Received: 4 November 2022 | Revised: 13 February 2023 | Accepted: 15 February 2023

DOI: 10.1111/jen.13109

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Bioconversion of poultry manure and vegetable waste mixes by *Hermetia illucens* (Diptera: Stratiomyidae)

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Funding information
South African Department of Trade and Industry through a Technology and Human Resource for Industry Programme, Grant/Award Number: THRIP/51/30/11/2017

Abstract

Development time, survival and final nutrient content of *Hermetia illucens* L. larvae depends on the substrates in which they develop. Mixing different waste types together can increase the performance and survival of the larvae, as well as their waste reduction. The main objective of this study was to evaluate the effect of different ratios of mixed fruit and vegetable waste with poultry manure on larval development time, size, biomass production, survival, bioconversion and waste reduction. Freshly hatched neonates (90 mg; approx. 6000 individuals) were placed on 12 kg of a mixture of fruit and vegetable waste and fresh, unprocessed poultry manure and held at 28 ± 0.5°C. Inclusion of fruit and vegetable waste varied from 0% to 100% in 10% increments. Initial temperature of the substrate was also measured. The individual mass of larvae increased significantly as more fruit and vegetable waste was included, from less than 81.3 ± 6.6 mg on poultry manure only to an average size of 211.6 ± 6.0 mg at 100% fruit and vegetable waste. After approximately 60% inclusion of fruit and vegetable waste the performance and survival of the larvae increased significantly while development time was reduced. A combination of high fruit and vegetable waste and low initial temperatures resulted in lower development time overall. The mixing of wastes can be applied in industry to further the goals of waste reduction and biomass production while incorporating low-quality wastes like poultry manure.

KEYWORDS
development time, moisture content, small industrial scale, survival, waste reduction

1 | INTRODUCTION

Organic waste such as excess manure and rotting fruit and vegetables poses problems for its disposal. A recent solution that has been proposed is to reduce waste and recover nutrients from it through bioconversion using fly larvae. The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) has mostly been investigated for bioconversion because the larvae have voracious appetites and naturally occur in, and are attracted to, several different organic waste types, including different types of manure. Fly larvae of *H. illucens* held at high density can reduce a large amount of waste in a short period of time while also reducing the presence and activity of other species of fly larvae in the waste, aerating the waste and increasing evaporation, improving the pH and increasing the temperature of the substrate, suppressing the growth of bacteria, reducing volatile emissions and eliminating pathogens (Beskin et al., 2018; Diener et al., 2015; Van Huis, 2013).

Development time, survival and final nutrient content of *H. illucens* larvae are highly dependent on the substrates used

(Barragan-Fonseca et al., 2018; Danieli et al., 2019; Nguyen et al., 2013; Tomberlin et al., 2002; Tschirner & Simon, 2015). Research regarding *H. illucens* larvae fed manure has focused on dairy, swine or poultry manures (Diener et al., 2009; Miranda et al., 2020; Oonincx et al., 2015; Zhou et al., 2013). Larvae can successfully develop on all three manure types but perform best on poultry manure (Miranda et al., 2020; Zhou et al., 2013), as poultry manure is lower in fibre and higher in protein than either swine or dairy manure (Chen, 2003). However, when an unbalanced diet is provided, such as a single waste type, metabolic costs may be experienced, reducing food intake, and prolonging development time (Gligorescu et al., 2018). Larvae feeding on an unbalanced diet can implement pre- and post-ingestive mechanisms to more effectively utilize the nutrients present in lower quality substrates (Cappellozza et al., 2019). For example, when *H. illucens* larvae are fed a low-quality substrate of fruit and vegetable waste, which is high in carbohydrates and low in protein, physiological and morphological modifications occur in their midgut, specifically an increase in the length of their microvilli for increased nutrient absorption (Cappellozza et al., 2019). *Hermetia illucens* larvae also have a larger arsenal of inducible digestive enzymes than other fly species such as *Musca domestica* L. (Kim et al., 2011), that play a role in coping with unbalanced diets.

Hermetia illucens larvae perform better when provided with a mixture of different substrates than when feeding on only one waste type at a time (Ur Rehman, Cai, et al., 2017). The majority of studies investigating diet blends have looked at the performance of *H. illucens* larvae fed specifically formulated artificial diets or on only one or at best a combination of two waste types at a time (Cammack & Tomberlin, 2017; Ur Rehman, Cai, et al., 2017). This may be practical for the purposes of waste reduction but not for the consistent production of an alternative protein source (Cammack & Tomberlin, 2017). The factors regulating or limiting *H. illucens* larval development, survival and nutrient acquisition on different waste streams are still poorly understood (Cammack & Tomberlin, 2017). The formulation of diets from a more variable mixture of different waste types with more targeted or balanced protein, lipid or carbohydrate content could lead to a vastly improved and more consistent nutrient profile and better biomass production by larvae while also maximizing waste reduction (Barragan-Fonseca et al., 2018; Cammack & Tomberlin, 2017; Danieli et al., 2019).

