IJIE Int. J. Indust. Entomol. 33(2) 54-58 (2016)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2016.33.2.54

Oviposition site preference in Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae), in Artificial Rearing System

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Abstract

In natural conditions, the 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. This species colonizes and oviposits in a wide variety of decomposing vegetable and animal matter. For mass rearing of black soldier flies, the egg deposition methods are dependent on sunlight and oviposition sites. In this study, we investigated the substrates and oviposition sites preferred by black soldier fly in artificial rearing system. Our results showed that as black soldier flies prefer mating under sunlight, they prefer ovipositing at sites where adequate sunlight and food substrate are available. Further detailed research is required to develop methods for artificially rearing black soldier fly throughout the year in Korea.

Int. J. Indust. Entomol. 33(2), 54-58 (2016)

Received : 4 Oct 2016
Accepted : 4 Nov 2016

Keywords:
Hermetia illucens,
oviposition,
black soldier fly,
egg,
mating

Introduction

The black soldier fly, *Hermetia illucens* (L.), is distributed throughout the tropics and warm temperate regions (James, 1935; McCallan, 1974). This 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). Larvae of black soldier fly decompose organic material efficiently, and therefore, have been used to transform organic waste matter to valuable fertilizer. Also, black Soldier fly prepupae can be used as a 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 a specialty feed, or marketed to exploit its other unique qualities (i.e., essential fatty acids and chitin), the value of the product might be relatively higher (Sheppard *et al.*, 1994). Females of black soldier fly mate once with one oviposition event in their lifetime, and mated females selectively oviposit 320~620 eggs in dry crevices near a moist food source approximately 2 d after successful copulation (Tomberlin *et al.*, 2002). Therefore, developmental studies, including mating behaviors, were conducted with a goal of maintaining a self-sustaining colony for year-round waste conversion (Booth and Sheppard 1984, Tomberlin and Sheppard, 2002). The study of oviposition is essential for developing methods for effective egg collection and year-round mass maintenance of black soldier fly.

The ability of organisms to select suitable habitats for their survival and reproduction can have a profound influence on

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their immediate Darwinian fitness and potential for further evolution (Thorpe, 1945). Habitat selection and, more specifically, oviposition site preference behaviors appear to have played an important role in the evolutionary divergence of several distinct species-groups in the genus *Drosophila* (Heed, 1971; Kambysellis and Heed, 1971; Fellows and Heed, 1972; Kaneshiro *et al.,* 1973). Adults of black soldier fly collected from the forest edge were primarily males (91.9%, n = 109) male, while those collected from the poultry facilities were primarily females (91.3%, n = 123) apparently seeking oviposition sites (Tomberlin *et al.*, 2001). Furman *et al.* (1959) and Sheppard *et al.* (1994) suggested black soldier flies living in a wild environment are mainly adults, whereas those observed in livestock facilities are newly emerged or ovipositing females. Despite the obvious importance of oviposition site preference in mass rearing system, relatively few laboratory studies of this behavior have been made.

The objective of this study was to investigate oviposition site preference by black soldier fly. We addressed two questions: How sunlight stimulates the gravid black soldier fly to oviposit and what substrates stimulate the oviposition of a gravid black soldier fly.

Materials and Methods

Source of black soldier fly and rearing conditions

Black soldier flies were obtained from a colony maintained year-round in an outdoor glasshouse at the National Institute of Agricultural Science, RDA, Wanju. A cage of size 2 × 2 × 4 m was placed at a site in the glasshouse where sunlight was available. Eggs from the colony were collected in egg traps made of floral foam square bars with holes. Blocks were laid 3 cm above the oviposition substrate. Food waste and calf feeds were used as oviposition substrates. The temperature and relative humidity (RH) were maintained at 28 ± 5°C and 60%, respectively. Eggs were held in a laboratory and placed in a plastic box (60 × 40 × 15 cm) containing food waste at 27°C and 60% RH.

