substantial reductions in population densities. The population densities rose again from late October, but the cold current in winter often reduced population densities considerably. The populations in sampling sites KS and YS showed a high similarity of 90.9% (Fig. 2).

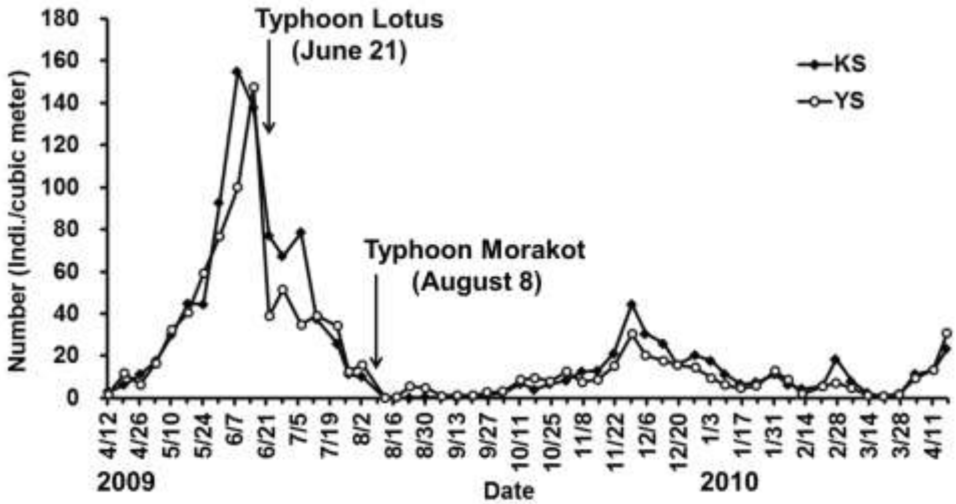


Figure 2: Population fluctuation of tiny leafhopper (*S. nigropunctata* Mahmood, 1967) on Burmese rosewoods (*Pterocarpus* spp.) from April 6, 2009 to April 17, 2010

During the year, the temperature at the KS site ranged from 12.3 to 35.9℃. In summer, the adults clustered in places with lower temperatures or in the shade. In winter, the adults clustered in places with higher temperatures and exposure to sunshine. The tiny leafhoppers showed a high mortality rate, low population density, and postponed reproduction during extreme weather, including high temperatures in summer, low temperatures in winter, and typhoons (Fig. 2).