The main objective of this study was to evaluate the effect of different ratios of mixed fruit and vegetable waste with poultry manure on larval performance in terms of development time, size, biomass production, survival, bioconversion and waste reduction. Poultry manure is a low-quality substrate for larvae to feed on (Chen, 2003), and it is likely that as the fraction of fruit and vegetable waste decreases and the poultry manure increases, the performance of the larvae will go down. However, the presence of some level of poultry manure may be beneficial to the larvae as it will introduce alternative nutrients that may be limited in the fruit and vegetable waste alone, leading to an improvement in larval performance. Determining an optimal combination of mixed fruit and vegetable waste with poultry manure with a high larval performance and a high waste reduction would assist in using *H. illucens* in protein production and waste reduction. The effect of initial temperature of the waste on the performance of the larvae was also evaluated in this study. Initial temperature of the waste may have an impact on early larval stage survival and performance, which will be valuable information as the temperature may vary between different batches. This is a variable that can also potentially be controlled for.

2 | MATERIALS AND METHODS

2.1 | Experimental insects

Freshly hatched *H. illucens* larvae, called neonates, were collected from an established culture held at Aegis Environmental Bioconversion Facility, Centurion, South Africa. The culture of adult flies is held under constant conditions in a climate-controlled room (30 ± 0.5°C, 70% RH) with 12:12 L:D cycle provided using UV fluorescent lights (Repti Glo 10.0 UVB 40 W). The eggs were collected once a day in egg traps and were kept in an incubator (26 ± 0.5°C, 65% RH) until hatching was completed. Egg traps are made from white, extruded twinwall fluted/corrugated polypropylene sheet (Correx, Duroplastic Technologies), cut into 3 cm × 8 cm sheets stacked in a group of eight and held together by an elastic band. Neonates were collected less than 3 h after hatching from containers placed underneath the egg traps. Neonates were weighed using a milligram scale (AE Adam® HCB123 120 g × 0.001 g) and weighing boats. A single neonate weighs approximately 0.015 mg (Cammack & Tomberlin, 2017, *personal observations*).

2.2 | Effect of fruit and vegetable waste inclusion with poultry manure on larval performance

Freshly hatched neonates (90 mg; approx. 6000 individuals), were placed on 12 kg of a mixture of fruit and vegetable waste and fresh, unprocessed poultry manure and held at an ambient temperature of 28°C ± 0.5°C, maintained using a combination of wall mounted bar heaters and fans. The fruit and vegetable waste was collected from a local fresh produce market in Lyttelton, Gauteng province, South Africa. The poultry manure was collected from a local layer hen farm in Rietvlei, Gauteng province, South Africa. The layer hens were over 20 weeks old and fed on standard layer hen diet with 18% protein provided by soya meal inclusion. Inclusion of fruit and vegetable waste varied from 0% to 100% (Table 1). Each treatment was repeated 16 times. At the point where neonates were added to the waste mix, initial temperature of the waste mix was measured using a combination pH meter with a temperature probe, Testo 206 (Entech Industrial Solution Co., Ltd.). The pH measurements that were taken were excluded from further analysis due to incorrect calibration of the pH meter that resulted in inaccurate results. Larvae were allowed to feed until they completed development and

TABLE 1 Quantity of fruit and vegetable waste and chicken manure in each substrate treatment.

Code	Fruit and vegetable waste (kg)	Manure (kg)	Replicates
0%	0	12	16
5%	1.2	10.8	16
10%	2.4	9.6	16
20%	3.6	8.4	16
30%	4.8	7.2	16
40%	6	6	16
50%	7.2	4.8	16
60%	8.4	3.6	16
70%	9.6	2.4	16
80%	10.8	1.2	16
90%	11.4	0.6	16
100%	12	0	16
Total[a]	1298.4	1065.6	192

Note: Treatments were formulated with a given percentage of fruit and vegetable waste relative to the total weight of the substrate. All experiments were conducted in a room held at 28 ± 0.5°C ambient temperature.

