2023

The Black Soldier Fly, Hermetia illucens Linnaeus (Diptera: Stratiomyidae): A Novel Approach to Combat Food Waste in Windsor-Essex.

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Recommended Citation

Okpara, Patricia Obianuju, "The Black Soldier Fly, Hermetia illucens Linnaeus (Diptera: Stratiomyidae): A Novel Approach to Combat Food Waste in Windsor-Essex." (2023). *Electronic Theses and Dissertations*. 8965.
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The black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae): a novel approach to combat food waste in Windsor-Essex.

By

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A Dissertation
Submitted to the Faculty of Graduate Studies
through the Department of Integrative Biology and the Department of Biomedical
Sciences in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy
at the University of Windsor

Windsor, Ontario, Canada

2023

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ABSTRACT

Within Ontario alone, approximately 3.7 million tonnes of organic food waste is generated yearly. This waste includes food scraps, wasted food, and plant wastes such as leaves, plant stems, and fruit. Most of it is transported to landfills for composting each year, but the use of landfills as a method of waste management is not sustainable. It adds strain to the environment by releasing harmful greenhouse gases and by demanding landfill space. The current protocols set by the Ontario government, while promising, do not address methods that support the safe disposal of organic waste and conversion into valuable end products that could contribute economic benefits, the black soldier fly presents an opportunity to address this lack.

The black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) has the potential to reduce organic waste, including kitchen waste and manure. My dissertation investigated the black soldier fly as a means of waste management within Windsor-Essex, and its role in converting food waste into economically valuable end products. I investigated the black soldier fly's ability to consume local municipal food waste from within this region. The flies reduced approximately 70% of the waste, and allowed me to develop a baseline for black soldier fly waste reduction within the area. As I measured slower development to adult and reduced waste reduction efficiency compared to a control diet of poultry feed, I investigated the potential role of pre-digestion and fermentation with beneficial microbes in the black soldier fly waste reduction process with the goal of improving the baseline waste reduction efficiency. Fermentation time impacted development and waste reduction efficiency of the black soldier fly. Diets fermented for 0 days had a positive influence on the survival and bioconversion efficiency of the black soldier fly, while diets fermented for 2 days had a positive influence on the relative growth rate and waste reduction efficiency. Diets fermented for longer than 2 days negatively affected the black soldier fly development, growth and waste conversion efficiency. The results show that using beneficial microbes is not straightforward and might depend on the purpose of the bioconversion process. Since larval density influences the waste conversion efficiency, it is essential to rapidly quantify egg numbers for introduction to waste streams. Thus, I developed a commercially-scalable model to quantify the number of eggs oviposited by female black soldier flies based on egg mass weight or volume. The model was created using linear regression of egg masses across a range of sizes and relating egg number to relate egg mass weight or volume. Once the linear equation was developed, it was validated with a new set of egg masses of varying sizes. Egg mass volume and weight were positively correlated to the number of eggs deposited in an egg mass, and either can be used to estimate the number of eggs within egg masses. Finally, I investigated the use of black soldier fly processing residue (residual wastes, shed exoskeletons, and frass) as a fertilizer for an economic value-added product. Tomato seeds were planted in an inert growing media (coconut coir) with three concentrations of the processing residue and compared to a control of slow-release fertilizer. The two highest concentrations of black soldier fly processing residue resulted in failed germination, whereas the lowest concentration supported germination and growth with larger root and shoot biomass, larger leaf area, and a higher number of flowering trusses compared to the slow-release fertilizer treatment. Together, my research provides valuable new insight into the black soldier fly's waste conversion ability, an essential tool for commercially-scalable methods of quantifying egg numbers in a non-destructive and timely manner that allows the establishment of optimized feed rates for the black soldier fly in waste management, and a useful end product to promote a circular economy.

DEDICATION

To my parents, Patricia Nwakaego Okpara and Simon Ndu Okpara
Thank you for all your unceasing Support, Guidance, and Love.

ACKNOWLEDGEMENTS

First, I would like to being by thanking my supervisor Dr. Sherah VanLaerhoven, thank you for allowing me to undertake a Ph.D. project and for all your mentorship during this time. Thank you for continuously allowing me to be independent and find my passion for research.

I want to thank my committee members, Dr. Oliver Love, Dr. Tanya Noel and Dr. Edwin Tam, for their support, guidance and suggestions that strengthened my research. Thank you to my collaborator Dr. Cameron Proctor for all your help with my last chapter and for allowing me to conduct research in your lab.

Next, I would like to thank my Lab mates Catalina, Madison, Carly, Theresa, Vanessa, Jeff and Paige for all the good laughs and encouragement. To Catalina, thank you for reading all my drafts and for your supportive comments. Maddie, thank you for all the work sessions that ultimately got me to where I needed to be. Carly and Jeff, thank you for all the advice and support. And Paige, thank you for all the adventures. To Theresa and Vanessa, thank you for being my friend and for all the coffee dates. To all my lab mates, thank you for teaching me to trust my instincts and for encouraging me to take chances.

Additionally, I would like to thank the stockroom ladies, Rodica Leu and Ingrid Churchill for all their patience and help. To my friends, Zea, Deola, Don, Theresia, Ellen, and Keshwana thank you for cheering me on all through, and for always being there for me.

Finally, I owe everything to my family, my parents for their unceasing financial and emotional support, to my father, you are one of my role models, and your memory will forever inspire me. To the rest of my siblings, thank you for believing in me, supporting me and cheering me on. I love you all to the moon and back.

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Analyzing the past to prepare for the future: a review of black soldier fly research

1.1 Waste and waste management.

One in nine people globally suffer from food insecurity, yet a third of all foods produced globally are wasted (Guo et al., 2020; Gustavsson et al., 2011; Hoornweg & Bhada-Tata, 2012). Waste is defined as materials and substances which are disposed of, intended for disposal, or required to be disposed of by law (Bontoux & Leone, 1997). Waste is in every ecosystem. However, the amount of waste in landfills reflects humanity's wastefulness. Waste is often composed of organic materials (e.g., food waste, yard waste) and inorganic materials (e.g., plastics, glass)(Ma & John Taylor, 2020). As the rate of urbanization, population, economic wealth, and disposable income increases, the consumption of goods and services and subsequent rate of municipal solid waste also increases (Hoornweg & Bhada-Tata, 2012; Ma & John Taylor, 2020; Ozbay et al., 2021; Vaverková, 2019). This leads to increasing concern for human and environmental health, as disease, pollution, and pest infestation can occur, unless waste is adequately managed (Ma & John Taylor, 2020). The management of waste residues begins with determining the rate at which waste is generated, waste collection, processing, and recovery, and ends with the final disposal (Ma & John Taylor, 2020; Sawell et al., 1996).

Waste management is an essential municipal service provided by every city government, and it is required for sustainable development, however, it is a costly undertaking (Hoornweg & Bhada-Tata, 2012). Currently, solid waste management practices cost 205.4 billion USD and are expected to increase worldwide to about 375.5 billion USD by 2025 (Hoornweg & Bhada-Tata, 2012). It cost approximately 3 billion CAD in 1992 to manage 33.76 million tonnes of waste generated annually by Canadians (Sawell et al., 1996). This amount averaged a waste generation rate of 3.38 kg/person/day (Sawell et al., 1996), which has likely increased over the last 20 years (Abdulla et al, 2013). These cost increases will be severe and devastating, especially in developing countries (Hoornweg & Bhada-Tata, 2012). Additionally, improperly managed waste leads to downstream costs that are greater than the value of an adequately managed waste (Hoornweg & Bhada-Tata, 2012).

1.2 Food waste

A waste stream that is gaining more attention recently is food waste. Food waste can be defined as any food, or parts of food, removed from the food value chain to be disposed of (Bellemare et al., 2017). One-third of all food produced globally is wasted, amounting to about 1.3 billion tons of food per year (Gustavsson et al., 2011; Hoornweg & Bhada-Tata, 2012). At this rate, it is expected that food waste will rise to 2.2 billion tonnes by 2025 worldwide (Hoornweg & Bhada-Tata, 2012). This massive loss in food is even more significant when placed in the context of all the resources and materials (i.e. land, water, energy, and nutrients) used in food production that are also wasted and the greenhouse gas emissions resulting from it (Gustavsson et al., 2011). When food is produced and goes directly to dumpsters and landfills, greenhouse gas emissions associated with the production are in vain (Schanes et al., 2018).

Food waste occurs throughout the food value chain, from harvesting techniques, improper food storage, poor processing and transportation, and lastly, consumer behaviour (Gustavsson et al., 2011). Most of the waste arises at the consumer level, especially in developed countries (Gustavsson et al., 2011). Food is often wasted when they are not considered aesthetic enough to comply with the market standards due to size, shape, or external appearance. Additionally, over-purchasing and lack of food planning by consumers contributes to food waste (Gustavsson et al., 2011). Food waste has been a constant feature throughout human history (van der Werf et al., 2018) and a growing concern among nations (Abdulla et al., 2013; Gustavsson et al., 2011). This careless/wasteful behaviour impacts not only economies but also the environment.

Canada invests resources to find ways to feed a growing population through increasing food production. However, fewer resources are invested into making appropriate use of already produced foods (Gooch et al., 2010). Canada's overall food waste is estimated at 27 billion CAD, which is more than Canadians spent going to restaurants in 2009, equals 40% of all foods produced and 2% of Canada's GDP (Gooch et al., 2010; Statistics Canada, 2010). The Canadian food value chain comprises farms, processing, distribution, hotels, restaurants, retail, and consumers. Food waste is often caused by overproduction, product aesthetics, inappropriate processing, and transportation delays that lead to increased spoilage (Gooch et al., 2010). Waste in Canada continues to grow much like in other countries, and waste generation is approximately 1.94 kg/capita/day (Assuah & Sinclair, 2021). This rate of increase is concerning, especially in Canadian communities that do not have the necessary tools and resources to properly manage such high amounts of waste generation such as many Canadian first nation communities, and particularly remote and on-reserve communities (Assuah & Sinclair, 2021). The benefits of properly managed waste impacts health, climate change, food security, poverty levels, and sustainable production. The state of waste management practices worldwide is sobering, with outdated protocols that fall short in the face of an increasingly growing population and decreasing available land for waste disposal. Moreover, the release of greenhouse gases (i.e. methane) from landfills is a significant contributor to climate change (Hoornweg & Bhada-Tata, 2012), which reinforces the urgency of finding alternative methods to manage solid waste.

1.3 Landfills

Landfills can be described as well-engineered waste facilities used for waste disposal (Ozbay et al., 2021). Landfills have existed for thousands of years and date back to 3000 BC in Greece where waste was dumped into pits, then covered when the pits were full. Landfills contain different kinds of waste but mainly consist of municipal solid waste. Waste disposal in landfills varies significantly among countries. In Sweden, landfills are not used for the disposal of municipal waste. According to the Swedish waste management and recycling association, less than 1% of municipal waste ends up in landfills; 49% is recycled whereas 50% is sent to waste to energy plants where waste is incinerated to power homes and buildings (Ozbay et al., 2021). In contrast, all municipal waste is disposed of in landfills in Bulgaria. In 2010, 3 million tonnes of waste were deposited in landfills, accounting for 98% of all waste generated (Ozbay et al., 2021). Waste management practices differ between EU countries, and some are still landfilling large amounts of municipal waste (e.g. Malta, Cyprus, Greece, Croatia, Romania, Bulgaria, and Slovakia).

Landfills are highly utilized in waste management systems in North America, despite being the least environmentally sustainable method of waste disposal (Vaverková, 2019). In Mexico, the official rate of municipal solid waste generation is 0.917 kg/person/day. In 1998, over 877, 000 tonnes of waste were dumped in controlled landfills and over 1 million tonnes in uncontrolled landfills (Buenrostro & Bocco, 2003). In the United States, 52.6% of municipal solid waste generated was disposed of in landfills in 2014 (Sun et al., 2019). In 1996, 83.9% of all municipal solid waste in Canada was disposed of in landfills (Sawell et al., 1996). This represented a waste generation rate of 1.76 kg/person/day of municipal solid waste that ended up landfilled (Sawell et al., 1996). In 2009, 205.000 tonnes of waste were dumped in landfills in London, Ontario, and it was approximately 60% of municipal waste generated (Asase et al., 2009).

Among sources contributing to anthropogenic greenhouse gas emission globally, landfills account for about 18% and is the third-largest contributor after agriculture and transportation (Sun et al., 2019). Among the most common by-products of landfill anaerobic decomposition of municipal waste are methane (55%), carbon dioxide (44%), and less than 1% of other hazardous gases (Humer & Lechner, 1999; Sun et al., 2019; Vaverková, 2019). Around 40 to 60 million tons of greenhouse gases are generated in landfills worldwide, and these emissions are caused by inadequate gas collection systems at landfill sites (Humer & Lechner, 1999). Landfill gas collection involves installing expensive landfill covers and gas collection systems, and even with that only 40% to 60% of greenhouse gases can be captured due to gas production before gas collection systems are installed and a less than 100% collection efficiency (Humer & Lechner, 1999; Sun et al., 2019). For instance, out of one million tons of methane emitted from Canadian landfills in 1990, only 20% was captured (Sawell et al., 1996); this number was set to reach 1.3 million tonnes by 2020 (Sawell et al., 1996). In an ideal world, all greenhouse gases would be captured due to their energy potential, however, this is often impossible. In 2015, Canada's greenhouse gas inventory determined that of 30 megatons (Mt) of carbon dioxide equivalent was generated in Canadian landfills, and approximately 11 Mt was captured (Mohsen & Abbassi, 2020). Due to the difficulties in monitoring methane emissions at landfill sites, the emission recovery efficiency is estimated to be approximately 63% of the total greenhouse gas emissions (Mohsen & Abbassi, 2020; Sawell et al., 1996). When methane is unmanaged, it has 20 times more global warming potential than carbon dioxide (Ozbay et al., 2021). Methane concentrations in the atmosphere have steadily increased by 1% since the 1970s and are considered the second most important greenhouse gas after carbon dioxide (Humer & Lechner, 1999). There is a correlation between methane and carbon dioxide concentrations in the atmosphere and rising air temperature (Humer & Lechner, 1999).

In addition to greenhouse gases, landfills also produce leachate. Leachate is the rainwater or moisture contained in the waste that accrues and becomes contaminated as it drains through the waste (Renou et al., 2008). This residual water contains polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (PFCs), considered organic pollutants (B. Li et al., 2012). It contaminates ground and surface water when they are in contact with leachate (B. Li et al., 2012). PBDEs and PFCs were detected in samples from 28 landfills across Canada, with greater concentrations in southern Canada compared to northern Canada (B. Li et al., 2012). Due to structural similarities, PBDEs mimic the function of thyroid hormones and affect the nervous endocrine and immune systems. At the same time, PFCs persist through the wastewater treatment process and are released into the environment (B. Li et al., 2012). Exposure to even small amounts of these pollutants can lead to nervous and reproductive system damage, congenital disabilities, and affect thyroid and sex hormone functions (B. Li et al., 2012).

1.4 Maximizing the value of waste: circular economy

A circular economy has been proposed as an alternative to the current linear economy model typically found in the economy. This unsustainable current model views our resources as limitless and our disposal as inexpensive. In contrast, the main objective of the circular economy is to keep materials and products in use within a product's life cycle to minimize the generation of waste and close the loop of materials through different methods of recycling (Salmenperä et al., 2021). Many waste management practices are yet to incorporate the circular economy model. New solutions for treating and managing waste are still being investigated and identified. Currently, we generate more annual waste than ever before, with 1.3 billion tons of food waste generated yearly, and this is projected to continue to increase (Romero-Hernández & Romero, 2018). Though waste management efforts have improved over the past decade, this improvement is mainly limited to the three Rs—reduce, reuse and recycle—and do not take full advantage of the potential value of food waste (Romero-Hernández & Romero, 2018). A circular economy strives to keep materials in use and create a closed-loop system. It provides an opportunity for companies to transform waste products into revenue streams by using by-products that would have been previously discarded. Adoption of a circular economy by government bodies and companies can have significant financial benefits, and one study estimated that a circular economy could generate billions in cost savings in materials (Esposito et al., 2017). It is essential to understand that a circular economy is not just about recycling but maximizing all aspects of a product's life cycle as it is in use, including the 'unusable' parts and converting it into a new resource (Esposito et al., 2017). The Ellen MacArthur Foundation, a leading voice in the circular economy movement, estimates that with a circular economy, the consumption of new materials and resources will decrease by as much as 53% by 2050 (Esposito et al., 2017). Proper waste management will reduce the use of landfills. It allows food waste to become a productive resource that can help transition toward a circular economy, where designing out waste from the system and regenerating and repurposing biological materials are vital principles. A circular approach is needed for food waste management, whereby waste is reduced, and its residue is returned to the system as a productive resource.