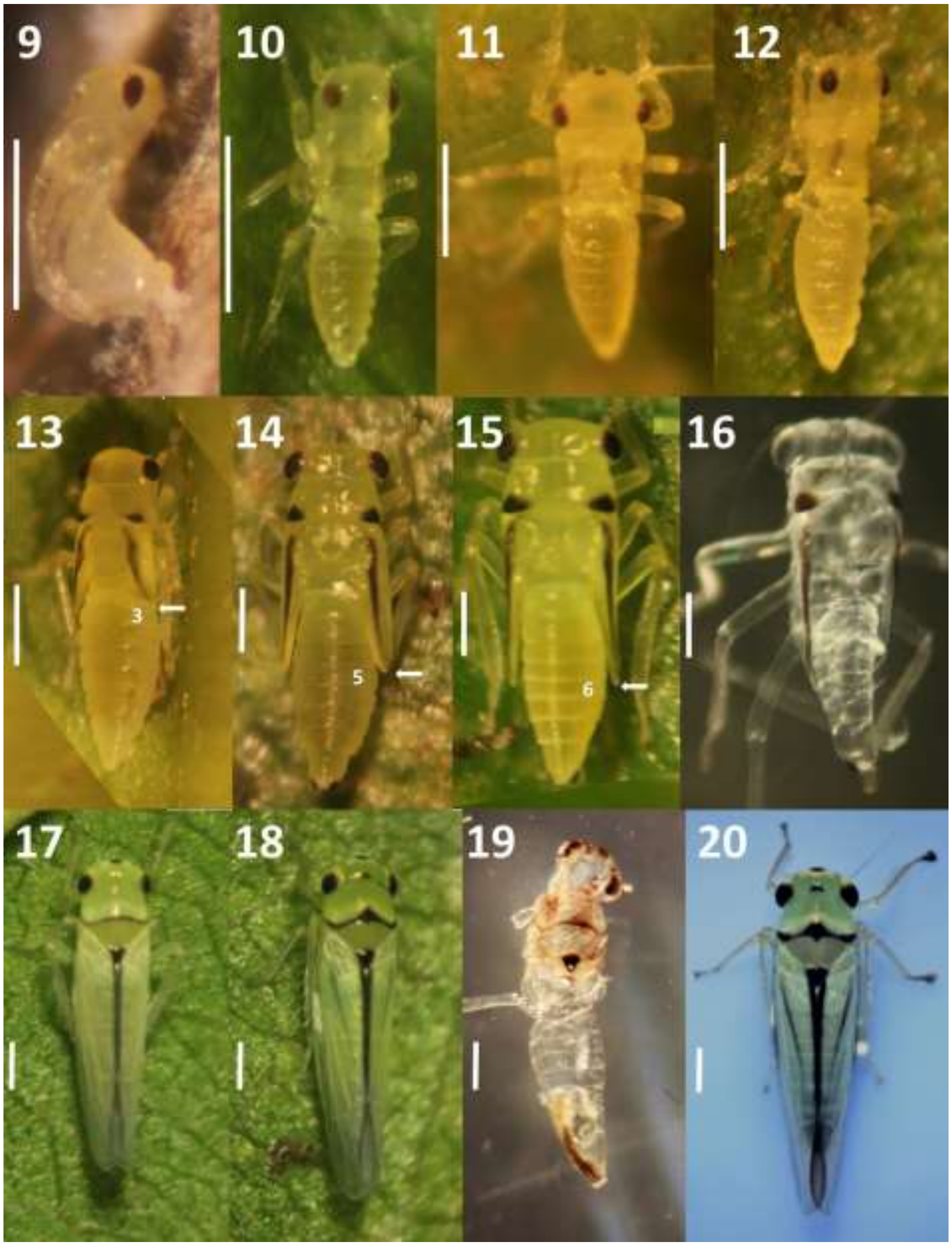
BL: The average BL of female leafhoppers was 3.36 ± 0.14 mm, and the average HW was 0.97 ± 0.05 mm (*n* = 30). The average BL of male leafhoppers was 2.95 ± 0.20 mm, and the average HW was 0.95 ± 0.06 mm (*n* = 22). Females were larger than males (Figs. 3–8).

Figures 3–8: Body appearance characteristics of females and males of *S. nigropunctata* Mahmood, 1967 (3-5) Female. (6-8) Male. (3,6) Dorsal view. (4, 7) Lateral view. (5, 8) Ventral view

Life history traits

At 30℃ ± 2℃, the number of eggs laid by a female was 91.5 ± 76.9 (*n* = 10), with a reproductive peak at 20.8 ± 27.6 on the third day. The average longevity of these females was 11.9 ± 6.7 days. The average egg-hatching time was 6.5 ± 2.4 d (*n* = 65).

