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Oviposition Activity of Black Soldier fly (*Hermetia illucens*) under Artificial Illumination

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Abstract

Under natural conditions, black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), colonizes in warm temperate regions, and is active in Korea from May through October. Information on black soldier fly rearing, which is affected by seasonal factors in Korea, is limited. Oviposition by black soldier flies is dependent on light intensity and wavelength. Therefore, continuous mass rearing of this fly requires determination of optimal artificial conditions of illumination. In this study, we compared the number of eggs laid under an artificial light source (750 watt HPL lamp) versus nature sunlight. Our results showed that compared to oviposition under natural sunlight, the use of one or two lamps for 7 hours, resulted in only 43 and 76%, of the total number of eggs laid under natural sunlight, respectively. We also investigated the hatchability of oviposited eggs under artificial illumination and under natural sunlight. The hatching rate under the former was much significantly lower than under the the latter. Further detailed research is required to develop methods for successful mass rearing of black soldier fly throughout the year by means of an indoor system.

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Introduction

Black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) is increasingly attracting so much attention worldwide as an effective means for reduction of food waste and its conversion of to organic materials, such as alternative feeds and valuable fertilizer, that an industry is evolving around it. The black soldier fly is distributed in the tropics and in warm temperate regions (Sheppard *et al*., 2002). Especially, black 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 nutritional value comparable to a menhaden fish meal. If used alive, as specialty feed, or marketed to exploit its other unique qualities (i.e., essential amino and fatty acids and chitin content), the value of the product might be higher (Sheppard *et al*., 1994). Females of the black soldier fly mate once with one oviposition event in their lifetime; mated females selectively oviposit 320~620 eggs in dry crevices near a moist food source about 2 days after successful copulation (Tomberlin and Sheppard, 2002). Therefore, developmental studies including mating behavior were conducted

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with the aim to rear a self-sustaining colony for year-round waste conversion (Booth and Sheppard, 1984; Tomberlin and Sheppard, 2002).

In Korea, the fly is active from May through October, and although it is a well-studied insect, with commonly known rearing techniques (Sheppard *et al*., 2002), data on how seasonal factors affect black-soldier-fly farming is limited (Park *et al*., 2010). In regions like Korea, where yearly climatic pattern comprises a well-defined winter season, mass production of black soldier fly needs much effort to maintain larvae and produce eggs under the prevailing low temperatures typical of Korean winter. Above all, mass production of eggs depends on successful adult mating adult's mating and oviposition. In nature, adult mating occurs under direct sunlight. An artificial light source capable of eliciting mating, would boost mass production; thus, facilitating use of this beneficial arthropod in a way that surpasses its regular life cycle and may promote even its import to non-native regions (Sheppard *et al*., 2002). A few laboratory studies available demonstrated that adult mating and oviposition may takes place indoors under artificial light systems. Previously, a 40-Watt, a 430-Watt Pro Ultralight Light System® and rare earth lamp were found to fail in eliciting mating behavior (Tomberlin and Sheppard, 2002). But a 500-Watt quartz-iodine lamp did succeed in stimulating mating and oviposition (Zhang *et al*., 2010). If mating and oviposition of the black soldier fly using artificial light did not require any supplementary direct sunlight, production costs it could be greatly reduced for uninterrupted mass rearing. On the other hand, we do not have enough information regarding oviposition promoting conditions, which a most important issue in a scheme for mass rearing of this fly. Understanding of mechanisms underlying mating and oviposition in the black soldier fly, which can be apply to commercial farming of this important insect (Park *et al*. 2016a). This study aimed to evaluate the effect on oviposition of black soldier fly under mass-rearing conditions using artificial illumination in insect farms. The results of this research shall contribute to adult fly maintenance to serve increase of female productivity.

Materials and Methods

Insect rearing

Adult population density was controlled by pupa number. Pupae were kept indoors at 26ºC and moved to a glass-house cage or into indoor cases upon emergence. Black soldier flies were obtained from a colony maintained year-round in an outdoor glasshouse at the National Institute of Agricultural Science, RDA, Wanju.

Conditions in oviposition cages

The cages were placed in the glasshouse and indoors, where sunlight and artificial light were available, respectively. Nylon-cage sizes 2.0 × 2.0 × 2 m were used in this study. The cages were placed inside a glass-house or indoors from May to August 2017. An HPL lamp (High performance lamp, 750W/Heat Sink 230V; Philips) was used as artificial light sources. The lamps were mounted on the outside of cages 1.5 m high, 50 cm apart. Cages were illuminated by either 2 HPL lamps for 7 hours;, 1 HPL lamp for 7 hours, or by 1 HPL lamp for 3.5 hours every day.

