A morphological indicator for estimating nutritional status in nymphs of the brown-winged green bug, Plautia stali Scott (Heteroptera: Pentatomidae)

メタデータ	言語: eng 出版者: 公開日: 2019-03-22 キーワード (Ja): キーワード (En): Stink bug, Fruit pest, Forecasting method, Synthetic aggregation pheromone, Starvation 作成者: 外山, 晶敏, 三代, 浩二, 井原, 史雄 メールアドレス: 所属:
URL	https://doi.org/10.24514/00002174

Original Paper

A morphological indicator for estimating nutritional status in nymphs of the brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae)

Masatoshi Toyama[1,*], Koji Mishiro and Fumio Ihara

Breeding and Pest Management Division, Institute of Fruit Tree Science,
National Agriculture and Food Research Organization (NARO)
Tsukuba, Ibaraki 305-8605, Japan

Summary

Sticky traps baited with the synthetic *P. stali* aggregation pheromone can lure and catch *P. stali* nymphs. If individuals with poor nutritional status than well-fed individuals in nymphs as well as in adults are more susceptible to the pheromone, monitoring nymphs caught by the trap might provide a simple method for assessing the food resource conditions of their forest habitat. However, the relationships between nutritional status and sensitivity to the pheromone have not yet been experimentally determined for nymphs. Therefore, we identified a morphological indicator of nutritional status for *P. stali* nymphs in order to assess the nutritional status of nymphs attracted to the synthetic *P. stali* aggregation pheromone. We first assessed several morphological characters in the fourth instar using nymphs that were extremely varied in nutritional status. Both body length at the longest point (BL) and body width at the widest point (BW) varied among them. On the other hand, the distance from the top of the right eye to the top of the left eye (EYES) was constant throughout the instar period, and thus, was regarded as a good estimator of fundamental body size. Based on these results, we propose the product of BL and BW (BODY) relative to EYES as the indicator. Next, we measured this indicator under several feeding treatments for individual instars, drawing regression lines for BODY versus EYES in each case. A comparison of indicator characters with the regression lines produced in this study allows us to estimate the food intake and nutritional status of a large number of nymphs captured in the field.

Key words: Stink bug, Fruit pest, Forecasting method, Synthetic aggregation pheromone, Starvation

Introduction

The brown-winged green bug *Plautia stali* Scott (Heteroptera: Pentatomidae) is a fruit-spotting bug that often causes extensive damage to various fruit crops in Japan (Umeya, 1976; Yasunaga et al., 1993). These crops include citrus (Ide, 1997), persimmon (Yanase, 1997), loquat (Katase et al., 2005), stone fruits, and Japanese pears (Sato, 1997). Since the first nation-wide outbreak in 1973 (Hasegawa and Umeya, 1974), large populations of this bug have periodically erupted in forests and then attacked orchards (e.g., Shiga, 1980; Inoue, 1986; Takagi,

(Received 3 October 2014, Accepted 13 January 2015)
1 果樹研究所ブドウ・カキ研究領域　広島県東広島市
Grape and Persimmon Research Division, NARO Institute of Fruit Tree Science Higashihiroshima, Hiroshima 739-2494, Japan
* Corresponding author. Email: tym@affrc.go.jp

1997).

Plautia stali feeds on various plants and moves from plant to plant as the season progresses from spring to autumn. It does not develop and reproduce on fruit crops, but irregularly moves into orchards looking for food when faced with food shortages in the surrounding vegetation.

Serious orchard infestations of this bug do not occur every year. In summer in Japan, it largely reproduces in the planted forests of Japanese cypress (*Chamaecyparis obtusa* Endl.) and Japanese cedar (*Cryptomeria japonica* D.Don), using their cones as food sources (Umeya, 1976; Yamada and Noda, 1985). The population levels fluctuate widely, depending on large annual variations in the cones produced in their forest habitat (Yamada and Noda, 1985; Morishita et al., 2007). If the bug population growth exceeds cone production, which is most likely to happen in autumn, the exhaustion of food resources leads the bugs to move from the forest to cultivated areas (Tsutsumi, 2001). Therefore, the monitoring of *P. stali* population dynamics and food resource conditions in the coniferous forests is a key practice in predicting orchard invasions and determining when to spray insecticides in the autumn (Takagi, 1997; Tsutsumi, 2001).

The numbers of stylet sheaths left on cypress cones after feeding can be used to assess food availability for *P. stali* and to make reliable predictions of orchard infestations (Tsutsumi, 2001). However, this approach requires considerable time and effort. Thus, there is a need to develop an alternative or supportive method. A simple monitoring system would enable us to perform further detailed assessments, resulting in further accurate predictions.

Sticky traps provide a simple, inexpensive, but effective method of monitoring pest levels, and therefore they are used to monitor various crop pests. We have also used sticky traps baited with the synthetic *P. stali* aggregation pheromone (Sugie et al., 1996) to lure and catch *P. stali* or its natural enemies (Mishiro and Ohira, 2002). One characteristic of this trap is that it can catch nymphs as well as adults.

