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Lower temperature threshold of black soldier fly (Diptera: Stratiomyidae) development

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RESEARCH ARTICLE

Abstract

The black soldier fly has shown great promise in addressing two environmental concerns: (1) waste management; and (2) protein supplementation for use as feed for livestock, poultry, and aquaculture. Thus, tremendous efforts have been placed on mass-production of the black soldier fly. Currently, little is known about the thermal tolerance limits of black soldier fly eggs and immatures. The objective of this study was to determine the lower temperature threshold for black soldier fly development. Development time, egg eclosion and adult emergence success were measured at 12, 16 and 19 °C. We determined that the lower threshold for egg hatch was between 12 and 16 °C, taking 15 days to hatch. Furthermore, we determined that the lower temperature threshold for larvae is between 16 and 19 °C with egg hatch in 7.75 days at 19 °C. Mean development time from egg to adult at 19 °C was 72 days.

Keywords: aquaculture, *Hermetia illucens*, insect-based protein production, sustainable protein, waste conversion

1. Introduction

By 2008, Canada was producing 34 million tonnes of municipal solid waste per year, in addition to 181 million tonnes of livestock manure waste (Statistics Canada, 2012). While on average, 25% of municipal solid waste is diverted to recycling and composting facilities across Canada, with organic materials comprising 32% by weight, there has been a 16% increase in livestock manure deposits, which is a large contributing factor of greenhouse gas emissions (Statistics Canada, 2012). In 2009, methane emissions attributed by agriculture, including all forms of manure management and deposits were 1000 kilo tonnes, with a total CO_2 emission equivalent of 56,000 kilo tonnes (Statistics Canada, 2012). On the other hand, methane emissions from solid waste disposal and handling on land and incineration were 980 kilo tonnes with a total CO_2 emission equivalent of 22,000 kilo tonnes (Statistics Canada, 2012). Public awareness of environmental consequences of greenhouse gas emissions has resulted in greater efforts to reduce such impacts by optimising product quality and durability while reducing consumption costs and minimising the use of raw materials (Sakai *et al.*, 1996). By adopting diversion strategies like recycling and composting of organics, Canada is making an effort to reduce waste destined for the landfills and incineration, as land use for solid waste disposal is a growing economic and environmental concern (Statistics Canada, 2012). However, despite such efforts, costs and further environmental damage due to the contributing organic waste emissions from both agriculture and solid waste management is becoming a prominent concern with the ever-growing municipal solid waste accumulations (Sakai *et al.*, 1996) and adopting more sustainable methods in waste disposal to reduce greenhouse gas emissions are essential.

Another industry of concern is aquaculture. As global human populations are on the rise, there is an increasing demand for livestock and aquaculture feeds (Henry *et al.*, 2015). Furthermore, a large proportion of world fishery production is processed into fishmeal and fish oil to supplement these feeds (FAO, 2014). Such efforts have resulted in negative impacts on the international fisheries worldwide due to overfishing. With the growing body of evidence shedding light on the depletion of international fisheries, researchers are exploring the use of insects as a protein supplement in fishmeal (see reviews of Henry *et al.*, 2015; Makkar *et al.*, 2014; Sánchez-Muros *et al.*, 2014). With a higher feed conversion efficiency and fecundity than livestock (Nakagaki and Defoliart, 1991), insects are nutritious, high in protein and can be mass reared in less space than traditional livestock (Rumpold and Schlüter, 2013). At the forefront of this endeavour is the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (Tomberlin *et al.*, 2015).

The black soldier fly is a wasp-like fly distributed worldwide prominently in equatorial tropics (Brammer and Von Dohlen, 2007). This species has a native range that includes Asia as far north as 45°N latitude, Europe (Leclercq, 1997; Martinez-Sanchez *et al.*, 2011), and the southeastern United States (Leclercq, 1997; Sheppard *et al.*, 2002; Tomberlin *et al.*, 2002). It has recently been documented in southern Canada, particularly in southern Ontario (M. Jackson and S. Paiero, University of Guelph, personal communication, S. VanLaerhoven, personal observations). The black soldier fly develops in decomposing organic material, including decayed fruits and vegetables, detritus and animal waste (Bondari and Sheppard, 1987; Booram *et al.*, 1977; Diener *et al.*, 2009; Larde, 1989; Myers *et al.*, 2008; Sheppard *et al.*, 1994; St-Hilaire *et al.*, 2007; Tomberlin *et al.*, 2002) and carrion (Tomberlin *et al.*, 2005). Females oviposit approximately 320-620 eggs in dry crevices near decaying organic waste (Tomberlin *et al.*, 2002).

