IJIE Int. J. Indust. Entomol. 37(2) 100-104 (2018)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2018.37.2.100

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)
[Note]

Influence of starvation on the larval development of the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae)

Kwanho Park[1], Heui-Sam Lee[1], and Tae-won Goo[2,*]

[1]*Industrial Insect Division, National Institute of Agricultural Sciences, Rural Development Association, 166 Nongsaengmyeong-ro, Wanju, Jeollabuk-do 55365, Republic of Korea*
[2]*Department of Anatomy, Graduate School of Dongguk University College of Medicine, 123, Dongdae-ro, Gyeongju-si, 38066, Republic of Korea*

Abstract

The rearing of black soldier flies in Korea is affected by seasonal factors such as temperature and sun light; for this reason, it requires a great deal of effort to rear and maintain black soldier flies during the winter. In this study, we investigated how starvation affects larval development. After hatching the larvae, they were fed for 5 or 10 d and then starved for a certain period to see how they recovered. The length and width of larvae were estimated to be 18.18 to 21.96 mm, 5.19 to 6.04 mm, respectively. Larvae survivorship to the pupal stage was significantly different between groups and periods of starvation. The groups of fed for 5 d then starved showed a high survival rate until 20 d starvation and then the survival of larvae rapidly decreased. The survival rate of the larvae was abruptly decreased for 20 d starvation in the groups of fed for 10 d and starved, and then gradually decreased until 60 d starvation thereafter. Our research attempted to influence larvae development through starvation and provides basic information on how to culture the black soldier fly effectively and economically throughout the year.

Int. J. Indust. Entomol. 37(2), 100-104 (2018)

Received : 26 Nov. 2018
Revised : 9 Dec. 2018
Accepted : 13 Dec. 2018

Keywords:
Black soldier fly,
Hermitia illucens,
larva,
starvation

Introduction

The black soldier fly, *Hermetia illucens* (L.), is distributed throughout the tropics and warm temperate regions (James, 1935; McCallan, 1974). The insect is mainly found in the vicinity of and in cattle sheds, manure sheds, living waste dump grounds, and food waste dump grounds (Kim, 1997).

Black soldier flies are increasingly attracting worldwide attention as an effective means for food waste reduction and converting waste to organic materials, such as alternative feeds and valuable fertilizer (Park *et al*., 2017). The black soldier fly is economically important in animal feed (Park *et al*., 2017). Soldier fly prepupae can be used as feed for a variety of animals, including fish (Bondari and Sheppard, 1981) and swine (Newton *et al*., 1977). Prepupae, when dried, have an estimated value comparable to menhaden fish meal. If used live, as specialty feed, or marketed to exploit its other unique qualities (i.e., essential fatty acids and chitin), the value of the product may be higher (Sheppard *et al*., 1994). In nature, black soldier flies colonize in warm temperate regions and are active in Korea from May through October (Park *et al*., 2017). For this reason, it is difficult to breed and maintain black soldier flies in winter. In addition,

***Corresponding author.**
Tae-won Goo
Department of Anatomy, Graduate School of Dongguk University College of Medicine, 123, Dongdae-ro, Gyeongju-si, 38066, Republic of Korea
Tel: +82-54-703-7801 / FAX: +82-54-770-2447
E-mail: gootw@dongguk.ac.kr

due to the mating rituals of black soldier fly adults, more effort is needed to aid in laying eggs in winter and the rainy season, where sunlight conditions are not appropriate for maintaining larvae. Although it is a well-studied insect with commonly known rearing techniques (Sheppard *et al*., 2002), data on how seasonal factors affect the black-soldier-fly farming is limited (Park *et al*., 2010). In areas where seasonal factors play a role in the breeding of black soldier fly, such as Korea, artificially controlling the period of larvae could help economically produce flies.

To produce black soldier fly economically throughout the year in Korea, we must control the larval period. In present study, we examined relationships between body composition and survival time of black soldier fly larvae after temporary starvation.

Materials and Methods

Source of Flies and maintenance

Black soldier flies were obtained from a colony maintained year-round in an outdoor glasshouse at the National Institute of Agricultural Sciences, RDA, Wanju. Eggs from the colony were collected in egg traps made of floral foam square bars. Eggs were held in a laboratory and hatched in food waste at 26°C and 60% R.H.

