Comparison of the morphology of Pseudoregma bambucicola in Southwest of China

Xiang Nong1,2,*, Yu Zhang1,2, Hua Yu3, Yao Jun Yang1,2, Zi Liang1,2, Qiang Hu1,2

1Bamboo Diseases and Pest control and Resources Development Key Laboratory of Sichuan Province, Sichuan, 614000, China
2College of Life Science, Leshan Normal University, Le’shan 614000, China
3Sichuan Entry-Exit Inspection and Quarantine Bureau, Chen’du 610041, China

*Corresponding author E-mail address: nongx2008@163.com

Abstract. The bamboo aphid, *Pseudoregma bambucicola*, is one of the most common insect pests of bamboo. It not only affects normal bamboo growth but also induces sooty molds, causing bamboo dead and hugely impacting the growth of bamboo. This study uses the LY-WN high-quality microscopic system to research and record the size of individual form of the *Pseudoregma bambucicola* from Leshan, Chengdu, Chongqing and Mianyang. The results show that the body length of adults are from 3.21 mm to 4.23 mm in Leshan, between 3.16mm to 4.47mm in Chengdu, from 3.05mm to 4.18mm in Mianyang and from 2.66mm to 3.87mm in Chongqing. What's more, the egg length from 1.14mm to 1.62mm and body wide from 0.60 mm to 0.77mm in Leshan. Larvas from Leshan grow faster at 2-3 days and 6-7 days than in other days. Above all, this study aims to distinguish the individual morphological character of the *Pseudoregma bambucicola* from different areas through comparative analysis, and to provide theoretical support for relevant studies.

1. Introduction

Aphid is one of the insect pests of many crops and plants. It damages crops, causing reduction in crop, production and quality [1-2]. *Pseudoregma bambucicola* are mainly distributed in southern and eastern parts of Asia, including China, Japan and Thailand. In China, it is widely distributed in Sichuan, Chongqing, Guangdong, Taiwan and other warm regions. This species parasitize in bamboo, mostly on bamboo’s stems or twigs near the ground. They reproduce in large numbers and form a dense population inside the bamboo [3-5].

*Pseudoregma bambucicola* is the main insect pest of bamboo. It is very harmful for bamboo's normal growth and its number usually peaks in early and middle May [4]. Usually, it sucks the juice of the infected twigs and stems. Having been infected, the bamboo twigs and stems are densely covered by such kind of insects. Moreover, the injured twig atrophy turns brown, inducing sooty molds and the growth of black molds, which hindering bamboo’s photosynthesis. The high fecundity of *Pseudoregma bambucicola* will cause substantial damage to twigs and lead to its death eventually. Nowadays, almost all studies on *Pseudoregma bambucicola* focus on its prevention. Yi et al (2003) studied prevention test with 10% Imidacloprid [5]. Deng et al (2009) studied the spatial pattern of the *Pseudoregma bambucicola* and its three major natural enemies [6]. In the same year, Zou et al studied...
the biological characteristics and morphology of the *Pseudoregma bambucicola* [7]. However, there is not a complete research on morphological differences. The purpose of this study is to expose the morphological differences of the *Pseudoregma bambucicola* from different areas, and provides the theoretical basis for further research and the prevention of the *Pseudoregma bambucicola*. Moreover, this study analyzes the individual morphological differences of adults in Leshan, Chengdu, Chongqing, and Mianyang through the statistical analysis. It aims to provide a scientific and theoretical basis for the identification, utilization and management of *Pseudoregma bambucicola*.

2. Materials and Methods

2.1. Experimental materials

2.1.1. Aphids

The adult aphids are from four different bamboo forests of natural infection: Leshan, Chongzhou of Chengdu, Mianyang and Chongqing.

2.1.2. Instruments

The microscope: LY-WN high-quality microscopic system with image interface, and the magnification is fifteen to two hundred and fifty times.

2.2. Experimental methods

2.2.1. Cultivation of Aphids’ Larvae

(1) Seven petri dishes that have been completely sterilized before being used, some gauze, part of which is cut to the petri dish’s size (round) so that it can be placed on the bottom of the dish; others can cover the dish[8].(2) Pour two milliliter artificial feed into every culture dish, and make the gauze in the bottom of culture dishes soaked.(3) Choose the adults from many living aphids picked from bamboo forest, and then divide them into seven groups of 30 aphids each. Next, put them in culture dishes separately, seal them with gauze and set a elastic on the outside of every culture dish so that the air can enter and avoid aphids climbing out.(4) Fostering culture dishes in the thermostat with eighteen degree Celsius.(5) Taking out the culture dishes to observe the growth of aphids every other day.