Black soldier flies have been studied globally for their potential use in waste management (Banks *et al.*, 2014; Cickova *et al.*, 2015; Nguyen *et al.*, 2013, 2015; Sheppard *et al.*, 2002) and bioconversion (Booram *et al.*, 1977; Lalander *et al.*, 2015; Li *et al.*, 2011, 2015; Oonincx *et al.*, 2015; Paz *et al.*, 2015). Their use in waste management systems has been demonstrated in the southeastern United States with larvae being able to reduce manure by 50% in large confined animal feeding operations, such as swine and poultry (Sheppard, 1983). More recently, they have been shown to reduce human food waste (Nguyen *et al.*, 2015; Oonincx *et al.*, 2015) and produce biofuel (Leong *et al.*, 2015; Li *et al.*, 2011, 2015). In a two-bird-one-stone philosophy, post-feeding larvae fed animal wastes, such as poultry and swine manure, are composed of approximately 40% protein and 30% fat (Newton *et al.*, 1977), which is a suitable replacement for 50% of the diet of rainbow trout (St-Hilaire *et al.*, 2007), as an additive in poultry feed (Hale, 1973) and swine feed (Newton *et al.*, 1977). For a more comprehensive outline of bioconversion rates, nutritional value, and biomass and by-product yields of black soldier fly development across various waste substrates, see Cickova *et al.* (2015). Furthermore, black soldier fly larvae consume and digest microorganisms, including *Escherichia coli* and *Salmonella* spp. (Erickson *et al.*, 2004; Lalander *et al.*, 2015; Liu *et al.*, 2008), thereby potentially reducing harmful bacteria easily transmitted by the house fly, *Musca domestica* L. (Diptera: Muscidae) (Liu *et al.*, 2008). Therefore, the use of the black soldier fly has potential to address two industries of environmental and economic concern, waste management and protein supplementation.

There is a growing body of literature on the black soldier fly's life-history across a series of environments and diets (Bondari and Sheppard, 1981, 1987; Gobbi *et al.*, 2013; Holmes *et al.*, 2012, 2013; Larde, 1989; Myers *et al.*, 2008; Nguyen *et al.*, 2013, 2015; Tomberlin *et al.*, 2002, 2009; Yu *et al.*, 2014; Zhou *et al.*, 2013). Development from egg eclosion to the post-feeding larval stage of development takes 19-20 days and 17-18 days at 27 and 30 °C, respectively (Tomberlin *et al.*, 2009), under 70% RH and a 12:12 (L:D) photoperiod. Their post-feeding development has been shown to differ in response to the pupation substrate available (Holmes *et al.*, 2013), with no substrate available taking ~2.5 days longer to pupate than post-feeding development in wood shaving or potting soil substrates. Relative humidity (RH) thresholds have also been found and have a profound influence on egg and pupal mortalities, specifically, eggs and pupae in less than 60% and 40% RH environments, respectively, had more than 50% mortality (Holmes *et al.*, 2012). Adults require a minimum light intensity of 60 $\mu mol/m^2/s$ sunlight to stimulate copulation, but mating has been achieved under artificial lights with 60% mating success compared with natural sunlight (Tomberlin and Sheppard, 2002; Zhang *et al.*, 2010).

Similarly, development time is also variable across diets (Gobbi *et al.*, 2013; Myers *et al.*, 2008; Nguyen *et al.*, 2013, 2015; St-Hilaire *et al.*, 2007; Zhou *et al.*, 2013). While black soldier fly larvae can develop on various organic compounds, they have been shown to develop poorly on meat meal compared to poultry feed meal (Gobbi *et al.*, 2013). However, Nguyen *et al.* (2013) demonstrated that while black soldier fly development is slowed when reared on swine manure, compared to swine liver, kitchen waste, fish-rendering waste and the control poultry feed diet, the fastest development to the pupal stage occurred on swine liver, with a median of 34.17±2.07 days. Development on kitchen waste and the control poultry feed diet took slightly longer compared to swine liver, but did not differ significantly (Nguyen *et al.*, 2013*).* Alternatively, development time has also been demonstrated to not vary across some diet types (Tomberlin *et al.*, 2002), further supporting the need to consider diet type in addition to environmental factors when proposing a black soldier fly facility for waste conversion and protein supplementation production.