Measurement of starvation resistance

Eggs and larvae were incubated at 26°C with a 16 h light-8 h dark cycle. Upon hatching in the food waste, groups were fed for 5 d or 10 d and then starved for a certain period. In each group, 1000 larvae underwent increased starvation days in 10 d intervals. After starving the larvae for each period, they were fed to measure the extent to which they recovered and were kept until they became prepupae. Prepupae were analyzed for nutritional composition. During starvation, only fresh water was supplied by spraying. The length, width, emergence rate and survival rate of starved larvae was investigated during each period. The remaining 30 prepupae were randomly selected and the length and width were measured. Individuals were weighed 24 h after food removal to ensure that most gut contents had been voided. To determine pupation of black soldier fly, 100 hatched larvae were reared in a plastic box (W×D×H = 60 × 40 × 15 cm) covered with net (200 mesh) and the Kim (1997) colony maintenance method was used. The experiment was replicated three times.

Statistical analysis

The mean and standard deviation (SD) of each experiment were compared to control. Values are means ± SD and were analyzed with a confidence interval of 95% by one-way ANOVA and Tukey's multiple comparison test.

Results and Discussion

Starvation resistance and larval development

Studies of black soldier flies have been carried out in tropical and warm-season temperate areas (Park *et al*., 2010). In Korea, the black soldier fly develops from late May to October (Kim *et al*., 2008). The black soldier fly has been the subject of multiple ecological studies in several fields, including exploration of its roles in animal feed and in decomposing organic matter (Park *et al*., 2010). In this study, we examined the differences in larval development under the starvation condition. It was necessary to control the larval period in order to raise flies that were influenced by seasonal factors, such as temperature and light condition. As such knowledge on many important aspects related to mass production technologies of black soldier fly remain poorly studied (Chia *et al*., 2018), especially starvation for larval development. Although information on development time, egg eclosion and adult emergence of black soldier fly is reported for temperatures (Harnden *et al*., 2016), no information is available on starvation-driven effects on the larval life history. We have identified the phenomenon that starved larvae stop growing. To investigate the effect of starvation on larval period, the larval weight, length, width, and survivor rates were observed for 60 d with different starvation periods. Numerous studies have implicated energy storage starvation resistance in *Drosophila* (Service, 1987; Blows and Hoffmann, 1993; Oudman *et al*., 1994; van Herrewege and David, 1997; Djawdan *et al*., 1998), but previously there was no study on the developmental status of black soldier flies. The larval length, width, weight, and emergence rate between the groups of fed for 5 d and starved or fed for 10 d and starved were measured. There were slight

Table 1. Larvae length and width of both groups. At intervals of 10-d starvation, larvae were fed until they become prepupae. Larval length and width were measured in each group. Values are means ± SD and analyzed with a confidence interval of 95% by one-way ANOVA and Tukey's multiple comparison test.

Period of starvation (day)	5-d feeding		10-d feeding	
	Larval length (mm)	Larval width (mm)	Larval length (mm)	Larval width (mm)
0	18.18±1.07 a	5.19±0.40 a	21.96±1.07 b	5.99±0.41 a
10	20.09±1.21 b	5.47±0.37 b	20.63±1.05 a	5.79±0.30 a
20	19.41±0.79 b	5.63±0.28 bc	20.55±1.10 a	5.74±0.37 a
30	19.65±0.94 b	5.66±0.40 bc	20.57±0.73 a	5.81±0.33 a
40	19.88±0.69 b	5.73±0.28 c	20.63±1.36 a	5.71±0.74 a
50	19.93±1.04 b	5.85±0.24 c	20.39±0.70 a	6.04±0.60 a
60	-	-	20.83±0.99 a	5.61±0.34 a

-, Data not collected in the study.

Table 2. Larvae weight of both groups. At intervals of 10-d starvation, larvae were fed until they became prepupae. One hundred larvae were weighed before they became prepupae in each group. Values are means ± SD and analyzed with a confidence interval of 95% by one-way ANOVA and Tukey's multiple comparison test.

Period of starvation (day)	Body weight (g/100 larvae)	
	5–d feeding	10-d feeding
0	18.75±0.08 a	19.94±0.28 d
10	18.35±0.33 c	18.99±0.08 c
20	17.31±0.42 b	18.91±0.16 a
30	18.50±0.40 b	19.56±0.39 cd
40	17.72±0.20 c	17.92±0.36 b
50	17.82±0.73 d	17.21±0.25 b
60	-	19.38±1.02 c

-, Data not collected in the study.

Table 3. Emergence rate of both groups. Values are means ± SD and analyzed with a confidence interval of 95% by one-way ANOVA and Tukey's multiple comparison test.

