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Relative Humidity Effects on the Life History of *Hermetia illucens* (Diptera: Stratiomyidae)

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ABSTRACT Black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) are of particular interest for their application in waste management such as reducing manure accumulations in confined feeding operations. Determining black soldier fly development time as a result of climatic variations will allow for optimizing their utilization as a waste management agent at landfill sites and confined animal feeding operations. To implement a black soldier fly waste management program in Canada, where seasonal variability does not support *H. illucens* development on a year round basis, determining maximum and minimum abiotic thresholds to sustain larval development is important. In Canadian winters, maintaining greenhouse temperatures necessary for black soldier fly development results in low relative humidity that could impact their development. The objective of this study was to determine relative humidity thresholds on egg eclosion and adult emergence. Egg eclosion success was measured at 25, 40, 50, 60, and 70% relative humidities and adult emergence success was measured at 25, 40, and 70% RH. Egg eclosion and adult emergence success increased with increasing relative humidities, while development time decreased with rising relative humidities.

KEY WORDS egg desiccation hypothesis, egg clustering, waste management, egg eclosion, adult emergence

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 mated females selectively oviposit 320–620 eggs in dry crevices near a moist food resource ~2 d after successful copulation (Tomberlin et al. 2002). Hatched neonate larvae quickly make their way to the resource (Booth and Sheppard 1984). Previous life history studies on the black soldier fly were primarily limited to their use in waste management. Black soldier flies efficiently convert organic waste and therefore have been used to reduce waste in large confined animal feeding operations (Sheppard et al. 2002) as well as to generate supplemental livestock feed (Booram et al. 1977). Therefore, developmental studies including mating behaviors (Booth and Sheppard 1984, Tomberlin and Sheppard, 2002) and the effects of temperature and diet on development (Sheppard et al. 1994; Tomberlin et al. 2002, 2009) were conducted with a goal of maintaining a self-sustaining colony for year-round waste conversion.

A neglected aspect of black soldier fly life history is the effect of relative humidity (RH) on black soldier fly development and survivorship. Upon egg eclosion, neonate larvae feed on moist decomposing organic matter until development to the postfeeding stage at which time they leave their food resource in search of a place to pupate and complete metamorphosis, this natural life-history of the black soldier fly occurs in confined animal feeding operations in the southeastern United States (Sheppard et al. 1994). As a result, successful manure management systems with the black soldier fly were in place in poultry feeding houses in 1994 without the use of separate facilities or special equipment to control the ambient environment (Sheppard et al. 1994). However, unlike the southeastern United States, indoor heating systems are necessary for greenhouses in regions experiencing cold weather resulting in low RH conditions.

Because low RH increases mortality in many insects, particularly during the egg stage (Sabelis 1985, as cited in Schausberger 1998), this could impede implementation of a year-round black soldier fly waste management program in Canada and other locations with a cold season. The objective of this study was to determine the effects of RH on black soldier fly development, including egg eclosion and successful

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adult emergence, with a goal of defining optimal conditions for black soldier fly production in cold-climate agricultural facilities.

Materials and Methods

Experiment: Egg Eclosion. *Source of Eggs.* Eggs were collected from a *H. illucens* laboratory colony housed in a cage (1.8 × 1.8 × 1.8 m and 1.5 mm mesh screen) maintained in a greenhouse, outside the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility located at Texas A&M University in College Station, TX. The laboratory colony originated from eggs used in Dr. Craig Sheppard's colony in Tifton, GA. Eggs for each replicate across RH treatments were collected within a 4 h window. Eggs were collected in a three layer, 3 × 5 cm corrugated cardboard, held together with Elmer white glue with 3 × 4 mm flutes used as an oviposition substrate, taped 5 cm above the oviposition substrate with the flutes perpendicular to the substrate. Oviposition attractant 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), developed for rearing house flies *Musca domestica* (L.) (Diptera: Muscidae) (Tomberlin et al. 2002, Hogsette 1992).