Black soldier flies rely on heat energy from their environment for growth and development, with ambient temperature largely regulating their metabolism and rate of development (Jarosik *et al.*, 2004). The direct dependence of temperature on insect development has been shown for a variety of dipteran species with several published life-history tables in response to changes in temperature (Byrd and Butler, 1996, 1997, 1998; Boatright and Tomberlin, 2010; Flores *et al.*, 2014; Grassberger and Reiter, 2001, 2002; Núñez-Vázquez *et al.*, 2013; Thomas *et al.*, in press; Warren and Anderson, 2013). However, insects are also bound by upper and lower temperature thresholds. Even across very large geographic distances, insects of the same species can have different physiological tolerances to the same unfavourable temperatures, with populations in the northern latitudes having better tolerances to low temperatures than populations in southern latitudes (Dixon *et al.*, 2009).

Temperatures at or below the lower developmental threshold, but above the lethal threshold, result in a cessation of development. For example, *Phormia regina* (Meigen) (Diptera: Calliphoridae) have successful egg hatch at 11.7 °C, but a cessation of larval development at or below 12 °C, with development proceeding at or above 14 °C (Nabity *et al.*, 2006). On the upper extreme, Tomberlin *et al.* (2009) reared black soldier flies at 30 and 36 °C and although 73% of larvae developed and survived to the post-feeding stage at 36 °C, only 0.1% of pupae successfully completed metamorphosis and emerged, suggesting an upper temperature threshold near 36 °C. Different stages of development have been demonstrated to have different minimum thresholds. For example, cessation of development in early instar development for *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae) is at or below 8.9 °C, whereas cessation of development at the pupal stage where metamorphosis occurs for *P. terraenovae* is 9.8 °C (Grassberger and Reiter, 2002).

The primary objective of this study was to determine the lower temperature developmental threshold of *H. illucens* eggs and larvae. Doing so will provide a valuable contribution to the field of waste management and the growing innovative use of insects as protein supplements in fishmeal and livestock feed. In climates that vary seasonally, particularly northern climates that experience seasonal temperatures below 20 °C for more than half of the year, it is important to understand the lower temperature bounds for black soldier fly development to maintain optimal bio-conversion and larval production regardless of season.

2. Materials and methods

Egg eclosion

Eggs were collected from a black soldier fly colony housed in a screen mesh cage (1.8×1.8×1.8 m with 1.5 mm mesh) maintained outdoors, outside the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) facility located at Texas A&M University in College Station, Texas, USA. Eggs were collected in a three layer, 3×5 cm corrugated cardboard rectangle with 3×4 mm flutes (tubular holes in cardboard), held together with glue such that the flutes acted as an oviposition substrate. These were taped with the flutes perpendicular to the oviposition substrate, 5 cm above the oviposition medium. Oviposition medium was composed of moist-to-liquefied Gainesville diet (5:3:2 hand mixture of wheat bran, alfalfa and corn meal, respectively), (Producers Cooperative Association, Bryan, TX, USA), developed for rearing houseflies (Hogsette, 1992; Tomberlin *et al.*, 2002).

Egg treatments

Each egg-containing corrugated cardboard flute was dissected to determine the number of egg clusters oviposited per flute. Egg clusters were randomly placed into 30 ml clear plastic soufflé cups (Dart P100; Dart Container Corporation, Mason, MI, USA) placed into 680 ml clear plastic sandwich containers (Rubbermaid® TakeAlongs, High Point, NC, USA). Each soufflé cup contained two egg clusters (egg contribution from two females) and cups were divided evenly among three separate growth chambers (Model I-36LLVLC8; Percival Scientific Inc., Perry, IA, USA). All three growth chambers were maintained at 70% relative humidity and a 14:10 L:D, but with unique temperature settings representative of each treatment (12, 16 and 19 °C, respectively). Each temperature treatment comprised 24 replicates, for an n=72. Temperatures in this study were chosen based on preliminary experiments on the black soldier fly using the egg stage of development only. It was evident that black soldier fly eggs could eclose at 19 °C and since the objective of this study was to elucidate the lower temperature threshold, 16 and 12 °C were chosen for lower temperature comparisons.