The response of adults to the pheromone varies according to their nutritional status; more than well-fed adults, adults with poor nutritional status are susceptible to the pheromone (Moriya and Shiga, 1984; Moriya, 1995). If this is also true for the nymphs, then monitoring nymphs using pheromone sticky traps might be useful for assessing the food resource conditions of their forest habitat. Since nymphs have a limited ability for sucking seeds inside cones due to their short proboscis, they could be more sensitive to food shortage.

We assume that most of the nymphs captured on the traps will be hungry or in a more severe nutritional status. If this is true, we will simply need to monitor the numbers of captured nymphs, without assessing their nutritional status each time. However, the relationship between nutritional status and sensitivity to the pheromone has not yet been experimentally determined for the nymphs.

Our purpose in this study is to develop a simple method for assessing the nutritional status of large numbers of nymphs caught in the field, with particular emphasis on the identification of poor nutritional status. To this end, we tried to find a morphological indicator for simplicity. It is often observed that nymphs have shrunken abdomens. We first assessed several morphological characters as candidates for the indicator of nutritional status. We aimed to identify a character that is strongly influenced by nutritional status but not by age, and relate it to one that is invariable during each instar period, because the sizes of most morphological characters are not only influenced by nutritional status but also depend on the fundamental body size in each instar. Then, we went on to measure these indicator characters in nymphs of multiple instars that had been experimentally subjected to a range of experimental feeding treatments (Toyama et al., 2013). Based on these results, we propose an indicator of nutritional status for *P. stali* nymphs and an application of the indicator to test the assumption regarding the monitoring of nymphs using pheromone sticky traps.

Materials and methods

1. Insects and growth conditions

This study was conducted from 2010 to 2011. The *P. stali* nymphs came from a laboratory colony that has been maintained for multiple generations at the Institute of Fruit Tree Science of the National Agriculture and Food Research Organization (NARO). The colony was founded using adults collected in Tsukuba, Japan (36°02′ N, 140°05′ E). The insects were raised following the methods of Moriya (1986) with some modifications. To prepare test nymphs, egg masses were isolated from the colony and individually maintained in transparent plastic dishes (90 mm diameter, 18 mm height) at 25 ± 1℃ under 16 h day-length conditions. Each sibling group from the same egg mass was reared together and fed on raw peanuts and dried soybeans, which are a favorable food for development and reproduction in *P. stali* (Kotaki et al., 1983; Shiga and Moriya, 1984), with distilled water, until they reached the focal instar for each test. Moderate humidity levels (within the range 60–75%) were maintained by setting the dishes in a sealed plastic container with small holes.

Upon reaching the focal instar, nymphs were individually separated into plastic petri dishes (55 mm diameter, 15 mm height) within 24 h after molting and assigned to 1 of the treatments. During the experiment, all of the dishes were placed within sealed plastic containers with small holes to provide moderate humidity (60–75%), and were maintained at 25 ± 1℃ under 16 h day-length conditions.

2. Candidate morphological characters

For the initial assessment, we selected the following morphological characters (Fig. 1) as candidates for composing a nutritional status indicator: the length of the needle-like proboscis (hereinafter referred to as "PRB"), body length at the longest point ("BL"), body width at the widest point ("BW"), abdomen length at the longest point ("AL"), distance from the top of the right eye to the top of the left eye ("EYES"), and body height at the highest point ("BH"). In addition, the product of BL and BW ("BODY") was calculated to represent the entire body size.

Images of specimens were captured with a digital microscope (×30: Keyence VHX-900) from the dorsal, ventral, and lateral angles (Fig. 1). The measurements were performed on the images using digital-image analysis software (supplied with the VHX-900). PRB was measured on the ventral side; EYES and AL were measured on the dorsal side; BL and BW were measured on both the ventral and dorsal sides, and BH was measured on the lateral angle.

3. Initial assessment of candidate morphological characters

The assessment was performed on fourth-instar nymphs. Newly molted nymphs (within 24 h after molting), which had been sufficiently fed before molting, were assigned to either of 2 groups with extremely different feeding conditions. In the first group, 15 nymphs were provided with raw peanuts, dried soybeans, and distilled water for the first 2 days after molting (hereinafter referred to as "2-day-fed nymphs"), and then preserved in 70% ethyl alcohol until their images were taken. The 15 nymphs in the other group were given water but no food until they died ("nymphs starved to death"), and then immersed in the alcohol within 24 h after death. Two of the nymphs were removed from the analyses because they molted into the next instar before death. Measurements on the images were done for each of the morphological characters mentioned above (EYES, PRB, BL, AL, BW, and BH).

