

# Development of Black Soldier Fly (Diptera: Stratiomyidae) Larvae Fed Dairy Manure

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**ABSTRACT** Black soldier flies, *Hermetia illucens* L., are a common colonizer of animal wastes. However, all published development data for this species are from studies using artificial diets. This study represents the first examining black soldier fly development on animal wastes. Additionally, this study examined the ability of black soldier fly larvae to reduce dry matter and associated nutrients in manure. Black soldier fly larvae were fed four rates of dairy manure to determine their effects on larval and adult life history traits. Feed rate affected larval and adult development. Those fed less ration daily weighed less than those fed a greater ration. Additionally, larvae provided the least amount of dairy manure took longer to develop to the prepupal stage; however, they needed less time to reach the adult stage. Adults resulting from larvae provided 27 g dairy manure/d lived 3–4 d less than those fed 70 g dairy manure. Percentage survivorship to the prepupal or adult stages did not differ across treatments. Larvae fed 27 g dairy manure daily reduced manure dry matter mass by 58%, whereas those fed 70 g daily reduced dry matter 33%. Black soldier fly larvae were able to reduce available P by 61–70% and N by 30–50% across treatments. Based on results from this study, the black soldier fly could be used to reduce wastes and associated nutrients in confined bovine facilities.

The black soldier fly is considered a beneficial insect in confined animal facilities for its tendency to colonize animal waste (Sheppard et al. 1994). Colonization of poultry and swine manure can reduce house fly, *Musca domestica* L. (Diptera: Muscidae), populations 94–100% and accumulated manure mass ≈50% (Sheppard 1983). Additionally, black soldier fly prepupae can be used as animal feed (Newton et al. 1977, Sheppard et al. 1994).

Three core laboratory studies examined fundamental developmental biology of the black soldier fly: Furman et al. (1959), May (1961), and Tomberlin et al. (2002). Black soldier fly larvae fed a Chemical Specialties Manufacturers' Association (CSMA) standard fly larval diet reach prepupal stage in ~2 wk at 30°C (Furman et al. 1959). Prepupae migrate from the food source to pupate (Sheppard et al. 1994). Adults emerge after 10–14 d at 27–30°C (Sheppard et al. 2002). Adult black soldier flies do not require food to survive but have been found to live longer when provided with a source of water (Tomberlin et al. 2002).

Two-day-old adults mate and females oviposit 2 d after copulation (Tomberlin and Sheppard 2002). Females tend to lay eggs in crevices near food sources, with eggs hatching after 4 d at 27°C (Booth and Shep-

pard 1984). Humidity >60% and temperature >26°C resulted in ≈80% egg hatch (Tomberlin and Sheppard 2002).

Development data available for the black soldier fly are limited to studies using an artificial diet (Furman et al. 1959, May 1961, Tomberlin et al. 2002). Consequently, predicting the black soldier fly life cycle under field conditions in confined animal facilities is based on extrapolations from these previous studies. The primary objective of this study was to examine the life history traits of black soldier flies fed four different feed rates of dairy manure, a previously unexamined dietary medium. Additionally, reduction of dry matter and associated nutrients in dairy manure by black soldier fly larvae was examined.

## Materials and Methods

**Acquisition of Flies.** The experiment was performed using a black soldier fly colony established at the Texas Agricultural Experiment Station (TAES), Stephenville, TX. Eggs used to establish the TAES colony originated in 2003 from a colony at the Coastal Plain Experiment Station, University of Georgia, Tifton, GA. Methods for collecting black soldier fly eggs and rearing the larvae were adapted from Sheppard et al. (2002). Eggs were collected and placed in a 0.5-liter plastic container, covered with a paper towel held in place with a rubberband, and stored in E-30B Percival Growth Chambers (Percival, Boone, IA; 27°C, 60% RH, and a photoperiod of 16:8 [L:D] h) until larvae emerged after 4 d. Once larvae were observed, 30 g Gainesville diet (Hogsette 1985) mixed with 51 ml

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**Table 1.** Percentage black soldier fly survival  $\pm$  SE to the pupal and adult stages when fed four daily dairy manure regimens under controlled conditions ( $N = 4$ )<sup>a</sup>

