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Egg-trapping of black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) with various wastes and the effects of environmental factors on egg-laying

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ABSTRACT: Black soldier fly (BSF), *Hermetia illucens* (L.) is widely used in bio-recycling of human food waste and manure of livestock. Eggs of BSF were commonly collected by egg-trapping technique for mass rearing. To find an efficient lure for BSF egg-trapping, this study compared the number of egg batch trapped by different lures, including fruit, food waste, chicken manure, pig manure, and dairy manure. The result showed that fruit wastes are the most efficient on trapping BSF eggs. To test the effects of fruit species, number of egg batch trapped by three different fruit species, papaya, banana, and pineapple were compared, and no difference were found among fruit species. Environmental factors including temperature, relative humidity, and light intensity were measured and compared in different study sites to examine their effects on egg-trapping. The results showed no differences on temperature, relative humidity, and overall light intensity between sites, but the stability of light environment differed between sites. BSF tend to lay more eggs in site with stable light environment.
Keywords: *Hermetia illucens*, environmental stability, volatile attraction, bio-recycling

Introduction

Hermetia illucens (L.) (Diptera: Stratiomyidae), the black soldier fly (BSF) is widely used on composting of various materials, and one of the reasons is that BSF larvae could feed on a variety of food. Many different organic wastes, including animal manures (Newton et al., 2005), fruits (Nguyen et al., 2013), and vegetables (Malloch, 1917), or even some indigestible food, such as coffee (Diener et al., 2009) and beeswax (Malloch, 1917) have been reported as food of BSF larvae. A high efficiency on digestion of manure is known for BSF larvae. They could assimilate 42.1% and 43.2% of the crude protein of poultry manure and swine manure (Newton et al., 2005), respectively. Thus, BSF becomes an economic way to recycle the nutrients from manures, and provide additional proteins for livestock, such as swine (Newton et al., 1977). BSF is also used as food for hen (Hale, 1973), and fish (Bondari and Sheppard, 1981).

The source of BSF eggs for mass production is usually collected from egg-trapping technique. Two major components in egg-trapping technique are baiting and trapping material. For example, Booth and Sheppard (1984) used chicken manure as baiting material, and put corrugated paper on chicken manure as trap of BSF egg, and BSF females laid eggs in the spaces of corrugated papers. Overall, the egg-trapping efficiency determines the efficiency of BSF production. Baiting material is a major factor affect the egg-trapping efficiency because BSF female may search for a specific food source for their offspring. Previous studies showed that BSF female lay egg on various materials, such as fruit, carrion (James, 1935), and manure (Tingle et al., 1975). In this study, we compared the differences

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on egg-trapping efficiency of different baiting materials, including animal manures, fruits, and household food wastes for searching the optimal baiting material. Results from this study could be used to improve the efficiency on BSF egg-trapping, which is helpful for any experiment and application of BSF in Thailand.

Many environmental factors, such as light intensity, light wavelength, temperature, and humidity, may affect the mating and ovipositing behaviors of BSF. Furthermore, a specific light intensity and light wavelength were considered as a crucial factor that stimulate the mating behavior of BSF (Tomberlin and Sheppard, 2002). For example, Tomberlin and Sheppard (2002) showed that increase of light intensity may increase the frequency of mating behavior of BSF, and the frequency of ovipositing behavior is positively correlated with temperature but negatively correlated with humidity. Considering the effects on mating and ovipositing behaviors, environmental factors may also affect the egg-trapping efficiency, thus, we tested the effects of environmental factors on egg-trapping efficiency in this study.

Materials and methods

1. Study site

All experiments were carried out in National Biological Control Research Center (NBCRC), Central Regional Center, Kasetsart University, Kamphaeng Saen Campus, in two sides of a building (14.033973°N; 99.975172°E).

2. Egg-trapping efficiency of different materials

An egg trap is designed to test the trapping efficiency of odors from different materials. The materials were put in a plastic box (36 × 43 × 18 cm) covered by nylon net, which allows the spread of odor but prevent the insects seeing and touching the trapping material (**Figure 1**). To trap the eggs of black soldier fly, two corrugated papers (3.5 x 47 x 0.5 cm) were placed on the top of plastic box (**Figure 1**). All of the experiments in this study used the same egg trap. The trapped eggs and larvae were kept in plastic box with fruits for observation on morphology. All of the trapped eggs were distinguished as BSF eggs based on the morphological descriptions of Oliveira et al., 2015 and Oliveira et al., 2016.