In order to identify characters that change depending on nutritional status and ones that remain relatively constant, comparisons of the characters between the 2 groups were made using a one-way analysis of variance (ANOVA). To evaluate the relation between EYES or PRB

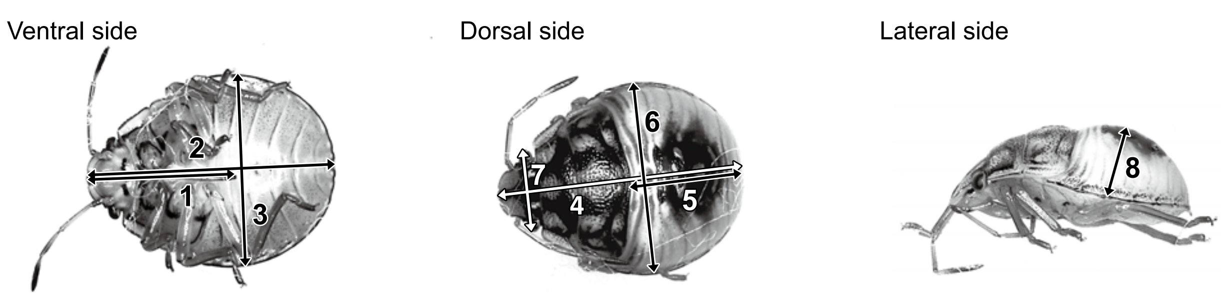

Fig. 1 Morphological characters that were tested as components of a nutritional status indicator in *Plautia stali* nymphs. The pictures of fourth-instar nymphs were taken from 3 different angles. Arrows with numbers indicate the characters measured in the initial assessment: 1) PRB, 2) BL (V = ventral side), 3) BW (V), 4) BL (D = dorsal side), 5) AL, 6) BW (D), 7) EYES, and 8) BH.

and the fundamental body size, Pearson correlation coefficients were calculated using the data for the 2-day-fed nymphs. Comparisons were made with BODY, which represents the entire body size. In addition, the correlation coefficients were applied to BL and BW using all nymphs of the 2 groups to test whether it is appropriate to allow measurement on either the dorsal or the ventral sides. All these analyses were performed using the JMP software version 8.0.2 for Windows (SAS Institute, 2009).

4. Determination of the indicator under various feeding conditions

The morphological characters selected in the initial assessment were measured using nymphs with different nutritional statuses in each instar from the second to the fifth instar (nymphs eat nothing during the first instar). Nymphs remain in each of the second, third, and fourth instars for around 5 days when kept at 25 ± 1℃ under 16 h day-length conditions. For these instars, in addition to the 2-day-fed and starved-to-death treatments (for the fourth instar we used the same samples as those used in the initial assessment), 3 treatments were added, in which the nymphs were fed for 0 or 4 days after molting ("newly-molted nymphs" and "4-day-fed nymphs") or provided with only water for 5 days after molting ("5-day-starved nymphs") to observe the changes in the relationship with nutritional status. The fifth instar generally lasts longer than the other instars. Under the same conditions, nymphs remain in the fifth instar for about 7 days. For this instar, therefore, the 4-day feeding treatment was replaced with a 5-day feeding treatment. After the feeding or fasting treatments, we preserved the nymphs in 70% ethyl alcohol until their images were taken. Nymphs that had been sufficiently fed before molting to the focal instar were used in this assessment.

We described the indicator, i.e., the association between the estimator of fundamental body size and that of variable body size depending on feeding conditions, for each group by fitting a least-squares regression line. Nymphs that died before the end of the experiment or that developed to the next instar during treatments were removed. The final numbers of nymphs that were used in each group ranged from 13 to 30 (see Appendix 1); we increased the number of observations for 4-day-fed nymphs (in the second, third and fourth instars) and 5-day-starved nymphs (in the fifth instar), which had large variations in measurements, to make statistical accuracy higher. The statistical analyses were performed using the JMP software version 8.0.2 for Windows (SAS Institute, 2009).

Results and discussion

1. Initial assessment of candidate morphological characters

In order to select characters that can give an indication of nutritional status in *P. stali* nymphs, 6 morphological characters were assessed using nymphs in the fourth instar. The comparisons between 2-day-fed nymphs and nymphs starved to death are shown in Table 1.

Table 1. Comparisons of candidate morphological characters (mean ± SD) between 2-day-fed *Plautia stali* nymphs and nymphs starved to death in the fourth instar.