Daily feed rate (g)	Percent survival to prepupal stage				Percent survival to adult stage		
	Total	Female	Male	No Sex	Total	Female	Male
27	77.33 $\pm$ 4.55	36.70 $\pm$ 3.86a	41.10 $\pm$ 3.47a	22.20 $\pm$ 6.63ABC,a	61.65 $\pm$ 2.74	46.80 $\pm$ 2.09	53.20 $\pm$ 2.09
40	84.50 $\pm$ 4.30	36.20 $\pm$ 1.39ab	36.50 $\pm$ 1.67a	27.30 $\pm$ 2.87AB,b	63.82 $\pm$ 4.92	49.90 $\pm$ 0.82	50.10 $\pm$ 0.82
54	78.67 $\pm$ 7.95	42.20 $\pm$ 2.53a	38.30 $\pm$ 2.97a	19.50 $\pm$ 3.50ABC,b	65.78 $\pm$ 6.22	52.50 $\pm$ 2.39	47.50 $\pm$ 2.39
70	71.25 $\pm$ 4.92	42.25 $\pm$ 1.88a	41.93 $\pm$ 0.59a	15.85 $\pm$ 1.70AC,b	62.03 $\pm$ 3.37	50.12 $\pm$ 1.32	49.88 $\pm$ 1.32

Means in a column followed by different capital letters are significantly different. Means for both sexes and no sex within a treatment with different lowercase letters are significantly different ( $P < 0.05$ , LSD; SAS Institute 1998). Values in columns or rows not followed by letters were not significantly different across treatments. Percentage data were arcsine transformed prior to analysis.

<sup>a</sup> 27°C, 60% relative humidity, and a photoperiod of 16:8 (L:D) h.

water was provided to the larvae. Larvae were fed 4 d before their use in the experiment. Voucher specimens were placed in the arthropod collection at the TAES, Stephenville, TX.

**Experimental Design.** The experiment was replicated four times from April through December 2005. One black soldier fly generation supplied eggs across treatments for each replicate. Three hundred 4-d-old larvae were placed in each of four containers (5.678-liter; Sterilite, Townsend, MA), covered with a 50- by 35-cm piece of T-310 100% nylon tulle (Wal-Mart Stores, Bentonville, AR), which was held in place with a rubberband. A lid with a 12- by 8-cm rectangle cut in its center also was used to cover each container. The double coverage was to prevent larvae from escaping. All containers were stored in the rearing chamber previously described with the aforementioned environmental parameters. Each container with larvae was randomly assigned one feeding regimen of (1) 27, (2) 40, (3) 54, or (4) 70 g of dairy cow, *Bos taurus* L., manure daily. Preliminary studies indicated 27 and 70 g manure daily were the thresholds for black soldier fly development and manure reduction. When 40% of the larvae in a treatment reached the prepupal stage, feeding was terminated for that particular treatment. However, daily observations were made until all larvae had reached the prepupal stage or died.

**Immature Life History Traits.** For each container, 10 larvae were randomly selected each day, individually weighed on an Adventurer Pro balance (Ohaus, Pine Brook, NJ), and returned to their respective containers. The mean larval weight recorded on the date that 40% of the larvae within a treatment reached the prepupal stage defined the final larval weight. To differentiate life stages, prepupae had a characteristic black cuticle, whereas larvae were white (May 1961).

Prepupae were removed daily from all treatments and individually weighed using the balance previously mentioned. Prepupae were placed individually in 35-ml cups, covered with a breathable lid (Bio-Serv, Frenchtown, NJ), and returned to the growth chamber. Each lid was labeled with information used for identification. Cups containing prepupae were monitored daily for adult emergence.

**Adult Life History Traits.** General adults were weighed individually, sex was determined, and pupation time was recorded for each treatment. Adult flies were returned to their respective 35-ml capped cup

and returned to the rearing chambers previously mentioned, and longevity was recorded. Adults were provided 0.125 ml water daily using a 1.25-cm needle inserted through the lid.