Hobo® U12-006 data loggers (Onset® Computer Cooperation, Bourne, MA, USA) with three temperature probes were placed in each growth chamber (one probe per shelf) to record potential temperature variation every 15 minutes within the growth chamber and across growth chambers.

Eggs were checked for eclosion twice daily. Upon first eclosion, eggs remained in their respective growth chambers under treatment conditions for an additional two days to allow the entire egg clutch to fully eclose. Two days after the first egg eclosion, empty and or non-viable egg clusters were stored in a -20 °C chest freezer for assessment of successful egg eclosion. Time to egg eclosion and percentage successful egg eclosion was recorded for each replicate within each temperature treatment.

Adult emergence

Eclosed larvae from the preceding effect of temperature on egg eclosion study were used to determine temperature effects on adult emergence. Upon egg eclosion, 10 g aliquots of Gainesville Diet (Hogsette, 1992) mixed with 18 ml of water (70% moisture) (Tomberlin *et al.*, 2002) was fed to the larvae as required until development to the post-feeding stage. Water was also added to day-old feed to prevent drying and desiccation.

Adult treatments and sampling

Larvae continued development under the same treatment conditions as those in the egg eclosion experiment (70% RH, 14:10 L:D and their respective temperature; 12, 16 and 19 °C) and monitored daily for pupation and adult emergence. Upon pupation, pupae were placed into 30 ml plastic soufflé cups and capped with a clear plastic lid, (Dart 100PCL25; Dart Container Corporation) pierced with a 10 ml (29 gauge) syringe. Upon emergence, adults were provided water in 0.125 ml aliquots daily using a 10 ml (29 gauge) syringe to maximise adult longevity (Tomberlin *et al.*, 2002). The syringe was inserted into the cup through the hole pierced in the lid of each cup and water was released into the cup. Flies were not injected with the syringe. Time to adult emergence, emergence success and sex was recorded for each individual. Adult longevity was also recorded for the first and last 10% of emerged adults per replicate per treatment.

Statistics

All statistics were computed using SAS JMP® version 8.0.1 statistical software (SAS, Cary, NC, USA). The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite box-cox transformation, thus a non-parametric one-way Wilcoxon/Kruskal-Wallis test (ranked sums) was used to test both treatment effects on the mean (± SE) time to egg eclosion (including only those replicates to eclose in the analysis) as well as the mean (± SE) percentage successful egg eclosion, independently. Differences among treatments were considered significant with P-values <0.05.

Statistical comparisons for adult emergence were not conducted due to the 19 °C treatment being the only treatment to complete development to the adult stage. Instead, mean (± SE) time to adult emergence and mean (± SE) successful adult emergence are reported for the 19 °C treatment. Mean (± SE) adult longevity was determined for the first and last 10% of the total number of adults to emerge. Sex ratio was compared using a non-parametric one-way Wilcoxon/Kruskal-Wallis (ranked sums) test and compared to α=0.05.

3. Results

Abiotic factors recorded

A Hobo® U12-006 data logger recorded the temperature within each growth chamber, a separate probe on each shelf for a total of 3 temperature recordings per treatment, every hour. The resulting output read a mean temperature of 11.71±0.02, 16.03±0.03 and 19.02±0.02 °C, for each treatment, 12, 16 and 19 °C, respectively. A nonparametric one-way Kruskal-Wallis was used to verify temperature treatments were significantly different, (χ^2=2,031.43, df=2, P<0.001).

Egg eclosion

Time to egg eclosion for those replicates reared at 16 °C was 15.26±0.11 days, taking longer to eclose than eggs reared at 19 °C, which eclosed in 7.75±0.02 days (χ^2=18.57, df=1, P<0.001). Successful egg eclosion for all replicates in the 12, 16 and 19 °C treatments was, 0.00±0.00, 12.85±3.32 and 75.40±2.88%, respectively. The percentage successful egg eclosion for eggs reared at 19 °C was higher than for eggs reared at 16 and 12 °C (χ^2=37.43, df=2, P<0.001). However, hatched neonate larvae in the 16 °C treatment suffered 100% mortality within three days of eclosion. After 30 days, eggs in the 12 °C treatment were observed under a dissection microscope to determine viability, however, the eggs were completely collapsed and deemed not viable.