Side	Character[z]	Two-day-fed[y]	Starved to death[x]	Difference (F-test)	
		n = 15	n = 13		
Ventral	PRB	3.48 ± 0.18	3.40 ± 0.15	$F = 1.48$,	$p = 0.23$
	BL	5.19 ± 0.43	4.62 ± 0.36	$F = 14.38$,	$p < 0.001$
	BW	3.94 ± 0.27	3.51 ± 0.18	$F = 22.87$,	$p < 0.0001$
	BODY	20.54 ± 3.01	16.28 ± 1.89	$F = 19.45$,	$p < 0.001$
Dorsal	EYES	1.86 ± 0.05	1.84 ± 0.08	$F = 0.25$,	$p = 0.62$
	BL	5.20 ± 0.43	4.55 ± 0.43	$F = 15.93$,	$p < 0.001$
	AL	1.95 ± 0.29	1.78 ± 0.38	$F = 1.91$,	$p = 0.18$
	BW	3.92 ± 0.27	3.49 ± 0.18	$F = 23.27$,	$p < 0.0001$
	BODY	20.48 ± 2.94	15.94 ± 2.02	$F = 21.94$,	$p < 0.0001$
Lateral	BH	1.44 ± 0.33	1.26 ± 0.26	$F = 2.73$,	$p = 0.11$

[z] See the text for details
[y] Nymphs were fed for the first 2 days after molting and then killed
[x] Nymphs were deprived of food after molting until their death

BL, BW, and BODY were significantly smaller in nymphs starved to death than those in 2-day-fed nymphs, indicating that the sizes of these characters are strongly influenced by nutritional status. However, because the nymph abdomen is soft and flexible, BL and BW are easily deformed, particularly in newly molted nymphs. On the other hand, although the differences in AL on the dorsal side and BH on the lateral side were not small, no significant differences were found, since the level of variation was relatively large in each group. The reasons for this probably included large measuring errors in these characters because of a difficulty in measuring them; for AL, the boundary between the abdomen and the thorax was somewhat difficult to identify, while for BH, it was a little difficult to stabilize the body on the lateral side. Thus, we concluded that BL, BW, and their product, BODY, are probably adequate estimators of nutritional status in nymphs, and that BODY must provide a particularly reliable estimate, reflecting the whole body size. In addition, we found strong positive correlations between measurements taken from different angles in BL ($n = 28$, $r = 0.969$, $p < 0.0001$) and BW ($n = 28$, $r = 0.979$, $p < 0.0001$). This indicates that measuring on either side is sufficient.

The variations in BL and BW are caused not only by nutritional status but also by the fundamental body size that results from genetic variations and/or various developmental conditions that the nymphs have experienced. Therefore, it is necessary to remove the influence of fundamental body size when using these characters as estimators of nutritional status. For this purpose, we selected a morphological character that was not affected by nutritional status but remained relatively unchanged during each instar period. In the comparison between 2-day-fed nymphs and nymphs starved to death, only small and insignificant differences were found in PRB and EYES (Table 1). This indicates that the variations in these characters are independent of nutritional status. However, in 2-day-fed nymphs, PRB showed no significant correlation with BODY, which represents the whole body size (with BODY on the dorsal side: $n = 15$, $r = 0.433$, $p = 0.11$), while EYES showed a significant positive correlation (with BODY on the dorsal side: $n = 15$, $r = 0.769$, $p < 0.001$). Based on these results, we selected EYES as an appropriate estimator of fundamental body size for nymphs in each instar.

2. Morphological indicator of nutritional status for individual instars

The selected characters, BL, BW, and EYES, were measured in fourth-instar nymphs that had been given 1 of 5 treatments ranging from 4-day-fed to starved to death. For each treatment, the linear regression lines of BL, BW, and BODY versus EYES are shown in Figure 2 (ref. Appendix 1).

Four-day-fed nymphs were larger than newly molted nymphs and 2-day-fed nymphs. On the other hand, there were few differences between newly molted nymphs (killed within 24 h of molting), 5-day-starved nymphs (killed 5 days after molting), and nymphs starved to death (which generally survived more than 5 days); any differences seen in BL or BW were reduced in their product, BODY (Fig. 2). This suggests that body length and width increase with food intake but not with ageing. The bodies of nymphs increase with feeding from those with few stocks in the body just after molting. This means that we can estimate the amount of food intake with the morphological characters for nymphs captured.

The feeding treatments and measurements were also applied to the second, third, and fifth instars. For each treatment, the linear regression lines of BODY versus EYES are shown for each instar in Figure 3 (ref. Appendix 1). Although low coefficients of determination (R^2) were seen in some regression lines due to large individual variations in the response of the indicator characters (probably because the food intake varied among individuals), each of these instars showed a similar pattern to the fourth in the relationships between the feeding treatments and the indicator characters. Again, there were no large differences between newly molted nymphs, 5-day-starved nymphs, and nymphs starved to death in any of the instars (Fig. 3; ref. Appendix 2).