[a]Total waste calculated by sum of each trial multiplied by the number of replicates.

were harvested just prior to becoming prepupae. Once larvae were harvested, the weight of all larvae, individual mass of 20 larvae, and the remaining frass was recorded. A 160-g sample of the larvae was collected and dried using a convection oven to constant weight to determine the moisture content of the larvae. The dry matter content of the initial waste and the frass were determined by taking the average from three samples of 5 g, dried in an oven for 8 h at 80°C. The final total biomass, final individual weight, total waste reduction, bioconversion and efficiency of conversion of digested feed (ECD) were calculated using the equations in Diener et al. (2009). Moisture content of larvae or waste weight was determined by subtracting final dry weight from the initial wet weight and dividing by the initial wet weight and converting it to a percentage of the original mass. This was used to determine total waste reduction Equation (1), bioconversion Equation (2) and ECD Equation (3) on a dry matter basis.

Waste Reduction (%) =

$$\frac{\textit{dry weight of waste mass}(i) - \textit{dry weight of waste mass}(f)}{\textit{dryweight waste mass}(i)} \times 100 \quad (1)$$

Bioconversion (DM basis) =

$$\frac{\textit{larval biomass}(f) - \textit{larval biomass}(i)}{\textit{waste mass}\ (i)} \times 100 \quad (2)$$

Efficiency of conversion of digested feed (ECD) =

$$\frac{\textit{larval biomass}(f) - \textit{larval biomass}(i)}{\textit{waste mass}\ (i) - \textit{waste mass}\ (f)} \times 100 \quad (3)$$

Generalized additive models (GAM) were used to evaluate the effects of both percentage fruit and vegetable waste and substrate initial temperature on development time, individual larval mass, final total biomass on a dry matter basis, biomass of individual larvae on a dry matter basis, survival, total waste reduction, bioconversion and ECD. The additive effect of percentage fruit and vegetable waste and substrate initial temperature was included in all models (i.e., y ~ FVW% + iTemp). All data analyses were run using R version 3.6.2 (R Core Team, 2020) using packages mgcv, gamair and lme.

3 | RESULTS

The total development time of the larvae decreased significantly, in a non-linear manner, as the inclusion of fruit and vegetable waste increased, from an average of 24.6 ± 1.0 days (mean ± SE) at 0% inclusion to an average of 20.3 ± 0.9 days at 100% inclusion of fruit and vegetable waste (Figure 1a). Initial temperature also had a significant effect on the total development time (Figure 2a), where higher initial temperatures of over 26°C led to longer development times. However, the adjusted R^2 value and percentage deviance explained (Table 2) by the GAM was low, indicating that fruit and vegetable waste and initial temperature only partially explain variation in total development time. A combination of high fruit and vegetable waste and low initial temperature results in lower development time overall, while low fruit and vegetable waste percentage and high initial temperatures results in longer development times (Figure 3a).

The individual mass of larvae increased significantly as more fruit and vegetable waste was included, from less than 81.3 ± 6.6 mg at 0% fruit and vegetable waste to an average size of 211.6 ± 6.0 mg at 100% (Figure 1b). Initial temperature also had a significant effect on the individual mass of larvae (Figure 2b), where higher initial substrate temperatures, especially temperatures from 29°C, led to lower individual larval mass of 122 ± 47 mg, reduced from 167 ± 46 mg at lower initial temperatures of between 23°C and 26°C. The GAM explained almost 69% of the variation in individual larval mass. Low fruit and vegetable waste and low initial temperature resulted in the largest larvae, while higher temperatures combined with low fruit and vegetable waste percentage resulted in much smaller larvae (Figure 3b).

The total biomass on a dry matter basis produced per tray increased from an average of 200 ± 21 g per tray to 266 ± 17 g per tray as fruit and vegetable waste inclusion increased from 0% through to 100% (Figure 1c). The GAM explained 58.8% of the variation in total biomass. However, initial temperature had no significant effect on total biomass (Table 2, Figure 2c).

In contrast, initial temperature did have a significant effect on the dry matter percentage of individual larvae (Figure 2d). Individual larvae harvested from a substrate with initial temperature of around 23°C–29°C had an average dry matter of 35.6 ± 4.0%, whereas temperatures above 29°C led to reduced dry matter of individual larvae of 31.1 ± 5.8%. The dry matter of individual larvae