Treatments. Each egg-containing corrugated cardboard flute was dissected allowing the removal of the egg clusters present. While maintaining egg cluster size and form, all egg clusters were combined and then distributed into 30 ml clear plastic cups (Dart P100, Dart Container Corporation, Mason, MI). Each cup contained one egg cluster (egg contribution from one individual female) composed of 1520 ± 43 eggs. Cups with eggs were placed into a 30-well clear plastic tray (Bio-Serv, Frenchtown, NJ) and assigned a RH treatment. RH treatments in this study were chosen based on Clark and Faeth's design (Clark and Faeth 1998) and preliminary experiments on adjusting the RH levels in the growth chambers (Model I-36LLVLC8, Percival Scientific Inc., Perry, IA). It was evident that the growth chambers could not maintain humidity levels below 25%; therefore, 25% RH was selected to test for the lower RH threshold and 70% RH was selected as the control as a result of successful development in Tomberlin et al. (2002). Later, a continuum of 40, 50, and 60% RH effects on egg eclosion was also examined.

This experiment was completed in three stages. First, 10 replicates of 25 and 70% RH effects on egg eclosion were simultaneously compared in two separate growth chambers. Second, 10 additional replicates of 25 and 70% RH treatment were compared by which the growth chambers were switched for each RH so that each treatment experienced each chamber. Finally, 20 replicates of 40, 50, and 60% RH effects on egg eclosion were compared in three separate growth chambers. Regardless of RH treatment, each growth chamber maintained a temperature of 27°C with a photoperiod of 12:12 (L:D) h. Photoperiod was chosen to mimic the rearing conditions used to maintain the parent stock colony.

A Hobo U12–012 data logger (Onset Computer Cooperation, Pocasset, MA) was placed in each growth chamber on the top shelf next to the tray of egg clusters. The data logger recorded RH, temperature and light intensity every 15 min. Eggs were monitored every 12 h for successful egg eclosion. Two days after egg eclosion, identified by observing neonatal larvae in each cup, egg clusters were stored at −25°C until the unhatched eggs were counted. Time to egg eclosion and per cent successful egg eclosion was recorded for each replicate within each RH treatment.

Experiment: Adult Emergence. *Source of Larvae.* Eggs were collected using the same methods as the egg eclosion experiment described previously. Each cup contained two egg clusters (egg contribution from two individual females to ensure the necessary survivorship required to proceed with adult emergence experiment) and cups were divided among three RH treatments (25, 40, and 70%). Because of limited growth chamber availability, all replicates for all treatments were placed in a single walk-in growth chamber maintained at 70% RH, 25°C and a photoperiod of 14:10 (L:D) h until the postfeeding stage of development. Upon egg eclosion, larvae were fed 10 g of dry Gainesville diet (Tomberlin et al. 2002, Hogsette 1992) mixed with 18 ml of water aliquots (≈70% moisture), ad libitum until larvae reached the postfeeding stage of development, indicated by their change in color from cream to black.

Treatments. To determine the effect RH has on adult emergence, all larvae from all three RH treatments (25, 40, and 70%) were reared in the same environmental conditions until growth to the postfeeding stage of development. Neonatal larvae live and feed on moist decomposing organic matter. While developing in this moist environment, feeding larvae are not readily exposed to the ambient RH. However, upon completion of the feeding stage, postfeeding larvae leave the moist environment in search of a safe place to pupate, however, by leaving the food resource; the postfeeding larvae are readily exposed to ambient environment, where RH may have a significant impact on pupation and adult emergence. As a result the effect of RH on adult emergence was conducted on postfeeding larvae. Thirty postfeeding larvae from each replicate per RH treatment were removed from their food in the walk-in growth chamber and placed individually into 30 ml clear plastic cups, without lids, for maximum RH exposure. Cups were placed in a 30-well tray and placed into their respective growth chambers (Percival Scientific Inc.) corresponding to their respective relative humidities (25, 40, or 70%) and 25°C and a photoperiod of 14:10 (L:D) h. The temperature and photoperiod used for this portion of the study was as a result of shared growth chamber space. The walk-in growth chamber used to rear the black soldier fly eggs to the postfeeding stage of development housed other research projects and therefore 25°C and a photoperiod of 14:10 (L:D) h was used instead of the conditions used to maintain the parent stock colonies. To maintain environmental consistency, the temperature and photoperiod used to

rear the black soldier fly from eggs to the postfeeding stage of development were also used in the treatment growth chambers.

