

## Research Article

# Effect of Larval Density on Development of the Coconut Hispine Beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae)

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The coconut hispine beetle, *Brontispa longissima* shows, aggregation in the field. To elucidate the effect of aggregation on larval developmental aspects, we examined the effects of larval density on various aspects of larval development and on survival rates. Recently we found that *B. longissima* was divided into two monophyletic clades by genetic analysis. Therefore, we also compared the results between two populations, from Ishigaki, Japan (ISH) and Papua New Guinea (PNG), which were representative of the two monophyletic clades of *B. longissima*. In both ISH and PNG, the larval developmental period was shorter and the survival rate higher with rearing under high-density conditions than under isolated conditions. Similarly, fewer instars were required before pupation under high-density conditions than under isolated conditions. *Brontispa longissima* therefore developed better under high-density conditions, and the trends in the density effect were similar between two monophyletic clades.

## 1. Introduction

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is one of the most serious insect pests of *Cocos nucifera* (L.) and other palms [1, 2]. This beetle is considered to be native to Papua New Guinea and Indonesia [3], but since the 1930s it has gradually invaded Australia and the Pacific Islands including Vanuatu and Samoa [3, 4]. Since heavy infestations of *B. longissima* were first found in the Mekong Delta of Vietnam in 2003, it has been spreading rapidly and widely throughout Southeast Asian countries such as the Maldives, Thailand, Cambodia, and the Philippines [1, 5, 6]. In these countries, the coconut palms are very important for food, fuel, and industrial materials, and as part of landscape at tourist destinations [7, 8]. It is therefore important to establish a method of protecting coconut palms in Southeast Asian countries from the damage caused by *B. longissima*. However, there is insufficient information on the fundamental ecology of this species.

For these reasons, we have been studying the beetle's ecological properties. Phylogenetic analysis based on the mtDNA sequences has revealed two monophyletic clades in *B. longissima* [9]. One group is referred to as the Pacific clade and is distributed in Australia, Papua New Guinea, Samoa, and Sumba Island in Indonesia. The other is the Asian clade and is distributed over a wide area of Asia and the Pacific region. Morphological traits do not differ between the two clades [9], but little has known whether there are any differences in their ecological properties.

In the field, *B. longissima* aggregates within the folded leaflets of the coconut palm [6, 10]. We therefore assumed that development at high density is promoted within the populations of this insect. Here, to clarify developmental differences in *B. longissima* during the immature developmental stages under different density conditions, we investigated the effects of changes in larval density on the length of the insect's developmental period and on the number of instars required before pupation, the survival rate, and body size.

Furthermore, we examined whether the effects of density differed between the two monophyletic clades.

## 2. Materials and Methods

**2.1. Insects.** *Brontispa longissima* populations were collected from Ishigaki Island, Okinawa Prefecture, Japan, and from East New Britain Province, Papua New Guinea. The populations from Ishigaki (ISH) and Papua New Guinea (PNG) have been, respectively, categorized according to genetic analysis into the Asian group and the Pacific group [9]. The two populations under the present experiment were same as the populations used in Takano et al. [9]. Larvae and adults were provided with fresh leaves of *C. nucifera*. The pieces of leaves (15 cm length) were bundled with elastic bands, because this beetle prefers to hide between the leaves. Until pupation, larvae were maintained with a bundle of leaves in a plastic container (15.5 cm long, 11.5 cm wide, 5.0 cm high) covered with a screened ventilated lid. Pupae were placed in a Petri dish and the adults were reared in a plastic container in the same way as the larvae. Rearing was conducted at  $25 \pm 1^\circ\text{C}$  under a 12:12-h (L/D) photoperiod and  $65\% \pm 5\%$  relative humidity.