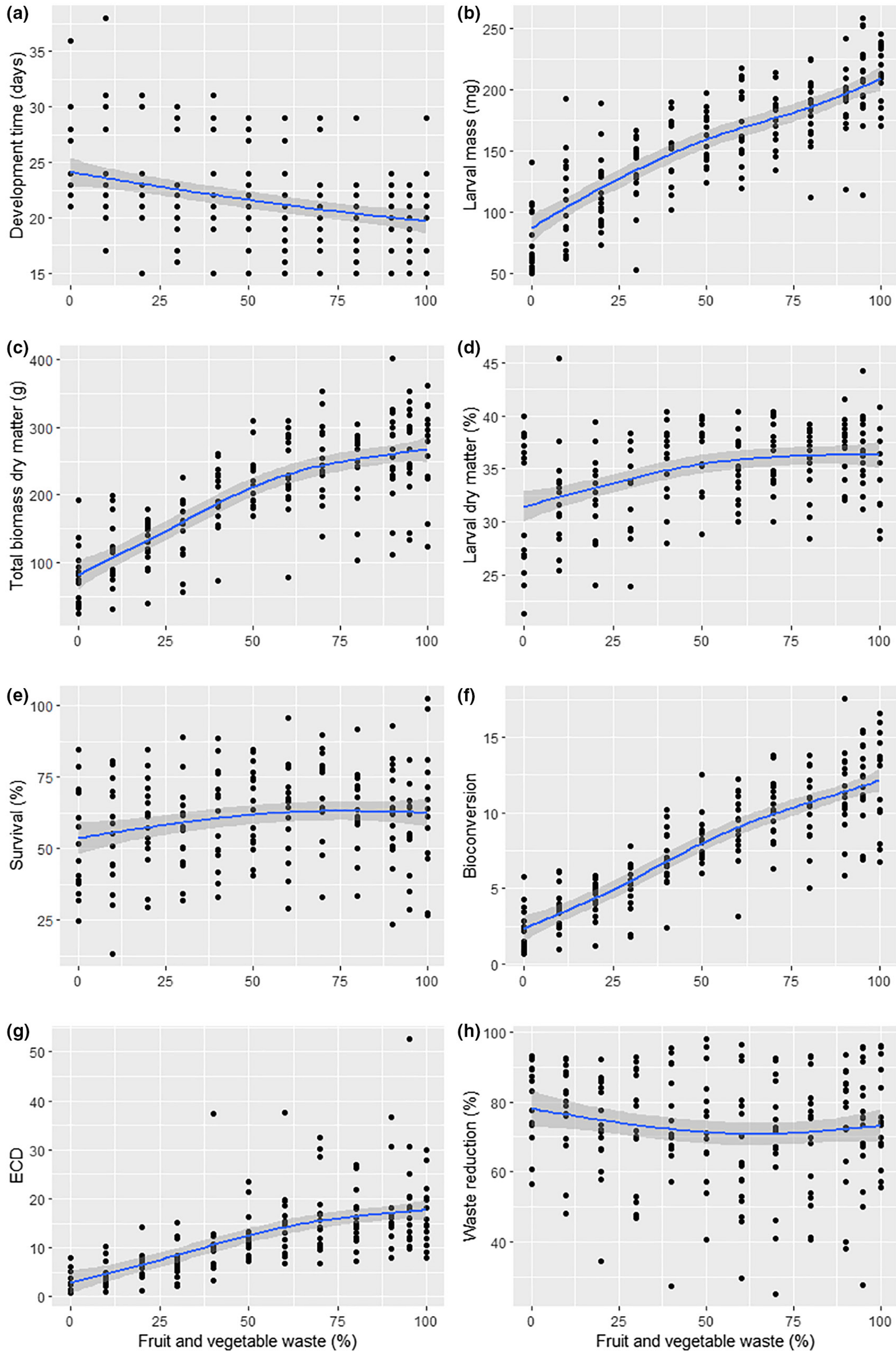

FIGURE 1 Effect of increasing fruit and vegetable waste in a substrate with chicken manure on different parameters of larval performance, bioconversion and waste reduction achieved by *H. illucens*. (a) Development time (days), (b) individual larval mass, (c) total biomass on a dry matter basis, (d) biomass of individual larvae on a dry weight basis (%), (e) survival (%), (f) bioconversion, (g) efficiency of conversion of digested material (ECD) and (h) waste reduction. Each graph depicts the curve fitted using a generalized additive model. The shaded region around the fitted line indicates the 95% confidence interval. [Colour figure can be viewed at wileyonlinelibrary.com]