Postfeeding larvae were observed every 12 h to determine time of pupation for each larva. Individual postfeeding larvae were kept in their original cup throughout the duration of the experiment. Upon pupation, pupae remained in their cups without lids for 5 d after pupation, to maximize RH exposure before adult emergence. Six-day-old pupae were capped with clear plastic lids (100PCL25 Dart Container Corporation), in anticipation of adult emergence and pierced once with a 29 gauge syringe to allow continuous RH exposure. Pupae were monitored every 12 h for successful adult emergence. By keeping each postfeeding larvae in their original cups until adult emergence, the length of the postfeeding and pupal stages of development, successful adult emergence and adult longevity were recorded for each larva. Dissections were completed on all pupae that did not emerge in the 25 and 40% RH treatments to observe any indication of desiccation. Hobo units were used to record temperature, RH, and light intensity as previously described.

Statistics. *Experiment: Egg Eclosion.* All statistics were computed using SAS JMP 8.0.1 statistical software (SAS Institute Inc., Cary, NC). The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts (Box Cox, arcsine and Log_{10} transformations). Thus, a nonparametric one-way Wilcoxon/Kruskal–Wallis (Ranked Sums) test was used for both analyses of time to egg eclosion and the percent successful egg eclosion. Pairwise comparisons using Wilcoxon/Mann–Whitney *U* tests were used on 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). A one way analysis of variance (ANOVA) was used to compare the number of eggs in each egg cluster across treatments and replicates.

Experiment: Adult Emergence. The data failed to meet the assumptions of normality (homogeneity of variance and normality goodness-of-fit) despite data transformation attempts (Arcsine and Box Cox transformations). Therefore, a nonparametric one-way Wilcoxon/Kruskal–Wallis test with Wilcoxon/Mann–Whitney *U* pairwise comparisons tests were used to determine postfeeding and pupal mortalities and the percent successful adult emergences with respect to treatment. Alpha values were adjusted using the Dunn–Sidak procedure (Quinn and Keough, 2002, p. 48). A one way ANOVA was used to compare the number of eggs in each egg cluster across RH and replicates.

A full factorial Cox Proportional Hazard test (model effects: sex, (RH) and sex*RH) was used to compare survivorship differences with respect to the length of the postfeeding and pupal stages of development and adult longevity between relative humidities.

Table 1. Mean (± SE) time to egg eclosion and per cent successful egg eclosion in each treatment, 25, 40, 50, 60, and 70% RH

RH (%)			Time to egg eclosion (hours)	Successful egg eclosion (%)
Experiment 1[a]	25	GC I[b]	124.43 ± 1.85A	8.44 ± 0.75a
		GC II	138.00 ± 0.00B	5.44 ± 1.40a
	70	GC I	87.63 ± 2.70C	64.67 ± 7.69b
		GC II	80.78 ± 3.75C	86.22 ± 2.91b
Experiment 2	40		90.11 ± 0.35A	19.86 ± 1.27a
	50		87.84 ± 1.30B	38.00 ± 2.29b
	60		71.21 ± 0.37C	72.74 ± 2.46c

Means for time to egg eclosion and percent of successful egg eclosion within the same exp followed by different letters are significantly different ($P < 0.05$, Wilcoxon/Kruskal–Wallis, SAS JMP 8.0.1).

[a] The 25% and 70% were analyzed separately from 40%, 50%, and 60%.

[b] GC = growth chamber, for purpose of depicting chamber effect.

