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Effect of adult population density on egg production in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae)

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Abstract

The black soldier fly is economically important because its prepupae are used as feed for many animals, including fish and swine. In Korea, black-soldier-fly farms have attempted to increase annual breeding and mass egg production for use in animal feed, as well as the decomposition of organic waste. Such efforts require an understanding of optimal mating and oviposition techniques. Specifically, adult densities and cage size may both improve the efficiency of mass egg production. Our study used four sizes of nylon cages (1.0 × 1.0 × 2.5 m, 1.5 × 1.5 × 2.5 m, 2.0 × 2.0 × 2.5 m, 2.5 × 2.5 × 2.5 m) and three density treatments (4 kg, 8 kg, and 10 kg of pupae) to investigate optimal habitat size and adult density. We found that cage size (independent of density) did not significantly influence female fecundity (number of egg clutches and egg weight), whereas higher densities increased egg number and weight regardless of cage size. Thus, we recommend manipulating adult density to enhance productivity in commercial black-soldier-fly farming. However, we also propose further detailed research to develop methods that account for seasonal changes and environmental conditions, as climatic variables (temperature, sunlight) likely influence female fecundity as well.

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Introduction

The black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) is a widespread and economically important insect that colonizes warm temperate regions. The fly's prepupae are a common feed ingredient for many animals worldwide, including fish (Bondari and Sheppard, 1981) and swine (Newton *et al.*, 1977). In Korea, black soldier flies have also attracted interest as a possible solution for the disposal of organic matter, such as food waste. However, this proposal's feasibility lies in the ability to continually produce numerous larvae that can process high volumes of organic waste. 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). Moreover, we do not have enough information regarding the physical environment necessary for mass rearing. Early research has indicated that cages of a certain size, as well as the location of those cages, may influence mating and oviposition. For example, mating and

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oviposition were observed often in a 3 × 6.1 × 1.8-m cage placed outdoors (Tingle *et al.*, 1975). Mating was also observed in a 0.76 × 1.14 × 1.37-m cage placed outdoors, but not in the greenhouse. Neither process occurred in two smaller cages (53 × 91 × 53 cm and 38 × 46 × 38 cm). Clearly, newer data are needed to clarify the most efficient means of farming black soldier flies.

Some hints of factors that may affect oviposition in black soldier flies can be gleaned from data on other insects. In *Musca domestica*, critical factors affecting egg number include temperature, quantity and quality of larval and adult diets, mating, adult population density, age, oviposition substrate, photoperiod, and genetics (Zvereva and Zhemchuzhina, 1988). Some of these factors and their effects on female fecundity have been determined to various degrees in many insect species (Pastor, Cickova, Kozanek, Martinez-Sanchez, Takac and Rojo 2011). In particular, high population densities produced more eggs in flies than low population densities. This pattern suggests that an efficient and cost-effective method to mass produce black solder flies may be to maintain an optimal adult density in appropriately sized cages.

This study aimed to evaluate the effect of adult population density on egg production in the black soldier fly under mass-rearing conditions. The results would define the optimal conditions for adult fly maintenance that serves to increase female productivity.

Materials and Methods

Four nylon-cage sizes were used in this study: 1.0 × 1.0 × 2.5 m, 1.5 × 1.5 × 2.5 m, 2.0 × 2.0 × 2.5 m, and 2.5 × 2.5 × 2.5 m. The cages were placed inside a greenhouse from May to September 2016.

Adult population density was controlled with pupal number. Pupae were kept indoors at 26ºC and moved to the greenhouse cages upon emergence. Three density treatments were created: 4 kg (approximately 26,000 pupae), 8 kg (approximately 52,000 pupae), and 10 kg (approximately 65,000 pupae). Eggs were collected for 12 ~ 14 d after adult emergence. Egg-clutch number and weight were recorded daily to determine the oviposition rate. Oviposition devices were made with floral foam and contained holes (5 mm diameter × 7 mm depth) for egg collection. These were placed above the oviposition substrate (50% food waste and 50% calf feed). The devices were replaced daily after removing oviposited eggs. All density treatments were replicated three times.