**2.2. Experiments.** ISH hatchlings less than 24 h after emergence were transferred into a Petri dish (5.5 cm diameter, 1.5 cm high) containing a bundle of fresh-cut leaves of *C. nucifera*. Some Petri dishes contained 1 individual (isolated conditions) and some contained 10 (crowded conditions). We considered a density of 10 individuals per dish to be crowded, but not overcrowded, because a preliminary experiment had shown that at this density there was still enough food for larvae of final instar which is the lifecycle stage at which the greatest amounts of food are ingested. The treatment for isolated condition was replicated 30 times (i.e., 30 individuals) and for crowded condition 10 times (i.e., 100 individuals). We transferred the larvae to a new Petri dish containing fresh leaves every day, and we checked the lengths of developmental periods of the larvae and the pupae. Survival rates and the occurrence of molting were recorded every day until adult emergence. Molting was checked by counting exuviae and measuring the head width of larvae under a stereomicroscope. Emerged adults were sexed and the length from the head to the tip of abdomen was measured under the stereomicroscope. The same procedures were followed with *B. longissima* PNG as with ISH. Experiments were conducted at  $28 \pm 1^\circ\text{C}$  under a 12:12-h (L/D) photoperiod and  $65\% \pm 5\%$  relative humidity.

**2.3. Statistical Analyses.** Differences in survival rate were statistically compared by using Fisher's exact probability test. The chi-squared test was used to examine differences between the two rearing densities in terms of the proportions of instars each number immediately before pupation. These analyses were conducted with version 2.11.0 of R software [11]. A *t*-test was used to compare the differences in length of the developmental period and body size. These analyses were conducted with version 5.1 of JMP software (SAS Institute Inc. Cary, NC, USA). Developmental period length and body

size were compared between isolated and crowded conditions within the same monophyletic group and between ISH and PNG under each density condition.

## 3. Results

In both populations the survival rate tended to decrease with developmental stage, and from the third instar larval stage onward it was higher under crowded conditions than under isolated conditions (Figure 1). The adult emergence rate under crowded conditions was 86% in both populations. In contrast, under isolated conditions the adult emergence rate was 70.0% in ISH and 53.3% in PNG (Figure 1).

We examined the lengths of the developmental periods in ISH and PNG populations at the two different densities (Table 1). The larval development period was significantly shorter under crowded conditions than under isolated conditions in both ISH and PNG. The length of the pupal development period did not differ significantly between the two density conditions in either population.

We then investigated the variations in the larval instar number at which pupation occurred (Figure 2). ISH larvae pupated at the fourth or fifth instar. In contrast, PNG larvae pupated at the fourth to sixth instars. The proportion of each of the instar numbers at which pupation occurred differed significantly between the two density conditions in each clade (chi-squared test, ISH:  $\chi^2 = 16.961$ ,  $P < 0.001$ ; PNG:  $\chi^2 = 9.122$ ,  $P = 0.010$ ). At pupation, the proportion of fourth instars under crowded conditions was greater than that under isolated conditions in both ISH and PNG.

The heads of last-instar larvae were significantly wider under isolated conditions than under crowded conditions (Table 2). In both ISH and PNG, body length in adult males was significantly greater under isolated conditions than under crowded conditions, whereas female body length did not differ significantly.

Within each density condition, the larval development period was significantly shorter in ISH than in PNG (Table 1). Body length in adult males under isolated conditions was significantly greater in PNG than in ISH (Table 2). The other data did not differ significantly between the two clades (Tables 1 and 2).

## 4. Discussion

Our results revealed that larval density influenced various ecological aspects in *B. longissima* and that the overall trends in the density effect were similar between the two populations, ISH and PNG.

Survival rates were higher under crowded conditions than isolated conditions. This trend has been observed in many other species of insects, including the leaf beetle, *Chrysolina aurichalcea* Mannerheim (Coleoptera: Chrysomelidae), and the pleasing fungus beetle *Dacne picta* Crotch (Coleoptera: Erotylidae) [12, 13]. Our results support the findings of these studies. Utida [14] described that low density causes stress in individuals, and in our study, the survival rates when only 1 individual was present were low.

