# Substrate Effects on Pupation and Adult Emergence of *Hermetia illucens* (Diptera: Stratiomyidae)

Author(s): L. A. Holmes , S. L. Vanlaerhoven , and J. K. Tomberlin
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# Substrate Effects on Pupation and Adult Emergence of *Hermetia illucens* (Diptera: Stratiomyidae)

L. A. HOLMES,[1,2] S. L. VANLAERHOVEN,[3] AND J. K. TOMBERLIN[4]

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**ABSTRACT** Black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), are of particular interest for their applications in waste management. Feeding on decaying organic waste, black soldier flies successfully reduce manure in confined animal feeding operations of poultry, swine, and cattle. To optimize waste conversion in confined animal feeding operations and landfill facilities, it is imperative to optimize black soldier fly development. Unfortunately, black soldier flies only convert waste during their larval feeding stages and therefore it is of interest to optimize the nonfeeding stages of development, specifically, the postfeeding and pupal stages. The time spent in these stages is thought to be determined by the pupation substrate encountered by the postfeeding larvae. The objective of this study was to determine the effect different pupation substrates have on postfeeding development time, pupation time, and adult emergence success. Five pupation substrates were compared: wood shavings, potting soil, topsoil, sand, and nothing. Postfeeding larvae took longer to reach pupation in the absence of a pupation substrate, although reaching pupation in the shortest time in potting soil and wood shavings. The time spent in the pupal stage was shortest in the absence of a pupation substrate. However, fewer adults emerged when a pupation substrate was not provided.

**KEY WORDS** waste management, rearing media, adult emergence, insect colony maintenance, postfeeding larvae

The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is a temperate and tropical species that develops in decomposing organic material with three generations per year in the southeastern United States (Tomberlin et al. 2002). Females mate once with one oviposition event in their lifetime and selectively oviposit their eggs in dry crevices near a moist food resource approximately 2 d after successful copulation (Tomberlin et al. 2002). Feeding on Gainesville Diet (Hogsette 1992), black soldier fly larvae require an average of 22 d of larval development (Tomberlin et al. 2002), with temperature dependent pupation averaging 15–17 d and temperature dependent adult longevity ranging from 12 to 17 d (Tomberlin et al. 2009). Postfeeding larvae of many dipteran species, including black soldier flies, search for suitable pupation substrates to bury themselves for protection from predation and desiccation while they undergo metamorphosis (Lima et al. 2009). This movement of postfeeding larvae away from feeding sites is initiated by an innate behavioral sequence and mediated by the abiotic environment including temperature, light, and soil moisture, such that the larvae tend to migrate toward cool, dark, and dry substrates (Gomes et al. 2006).

Insects colonizing patchily distributed resources often encounter a diversity of available pupation substrates. The black soldier fly's habitat is patchily distributed, and includes various organic compositions including animal waste, spoiled fruits and vegetables, fish rendering waste, and carrion (Lord et al. 1994; Sheppard et al. 1994; Tomberlin et al. 2002, 2005; Erickson et al. 2004; St-Hilaire et al. 2007; Liu et al. 2008; Myers et al. 2008; Pujol-Luz et al. 2008; Diener et al. 2009). These habitats can facilitate or impede larval development (Lord et al. 1994; Tomberlin et al. 2002, 2005).

Previous research on black soldier flies has been limited to their use in waste management (Booram et al. 1977; Booth and Sheppard 1984; Sheppard et al. 1994, 2002; Tomberlin and Sheppard 2002; Tomberlin et al. 2002, 2009; Holmes et al. 2012) with a goal of maintaining a self-sustaining colony for year-round waste conversion. A neglected aspect in previous research is the effect pupation substrate has on black soldier fly development and survivorship. The objective of this study was to determine the effect of pupation substrate on black soldier fly development, specifically the time spent in the postfeeding and pupal stages of development with an emphasis on suc-

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[1] Department of Biology, Queens University, 99 University Ave., Kingston, ON, Canada, K7L 3N6.

[2] Corresponding author: Leslie Holmes, Department of Biology, Queen's University, 116 Barrie St., Kingston, ON, Canada, K7L 3N6. (e-mail: 12lh22@queensu.ca).