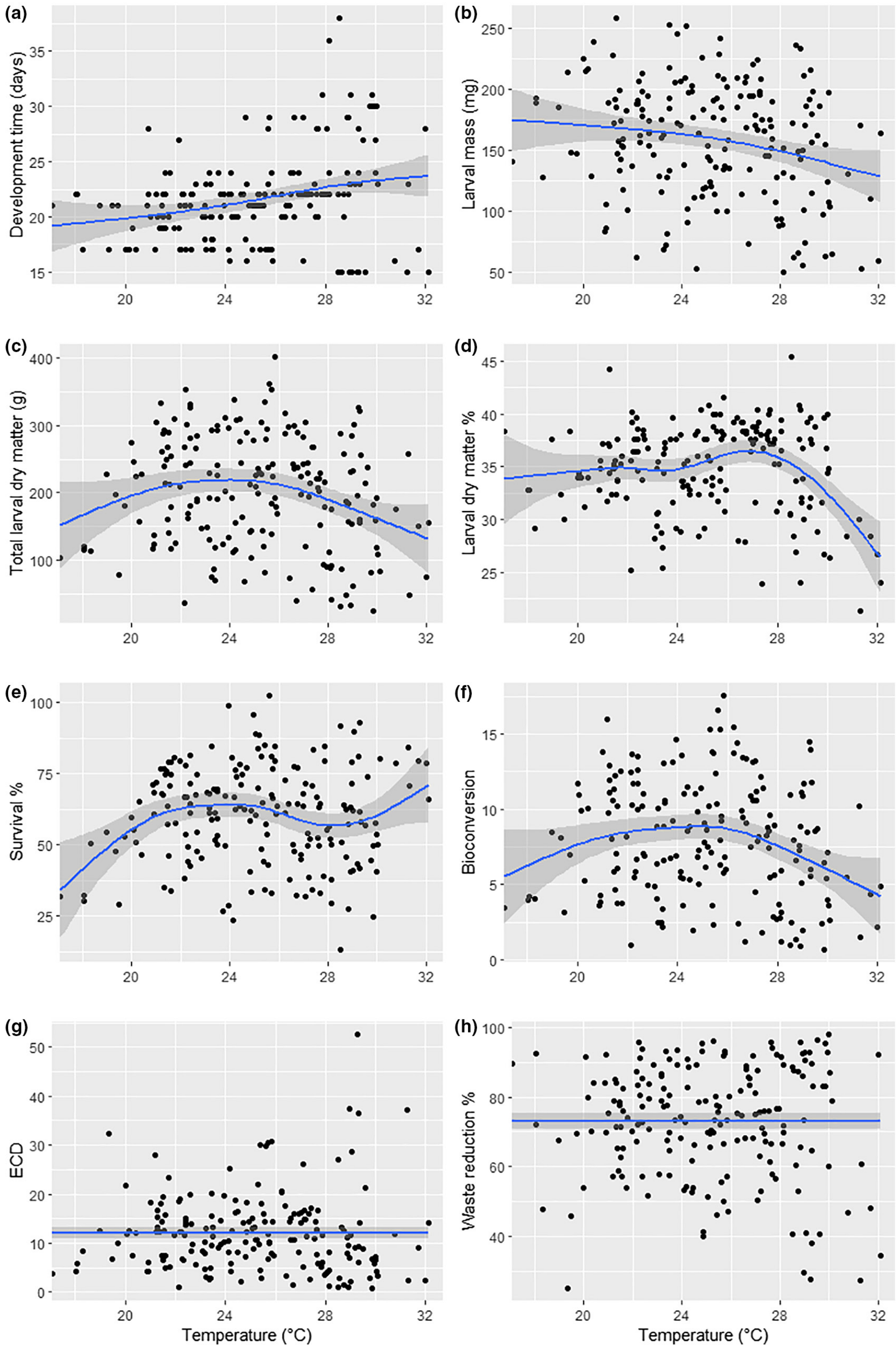

FIGURE 2 Effect of initial temperature of substrates comprising fruit and vegetable waste and chicken manure on different parameters of larval performance, bioconversion and waste reduction. (a) Development time (days), (b) individual larval mass (mg), (c) total biomass on a dry matter basis, (d) biomass of individual larvae on a dry matter basis (%), (e) survival (%), (f) bioconversion, (g) efficiency of conversion of digested material (ECD) and (h) waste reduction. Each graph depicts the line fitted using a generalized additive model. The shaded region around the fitted line indicates the 95% confidence interval. [Colour figure can be viewed at wileyonlinelibrary.com]

Response variables	Edf	Ref. df	*F*	Approximate *p*-value	R^2 (adj)	Deviance explained
Development time						
FVW%	1.004	1.008	33.179	**0.001**	0.244	26.4%
iTemp	3.929	4.900	5.442	**0.001**		
Individual larval mass						
FVW%	2.366	2.934	121.348	**0.001**	0.679	68.8%
iTemp	2.868	3.640	5.594	**0.001**		
Total biomass dry matter						
FVW%	2.715	3.368	74.220	**0.001**	0.580	58.8%
iTemp	1.003	1.005	1.450	0.230		
Dry matter of larvae						
FVW%	2.144	2.661	9.186	**0.001**	0.251	27.9%
iTemp	4.871	5.972	3.717	**0.010**		
Survival						
FVW%	2.241	2.780	3.525	**0.016**	0.040	5.7%
iTemp	4.584	5.650	3.560	**0.010**		
Bioconversion						
FVW%	2.009	2.495	181.901	**0.001**	0.713	71.8%
iTemp	1.000	1.001	0.949	0.331		
ECD						
FVW%	3.259	4.041	72.330	**0.001**	0.627	64.0%
iTemp	3.116	3.939	1.560	0.163		
Waste reduction						
FVW%	2.445	3.034	4.289	**0.010**	0.100	12.9%
iTemp	3.590	4.503	2.994	0.134		