Two trials were done in this study to test the egg-trapping efficiency of odors from different materials. The first trial was carried out from 1 April to 30 April in 2016. In the first trial, egg-trapping efficiencies of five materials, including fruit waste, household food wastes, chicken manure, pig manure, and dairy manure, were compared. The fruit waste was a mixture of fresh fruits including wax apple, pineapple, apple, water melon, and melon from fruit market. Household food wastes was obtained from food center of Kasetsart University, Kamphaeng Saen Campus, was a mixture of leftover such as rice, vegetable and meat. Chicken manure, pig manure, and dairy manure were collected from experimental farm of Department of Animal Science of Kasetsart University, Kamphaeng Saen Campus. The second trial starts from 13 April to 13 May in 2016. Egg-trapping efficiency of three common fruits, pineapple, papaya and banana, which could be bought all year around in Thailand, were compared in the second trial. These materials were fresh fruits bought from fruit market. In both trials, two traps of each material were placed in western and eastern sides of the building, respectively. The relative positions of traps and building in the two trials are shown in **Figure 2.** In each trap, number of egg batch and egg number in each batch were counted daily.

3. Effects of environmental factors

To examine the effects of environmental factors on egg-trapping, the temperature (ºC), relative humidity (%), and light intensities (Lux) were measured at two time periods of afternoon, 12:00-15:00 and 15:00-18:00, for every 2-3 days in both sides of the building from 13 April to 20 April in 2016. Environmental factors were only measured in the afternoon because we have observed BSF oviposition in the afternoon for more than three times, and never observed them ovipositing in the morning in our previous egg-trapping process which was carried out for two months (unpublished data). Temperature and relative humidity were measured by a thermo-hygrometer (Model: HTC-1, Guangzhou Lexiang Electronic Co. Ltd). The light intensity was measured using a Lux meter (Model: Victor1010A, Shenzhen Yisheng Victor Tech Co. Ltd).

4. Statistical Analysis

Numbers of egg batch trapped by egg traps were pooled by weeks, and were compared using Mood's median test. Temperatures (ºC), relative humidity (%), light intensity (Lux), and egg number in egg batches trapped in west and east sides were compared by Mann-Whitney *U* test. All statistical analyses were done using the software PAST (version 3.12).

Results and discussion

Egg-trapping efficiency of different materials

In the first trial, the number of egg batch trapped by two traps with fruit waste are 2.75 ± 3.11 and 5.50 ± 3.20/week, respectively, which were significantly higher than those with household food wastes, chicken manure, pig manure, and dairy manure (Table 1). Eggs were trapped only by traps with fruit waste (Table 1). In the second trial, the number of egg batch trapped by papaya, banana and pineapple were low (papaya: 0.75 ± 1.30/week; banana 0.50 ± 0.50/week; pineapple 0/week) and were not significantly different (Table 2). These results supported that the odor of fruit was more attractive to BSF or more likely to stimulate the egg-laying behavior of BSF. However, it does not mean that BSF would always lay eggs on fruits, because the feeding preference of Diptera insects maybe affected by prior exposure of food. For example, the feeding preference of adult of vinegar fly *Drosophila melanogaster* is affected by the diet of their larval stage (Jaenike 1983). Considering that induction of feeding preference is possible, we propose that BSF are more likely to lay eggs on the waste which is abundant and was most utilized by the field BSF population in the environment. This hypothesis explains why BSF laid eggs only on fruits in this study, but laid eggs on manures, such as chicken manure in previous study (Booth and Sheppard 1984). In our study site, there are no chicken, pig, and dairy farms surrounding the traps, and the fruits seem to be the only food source of field BSF population. In contrast, the study site of Booth and Sheppard 1984 was nearby a chicken farm, and the chicken manure is more likely to be the food of field BSF population. We propose that BSF recognize and were attracted by the volatile compounds emitted from the rotten food. For example, the compounds released during the fermentation of fruit, such as ethanol, acetic acid, ethyl acetate, and acetaldehyde (Barrows, 1907; Hunter et al., 1937; West, 1961), which were known to attract the adults of vinegar fly (Zhu et al., 2003).

Effects of environmental factors

In two sides of building, there were no difference on temperature and relative humidity (**Figure 3AB**). Thus, these two factors are not likely to be the major factors affect mating and ovipositing behavior in this study. The overall light intensities in two sides were not significantly different (Mann-Whitney U test: U = 107, p = 0.84), but two sides showed different patterns of light environments (**Figure 3C**). In east side, light intensities at 12:00-15:00 were significantly higher than that at 15:00-18:00, but no difference were found between two time periods in west side (**Figure 3C**). It supports that light environment of east side is not stable, decrease quickly in the afternoon, and that of west side is stable in the afternoon. The difference on stability of light environment is not likely to affect the frequency of ovipositing of BSF because there is no difference on number of egg batch catch in two sides (**Tables 1, 2**); however, the egg number per batch of west side is significantly higher than that in east side (**Figure 4**), which indicated BSF females laid more eggs for each egg batch in stable light environment. The effects of stability of light environment on ovipositing behavior of BSF has never been reported in previous studies. We proposed that, BSF females in field may likely to search for stable light environment for their offspring, for example, the shade under trees.