In 2017 and 2018, we repeated the experiment or the life history of immature S. *nigropunctata*. The characteristics of nymphs at different stages were as follows. The nymphs crawled out from eggs, with thin, pale yellowish bodies and red eyes (Fig. 9); they underwent their first molting within 2 h. The first nymphal instar had BL of 0.72 ± 0.06 mm and HW of 0.17 ± 0.01 mm (*n* = 2). The second nymphal instar had BL of 0.93 ± 0.16 mm and HW of 0.27 ± 0.03 mm (*n* = 15); the forewing buds were light gray, and the hindwing buds were yellow (Fig. 10). **T**he third instar stage had BL of 1.23 ± 0.19 mm and HW of 0.34 ± 0.06 mm (*n* = 14); a black spot was noticed on the ventral head, along with hindwing buds to the chest and abdomen junction (Fig. 11). The fourth instar stage had BL of 1.48 ± 0.23 mm and HW of 0.44 ± 0.08 mm (*n* = 16). The forewings extended to the chest complex joint, and the hindwings extended to the first abdominal segment; the forewings and hindwings were of approximately the same length (Fig. 12). The fifth instar stage had BL of 1.99 ± 0.26 mm and HW of 0.59 ± 0.09 mm (*n* = 26); the forewing pads extended to the second abdominal segment, and the hindwing pads extended to the third abdominal segment (Fig. 13). The sixth nymphal instar had BL of 2.52 ± 0.32 mm and HW of 0.79 ± 0.09 (*n* = 48); the forewing pads extended to the fourth abdominal segment, and the hindwing pads extended to the fifth abdominal segment (Fig. 14). The seventh nymphal instar had BL of 2.753 ± 0.28 mm and HW of 0.82 ± 0.07 (*n* = 34); the forewing pads extended to the fifth abdominal segment, and the hindwing pads extended to the sixth abdominal segment (Figs. 15–16). The eighth nymphal instar had BL of 3.29 ± 0.318 mm and HW of 0.96 ± 0.08 mm (*n* = 30); the wings were light green and extended over the entire abdomen (Fig. 17). The ninth nymphal instarof male had BL of 2.80 ± 0.23 mm and HW of 0.96 ± 0.08 mm (*n* = 43), female had BL of 2.99 ± 0.29 mm and HW of 0.90 ± 0.08 mm (*n* = 15). The forewings were much longer than the tail but stuck to the abdomen and did not allow flight; the forewings were nearly transparent, and the hindwings were light green dark spots between compound eyes, pronotum has triangular spots, subgenital plate separated form a J-shap in lateral view, aedeagus of male and ovlpsoltor of female are shorter than parame (Figs. 18–19, 22–24). The tenth nymphal instar of male had BL of 2.91 ± 0.12 mm and HW of 0.94 ± 0.05 mm (*n* = 42), female had BL of 3.18 ± 0.23 mm and HW of 0.94 ± 0.06 mm (*n* = 32). The forewings were much longer than the tail but stuck to the abdomen and did not allow flight; the forewings were nearly transparent, and the hindwings were light green (Figs. 20, 25–26). On the 12th to 14th day after hatching, the tenth molting turned the nymphs into subadults. The subadults were not yet able to fly, because their wings were not completely mature and hindwings were wrinkled; therefore, they were maintained flat near the hind legs. On the following day, they became fully mature as adults and were able to fly and mate. The aedeagus of adut is longer than paramer (Figs. 27–28) (Table 1). The each shape-winged instar nymph was shown in the ninth to 26thphotos.



Figures 9–20: Immature stages of *S. nigropunctata* Mahmood, 1967. (9) First instar. (10) Second instar. (11) Third instar. (12) Fourth instar. (13) Fifth instar. (14) Sixth instar. (15) Seventh instar. (16) Molt of seventh instar. (17) Eighth instar. (18) Ninth instar. (19) Molt of ninth instar. (20) Tenth instar. Bar, 0.5 mm

Figures 21–28: Pygofer of *S. nigropunctata* Mahmood, 1967. 21-23, 25, 27 in lateral view, 24, 26, 28 in ventral view. (21, 23–28) Male. (22) Female. (21) Eighth instar. (22–24) Ninth instar. (25–26) Tenth instar. (27–28) Adult. Bar, 0.2 mm

Table 1: Body sizes and appearance characteristics of each instar of *Singapora nigropunctata* Mahmood, 1967

Instar**s**	Body length, BL (mm)	Head width, HW (mm)	n	**Type feature**
1st	0.72 ± 0.06	0.17 ± 0.01	2	Body thinner, pale yellowish, and eyes red.
2nd	0.93 ± 0.16	0.27 ± 0.03	15	Forewing pads light gray, hindwing bud yellow.
3rd	1.23 ±0.19	0.34 ± 0.06	14	Wing pads grey, hindwing pads extend to the chest and abdomen junction.
4th	1.48 ± 0.23	0.44 ± 0.08	16	Forewing pads extend to junction of the chest and abdomen, hindwing pads extend to first abdominal segment.
5th	1.99 ± 0.26	0.59 ± 0.09	26	Forewing pads extend to second abdominal segment, hindwing pads extend to third abdominal segment.
6th	2.52 ± 0.32	0.79 ± 0.09	48	Forewing pads extend to fourth abdominal segment, hindwing pads extend to fifth abdominal segment.
7th	2.75 ± 0.28	0.82 ± 0.07	34	Forewing pads extend to fifth abdominal segment, hindwing pads extend to sixth abdominal segment.
8th	2.97 ± 0.16	0.91 ± 0.08	28	Wings green and extend over bdominal.
9^{th} Male	2.80 ± 0.23	0.96 ± 0.30	43	Dark spots between compound eyes, the subgenital plate separated form a J-shap in lateral view, aedeagus is shorter than paramer.
9^{th} Female	2.99 ± 0.29	0.90 ± 0.08	15	Dark spots between compound eyes, pronotum has triangular spots.
10^{th} Male	2.91 ± 0.12	0.94 ± 0.05	42	Aedeagus extend to the paramer.

