

# Factors Influencing Mating and Oviposition of Black Soldier Flies (Diptera: Stratiomyidae) in a Colony<sup>1</sup>

Jeffery K. Tomberlin<sup>2</sup> and D. Craig Sheppard

Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Tifton Campus, P.O. Box 748, Tifton, GA 31793-0748 USA

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**Abstract** Most information on the black soldier fly, *Hermetia illucens* (L.), is limited to its use as a biological control and waste management agent. Little is known about its mating and oviposition activities. Latency from emergence to mating and oviposition for colony-reared black soldier flies placed in a 1.5 × 1.5 × 3 m nylon cage located in a greenhouse was determined. Sixty-nine percent of mating occurred 2 d after eclosion and 70% of oviposition 4 d after eclosion. Time of day and light intensity significantly correlated with mating ( $r^2 = 0.49$ ;  $P < 0.0001$ ), while time of day, temperature, and humidity significantly correlated with oviposition ( $r^2 = 0.58$ ;  $P < 0.0001$ ). Latency after emergence significantly correlated with mating ( $r^2 = 0.99$ ;  $P < 0.0001$ ) and oviposition ( $r^2 = 0.99$ ;  $P < 0.0001$ ). A second experiment was conducted to examine oviposition preference of the black soldier fly. Adults were allowed to oviposit in Gainesville house fly, *Musca domestica* L., larval media with and without 5-d-old black soldier fly larvae. Based on sign non-parametric *t*-tests, numbers of egg clutches deposited in each treatment were not significantly different.

**Key Words** Black soldier fly, Stratiomyidae, *Hermetia illucens* (L.)

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Colonization of manure in poultry facilities by the black soldier fly, *Hermetia illucens* (L.), can result in 94 to 100% suppression of the house fly, *Musca domestica* L. (Diptera: Muscidae) and a 50% reduction in manure accumulation (Sheppard 1983). Their prepupae also can be self-harvested and used as a feed for livestock, such as swine (Newton et al. 1977) and fish (Bondari and Sheppard 1981).

The black soldier fly has a worldwide distribution in the tropics to warm temperate regions and is active in the southeastern United States from April through October. It will oviposit in a variety of decomposing materials, such as fruit, carrion (James 1935), and manure (Tingle et al. 1975). Eggs are deposited along the edge of manure in poultry facilities (Sheppard 1983) and hatch after approximately 4 d when held at 27°C (Booth and Sheppard 1984). Larvae feed for about 2 wk before becoming prepupae (Tomberlin et al. 2002). Prepupae migrate from the larval habitat to pupate (Sheppard et al. 1994), and adults emerge 2 wk later depending on environmental conditions (Tomberlin et al. 2002).

Other than knowing emergent adult black soldier flies disperse into the area surrounding their pupation site (Axtell and Arends 1990) and that they will lek with males

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<sup>1</sup>Received 27 September 2001; accepted 31 March 2002.

<sup>2</sup>Current address: Department of Biological and Agricultural Engineering; To whom offprint requests are to be addressed (email:jtomber@tifton.cpes.peachnet.edu).

attempting to mate with females entering the aggregation site (Tomberlin and Shepard 2001) little behavioral information has been documented. Furman et al. (1959) indicated that gravid adults were attracted to poultry manure containing conspecific larvae. Kemppinen (1998) attempted to address this hypothesis. However, her data were inconclusive.

The objectives of this study were: (1) to determine time from emergence to mating and oviposition for black soldier flies maintained in a colony, as well as the environmental factors influencing these behaviors; (2) to determine if black soldier flies preferred to oviposit in media inoculated with or without soldier fly larvae. Information recorded for both objectives is important for mass rearing the black soldier fly for biological control of fly pests and waste management in livestock and poultry facilities.

### Materials and Methods

**Factors influencing mating and oviposition.** The experiment was replicated three times from February through June 2000. For each replicate, approximately 750 newly-emerged (<15 h old) adult black soldier flies from a colony maintained at the Coastal Plain Experiment Station, Tifton, GA, were released into an empty 1.5 × 1.5 × 3 m Lumite® screen (5.5 mesh per cm) cage (Bioquip® Products, Gardena, CA) located inside a 6 × 9.4 × 5 m greenhouse. Flies rested on the cage walls and were watered by two Aqua Cool® Fog Nozzles #WF4025 (Farm Tek®, Dyersville, IA) that delivered 30 Lr/h at 7 kg/cm<sup>2</sup>. Environmental conditions and the number of pairs mating were recorded for 10 min periods at 1 h intervals (time of day) from 0800 to 1800 h each day. Observations were initiated at adult release and terminated when a complete day passed without observing mating and ovipositing. Light intensity was measured with a Basic Quantum Meter Model BQM® (Spectrum Technology Inc, Plainfield, IL), while temperature and humidity were measured with a Hanna HI 9161C® Portable Microprocessor Thermohygrometer (Hanna Instruments Inc, Woonsocket, RI). The Basic Quantum Meter measured the intensity, photosynthetic photon flux ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ ), of visible light (400 and 700 nm). All environmental readings were recorded in the center of the cage approximately 30 cm above the ground. Mating was defined as a male and female observed *en copula*.