Results

Experiment: Egg Eclosion. *Lower RH Threshold: 25 and 70%.* Regardless of switching the growth chambers, eggs subjected to 25% RH had higher mortality (Table 1; $\chi^2 = 27.8882$, df = 1, $P < 0.001$) and slower development (Table 1; $\chi^2 = 28.7834$, df = 1, $P < 0.001$) than eggs in 70% RH. After repeating the experiment and switching growth chambers for each treatment, there was a difference with respect to time to egg eclosion for the 25% RH treatment ($\chi^2 = 12.5057$; df = 1; $P = 0.0004$; Dunn–Sidak adjusted $\alpha = 0.0253$). Time to egg eclosion for the 25% RH treatments in chamber 1 was delayed 10.3% compared with chamber 2. In contrast, there was no difference with respect to time to egg eclosion for the 70% RH treatment when switching the growth chambers ($\chi^2 = 0.1330$; df = 1; $P = 0.7154$). There was no differences observed when switching the growth chambers with respect to mean (±SE) percent successful egg eclosion for 25 and 70% RH ($\chi^2 = 3.9153$, df = 1, $P = 0.0478$ and $\chi^2 = 3.9637$, df = 1, $P = 0.0465$, respectively; Dunn–Sidak adjusted $\alpha = 0.0253$). The number of individual eggs in each egg cluster (contribution by one female) was not different across treatments ($F_{1,36} = 0.1435$; $P = 0.7070$).

Hobo data loggers recorded the temperature, RH and light intensity every 15 min. The mean (±SE) temperature, RH and light intensity in the 25% RH treatment for chambers 1 and 2 were 28.45 ± 0.02°C, 24.42 ± 0.01% RH, 2698.07 ± 41.25 Lux and 28.79 ± 0.02°C, 24.12 ± 0.01% RH, 3001.29 ± 68.32 Lux, respectively. The mean (±SE) temperature in the 70% RH treatment for chambers 1 and 2 were 27.63 ± 0.02°C, 70.19 ± 0.01% RH, 3127.42 ± 43.92 Lux and 27.59 ± 0.04°C, 69.17 ± 0.01% RH, 3120.02 ± 123.23 Lux, respectively.

RH Continuum: 40, 50, and 60%. Eggs subjected to 40% RH had higher mortality and slower development than eggs in 50% and 60% RH (Table 1, $\chi^2 = 50.2911$, df = 2, $P < 0.001$ and $\chi^2 = 43.4409$, df = 2, $P < 0.001$, respectively). Additionally, eggs in the 50% RH treatment also had higher mortality and slower development than eggs in 60% RH (Table 1, $\chi^2 = 28.7371$, df = 1, $P < 0.001$ and $\chi^2 = 24.1606$, df = 1, $P < 0.001$,

Table 2. RH effects on length of development, mortality, adult emergence, and adult longevity

RH (%)	Length of development stage (d)		Mortality (%)		Successful adult emergence (%)	Adult longevity (d)
	Postfeeding	Pupal	Postfeeding	Pupal		
25	10.36 ± 0.09A	8.92 ± 0.15A	62.00 ± 0.06a	65.00 ± 5.9a	16.00 ± 3.6A	5.17 ± 0.14a
40	9.72 ± 0.08B	8.57 ± 0.06B	26.00 ± 0.05b	23.00 ± 4.2b	59.00 ± 5.9B	6.68 ± 0.10b
70	9.48 ± 0.09C	8.41 ± 0.06C	3.00 ± 0.01c	2.00 ± 0.5c	93.00 ± 1.2C	7.94 ± 0.09c

Means for development times, mortalities, successful adult emergence, and adult longevity 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).

respectively). The number of individual eggs in each egg cluster (contribution by one female) was not different across treatments ($F_{2,57} = 2.5797$; $P = 0.0846$).

Hobo data loggers recorded the temperature, RH and light intensity every 15 min. The mean (±SE) temperature, RH and light intensity for the 40, 50, and 60% RH treatments were, 27.36 ± 0.01°C, 35.79 ± 0.03% RH, 2302 ± 56.34 Lux, 27.82 ± 0.02°C, 48.89 ± 0.07% RH, 2726 ± 66.80 Lux and 28.81 ± 0.02°C, 58.59 ± 0.07% RH, 3736 ± 91.55 Lux, respectively.