10th Female	3.18 ± 0.23	0.94 ± 0.06	32	Pronotum has triangular spots on both sides.

At a constant temperature of 30℃, the development time was shorter and the survival rate was higher than in winter or summer. In these three environments, the development time from hatching to the mature adult was 13.5 to 15.2 d. Table 1 displays the growth times for each nymphal instar. In the 30℃ experiment, the mortality rate was 47.8% (n = 69). The average time from hatching to the mature adult stage was 13.9 d. In the winter experiment, the average room temperature was 23.5℃ (15.9℃ to 29.2℃), and the mortality rate was 80% (n = 25). The average time from hatching to the mature adult stage was 15.2 d. In the summer experiment, the average room temperature was 30℃ (27.0℃ to 30.5℃), and the mortality rate was 69% (n = 55). The average time from hatching to the mature adult stage was 13.5 d.

Discussion

There is limited previous research on the tiny leafhopper (*S. nigropunctata*) and its cospecies. This study is the first to examine its life cycle and development. In the laboratory, the development process from hatching to maturity, including ten nymphal instars and the growth of mature wings, took 13.5 to 15.2 d. Although the first five nymphal instars description of Sosa et al. (2005) is similar to this study, but there is no record at sixth to tenth nymphal instars (Sosa et al. 2005).

In the field, from May to June, the tiny leafhoppers had sufficient food, and the temperatures (28.5℃–29.5℃) were ideal for their survival. Therefore, populations grew rapidly. During this period, the tiny leafhoppers could produce subsequent generations within a short time, resulting in a high population growth rate. By contrast, in July and August, the population densities decreased considerably. During this hotter period, the adults flew to cooler places; if they were unable to leave the host, the insects died in the high temperature environment. Similarly, after rain, the adults flew to drier places. Therefore, the population fluctuation of the tiny leafhoppers was distinctly influenced by environmental factors such as typhoons, rainfall, and temperature.

The tiny leafhopper population densities were the highest in June 2009. Typhoon Lotus caused a decrease on June 21, 2009. Plants changing their leaves on December 5, 2009, caused the third major change in population densities, a decrease of 33.7%. Thus, changes in temperature, rainfall, typhoons, and the changing of leaves can cause the numbers of tiny leafhoppers to decrease rapidly.

Before the old leaves had fallen, new leaves started growing on the ends of the branches. The food supply was limited because few mature leaves were available for the tiny leafhoppers. The trees shed their leaves completely prior to the slow growth of new leaves. This led to widespread deaths of adults and nymphs.

In summary, the present study used laboratory and field experiments to determine the developmental trends and population dynamics of the tiny leafhopper (*S. nigropunctata*). Understanding the life cycle, development, and population dynamics of this agricultural insect pest may facilitate the determining of optimal times to implement control measures to limit insect numbers and protect crops.

These studies were initiated to evaluate relationships among reproduction, feeding, number and size of nymphal instars and growth rates of nymphs. Their life cycle and development increased the tiny leafhopper's population growth rapidly.

The tiny leafhopper *S. nigropunctata* is a parasitic insect of tropical plants. Therefore, the mortality rate was extremely high at 80% under winter conditions and remained high at 69% under summer conditions. This may have resulted from the laboratory conditions (handling) and may have affected how representative the data were. During the experiment in the feeding box, the food (leaves) easily lost water and affected the instar stages. Some individuals only experienced eight or nine instars before becoming subadults.

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