[3] Department of Biology, University of Windsor, 401 Sunset Ave., Windsor, ON, Canada, N9B 3P4.

[4] Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX, 77843–2475.

cessful adult emergence, and to define an optimal pupation substrate for rearing black soldier fly.

## Materials and Methods

**Ethical Declaration.** On behalf of the University of Windsor in Windsor, ON, Canada, all experimental methodologies in this research were performed in compliance with all legalities in Canada. The authors on this research have no conflict of interest with the funding agencies; however, L. A. Holmes was employed as a teaching assistant by the University of Windsor throughout the duration of this study.

**Source of Larvae.** Eggs were collected from an *H. illucens* laboratory colony maintained in a greenhouse at the University of Windsor, Windsor, ON, Canada. The laboratory colony originated from eggs used in Dr. Craig Sheppard's colony in Tifton, GA, USA. The greenhouse was maintained on varied temperatures (20–40°C), at ambient RH (20–50%), a photoperiod of 14:10 (L:D) h, and depending on season. Eggs for each replicate across pupation substrate treatments were collected within a 4-h window. Eggs were collected in a three layer, 2- by 5-cm roll of corrugated cardboard, held together with masking tape (3M, St. Paul, MN) with 3- by 4-mm flutes used as an oviposition substrate, taped 5 cm above the oviposition attractant with the flutes perpendicular to the attractant. Oviposition attractant was composed of moist-to-liquefied 18% protein Purinature Layena poultry laying chow, (code number 6498: corn meal, soybean meal, wheat, canola meal, and pork meal) (Essex Feed Warehouse, Essex, ON, Canada).

**Larval Development.** Collected eggs were homogenized and placed into a 946-ml clear plastic Gladware container (The Clorox Company of Canada, Brampton, ON, Canada) covered with a laboratory paper towel and maintained in a growth chamber at 28°C, 70–80% RH, and a photoperiod of 14:10 (L:D) h until egg eclosion. An iButton data logger (Alpha Mach Inc., MT. St-Hilaire, QC, Canada) was placed in the growth chamber to record temperature every 30 min throughout the duration of the experiment. Upon egg eclosion, larvae were fed 20 g of dry poultry laying chow mixed with 42 ml of water for 70% moisture content ad libitum for 4 d to allow larvae to develop to a more manageable size. Three hundred 4-d-old larvae then were transferred into 0.5-liter mason jars with a layer of black landscaping cloth (Home Depot, Windsor, ON, Canada) as a breathable lid held in place by the hollow metal screw cap to prevent larvae from escaping and returned to the growth chamber. Feeding continued ad libitum until the postfeeding stage was reached (≈20 d from oviposition), which is visually identified by the larvae changing color from cream to black (Tomberlin et al. 2005).

**Treatments.** The aforementioned jars of feeding larvae were removed from their food and combined into one container upon reaching the postfeeding stage. The postfeeding larvae then were divided into five pupation substrate treatments: wood shavings (NEPCO, Warrensburg, NY), topsoil (Maidstone Garden Products, Maidstone, ON, Canada), potting soil (Premier Pro-Mix BX, Essex, ON, Canada), playing sand (Canadian Tire, Windsor, ON, Canada), and no substrate, such that each larvae had an equal opportunity of being selected for each treatment. Pupation substrates were chosen based on current laboratory practices such as the use of wood shavings (the laboratory standard) and previous research in our laboratory on the effect of pupation substrate on blow fly development. The wood shavings used were a virgin hardwood blend beta chip (maple, beech, birch, or poplar). The topsoil used contained Canadian sphagnum peat moss (80–85%/vol), Endomycorrhizae (fungi symbionts), perlite (to increase air space and water drainage), vermiculite (to hold water and fertilizer), dolomitic calcitic limestone (to regulate pH), macronutrients (a proprietary composition of nitrogen, phosphorus and potassium), micronutrients (a proprietary composition of calcium, zinc and magnesium), and a wetting agent. The potting soil used contained Canadian sphagnum peat moss, soy-based natural fertilizer, limestone, perlite, gypsum, and mycorise premier tech. The sand used was prewashed and contained <3% crystalline silica.