Experiment: Adult Emergence. Postfeeding larval and pupal mortalities differed between RH (Table 2, $\chi^2 = 37.0437$, df = 2, $P < 0.001$ and $\chi^2 = 42.2135$, df = 2, $P < 0.001$, respectively). Postfeeding larvae and pupae in 25% RH had higher mortality than those in the 40% ($\chi^2 = 14.7220$, df = 1, $P < 0.001$ and $\chi^2 = 16.8152$, df = 1, $P < 0.001$, respectively) and 70% RH ($\chi^2 = 26.7192$, df = 1, $P < 0.001$ and $\chi^2 = 27.1328$, df = 1, $P < 0.001$, respectively) and those in 40% RH had higher mortality than those in 70% RH ($\chi^2 = 17.4448$, df = 1, $P < 0.001$ and $\chi^2 = 24.8949$, df = 1, $P < 0.001$, respectively). Successful adult emergence differed between the 25, 40, and 70% RH treatments (Table 2, $\chi^2 = 41.2807$, df = 2, $P < 0.001$). Pupae in 25% had lower successful adult emergences than those in the 40 and 70% RH treatments ($\chi^2 = 18.4713$, df = 1, $P < 0.001$ and $\chi^2 = 26.4919$, df = 1, $P < 0.001$, respectively) and pupae in the 40% RH had lower successful adult emergences than those in the 70% RH ($\chi^2 = 21.3952$, df = 1, $P < 0.001$).

There was no effect of sex and the interaction of RH and sex on time spent in the postfeeding stage of development ($\chi^2 = 1.5194$, df = 1, $P = 0.2177$ and $\chi^2 = 1.1851$, df = 2, $P = 0.5529$, respectively). RH independently affected the time spent in the postfeeding stage of development (Fig. 1a, $\chi^2 = 16.4343$, df = 2, $P < 0.001$), such that a greater proportion of larvae in the 25% RH treatment spent more time in the postfeeding stage of development than larvae in other relative humidities. Similarly, there was no effect of sex and the interaction of RH and sex on the time spent in the pupal stage of development ($\chi^2 = 3.0472$, df = 1, P = 0.0809 and $\chi^2 = 1.6552$, df = 2, $P = 0.4371$, respectively). RH independently affected the time spent in the pupal stage of development (Fig. 1b, $\chi^2 = 10.5336$, df = 2, $P < 0.005$), such that a greater proportion of larvae in the 25% RH treatment spent more time in the pupal stage of development than larvae in other relative humidities.

The sex ratio was 76.74, 67.60, and 71.98% male in the 25, 40, and 70% RH treatments, respectively. The proportion of males was higher than females for each RH ($\chi^2 = 165.9885$, df = 1, $P < 0.0001$); however, RH was not a factor on the sex ratio ($\chi^2 = 3.441$, df = 2, $P = 0.1790$). The mean (±SE) longevity for males and females was 5.08 ± 0.18 d and 5.3 ± 0.21, 6.74 ± 0.13, and 6.51 ± 0.16 d and 8.00 ± 0.11 and 7.77 ± 0.14 d, respectively in the 25, 40, and 70% RH treatments, respectively. Sex did not have an effect on the timing of adult emergence, ($\chi^2 = 2.7546$, df = 1, $P = 0.0970$), nor adult longevity ($\chi^2 = 0.5388$, df = 1, $P < 0.4629$). There was no effect of sex and the interaction of RH and sex on adult longevity ($\chi^2 = 1.5659$, df = 1, $P = 0.2108$ and $\chi^2 = 0.3779$, df = 2, $P < 0.8278$, respectively). RH independently affected adult longevity (Fig. 1c, $\chi^2 = 103.6544$, df = 2, $P < 0.001$), such that adults in 25% RH survived fewer days than those in 40 and 70% RH, and the adults in 40% RH survived fewer days than those in 70% RH.

Hobo data loggers recorded the temperature, RH and light intensity every 15 min upon being placed in their treatment growth chambers at the postfeeding stage of development. The mean (±SE) temperature, RH and light intensity for the 25, 40, and 70% RH treatments were, 26.23 ± 0.01°C, 30.04 ± 0.11% RH, 3034.67 ± 25.71 Lux, 26.23 ± 0.02°C, 41.32 ± 0.06% RH, 3642.31 ± 29.19 Lux and 26.96 ± 0.02°C, 69.53 ± 0.07% RH, 3665.63 ± 29.94 Lux, respectively.