Note: Significant *p*-values ($p < 0.05$) are in bold type.
Abbreviation: ECD, Efficiency of conversion of digested material.

TABLE 2 Approximate significance of smooth terms from generalized additive models predicting the effects of fruit and vegetable waste percentage (FVW%) and initial temperature (iTemp) on larval performance parameters, bioconversion and waste reduction achieved by *H. illucens*.

also increased from an average of 31.2 ± 1.6% dry matter to an average of 35.1 ± 1.0% at 100% fruit and vegetable waste inclusion (Figure 1d). The GAM only explained 27.9% of the variation present in the percentage dry matter of larvae. The combination of low fruit and vegetable waste and low initial temperatures was where larvae with the highest dry matter content were produced, whereas higher initial temperatures masked the effects of fruit and vegetable waste % (Figure 3c).

The survival of the larvae in relation to the percentage of fruit and vegetable waste in the substrate was not linear. It increased significantly from 0 to 60%, with an average survival of 51.0 ± 4.6% for 0% fruit and vegetable waste. Survival was very similar for trays that had between 60% and 100% fruit and vegetable waste, with average survival of 62.6 ± 5.7% to 64.5 ± 4.3%, respectively (Figure 1e). Initial temperature had a significant effect on survival as well, with a non-linear relationship (Figure 2e). However, the adjusted R^2 value and percentage deviance explained were extremely low, indicating that fruit and vegetable waste percentage and initial temperature only weakly describe variation in survival. Survival of larvae was very strongly impacted by the initial temperature and less affected by the fruit and vegetable waste, before reaching 60%, when viewed in combination (Figure 3d). Higher initial temperatures resulted in higher survival rates across different fruit and vegetable waste percentages.

Both bioconversion (Figure 1f) and ECD (Figure 1g) values increased significantly, from an average of 2.38 ± 0.36 to 12.14 ± 0.81, and 3.13 ± 0.52 to 16.83 ± 1.65, respectively, as the inclusion of fruit and vegetable waste increased in the substrate. The relationship between fruit and vegetable waste inclusion and waste reduction was not linear and the lowest waste reduction value was at 60% fruit and vegetable waste inclusion, at 65.89 ± 4.78% and the highest was at 0% fruit and vegetable waste, or 100% poultry manure, at 79.84 ± 2.08% total waste reduction on a dry matter basis (Figure 1h). Initial temperature had no significant effect on bioconversion, ECD or waste reduction (Figure 2f–h; Table 2). The GAM explained 64% of variation in ECD, but the additive effects of percentage fruit and vegetable waste inclusion and initial temperature of the substrate only weakly explained variation in waste reduction.

4 | DISCUSSION

After approximately 60% inclusion of fruit and vegetable waste the performance and survival of the larvae increased significantly while