1.5 Managing food waste

Food waste can be managed in a number of ways. One way to manage food waste is by preventing food waste from occurring in the first place through educational resources, and another involves providing measures that ensure the safe donation of excess food (Närvänen et al., 2020). Other methods involve recycling food waste into compost, and lastly, disposal of food waste in landfills (Närvänen et al., 2020). It is important to note that no one solution can solve the whole problem, but each solution has a part to play and changes how food waste can be viewed. Solutions can view food waste as a problem that needs to be addressed or as a resource to be processed further (i.e., circular economy), as is the case with composting. Narvanen et al. (2020) describe food waste as a “relentless and wicked problem which requires many factors to be engaged to solve it through different activities and at different levels”. Innovating practical solutions, such as utilizing insects to process food waste into valuable end products like compost and larvae for animal feed, provides an opportunity to view food waste as a valuable material.

Insects are a diverse class of organisms and include groups highly specialized in their ability to feed and develop on different organic materials (Fowles & Nansen, 2020). This is known as insect bioconversion. It presents an opportunity to view food waste as a resource that can be further processed, via insects, to result in valuable materials, such as feed for animals and nutrient-rich soil amendments. Insect bioconversion of food waste can be defined as the breakdown of food waste into insect biomass and frass (Barry, 2004). This process closely resembles the natural breakdown of organic matter within ecosystems, where insects and microorganisms colonize food waste and break it down, utilizing it for their own metabolic and reproductive needs (Fowles & Nansen, 2020). The bioconversion process can be regulated and optimized to encourage the growth of the species required for the bioconversion process and the breakdown of the food waste. Commercialization of this process presents an alternative for food waste reduction and nutrient recycling. This is a relatively new industry that has significant growth potential, with the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae)(Linnaeus, 1758), being one of the most commonly used insect species.

1.6 The black soldier fly

The black soldier fly belongs to the subfamily Hermetiinae of the family Stratiomyidae (Rohácek & Hora, 2013). The fly can often be confused with a wasp due to its Batesian mimicry. However, as is characteristic of all Dipterans, the black soldier fly has hind wings modified into halters that act as essential mechanosensory organs for flight (Yarger & Fox, 2016). The insect is distributed throughout the western hemisphere and is abundant during late spring and early fall in the southeastern United States, with three generations a year in that environment (Diclaro & Kaufman, 2009; Tomberlin & Sheppard, 2001). The adults range from 13 mm to 20 mm in length (Diclaro & Kaufman, 2009; Sheppard et al., 2002; Tomberlin & Sheppard, 2001), have two translucent body segments on the first abdominal segment (Diclaro & Kaufman, 2009; personal observation), and possess three antennae segments (Diclaro & Kaufman, 2009).

Mating occurs year-round in tropical regions and begins two days after adult emergence (Tomberlin & Sheppard, 2001, 2002), with the male and female facing opposite directions (Tomberlin & Sheppard, 2001). Males display lekking behaviour, characterized by males grappling with other males that invade their territory (Tomberlin & Sheppard, 2001). This results in an aerial spiral and ends with the defeated male leaving the area (Tomberlin & Sheppard, 2001). Females are welcomed similarly, until the males grasp the females, and they descend into copula (Tomberlin & Sheppard, 2001). Individual female black soldier flies lay 62 to 620 eggs in dry crevices near moist decomposing organic matter.

1.6.1 Egg

Eggs hatch in ca. four days at 24°C, with red eyespots visible at 72 h and embryonic movement at 84 h (Sheppard et al., 2002). Eggs look like cooked rice grains about 1 mm long and creamy white in colour (Figure 1.1).

1.6.2 Larvae

Black soldier fly larvae are voracious feeders and moult through six instars, reaching ca. 27 mm in length and 6 mm in width (Diclaro & Kaufman, 2009) (Figure 1.2). The larval stage can last up to 215 days, depending on the larval diet (Oonincx et al., 2015). The sixth instar is the post-feeding wandering stage, and moves away from the larval diet to find a suitable dry site for pupation.

1.6.3 Pupae

Pupae are non-moving (Figure 1.3), and this life stage can last weeks to months, depending on the larval diet and developmental temperature.

1.6.4 Adults

Adults are non-feeding and rely on fat stores gained during the larval stage, although they do drink water (Figure 1.4).

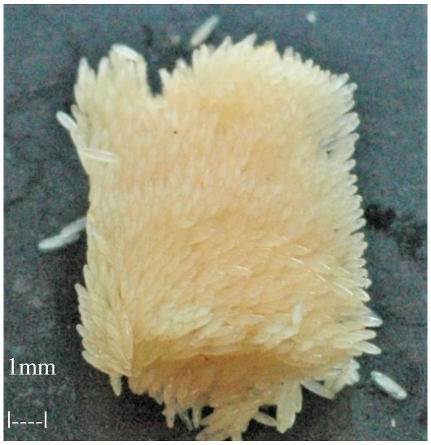

Figure 1.1. Egg mass of the black soldier fly*, Hermetia illucens* Linnaeus (Diptera: Stratiomyidae)

Figure 1.2. Larvae of the black soldier fly*, Hermetia illucens* Linnaeus (Diptera: Stratiomyidae)

Figure 1.3. Pupae of the black soldier fly*, Hermetia illucens* Linnaeus (Diptera: Stratiomyidae)

Figure 1.4. Adult of the black soldier fly*, Hermetia illucens* Linnaeus (Diptera: Stratiomyidae)

1.7 Factors affecting black soldier fly life-history traits

Insects are poikilotherms and rely on abiotic factors that dictate their life history parameters, especially temperature and humidity. These factors affect their distribution, abundance, and development (Damos & Savopoulou-Soultani, 2012; Ratte, 1985). As a general rule for insects, as temperature decreases, development time increases and developmental rate slows and eventually ceases at the lowest critical temperature the insect can tolerate (Damos & Savopoulou-Soultani, 2012). Likewise, as temperature increases, development time decreases and developmental rates increase until an optimum, then as temperature continues to increase, developmental rate slows then ceases as temperature surpasses an insect's developmental maximum threshold (Damos & Savopoulou-Soultani, 2012). Insects are adapted to specific temperature ranges, within which development is optimized (Ratte, 1985). Relative humidity, like temperature, affects development of an insect. All insects have a waxy layer on their cuticle that prevents excessive water loss. However, eliminating all water loss is impossible due to respiration through the spiracles. Egg and neonate stages are particularly susceptible to low relative humidity due to desiccation and an inability to eclose.

Black soldier fly development is variable and depends on factors such as temperature, humidity, and larval diet (Holmes et al., 2012; Nguyen et al., 2013; Tomberlin et al., 2009). Development time takes approximately 19-20 days at 27°C and 17 -18 days at 30°C, with longer development time for females than males (Tomberlin et al., 2009). The upper thermal limit for the black soldier fly is 36°C, with only 0.1% adult emergence when larvae were reared at that temperature (Tomberlin et al., 2009). Larval development time and larval weight were strong predictors of adult longevity (Tomberlin et al., 2009). Relatively humidity below 50% results in less than 40% egg eclosion of black soldier flies (Holmes et al., 2012). Time to egg eclosion decreases with increasing relative humidity (Holmes et al., 2012).

In the early 90s, the black soldier fly was implicated in the management of manure accumulation in poultry farms (Sheppard et al., 1994). It was later established that the black soldier fly larvae can feed on a wide range of diets (Diener et al., 2009, 2011; Nguyen et al., 2013, 2015; Oonincx et al., 2015). Larvae fed on manure generally take the longest to develop, at about 73 days to 215 days (Nguyen et al., 2013; Oonincx et al., 2015). Oonincx et al. (2015) observed that larvae feeding on chicken and pig manure had a development time of about 144 days, while larvae feeding cow manure had a development time of about 215 days. Nguyen et al. (2013) observed differences in the mean larval weight of larvae fed six different larval diets. Larvae weighed highest when fed on kitchen waste and chicken feed and the lowest when fed on manure and fruits and vegetables (Nguyen et al., 2013). Additionally, mortality was highest for larvae fed on the liver and fish diets; this could be due to heavy metal contamination in the fish diet and low fat and energy content in the liver diet (Nguyen et al., 2013). The different combinations of macronutrients present in different diet compositions may explain why researchers observe diet-dependent growth variations (De Smet et al., 2018). As stated earlier, black soldier fly adults have reduced mouthparts and are non-feeding. This means they rely on their fat stores gained during the larval stage for survival. Larvae take longer to develop on diets low in nutrients, as seen by Nguyen et al (2013). The effect of the larval diet on the black soldier fly is not restricted to its development, but also affects the nutritional components of the larvae (Nguyen et al., 2013; Spranghers et al., 2017; Tschirner & Simon, 2015), impacting their use as food for animals in a circular economy.

Mating and oviposition are influenced by time of day, light intensity, and humidity (Tomberlin & Sheppard, 2002). Approximately 85% of mating occurs during the mornings when light intensities are about 110 μmolm^{-2}s^{-1} (Zhang et al., 2010). However, the number of mating pairs observed decreases throughout the day (Zhang et al., 2010). Pupation medium also affects pupation and adult longevity of black soldier flies, as post-feeding larvae placed in lower compaction pupation mediums took less time to pupate and had higher adult longevity (Holmes et al., 2013).

1.8 Interaction with microbes

The gut microbiota of insects houses a large number of bacteria. These microbes play an essential role in digesting foods, synthesizing vitamins, and distorting the sex ratio in some insect species (De Smet et al., 2018). This is a less studied factor in the black soldier fly, yet larvae feed on decomposing organic matter that also has a microbial community within the substrate, and it raises important questions about the role the substance microbial community or gut microbes play in affecting black soldier fly life history traits. Some studies have shown that the gut microbiota of the black soldier fly is divided into three parts—the foregut, the midgut, and the hindgut—all hosting different bacterial communities (De Smet et al., 2018). The roles of these bacteria could mirror that observed in other animals, such as digesting and fermenting large compound plant polymers, conversion of toxic compounds, stimulating the immune system, and preventing colonization by pathogens (De Smet et al., 2018).

Little work has been done to examine the effect of microbes on the development of the black soldier fly. Most studies investigate the impact of diet and abiotic factors on development while ignoring the roles of microorganisms on the growth performance of the black soldier fly. The diversity of microbes found in the gut of the black soldier fly is linked to the nutritional complexity of the larval diet (Jeon et al., 2011). Jeon et al. (2011) identified 176 bacterial species in larvae fed with food waste compared to 36 species in larvae fed with cooked rice. Interestingly, not all bacterial species are present during all life cycle stages of the fly (Zheng, Crippen, Singh, et al., 2013). In a study by Zheng et al. (2013), 20.5% of the bacterial genera found were present during the larval, prepupal and pupal stages; however, only 11.5% were present in larval, prepupal, pupal and adult stages. The major phylum of bacteria found in the gut of the black soldier fly during development includes: Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria and Fusobacteria (Bruno et al., 2019; Jeon et al., 2011; Zheng, Crippen, Singh, et al., 2013).

The presence/absence of bacteria also mediates oviposition by female black soldier flies (Zheng, Crippen, Holmes, et al., 2013). The presence of bacteria species from insect competitors (e.g., *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae)) or sterile eggs devoid of microbes resulted in reduced oviposition by female black soldier flies (Zheng, Crippen, Holmes, et al., 2013). Interestingly, the presence of bacteria isolated from the eggs of the black soldier fly and the hairy maggot blowfly (*Chrysomya rufifacies* Macquart (Diptera: Calliphoridae)) enhanced oviposition. *Chrysomya rufifacies* is an opportunistic predator, in addition to feeding on decomposing flesh, in its later larval stages, and perhaps possesses microbes that encourage oviposition from other flies that it can prey upon, such as the black soldier fly.

The larvae of the black soldier fly feed on a wide range of diets ranging from an all-vegetable to an all-meat diet, and hence, their gastrointestinal tract shows high levels of amylase, protease and lipase enzymes (Kim et al., 2011). Understanding how microbes influence the growth and development of the black soldier fly is crucial, particularly for industrial applications and waste conversion optimization. One study suggests that anaerobic bacterial growth during black soldier fly larval feeding competes for nutrients (Tomberlin et al., 2002). Tomberlin et al. (2002) stated that wild populations of the black soldier fly had superior growth and survival due to their ability to source fresh resources during their larval development. The authors hint at the old/aging diet reducing larval growth and survival as larvae fed old manure developed slower than larvae fed with fresh manure. These observations suggest that the old/aging manure microbial community could play a crucial role in limiting black soldier fly larval development.

1.9 Larval density and bioconversion of waste

As discussed above, a number of factors can affect the bioconversion process including food waste type, temperature, humidity, and the nutritional components of the waste. The bioconversion process by the black soldier fly is affected by the type of food waste, the quantity and the nutritional component of the food waste, as well as environmental factors such as temperature. Industrial-Commercial application of black soldier fly bioconversion technology requires an understanding of the system load capacity and feed rate optimization. These depend on a ratio of larval density to the amount of waste. Several studies have found feed rates of 200 mg/larva/day to 250 mg/larva/day show higher biomass production and reduction efficiency of faecal sludge waste compared to other feed rates tested (Diener et al., 2009; Nyakeri et al., 2019). A system load capacity of approximately 1.2 larvae/cm^2 and a feed rate of 163mg/larva/day were determined to be ideal conditions for the bioconversion of vegetable waste (Parra Paz et al., 2015). Thus it is clear that initial population starting size must be adjusted depending on the amount of waste, type of waste, and environmental conditions. The proper combination of these parameters improves the bioconversion process in terms of optimal biomass production and reduced bioconversion time. Initial population starting sizes are measured by introducing egg masses. Accurately predicting the number of eggs within an egg mass is a necessary step in optimizing black soldier fly bioconversion and such a tool would be immediately useful in a commercial/industrial setting.

1.10 Use of black soldier fly products

A by-product of optimizing the black soldier fly larvae waste conversion system will be an abundance of black soldier fly prepupae; this is the non-feeding larval stage as they prepare to pupate before emerging as adults. These prepupae are approximately 63% - 37% protein and 40% - 20% fat (Newton et al., 2005), making them a suitable source of alternative proteins for livestock, poultry, and commercially raised fish. Insect production leaves a small ecological footprint, and black soldier fly prepupae are good sources of minerals such as calcium, iron, potassium, magnesium, phosphorus and zinc, as well as vitamins such as B12, thiamine and riboflavin and essential amino acids such as lysine, isoleucine, threonine, valine and methionine (Spranghers et al., 2017). One study found that replacing 25% of the rainbow trout diet with black soldier fly prepupae resulted in the same weight gain as fish on a 100% diet over a nine-week trial period (St-Hilaire et al., 2007). Bodyweight, egg laying rate, meat colour, pH, meat composition, and sensory traits in laying hens and broiler quails were the same when black soldier fly prepupae was used in their diet compared to those on regular feed (Cullere et al., 2016; Kawasaki et al., 2019). Hens and quails improved amino acid levels and saturated fatty acids on the black soldier fly prepupae diet suggesting more nutritious meat (Cullere et al., 2016). It is suggested that chitin possibly increases eggshell thickness and microbiota diversity values in the cecum of hens supplemented with black soldier fly prepupae (Kawasaki et al., 2019). Black soldier fly prepupae raised on pre-consumer waste has been approved as feed for pet reptiles, poultry, and fish in several countries, including the USA, Canada, Mexico, Australia, China, South Africa, Kenya, and Uganda (Gold et al., 2018).

In addition to their utility as animal feed, black soldier fly prepupae can be processed into biodiesel. Biodiesel has been considered a more environmentally friendly option to reduce the use and consumption of petroleum, however, the cost of production is a major problem (Q. Li et al., 2011). The fatty acids within the insect fat body are a substrate for biodiesel production. The black soldier fly prepupae can be harvested, dried and mechanically pressed to produce crude oil, which can be further processed into biodiesel. Lauric, palmitic, oleic, linoleic, and myristic acids are the most common fatty acids derived from black soldier fly prepupae (Surendra et al., 2016). These fatty acids are more dominant in black soldier fly prepupae than in crops commonly used for biodiesel production, such as soybean (Surendra et al., 2016), making black soldier fly prepupae highly suitable for biodiesel production (Leong et al., 2016).