**Dairy Waste Analysis.** Manure <6-h-old was collected from a commercial dairy located in Stephenville, TX. This local dairy is a commercial production facility where cows are fed a standard, total mixed ration diet ad libitum. Collected manure was placed in 3.8-liter Ziploc bags and stored frozen in a -20°C Kenmore Elite heavy duty commercial chest freezer (Sears, Roebuck and Company, Chicago, IL) at the TAES, Stephenville, TX, for 1 wk before use. Each bag of manure used for feeding was allowed to thaw for  $\approx$ 24 h before use. One hundred-gram samples were taken from each bag and returned to the freezer. These "predigested" samples and the total "digested" manure volume from each treatment were shipped to the Soil, Water, and Forage Testing Laboratory in College Station, TX, for analysis of dry matter, N, and P mass. Digested manure was defined as the waste product remaining in a container after removal of all larvae. Percentage dry matter was determined using the gravimetric method (Franson 1989). Total P was measured using the nitric acid digest method (Feagley et al. 1994). Nitrogen was measured using the Dumas combustion method (Sheldrick 1986).

**Statistical Analysis.** Larval and pupal development times, final larval, prepupal, and adult weights, and adult longevity were analyzed using the PROC Mixed (SAS Institute 1998). The least significant difference (LSD) test (SAS Institute 1998) was used after a significant  $F$  test ( $P \leq 0.05$ ) to separate mean differences for each variable. All percentage data were arcsine-transformed before analysis.

## Results

**Immature Life History Traits.** Total black soldier fly larval survival was >71% throughout these experiments (Table 1). Less than 20% failed to reach the prepupal stage for those fed between 27 and 54 g dairy manure, whereas those fed 70 g/d suffered 29% mortality. Individuals reaching the prepupal stage but failing to emerge as adults were placed in a separate category including no sex. Larvae fed either 54 ( $F = 10.60$ ,  $df = 2$ ,  $P = 0.0107$ ) or 70 g ( $F = 62.84$ ,  $df = 2$ ,  $P < 0.0001$ ) had a significant difference in survivorship

to the prepupal stage across sexes including no sex. Percentage female survivorship to the prepupal stage was not significantly different across treatments ( $F = 2.08$ ,  $df = 3$ ,  $P = 0.1737$ ). Similar results were determined for males ( $F = 1.33$ ,  $df = 3$ ,  $P = 0.3245$ ), as well as those individuals within the no sex ( $F = 2.27$ ,  $df = 3$ ,  $P = 0.1497$ ) category.

Treatment ( $F = 18.90$ ,  $df = 3$ ,  $P < 0.0001$ ) and sex ( $F = 10.24$ ,  $df = 1$ ,  $P = 0.0038$ ) significantly influenced larval development (Table 2). Replicate was significant ( $F = 15.11$ ,  $df = 3$ ,  $P < 0.0001$ ) in addition to a three-way interaction between replicate, treatment, and sex ( $F = 1.97$ ,  $df = 9$ ,  $P = 0.0389$ ).

Sex-based data were determined for individuals successfully reaching the adult stage. Male and female larvae reared on 54 and 70 g dairy manure required ~1–5 d less to reach the prepupal stage than those at lower feed rates. Larvae reached the prepupal stage in 25–31 d. Final larval weight was significantly influenced by treatment ( $F = 10.07$ ,  $df = 3$ ,  $P = 0.0031$ ) but not replicate ( $F = 2.55$ ,  $df = 3$ ,  $P = 0.1206$ ).

Prepupal weight was significantly different across treatments ( $F = 84.67$ ,  $df = 3$ ,  $P < 0.0001$ ) and sex ( $F = 52.44$ ,  $df = 1$ ,  $P < 0.0001$ ). Replicate was significantly different ( $F = 11.33$ ,  $df = 3$ ,  $P < 0.0001$ ). An interaction between replicate, treatment, and sex was determined ( $F = 2.97$ ,  $df = 9$ ,  $P = 0.0016$ ). Male and female prepupae fed 70 g weighed ~35% more than those fed 27 g.