The density of adults was maintained by 10 kg pupae (approximately 65,000 pupae). Oviposition devices which were made with floral foam and contained holes (5 mm diameter × 7 mm depth) for egg collection were set inside the cages. These were then placed above the oviposition substrate (50% food waste and 50% calf feed). Temperature and relative humidity (RH) in the glass-house and in the indoor cages were maintained at 27 ± 5°C and 50± 10%, respectively.

Fecundity and hatching rate

Eggs were collected every day at the end of the experimental photoperiod. Egg-clutch number and weight were recorded daily to determine oviposition rate. Thirty-egg clutches which were removed out of the floral forms everyday were used to determine hatchability. Each egg clutch was placed in a Petri dish and kept in the laboratory at 27°C and 60% RH.

Statistical analyses

Fecundity and hatchability data were subjected to a one-way ANOVA and t-test to determine if the differences were significant; Tukey's HSD test was used for post-hoc analysis. All tests were performed using SPSS PASW 22.0 software for windows (IBM, USA).

Results and Discussion

Fecundity

Tomberlin and Sheppard (2002) showed that maintenance of a black soldier fly colony determined that time of day, light source and light intensity significantly correlated with mating; whereas time of day, temperature, and relative humidity, significantly correlated with oviposition. Light intensity positively regressed with number of black soldier flies mating. Also, the black soldier fly adults needed appropriate light to oviposit after mating (Park *et al*. 2016b). In this study, we used 750W HPL lamps for an artificial light source to stimulate mating of adults. In order to test this study at different light intensities, illuminating time (7 and 3.5 h) and number of lamps (one and two) were altered. Tomberlin and Sheppard (2002) observed that under the artificial

Table 1. Total number and weight of egg batches of different illumination treatments by sunlight, two lamps (7 h), one lamp (7 h) and one lamp (3.5 h). Differences between treatments from the Tukey HSD test are shown with letters.

Total no. and weight of egg batches				
Illuminating time	Two 750w Lamp	One 750w Lamp		Sunlight
	7	7	3.5	7
No. of egg batches	4706±44b	2672±244c	1320±110d	6192±158a
Weight of egg batches(g)	172.1±13b	95.6±7c	44.1±5.7d	218.2±24.1a