2.2.2. Measures

2.2.2.1 Survey the adults from different areas

We took out twenty adult samples of different areas from the glass bottle, and dried them up. Then, we lay them on the stage with their abdomen upwards and used pins to fasten their antennae and three pairs of feet in order to make it extended on the stage and look three pairs of feet clearly. After a period of time, we removed the pins, put it under the microscope and adjusted the magnification. After repeated experiments, we found images were clear in the sixty times and the whole body could be looked in a picture. When we finished all the steps, we got twenty microscopic images of adult from every area and found the aphid have a pair of feeler and three pair of feet by observing the image. Then, we used the measurement software to measure and save the antennae length, body length and all foot length of adults. At last, we recorded the data of the one to twenty adults from four zones in a table.

2.2.2.2 Measure the nit born one to seven days in Leshan

At first, we got the Leshan aphids which brought one to seven days out. Secondly, we killed them in the 90% alcohol, in order to make them static under the microscope and good for getting clear pictures and fastening their shape. Thirdly, we used pins to fasten the larva with abdomen upward on the stage. After a while, we pulled pins out and watched them. Because they were too small, we discovered that
the most clear image appeared in the ninety times through many experiments. We can observe clearly the antennae and three pairs of feet of larvae from this image. Finally, we measured the length of the antennae and the front, middle and back of three pairs of feet of nits, using the measurement software. Finally, we saved the measured images and recorded data in a table.

2.2.2.3 Pseudoregma bambucicola eggs of Leshan
We placed the egg on the stage and watched them in the ninety times. Then, we measured the length of eggs by using the measurement software and saved the data in a form.

3. Results and Analyses

3.1. The length of eggs
Mainly analyze data and calculate the standard deviation of eggs’ length. The results are shown in table 1:

Table 1. Eggs length standard deviation calculation of Pseudoregma bambucicola from Leshan city

| Total length/L | Total length square sum/T | Degrees of freedom | Variance/S^2 | Standard Deviation |
|----------------|---------------------------|--------------------|--------------|--------------------|
| ΣX=26.53       | ΣX^2=35.5323              | 19                 | S^2=0.02mm   | S=0.14mm           |

By analyzing the table, we know that the coefficient of variation of egg length is 10.5%. It is bigger, so the egg length is not closed to the sample mean. This shows a big discrete degree and a wide variation of egg size.

3.2. Comparison of the growth of Pseudoregma bambucicola larvae (1-7 days)
The standard deviation is smaller, with the variable quantity distributing in near mean value. The standard deviation is bigger. It distributes dispersedly. The standard deviation of body length of larvae (1-7 days) is small, which indicates that the data concentrates near the mean of daily body length. Above all, larvae grow faster in 2-3 days and 6-7 days than other days by analyzing the changing trend of mean (Table 2).

Table 2. Body long standard deviation calculations of Leshan larvae (1-7 day)

|       | 1d   | 2d   | 3d   | 4d   | 5d   | 6d   | 7d   |
|-------|------|------|------|------|------|------|------|
| Average | 1.37 | 1.38 | 1.55 | 1.57 | 1.59 | 1.59 | 1.68 |
| SD     | 0.102| 0.124| 0.153| 0.116| 0.097| 0.130| 0.384|

3.3. Comparison of the individual differences of adult pseudoregma bambucicola from different city
The results show that the difference of body length of adult aphids is obviously between Chongqing and other areas, which are Leshan, Chengdu and Mianyang. However, it is not obvious among Leshan, Chengdu and Mianyang. Besides, most of body length of adult aphids is distributed in 3.85mm because the standard deviation is the least in Chengdu (Table 3).
Table 3. Mean differences significant of length for four local adult aphids

| Sources of variation | Average | Standard deviation | α=0.05 |
|----------------------|---------|--------------------|--------|
| Chengdu              | 3.85    | 0.27               | a      |
| Leshan               | 3.82    | 0.42               | ab     |
| Mianyang             | 3.64    | 0.35               | ab     |
| Chongqing            | 3.15    | 0.36               | c      |

Annotation: different letters express significant difference.

The results show that the difference of adult aphids’ antenna length is obviously between Leshan and Chengdu. Meanwhile, the difference of adult aphids’ antenna length is obviously between Chengdu and Chongqing (Table 4).