Pupal developmental time differed significantly across treatment ( $F = 6.80$ ,  $df = 3$ ,  $P = 0.0018$ ) but not sex ( $F = 0.35$ ,  $df = 1$ ,  $P = 0.5607$ ). Replicates differed significantly ( $F = 8.69$ ,  $df = 3$ ,  $P = 0.0004$ ) within treatment. Days for pupal development increased with greater feed rates but decreased at the 70-g feed rate. Individuals reached the adult stage between 15.8 and 17.2 d regardless of feed rate.

**Adult Life History Traits.** Mean percentage failing to survive from larva to adult was ~37% (Table 1). No significant difference was determined between percentage survival of male ( $F = 1.44$ ,  $df = 3$ ,  $P = 0.2955$ ) and female ( $F = 1.44$ ,  $df = 3$ ,  $P = 0.2955$ ) larvae to the adult stage across treatments, respectively. Percentage male survival was slightly higher than female at 27 and 40 g, whereas percentage female survival was greater than male survival at the two greater feed rates.

Adult weight differed significantly across treatment ( $F = 74.13$ ,  $df = 3$ ,  $P < 0.0001$ ), sex ( $F = 84.53$ ,  $df = 1$ ,  $P < 0.0001$ ), and replicate ( $F = 6.81$ ,  $df = 3$ ,  $P = 0.0018$ ; Table 2). Interactions between sex and treatment ( $F = 9.58$ ,  $df = 3$ ,  $P < 0.0001$ ) and sex and replicate ( $F = 4.15$ ,  $df = 3$ ,  $P = 0.0061$ ) were determined. As with prepupal weight, greater adult weight was recorded with increased daily feed rate (Table 2). Males weighed ~18% less than females within treatment.

Daily feed rate ( $F = 32.50$ ,  $df = 3$ ,  $P < 0.0001$ ) and replicate ( $F = 28.64$ ,  $df = 3$ ,  $P < 0.0001$ ) significantly influenced male and female longevity, but sex did not ( $F = 0.21$ ,  $df = 1$ ,  $P = 0.6487$ ). An interaction between

Table 2. Adult and larval black soldier fly life-history traits ± SE fed four daily dairy manure feeding regimens<sup>a</sup>

Daily feed rate (g)	Sex	Final larval weight (g)	Larval development (d)	Prepupal weight (g)	Pupal development (d)	Adult weight (g)	Adult longevity (d)
27	F	—	31.49 ± 0.1564 (366) <sup>b</sup>	0.0966 ± 0.0010 (366)	15.87 ± 0.1328 (366)	0.0409 ± 0.0004 (361)	10.21 ± 0.3937 (360)
	M	—	29.25 ± 0.1627 (346)	0.0820 ± 0.0010 (346)	15.93 ± 0.1381 (346)	0.0335 ± 0.0004 (345)	9.72 ± 0.4073 (343)
	Combined	—	30.39 ± 0.1128A (928)	0.0893 ± 0.0007A (928)	15.90 ± 0.0958A (717)	0.0372 ± 0.0003A (706)	9.96 ± 0.2833A (703)
40	F	—	28.19 ± 0.1570 (367)	0.1107 ± 0.0010 (367)	16.33 ± 0.1333 (367)	0.0449 ± 0.0004 (366)	11.81 ± 0.3930 (365)
	M	—	26.83 ± 0.1568 (371)	0.0958 ± 0.0010 (371)	16.19 ± 0.1331 (371)	0.0375 ± 0.0004 (369)	11.90 ± 0.3933 (368)
	Combined	—	27.51 ± 0.1110B (1014)	0.1033 ± 0.0007B (1014)	16.26 ± 0.0942B (740)	0.0412 ± 0.0003A (735)	11.86 ± 0.2780AB (733)
54	F	—	26.45 ± 0.1517 (392)	0.1323 ± 0.0009 (392)	17.07 ± 0.1328 (392)	0.0543 ± 0.0004 (389)	14.96 ± 0.3802 (388)
	M	—	25.50 ± 0.1617 (363)	0.1145 ± 0.0010 (363)	17.20 ± 0.1373 (363)	0.0446 ± 0.0004 (360)	14.47 ± 0.4047 (360)
	Combined	—	26.17 ± 0.1108B (944)	0.1234 ± 0.0007C (944)	17.13 ± 0.0941BC (763)	0.0494 ± 0.0003B (750)	14.71 ± 0.2776BC (749)
70	F	—	26.43 ± 0.1581 (358)	0.1466 ± 0.0010 (358)	16.56 ± 0.1343 (358)	0.0611 ± 0.0004 (358)	12.86 ± 0.3945 (358)
	M	—	25.26 ± 0.1591 (359)	0.1274 ± 0.0010 (359)	17.01 ± 0.1351 (359)	0.0504 ± 0.0004 (358)	14.42 ± 0.3969 (359)
	Combined	—	0.1786 ± 0.0007B (4)	25.84 ± 0.1122B (855)	0.1370 ± 0.0007D (855)	16.78 ± 0.0952C (723)	13.64 ± 0.2798C (717)