Another by-product of optimizing the black soldier fly larvae waste conversion system will be an abundance of black soldier fly post-processing residue, which some have suggested could be used as compost (Chiam et al., 2021; Nguyen et al., 2015; Tan et al., 2021). This post-processing residue is composed of insect frass, pupal casings and food residue and the chemical and physical properties this post-processing residue can be likened to commercial fertilizer (Fowles & Nansen, 2020). Additionally, the benefits of using insect frass for crop growth include a reduction of pathogenic microbes, or need for some pesticides (Fowles & Nansen, 2020; Lalander et al., 2019). Only a few studies have tested this proposed ability to act as a fertilizer for plant growth, and thus far, the results are variable. Chiam et al (2021) tested black soldier fly frass from soy pulp on lettuce growth. They observed that lettuce plant dry weight decreased with increasing concentration of frass added, with treatment with the highest frass composition performing the worst compared to treatment with the least frass. In contrast, other researchers found increased yields in lettuce, bok choi, and potatoes when black soldier fly frass was applied at different rates (Temple et al., 2013). Interestingly, the yield of beans decreased with increasing frass application (Temple et al., 2013). Frass from brewery waste performed better than frass from poultry manure on chilli pepper and shallots (Quilliam et al., 2020). This could suggest plant-specific benefits with black soldier fly frass applications; however, more research is needed in this area.

1.11 Conclusion

Overall, the black soldier fly has been promoted in waste management as a resource for reducing organic waste due to its ability to feed on a wide range of diets (Nguyen et al., 2015). Canadians produced 30.4 million tons of waste sent to landfills for decomposition in 2002 (Cameron et al., 2005). Due to the continuous increase in organic waste production, there is a growing need for new landfills to be opened as older landfills reach their capacity (Cameron et al., 2005). Black soldier fly larvae can reduce waste by greater than 50% (Sheppard et al., 1994) and present an opportunity for Canada to transition to a circular economy framework. The circular economy offers reuse, recycling and reintegrating of otherwise wasted materials back into the system and is an approach to sustainable environmental and economic development (Schulze, 2016). This is a broad idea and can be achieved by reducing waste and reintroducing output materials (e.g., waste) back into the economy as inputs (e.g., compost)(Schulze, 2016). The rate of waste conversion is dependent on larval diet composition (Nguyen et al., 2013), rate of consumption (Diener et al., 2009), temperature (Tomberlin et al., 2009), and potentially microbes introduced to accelerate the rate of consumption. Most research focuses on the effect of diet substrates and abiotic factors on the life history traits (Holmes et al., 2012, 2012, 2013; Nguyen et al., 2015). The work outlined in this thesis aims: 1) to investigate the waste conversion capabilities of the black soldier fly when fed with food waste from the Windsor-Essex region in Ontario, Canada; 2) to examine waste conversion optimization through the introduction of microbes; 3) to generate a commercially scalable model to quickly measure the number of eggs in black soldier fly egg masses; and 4) to test black soldier processing residue as a compost for growing the most economically important vegetable in Canadian greenhouse production. It is imperative to not only use the black soldier fly as a waste management alternative in keeping with a circular economy framework, but to seek ways to improve and optimize its waste conversion capabilities.

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Chapter 2

The suitability of the black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) as a waste management strategy for municipal food waste.

2.1 Introduction

2.1.1 Waste in Ontario

Landfills are the most used strategy of waste disposal in Canada (Mohsen et al., 2019). In 2016, 33 million tons of waste were generated, and 26 million tons of municipal solid waste were sent to landfills (Mohsen et al., 2019). According to the Ministry of the Environment and Climate Change (MOECC), there are about 3.7 million tonnes of organic food waste generated in Ontario yearly (Ministry of the Environment and Climate Change., 2017). Consumers generate most of this food waste, and the rest accumulates along the food supply chain where food is grown, processed, and transported. Approximately 70% of this waste is sent to landfills for disposal (Mohsen et al., 2019). The residential sector generates 55% of all food waste in Ontario, while the industrial and commercial (IC) (i.e., greenhouses) sector is responsible for 45% (Ministry of the Environment and Climate Change., 2017). The goal of the province of Ontario is to shift towards a circular economy; this means a system in which nothing is discarded, but instead, reused, recycled, and reintegrated into the market (Ministry of the Environment and Climate Change., 2017). In 2015, Ontario successfully diverted 50% of residential waste and 25% of IC waste from landfills (Ministry of the Environment and Climate Change., 2017). The use of landfills as a strategy for waste management is unsustainable and adds strain to the environment by demanding landfill space, and releasing harmful greenhouse gases (GHGs) that contribute to climate change (Bogner et al., 2008; Scheutz et al., 2009; Yusuf et al., 2012; Balcombe et al., 2018), and leachate that pollutes water, impacts aquatic flora and fauna, contributes to ammonia toxicity and pollutants with severe health and environmental concerns (De Wit, 2002;Baun & Christensen, 2004; Darnerud, 2008; Jensen & Jeffers, 2008; Lavrova & Koumanova, 2010; B. Li et al., 2012; Kamaruddin et al., 2015). The Windsor-Essex regional landfill is approximately 123 hectares with a waste footprint of 58 hectares (Essex-Winsor Solid Waste Authority 2015).

2.1.2 Ecosystem services: Insects for waste management

Ecosystem services are mechanisms by which species within natural ecosystems support and satisfy human life (Daily, 1997). They can be grouped into four main classes: supporting, cultural, provisioning, and regulating services (Millennium ecosystem assessment, 2005). As insects are the most diverse and successful group of organisms on earth, they play a key role in contributing to vital ecosystem services under the categories of regulating services (i.e. pollination, biological control), supporting services (i.e. nutrient cycling through decomposition), and provisioning services (i.e. food provisioning) (Daily, 1997; Losey & Vaughan, 2006; Weisser & Siemann, 2013). Pollinators, herbivores, predators, parasitoids, and decomposers are estimated to provide USD 57 billion worth in ecosystem services (Losey & Vaughan, 2006). However, insects are less extensively studied than other organisms and are often under-represented in their contribution to ecosystem services (Losey & Vaughan, 2006). Consequently, there needs to be a more comprehensive understanding of insect contribution to ecosystem function and services (Losey & Vaughan, 2006). Pollination is a valuable ecosystem service that results in the pollination of 60-90% of plant species necessary to produce crops (Losey & Vaughan, 2006). Biological control is another ecosystem service extensively studied, and it is the practice of using natural organisms to reduce pest populations and is a crucial component in integrated pest management (Losey & Vaughan, 2006). Decomposition by insects involves the degradation of organic matter and is the least studied of the ecosystem services performed by insects. As insects degrade organic matter, nutrients are released into the ecosystem for processes such as nutrient cycling (Foster & Bhatti, 2005; Magcale-Macandog et al., 2018). This keeps the availability of elements such as nitrogen (N), phosphorus (P), carbon (C), hydrogen (H), oxygen (O), potassium (K), calcium (Ca) and magnesium (Mg) in an ecosystem at balance (Magcale-Macandog et al., 2018). Insect decomposers that feed on dead organic matter are known as saprophages, and entomologists recognize three major groups within this class: dead plant tissue feeders, carrion feeders, and dung feeders. Organic matter decomposition is important to ecosystem functioning (Weisser & Siemann, 2013).

Due to their ability to feed on dead organic matter, the use of insects for recycling organic waste was first proposed in 1919 by P. Lindner (Čičková et al., 2015). Following this, it was shown that Muscidae (Diptera) larvae could reduce manure into an odourless material, and the larvae could be processed into feed for poultry (B. F. Miller et al., 1974). The selection of insect species used for recycling waste is vital in determining the success of the recycling process. Several life history characteristics such as fecundity, development time, size, pest status, adaptability to controlled mass-rearing, and behavioural characteristics must be understood to appropriately manage a sustainable biodegradation process using insect species.

Several dipteran species have been studied for this purpose (Barnard et al., 1998). *Musca domestica* Linnaeus (Diptera: Muscidae) feeds on a wide range of organic matter and develops through three larval stages (Barnard et al., 1998; Čičková et al., 2015; Pastor et al., 2011). Under ideal conditions, larval development ranges from 7-10 days, and females have high fecundity laying up to 730 eggs during their lifetime (El Boushy, 1991; Fletcher et al., 1990). Due to the high reproductive potential, this species is highly adaptable to mass rearing (Čičková et al., 2015; Fletcher et al., 1990). However, *M. domestica* is a pest and a vector for many diseases (Blazar et al., 2011; Förster et al., 2007, 2009; Khoobdel et al., 2009). Similarly, *Lucilia sericata* Meigen (Diptera: Calliphoridae) has also been used in waste management and is commonly found feeding on manure (Čičková et al., 2015). The development time of *L. sericata* ranges from 7.8-46.1 days depending on developmental temperatures (Grassberger & Reiter, 2001; M. Wang et al., 2020; Y. Wang et al., 2016). However, *L. sericata* is a well-studied species, a pest, and ectoparasite known for causing myiasis in sheep (Wall et al., 2001).

2.1.3 Study organism: The black soldier fly

The black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) is originally native to the Americas but occurs in both temperate and tropical regions (Čičková et al., 2015; Rozkošný & Nartshuk, 1988; Y.-S. Wang & Shelomi, 2017). In the southern United States, the black soldier fly has three generations, from April to November (Sheppard et al., 1994, 2002). The adults are a large, wasp-like form, ca. 15-20 mm in length. The larvae develop through six larval stages, each increasing in size (Tomberlin et al., 2009; Tomberlin & Sheppard, 2001). Black soldier fly males demonstrate lekking behaviour, which involves resting on the leaves of plants as territories and fighting off other males that intrude, while welcoming females (Alcock, 1990; Tomberlin & Sheppard, 2001). This behaviour has been observed in other Stratiomyid species (Alcock, 1990). Adult flies mate two days after eclosion and oviposit two days after mating at temperatures above 26^{o}C (Tomberlin & Sheppard, 2002). Tomberlin & Sheppard (2002) determined that light intensity and time of day influence mating, and environmental temperatures influence oviposition. Adults need temperatures above 26°C for successful mating and light intensities of at least 63 $umolm^{-2}s^{-1}$ (Tomberlin & Sheppard, 2002). Additionally, more egg masses are collected at relative humidities over 60% (Holmes et al., 2012; Tomberlin & Sheppard, 2002), and egg masses are fragile and susceptible to desiccation at lower relative humidities (Holmes et al., 2012). Females oviposit clutches of 206-620 eggs in dry crevices close to decomposing organic matter (Booth & Sheppard, 1984; Tomberlin & Sheppard, 2002). At 27°C and 60% relative humidity, eggs eclose approximately four days after oviposition and can develop on a wide range of decomposing organic waste such as manure (Mazza et al., 2020; Rehman et al., 2017; Sheppard et al., 1994; Xiao et al., 2018), rice straw (Zheng et al., 2013), municipal waste (Diener et al., 2011), fecal sludge (Banks et al., 2014; Lalander et al., 2013), kitchen waste, and fish offal (Nguyen et al., 2013, 2015). Adults lack mouthparts and do not feed except for drinking water; they are weak fliers and are not considered a pest or disease vector (Furman et al., 1959; Sheppard et al., 2002). Adults survive on the fat reserves obtained as maggots and die when this is depleted (Myers et al., 2014; Tomberlin et al., 2009).

The nutritional content of a resource affects the developmental rate of an insect. Some Dipteran species have shown developmental differences of up to seven days when reared on resources lacking in essential nutrients; *Calliphora vomitoria* Linnaeus (Diptera: Calliphoridae) (Ireland & Turner, 2006), *Calliphora vicina* Robineau-Desvoidy

(Diptera: Calliphoridae) (Kaneshrajah & Turner, 2004), and *Lucilia sericata* (El-Moaty & Abd Elmoneim, 2013). In the black soldier fly, development time from egg to adult ranges from 40 days to months depending on environmental temperatures, food availability, and nutrient content (Furman et al., 1959; Nguyen et al., 2013, 2015). Nguyen et al. (2013) found that larvae of the black soldier fly reared on diets low in fat had longer development times. Larvae of the black soldier fly reared on diets with high fat, and calorie content weighed significantly more than larvae reared on a low-fat diet (Nguyen et al., 2013, 2015).

The work outlined in this chapter investigated the suitability of the black soldier fly as an alternative waste management strategy for municipal food waste using the Windsor-Essex region as a case study. This suitability is measured by assessing the waste reduction percentage (i.e., percentage of waste reduced), the waste reduction index (i.e., waste reduction as a function of time), adult survival (i.e., percentage of adult survival), and development time (i.e., development form egg to adult). Based on the findings from Nguyen et al. (2013; 2015), it is expected that adults will develop longer when fed with municipal solid waste compared to a control diet of chicken feed. Based on Diener et al. (2011), it is expected that the waste reduction percentage will not differ from the control poultry feed diet, and the waste reduction index will be lower compared to the control diet due to slower fly development on municipal food waste. No differences are expected in survival to adult between individuals reared on chicken feed versus municipal food waste.

2.2 Materials and Methods

2.2.1 Colony maintenance

A black soldier fly colony was established in 2018 from prepupae sourced from the worm lady (wormlady.myshopify.com, Ontario, Canada) and maintained at the University of Windsor, Windsor, Ontario, Canada. The colony was supplemented with commercially acquired larvae every year (wormlady.myshopify.com, Ontario, Canada). Adults were held in a black mesh cage (1.5 mm) constructed with polyvinyl chloride (PVC) pipes (1.8 m x 1.8 m x 1.8 m). The enclosure was provided with plastic golden pothos plants as a lekking site for males (Tomberlin and Sheppard 2001). Lighting was provided using 150-W high pressure LED lights (Model: BSF-4C-200-3030, Eco Conversion Systems LLC, Texas, USA) to maintain a photoperiod of 16:8 (L:D) cycle and light intensity required for mating (Tomberlin & Sheppard, 2002). Colonies were maintained at ca. 26.7°C ± 0.9 and relative humidity (RH) greater than 20%. A water misting system (set for 30-sec intervals twice a day) helped maintain humidity in the enclosure and provided water droplets for adult consumption. An oviposition site was provided by using corrugated cardboard taped to the side of a Tupperware container (24.43cm x 16.81cm x 8.55cm) (Snaptite, ID: 10-1001012). The Tupperware container was filled with poultry feed saturated with water (Purina Gold'N start & grow crumbles, product number: 6040, Mississauga, Ontario). Poultry feed has been established as a standard diet for rearing black soldier flies (Sheppard et al., 2002), this feed contained approximately 20% crude protein, 3% fat, and 5% crude fibre saturated. Females were allowed to oviposit for 24 h, and then the Tupperware container, with the corrugated cardboard now filled with eggs, was moved into a growth chamber set at 27°C, 70% RH, and 16L: 8D and monitored daily until eclosion. After eclosion, larvae were transferred into a Rubbermaid bin (82 cm x 51.8 cm x 42.4 cm) and fed poultry feed *ad libitum* until they reached the prepupae stage, at which point they stopped feeding. Prepupae were then transferred into a container filled with wood chips for pupation and placed into the colony cage until emergence.

2.2.2 Experimental design

To assess the overall suitability of the black soldier fly as an alternative waste management strategy, the waste reduction, waste reduction index, efficiency of bioconversion, female body weight, survival to adult, and development of larvae were measured on two different diets. Larvae were fed either a municipal food waste diet or a poultry feed diet (control). Food waste was obtained from Greener Bins, a local compost farm in Kingsville, Ontario and was composed of plant and animal material. Waste samples were sent to Bureau Veritas, Mississauga, Ontario, Canada, for nutritional analysis. It was shown to contain 50 calories/100g, 7g/100g of carbohydrate, 3g/100g of protein and 3g/100g of fat. To ensure consistency, both diets were ground and homogenized using a mixer with a moisture content of approximately 70%. The waste was then portioned, pre-packaged, sealed in sandwich bags, frozen, and stored at -20ºC until needed, and thawed 24 h before use.

Egg masses were collected from the colony cages over a 24 h period using corrugated cardboard and poultry feed as an oviposition attractant. Masses were placed in a growth chamber (Conviron Adaptis A1000) set at 27°C, 90% RH and 16L:8D until hatching. Newly hatched larvae were put into a clear plastic container (475ml) fed poultry feed *ad libitum* and placed back into the growth chamber for four days. One hundred and fifty 4-day old larvae were randomly selected per treatment, and using a paintbrush, transferred into separate clear plastic containers (475ml) with 150 g of the diets (food waste or poultry feed, N = 12). Containers were covered with perforated lids for gas exchange and put into the growth chamber programmed to 90% RH, 27°C and 16L:8D.

Containers with both larval diets were checked every two days, and five larvae from each treatment were selected, weighed, and returned to the container to obtain larval weight during development until the prepupal stage was reached. Development time was measured as the time from egg eclosion to adult emergence. Survival was determined by counting the number of successfully emerged adults from each treatment and based on the proportion of adults at the end and the beginning of the experiments (equation 1) (Van De Fels-Klerx et al., 2016; Gold et al., 2020). To determine the amount of food that the larvae consumed, waste reduction percent was calculated as the ratio of residue wet weight at the end of the experiment to food waste provided at the beginning (equation 2) (Diener et al., 2009). The waste reduction index is defined as waste reduction percentage as a function of time. It was calculated by dividing the waste reduction percentage by the larval development time (equation 3) (Diener et al., 2009). A high waste reduction index is indicative of a high waste reduction efficiency, as larvae were able to reduce a significant amount of waste in a relatively short time. The efficiency of bioconversion was calculated as the difference between the final larval fresh weight and the initial larval fresh weight at the beginning of the experiment divided by the ingested food and multiplied by the number of larvae at the end of the experiment (equation 4). This is a measure of how efficiently black soldier fly larvae can convert larval diet into larval biomass. Female body size was measured as the dry weight of adult females (g) using a Gemini 20 scale (Model no: GEM20, manufacturer: smart weigh, amazon.ca).