Discussion

Our predictions are conclusive; RH has significant effects on successful black soldier fly egg eclosion and adult emergence. In low RH environments, water loss through the egg and pupal membranes can be detrimental to the survivorship of holometabolous insects, resulting in desiccation (Wigglesworth 1984). We believe the deleterious effects 25% RH had on egg eclosion success was attributed to desiccation. Stamp (1980) was the first to coin the egg desiccation hypothesis, an oviposition strategy used in Lepidoptera to prevent egg desiccation. Clark and Faeth (1997, 1998) followed up on Stamp's hypothesis with reference to egg cluster size and composition, where they observed eggs on the surface of egg clusters desiccated, while eggs within the egg cluster did not. We observed 6.84% successful egg eclosion at 25% RH and similar to Clark and Faeth (1998), the eggs located on the surface of egg clusters appeared desiccated, while

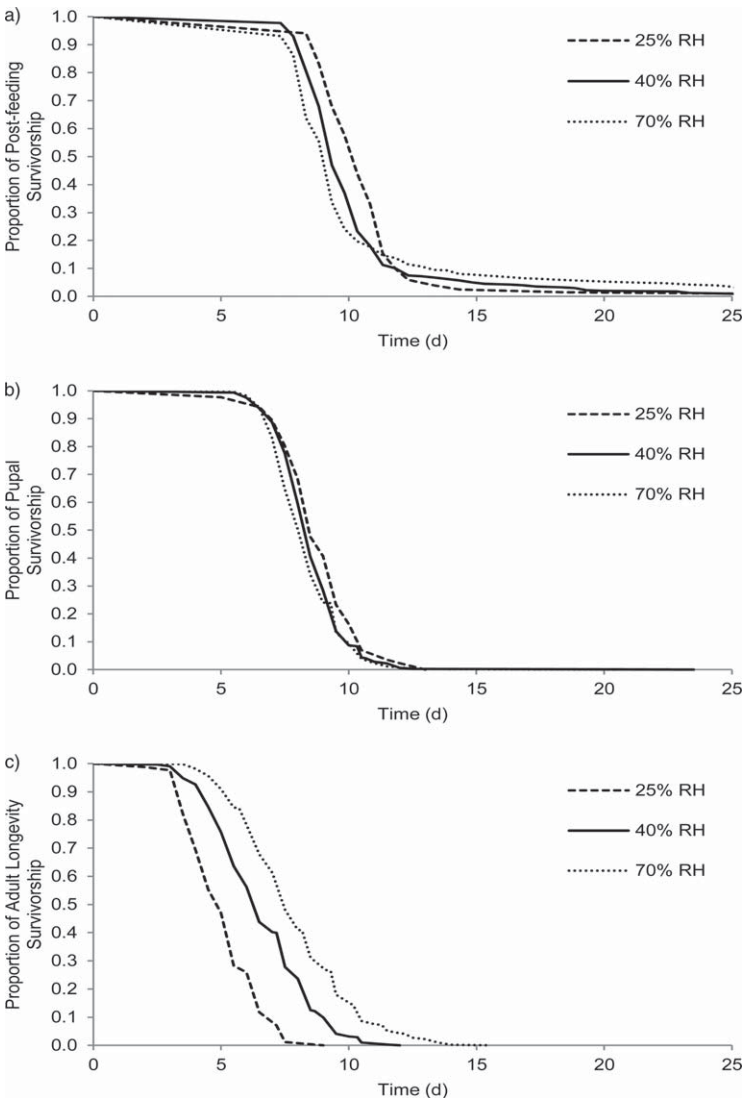

Fig. 1. Proportion of survivorship at each stage of development over time. (a) The effect of RH on time spent in the postfeeding stage of development was different across treatments. (b) The effect of RH on time spent in the pupal stage of development was different across treatments. (c) The effect of RH on adult longevity was different across treatments. ($P < 0.05$, Wilcoxon/Kruskal–Wallis, Cox Proportional Hazard, SAS JMP 8.0.1).