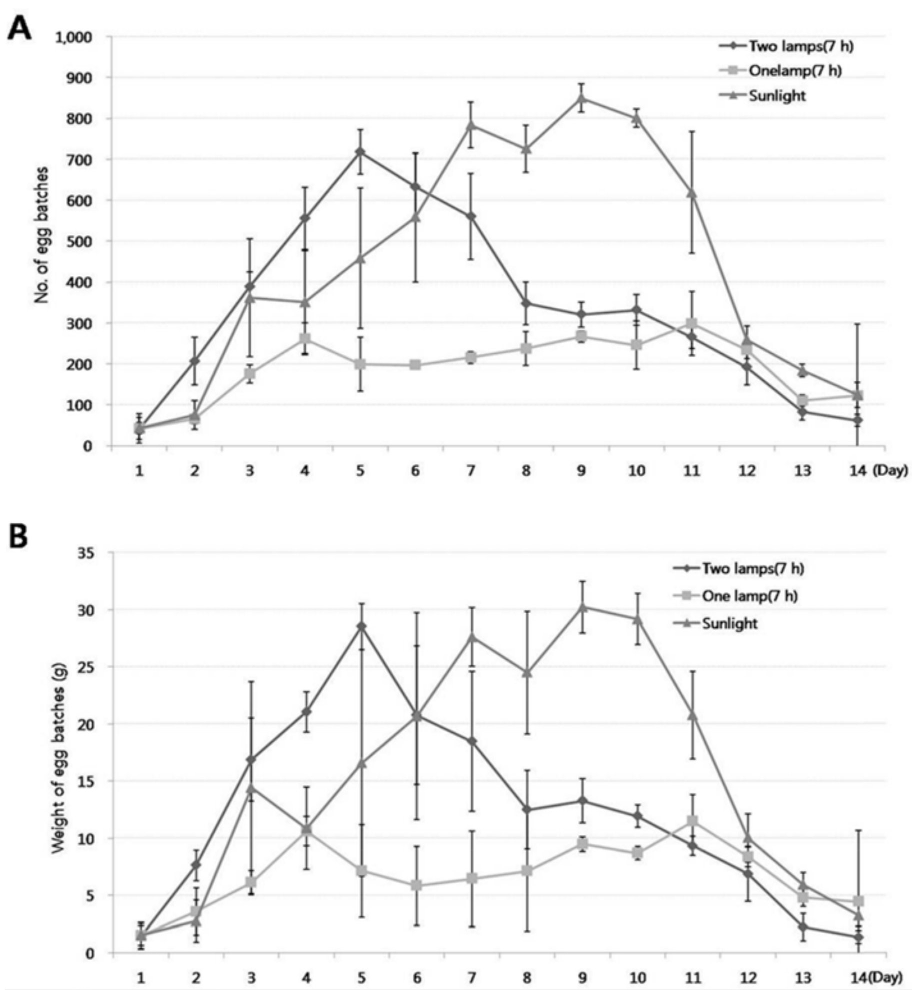

Fig. 1. The number (A) and weight (B) of egg masses laid in sunlight, under two (7 h) or one lamp (7 h) illuminating treatments.

light source, which they used to promote mating, no mating occurred, and only infertile eggs were oviposited. Artificial lamps (such as a 430-Watt Pro Ultralight light system and a 40-Watt Sylvania Gro Lux system) failed to stimulate mating and oviposition (Tomberlin and Sheppard, 2002). In contrast, a 500-Watt, quartz-iodine lamp did succeed in stimulating mating and oviposition (Zhang *et al*., 2010). In our experiment, beginning of with adult emergence, the amount of egg batches began to increase. Significant differences ($P>0.05$) were found in the number and weight of egg masses laid in sunlight, under two (7 h) or one lamp (7 h) illuminating treatments (Table. 1). Overall, we observed 6,192 (218.2 g), 4,706 (172.2 g), 2,672 (95.6 g) and 1,320 (44.1 g) egg batches in the glasshouse under sunlight, in cages illuminated by two lamps (7 h), one lamp (7 h) and one lamp (3.5 h), respectively (Figs. 1, 2). The maximum number of egg batches laid within one day was 849 and was observed on the 9th day in the glasshouse, 718 eggs were laid on the 5th day where two lamps illuminated the oviposition cage for 7 hours and 299 eggs were laid on the 5th day underwhere one lamp illuminated the oviposition cage for 7 hours (Fig. 1)(One way ANOVA test: $F_{2,6} = 323.162$, $p = 0.0001$). Also, the number and weight of egg masses differ statistically between the 7 h and 3.5 h treatments (Fig. 1, 2) (T-test: $t(4)=8.717$, $p=0.001$). The differences in time interval for oviposition to peak may be due to variation in environmental conditions in the greenhouse (Tomberlin and Sheppard, 2002). The artificial light conditions indoors were maintained at a stable temperature of 26ºC, and it is presumed that the adult emergence has progressed steadily. When 750-Watt lamps were used, there was a large difference in fecundity depending on the number of lamps and illuminating time. It is presumed that adults are stimulated to oviposit light intensity and the illuminating hours using this lamp. We did not determine a