Substrate tests were conducted in clear plastic 2.2-liter Ziplock tubs (SC Johnson Canada, Brantford, ON, Canada) with their corresponding lids and a 15-cm depth of their respective pupation substrate. The centers of the lids were removed and replaced with black landscaping cloth to allow air exchange and prevent escape; 50 ml of water to maintain moisture was added to each pupation substrate, with the exception of the no substrate control treatment, before the addition of larvae. Five replicate tubs were established for each substrate, each containing 100 postfeeding larvae. All treatment containers were placed in the growth chamber until adult emergence.

Postfeeding larvae were monitored every 12 h to record the time of pupation, indicated when the puparium reached a completely rigid state with no elasticity. Upon pupation, pupae were placed into 100- by 25-mm Fisherbrand petri dishes (Fisher, Ottawa, ON, Canada) with a layer of their respective pupation substrate on a piece of moist filter paper (Fisher, Ottawa, ON, Canada) to prevent desiccation. Petri dishes of pupae were returned to the growth chamber, with 12-h observations for adult emergence.

## Statistics

**Software.** All statistics were computed using SAS JMP 8.0.1 statistical software.

**Postfeeding and Pupal Development.** The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts ($\log_{10}$ and square root transformations). Thus, a nonparametric one-way Wilcoxon and Kruskal–Wallis (ranked sums) test was used to test both treatment effects on the mean time to pupation as well as the mean time to adult emergence, independently. Pairwise comparisons using Wilcoxon and Mann–Whitney *U*-tests were used on

**Table 1. Pupation substrate effects on length of postfeeding and pupal development and successful adult emergence**

| Pupation substrate | Length of development Stage (d) | | Successful adult emergence (%) |
|---|---|---|---|
| | Post-feeding | Pupal | |
| No substrate | 10.28 ± 0.17A | 6.74 ± 0.03A | 87.60 ± 5.50A |
| Sand | 8.44 ± 0.12B | 8.86 ± 0.64B | 96.80 ± 2.95B |
| Topsoil | 7.93 ± 0.072B | 6.89 ± 0.69B | 98.00 ± 2.12B |
| Wood shavings | 7.71 ± 0.09C | 6.93 ± 0.64B | 95.60 ± 4.50B |
| Potting soil | 7.56 ± 0.05C | 6.92 ± 0.62B | 97.20 ± 2.28B |

Means for development times and successful adult emergence followed by different letters within the same column are significantly different, ($P < 0.05$, Wilcoxon/Kruskal–Wallis, Cox proportional hazard, SAS JMP 8.0.1).

significant results. Alpha values were adjusted using the Dunn–Sidak procedure to correct for type I error as a result of multiple comparisons (Quinn and Keough 2002).

**Successful Adult Emergence.** A one-way analysis of variance with the Tukey–Kramer HSD to compare means was used to test the significance of treatment effects on percent successful adult emergence.

## Results

**Postfeeding Development.** The effect of pupation substrate on the mean time to reach the pupal stage varied between pupation substrates (Table 1; $\chi^2 = 253.69$, df = 4, $P < 0.001$), with postfeeding larvae in no substrate taking longer to pupate than all other substrates. There was no difference in the length of the postfeeding stage between larvae placed in sand or topsoil ($\chi^2 = 1.2108$, df = 1, $P = 0.2712$). Also, postfeeding larvae in potting soil and shavings did not differ ($\chi^2 = 0.7994$, df = 1, $P = 0.3713$); however, postfeeding larvae in both sand and topsoil took longer to reach pupation than postfeeding larvae in potting soil and shavings ($\chi^2 = 31.4185$, df = 3, $P < 0.001$).

**Pupal Development.** Pupae in no substrate took less time to eclose than any of the other treatments (Table 1; $\chi^2 = 27.97$, df = 4, $P < 0.001$) and there were no differences between the other treatments in the amount of time required to complete the pupal stage of development ($\chi^2 = 0.19244$, df = 3, $P = 0.2414$).

**Successful Adult Emergence.** Fewer adults emerged in no substrate than any of the other pupation substrates (Table 1, $F_{4,20} = 4.62$, $p = 0.0008$). The remaining substrates did not differ in the number of emerged adults.