Measurement of oviposition rates

The rate of oviposition was determined by recording egg clutches deposited daily. To make this measurement, a plastic pot (25 cm in diameter × 10 cm in depth)containing 1 kg of 50% food waste and 50% calf feeds was placed in the center of the cage. Individuals oviposited in the holes (5 mm in diameter × 7 mm in depth) of two floral foam square bars (egg collecting units) that were approximately 3 cm above the moist media. The floral foam square bars were replaced daily, and oviposited eggs were removed out of the floral foams. The numbers and weights of egg clutches were recorded. The floral foams were kept under direct sunlight, and the others were placed in the shade between 13:00 to 16:00 h. The floral foams of the shady side were placed in the shade open on all sides. To investigate the oviposition preference by induction medium, we used mixed media containing different concentrations of (10, 20, 30, 40, and 50%) of calf feed and food waste. Three replicates of each experiment were conducted for each of the three preference tests using approximately 10,000 flies in each cage.

Results and Discussion

Oviposition site preference

Tomberlin and Sheppard (2002) have reported that the intensity of sunlight plays a major role in determining the mating time of black soldier fly. They found that light intensity positively regressed with the number of black soldier flies mating but not ovipositing. Our results showed that as black soldier flies prefer mating under the condition of sun light intensity the

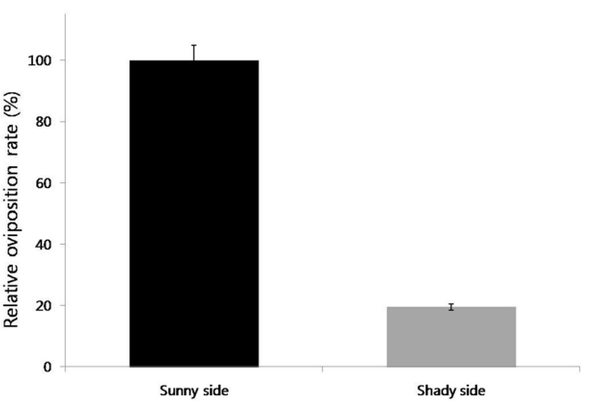

Fig. 1. Relative oviposition rate to the laying of floral foam square bar in the sunny side and the.shady side. Error bars represent the standard deviation.

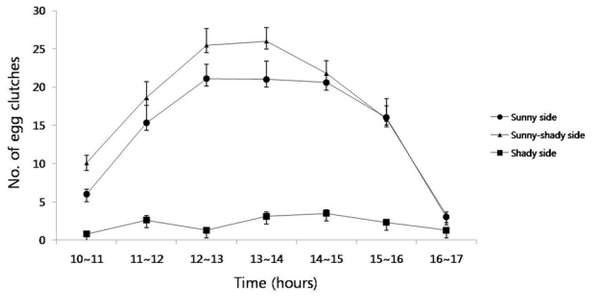

Fig. 2. The influence of different sites on the ovipositing of black soldier fly and the number of egg clutches were recorded in one day.

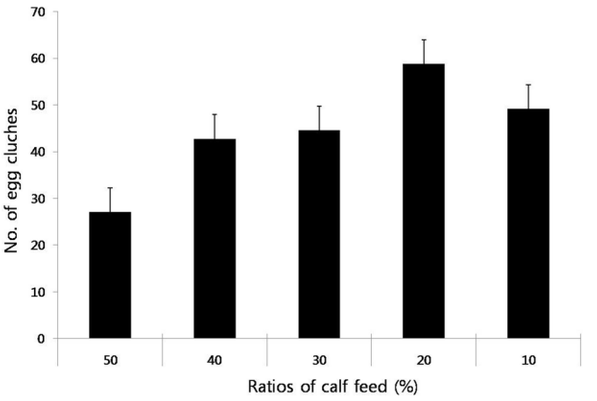

Fig. 3. Total number of egg clutches of black soldier fly laid in each of the substrates. The means and standard errors were tested at 95% confidence level by Least Significant Difference (LSD) test.