The effects of the feeding treatments on nymph development were explored in a previous study (Toyama et al., 2013). This should be a reference for the evaluation of the nutritional status of nymphs captured in the field. Nymphs that were given no food after molting survived on average 5.3 ± 1.0 (SD), 10.3 ± 1.7, 8.8 ± 2.8, and 10.1 ± 2.8 days in the second, third, fourth, and fifth instars, respectively. Many of them failed to develop to

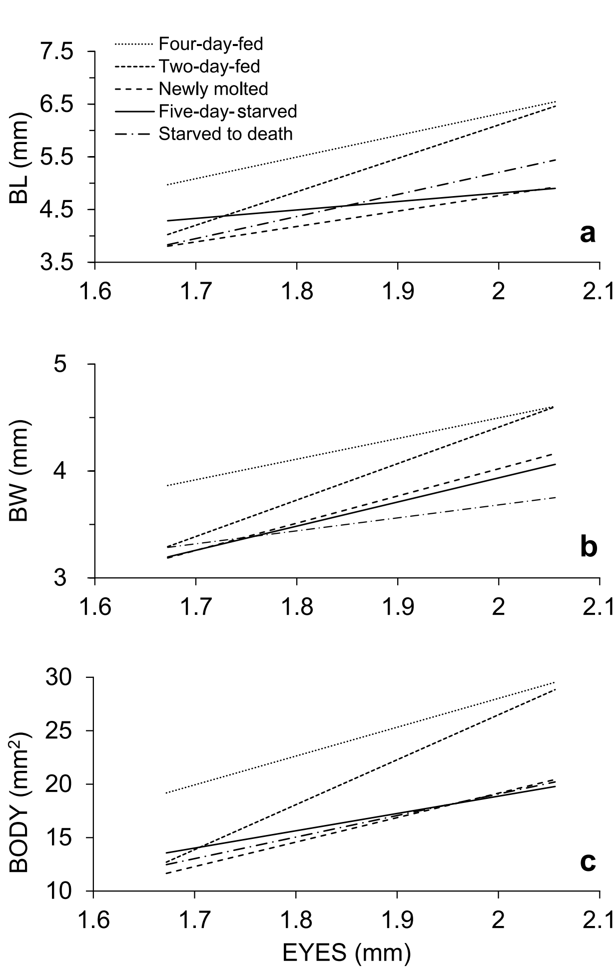

Fig. 2 Regression lines of a) BL, b) BW, and c) BODY versus EYES in nymphs given a range of feeding treatments for the fourth instar: newly molted nymphs (killed within 24 h after molting); 2-day-fed nymphs and 4-day-fed nymphs (fed without any restrictions); 5-day-starved nymphs and nymphs starved to death (deprived of food after molting).

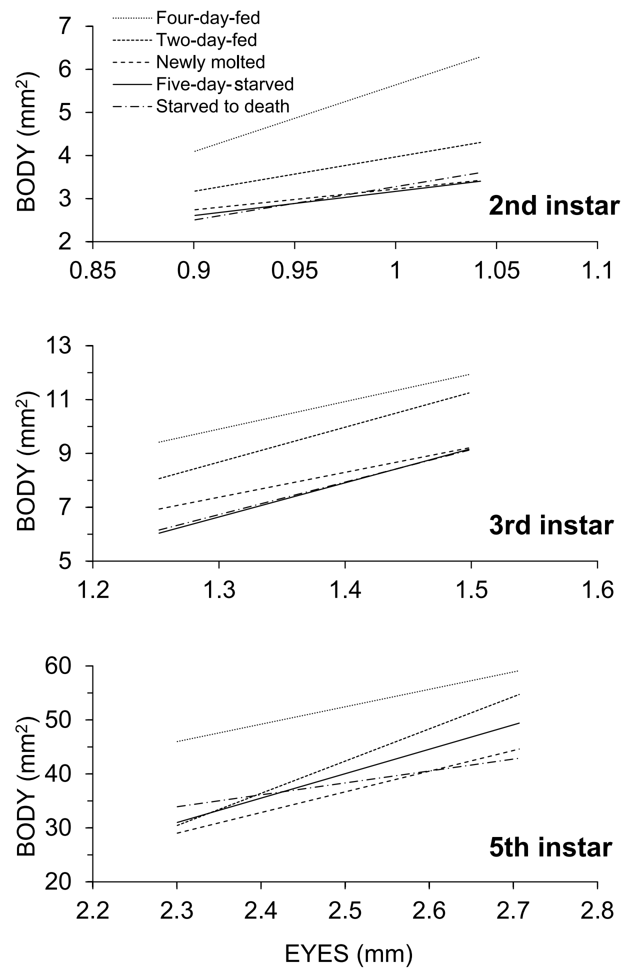

Fig. 3 Regression lines of BODY versus EYES in nymphs given a range of feeding treatments for the second, third, and fifth instars. The treatments were as described for Figure 2 except that in the fifth instar a 5-day feeding treatment replaced the 4-day treatment.

their next instars (0%, 32%, 35%, and 20%developed to the next instar in the second, third, fourth, and fifth instars, respectively). Feeding encouraged nymphal development even when food intake did not fill the nymphs. When fed for 2 days after molting, the percentages of nymphs that developed to the next instar reached 60%, 94%, 94%, and 45%in the successive instars. In addition, for nymphs given the 4-day or 5-day-feeding treatment, the percentages increased to 80%, 100%, 100%, and 95%.