Level of oviposition was determined by recording the number of egg clutches deposited during each interval between consecutive observations. In order to make this measurement, a 5-L white bucket containing 1 kg of saturated Gainesville house fly larval diet (Hogsette 1985) was placed in the center of the cage on a 40-cm high cement block for the duration of the experiment. Females oviposited in the flutes of two corrugated cardboard rolls (egg collecting units), measuring 2.54 cm diam × 4 cm length, taped to the inside of the bucket approximately 3 cm above the moist media. Each flute opening measured 2 × 3 mm. A flute containing more than 100 eggs was considered an egg clutch. Cardboard rolls were replaced each observation and the number of egg clutches recorded.

A stepwise regression (SAS Institute 1992) was used to determine the model ( $P < 0.05$ ) that best describes the relationship between the number of pairs observed mating and oviposition with environmental conditions and time of day. Mating, oviposition, and light intensity data were  $\log_{10}(n + 1)$  transformed prior to analysis in order to normalize the data.

Total numbers of pairs observed mating and egg clutches recorded during the experiment were tabulated and percent occurrence per day determined and re-

gressed with latency after emergency to determine if a significant relationship ( $P < 0.05$ ) occurred (SAS Institute 1992). Additionally, for mating and oviposition data, respectively, Least Significant Difference test (SAS Institute 1992) was used following a significant F test ( $P < 0.05$ ) to separate mean differences between percent to occur each day. Percent data were arcsine transformed to normalize data prior to analysis.

**Influence of conspecific larvae on oviposition preference.** In the greenhouse, two treatments, each consisting of a 5-L white bucket containing 300 g of Gainesville house fly larval media saturated with water, were examined for oviposition preference by black soldier flies. Two egg-collection units, as previously defined, were placed in each bucket approximately 3 cm above the media. According to Booth and Sheppard (1984), black soldier flies prefer to oviposit in dry sites above the larval medium. Therefore, 40 to 50 ml of water were mixed daily with the media in each bucket to inhibit soldier fly oviposition in it. Media in one treatment was inoculated with approximately 500 5-d-old black soldier fly larvae 15 min prior to initiating the study, while the other had no larvae added. Paired treatments were placed side by side on 40-cm high cement blocks at three sites separated by approximately 2 m in the greenhouse near its center (Fig. 1). A white plastic sheet (12 cm  $\times$  6 cm  $\times$  1 mm) was placed on top of each bucket and covered approximately 75% of the opening to shade the oviposition sites from direct sunlight. Treatments were replaced every 8 d. Adult flies were reared and released into the greenhouse colony daily. Because of the inability to regulate adult emergence, fly numbers in the colony were variable ranging from 500 to 2000 individuals on any given day. This variation in fly numbers was due to fluctuating daily fly emergence in the colony. The experiment was replicated 8 times from 26 July to 20 September 2000.

Egg-collecting units from each treatment were replaced and the number of egg clutches per treatment recorded daily. The data were  $\log_{10}(n + 1)$  transformed prior to analysis (SAS Institute 1992), but did not meet the requirement of homogeneity of variance. Therefore, a sign non-parametric  $t$ -test was used to determine if significantly more clutches were oviposited in media inoculated with or without soldier fly larvae each day of the experiment (SAS Institute 1992).