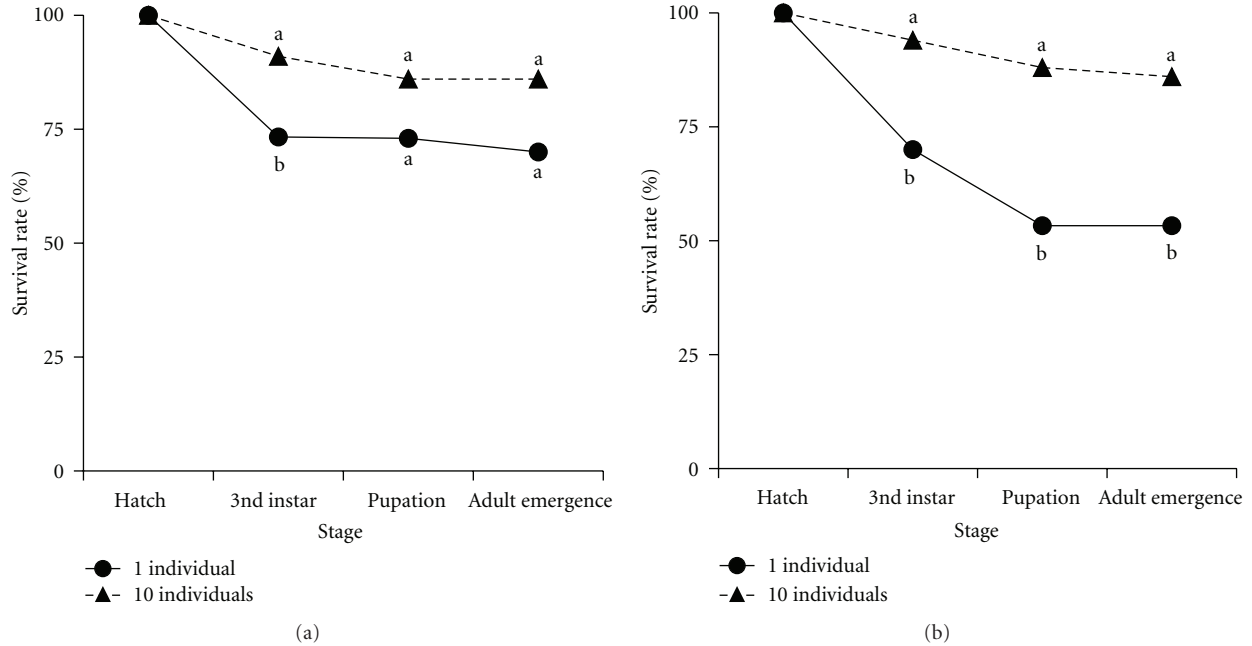


FIGURE 1: Survival rates from hatching to adult emergence in *B. longissima* reared under isolated conditions (1 individual) or crowded conditions (10 individuals). (a) ISH (individuals obtained from a stock culture initiated from insects collected on Ishigaki Island). (b) PNG (obtained from a stock culture of insects collected in Papua New Guinea). Values with the same letters do not differ significantly (Fisher's exact probably test,  $P > 0.05$ ).

TABLE 1: Developmental periods (mean  $\pm$  SE, days) of *B. longissima* reared under the density of one individual or 10 individuals.

Developmental stage	Density		Population		<i>t</i> -value	<i>P</i>	
			ISH	PNG			
Larva	1 individual	22	24.8 ± 0.7	16	28.1 ± 0.8	−2.85	0.0073
	10 individuals	86	20.5 ± 0.3	88	25.8 ± 0.8	−13.88	<0.0001
	<i>t</i> -value		−6.83		−3.07		
	<i>P</i>		<0.0001		0.0027		
Pupa	1 individual	21	5.1 ± 0.1	16	4.9 ± 0.1	1.42	0.1647
	10 individuals	86	4.9 ± 0.1	86	4.8 ± 0.1	1.20	0.2300
	<i>t</i> -value		−1.73		−0.75		
	<i>P</i>		0.0873		0.4563		

One reason is might therefore be that extremely low density is a stressor for *B. longissima* larvae.

The length of the larval development period was clearly influenced by the density conditions, but larval density had no effect on the length of pupation in *B. longissima*. This effect of crowding on larval development is found in some other species of insects such as *Diploptera punctata* (Eschscholtz) (Blattodea: Blaberidae), and *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) [15, 16]. Applebaum and Heifetz [17] described that food consumption, metabolic rate, and general activity are enhanced by crowded conditions in the insects that show density-dependent responses. We did not examine the quantities of leaves ingested in each dish. Possibly, larvae under crowded conditions might grow more quickly than isolated larvae because of competitive need to feed more actively. Moreover, during

the experiments, we found that several larvae overlapped with, or touched, each other, even though there was enough space for them to eat on the leaves while apart. In *B. longissima*, this body-touching behavior and/or the presence of feces from other individuals might shorten the larval development period under crowded conditions. However, we cannot make this inference from our current data.