3. Estimation of nutritional statuses of wild nymphs captured

Plautia stali synthetic aggregation pheromone can attract nymphs as well as adults. We hope that monitoring the numbers of nymphs captured on sticky traps baited with the synthetic aggregation pheromone will enable us to evaluate the food resource conditions in their habitats because it is believed that nymphs (like adults) will generally respond to this odor only when their nutritional statuses are low and they are searching for food. However, this assumption has not yet been tested scientifically, so further research is needed on the sensitivity of nymphs to the pheromone traps.

Using the results of the current study, we can assess the nutritional status of large numbers of nymphs captured in the field. The BODY and EYES measurements for collected nymphs can be plotted over the regression lines for the experimental groups. They will be somewhere between those of the well-fed nymphs and nymphs starved to death. If the nymphs are located near lines derived from a group of nymphs starved to death, they can be estimated to have a very low nutritional status and to need much more food before they can develop to the next instar. We predict that most of the nymphs captured on the trap will have poor nutritional status, and be close to these lowest lines. On the other hand, if nymphs are attracted to the synthetic aggregation pheromone regardless of nutritional status, their sizes should be distributed throughout the area between those of the well-fed nymphs and nymphs starved to death.

Nymphs captured in the field will have slightly wider variation in fundamental body size than that found in this study. The food conditions that an individual nymph has experienced before molting determine its post-molt fundamental body size. In this study, sufficient food was given to experimental nymphs before testing, but wild nymphs would have individually different food conditions as a background. However, in general, basic relationships between morphological characters are relatively stable (Stern and Emlen, 1999). In addition, extremely poor conditions inhibit development and survival in nymphs, thus, in many captures, growth should be within the normal range that was obtained in this study. Even for wild nymphs with unknown backgrounds, it is not necessary to consider the variation so much and to hesitate with extending the application of the indicator.

In the near future, we plan to report an evaluation of the nutritional status of wild nymphs attracted to the synthetic aggregation pheromone using this method.

Acknowledgements

This work was financially supported by the Ministry of Agriculture, Forestry, and Fisheries, Japan through a project entitled "Revision of forecasting systems for crop pests and diseases."

References

Hasegawa, H. and K. Umeya. 1974. Outbreak of stink bugs in fruit trees. Plant Prot. 28: 279–286 (in Japanese).

Ide, Y. 1997. Damage of citrus by the outbreak of the stink bugs attacking fruit trees in 1996. Plant Prot. 51: 155–157 (in Japanese).

Inoue, K. 1986. Outbreak of the stink bug attacking fruit trees in 1985 and its cause. Plant Prot. 40: 289–292 (in Japanese).

Katase, M., K. Shimizu, H. Nagasaki and I. Adachi. 2005. Application of synthetic aggregation pheromone of *Plautia crossota stali* Scott to the monitoring and mass trapping of fruit-piercing stink bugs. Bull. Chiba Agric. Res. Cent. 4: 135–144 (in Japanese with English summary).

Kotaki, T., K. Hata, M. Gunji and S. Yagi. 1983. Rearing of the brown-winged green bug, *Plautia stali* SCOTT (Hemiptera: Pentatomidae) on several diets. Jpn. J. Appl. Entomol. Zool. 27: 63–68 (in Japanese with English summary).

Mishiro, K. and Y. Ohira. 2002. Attraction of a synthetic aggregation pheromone of the brown-winged green bug, *Plautia crossota stali* Scott to its parasitoids, *Gymnosoma rotundata* and *Trissolcus plautiae*. Kyushu Pl. Prot. Res. 48: 76–80 (in Japanese with English summary).

Morishita, M., E. Masao, T. Minamikata, H. Komatsu, H. Tsuda, Y. Wada and Y. Tajima. 2007. Forecasting the damage to persimmon fruit by stink bugs (Heteroptera: Pentatomidae), by using airborne pollen counts of Japanese cypress, *Chamaecyparis obtuse*. Jpn. J. Appl. Entomol. Zool. 51: 21–27 (in Japanese with English summary).

Moriya, S. 1986. A method for successive rearing of *Plautia stali* Scott and its technical problems. Plant Prot. 40: 195–199 (in Japanese).

Moriya, S. 1995. Ecological studies on the brown-winged green stink bug, *Plautia stali* Scott, with special reference to its occurrence and adult movement. Bull. Okinawa Agric. Exp. Stn. Suppl. 5: 1–135 (in Japanese).

Moriya, S. and M. Shiga. 1984. Attraction of the male brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae) for males and females of the same species. Appl. Entomol. Zool. 19: 317–322.

Sato, R. 1997. Outbreak of the brownwinged green stink bug and its damage to pear in 1996. Plant Prot. 51: 158–159 (in Japanese).