2.2.3 Equations

Equation 1: $\text{Survival (\%)} = \frac{\text{Number of adults at the end of experiment}}{\text{Number of larvae at the beginning of experiment}} \text{ x } 100$

Equation 2: $\text{Waste reduction (\%)} = \frac{\text{Waste IN} - \text{Waste OUT}}{\text{Waste IN}} \text{ X } 100$

Equation 3: $\text{Waste reduction index (WRI)} = \frac{\text{Waste reduction percent}}{\text{Larval development time}}$

Equation 4: $\text{Efficiency of Bioconversion} = \frac{\text{Change in larval weight}}{\text{Ingested food}} \text{ X } 100$

2.3 Statistical Analyses

All analyses were completed in JMP (version 16.1.0). Normality was tested using the Shapiro-Wilk test. Homogeneity of variance was assessed using Levene’s test. Variables that did not meet the assumptions of normality were analyzed using the Kruskal Wallis test while an ANOVA was used to analyze variables that met the assumptions of normality. The effect of larval diet on adult survival, waste reduction index, female body size, development time, and efficiency of bioconversion was analyzed using the Kruskal-Wallis test. An ANOVA was used to analyze the effect of the larval diet on the waste reduction percentage. The significance level was set at $\alpha = 0.05$.

2.4 Results

Type of larval diet had an effect on adult survival ($X^2 = 4.096$; df = 1; $p = 0.043$) such that mean survival was 16.3% higher in treatments reared on poultry feed compared to food waste (Table 2.1). Female body mass, often used as a proxy for female fecundity, was affected by larval diet ($X^2 = 14.02$; df = 1; $p = 0.0002$), with females emerging from treatments fed with food waste weighing 1.2 times more than females emerging from treatments fed with chicken feed (Table 2.1). This is likely a reflection of prepupal weight, as larvae gained weight faster, but pupated sooner at a lower mass when fed chicken feed, compared to those on food waste (Figure 2.1). This effect of larval diet also impacted the overall development time ($X^2 = 18$; df = 1; $p < 0.0001$), with the first adults emerging 5 days sooner on poultry feed than on food waste (Table 2.1).

Although larval diet had no effect on mean waste reduction percentage ($F_{1, 22} = 0.0473$; p = 0.8; Figure 2.2a), with both types of waste reduced by approximately 65%, mean waste reduction index was affected by larval diet (X^2=8.333; df = 1; $p = 0.004$), as larvae fed on poultry feed had a higher mean waste reduction index than larvae fed food waste (Figure 2.2b). Additionally, the mean efficiency of bioconversion was influenced by larval diets ($X^2 = 8.34$; df = 1; $p = 0.043$), such that larvae fed food waste were better able to convert ingested food into larval biomass when compared to larvae fed poultry feed (Figure 2.2c).

2.5 Discussion

Insect nutrition can be defined in different ways. In this study, it is defined as the conversion of larval diet into insect life history traits such as development, survival, and body mass. The nutritional requirements of an insect are affected by the type (qualitative), including diet digestibility, and amounts (quantitative) of essential nutrients necessary and available for appropriate development (House, 1969). The composition of the organic material can affect the physiology and development of decomposer insects. The development of *Calliphora vomitoria* Linnaeus (Diptera: Calliphoridae) differed when raised on different pig organs (i.e., liver, brain, and muscle) (Ireland & Turner, 2006). Adult black soldier flies are non-feeding and thereby accumulate a fat body during their larval stage from their diet to survive as adults (Sheppard et al., 1994). As a result, it is reasonable to assume that larvae will take longer to develop and might have reduced survival and body size on diets with low nutritional components (Nguyen et al., 2013, 2015).

Our study demonstrates that black soldier fly successfully develop from egg to adult on municipal food waste. However, the larval diet affected the development time of the black soldier fly. It was expected that larvae reared on poultry feed would have a shorter development time than those reared on municipal food waste, and this occurred.

This is expected as poultry feed is an industrially manufactured diet for young poultry birds to ensure proper development. At the same time, municipal food waste is often composed of plant detritus, leftovers, and generally organic materials low in calories. An analysis of municipal food waste from our study was sent to Bureau Veritas and showed it had 50 calories/100g, 7g/100g of carbohydrate and 3g/100g of protein compared to previous analysis of poultry feed with 77 calories/ 100g, 13.3g/100g carbohydrate and 4.47g/100g protein (Nguyen et al., 2013).

Insects utilize carbohydrates as building blocks and fuel (Cohen, 2003). They can be converted into lipids and can contribute to the synthesis of amino acids (Genç, 2006). These carbohydrates in insect diets also serve as components of glycoproteins which act as sites of recognition sites for proteins that form channels and receptors for the movement of materials in and out of cells (Cohen, 2003). An insect's ability to use carbohydrates, however, depends on the hydrolyzed polysaccharides. Some species can utilize a broad range of carbohydrates, such as *Tribolium sp*., Macleay (Coleoptera: Tenebrionidae), which can utilize starch, mannitol, sucrose and other monosaccharides (Chapman et al., 2013). One study revealed that *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) larvae reared on a carbohydrate-rich diet survived when cold-shocked compared to larvae reared on a protein-rich diet (Andersen et al., 2010). Insects use proteins are their primary source of nitrogen (Cohen, 2003). Proteins are broken down into amino acids and then absorbed and resynthesized into proteins that make up insect bodies such as muscles, enzymes and hormones (Chapman et al., 2013; Cohen, 2003). Insects require certain essential amino acids such as methionine, threonine, tryptophan, valine, isoleucine, phenylalanine, lysine, arginine and histidine as part of their diets to thrive (Cohen, 2003). Protein is required for ovary development and egg maturation, and it is also crucial for the secretion of the juvenile hormone (JH) necessary for ovary and egg development (Genç, 2006). Therefore, it is safe to assume that the lower carbohydrate and protein amounts present in municipal food waste could explain the slower development of the black soldier fly,

Larvae need to consume a nutritionally balanced diet during the larval stage to produce reproductively competitive adults. Although the abundance of food is generally recognized as one of the major biotic factors affecting larval growth, deficiencies in the larval diet can impose constraints on the life history traits of the insect as it develops (House, 1969). The wild bee *Osmia bicornis* Linnaeus (Hymenoptera: Megachilidae) had reduced body mass, survival, and underdevelopment of cocoons when larval diets were deficient in nutrients sodium (Na)and potassium (K) (Filipiak & Filipiak, 2020). A nutritionally balanced diet has to supply all the nutrients necessary in appropriate amounts and proportions to each other for optimum development (House, 1969). Failure to grow and develop on a particular food diet may mean that the food intake is low due to due to absence of phagostimulants essential for regular feeding, the presence of phagodeterrents that inhibit feeding, poor digestion of ingested food due to lack of lytic enzymes, or absorbed food cannot be converted into necessary sustenance due to deficiency in essential nutrients or vitamins (Gordon, 1968). Phagostimulants are chemical attractants that factor into the acceptance of potential foods by insects, and these chemical compounds stimulate feeding in insects (Genç, 2006), while phagodeterrents are chemical deterrents that have an inhibitory effect on insect feeding (Genç, 2006). In this study, larvae reared on food waste weighed 1.2 times more than larvae reared on poultry feed. This was surprising since larvae took longer to develop and had lower survival. The lower survival on food waste was unexpected, but is perhaps explained by missing nutrients or the possible presence of phagodeterrents in the food waste.

The percentage of waste reduced by the black soldier fly was the same regardless of diet type. Municipal food waste was reduced by 66%-65%, which is comparable to the reduction observed by other studies. One study observed waste reduction of 66.4%-78.9% (Diener et al., 2011), while another study found 32.7%-58.4% (X. Li et al., 2021). However, the waste reduction index, defined as the waste reduction percentage as a function of larval development time, differed between food waste and chicken feed because larvae fed on municipal food waste took four days longer to reach the prepupal stage when compared to larvae fed with poultry feed. Again, this difference could be attributed to the difference in the nutritional composition of the different diets.

The bioconversion rate was also higher in the waste-fed group than in the control group, due to the effect of body mass, with larger body size recorded for larvae reared on food waste. It is also possible the lower survival in the food waste group lowered the overall larval density and thereby reduced competition for nutritional resources. This would allow more food to be available to the larvae fed on municipal food waste. The effect of larval density on body size has been extensively studied. Researchers observed smaller adults with increasing larval densities in experimental populations of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) due to increasing levels of competition (R. S. Miller & Thomas, 1958). Another study also found similar trends of decreasing body size of the seed-feeding beetle, *Stator limbatus* Horn (Coleoptera: Chrysomelidae), with increasing larval density and decreasing seed size (Amarillo-Suárez et al., 2011; Fox et al., 1999).

Body size varies considerably among a population and is vital because it affects almost all physiological and life history traits of organisms. It is an essential indicator of fitness in insects where overall larger individuals have greater longevity and higher fecundity than smaller individuals (Beukeboom, 2018). Larger males have greater access to females through a competitive size advantage when fighting amongst males, and larger females produce and lay more eggs (Beukeboom, 2018). Fecundity in most insects is related to female body size; under constant environmental conditions, the body size is positively correlated with fecundity (Honěk & Honek, 1993). For example, fecundity favours larger females, and sources suggest sexual selection favours larger males (Preziosi et al., 1996). A larger body size is particularly important in the black soldier fly because females have one oviposition event in their lifetime, and males engage in lekking behaviours where they guard territories and fight off intruding males (Tomberlin & Sheppard, 2001). This behaviour would be more successful in larger males than smaller males. One study investigated the effect of larval diet on male body size and mating success of the melon fly, *Zeugodacus cucurbitae* Coquillett (Diptera:Tephritidae) and found that males reared on zucchini grew larger, faster and dominated male-male interactions, making them more reproductively successful (Shelly, 2018). Using the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), a study found the proportion of smaller alate individuals increased with crowding and lower host plant quality (Ríos Martínez & Costamagna, 2018). Hence, in this study, we can assume that larvae fed municipal solid food waste would have higher fecundity than larvae fed poultry feed, due to the difference in body size. Taken together, there are apparent effects of larval nutrition on adult body size and fitness. The black soldier fly is mass-reared for waste reduction, and the success of controlled mass rearing depends on the reproductive fitness of the adults. This study shows that adult body size is influenced by the nutritional content of the larval diet, and potentially density. We stress the importance of optimal conditions during mass rearing.

Although taking longer to develop, the black soldier flies in this study were able to reduce waste by almost 70%, making it a viable alternative strategy to landfilling in the Windsor-Essex region. It is important to note however that seasonal variations from summer to winter could impact the waste composition, quality, and the decomposition of organic waste collected. As stated earlier, the Windsor-Essex landfill has a waste footprint of 58 hectares taking up landfill space and releasing toxins in its leachate. More than 16 new landfills will be needed, given the current population growth rate, if more effort is not made towards diverting food waste from landfills (Ministry of the Environment and Climate Change., 2017). This is a call to action to evaluate and determine other means of waste management within Windsor-Essex, and the black soldier fly is a promising candidate for removal of organic wastes from landfills.

2.6 Reference

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Table 2.1. Mean (± SE) life history traits measured for the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) reared on poultry feed or food waste. Values within rows followed by the same letter are not significantly different ($p < 0.05$) (n = 12).

	Poultry feed	**Food waste**
Survival rate to adult %	82.05 ± 4.41^{a}	65.83 ± 5.81^{b}
Female body mass (g)	0.0299 ± 0.0016^{b}	0.037 ± 0.0008^{a}
Prepupal body mass (g)	0.89 ± 0.014^{a}	1.68 ± 0.038^{b}
Time to first adult emergence (d)	26.42 ± 0.29^{a}	31.55 ± 0.19^{b}
Time to first prepupal (d)	15	19

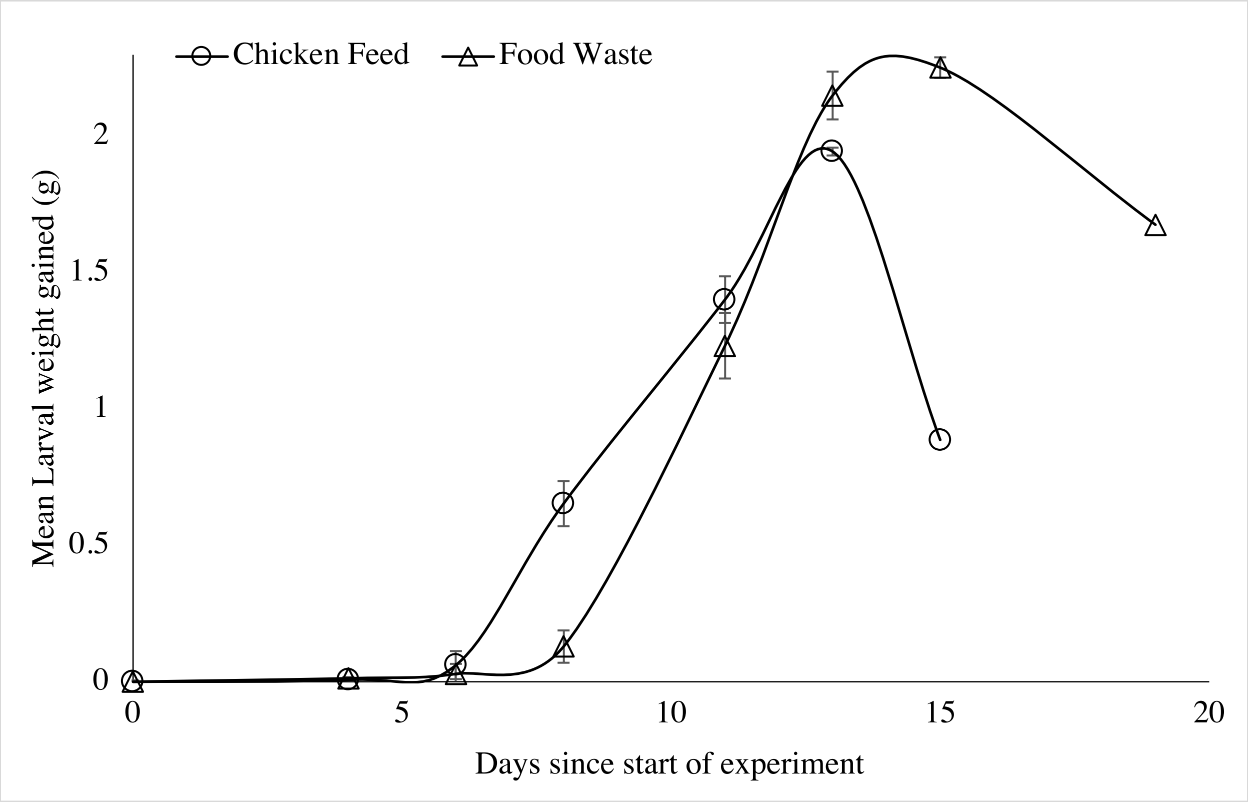

Figure 2.1. Mean (±SE) Larval Mass (g)(per 5 larvae, n=12) over time (days) for black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) larvae fed chicken feed or food waste.