## Results and Discussion

**Factors influencing mating and oviposition.** Stepwise regression analysis indicated that environmental conditions significantly influenced time of mating ( $F = 164.8$ ;  $df = 4, 5$ ;  $P < 0.0001$ ) and oviposition ( $F = 238.4$ ;  $df = 4, 5$ ;  $P < 0.0001$ ) (Table 1). Light intensity positively regressed with number of black soldier flies mating but not ovipositing (Table 1). We observed 268 mating pairs during 53 of 134 observations made and none occurred when light intensity was less than  $63 \mu\text{mol m}^{-2}\text{s}^{-1}$ . In contrast, 75% of the mating pairs occurred when light intensity was greater than  $200 \mu\text{mol m}^{-2}\text{s}^{-1}$ , which was recorded during 60% of the observations that had mating occur. Additionally, approximately six mating pairs were observed per observation when light intensity exceeded  $200 \mu\text{mol m}^{-2}\text{s}^{-1}$ , which was 50% greater than that observed when light intensity was below that value. Time of day negatively regressed with mating indicating mating tended to occur more frequently early in the day (before 1500 h) and decreased as the day progressed.

While developing a method for maintaining a black soldier fly colony in a room maintained at approximately  $22^{\circ}\text{C}$  and 60 to 70% RH it was determined that light source was important (D. C. S., unpubl. data). Initially, a 40-watt Sylvania Gro Lux®

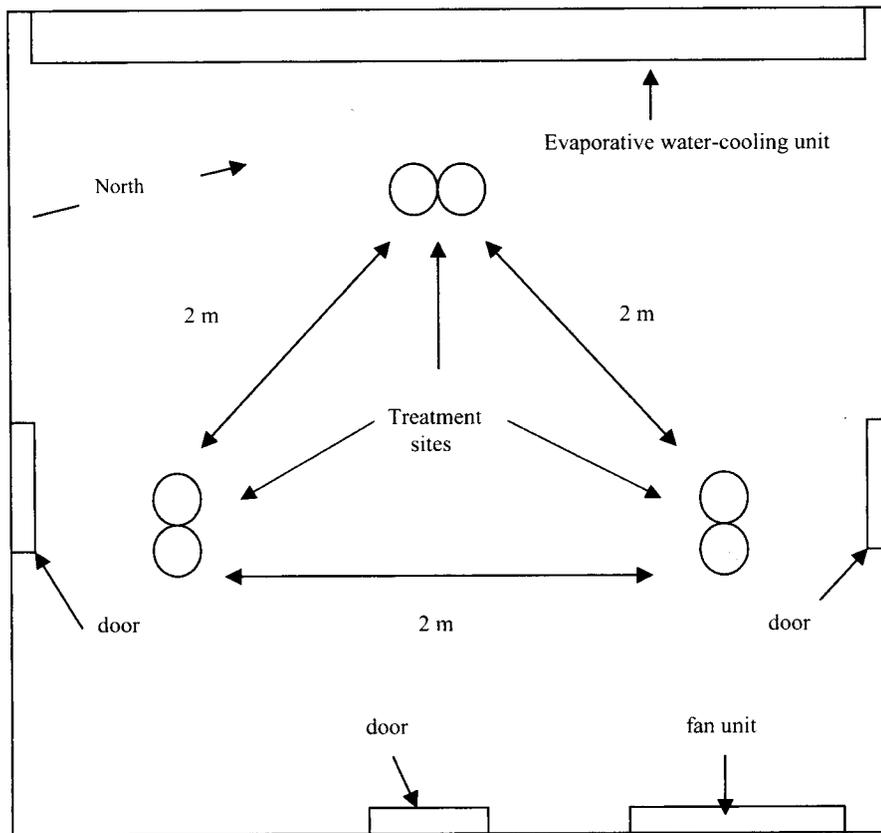


Fig. 1. Diagram of the greenhouse (not to scale) used to house the black soldier fly colony and to examine oviposition site finding behavior. Two treatments were paired at three sites.

light (Osron Sylvania Inc., Danvers, MA) was used and then subsequently a 430-watt Pro Ultralight Light System® (Hydrofarm Inc., Petaluma, CA) as the light source, but mating was not observed with either light used and infertile eggs were oviposited (D. C. S., unpubl. data), which also demonstrates light is not important for soldier fly oviposition. However, once a 60 × 90 cm window was placed in the wall of the room thereby exposing the soldier flies directly to sunlight, mating was observed and consequently fertilized eggs deposited.