eggs located within the egg cluster had successfully eclosed (unpublished data). Black soldier fly lay their eggs in clusters composed of multiple layers, taking on a three dimensional form of the crevice in which they were oviposited in; however, when no oviposition substrate is provided, females will lay spherical clusters composed of multiple layers which are loosely packed compared with using confined crevices (unpublished data). Therefore, egg cluster shape, size, and layer composition is manipulated by either the oviposition substrate provided or the oviposition site selected by the female. In agreement with Clark and Faeth (1997, 1998), egg clustering may be an oviposition strategy for the black soldier fly; however, conducting choice experiments on oviposition site selection under different RH conditions would provide further insight.

Furthermore, time to egg eclosion was inversely proportional to successful egg eclosion at low relative humidities compared with high relative humidities. Eggs in 25% RH took between 1.5 and 2.5d longer to eclose than eggs in 70% RH. Why low RH conditions delays egg eclosion is not known (Smith 1993; Guarneri et al. 2002, as cited in Han et al. 2008); however, Willmer (1982) suggested longer development times are a result of an increase in energy costs for conserving water because of transpiration. When insects take in oxygen through their spiracles, they concomitantly lose water (Wigglesworth 1984). Unlike juveniles and adults who have the physiology to regulate water loss

trade-offs with respiration, eggs are often immobilized and without mechanisms of controlling water loss during respiration, a requirement of embryo development (Zrubek and Woods 2006). The rate of oxygen conductance through the egg cuticle is very complex (Woods et al. 2005) and the relationship between oxygen conductance and ambient relative humidity and its effect on egg development is unclear.

Interestingly, eggs in the 60% RH treatment eclosed before eggs in the 70% RH treatment with a comparable eclosion success; however, because the mean temperature in the 60% RH growth chamber was recorded a degree higher than in the mean temperature in the 70% RH growth chamber, we cannot attribute this difference solely on the effects of RH. Insect development is directly proportional to temperature with lower temperatures slowing the rate of development and higher temperatures increasing the rate of development in many insects (Gullan and Cranston 2010). Tomberlin et al. (2009) demonstrated that black soldier fly development at 27°C was delayed by 11% when compared with development at 30°C. It would be necessary to run a replicated study with the same experimental design, introducing growth chamber as an added treatment to decipher if a growth chamber effect with respect to temperature is evident.

Because eggs successfully eclosed at 25% RH, we cannot yet decipher the lower RH threshold for black soldier fly egg eclosion. To determine the lower RH threshold, alternative measures in achieving lower RH conditions in the growth chambers, possibly using saturated salt solutions (Pappas, Broufas, and Koveos 2008, Clark and Faeth 1998) would be necessary because the growth chambers used cannot maintain relative humidities below 25% under normal automated programming (unpublished data). Furthermore, eggs developing at 25% RH in chamber 1 eclosed 12 h before those developing in chamber 2. The mean temperature and RH only differed by 0.3°C and 0.3% RH, respectively, between the two growth chambers; however, the mean light intensity in chamber 2 was 10.6% higher than in chamber 1. In *Drosophila melanogaster* (Meign) (Diptera: Drosophilidae), increased light intensity slows development (Bruins et al. 1991), therefore, the delay in development in chamber 2 may be attributed to the increased light intensity.

Because black soldier flies are found more commonly in tropical and subtropical humid environments (Booth and Sheppard 1984), they probably face little selection for tolerating low RH. However, because some eclosion success was evident at lower relative humidities, it may be possible that low RH toleration may have evolved in our black soldier fly colony as a result of mass rearing in a controlled environment for eight years in Tifton, GA. Evolution of low RH tolerance is evident in other arthropods, Ferrero et al. (2010) reared a low RH tolerant laboratory strain of mites over several generations and, therefore, it may be possible to develop a black soldier fly strain that can thrive in low RH conditions. The black soldier fly's natural tropical and subtropical habitats have likely been crucial selective pressures through evolutionary time in their tolerance of RH variation. For the purpose of our objective, although the lower RH threshold could not be determined for egg eclosion or adult emergence, mortality because of desiccation provides sufficient evidence for using higher relative humidities (≥50% RH) in colony maintenance. Although, higher successful egg eclosion was evident in the 50, 60, and 70% RH treatments, demonstrating that egg eclosion and adult emergence success increases with RH, there was successful egg eclosion and adult emergence in the lower RH treatments, leading us to believe that developing a low RH strain of black soldier fly may be possible.