Results and Discussion

Our goal in this experiment was to find the adult population density that was optimal for egg production in mass-reared black soldier flies. We introduced adult flies into a limited space (cages) with differing population densities and examined whether space and adult mass increased egg production. We chose population density as a factor because high population density is a typical feature of mass rearing and negatively influences fecundity in many insects (e.g., Ohnisni, 1976; Wardhaugh & Didham, 2005; Hari *et al.*, 2008). This influence may occur directly during adult life and indirectly during the larval stage, resulting in high larval mortality as well as small adults with low reproductive potential (Black & Krafsur, 1987; Honek, 1993). Although we did indeed observe an effect of population density on female fecundity, with the number of adults significantly influencing mean egg number and weight, our results contradicted previous reports. Specifically, oviposited eggs increased as adults increased, given the same cage size. Moreover, under identical adult density, cage size did not noticeably affect egg production (Tables 1 and 2). The highest mean number of eggs (9016 clutches or 368 g) was recorded in the 2 × 2 × 2.5-m cage with 10 kg of pupae. Thus, our results failed to show that habitat space is influential in the mating and oviposition of black soldier flies, at odds with earlier, small-scale laboratory studies (Tingle *et al.*, 1075) that reported an effect of cage size and location. Our results provide guidance for determining optimal space and adult density to maximize egg production and retain adults in a mass-rearing system. Because it is costly to maintain suitable spaces in a greenhouse year-round, successful commercial breeding of black solder flies requires an understanding of the ideal conditions for improved productivity.

Based on our data, we concluded that the 1.5 × 1.5 × 2.5-m and 2 × 2 × 2.5-m cages housing 65 000 pupae were the most appropriate for mass rearing. Additionally, our results clearly show that adult number is critical to the continuous production of eggs daily. Thus, pupae should be introduced into housing cages at regular intervals for steady adult emergence. The difference between studies may be due to the fact that our density treatments had not reached the upper limit where suppression begins. Besides population density, other factors likely affect

Table 1. Total number of egg clutches over 12~14 d (mean ± SD) depending on the cage size and number of flies (pupae) in the cages. (-) Data not collected in the study.

	(W×L×H, m)	1×1×2.5	1.5×1.5×2.5	2×2×2.5	2.5×2.5×2.5
Total no. of egg clutches	4 kg Pupae	1428 ± 31	1373 ± 42	1420 ± 25	1542 ± 22
	8 kg Pupae	2944 ± 20	3467 ± 13	3047 ± 25	2900 ± 22
	10 kg Pupae	—	8132 ± 85	9016 ± 154	—

Table 2. Total weight of eggs over 12~14 d (mean ± SD) depending on the cage size and number of flies (pupae) in the cages. (-) Data not collected in the study.

	(W×L×H, m)	1×1×2.5	1.5×1.5×2.5	2×2×2.5	2.5×2.5×2.5
Total weight of eggs (g)	4 kg Pupae	38.76 ± 1.78	33.08 ± 1.48	34.42 ± 2.57	42.87 ± 1.84
	8 kg Pupae	101.11 ± 4.57	127.63 ± 3.83	113.59 ± 2.62	105.1 ± 4.05
	10 kg Pupae	—	281.9 ± 19.8	368.7 ± 24.1	—

egg productivity, either directly or indirectly. For example, sex ratios significantly influence adult longevity and emergence time (Tomberlin, Adler and Myers 2009). For this reason, it is important to maintain a large number of both sexes receiving the same treatment simultaneously. Furthermore, direct sunlight, temperature, and substrate are all known to affect oviposition of the black soldier fly. Some evidence also exists to show that diets may affect life history traits in this insect, including female fecundity (Tomberlin *et al.*, 2002). Thus, to effectively mass-rear insects like the black soldier fly, factors influencing fecundity should be reassessed, and traits or procedures that do not enhance reproduction should be avoided (Pastor *et al.*, 2011). While our study was unable to examine these other factors, we clearly demonstrated the effects of adult density on fecundity. Therefore, we contributed to an improved understanding of mechanisms underlying mating and oviposition in the black soldier fly, which can be apply to commercial farming of this important insect.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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