In some species of insects, body size diminishes with increasing population density [18]. Besides, Goulson and Cory [19] explained that this phenomenon occurs unrelated to a direct shortage of food. Also, here, we provided enough leaves for larvae every day, but we observed an inverse relationship between larval density and body length of adult males. By comparison, female body length was not influenced by density. These findings suggest that males of *B. longissima*, but not females, are susceptible to density effects.

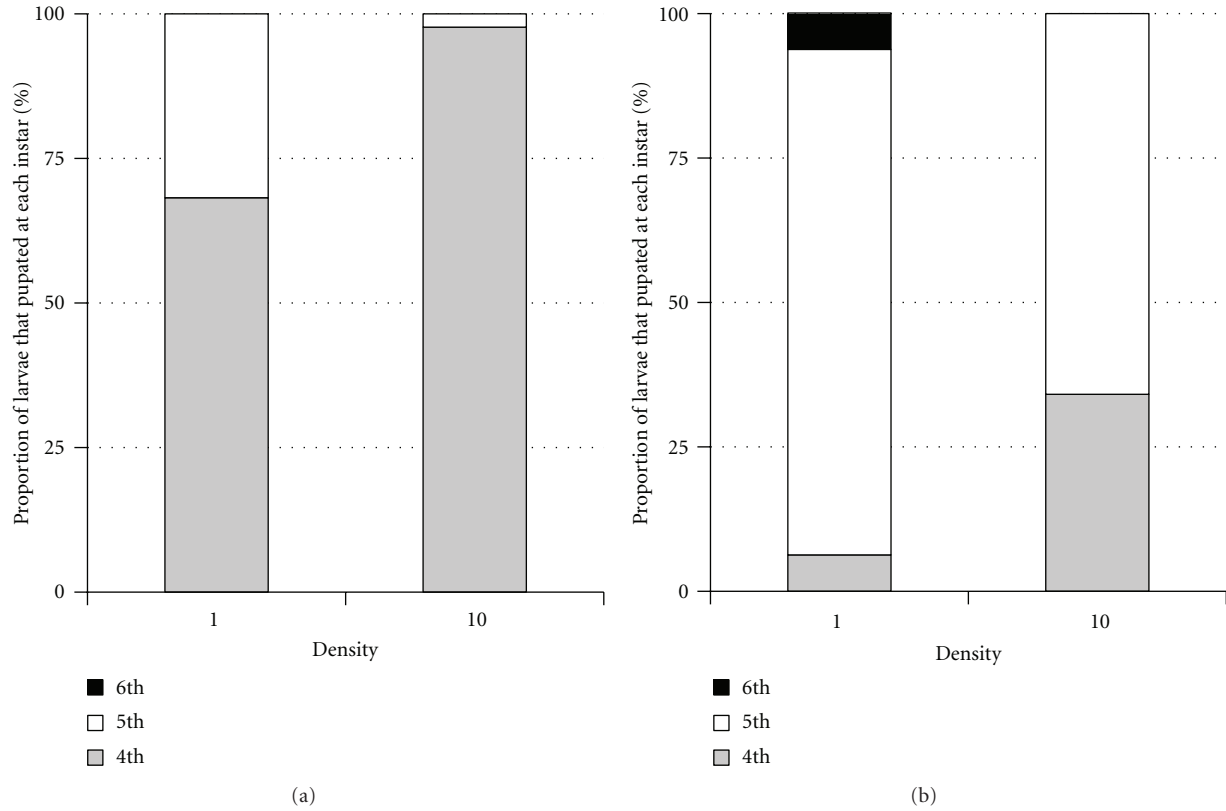


FIGURE 2: Variations in the proportions of pre-pupation larval instars (fourth, fifth, and sixth) in *B. longissima* reared under isolated conditions (density, 1 individual per Petri dish) or crowded conditions (10 individuals) (a) ISH, (b) PNG.

TABLE 2: Head width of last-instar larvae and body length of adults (mean  $\pm$  SE, mm) of *B. longissima* reared under the density of one individual or 10 individuals.