Stern, D. V. and D. J. Emlen. 1999. The developmental basis for allometry in insects. Development. 126: 1091–1101.

Shiga, M. 1980. Current topics and problems of stink bugs infesting fruits. Plant Prot. 34: 303–308 (in Japanese).

Shiga, M. and S. Moriya. 1984. Utilization of food plants by *Plautia stali* Scott (Hemiptera, Heteroptera, Pentatomidae), an experimental approach. Bull. Fruit Tree Res. Stn. A. 11: 107–121 (in Japanese with English summary).

Sugie, H., M. Yoshida, K. Kawasaki, H. Noguchi, S. Moriya, K. Takagi, H. Fukuda, A. Fujiie, M. Yamanaka, Y. Ohira, T. Tsutsumi, K. Tsuda, K. Fukumoto, M. Yamashita and H. Suzuki. 1996. Identification of the aggregation pheromone of the brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae). Appl. Entomol. Zool. 31: 427–431.

Takagi, M. 1997. The strategy of the control for brown-winged green bug. Plant Prot. 51: 8–12 (in Japanese).

Toyama, M., K. Mishiro, R. Nakano and F. Ihara. 2013. Effects of food shortage on nymphal life history of the brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae). Appl. Entomol. Zool. 48: 461–468.

Tsutsumi, T. 2001. A method for inspecting stylet sheaths of stink bugs on the cone of Japanese cypress. Plant Prot. 55: 560–562 (in Japanese).

Umeya, K. 1976. Outbreak of stink bugs in fruit trees. Plant Prot. 30: 133–141 (in Japanese).

Yamada, K. and M. Noda. 1985. Studies on the forecasting of the occurrence for stink bugs infesting fruits. Bull. Fukuoka Agric. Res. Cent. B-4: 17–24 (in Japanese with English summary).

Yanase, N. 1997. An analysis of damage caused by extraordinary outbreak of fruit stink bugs in 1996 －persimmon－. Plant Prot. 51: 160–162 (in Japanese).

Yasunaga, T., M. Takai, I. Yamashita, M. Kawamura and T. Kawasawa. 1993. Damage of crops by bugs. In: Tomokuni, M. (ed.). A field guide to Japanese bugs. pp. 243–300. Zenkoku Nouson Kyouiku Kyoukai, Tokyo (in Japanese).

チャバネアオカメムシ幼虫の栄養状態を推定する形態指標

外山晶敏・三代浩二・井原史雄

独立行政法人　農業・食品産業技術総合研究機構
果樹研究所品種育成・病虫害研究領域
305-8605　茨城県つくば市

摘　　要

チャバネアオカメムシ幼虫について，野外で採集した多数個体の栄養状態を，形態形質から簡便に推定する手法を確立するため，まず栄養状態が著しく異なる4齢幼虫を用いて，栄養状態によりサイズが変化する形態形質と，脱皮以降ほとんど変化しない形態形質について検討した．結果，採餌量に応じて変化する形質として体長（BL）と体幅（BW），変化しない形質として眼間長（EYES）が選抜され，EYESに対するBLとBWの積（BODY）の相対的サイズにより，採餌量，さらに個体の栄養状態の推定が可能と考えられた．そこで，2～5齢の各齢について，これらの形質を5つの異なる栄養状態にあるグループで測定し，状態ごとに形質間の関係を直線回帰により求めた．野外採集虫を，これら回帰直線とともにBODY–EYESグラフに図示することにより，サイズの分布パターンや回帰直線との比較から，栄養状態を集団レベルで評価する方法を提示した．

Appendix 1. Linear regression equations of BODY versus EYES for *Plautia stali* nymphs given various feeding treatments.