Although visible light positively regressed with percentage of adults to mate, it might not be the primary variable regulating this behavior. The eyes of male black soldier flies might cue in on specific wavelengths in sunlight other than those measured in our experiment. The absence of these other wavelengths in the artificial lights described previously might explain why mating did not occur and infertile eggs were deposited. The ability of other insects to detect wavelengths of light not visible to humans has been documented. Males of the black fly, *Cnephia dacotensis* (Dyar and

**Table 1. Regression equations for black soldier fly mating and oviposition response with temperature, humidity light intensity, time of day, latency after emergence for individuals maintained in a 1.5 x 1.5 x 3 m nylon cage placed in a greenhouse in Tifton, GA**

Number per observation period	Equation of fitted response
mating	$y = 0.51 - 0.01(h) + 0.31(\log_{10}[L + 1]); r^2 = 0.49; P < 0.0001$
oviposition	$y = -0.78 + 0.01(h) - 0.01(\text{hum}) + 0.03(t); r^2 = 0.58; P < 0.0001$
Percent* per day	
mating	$y = -579.33 + 1006.40(x) - 530.38(x^2) + 112.01(x^3) - 8.3(x^4); r^2 = 0.99; P < 0.0001$
oviposition	$y = -387.2 + 789.04(x) - 531.89(x^2) + 143.15(x^3) - 13.03(x^4); r^2 = 0.99; P < 0.0001$

\* Percent data arcsine transformed prior to analysis; h, time of day; L, light intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); hum, humidity; t, temperature; x, day after emergence.

Shannon) (Diptera: Simuliidae), are dependent on sunlight to locate mates (McIver and O'Grady 1987). Specifically, their ommatidia are specialized for detection of ultraviolet light (Gates 1980). Therefore, in order to better understand the biology of the black soldier fly, additional research is needed to determine which parts of the light spectrum are influencing its mating behavior.

During our experiment 263 egg clutches were collected during 56 of the 134 intervals between observations and temperature and humidity were determined to positively regress with clutches oviposited. All clutches were deposited when the temperature was greater than 26°C. As mentioned above, the black soldier fly flourishes during warm temperatures and has a known distribution throughout the tropic and warm temperate regions. However, the lower temperature limits for black soldier fly activity are not known only that a colony can be maintained at 22°C (D. C. S., unpubl. data). Additionally, unlike mating, oviposition positively regressed with observation period (time of day) with more clutches deposited later in the day.

Humidity positively regressed with oviposition (Table 1). Eighty percent of the clutches were deposited when humidity exceeded 60%, which was recorded during 75% of the observations when oviposition occurred. Additionally, approximately 5 clutches were laid per interval between observations when the humidity exceeded 60%, which was 40% greater than that recorded during lower humidity levels. Humidity is known to affect oviposition of other insect species. Canyon et al. (1999) determined that low humidity in association with food significantly delayed *Aedes aegypti* (L.) (Diptera: Culicidae) oviposition. Christopher (1960) suggested this delay is an advantage because eggs deposited during periods of low humidity are susceptible to desiccation. This explanation might also be true in the case of the black soldier fly, which is active during the drier months in the southeastern United States (Sheppard et al. 1994).

It is evident from the stepwise regression analysis (SAS Institute 1992) of the data

that mating and oviposition are not a linear function of latency after emergence. Models simpler than the selected fourth-degree polynomial equation (Table 1) were not significant ( $P < 0.05$ ) and had lower  $r^2$  values. During the experiment, mating and oviposition were primarily restricted to one day each, which were explained by the curvilinear nature of the selected models. Sixty-nine percent of mating occurred 2 d, and 72% of oviposition 4 d, post-emergence (Table 2).

Early mating and oviposition by the black soldier fly might be due to several biological factors. Black soldier fly adults provided water, but not food, have short lives (10 to 14 d) (Tomberlin et al. 2002), but are still able to reproduce in a colony (Sheppard et al. 2002). It is hypothesized that adults in the wild may not need to feed but rely on energy stored in their fat body during the larval stage (Tomberlin et al. 2002), which may explain their short life span. Prepupae collected from the field are approximately 35% fat and 42% protein dry matter (Newton et al. 1977). In contrast, house fly pupae are approximately 9% (Teotia and Miller 1974) to 15% fat (Calvert et al. 1969), and resulting adults have to feed in order to reproduce. Additionally, Tomberlin et al. (2002) dissected virgin and mated black soldier fly females and determined that oocytes were not present until 2 d after emergence and completely formed eggs 2 d after mating. Individuals dissected 3 d after ovipositing contained no visible fat or developing ovaries (Tomberlin et al. 2002). Accordingly, individuals collected from the wild typically die after depositing their egg clutch (D. C. S., unpubl. data), which may be a consequence of the fat body having been depleted (Tomberlin et al. 2002). If the hypothesis listed above is accurate, soldier flies failing to mate early after emergence risk reduced clutch size due to oocytes being reabsorbed and utilized to sustain respiration instead of being fertilized and deposited.