Site	Density		Population		PNG	<i>t</i> -value	<i>P</i>
			ISH				
Head width of last-instar larvae	1 individual	22	1.24 ± 0.02	16	1.25 ± 0.01	−0.51	0.6098
	10 individuals	86	1.19 ± 0.01	88	1.20 ± 0.01	−0.96	0.3375
	<i>t</i> -value		−3.08		−2.67		
	<i>P</i>		0.0027		0.0089		
Male adult body length	1 individual	11	8.6 ± 0.1	8	9.1 ± 0.2	−2.47	0.0246
	10 individuals	44	8.3 ± 0.0	45	8.3 ± 0.0	0.33	0.7423
	<i>t</i> -value		−2.02		−6.44		
	<i>P</i>		0.0487		<0.0001		
Female adult length	1 individual	10	9.2 ± 0.1	8	9.3 ± 0.1	−0.70	0.4940
	10 individuals	42	9.3 ± 0.0	41	9.3 ± 0.0	−0.10	0.9205
	<i>t</i> -value		1.50		0.30		
	<i>P</i>		0.1391		0.7628		

Regarding head width, it seems that the wider heads under isolated conditions were due to the size of male larvae.

The last-instar larvae of *B. longissima* included more 5th or 6th instars under isolated conditions than under crowded conditions. Generally, insects initiate to pupation when the larvae have reached a critical body size [20, 21]. Some species of insects increase the number of prepupation

instars as a form of compensatory growth when the larvae fail to reach their threshold size for metamorphosis under adverse conditions [22]. In our experiments, the head width of the penultimate instar under isolated conditions was smaller than that of the final instar under crowded conditions (data not shown). We therefore consider that *B. longissima* larvae under isolated conditions needed to undergo more

larval molts to reach the threshold size for pupation. The number of pre-pupation instars in *B. longissima* has been reported as five to six by Waterhouse and Norris [3] and four to six by Yamauchi [23]. In many species of insects, the number of pre-pupation instars is affected by rearing conditions, including food quality, density, temperature, and humidity [22]. Therefore, any differences between our results and these past reports are likely associated with differences in environmental factors, including temperature and food. Furthermore, we observed the differences in proportions of last instars (fourth, fifth, and sixth) between ISH and PNG. We therefore think that the number of pre-pupation instars varies among not only density conditions but also monophyletic clades in *B. longissima*.

Our findings suggest that *B. longissima* reared under high-density conditions has a survival advantage, and therefore high-density conditions allowed *B. longissima* to increase the number of its generations in the field. In other words, once *B. longissima* invades into the coconut palm field, it might be able to increase acceleratingly as the population increases and spread in the field. The density-dependent phenomena have the potential to influence fecundity or dispersion during the adult stage. In future, we need to investigate the effect of population density on ecological aspects in *B. longissima* adults. Our findings will help to elucidate the factors involved in the rapid spread of *B. longissima* and its damage, in the coconut palm field in Southeast Asian countries.