Adult emergence

Time to adult emergence was 72.07±0.08 days for the 19 °C treatment. With a larval mortality and pupal mortality of 63.53±7.16 and 16.47±2.76%, respectively, the overall percentage successful adult emergence was 31.90±6.56% at 19 °C.

Male and female emergence distributions were not different, however, the total emerged adult population (n=2,426) was male biased with 62.98% males, (χ^2=27.16, df=1, P<0.0001). The adult longevity calculated for the first and last 10% of emerged females and males (n=233) was 25.37±0.58 and 25.47±0.53 days, respectively. Male and female longevity did not differ (χ^2=0.001, df=1, P=0.97), however, a male biased sex ratio of 61.37% was also evident within this sub population (χ^2=9.60, df=1, P=0.002).

4. Discussion

Black soldier flies successfully developed from egg to adult at 19 °C, failing to survive beyond the neonatal larval stage at 16 °C and failing to even hatch at 12 °C. It is not surprising that temperature has a significant impact on black soldier fly development. Tomberlin *et al.* (2009) determined the maximum developmental temperature threshold for black soldier flies is between 30 and 36 °C, with optimal rearing at 27 °C with respect to development time and mortality rates. Our results indicate two distinct lower developmental thresholds: one for successful egg eclosion and one for successful adult emergence. Eggs successfully eclosed at 16 °C and 19 °C, however hatched neonate larvae reared at 16 °C died three days after egg eclosion. Although not measured, three day old larvae were noticeably larger than one day old larvae (L.A. Holmes, personal observation), suggesting some development. Unexpectedly, successful egg eclosion at 19 °C was comparable to egg eclosion success at 27 °C (Holmes *et al.*, 2012), which had a mean percentage of 76±2.28. Although, the time to egg eclosion took twice as long at 19 °C compared to egg eclosion time in Holmes *et al.* (2012) at 27 °C. With successful adult emergence at 19 °C, we predict the lower developmental threshold for *H. illucens* is between 16 and 19 °C for all life stages of development.

The length of development from egg to adult was drastically delayed when reared at 19 °C when compared with previous studies under similar diets, but various temperatures (Table 1), (Holmes *et al.*, 2012, 2013; Nguyen *et al.*, 2013, 2015; Tomberlin *et al.*, 2002, 2009). Because black soldier flies do not require food as adults, their energy is acquired via feeding during the larval stages. Insects undergoing slow development typically accumulate more weight, with greater fat reserves allocated to adult fitness, resulting in longer adult longevities (Andrewartha, 1952). With longer development times, adult longevity was also longer in 19 °C versus rearing at higher temperatures in other studies (Table 1). In agreement, Tomberlin *et al.* (2009) noted that length of larval development is an excellent predictor of adult longevity for the black soldier fly such that the longer the larval development, the longer the longevity of the emerged adult.

For future research in waste management and bioconversion industries, it would be worth investigating the effect temperature has on feeding rates and time to pupation, across a gradient of temperatures to determine the optimal thermal environment for increased waste conversion and post-feeding larval biomass yields. Regretfully, larval biomass was not determined in this study. However, Diener *et al.* (2009) determined an optimal feeding rate of 100 mg of chicken feed per larva, resulting in an optimal trade-off of food reduction and biomass gained. Using the same feeding rate of 100 mg of feed per larva as Diener *et al.* (2009), Lalander *et al.* (2015) fed black soldier fly larvae a mixed diet of swine manure, dog food, and human feces and recorded a 55.1% waste reduction, similar to other studies (Myers *et al.*, 2008; Sheppard, 1983; Sheppard *et al.*, 1994) and a conversion rate of 11.8%, similar to Diener *et al.* (2011), noting however that discrepancies in conversion rates with other studies are likely a result of differences in larval densities and feeding regimes (Lalander *et al.*, 2015). However, these studies were conducted at a constant temperature regime and did not address the different rates of bio-conversion on different diets or at different temperatures. Alternatively, Nguyen *et al.* (2015) did demonstrate that larval weight consumption differs across food types, with larvae fed kitchen waste weighing the most at 226.63 mg per larva. Again, this study was conducted at a constant thermal environment (Nguyen *et al.*, 2015).