Period of starvation (day)	Emergence rate (%)	
	5-d feeding	10-d feeding
0	99.00±0.00 ab	97.00±2.00 a
10	99.00±0.58 b	96.67±0.58 a
20	98.00±1.00 ab	97.33±0.58 a
30	100.00±0.58 b	97.67±1.15 a
40	98.00±1.00 ab	98.00±0.00 a
50	97.00±0.58 a	97.00±1.73 a
60	-	97.33±2.08 a

-, Data not collected in the study.

differences between mean length, width, weight, and emergence rate compared with controls (Table. 1, 2, 3). The length and width of larvae were estimated to be 18.18 to 21.96 mm, 5.19 to 6.04 mm (5 d feeding length; ANOVA $F(5,174)=15.512$, $p=0.000$, 10 d feeding length; ANOVA $F(6,203)=8.063$, $p=0.000$; Table. 1), respectively. The weight of the final 100 larvae across the treatments ranged from 17.72 to 19.94 g (5 d feeding; ANOVA $F(5,12)=8.231$, $p=0.021$, 10 d feeding; ANOVA $F(6,14)=12.514$, $p=0.017$; Table 2). However, it is believed that there is no significant difference in larval development levels in black soldier fly rearing farms. The difference in larval development between groups is attributed to the change of food waste used as feed. The emergence rate for all groups was more than 96%, though there were no significant differences (5 d feeding; ANOVA $F(3,14)=3.721$, $p=0.037$, 10 d feeding; ANOVA $F(6,14)=2.051$, $p=0.135$; Table. 3). In this experiment, we did not test the extent of larval tolerance to starvation after hatching in eggs, or whether they recovered afterward.

Larvae survivorship to the pupal stage was significantly different between groups and periods of starvation (5 d feeding; ANOVA $F(6,14)=3717.963$, $p=1.37E-21$, 10 d feeding; ANOVA $F(6,14)=582.043$, $p=5.78E-16$; Fig. 1). The groups of fed for 5 d then starved showed a high survival rate until 20 d starvation and then the survival of larvae rapidly decreased. However, the survival rate of the larvae was abruptly decreased for 20 d starvation in the groups of fed for 10 d and starved, and then gradually decreased until 60 d starvation thereafter. These results seem to suggest that larvae accumulate energy in the

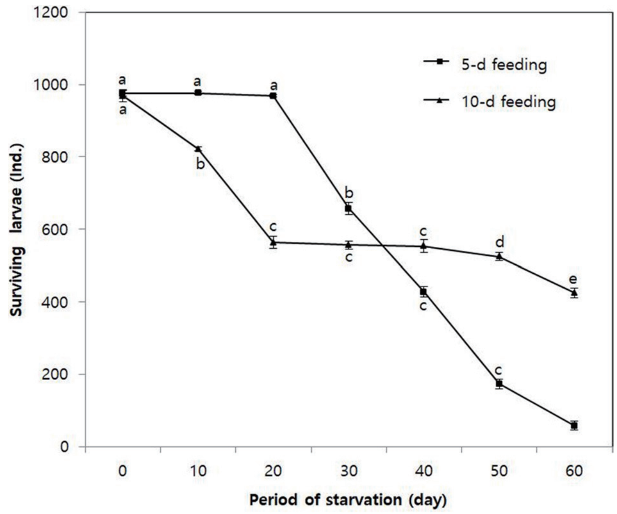

Fig. 1. Mortality of both groups. We counted the number of individuals that progressed to the pupa stage in each group. Values are means ± SD and analyzed with a confidence interval of 95% by one-way ANOVA and Tukey's multiple comparison test. Graphs with different letters are significantly different within groups.

body to the next stage and survive. Although death by starvation is undoubtedly a complex physiological process, depletion of reserves used in energy metabolism plays a major role (Brian, 1991). Energy metabolism during starvation stress has been examined in only a few species of insects (Marron *et al*., 2003). Locusts (*Locusta migratoria*) and fruit beetles (*Pachnoda sinuata*) metabolize glycogen stores during the initial stages of starvation, then switch to lipid and protein metabolism when carbohydrates are not present (Hill and Goldsworthy, 1970; Jutsum *et al*., 1975; Auerswald and Gade, 2000).

Before Tomberlin *et al*. (2002), researchers reported that the fat body of black soldier fly larva is mainly composed of trophocytes, which are rich in lipid droplets and protein granules. The low protein content in the diet affects both protein and lipid accumulation in these cells, leading to a lower abundance of protein granules and larger, more abundant, lipid droplets (Sheppard *et al*., 2002). Starvation resistance of gypsy moth larvae was significantly associated with body mass prior to starvation and diet nitrogen concentration, and initial body mass was positively correlated with starvation resistance for both diet nitrogen concentrations and aspen so that within any one diet treatment, large larvae survived longer (Brian, 1991). Starvation tolerance is also extremely variable among species and their energy metabolism. By keeping the larval period longer, the timing of the feeds used in the energy metabolism could be detected and compared among starving larvae reared on different diets. Studies on the nutritional composition of animal feed and egg productivity of starving larvae should be examined in future studies.