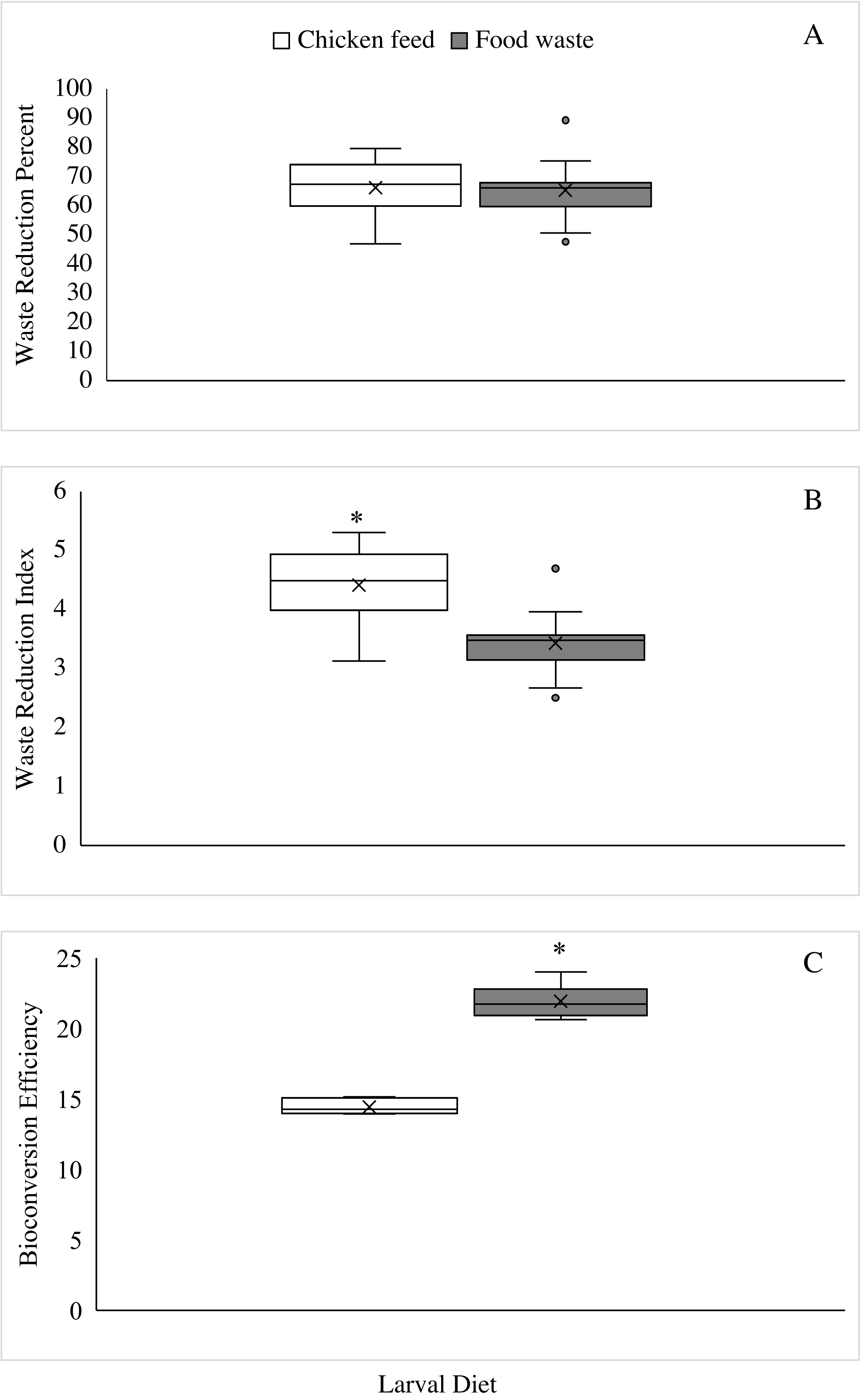
☐ Chicken feed ▨ Food waste
A
Waste Reduction Percent
100
90
80
70
60
50
40
30
20
10
0
B
Waste Reduction Index
6
5
4
3
2
1
0
*
C
Bioconversion Efficiency
25
20
15
10
5
0
*
Larval Diet

Figure 2.2. Boxplot showing the comparison of black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) larvae fed chicken feed or food waste across three waste metrics (n=12). A) Mean (±SE) Percent Waste Reduction calculated as the ratio of residue wet weight at the end of the experiment to food provided at the beginning. B) Mean (±SE) Waste Reduction Index calculated by dividing the waste reduction percentage by the larval development time. C) Mean (±SE) Bioconversion Efficiency calculated as the difference between the final larval fresh weight and the initial larval fresh weight at the beginning of the experiment divided by the ingested food and multiplied by the number of larvae at the end of the experiment. Plots with an asterisk (*) refers to the difference being statistically significant ($p < 0.05$).

Chapter 3

Optimizing black soldier fly food waste conversion by pre-digestion.

3.1 Introduction

Within an ecosystem, three types of organisms exist, the producers, consumers, and decomposers. The functioning and integrity of an ecosystem is maintained through interactions between these three distinct sub-systems (Swift et al., 1979). Producers use solar energy to fix CO_2, producing organic matter rich in nutrients and energy (e.g. plants). Consumers are heterotrophic organisms that obtain food from producers (i.e., herbivores) or other consumers (i.e., predators), with possible trophic level complexities within the ecosystem. Finally, decomposers are saprophytic organisms that feed on dead organic matter from producers and consumers, gaining energy from both. Within the decomposition system, organic matter is broken down by a community of decomposers composed of microorganisms (bacteria and fungi) and invertebrate animals. These organisms feed on organic matter and utilize the energy and nutrients for their growth and development. Decomposers function at all levels of the ecosystem, utilizing all the energy not used by the consumers and producers, as well as their excretory products, and recycling them back into the ecosystem. Decomposition is one of the most critical functions within ecosystems and can be defined as the process by which dead organic matter is broken down to the components it is made up of, and their complex organic structures fragmented from complex organic matter into simple inorganic elements.

Among invertebrate decomposers are insect decomposers. Insects associated with decomposition can be assigned to various functional groups, including wood feeders, carrion feeders, detritus feeders, and predators (Ulyshen et al., 2016). Different species are active at different stages of decomposition, and all have the ability and capacity to influence decomposition, directly or indirectly (Ulyshen et al., 2016). In wood feeders, ants and termites commonly dominate this functional group and considerably influence wood decomposition (Ulyshen et al., 2016). Carrion feeders are primarily composed of insects in the orders Diptera and Coleoptera (Moretti et al., 2008). They arrive within minutes or hours after death, with the earliest arrivals from the families Calliphoridae, Muscidae, and Sarcophagidae (Tomberlin et al., 2017). Detritus feeders feed on detritus, which is defined as non-living organic matter, including plant tissue, animal tissue, faeces, as well as products and compounds exuded from other organisms (Moore et al., 2004). The chemical composition of detritus includes compounds such as cellulose, hemicellulose, and lignin, as well as biomolecules such as fats, nucleic acids, and proteins (Moore et al., 2004). Municipal waste is made up of some of these components.

The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) is a generalist decomposer that can consume a wide range of organic waste from vegetables to manure (Nguyen et al., 2013; Sheppard et al., 1994). A large wasp-like fly, the black soldier fly has been promoted for waste treatment and bioconversion, and is commercially reared by a few companies (De Smet et al., 2018; Sheppard et al., 1994; Tomberlin et al., 2002). Adult black soldier flies have limited functional mouthparts, only able to drink; they do not go into residences seeking food and shelter, and hence, are not considered vectors for diseases or nuisance pests. As a result of their non-feeding adult stage, adult survival, dispersal, and reproduction relies on fats and proteins obtained during the larval development (Newton et al., 2005). Rearing diets significantly affect the development of the black soldier fly (Nguyen et al., 2013, 2015), and larvae reared on fruits and vegetables perform poorly, perhaps due to low protein content and low-fat content (Nguyen et al., 2013). On the other hand, poor development could be due to the black soldier fly's inability to break down cellulose, hemicellulose, and lignin in a vegetable diet (Douglas, 2009), as these components are not readily accessible to enzymes for breakdown (Douglas, 2009).

When organic materials are recycled, various organic compounds undergo decomposition. Organic matter decomposition is essentially an enzymatic process (Khatoon et al., 2017). It is carried out by microorganisms comprising bacteria, fungi, actinomycetes, and protozoa (Janzen, 1977). Organic matter decomposition provides energy for growth, carbon for cell formation, and other nutrients for growth (Khatoon et al., 2017). Microbes can release intracellular or extracellular enzymes; extracellular enzymes are necessary for the breakdown of polysaccharides, whereas monosaccharides, such as glucose, are metabolized by intracellular enzymes (Khatoon et al., 2017). Organic residues are first broken down from their complex forms by extracellular enzymes secreted by microbes, and then their primary components are utilized by intracellular enzymes (Khatoon et al., 2017). The by-product of aerobic decomposition is carbon dioxide (CO_2), whereas for anaerobic it is CO_2, methane (CH_4), and various other organic acids. Decomposition occurs fast at the beginning, but slows down as the availability of decomposable organic matter decreases.

Compounds, such as sugars, amino acids, and lipids, are easily decomposable and break down rapidly. In contrast, complex compounds, such as cellulose, hemicellulose, lignin, and proteins, are broken down slowly. Cellulose is an abundant carbohydrate source present in plant residues/organic matter, which is organized into crystalline microfibrils, and enclosed within a matrix of hemicelluloses, pectins, and lignin (Douglas, 2009). Cellulose decomposition occurs in two stages; first, the hydrolysis of cellulose to glucose, and then the oxidation of glucose to CO_2 and water (Khatoon et al., 2017). Microbes, such as *Penicillium* Link*, Aspergillus Micheli* (Eurotiales: Trichocomaceae)*, Streptomyces* Waksman & Henrici (Streptomycetales: Streptomycetaceae), and *Pseudomona*s Migula (Pseudomonadales: Pseudomonadaceae), are significant players in the breakdown of cellulose (Khatoon et al., 2017). Hemicelluloses are polysaccharides and are major components of plants, second in quantity to cellulose. Hydrolysis of hemicellulose is a function of hemicellulases secreted by microbes; hemicelluloses are converted into soluble sugars, which are further broken down into organic acids, CO_2, and water (Khatoon et al., 2017). Microbes, such as fungi, bacteria, and actinomycetes, are necessary to decompose hemicelluloses (Khatoon et al., 2017). Lignin is the third most abundant component of plant tissues and is one of the most resistant organic substances for microbes to degrade. However, certain fungi are known to degrade lignin at slow rates. During decomposition, proteins are immediately hydrolyzed into polypeptides; these are then further broken down into individual amino acids, through a process known as ammonification, by the action of proteases secreted by various microorganisms (Khatoon et al., 2017). The decomposition of organic matter is primarily driven by the action of microbes, such as bacteria and fungi.

The interactions between microorganisms and insect decomposers are often complex to understand. Microbes present on the surface of decomposing organic matter, such as manure, rotting fruits, or carrion, function as more than nutrient recyclers, but also as resource competitors as well (Janzen, 1977). Decomposing resources are rapidly colonized by both animals and microbes (Janzen, 1977). Microbes release antibiotic chemicals to prevent competitive exclusion by other competing microbes, but also use these chemicals to reduce competition from animal competitors (Janzen, 1977). Microbial activity can reduce competition for decomposing organic matter, an ephemeral resource, by producing chemicals that repel animal competitors (Burkepile et al., 2006). Burkepile et al. (2006) reported that fresh carrion, contaminated with fewer microbes, attracted a more comprehensive range of animal scavengers for a longer time, than microbe-laden carrion aged for two days. This study proposed that microbes release noxious chemicals that deter colonization by other scavengers. Interestingly, microbial activity can also drive the colonization of decomposing organic matter by arthropods and suppress the growth of pathogenic fungi (Ponnusamy et al., 2008). Several studies have found that feeding and oviposition by Dipteran species are driven by microbially mediated odours (Tomberlin et al., 2017). Inoculation from cultures of *Pseudomonas aeruginosa* Migula (Pseudomonadales: Pseudomonadaceae), *Bacillus subtilis* Ehrenberg (Bacillales: Bacilllaceae), *Proteus mirabilis* Hauser, and *Enterobacter cloacae* Jordan (Enterobacteriales: Enterbacteriaceae) induced oviposition by *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae), an agent of sheep myiasis (Emmens & Murray, 1982). Studies have determined that the attraction of gravid females to resources inoculated with bacteria results from volatile organic compounds (VOCs) emitted from the bacteria-inoculated resource during decomposition (Chaudhury et al., 2010). For example, ammonia released during carrion decomposition facilitates oviposition by several blow flies (Diptera: Calliphoridae) (Holdway, 1930; Seddon, 1931). A recent study by Zheng et al. (2013) showed that gravid black soldier fly females were repelled when the oviposition medium was inoculated with *Acinetobacter sp*. Brisou & Prevot (Pseudomonadales: Moraxellaceae), but were attracted when the medium was inoculated with *Providencia sp*. Ewing (Enterobacteriales: Enterobacteriaceae) In addition, Zheng et al. (2013) determined that a mixed bacterial culture isolated from egg masses of the black soldier fly resulted in larger egg mass from gravid females when the oviposition medium was inoculated with the bacterial culture. A significant component of the bacterial culture was identified as genus *Cellulomonas,* an actinobacteria capable of degrading cellulose (Zheng et al., 2013). Yu et al. (2011) inoculated chicken manure with *Bacillus subtilis* strains isolated from the gut of the black soldier fly*,* and this increased larval weight and decreased development time (Yu et al., 2011). Inoculating organic waste with beneficial microbes for pre-digestion has the potential to improve the biodegradation of wastes by the black soldier fly larvae. Additionally, studies determined that black soldier fly larvae can suppress the development and growth of *Escherichia coli* Migula and *Samonella enterica* Le Minor & Popoff (Enterobacteriales: Enterobacteriaceae) (Erickson et al., 2004; C. H. Lalander et al., 2015; Q. Liu et al., 2008).

Due to their ability to consume a wide range of organic waste, the black soldier fly has been instituted as a waste management alternative in several countries; United States (Newton et al., 2005; Sheppard et al., 1994), Switzerland (Diener et al., 2009, 2011), China (Zhou et al., 2013), Ghana (Ewusie et al., 2018), Republic of Guinea (Hem et al., 2008) to name a few. Using the black soldier fly larvae for waste management requires the bioconversion of organic waste at a stable rate, with a high waste reduction efficiency. It is possible that pre-digestion of organic wastes with microbes could increase development and waste conversion efficiency of the black soldier fly larvae.

Effective microbes (EM) is a microbial inoculant developed by Dr. Teruo Higa at the University of Ryukyus, Okinawa, Japan (Higa, 1991; Higa & Wididana, 1991). EM comprises naturally occurring non-pathogenic microbes that can be applied to soils and plants to increase microbial diversity (Higa & Parr, 1994). Additionally, adding EM to organic waste was shown to promote the breakdown and accelerate the composting process (Zakarya et al., 2021). EM inoculant is often composed of lactic acid bacteria, yeast, actinomycetes, fungi, and photosynthetic bacteria (Xu, 2001). Lactic acid bacteria inhibit the growth of pathogenic microbes through lactic acid production, which acts as a sterilizing compound (Xu et al., 2001). Yeast produces amino acids and polysaccharides, which can feed other microbes, while promoting the fermentation of organic matter (Xu et al., 2001). Lastly, actinomycetes produce antibacterial substances, while fungi promote the decomposition of organic matter (Xu, 2001; Xu et al., 2001).

This chapter will measure effects of pre-digesting organic waste using EM before it is inoculated with the black soldier fly larvae. This chapter will: 1) compare the development of the black soldier fly on pre-digested waste versus non-digested; 2) measure the fermentation period required to elicit a beneficial effect on the black soldier fly; and 3) establish the volume of EM required to optimize waste conservation of organic waste in conjunction with black soldier fly. We expect the development time to be reduced for black soldier flies reared on predigested waste compared to black soldier flies reared on non-digested waste. We expect increased survival of black soldier flies raised on predigested organic waste compared to non-digested. Finally, we expect that longer fermentation times will improve the waste reduction and waste conversion efficiency of the black soldier fly through a combination of faster decomposition and increased consumption by the black soldier fly.

3.2 Materials and methods

3.2.1 Colony maintenance

A colony of black soldier fly was established in 2018 and maintained at the University of Windsor, Windsor, Ontario, Canada. New larvae were added yearly from orders purchased from the worm lady (wormlady.myshopify.com) located in Ontario, Canada. Adults were held in a black mesh (1.5 mm) cage constructed with PVC pipes (1.8 m x 1.8 m x 1.8 m). The enclosure was provided with golden pothos plants as a lekking site for males (Tomberlin & Sheppard 2001). Lighting was provided using 150-W high-pressure LED lights (Model: BSF-4C-200-3030, Eco Conversion Systems LLC, Texas, USA) to maintain a photoperiod of 16:8 (L:D) cycle. Colonies were maintained at approximately 26.7°C and relative humidity (RH) of at least 20%. A water misting system (set for 30-sec intervals twice a day) helped maintain humidity in the enclosure and provided droplets of water for adult consumption. An oviposition site was provided by using corrugated cardboard taped to the side of a Tupperware container (24.43 cm x 16.81cm x 8.55 cm) (Snaptite, ID: 10-1001012), and the Tupperware was filled with poultry feed (Purina Gold'N start & grow crumbles, product number: 6040, Mississauga, Ontario) saturated with water. Poultry feed has been established as a standard diet for rearing black soldier flies (Sheppard et al., 2002); this feed contains approximately 20% crude protein, 3% fat, and 5% crude fibre saturated. After oviposition, the Tupperware container with the corrugated cardboard now filled with eggs is moved into a growth chamber set at 27°C, 70% RH, and 16L: 8D until eclosion. Larvae were fed poultry feed until they reached the prepupae stage when they stopped feeding. Prepupae were then transferred into a container filled with wood chips (used as a pupation medium) and placed into the colony cage until emergence.