Table 4. Comparison of the antennae long mean of adult Pseudoregma bambucicola from four city

| Sources of variation | Average | Standard deviation | α=0.05 |
|----------------------|---------|--------------------|--------|
| Chengdu              | 0.649   | 0.057              | ac     |
| Leshan               | 0.593   | 0.060              | b      |
| Mianyang             | 0.656   | 0.072              | a      |
| Chongqing            | 0.635   | 0.068              | c      |

Annotation: different letters express significant difference. (P<0.05)

The results show that the front foot length in Leshan has palpable differences with Chengdu. Meanwhile, the front foot length in Leshan has palpable differences with Chongqing and Mianyang (Table 5).

Table 5. Analysis of the mean difference significantly of forefoot length from adults Pseudoregma bambucicola

| Sources of variation | Average | Standard deviation | α=0.05 |
|----------------------|---------|--------------------|--------|
| Chengdu              | 1.9145  | 0.111              | a      |
| Leshan               | 1.7315  | 0.131              | b      |
| Mianyang             | 1.898   | 0.125              | ac     |
| Chongqing            | 1.8895  | 0.104              | c      |

Annotation: different letters express significant difference. (P<0.05)

The difference significantly shows that the mid-leg length in Leshan has palpable differences with other areas. Meanwhile, the mid-leg length in Leshan and Mianyang has palpable differences with Chengdu (Table 6).

Table 6. Analysis of the mean difference significantly of mid-leg length of adults Pseudoregma bambucicola

| Sources of variation | Average | Standard deviation | α=0.05 |
|----------------------|---------|--------------------|--------|
| Chengdu              | 2.026   | 0.146              | a      |
| Leshan               | 1.84    | 0.188              | b      |
| Mianyang             | 2.049   | 0.155              | ac     |
| Chongqing            | 2.1585  | 0.168              | c      |

Annotation: different letters express significant difference. (P<0.05)
The mean of adult metapedes foot length has palpable differences within the four areas by difference analysis (Table 7).

**Table 7.** Analysis of the mean difference significantly of metapedes length from adults Pseudoregma bambucicola

| Sources of variation | Avarage | Standard deviation | α=0.05 |
|----------------------|---------|--------------------|--------|
| Chengdu              | 2.6125  | 0.223226932        | a      |
| Leshan               | 2.462   | 0.215738633        | b      |
| Mianyang             | 2.8775  | 0.290586974        | c      |
| Chongqing            | 2.658   | 0.19138278         | d      |

Annotation: different letters express significant difference. (P<0.05)

3.4. Morphologic observation

By contrasting Leshan, Chengdu, Mianyang and Chongqing, we can find the foot length of *pseudoregma bambucicola* from Leshan is relatively short, while those in the Mianyang area have longer foot than those in other areas. In comparison, the body length of Leshan *pseudoregma bambucicola* is longer than those other areas(Figure 1-3).

**Figure 1.** *P. bambucicola* eggs length measurements of Leshan city

**Figure 2.** Observation of the growth of Pseudoregma bambucicola larvae (1-7 days)

Notes: A1 and A2 were the first day of the growth of larvae; B1 and B2 were the second day of larvae; C1 and C2 were the third day; D1 and D2 were the fourth day; E1 and E2 were the fifth day; F1 and F2 were the sixth day; and G1 and G2 were the seventh day, respectively.

**Figure 3.** Measurement of various indexes (foot and body length) of adult pseudoregma bambucicola in different places

Notes: a1 and a2 are adult from Chengdu; b1 and b2 from Leshan; c1 and c2 are measurements of adult in Mianyang; d1 and d2 are measurements of adult in Chongqing.
4. Discussions

4.1. Morphology of Pseudoregma bambusicola larval growth stage

The larva grows slower in 1-2 days, but faster in 2-3 days and 6-7 days. Besides, it grows slowly at other times. The mean of larval body length at 1-7 days is between 1.37 mm and 1.68 mm. The standard deviation of mean is smaller, which shows the sample average concentrates near the mean. Therefore, we can judge its growing days by the body length of sample and provide evidence for studying the morphological character of the 1-7days’ larva in the future.