Significant difference, indicated by different capital letters within column, was determined only for combined sex data because the sex × feed rate interaction was not significant for any variable. All data were analyzed using Proc Mixed ( $P \leq 0.05$ ; LSD; SAS Institute 1998).

<sup>a</sup> 27°C, 60% relative humidity, and a photoperiod of 16:8 (L:D) h.

<sup>b</sup> n = sample size.

<sup>c</sup> N = replicate.

**Table 3.** Mass reduction of dairy waste fed  $\pm$  SE for four dairy regimens fed to black soldier fly larvae ( $N = 4$ )<sup>a</sup>

Daily feed rate (g)	Percent reduction		
	Dry matter	P	N
27	58.20 $\pm$ 5.26A	70.39 $\pm$ 10.04	46.40 $\pm$ 13.63
40	54.95 $\pm$ 5.05A	70.90 $\pm$ 9.31	50.42 $\pm$ 9.04
54	50.08 $\pm$ 1.67A	67.42 $\pm$ 6.91	44.30 $\pm$ 5.46
70	33.18 $\pm$ 3.34B	61.53 $\pm$ 8.10	30.49 $\pm$ 7.53

Means in a column followed by different letters are significantly different ( $P \leq 0.05$ ; LSD; SAS Institute 1998). Values in columns not followed by capital letters were not significantly different across treatments. Percentage data were arcsine-transformed before analysis.

<sup>a</sup> 27°C, 60% relative humidity, and a photoperiod of 16:8 (L:D) h.

sex and treatment was determined ( $F = 2.99$ ,  $df = 3$ ,  $P = 0.0300$ ).

Males and females fed either 54 or 70 g lived between 2 and 5 d longer than those on lower feed rates. Longevity of males and females in the same treatment were usually similar, except at 70 g, where males lived  $\approx$ 1.5 d longer than females.

**Dairy Waste Analysis.** Nutrient analysis and dry matter reduction data are presented in Table 3. Percentage dry matter reduction was significantly different across treatment ( $F = 10.07$ ,  $df = 3$ ,  $P = 0.0031$ ). Replicate had no effect ( $F = 2.55$ ,  $df = 3$ ,  $P = 0.1206$ ). Larvae fed 70 g dairy manure/d reduced dry matter  $\approx$ 25% less than those fed 27 g. Phosphorus reduction in dairy waste was not significantly different across treatments ( $F = 1.68$ ,  $df = 3$ ,  $P = 0.2409$ ), but replicate was significant ( $F = 20.64$ ,  $df = 3$ ,  $P = 0.0002$ ). Approximately 67% of the P present in the dry matter dairy manure was removed by black soldier fly larvae for all treatments. N reduction was significantly different across replicate for each treatment ( $F = 10.11$ ,  $df = 3$ ,  $P = 0.0031$ ) but not within treatment ( $F = 2.71$ ,  $df = 3$ ,  $P = 0.1079$ ). Approximately 43% reduction in N mass associated with dairy manure was recorded.

## Discussion

All prior studies examining the life history traits of black soldier fly larvae and adults used artificial diets. CSMA (Furman et al. 1959) and Gainesville diets (Hogsette 1985), which were developed for raising house flies, as well as a 15% protein layer ration (Tomberlin et al. 2002), have been used to raise black soldier flies. May (1961) also examined the development of the black soldier fly but failed to define the diet composition implemented. Data presented here are the first for black soldier flies raised on animal manure.