Table 1. Life-history traits of the black soldier fly (mean ± standard error) compared across temperatures and studies performed under similar diets and environments.

Temperature (°C)	Length of development (days) ± SE		Successful percentage adult emergence ± SE	Adult longevity (days) ± SE	Study
	Larval	Pupal			
12	–	–	–	–	this study
16	–	–	–	–	this study
19	60.96±0.03	11.52±0.06	31.90±6.56	25.37±0.58[1]	this study
27	13.0±0.95	8.41±0.06	93.00±1.2	7.94±0.09	Holmes *et al.*, 2012
28	10.28±0.17[2]	6.74±0.03	87.60±5.50	–	Holmes *et al.*, 2013[4]
28	31.67±1.17	7.16±2.15[3]	75.56±SE	–	Nguyen *et al.*, 2013[4]
27	20.1±0.32[1]	17.80±0.74[1]	83.2-91.8[5]	14.00±0.69[1]	Tomberlin *et al.*, 2009
30	18.5±0.50[1]	15.50±0.58[1]	74.2-96.7[5]	12.40±0.70[1]	Tomberlin *et al.*, 2009
36	25.9±0.23	–	0.1	–	Tomberlin *et al.*, 2009

[1] Data for females only.
[2] Length of post-feeding stage.
[3] Value calculated from subtracting the mean time to reach the pupal stage of development from the mean time to adult emergence (Nguyen, 2010).
[4] Diet is chicken feed pellets (Agribrands Purina Canada Inc.) not Gainsville Diet (Hogsette, 1992) used in all other studies.
[5] Range accounts for successful adult emergence over three temporally distinct replicates.

Despite some studies demonstrating the interactive effects of diet and temperature on growth rate and nutrition acquisition (Clissold *et al.*, 2013; Kingsolver, 2006; Lee and Roh, 2010; Stillwell *et al.*, 2007), we have found no study where the interaction of temperature and diet types on black soldier fly feeding rates, resource utilisation and thus development is investigated. Miller *et al.* (2009) demonstrated that at high temperature (38 °C), growth rate increases, but nutrient utilisation efficiency decreased, whereas at lower temperatures (32 °C), development slowed, but nutrient utilisation efficiency was maximised. Interestingly, in the same study, they showed that when placed in a stadium with a temperature gradient (32-38 °C), locusts fed *ad libitum* chose body temperatures that maximised growth rate at a cost of nutrient utilisation (Miller *et al.*, 2009). With further investigation, nutrition acquisition has been shown to differ by temperature in locusts (Clissold *et al.*, 2013). Specifically, at lower temperatures, carbohydrates are absorbed with higher efficiency compared to protein, whereas at higher temperatures, proteins are absorbed with higher efficiency (Clissold *et al.*, 2013). Therefore, it is worth investigating the interaction of diet type and temperature on black soldier fly feeding rates and development to optimise waste reduction and bioconversion biomass.

Lastly, it is not known if mating and oviposition are successful at 19 °C. Light intensity is known to play a role in successful mating (Zhang *et al.*, 2010), and that mating and oviposition is successful under artificial lighting, but it is worth investigating the role temperature versus light intensity has on mating and oviposition. For the purpose of using the black soldier fly year-round in waste management and bio-conversion industries, it would be necessary to subject adult black soldier flies reared at 19 °C and a gradient in between it and the optimal 27 °C to determine if other life history traits are affected by low temperatures, such as mating and oviposition. Of particular essential interest in northern latitudes, is insight into potential diapause cues and mechanisms. It is not clear if *H. illucens* enter diapause, as evidence of diapause in the family Stratiomyidae is very limited with only one published record found (Rozkosny and Kovac, 1998). As such, results from such a study and many other studies on the effects environment and diet have on black soldier fly development are needed to further the global production of black soldier flies and their use in waste management and protein supplementation for agriculture and aquaculture industries.

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