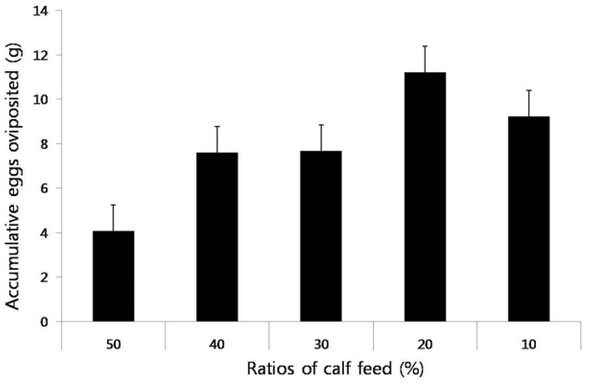

Fig. 4. Total weights of eggs of black soldier fly laid in each of the substrates. The means and standard errors were tested at 95% confidence level by Least Significant Difference (LSD) test.

black soldier flies prefers the sun is shining place to oviposit (Fig. 1). Relative oviposition rate to the laying of floral foam square bar in the shady side was only 19.5% of the oviposition rate on the sunny side. It appeared that the black soldier fly adults needed appropriate light to oviposit after mating. Large-scale artificial rearing of *H. illucens* has sometimes been carried out with no sunlight, resulting in low mating and oviposition rates (Zhang *et al.* 2010). Moreover, Tomberlin and Sheppard (2002) have reported that under artificial light sources, no mating was observed and infertile eggs were oviposited. In our experiment, adult females preferred to oviposit on the sunny side than on the shady side. In addition, they preferred to oviposit on the other side (sunny-shady) than where the direct sunlight was available (Fig. 2). We observed the numbers of egg clutches at the shady side, sunny side, and sunny-shady side during 10:00 ~ 17:00 h. The numbers of egg clutches were significantly different between the sunny side, sunny-shady side, and shady side (Fig. 2).The number of egg clutches recovered from the sunny-shady side was slightly higher than that of the egg clutches recovered from the sunny side. We observed a significant difference in the oviposition rate with the lapse of time between the sunny side and shady side, but no significant difference was observed on the sunny-shady side. As sunlight affects mating, it was expected to affect oviposition. We presumed that the wavelength of light was not adequate for oviposition under shaded conditions. Sensory organs of insects might significantly affect the selection of oviposition site (Davis, 1976; Takamura and Fuyama, 1980).

Substrate preference

Organic material in oviposition sites played an important role in attracting gravid black soldier fly females. The gravid black soldier flies exhibit scattering behavior for two possible reasons: one is to oviposit at sites with a small gap between them and the other is to oviposit at sites where the organic material is available. If the two requirements are not met, the gravid black soldier fly does not oviposit. When oviposition was driven by a medium containing either food waste or calf feed, insufficient oviposition effect was observed (data not shown). In this study, we used mixed medium containing both food waste and calf feed to drive oviposition in gravid black soldier fly (Fig. 3, 4). Our results showed that the oviposition effect depended on the ratio of calf feed in the medium. Colonization attempts when volatiles were above a threshold resulted in reduced survival of deposited eggs, while the opposite was observed for cohorts deposited with eggs emitting volatile concentrations below threshold levels (Lam *et al.* 2007). The same has been determined for other species including blow flies (Ashworth and Wall, 1994; Vass

et al., 2002). The attraction and repulsion of conspecifics for oviposition behavior depends upon the threshold concentrations of these volatile substances (Lam *et al.*, 2007). It seems that the most important factor driving oviposition is the medium for mass egg deposition. Significant differences were observed in the number of laid egg clutches with media containing different ratios of calf feed. The mixed medium containing 20% ratio of calf feed was found to be the most effective substrate for maximum egg production. Our results indicate that oviposition behavior is mediated by environmental factors such as sunlight and food medium. We were not able to conduct an in depth study of volatile gases emitting from the medium and bacteria-related factors and sunlight with black soldier fly oviposition. Future studies should be conducted to better understand the behavioral ecology of black soldier fly. Our result would provide useful information on improving the current methods of black soldier fly mass rearing.

Acknowledgments

This work was supported by a grant (No. PJ0108432016) from the National Institute of Agricultural Science, Rural Development Administration, Republic of Korea.

Conflicts of interest

The authors have no conflicts of interest to declare.

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