Instar	Status of nymphs	*n*	Linear regression line[a]	*p* (*F*-test)	R^2
2nd	Four-day-fed[b]	13	BODY = − 9.86 + 15.50 * EYES	0.0012	0.629
	Two-day-fed[c]	15	BODY = − 4.02 + 7.98 * EYES	0.0457	0.273
	Newly molted[d]	15	BODY = − 1.61 + 4.83 * EYES	0.0326	0.305
	Five-day-starved[e]	13	BODY = − 2.45 + 5.61 * EYES	$<$0.0001	0.793
	Starved to death[f]	15	BODY = − 4.46 + 7.74 * EYES	$<$0.0001	0.822
3rd	Four-day-fed	30	BODY = − 3.39 + 10.23 * EYES	$<$0.0001	0.577
	Two-day-fed	15	BODY = − 8.16 + 12.96 * EYES	0.1010	0.193
	Newly molted	15	BODY = − 4.65 + 9.25 * EYES	0.0040	0.484
	Five-day-starved	14	BODY = − 9.86 + 12.69 * EYES	0.0052	0.491
	Starved to death	13	BODY = − 8.96 + 12.07 * EYES	$<$0.0001	0.890
4th	Four-day-fed	28	BODY = − 25.93 + 26.98 * EYES	$<$0.0001	0.744
	Two-day-fed	15	BODY = − 57.63 + 42.06 * EYES	0.0015	0.551
	Newly molted	15	BODY = − 26.63 + 22.89 * EYES	$<$0.0001	0.699
	Five-day-starved	15	BODY = − 13.51 + 16.20 * EYES	0.0010	0.579
	Starved to death	13	BODY = − 21.25 + 20.17 * EYES	0.0005	0.681
5th	Five-day-fed[g]	30	BODY = − 28.31 + 32.30 * EYES	$<$0.0001	0.500
	Two-day-fed	15	BODY = − 106.85 + 59.69 * EYES	$<$0.0001	0.800
	Newly molted	15	BODY = − 59.13 + 38.32 * EYES	$<$0.0001	0.692
	Five-day-starved	15	BODY = − 73.14 + 45.27 * EYES	0.0002	0.671
	Starved to death	15	BODY = − 16.93 + 22.11 * EYES	0.0007	0.601

[a] See the text for details
[b] Nymphs were fed for the first 4 days after molting and then killed
[c] Nymphs were fed for the first 2 days after molting and then killed
[d] Nymphs were killed within 24 h after molting
[e] Nymphs were deprived of food for the first 5 days after molting and then killed
[f] Nymphs were deprived of food after molting until their deaths
[g] Nymphs were fed for the first 5 days after molting and then killed

Appendix 2. Morphological character measurements (mean ± SD) of *Plautia stali* nymphs given various feeding treatments in each instar.

Instar	Character[a]	More-fed[b]	Two-day-fed[c]	Newly molted[d]	Five-day-starved[e]	Starved to death[f]
2nd		n = 13	n = 15	n = 15	n = 13	n = 15
	EYES	0.97 ± 0.02	0.96 ± 0.03	0.96 ± 0.03	0.98 ± 0.04	0.97 ± 0.04
	BL	2.71 ± 0.10	2.24 ± 0.21	1.99 ± 0.12	1.96 ± 0.08	2.01 ± 0.14
	BW	1.92 ± 0.07	1.64 ± 0.09	1.52 ± 0.07	1.57 ± 0.08	1.53 ± 0.08
	BODY	5.20 ± 0.34	3.67 ± 0.50	3.04 ± 0.28	3.08 ± 0.24	3.08 ± 0.34
3rd		n = 30	n = 15	n = 15	n = 14	n = 13
	EYES	1.36 ± 0.06	1.36 ± 0.03	1.41 ± 0.05	1.40 ± 0.04	1.36 ± 0.07
	BL	3.75 ± 0.19	3.49 ± 0.19	3.18 ± 0.19	3.14 ± 0.25	3.01 ± 0.26
	BW	2.80 ± 0.10	2.72 ± 0.14	2.62 ± 0.07	2.53 ± 0.09	2.45 ± 0.14
	BODY	10.49 ± 0.81	9.49 ± 0.92	8.36 ± 0.62	7.94 ± 0.74	7.40 ± 0.96
4th		n = 28	n = 15	n = 15	n = 15	n = 13
	EYES	1.78 ± 0.07	1.86 ± 0.05	1.92 ± 0.07	1.89 ± 0.07	1.84 ± 0.08
	BL	5.45 ± 0.37	5.20 ± 0.43	4.53 ± 0.29	4.64 ± 0.32	4.55 ± 0.42
	BW	4.09 ± 0.20	3.92 ± 0.27	3.82 ± 0.20	3.70 ± 0.19	3.49 ± 0.18
	BODY	22.32 ± 2.29	20.48 ± 2.94	17.34 ± 1.85	17.16 ± 1.50	15.94 ± 2.02
5th		n = 30	n = 15	n = 15	n = 15	n = 15
	EYES	2.40 ± 0.07	2.56 ± 0.08	2.45 ± 0.07	2.44 ± 0.09	2.46 ± 0.07
	BL	8.70 ± 0.33	8.16 ± 0.53	6.59 ± 0.36	6.95 ± 0.67	6.69 ± 0.37
	BW	5.70 ± 0.21	5.62 ± 0.40	5.30 ± 0.21	5.33 ± 0.29	5.59 ± 0.13
	BODY	49.21 ± 3.12	45.94 ± 5.48	34.93 ± 3.02	37.26 ± 4.75	37.35 ± 2.07

[a] See the text for details
[b] Nymphs were fed for the first 4 (2nd-4th instar) or 5 (5th instar) days after molting and then killed
[c] Nymphs were fed for the first 2 days after molting and then killed
[d] Nymphs were killed within 24 h after molting
[e] Nymphs were deprived of food for the first 5 days after molting and then killed
[f] Nymphs were deprived of food after molting until their deaths