3.2.2 Experimental design

Treatments were a complete factorial design with five diets, each with 100 g of food waste, inoculated with different volumes of effective microbes purchased from the gardener's pantry (gardnerspantry.ca)(0 mL, 5 mL, 10 mL, 15 mL, and 25 mL), and three post-fermenting diet treatments of 0 days, 2 days, and 7 days. Diets were chosen to measure the development of the black soldier fly on predigested food waste while using an uninoculated diet as a control. The survival to adult and development time of larvae fed food waste inoculated with different levels of microbes and fermented for different times were measured. Development time was measured as the time from the start of the experiment with 4-day-old larvae to the time of adult emergence. Treatments were checked every two days, and five larvae per replicate were randomly selected using forceps, weighed together, and returned to obtain larval weight during development, until the prepupal stage was reached. One hundred 4-day-old larvae were randomly selected per treatment and transferred into separate clear plastic containers (475ml) with the diets using a paintbrush covered with a lid, perforated with holes for gas exchange and put into the growth chamber programmed to 90% RH, 27°C and 16L:8D. Food waste was obtained from a local composting farm, Greener Bins in Kingsville, ON and was composed of plant and animal material. Waste samples were sent to Bureau Veritas, Mississauga, Ontario, Canada, for nutritional analysis. It was shown to contain 50 calories/100g, 7g/100g of carbohydrate, 3g/100g of protein and 3g/100g of fat. To ensure consistency, diets were ground using an industrial blender and homogenized using a mixer with a moisture content of approximately 70%. The waste was then portioned, pre-packaged, sealed in sandwich bags, frozen, and stored at -20ºC until needed, and thawed 24 h before use. Inoculated diets were left to ferment at room temperature at 19°C to 21°C for the assigned time and used accordingly.

3.2.3 Equations

Survival was determined by counting the number of successfully emerged adults from each treatment and comparing the ratio of larvae at the end and the beginning of the experiments (Gold et al., 2020).

Equation 1: $\text{Survival}(\%) = \frac{\text{Number of adults at the end of experiment}}{\text{Number of larvae at the beginning of experimentt}} \text{ x } 100$

The relative growth rate per larvae per day was calculated using the equation below (Cai et al., 2019).

Equation 2: Relative growth rate per larvae per day =

$$\frac{\text{Larval weight at the end} - \text{inital larval weight}}{\text{larval development time X inital larval weight}}$$

Waste reduction percent was calculated using the equation below as the ratio of residue wet weight at the end of the experiment to food waste provided at the beginning (Diener et al., 2009).

Equation 3: $\text{Waste reduction }(\%\text{ww}) = \frac{\text{Waste IN} - \text{Waste OUT}}{\text{Waste IN}} \text{ X } 100$

The waste reduction index was calculated using the equation below as the waste reduction percentage calculated above divided by larval development time (Diener et al., 2009).

High waste reduction index = High waste reduction efficiency

Equation 4: $\text{Waste reduction index (WRI)} = \frac{\text{Waste reduction percent}}{\text{Larval development time}}$

The efficiency of bioconversion was calculated using the equation below as the difference between the final larval fresh weight and the initial larval fresh weight divided by ingested food and multiplied by the number of larvae at the end of the experiment.

Equation 5: Efficiency of Bioconversion = $\frac{\text{Change in larval weight}}{\text{Ingested food}}$ X 100

3.3 Statistical Analyses

All analyses were completed in R 4.1.3 (R Project for Statistical Computing, http:/www.R-project.org/). Normality was tested using the Shapiro-Wilk and homogeneity of variance was tested using Bartlett's test. The effects of microbial amount and fermentation time on relative growth rate, waste reduction, waste reduction index, development time, and bioconversion rate were modelled using Linear Models with the stats (glm) package (family = gaussian, link = identity). Significant results were followed by a means comparison post hoc test (Tukey post hoc comparisons) using the rstatix (tukey_hsd) package. The effect of microbial amount, fermentation time on survival, and the interaction between these factors were analyzed using a Generalized Linear Model (glm) (GLZ, Family = Binominal, link = logit). Significant results were followed by a means comparison post hoc test (Tukey post hoc comparisons) using the rstatix package.

3.4 Results

An interaction between fermentation time and microbial amount influenced adult survival ($X^2 = 843.78$; df = 8; $p < 0.001$; GLM; Figure 3.1). For all treatments with microbes added and across fermentation times tested, survival had a decreasing trend. With the exception of 25 ml where survival did not differ with fermentation time, survival was highest at 0 days and lowest at 7 days, intermediate survival at 2 days, and not different from 0 days. In contrast, the treatment without microbials added had the highest survival at 0 and 7 days, with survival at 2 days lower than 0 days, but not different from 7 days. Overall, the lowest survival was observed at 15 ml at 7 days fermentation time.

The relative growth rate per larvae per day was influenced by an interaction between fermentation time and microbial amount ($F_{8,93} = 2.48$; $p = 0.019$; ANOVA; Figure 3.2). Across all microbial amounts and without microbials added, growth rate was highest at 2 days, except at 15 ml of microbes added where growth rate at 2 days did not differ from that of 0 or 7 days. Regardless of whether microbials were added or not, growth rate did not differ between 0 and 7 days.

Development time was influenced by an interaction between fermentation time and microbial amount ($F_{8,93} = 68.82$; $p<0.001$; ANOVA; Table 3.2). Mean development time varied from 28-33 days. Without microbials added, development time did not differ with fermentation time, and did not differ from the intermediate and longest development times exhibited by any treatments with microbials added. With microbials added, the longest developmental times were at 7 days fermentations, with this not differing from development time at 0 days fermentation for the two highest levels of microbials (15 ml, 25 ml). The shortest development times were at 2 days of fermentation for all treatments with microbials added, as well as at 0 days for 10 ml of microbials added. However, development time of these 2 day fermentation treatments with microbials added were intermediate, and not different from 0 days fermentation for microbial amounts of 5 ml, 15 ml and 25 ml. At 0 days with 10 ml of microbials added, mean (+/- SE) development time was the shortest at 28.17 ± 0.20 days.

Fermentation time and microbial amount interacted to influence the percentage of waste reduced ($F_{8,93} = 9.79$; $p<0.001$; ANOVA; Figure 3.3). Variation in waste reduction percentage can be explained predominately by fermentation time (MS= 1794.94), then by an interaction with the microbial amount (MS=365.44) and microbial amount explains the least amount of variation (MS=230.31)(Table 3.1). Without microbials added, waste reduction was highest at 0 days, and lowest at 2 and 7 days. With microbials added, waste reduction was lowest at 7 days of fermentation except at 25 ml, where waste reduction didn’t differ with fermentation time. At 5 ml and 15 ml, waste reduction at 0 days was intermediate between 2 days and 7 days, not differing from either. At 10 ml, waste reduction was higher at 0 days and 2 days, compared to 7 days. Across all treatments with microbials added, equivalent waste reduction occurred at 2 days, which was also the highest waste reduction percentage, except as noted for 10 ml microbials added.

Fermentation time and microbe amount interacted to influence the waste reduction index ($F_{8,93}$ = 9.63; p<0.001; ANOVA; Figure 3.3). Fermentation time had the largest effect (MS = 7.09), accounting for four times more variation than the interaction effect (MS = 1.64) and over seven times more variation than the microbial amount (MS = 0.97) (Table 3.1). With no microbials added, fermentation time did not affect the waste reduction index. When microbials were added, the waste reduction index was highest overall at 2 days fermentation, except at 10 ml with no difference between 0 days and 2 days fermentation. The lowest waste conversion index was 10 ml microbial added at 7 days of fermentation. For other levels of microbials added, the waste reduction index did not differ between 0 days and 7 days.

The bioconversion rate was affected by the interaction between fermentation time and microbial amount ($F_{8,93}$ = 5.31; ANOVA; p<0.001; Figure 3.4). Fermentation time accounted for the most variability (MS = 0.067), followed by the interaction effect (MS = 0.057) and microbial amount (MS = 0.026) (Table 3.1). With no microbials added, the bioconversion rate did not differ across fermentation time. This was also true at the highest level of microbials added, 25 ml. At 5 ml, 10 ml and 15 ml, the bioconversion rate decreased with increasing fermentation times. At these levels, the bioconversion rate was lowest at 7 days and highest at 0 days. Within these levels of microbials added, bioconversion rate at 2 days of fermentation varied between not different from 0 days or not different from 7 days.

3.5 Discussion

Organic matter decomposition is an ecological process that involves the breakdown of organic materials and recycling its nutrients and energy through other organisms. It involves the breakdown of biological matter into its smaller constituents and occurs through the action of microbes through microbial breakdown and consumption by invertebrates such as insects. Understanding the ecology of decomposition is crucial to understanding ecosystem functioning, as when organic matter begins to decompose, it becomes an ephemeral resource utilized by many organisms, including the microbial community, invertebrates, and scavengers.

Most of the research conducted with black soldier flies and microbial interaction has focused on the gut microbiota, and how the foregut, midgut, and hindgut host different bacterial communities (De Smet et al., 2018). The diversity of microbes found in the gut of the black soldier fly is related to the nutritional complexity of the diet the larvae are feeding on (Jeon et al., 2011). Other studies have investigated how oviposition can be mediated by the presence of bacteria isolated from the eggs of the black soldier fly enhanced oviposition (Zheng et al., 2013). This study is the first of its design and one of the few studies investigating the pre-treatment of organic waste to improve the black soldier fly bioconversion (Isibika et al., 2019; Lindberg et al., 2022; Raksasat et al., 2022)

Microbe treatment of different fermentation times and microbial amounts resulted in differences in survival, development, waste reduction, and bioconversion. In most metrics, adding microbes with the larvae at the start of the experiments resulted in higher fitness (higher survival and shorter development time) compared with the control of no microbes added and longer fermentation times. However, other metrics measured, such as relative growth rate and waste reduction index, were higher at 2 days fermentation time compared to 0 days and 7 days. All metrics measured were negatively impacted at 7 days of fermentation. These results contradict the initial hypothesis that longer fermentation times would result in greater fitness and more resource availability to larvae due to microbial breakdown. Other studies with pre-digestion without black soldier fly treatment have found that the efficiency of reduction and release of molecules, such as glucose in organic waste, was improved with longer pre-treatment durations (Izaguirre et al., 2019). Pre-treatment with enzymes resulted in faster glucose breakdown compared to no added enzymes (Izaguirre et al., 2019). The addition of an alkaline peroxide pre-treatment improved the digestibility of rice straw by 18% largely because pre-treatment resulted in the breakdown of macromolecules such as cellulose, hemicellulose and lignin (C. Liu et al., 2021).

Overall, survival decreased as fermentation time increased. This means that the highest survival was observed when effective microbes were inoculated at the same time as black soldier fly larvae. Other studies have inoculated organic waste with microbes and observed no difference in the survivorship (Yu et al., 2010, 2011), although both of these studies inoculated wastes with microbes isolated from the gut of the black soldier fly. Adult survival is seen as a measure of fitness in this study, as biological fitness means to survive to adult reproductive age. Survival in larval development can be affected by larval diet, developmental temperature, and relative humidity (Chia et al., 2018; Holmes et al., 2012; Nguyen et al., 2013). However, since developmental temperature and humidity was kept constant in this study, the variation in survival was due to larval diet, particularly changes in larval diet due to diet fermentation. As decomposition progresses the quality of organic matter decreases. The total carbohydrates and proteins available decreases, suggesting that the microbes consumed the monomers produced (Lindberg et al., 2022). Polysaccharide concentrations increased during the first 12 h of decomposition when organic waste was pre-treated with enzymes (Hou et al., 2021). An increase in polysaccharides during the first 12 h could explain why longer fermentation times did not result in higher survival in the present study, while immediate microbe addition increased survival. Monomers of proteins and carbohydrates produced are necessary for insect development and act as building blocks and fuel (Cohen, 2003). Proteins provide the amino acids necessary to build tissues and enzymes, while carbohydrates function as fuel that drives this biosynthesis (Le Gall & Behmer, 2014). One study suggests that old or aging manure reduced the growth and survival of the black soldier fly compared to fresh manure (Tomberlin et al., 2002).

Additionally, at longer fermentation times, microbes may switch from beneficial to antagonistic. When organic matter decomposes, a diverse decomposer microbial community forms, including nematodes, fungi and bacteria (Lauber et al., 2014). Microbes play an important role in decomposition and play a critical role in mineralizing organic compounds in terrestrial ecosystems, such that in the absence of soil microbial community, carrion decomposition was significantly slower compared to when the microbial community was present (Lauber et al., 2014). During organic decomposition, a succession of organisms occurs, which is represented by the regulating structure and interaction of biological communities involved in decomposition (Dilly et al., 2001). Different catabolic processes are required to complete the decomposition process (Dilly et al., 2001). Most studies investigating microbial succession show that zymogenous organisms dominate earlier stages of decomposition while autochthonous organisms dominate the later stages of decomposition, and with this, the decrease in biomass content and respiration rate suggest a decline in organic matter quality over time (Dilly et al., 2001). Thus, there is the potential for not just a change in nutrients, but also a change in the microbial community itself that could affect the black soldier fly. This is supported by the overall fermentation time seeming to be more important for survival than the amount of microbe added to the food waste.

Unsurprisingly, larval development time was also likely influenced by nutrient availability within the larval diet. When the larval diet is not nutritionally balanced, larval development and survival are severely impacted (Nguyen et al., 2013, 2015). This idea is also supported by the longest development time recorded for larvae fed the 7-day fermented diet. As proposed previously, low nutrient availability due to microbes depleting nutrients over the longer fermentation period would mean fewer nutrients for growth and development. With a diet lacking in essential nutrients, larvae likely utilized compensatory feeding, delaying their development to attempt to gain the requisite nutrients.

Yet the trend in larval growth rate was not the same as that survival. Whereas survival was highest at the shortest fermentation time, declining with fermentation time, larval growth rate first increased between 0 to 2 days, then decreased, with fermentation time. Similarly, another study found that pre-treatment of waste for 7 days and 14 days increased the larval growth rate, however a 21-day pre-treatment fermentation resulted in a lower growth rate (Isibika et al., 2019). The reason for this trend could be that at first microbial action aids in the pre-digestion of large polymers, breaking them down into smaller monomers that are easily assessable to the larvae. Still, over time the microorganisms themselves consume the nutrients required by the black soldier fly larvae. It is possible that while polysaccharide concentrations increase in the first 12 h providing a boost to larval growth rate in the short term, the overall concentration of easily digestible monomers are still gradually depleted by microorganisms (Hou et al., 2021), resulting in the reduction in survival and reduction in growth rate for larvae grown on a 7-day fermented diet. Overall, it seems like the microbes play a beneficial and antagonistic role which is defined by the fermentation time.

As would be expected, the contrasting effects of fermentation time on survival compared to growth rate, influenced the waste metrics calculated from life history traits. In the present study, larvae reared on diets fermented for 0 days and 2 days had similar waste reduction percentages, however, 7 days fermentation had low waste reduction percentages. It is likely due to low survival observed in black soldier fly fed 7 days fermented waste, as outlined previously. Depending on the timing of black soldier fly mortality in this treatment, the larval density and larvae available to feed on and reduce the waste would also change. More interesting is the lack of difference in waste reduction between 2 days and 0 days, even though survival, and therefore larval density, was reduced in the 2-day fermentation treatments. Perhaps there is still a synergistic effect of microbe and larvae within that timeframe that results in waste reduction comparable to waste reduction at 0 days. It is also possible that a greater proportion of the mortality occurred after waste consumption, such as during the pupal stage, therefore not impacting the density of larvae available to consume waste within the 2-day fermentation treatment.

The waste reduction index is waste reduction as a function of larval development time. The lowest waste reduction index was observed in black soldier fly fed the 7-day fermentation treatment. This is expected since waste reduction percentage was also lowest at this fermentation, and as previously proposed, is likely explained by low survival observed in these treatments, resulting in fewer larvae available to reduce the waste. Those that did survive, also took the longest to develop. Lindberg et al. (2022) also observed reduced material reduction at longer fermentation times.

Despite overall waste reduction not differing between larvae fed 0-day and 2-day fermented diets, the overall waste reduction index differed such that 2-day fermentation treatments had a higher waste reduction index than any other treatments. This is likely due to the similar larval development between 0 and 2-day fermentation treatments. It is important to note that a higher waste reduction index is equal/related to a higher waste reduction efficiency. This means that larvae reduced a higher percentage of waste in a shorter time period.

Bioconversion rate is the ratio of the weight of the pre-pupae to the weight of the larval diet provided at the beginning of the experiment. In this study, the bioconversion ratio decreased with fermentation time, meaning the black soldier fly larvae were less capable of converting older fermenting microbial-laden waste into larval biomass. As previously proposed, this may be due to depleted nutrients as the diet ferments.