## Discussion

Pupation substrate, or lack thereof, significantly impacts black soldier fly development, specifically the larva's ability to pupate, emerge, and survive as an adult until copulation events. Postfeeding larvae took the longest to pupate in no substrate, as expected. A likely explanation for this is the larva's innate behavioral strategy to bury itself as a protection mechanism from predators and desiccation (Lima et al. 2009). In the absence of a pupation substrate, postfeeding larvae proceeded to disperse in what was assumed a search for a substrate to bury themselves in, which appeared to delay their pupation. Furthermore, postfeeding larvae in no substrate aggregated together (L.A.H., unpublished data). This behavior may have been an effort to increase the temperature, because of the lack of a pupation substrate to serve as an abiotic barrier blanket, or perhaps a thigmotatic response in an effort to try to bury themselves in each other.

Unexpectedly, pupae developing in no substrate emerged faster than those in the presence of a pupation substrate, regardless of the type of substrate. Insects that do not feed as adults, including the black soldier fly, have a limited amount of stored energy that is allocated to adult fitness, including mating, flight, and ovarian development (Nilssen 1997). Schmolz and Lamprecht (2000) suggested that energy depletion is highest in the postfeeding stage of development because of catabolism instead of anabolism. Therefore, it is likely postfeeding larvae in the no substrate treatment that did not emerge, expended too much energy and as a result, could not energetically afford metamorphosis and died. However, this does not support why those that were able to complete metamorphosis, were able to do so before those in the presence of a pupation substrate. As discussed above, a pupation substrate provides protection from predation (Lima et al. 2009). Therefore, we postulate that pupae developing in no substrate may have completed metamorphosis in less time to reduce their risk of predation, however further studies would be necessary to support this theory.

Two factors we observed differing among the selected substrates were moisture content and compaction. We suspect these two factors may have impacted postfeeding larval dispersal and pupation. Substrate compaction can have significant effects on a postfeeding larva's ability to pupate; the denser the substrate, the more impenetrable it may be for postfeeding larvae (Dimou et al. 2003). In our experiment none of the pupation substrates used was mechanically compacted during the course of the experiment. However, each substrate had a different natural compaction density. Although this trait was not measured, it was observed qualitatively that sand had the highest compaction density with very little free space between sand aggregates, followed by topsoil, wood shavings, and potting soil. Based on our results, postfeeding larvae in lower compaction density substrates (wood shavings and potting soil) took less time to pupate than those in high compaction density substrates (sand and topsoil), suggesting that low density substrates are more penetrable, facilitating pupation, whereas high density substrates are less penetrable for postfeeding larvae and therefore impede pupation.

Moisture content of the pupation substrate can also have significant impacts on pupation and emergence success. Of the substrates used in this experiment, topsoil and potting soil retained water longer than sand and wood shavings, with sand quickly becoming dry and dusty. This was observed at the beginning of the experiment when topsoil aggregates formed as a result of adding water. As well, the perlite and vermiculite aggregates in the potting soil enabled it to retain moisture longer than all other substrates. Several studies have demonstrated that moist soil substrates support higher pupation success than dry soil substrates, regardless of soil type (Alyokhin et al. 2001, Dimou et al. 2003, Ellis et al. 2004, Chen and Shelton 2007). The ability of potting soil to retain some degree of moisture may have had a significant impact on the larva's reduced search for a pupation site in the substrate.

Interestingly, with potting soil appearing less compact and able to retain more moisture than wood shavings, but the larvae in both treatments did not differ in their times to pupation, it is possible that moisture content and compaction have interacting properties that may affect pupation in the black soldier fly. It is also possible that lack of moisture in the no substrate treatment may have caused desiccation; however, because the growth chamber was maintained at 70–80% RH throughout development, and our previous research demonstrates that black soldier flies reared in 1-oz cups at 70% RH with no pupation substrate have 93% emergence success (Holmes et al. 2012), it is unlikely these pupae desiccated. However, dissections on all pupae that failed to emerge were not done to confirm this deduction.

In summary, the presence of a pupation substrate has a significant impact on facilitating postfeeding larvae to pupate, regardless of the type of substrate. In the absence of a pupation substrate, postfeeding larvae are slightly delayed from pupating. For the purpose of our objective, wood shavings, the current laboratory standard for blow fly rearing would be considered more feasible for mass rearing, with efficient pupation and increased successful adult emergence and longevity.

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