In our research, such information is necessary for developing larvae as animal feeds and as waste management agents.

Acknowledgements

We are grateful to Inim Kim and Seunghwan Jo for their valuable help in rearing the black soldier fly. This work was supported by a grant from RDA (PJ 0108432018).

References

Auerswald L, Gade G (2000) Metabolic changes in the African fruit beetle, *Pachnoda sinuata*, during starvation. J Insect Physiol 46, 343-351.

Blows MW, Hoffmann AA (1993) The genetics of central and marginal populations of Drosophila serrata. 1. Genetic variation for stress resistance and species borders. Evolution 47, 1255–1270.

Bondari K, Sheppard DC (1981) Soldier fly larvae as feed in commercial fish production. Aquaculture 24, 103-109.

Brian A (1991) Starvation resistance of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae): tradeoffs among growth, body size, and survival. Oecol 88, 422-429

Chia SY, Tnaga CM, Khamis FM, Mohamed SA, Salifu D, Sevgan S, *et al.* (2018) Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. PLoS One 13(11), e0206097

Djawdan M, Chippindale AK, Rose MR, Bradley TJ (1998) Metabolic reserves and evolved stress resistance in Drosophila melanogaster. Physiol Zool 71, 584-594.

Harnden LM, Tomberlin JK (2016). Effects of temperature and diet on black soldier fly, Hermetia illucens (L.)(Diptera: Stratiomyidae), development. Forensic Sci Int 266, 109-16

Hill L, Goldsworthy GJ (1970) The utilization of reserves during starvation of larvae of the migratory locust. Comp Biochem Physiol 36, 61-70.

James MT (1935) The genus Hermetia in the United States (Diptera: Stratiomyidae). Bull Brooklyn Entomol Soc 30, 165-170.

Jutsum AR, Agarwal HC, Goldsworthy GJ (1975) Starvation and haemolymph lipids in Locusta migratoria migratorioides (R. & F.). Acrida 4, 47-56.

Kim JG, Choi YC, Choi JY, Kim WT, Jeong GS, Park KH, *et al.* (2008) Ecology of the black soldier fly, *Hermetia illucens* (Diptera: Stratmyidae) in Korea. Kor J Appl Entomol 47, 337-343.

Kim JI (1997) Newly recording two exotic insects species from Korea. J Kor Biota 2, 223-225.

Marron MT, Markow TA, Kain KJ, Gibbs AG (2003) Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila.* J Insect Physiol 49, 261-270

McCallan E (1974) *Hermetia illucens* (L.) (Diptera: Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. Entomol Mo Mag 109, 232-234.

Newton GL, Booram CV, Barker RW, Hale OM (1977) Dried *Hermetia illucens* larvae meal as a supplement for swine. J Anim Sci 44, 395-400.

Oudman L, van Delden W, Kamping, A, Bijlsma R (1994) Starvation resistance in Drosophila melanogaster in relation to the polymorphisms at the adh and alpha-gpdh loci. J Insect Physiol 40, 709-713.

Park K, Kim W, Lee S, Choi Y, Nho S (2010) Seasonal pupation, adult emergence and mating of black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae) in artificial rearing system. Int J Indust Entomol 21, 189-191.

Park KH, Han MH, Lee S, Kim ES, Song MH, Kim WT, *et al.* (2017) Oviposition Activity of Black Soldier fly (*Hermetia illucens*) under Artificial Illumination. Int J Indust Entomol 35(2), 100-105.

Service PM (1987) Physiological mechanisms of increased stress resistance in Drosophila melanogaster selected for postponed senescence. Physiol Zool 60, 321-326.

Sheppard DC, Newton GL, Thompson SA (1994) A value added manure management system using the black soldier fly. Bio Resource Tech 50, 275-279.

Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM. (2002) Rearing methods for the black soldier fly (Diptera: Stratiomyidae). J Med Entomol 39, 695-698.

Tomberlin JK, Sheppard DC, Joyce JA (2002) Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. Ann Entomol Soc Am 95(3), 379-3386.

van Herrewege J, David JR (1997) Starvation and desiccation tolerances in Drosophila: comparison of species from different climatic origins. Eco Sci 4, 151-157.