**Influence of conspecific larvae on oviposition preference.** We determined that oviposition preferences within treatments differed significantly across replicates ( $F = 4.61$ ;  $df = 7, 48$ ;  $P < 0.0001$ ) which may be due to variation in environmental conditions recorded in the greenhouse. Humidity ranged from 30 to 85%, while light intensity varied from 3 to 800  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Temperature also varied from 24.8 to 38.4°C. Additionally, as described above, number of adult flies in the colony during the experiment varied from 500 to 2000. Considering that approximately 60% of emergent adults are typically female (Tomberlin et al. 2002), the number of potential clutches

**Table 2. Days from emergence to mating and oviposition  $\pm$  SE (n = 3) for 750 adult black soldier flies released in a 1.5 x 1.5 x 3 m nylon cage placed in a greenhouse in Tifton, GA**

Days after emergence	% Mating	% Ovipositing
1	7.8 $\pm$ 7.5ab	0 $\pm$ 0a
2	68.8 $\pm$ 6.8c	0 $\pm$ 0a
3	16.70 $\pm$ 2.1a	12.9 $\pm$ 10.3b
4	2.9 $\pm$ 1.2b	72.9 $\pm$ 11.12c
5	3.7 $\pm$ 0.2b	12.9 $\pm$ 3.2b
6	0 $\pm$ 0b	1.2 $\pm$ 1.2a

Means within a column followed by different capital letters differ significantly ( $P < 0.05$ ; LSD, SAS Institute 1992).

deposited per replicate could vary from 300 to 1200, which would also contribute to the variation in number of egg clutches deposited across replicates.

Similar to Kemppinen (1998), we did not determine a significant difference ( $t = 0.33$ ;  $df = 108$ ;  $P = 0.7433$ ) in oviposition preference for resources with and without conspecific larvae (Table 3), which contradicts other published information. Booth and Sheppard (1984) determined that larval resource type significantly influenced oviposition-site selection by gravid adults. They suggested that the presence of soldier fly larvae could also influence site selection. Furman et al. (1959) reported that black soldier fly adults were attracted to freshly exposed conspecific larvae in hen manure and oviposition occurred. The manure that initially contained soldier fly larvae had "large numbers" of young larvae 3 wk later and nearby manure that did not initially contain soldier fly larvae "remained negative for larvae".

In conclusion, our results indicate that mating and oviposition behaviors are mediated by environmental cues, as well as time of day. Intensity of sunlight plays a major role in determining when soldier flies will mate, while temperature and humidity significantly regress with oviposition behavior of the soldier fly. Latency after emergence significantly regressed with mating and oviposition. However, optimal conditions for these behaviors to occur are still not known. Such information would be useful in improving current black soldier fly rearing methods (Sheppard et al. 2002) and would provide insight to cues regulating mating and oviposition behaviors. Additionally in this study, we recorded no discernible differences for number of egg clutches laid in Gainesville larval media inoculated and not inoculated with black soldier fly larvae. Although anecdotal, personal observations (J. K. T., unpubl. data) and those by Furman et al. (1959) indicate that conspecific larvae are attractive to ovipositing adults. If true, such information could prove vital for maintaining a colony, as well as initiating colonization of manure in livestock and poultry facilities by wild populations. Therefore, additional research in a more controlled setting (i.e., set adult numbers and environmental conditions) is needed to determine if ovipositing black soldier flies are truly attracted to conspecific larvae.

**Table 3. Mean number of egg clutches  $\pm$  SE deposited by black soldier flies in Gainesville house fly larval diet inoculated, and not inoculated with, conspecific larvae**

Day	n	Egg clutches collected from media inoculated with soldier fly larvae	Eggs collected from media not inoculated with soldier fly larvae
1	8	10.57 $\pm$ 4.95a	4.43 $\pm$ 2.64a
2	8	29.14 $\pm$ 9.13a	22.86 $\pm$ 10.06a
3	8	27.86 $\pm$ 9.56a	27.28 $\pm$ 12.32a
4	8	31.71 $\pm$ 8.27a	30.85 $\pm$ 9.19a
5	8	25.14 $\pm$ 6.65a	41.43 $\pm$ 15.69a
6	8	23.43 $\pm$ 7.28a	26.29 $\pm$ 8.89a
7	7	31.00 $\pm$ 9.27a	23.33 $\pm$ 6.68a
8	8	15.43 $\pm$ 3.24a	28.00 $\pm$ 5.83a

Means across treatments on the same day followed by different lower case letters differ significantly ( $P < 0.01$ ; LSD, SAS Institute, 1992).

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