Food availability can affect larval and adult life history traits (Giberson and Rosenberg 1992, Hunter and McNeil 2000). Black soldier fly larvae are suspected to develop optimally on fresh manure introduced at a low rate (Sheppard 1983); however, no supporting data have been provided. Furman et al. (1959) stated a reduced food supply can extend black soldier fly larval development time up to 4 mo. Before

this study, the appropriate rate to feed black soldier fly larvae dairy manure was not known. Life history traits of black soldier flies fed 54–70 g dairy manure/d were improved compared with larvae fed the lesser feed rate and therefore might be optimal for raising black soldier fly larvae on dairy manure.

Food quality affects insect growth rates and positively correlates with larval length and percentage survival (De Haas et al. 2006). Wild black soldier fly adult weights are typically greater than for specimens reared in the laboratory (Tomberlin et al. 2002). These differences are likely because of nutritional value of the larval resource (Tomberlin et al. 2002). Percentage survival to the prepupal stage was 12–25% less in our study than that recorded by Tomberlin et al. (2002). Larvae held in containers with their own waste might have contaminated and consequently lowered the nutritional value of newly provided manure (Tomberlin et al. 2002).

Parental effects on offspring morphology, development, and population dynamics have been shown for other species (Hunter and McNeil 2000). Temperature and photoperiod are considered to be key variables responsible for parental influence on resulting offspring life histories (Hunter and McNeil 2000). Accordingly, our colony was maintained in a greenhouse, and these same variables could be responsible for the significant differences across generation observed in our study. Many studies, however, do not replicate over time to encompass multiple generations, representing a significant loss of variability. Although replicates in our study were reared under identical conditions, the parents of these flies were housed in an outdoor greenhouse where they experienced light cycles and temperature regimens reflective of seasonality in northcentral Texas.

Adults in this study lived up to 5 d longer than those raised on artificial diets (Tomberlin et al. 2002). This increased longevity may be a result of extended larval development periods, which allowed greater time for storing nutrients. This is not the case with other insects. For example, adult *Schistocerca americana* Drury (Orthoptera: Acrididae) rely only partially on reserves derived during larval development (Hahn 2005). In the case of *H. illucens*, adults are not known to feed but solely depend on nutrients stored during the larval stage (Sheppard et al. 2002).

Black soldier fly development is influenced by moisture of the larval resource (Fatchurochim et al. 1989). Fresh dairy manure can be as high as 87% moisture, as reported by the American Society of Agricultural Engineers (ASAE) (Adhikari et al. 2005). Average percentage moisture of manure in this study was  $\approx$ 70%, whereas the artificial diet studies ranged from 52 to 70% moisture (Furman et al. 1959, Tomberlin et al. 2002). Fatchurochim et al. (1989) determined black soldier flies optimally developed in manure with 40–60% moisture and percentage survival at greater moisture levels was significantly reduced.

Reduction of P and N by black soldier fly larvae is promising for the development of a sustainable manure digestion system in confined animal facilities.

Because the larvae of this species naturally colonize manure, it may prove economically more efficient than usage of lagoons or spreading the manure as fertilizer and possibly increasing P and N levels in the soil and nearby watersheds. The Texas Institute for Applied Environmental Research (TIAER) reported dairy facilities in the Erath County, TX, are responsible for 65% of the N and P in the upper Bosque River (Adhikari et al. 2005). Reducing the mass of these nutrients at the rates found in our study could have a great impact on water quality problems.

Clearly delineating the expected outcome is important when devising a sustainable dairy waste management plan with the black soldier fly. If larvae production for sale as feed was considered the primary objective, higher manure introduction feed rates have to be used to acquire the most nutritious value added product. In contrast, if greater dry matter reduction is desired, the lower manure introduction rates need to be implemented. Regardless, additional research examining optimal development of black soldier fly larvae on animal manure is needed to develop an efficient animal digestion system with this insect.

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