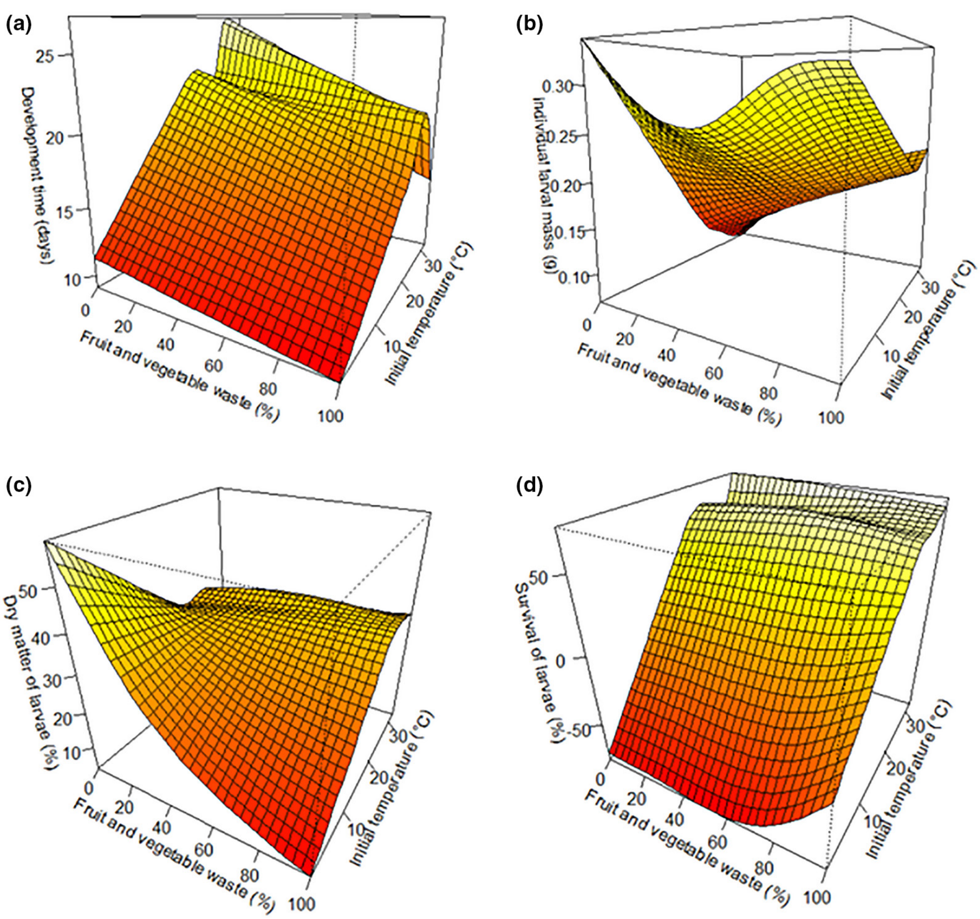

FIGURE 3 Response surfaces depicting the generalized additive model effects of fruit and vegetable waste and initial temperature on (a) development time (days), (b) individual larval mass (g), (c) dry matter of larvae (%), (d) survival (%). [Colour figure can be viewed at wileyonlinelibrary.com]

development time was reduced. The inclusion of poultry manure, even at lower inclusion percentages, did not improve the survival or performance of the larvae. This indicates that a mix of just fruit and vegetable waste is sufficient for *H. illucens* larvae to grow and survive, but that the inclusion of up to 40% poultry manure is acceptable for waste reduction purposes, as sufficient fruit and vegetable waste is still present for the larvae to reach full maturity.

Higher bioconversion values indicate effective uptake of nutrients from the waste, while higher ECD values indicate efficient conversion of waste into biomass. In this study, both bioconversion and ECD values increased as fruit and vegetable waste inclusion increased, indicating that the larvae were more able to access and take up the nutrients that were present in the waste mix as the poultry manure fraction decreased. Mixing of different waste types has been found in previous studies to improve overall waste reduction and bioconversion (Ur Rehman, Cai, et al., 2017; UR Rehman, Rehman, et al., 2017). This may be due to increased nutrient balance, and improved balance of pH, or buffer capacity, of the waste. However, the values of waste reduction that were achieved in this study were higher than have been previously reported, even for 100% poultry manure, where up to 80% waste reduction on a dry matter basis was achieved. Sheppard et al. (1994) reported an observed reduction of 50% of poultry manure, while Oonincx et al. (2015) achieved 37% reduction on a dry matter basis. The higher waste reduction achieved in this study may be due to the use of fresh manure that had not been dried or frozen prior to use. Drying and rehydrating or freezing and thawing manure could lead to destruction of nutrients, such as heat-labile vitamins, and reduction of the microbial load in the waste (Oonincx et al., 2015). In addition, certain bacteria present in waste that promote growth and survival of *H. illucens* larvae (Oonincx et al., 2015; Yu et al., 2011), and may also be affected by different means of waste storage. The potential for freezing of waste to affect parameters associated with *H. illucens* larval performance should be explored for a variety of waste types, especially when there are lower nutrient waste types involved.

Mixing of different waste types can have a beneficial impact on the survival and performance of larvae. In this study, survival decreased as poultry manure inclusion increased but even with little poultry manure in the substrate mean survival never surpassed 65%. The results presented in this study differ from previous results where larvae that were grown on poultry manure usually experienced reduced biomass but high survival (>85%) (Ur Rehman, Cai, et al., 2017). One of the major differences in this study from many others is the seeding of neonates directly on the waste rather than larvae that had been initially grown on a starter feed (Beskin et al., 2018; Miranda et al., 2019, 2020) before being placed on the waste. Starter diets are usually a standard or formulated artificial diet, such as the Gainesville diet (Hogsette, 1992), or another diet that is not disclosed in the study (Li et al., 2011; Ur Rehman, Cai, et al., 2017), that is as close to nutritionally balanced, with the correct moisture content, as possible. This strongly indicates the benefits of using starter feeds, which reduces larval mortality during their most vulnerable stage. As larvae grow significantly during the first two to 3 days after hatching, they are more robust and can navigate the conditions that they experience on the waste more effectively and efficiently. Challenging or detrimental conditions, such as imbalanced nutrient availability, variable pH, variable moisture, interspecific competition or high microbial load, can also reduce larval survival during early development.