The egg length of Pseudoregma bambusicola

The experimental data shows that the size of Pseudoregma bambusicola eggs from Leshan was 1.19mm to 1.62mm, and it has a little difference with the first size of larva from 1.20mm to 1.64mm. There is a little change with the body length from eggs to the first day of larvae or grow very slowly. However, the Coefficient of Variation of eggs is biggish by calculating standard deviation and Coefficient of Variation, so the eggs length does not concentrate in the vicinity of sample mean 1.32mm. There is a big diversity on the length of every egg.

4.2. Morphological differences of adult in four places

By contrasting Leshan, Chengdu, Mianyang and Chongqing, we can find the foot length of pseudoregma bambusicola from Leshan is relatively short, while those in the Mianyang area have longer foot than those in other areas. In comparison, the body length of Leshan pseudoregma bambusicola is longer than those other areas. In a word, the body size of Leshan pseudoregma bambusicola is longer than other three places. The results of analysis of variance display the mean of three pairs of adult feet in four regions are hugely different in Leshan and Chengdu. Consequently, the foot length can be offered as one of the criterion to distinguish aphids in different areas. In previous studies, there were some studies on the morphology of the pseudoregma bambusicola, and they concluded that the body length of adults was 1-4mm, which was not accurate. Meanwhile, there is no research on aphids from different parts. This experiment did multiple and particular experiments on the morphological character of the Pseudoregma bambusicola based on previous studies. This experiment aims to provide theoretical basis for further study related to characters of the pseudoregma bambusicola. The results of significant difference analysis also provide a certain basis for differentiating aphids from the four following regions: Chongqing, Leshan, Mianyang and Chengdu. This study indicates that the growth rate of aphids in different periods is different, and the size is with little diversity between eggs and one day old larvae in Leshan. The body length of adults in Leshan is longer than in other areas, but their three pairs of feet is shorter than other three places. We draw this conclusion by comparing aphids from Chengdu, Chongqing, Mianyang and Leshan. What’s more, all of their individuals are oval.

Acknowledgments

This study was supported by the Scientific Research Fund of Sichuan Provincial Education Department (No. 18TD0032), the Science & Technology Department of Sichuan Province (No.2019JY0290), and Bamboo Diseases and Pest control and Resources Development Key Laboratory of Sichuan Province (No.17ZZ002).

References

[1] Baek, M. Y., Park, H. J., Kim, G. M., Lee, D. Y., Lee, G. Y., Moon, S. J., Ahn, E. M., Kim, G. S., Bang, M. H., Baek, N. I., 2013. Insecticidal Alkaloids from the Seeds of Macleaya cordata on Cotton Aphid (Aphis gossypii). J Korean Soc Appl Biol Chem (2013) 56, 135–140.

[2] Kim, S. K., Kim, Y.C., Lee, S., Kim, J.C., Yun, M. Y., Kim, I. S., 2011. Insecticidal Activity of Rhamnolipid Isolated from Pseudomonas sp. EP-3 against Green Peach Aphid (Myzus persicae). J. Agric. Food Chem.2011,59,934–938.
[3] Yi Long. The control experiments of 10% imidaclorpid against Pseudoregma bambucicola. Plant doctor. 2003.6:25-26.

[4] Petitt, F. and Smilowitz, Z. (1982) Green peach aphid feeding damage to potato in various plantgrowth stages. Journal of Economic Entomology, 75, 431-435.

[5] Zou Hai-Kui, Jiang Gui-Hua, Tang Sheng-Wu, Yang Xi, Chen Xiao-Yu. Biological characteristics and morphology of the Pseudoregma bambucicola. Chinese Journal of Ethnomedicine and Ethnopharmacy. 2009(11):11-13.

[6] Fukatsu, T., Shibao, H., Nikoh, N., Aoki, S., 2001. Genetically Distinct Populations in an Asian Soldier-Producing Aphid, Pseudoregma bambucicola (Homoptera: Aphididae), Identified by DNA Fingerprinting and Molecular Phylogenetic Analysis. Molecular Phylogenetics and Evolution. Vol. 18, No. 3, March, pp. 423–433, 2001

[7] Deng Shun, Wang Peng, Shu Jian-Ping, Wang Hao-Jie. The studies on spatial distribution of bamboo aphid (Pseudoregma bambucicola) and its three natural enemies. Journal of Environmental Entomology. 2009(4):300-305.

[8] Pan Ke, Huang Bing-Qiu, Hou Xue-Wen. A modified practical method for rearing Aphis craccivora with meridic liquid nutrients. Chinese Bulletin of Entomology. 2006 43(6):728-729.