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## References

- [1] S. Nakamura, K. Konishi, and K. Takasu, "Invasion of the coconut hispine beetle, *Brontispa longissima*: current situation and control measures in Southeast Asia," in *Proceedings of the International Workshop on Development of Database (APASD) for Biological Invasion*, T. Y. Ku and M. Y. Chiang, Eds., vol. 3, pp. 1–9, Taiwan Agricultural Chemicals and Toxic Substance Research Institute, Taichung, Taiwan, Food and Fertilizer Technology Center (FFTC) for the Asia and Pacific Region, Taipei, Taiwan, 2006.
- [2] P. Rethinam and S. P. Singh, "Current status of the coconut beetle outbreaks in the Asia-Pacific region," in *Developing an Asia-Pacific Strategy for Forest Invasive Species: the Coconut Beetle Problem—Bridging Agriculture and Forestry*, S. Appanah, H. C. S. Sim, and K. V. Sankaran, Eds., pp. 1–23, Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok 2007, RAP publication, Bangkok, Thailand, 2007.
- [3] D. F. Waterhouse and K. R. Norris, "*Brontispa longissima* (Gestro)," in *Biological Control Pacific Prospects*, pp. 134–141, ACIAR. Inkata Press, Melbourne, Australia, 1987.
- [4] J. M. Voegelé, "Biological control of *Brontispa longissima* in Western Samoa: an ecological and economic evaluation," *Agriculture, Ecosystems and Environment*, vol. 27, no. 1–4, pp. 315–329, 1989.
- [5] W. Liebrechts and K. Chapman, "Impact and control of the coconut hispine beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae)," in *Report of the Expert Consultation on Coconut Beetle Outbreak in APPPC Member Countries*, FAO, Ed., pp. 19–34, Regional Office for Asia and the Pacific, FAO, Bangkok, Thailand, 2004.
- [6] K. Takasu, S. Takano, K. Konishi, and S. Nakamura, "An invasive pest *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) attacks an endemic palm in the Yaeyama Islands, Japan," *Applied Entomology and Zoology*, vol. 45, no. 1, pp. 137–144, 2010.
- [7] L. Guarino, "The coconut in the Pacific: the role of the secretariat of the pacific community, coconut revival: new possibilities for the "tree of life"," in *Proceedings of the International Coconut Forum Held in Cairns, Australia (ACIAR '06)*, S. W. Adkins, M. Foale, and Y. M. S. Samosir, Eds., no. 125, pp. 28–30, 2006.
- [8] P. Naka, "Appropriate processing technologies for value addition in coconut," *Indian Coconut Journal*, vol. 37, no. 3, pp. 11–20, 2006.
- [9] S. Takano, A. Mochizuki, K. Konishi, and K. Takasu, "Two cryptic species in *Brontispa longissima* (Coleoptera: Chrysomelidae): evidence from mitochondrial DNA analysis and crosses between the two nominal species," *Annals of the Entomological Society of America*, vol. 104, no. 2, pp. 121–131, 2011.
- [10] E. S. Brown and A. H. Green, "The control by insecticides of *Brontispa longissima* (Gestro) (Coleopt., Chrysomelidae-Hipinae) on young coconut palms in the British Solomon Islands," *Bulletin of Entomological Research*, vol. 49, no. 2, pp. 239–272, 1958.
- [11] R Development Core Team, "R: a language and environment for statistical computing," R Foundation for Statistical Computing, Vienna, Austria, 2010, <http://www.R-project.org/>.
- [12] S. Fujiwara and K. Miyachi, "The effect of larval density on survival rate and development of *Chrysolina aurichalcea* Mannerheim (Coleoptera: Chrysomelidae)," *Bulletin of Environmental Conservation*, vol. 7, pp. 61–65, 1985.
- [13] T. Sato, N. Shinkaji, and H. Amano, "Effects of larval density on larval survivorship and imaginal fecundity of *Dacne picta* (Coleoptera: Eurytomidae)," *Applied Entomology and Zoology*, vol. 39, no. 4, pp. 591–596, 2004.
- [14] S. Utida, *The Theory of Animal Population*, NHK, Tokyo, Japan, 1972, (in Japanese).
- [15] S. Iwao, "Phase variation in the armyworm, *Leucania unipuncta* Haworth II. "Effect of population density on the larval growth pattern"," *Japanese Journal of Applied Entomology and Zoology*, vol. 2, no. 4, pp. 237–243, 1958 (Japanese), (with English summary).
- [16] A. P. Woodhead and C. R. Paulson, "Larval development of *Diploptera punctata* reared alone and in groups," *Journal of Insect Physiology*, vol. 29, no. 9, pp. 665–668, 1983.
- [17] S. W. Applebaum and Y. Heifetz, "Density-dependent physiological phase in insects," *Annual Review of Entomology*, vol. 44, pp. 317–341, 1999.
- [18] T. M. Peters and P. Barbosa, "Influence of population density on size, fecundity and developmental rate of insects in culture," *Annual Review of Entomology*, vol. 22, pp. 431–450, 1977.
- [19] D. Goulson and J. S. Cory, "Responses of *Mamestra brassicae* (Lepidoptera: Noctuidae) to crowding: interactions with disease resistance, colour phase and growth," *Oecologia*, vol. 104, no. 4, pp. 416–423, 1995.

- [20] G. Davidowitz, L. J. D'Amico, and H. F. Nijhout, "Critical weight in the development of insect body size," *Evolution and Development*, vol. 5, no. 2, pp. 188–197, 2003.
- [21] H. F. Nijhout, "A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L.)," *Biological Bulletin*, vol. 149, no. 1, pp. 214–225, 1975.
- [22] T. Esperk, T. Tammaru, and S. Nylin, "Intraspecific variability in number of larval instars in insects," *Journal of Economic Entomology*, vol. 100, no. 3, pp. 627–645, 2007.
- [23] S. Yamauchi, "Some biological notes of the *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae)," *Okinawa Nogyo*, vol. 20, no. 1-2, pp. 49–53, 1985 (Japanese).