Despite the benefits of using starter diets for *H. illucens* neonates, their use also poses challenges for comparing results across studies. Larvae initially fed starter diets are often introduced in terms of age (4 days old to up to seven or 10 days old) rather than mass (Beskin et al., 2018; Miranda et al., 2019, 2020; Spranghers et al., 2017; Ur Rehman, Cai, et al., 2017). The size of the larvae does not relate directly to their age, as growth rate is highly dependent on the density, ambient temperature, moisture content, nutrients and other functional properties of the substrate. Therefore, there is limited capacity to compare performance, in terms of development time, size and survival of larvae between different studies that do not follow the same procedures. The values that are provided for waste reduction and bioconversion are also difficult to compare, as the data available from other studies do not indicate the overall size change that the larvae achieved from the start to the end of an experiment. Other factors to consider when looking at the use of neonates directly on the waste compared to the use of starter feeds are the economic costs and benefits. Lower survival and longer development times can translate into reduced yield and increased cost respectively (Miranda et al., 2019). However, the cost of a starter feed may be high as well.

Initial temperature of the substrate had a significant effect on larvae performance. Neonates, which are extremely small and therefore extremely vulnerable to outside factors, are significantly affected by the initial temperature of the substrate (Putra et al., 2022; Vogel et al., 2022). This may lead to differences in survival, which will translate into differences between larvae, such as total development time and individual larval mass near the end of development. Higher initial temperatures of over 29°C may introduce a stress factor to neonates, contributing to increased development time and decreased individual larval mass. Chia et al. (2018) found that black soldier fly larvae thrive best at intermediate temperatures. Further research should look into the importance of the initial temperature that neonates experience when seeded on a substrate, the temperature of the substrate during development and the difference between the temperature of the substrate and the ambient temperature. Starter diets, that have a lower initial amount of substrate, can likely start at and be maintained at a close to optimized initial temperature.

More research is also required on the impact of substrate temperatures on performance of *H. illucens* larvae (Chia et al., 2018). Substrate temperatures can become elevated due to the activity of larvae or microbes in the waste (Kotzé et al., 2016). This increased temperature in the substrate can lead to improved larval development over time and decreased mortality but could also lead to thermal stress, less efficient nutrient assimilation and stunted growth (Chia et al., 2018; Shumo et al., 2019). Substrates may also have lower than ambient temperatures due to high water loss, leading to evaporative cooling that could suppress development of the larvae and reduce survival (Erbland et al., 2021), especially during the early stages of development.

There are several benefits of using *H. illucens* larvae to reduce waste, especially environmentally problematic waste such as manure. Larvae successfully reduce the waste, the presence of pathogens (Lalander et al., 2013; Liu et al., 2008) and emission of volatile organic compounds (Beskin et al., 2018); recover nutrients; and produce biomass that can be processed into valuable products. However, poultry manure is a low-quality substrate that is low in nutrients. Mixing different waste types together can improve potential nutrient limitation, or other functional properties of the substrates, and increase the performance and survival of larvae, as well as reducing waste r (Ur Rehman, Cai, et al., 2017; UR Rehman, Rehman, et al., 2017). The mixing of wastes is also something that can be applied in industry to further the goals of waste reduction and biomass production without having to resort to incorporating expensive ingredients in the substrate, such as bran or chicken feed.

AUTHOR CONTRIBUTIONS

Nina Jennifer Parry: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; writing – original draft; writing – review and editing. **Christopher W Weldon:** Conceptualization; methodology; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGEMENTS

This research was supported by the South African Department of Trade and Industry through a Technology and Human Resource for Industry Programme grant (THRIP/51/30/11/2017) to Aegis Environmental (Pty.) Ltd., the University of Pretoria (C.W.W.) and the University of Venda. N.J.P. received a PhD scholarship associated with the grant.

CONFLICT OF INTEREST STATEMENT

No conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the results of this study are available at the following link: https://doi.org/10.25403/UPresearchdata.21404889

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How to cite this article: Parry, N. J., & Weldon, C. W. (2023). Bioconversion of poultry manure and vegetable waste mixes by *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Applied Entomology*, *147*, 279–288. https://doi.org/10.1111/jen.13109