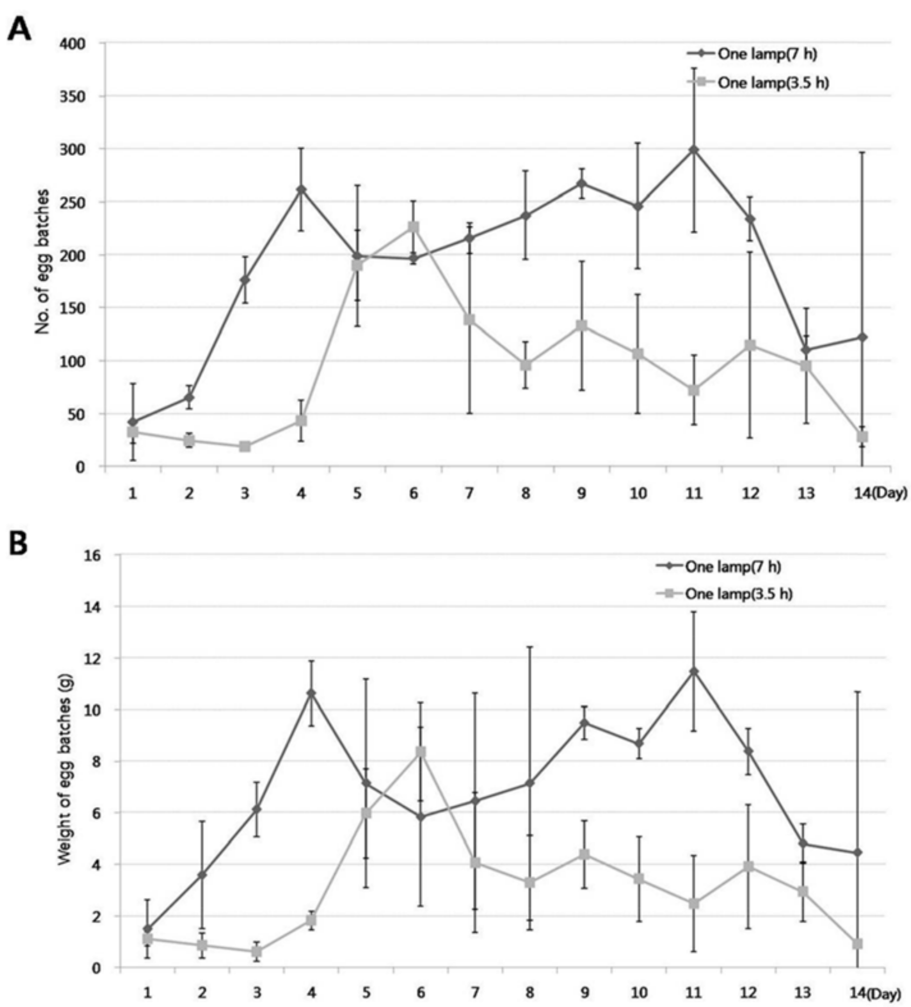

Fig. 2. The number (A) and weight (B) of egg masses laid under one (7 h) or one lamp (3.5 h) illuminating treatments.

Table 2. Egg hatching percentage of different illumination treatments by sunlight, two lamps (7 h), one lamp (7 h) and one lamp (3.5 h). Differences between treatments from the Tukey HSD test are shown with letters.

Hatchability				
	Two 750w Lamp	One 750w Lamp		Sunlight
Illuminationg TIME	7	7	3.5	7
%	55.1±1.6b	50±0.6c	21.6±1d	88.5±2.4a

significant difference in individual egg weight as a result of light conditions (t-test: t (4) = 0.464, p = 0.667). Most of the biological studies one *H. illucens* have not exclusively addressed the effect of light. A previous study provided some information on mating and oviposition of *H. illucens* exposed to different light sources and demonstrated the successful use of artificial illumination to induce mating in this insect (Tomberlin and Sheppard, 2002; Zhang *et al*., 2010). Our results are supported by previous studies in which mating and oviposition behaviors were found to be affected by illuminating radiation wavelengths and intensity. Our results shall be useful in improving current methods for mass rearing of *H. illucens* during winter and under cloudy conditions by using supplementary artificial light. Although illuminating conditions affect mating and oviposition, reliable information on the details involved is scarce. Therefore, additional research in a more controlled setting is needed to significantly improve continued mating rate throughout the seasons.

Hatching rate

Egg hatching percentage was reduced in artificial illumination treatments with two lamps (7 h), one lamp (7 h), one lamp (3.5 h) and sunlight. We registered 55.1 %, 50 %, 21.6% and 88.5 % egg hatching rates in the two lamps (7 h), one lamp (7 h), one lamp (3.5 h) and glasshouse experimental treatments, respectively (Table. 2)(One way ANOVA test: F (2,123)=113.373, p=0.0001, T-test: t(82)=11.042, p=0.0001). There was a difference in hatching percentage depending on sunlight and artificial illuminating conditions. Under conditions of artificial illumination, egg hatching percentage increased increasing photoperiod duration. In our study, under artificial illumination, stimulation of oviposition by female adults seemed to precede stimulation to mate. In 2002, Tomberlin and Sheppard speculated that the eyes of male black soldier flies might cue in at specific wavelengths in sunlight; thus, the absence of certain wavelengths from artificial light used in these studies might explain why mating did not occur, causing infertile eggs to be laid. We were not able to clearly explain the low production of fertilized eggs by artificial light quality. Finding suitable illumination conditions may improve oviposition rate of fertilized eggs by an effect; although this information has not been verified yet, it may influence the male adults, to mate. Our results provide useful information on improving current mass rearing methods for oviposition and hatching of black soldier fly in an indoor system for continuous colony maintenance year around. Future studies should be conducted to better understand the behavioral ecology of black soldier fly.

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