Waste conversion for municipal food waste tends to be lower due to the heterogeneous nature of the waste (Gold et al., 2020; C. H. Lalander et al., 2015). This can be due to high carbon content and low availability of lignin and hemicellulose-rich materials to larvae, as well as reduced protein content (Gold et al., 2018; Lindberg et al., 2022; Nguyen et al., 2015). The nutritional parameters required to ensure efficient and high waste-to-larval biomass conversion are high protein content (C. Lalander et al., 2019), an equal ratio of protein to carbohydrate content (Cammack & Tomberlin, 2017), and low lipid and fibre contents (Gold et al., 2020). Yet, these nutritional parameters are impossible to ensure when dealing with municipal food waste. One way of dealing with this issue is pre-digestion and fermentation with microbes to improve the digestibility of the food waste that does not meet the requirements needed for high waste conversion. For example, pre-treating banana peels with ammonia solution combined with fungi for a period of 7-14 days resulted in the increase of waste reduction by 15% (Isibika et al., 2019). This is likely due to the ammonia solution promoting the breakdown of macromolecules and the enzymes secreted by fungi degrading the peels into more easily available nutrients for the larvae (Isibika et al., 2019). Fungi produce cellulolytic and oxidative enzymes that break down different components of organic matter (Sigoillot et al., 2012).

The interactions between microbes and insects are often difficult to characterize. This is more apparent in systems with ephemeral resources. As evidenced in this study, the effects of microbial fermentation of larval diets on the development of the black soldier fly are difficult to understand and remain unclear. Fish carrion contaminated with fewer microbes was more attractive than the microbial-laden fish carrion (Burkepile et al., 2006). Different life history traits may benefit from different fermentation times of the larval diet.

The goal of this experiment was to find the waste fermentation time and the microbial amount that was optimal for waste conversion, while also supporting black soldier fly development. Based on this study, it seems that 2 days of fermentation time elicits positive trends in the black soldier fly development, such as a higher growth rate and better waste reduction index. However, 0 days of fermentation elicits better survival and a high bioconversion ratio. All life history traits tested at 7 days had negative impacts such as severely reduced survival, bioconversion ratio, waste reduction index and overall longer development. All of this suggests that black soldier fly larvae are negatively impacted by old microbial-laden waste, and if the goal of the waste conversion system is to optimize waste reduction efficiency while simultaneously improving larval growth rate then pre-fermenting the waste for 2 days could achieve this goal. This goal could also be achieved by continuous feeding (i.e. continuously adding fresh waste to prevent the issue of nutrient depletion such as that seen in old/aging waste). In addition to continuous feeding, co-digestion of highly nutritious waste with poor heterogeneous waste could improve waste reduction efficiency and larval growth rate (Rehman et al., 2017). Further research should consider these methods and perhaps test adding pure enzymes to municipal food waste, which could provide more control over the process instead of waiting for the microbes present to synthesize enzymes that could break down the complex molecules.

3. 6 Reference

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Table 3.1. Analysis of Variance (ANOVA) results to determine the effects of microbial amount, fermentation time, and the interaction of these effects on the waste reduction, waste reduction index, and bioconversion rate of the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). Significant effects are indicated in bold font; α = 0.05 for all effects.

Treatment	**df**	**Sum of Squares**	**Mean Square**	**F ratio**	***p*-value**
		Waste reduction			
Fermentation time	2	3589.88	1794.94	49.86	**<0.0001**
Microbial amount	4	921.24	230.31	6.39	**0.0002**
Fermentation time X Microbial amount	8	2923.55	365.44	10.15	**<0.0001**
		Waste reduction index			
Fermentation time	2	14.18	7.09	41.93	**<0.0001**
Microbial amount	4	3.89	0.97	5.75	**0.0004**
Fermentation time X Microbial amount	8	13.15	1.64	9.72	**<0.0001**
		BCR			
Fermentation time	2	2229.80	1114.9	20.51	**<0.0001**
Microbial amount	4	1346.75	336.68	6.19	**0.0002**
Fermentation time X Microbial amount	8	2310.53	288.81	5.31	**<0.0001**

Table 3.2. Mean Development time (± SE) of black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) adults reared on diets with different fermentation times and microbial amounts. Waste reduction was affected by an interaction between fermentation time and microbial amounts ($p < 0.0001$). Means with the same letters indicate no significant differences in development time between fermentation times and across microbial amounts tested ($p < 0.05$)

Microbial Amount	**Fermentation time** **0 Days**	**2 Days**	**7 Days**
0 ml	30.83 ± 0.31^{abcd}	32.5 ± 0.35^{ab}	31.67 ± 0.16^{abc}
5ml	30.67 ± 0.72^{bcd}	30.17 ± 0.28^{cde}	32.6 ± 0.25^{ab}
10ml	28.17 ± 0.20^{e}	30.33 ± 0.15^{bcde}	33 ± 0.55^{a}
15ml	31.33 ± 0.67^{abc}	30.33 ± 0.15^{bcde}	33 ± 0.63^{a}
25ml	31 ± 0.78^{abcd}	28.83 ± 0.22^{de}	31.17 ± 0.12^{abc}

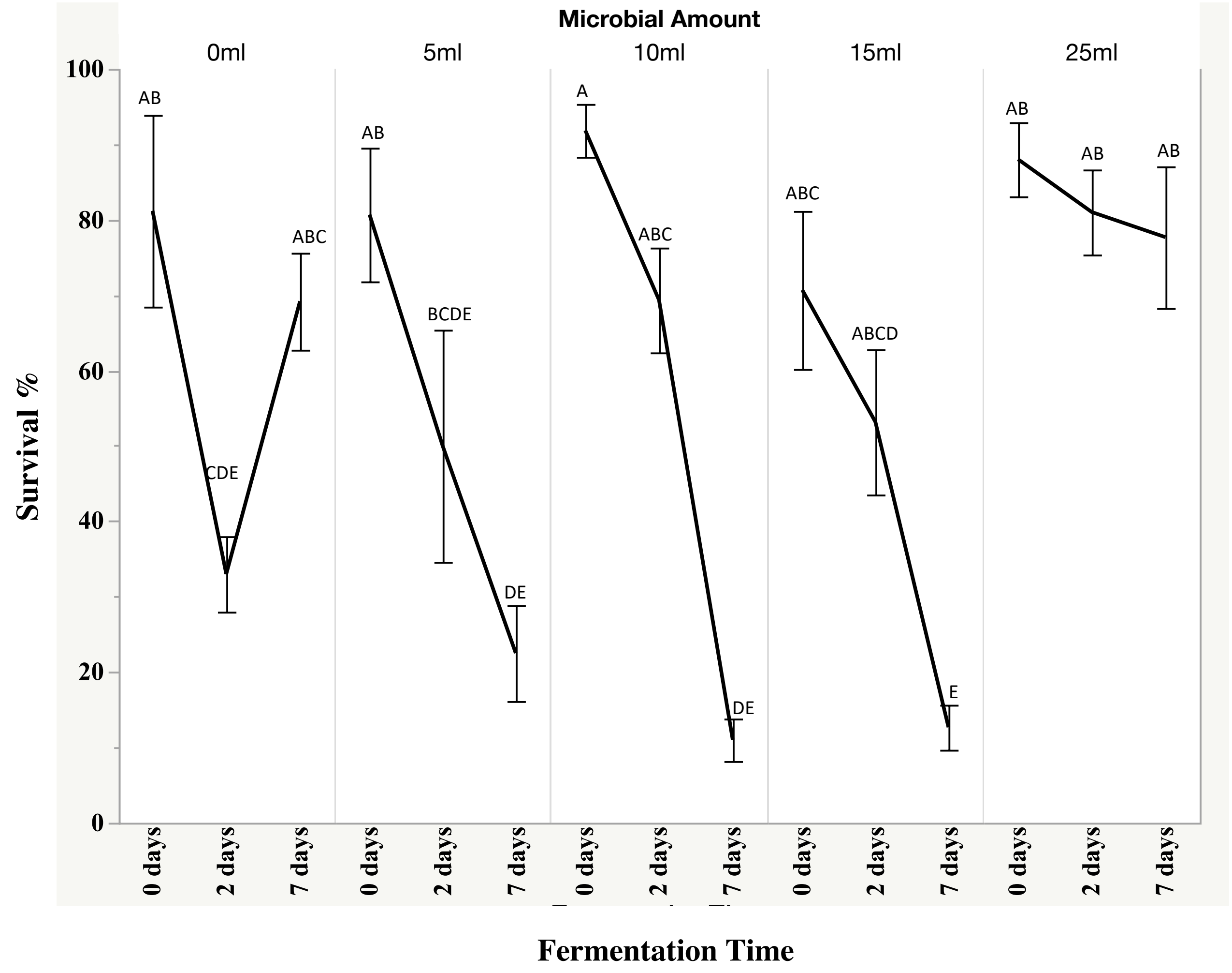

Figure 3.1. Mean (±SE) survival percent of black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) adults reared on diets with different fermentation times and microbial amounts. Survival of the black soldier fly was affected by an interaction between fermentation time and microbial amounts ($X^2 = 843.78$; df = 8; $p < 0.0001$). Means with the same letters indicate no difference in survival between fermentation times and across microbial amounts tested ($p < 0.05$).

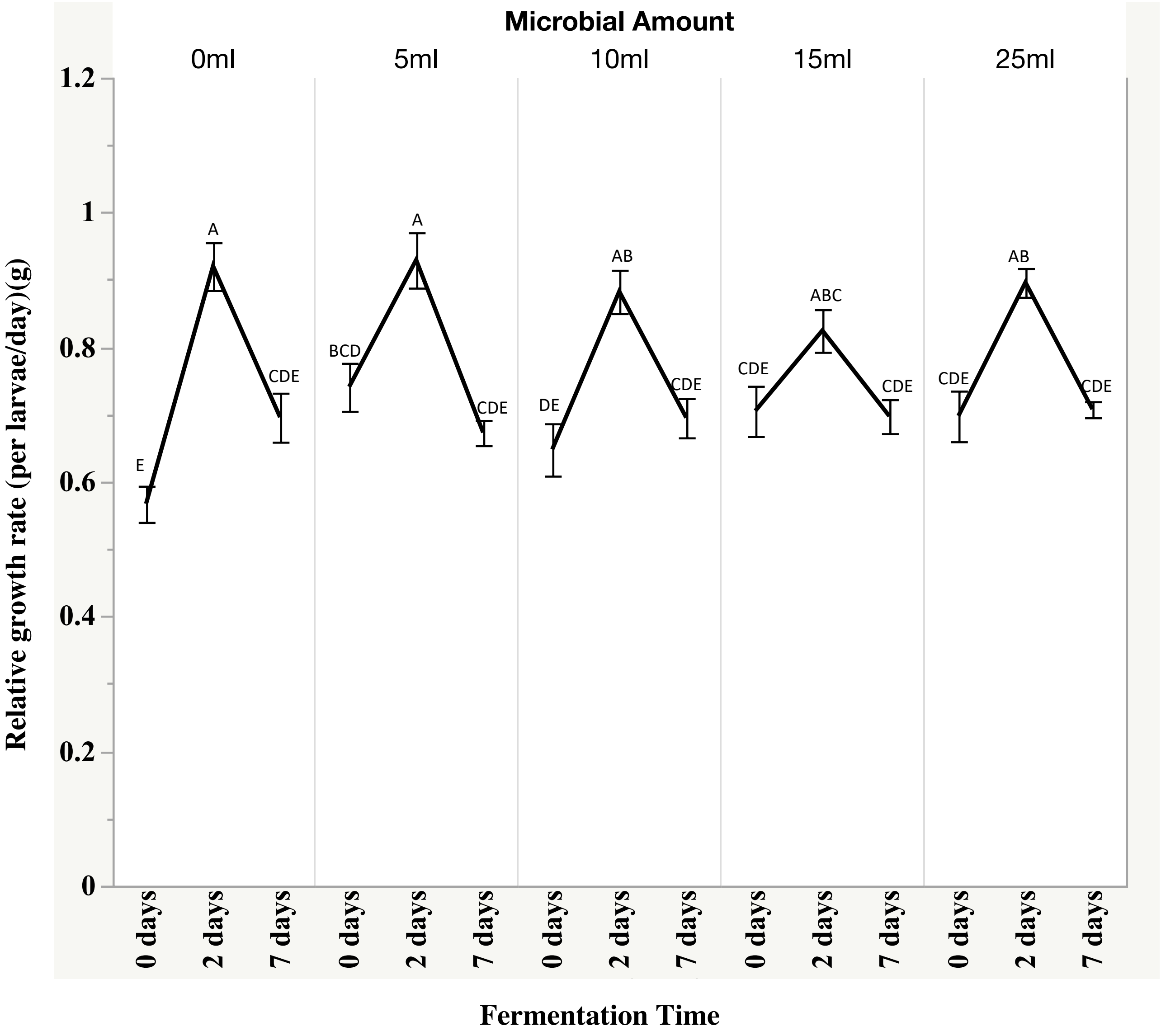

Figure 3.2. Mean (±SE) relative growth rate (per larvae/day)(g) of black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) reared on diets with different fermentation times and microbial amounts. Relative growth rate was affected by an interaction between fermentation time and microbial amounts ($F_{8,93} = 2.48$; df = 8; $p = 0.019$). Means with the same letters indicate no difference in relative growth rate between fermentation times and across microbial amounts tested ($p < 0.05$).

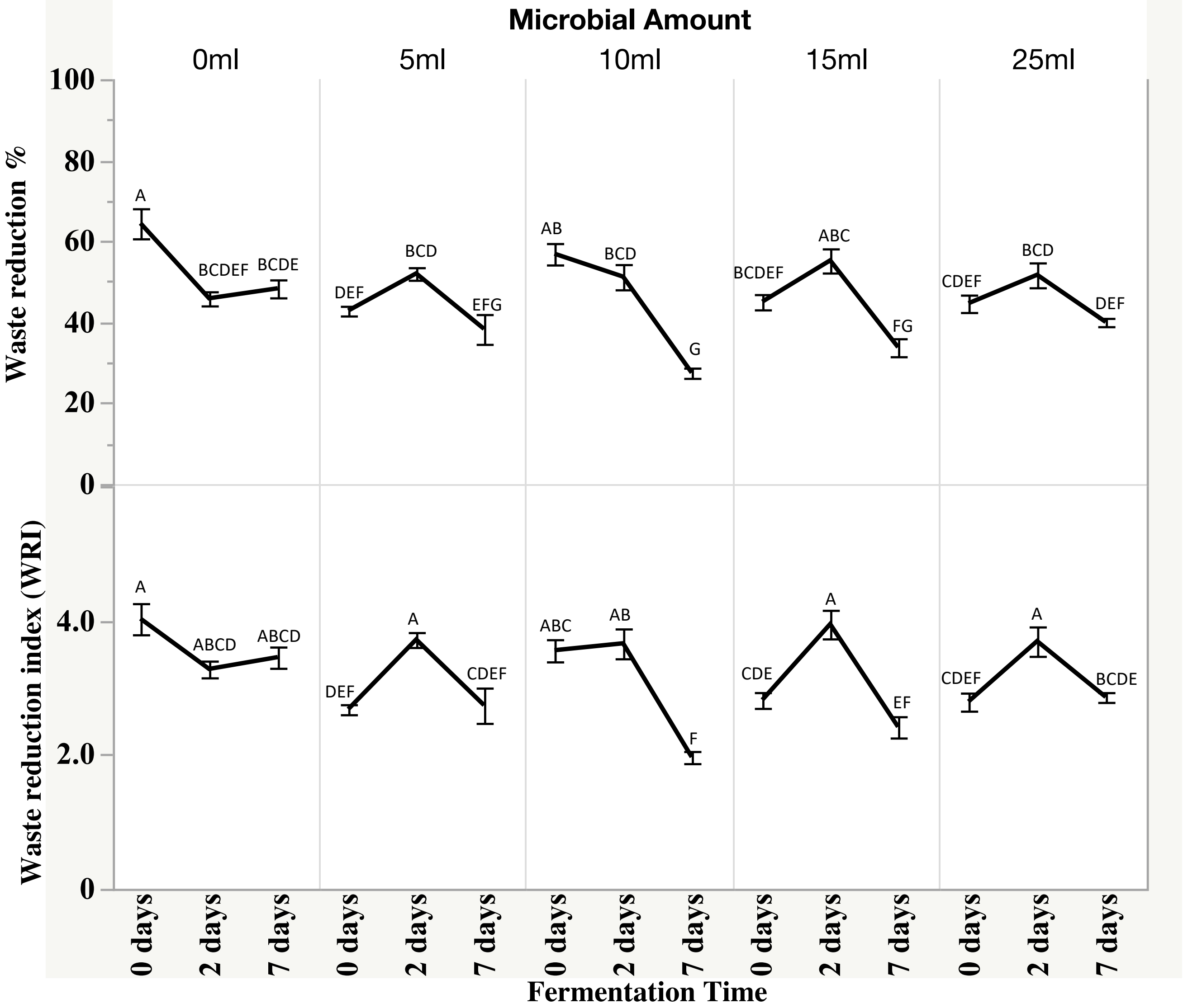

Figure 3.3. Mean (±SE) waste reduction percentage is calculated as the ratio of residue wet weight at the end of the experiment to food waste provided at the beginning and mean (±SE) waste reduction index calculated by dividing the waste reduction percentage by the larval development time of black soldier fly *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) reared on diets with different fermentation times and microbial amounts. Waste reduction ($F_{8,93} = 9.79$; df = 8; $p < 0.001$) and waste reduction index ($F_{8,93} = 9.63$; df = 8; $p < 0.001$) was affected by an interaction between fermentation time and microbial amounts. Means with the same letters indicate no difference in waste reduction and waste reduction index within and between fermentation times and across microbial amounts tested ($p < 0.05$).