Table 1 Comparing the number of egg batch trapped by different trap types in four weeks

Trap type	Trap code (comparative light intensity)	Week 1	Week 2	Week 3	Week 4	Mean ± SD[1]
Fruit waste	1 (East)	2	8	1	0	2.75 ± 3.11a
	2 (West)	4	11	3	4	5.50 ± 3.20a
Food waste	1 (East)	0	0	0	0	0b
	2 (West)	0	0	0	0	0b
Chicken manure	1 (East)	0	0	0	0	0b
	2 (West)	0	0	0	0	0b
Pig manure	1 (East)	0	0	0	0	0b
	2 (West)	0	0	0	0	0b
Dairy manure	1 (East)	0	0	0	0	0b
	2 (West)	0	0	0	0	0b

[1] Means followed by the same lowercase letter within a column are not significant different at $p < 0.05$ (Mood's median test).

Table 2 Comparing the number of egg batch trapped by different fruits in four weeks.

Fruit type	Trap code (comparative light intensity)	Week 1	Week 2	Week 3	Week 4	Mean ± SD[1]
Papaya	1 (East)	3	0	0	0	0.75 ± 1.30a
	2 (West)	0	0	0	0	0a
Banana	1 (East)	0	1	1	0	0.50 ± 0.50a
	2 (West)	0	0	0	0	0a
Pineapple	1 (East)	0	0	0	0	0a
	2 (West)	0	0	0	0	0a

[1]Means followed by the same lowercase letter within a column are not significant different at $p < 0.05$ (Mood's median test).

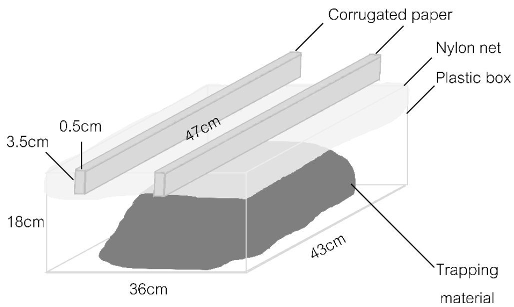

Figure 1 Egg trap of black soldier fly

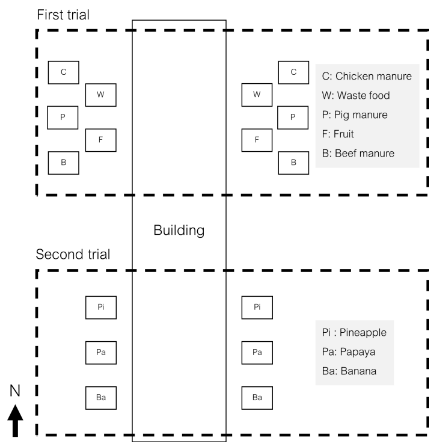

Figure 2 Study site and experimental design of comparing egg-trapping efficiency of different types of wastes in two light environments

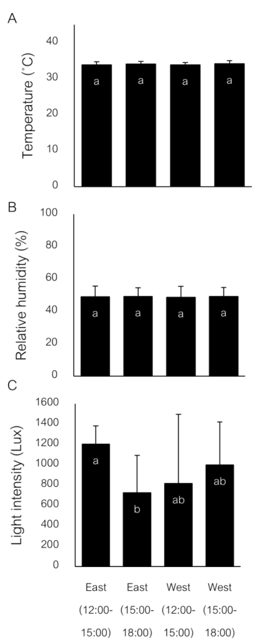

Figure 3 Comparing the temperature (ºC), relative humidity (%), and light intensity (Lux) in west and east sides of study site. Bars with the same lowercase letter on top are not significantly different at $p < 0.05$ (Mann-Whitney U test).

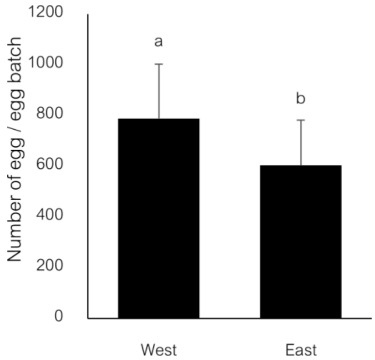

Figure 4 Comparing the number of egg in egg batches trapped by fruit waste in west and east sides. Bars with the same lowercase letter on top are not significantly different at $p < 0.05$ (Mann-Whitney *U* test).

Conclusions

This study supports that BSF females more likely to lay eggs nearby fruit wastes, and they would lay more eggs in sites with stable light environment. The results would be helpful for choosing materials and sites to trap BSF eggs for mass rearing purpose.

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