Our results confirm desiccation is highly evident on postfeeding larvae and pupae under low relative humidities with only 16% adult emergence at 25% RH. Desiccation was relatively equally evident in both postfeeding larvae and pupae at 25% RH. Desiccated postfeeding larvae were identified by their brittle, truncated, and flattened appearance. Desiccated pupae were identified at the end of the experiment; 1 mo after the pupae in the 70% RH emerged, when emergence did not occur. Desiccated pupae in the 25 and 40% RH treatments were dissected to assess evidence of dehydration. High proportions of hollowed cavities within the puparium were evident and fat bodies were completely dehydrated. However, the 7% of pupae in the 70% RH treatment that did not emerge were not dissected for evidence of desiccation, because this mortality rate was similar to the mortality rate in Tomberlin et al. (2009) under similar conditions.

An insect's metabolic response to desiccation involves an up regulation of the desiccation stress protein 28 (dsp28) and juvenile hormone (JH) (Kroeker and Walker 1991a). Kroeker and Walker (1991b) studied the correlation of up regulated dsp28 and JH in the common mealworm *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae). They found that JH and dsp28 are up regulated during ecdysis, down regulated just before pupation and not up regulated again until adult reproduction (Kroeker and Walker 1991b). Should this mechanism be similar in the black soldier fly, a desiccation response to low RH may involve an up regulation of dsp28 and JH during the postfeeding stage of development, down regulation in the pupal stage and up regulation again in the adult stage during mating and reproduction. This would explain why postfeeding larvae were able to successfully pupate; however, inevitably succumb to desiccation in the pupal stage of development because of a down regulation of dsp28. On the contrary, hollow body cavities observed in the dissected pupae may not be a result of desiccation, but instead, exhausted fat bodies because of the postfeeding larvae's delayed time to pupation. Regardless, with 16 and 59% successful adult emergences in the 25 and 40% RH, respectively, RH did significantly impact *H. illucens* development.

One drawback to this study is the lack of observations on adult fecundity. Although a proportion of adults successfully emerged in each RH treatment, RH effects on successful mating and oviposition are unknown. RH effects on fecundity have been studied by

several authors in several arthropod systems (Cunnington 1985, Wilson 1982, Kitayama 1979, Coombs 1978, Richards 1947). For example, low RH environments delays oviposition in *Acarus siro* (L.) (Acarina: Acaridae) (Cunnington 1985). Similarly, adult weight and oviposition increased with higher RH in *Crvptolestes pusillus* (Schönherr) (Coleoptera: Cucujidae) (Currie 1967). On the contrary, various species of spider mites have higher oviposition and longer longevity at lower RH (Kramer and Hain 1989, Boudreaux 1957). In our study, the only indicator of adult fitness we have is longevity, with adults in 70% RH living 2 to 3 d longer than adults subjected to lower RH environments. Despite longevity trends, we have no evidence in this study that ovarian development and successful mating followed by viable egg deposition, is correlated to adult longevity. Additionally, the greenhouse conditions with surrounding outbuildings cannot be excluded as potentially impacting the results generated from this study.

Reduced indoor ambient RH throughout Canadian winters is at a direct consequence to heating systems in greenhouses and homes. Before commencing this study, unsuccessful rearing of the black soldier fly was attempted in a greenhouse without the addition of humidifiers in Windsor, Ontario, during the winter season. The results of this study might explain prior unsuccessful attempts of rearing the black soldier fly and suggests that in Canada and similar locations experiencing cold weather, year-round waste management using the black soldier fly will require that steps be taken to increase RH in heated indoor facilities.

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