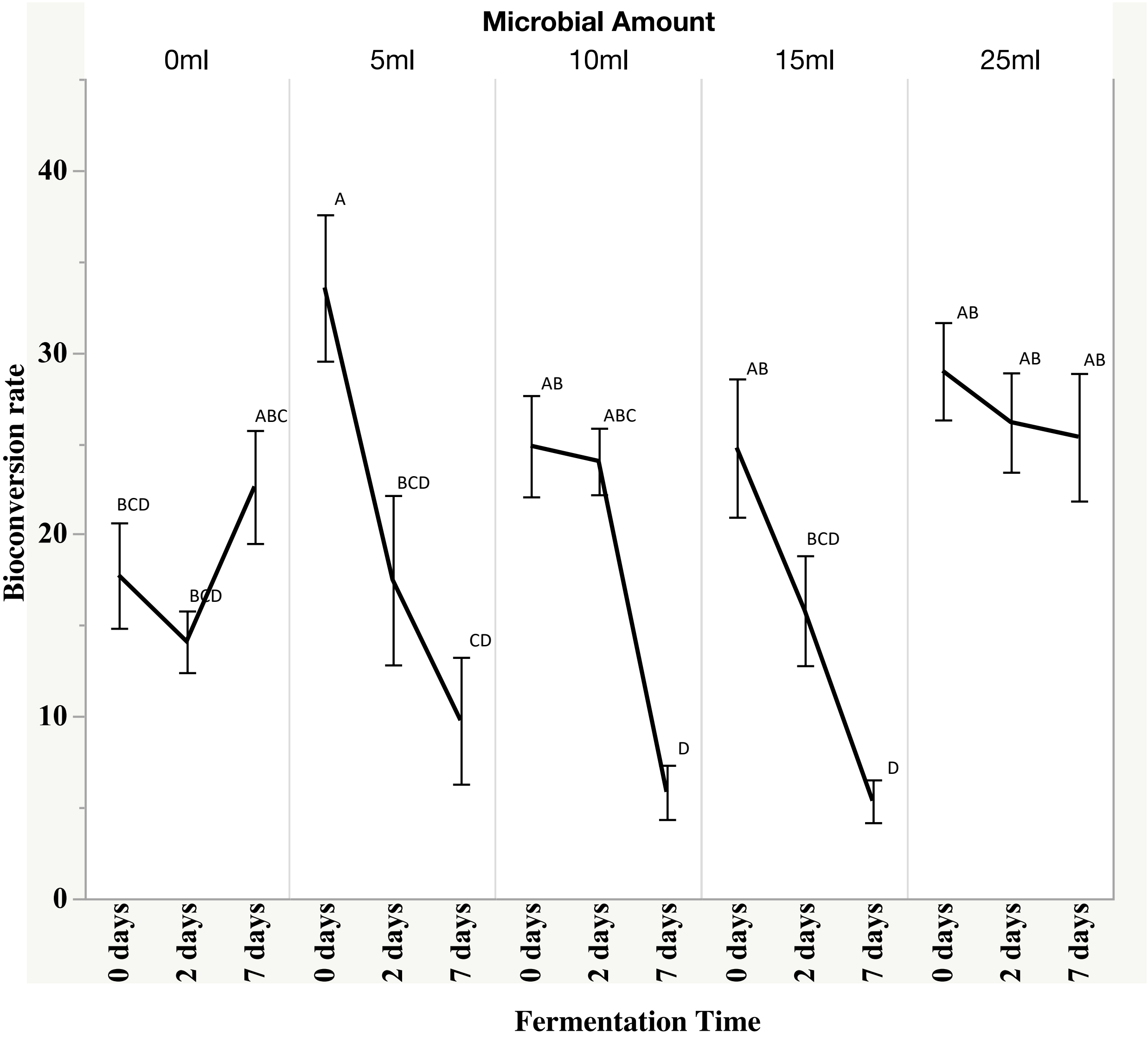

Figure 3.4. Mean (±SE) bioconversion rate calculated as the difference between the final larval fresh weight and the initial larval fresh weight at the beginning of the experiment divided by the ingested food and multiplied by the number of larvae at the end of the experiment, of black soldier fly *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) reared on diets with different fermentation times and microbial amounts. The bioconversion rate was affected by an interaction between fermentation time and microbial amounts ($F_{8,93} = 5.31$; df = 8; $p < 0.001$). Means with the same letters indicate no difference in bioconversion rate between fermentation times and across microbial amounts tested ($p < 0.05$).

Chapter 4

Using models to simplify quantification of black soldier fly (Diptera: Stratiomyidae) egg mass.

4. 1 Introduction

An alternative strategy for diverting waste away from landfills is valorization by insect farming. In waste management, the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), has gained traction due to its ability to reduce large amounts of food waste within a relatively short period during its larval stage (Diener et al., 2009, 2011; Newton et al., 1992.; Nguyen et al., 2013, 2015). Utilizing the black soldier fly in an industrial setting for waste management is currently being attempted in several countries, including Canada, the United States, and China. However, the waste conversion system still requires further optimization to scale up commercially. Type of diet (Nguyen et al., 2013), feed rate (Diener et al., 2009; Manurung et al., 2016; Parra Paz et al., 2015), larval density (Parra Paz et al., 2015), and abiotic conditions (Cheng et al., 2017; Holmes et al., 2012) are all factors that influence the waste reduction system using black soldier flies. All these factors can directly or indirectly affect the commercial use of the black soldier fly in waste reduction by changing fly development, which may alter adult reproductive success, thereby impacting the waste reduction capability and success of the waste conversion system, or by changing the egg hatching success, which may impact the establishment of a thriving colony that is necessary for the success of the bioconversion system.

Diet affects the black soldier fly by providing the nutrients necessary for larval growth, and depending on the nutritional component, can result in longer development (i.e., processing time) or reduced survival (i.e larval density available for waste reduction)(Nguyen et al., 2013). Larval survival varies greatly depending on the larval diet. For instance, on rice straw, black soldier fly larvae had a survival range of 51.2%-98.3% and a development range of 38-54 days, depending on the feeding rate (Manurung et al., 2016). In contrast, on cow manure, chicken manure, and pig manure, black soldier fly larvae had a survival rate of 87.8%, 82.2%, and 97%, respectively and a mean development times of 214.5 days, 144 days, and 144 days, respectively (Oonincx et al., 2015). Understanding how the larval diet might affect the development of the black soldier fly is the first step in bioconversion system optimization. Optimizing bioconversion will depend on finding a balance between the type of diet provided, the amount of diet provided, and the larval density present. The larval diet influences larval survival, thereby changing the overall larval density and the number of individuals available for the waste conversion process.

The second step in system optimization is determining a feeding rate for the black soldier fly. The optimum feeding rate should be the rate that provides a balance between larval weight and waste reduction efficiency. An optimum feeding rate of 100 mg/larva/day was established by Diener et al. (2009) when larvae were provided with chicken feed. In contrast, Manurung et al. (2016) found that larvae fed with rice straw at a rate of 12.5 mg/larva/day had the highest waste reduction efficiency, whereas larvae fed 200 mg/larva/day had the highest prepupal weight. Additionally, Parra Paz et al. (2015) found a feed rate of 163 mg/larva/day is optimum and produces 1.1 kg/m^2/day of compost and 59 g/m^2/day of larval biomass when insects ate vegetable waste. The variability in feeding rate between these studies may be due to different nutritional components contained in the diets. Knowing the initial larval density available for waste reduction helps determine the feed rate required for efficient waste conversion.

Abiotic factors such as temperature, relative humidity, moisture level, and pH could significantly influence the development, and thereby the colony establishment and waste reduction capability, of the black soldier. Research suggested an optimum temperature of 27°C resulted in the most efficient development for both males and females, and higher temperatures resulted in smaller adults and decreased longevity (Tomberlin et al., 2009). Higher developmental temperatures can affect the success and establishment of a successful black soldier fly colony. The amount of moisture in the waste can affect the conversion process as it affects particle size distribution and performance of the black soldier fly. High moisture content makes it challenging to separate processing residue from insect biomass (Cheng et al., 2017) when waste particles aggregate more closely together, making it difficult to separate them from the larvae (i.e., sieving efficiency). Yet, higher moisture content resulted in faster growth rates of black soldier fly (Cheng et al., 2017). This represents a trade-off between larval growth rate and sieving efficiency; maximizing larval growth could lead to difficulty separating the waste residue from the larvae. Relative humidity affects the egg eclosion and adult emergence, such that at low relative humidities, water loss through the egg and pupal membranes is deleterious and results in desiccation (Holmes et al., 2012). This desiccation results in death, affecting larval density and reducing the number of individuals available for waste conversion and establishing a successful colony. At low relative humidities egg desiccation is high, meaning fewer individuals hatch, and this reduces the larval density necessary for waste conversion. Optimizing the rearing conditions can ensure industry operators introduce the appropriate number of larvae to achieve a high waste reduction efficiency.

Waste pH is another parameter affecting the lifecycle, and therefore commercial establishment and the waste reduction of the black soldier fly. Waste pH above 6 and up to 10 was the most efficient for larval growth performance, with larvae within this pH range weighing more than larvae subjected to pH of 4 or 2 (Ma et al., 2018). The high alkalinity of the larval diet helps reduce volatile fatty acids and increase larval production (Ma et al., 2018). Ma et al. (2018) recommends a pH of 6 to 8 for the bioconversion process due to the survival rate of the larvae, since prepupa and prepupal weight were all significantly higher in feeding substrates within that range. Knowing the initial larval density and the high survival rate within this pH range means more efficient waste bioconversion. Industry operators will want to keep the pH range between 6 to 8 to ensure high larval survival and an efficient bioconversion.

Sub-optimal temperature, moisture level, pH, and relative humidity can affect the success of the waste conversion process by increasing the development time (i.e. waste conversion time), increasing larval mortality (i.e. the number of individuals available for waste conversion), mating success (i.e. ability to maintain a thriving colony) and overall success of the bioconversion system. Since all these factors can affect the larval density necessary for waste reduction and the success of a bioconversion system, it is important to keep them in optimal conditions during the waste conversion process. Another vital step in continuing optimization and the focus of this chapter is egg mass quantification. Optimizing waste conversion on a large scale requires knowing the number of eggs contained in a single egg deposition. It requires knowing the initial number of larvae available so the feed rate and environmental conditions can be optimized. This procedure can be tedious and time-consuming if done manually.

It is necessary to develop a methodology that can reliably predict the number of eggs laid in individual egg masses without affecting the integrity of the eggs. If achieved, it would significantly facilitate the study of life-history traits in this species and improve current methods applied in black soldier fly waste reduction efficiency. In laboratory settings, it is easy to count individuals needed for an experiment; however, in an industry setting, knowing the number of individuals contained in single egg masses would give quick estimates for insect farming and a potential reduction efficiency. However, egg-hatching success depends on the interaction between various abiotic variables such as humidity and temperature. For example, egg hatching success depends on relative humidity, such that eggs kept at 70% RH had 93% eclosion success compared to eggs maintained at a lower humidity of 25% RH, which had a 16% eclosion success (Holmes et al., 2012). With this in mind, developing an appropriate technique for reliably determining the number of eggs in a black soldier fly egg mass is essential. Hence, in this study, I utilize ImageJ (https://imagej.nih.gov/ij/), a license-free program capable of analyzing and accepting a wide range of image formats, to analyze and quantify the number of eggs within black soldier fly egg masses using egg mass volume and egg mass weight as a proxy. ImageJ has previously been used to quantify egg deposition in blow fly species (Diptera: Calliphoridae) (Rosati et al., 2015) and estimate reproductive rates in mosquitoes (Diptera: Culicidae) (Mains et al., 2008). Egg weight was used to determine the number of eggs with an egg mass; this was done for ease in industry settings where volumetric ImageJ analysis would be less feasible.

4.2 Methods

4.2.1 Insect source and egg collection

Black soldier fly colonies were established in 2018 and continuously maintained at the University of Windsor, Windsor, ON, Canada. Individuals were initially sourced from (wormlady.myshopify.com, Ontario, Canada) and maintained at approximately 26.7°C, and relative humidity of at least 20% was achieved using a water misting system set for 30-sec intervals twice per day. The misting system also provided drinkable water droplets for adult flies. Corrugated cardboard was provided as an oviposition site to obtain egg masses for quantification. The cardboard was taped to the side of a plastic container (50 cm x 25 cm x 20 cm), the plastic contained poultry feed (Purina Gold'N start & grow crumbles, 6040 - 20% crude protein, 3% fat, 5% natural fibre) saturated with water. The cardboard was changed every 24 h, and egg masses were collected to record weight measurements ($N = 37$) and volume ($N = 30$).

4.2.2 Egg volume

The method outlined below was adapted from (Rosati et al., 2015). A photograph of each egg mass was taken for egg volume using a Huawei P30 Pro camera lens at a 90° angle with a 15 cm photo evidence ruler (Fischer scientific). A depth measurement was taken at the deepest point. Within the ImageJ program, the photos were calibrated using the ruler in the image and the straight-line tool in the menu of the ImageJ program (Rosati et al., 2015). A 1 mm line was overlaid on the ruler in each photo using the ANALYZE>SET scale function to calibrate the image. The "freehand selections" tool was used to trace the egg mass, and the ANALYZE>MEASURE function was used to measure the surface area of the egg mass (Rosati et al., 2015). The surface area (mm^2) measurements were multiplied by the depth (mm) measurements for a specific egg mass and recorded. Once all measurements were taken, each egg mass was placed in a glass vial filled with 70% ethanol and sparkleen detergent to promote the separation of eggs within the group. This made the eggs more visible and easier to count afterward.

4.2.3 Egg weight

Egg masses used to determine egg numbers were weighed within 6 h of collection. Each egg mass was weighed using a digital scale (American weigh scales: Gemini 20, amazon.ca). Once all measurements were taken, each egg mass was placed in a glass vial filled with 70% ethanol and sparkleen detergent to promote the separation of eggs within the group. All eggs from the egg masses collected to measure volume and weight were manually counted using a Meiji EMZ zoom stereomicroscope. The ethanol solution was drained from the vials and the eggs were carefully placed in a petri dish using a paint brush. The dish was divided into grids to facilitate visibility and egg counting.

4.3 Statistical analyses

All analyses were completed in JMP (version 16.1.0). Normality was tested using the Shapiro-Wilk test. Homogeneity of variance was assessed using Levene's test. A simple linear regression was used to evaluate the effect of egg mass volume (V) on egg number (N) using the model: $N \propto V$. Additionally, the effect of egg weight (W) on egg number (N) was evaluated with a simple linear regression using the model: $N \propto W$. The slope and intercept parameters from the regression analysis were used to determine predictive equations. All P-values were compared with $\alpha = 0.05$.

4.4 Results

4.4.1 Model

Females laid eggs in clumps that took on the shape of the corrugated cardboard used to collect the egg masses. The mean (±SE) for the number of eggs per egg mass for volume and weight was 842 ± 83 and 694 ± 76, respectively. Egg mass numbers ranged from 178 to 1746 for egg volume and 72 to 2081 for egg weight. Egg mass volume ranged from 32.44 mm^3 to 160.05 mm^3, while egg mass weight ranged from 0.001 g to 0.048 g. The mean (± SE) egg mass volume was 90.75 mm^3 ±7.88 mm^3, and the mean (± SE) mass weight was 0.0154 g ± 0.0018 g. The linear models were significant and linearly correlated for egg mass weight ($F_{1,36} = 437.9$; $P < 0.0001$) and egg mass volume ($F_{1,29} = 386.0$; $P < 0.0001$) as significant predictors of egg number. The volume model, $N \propto V$ is acceptable based on the results of the regression analysis where egg number is directly proportional to egg mass volume and increases as egg mass volume increases. In the same way, in the weight model, $N \propto W$ is acceptable and egg number is directly proportional to egg mass weight and increases as egg mass weight increases. The results from the model show that both egg mass weight ($R^2 = 0.924$) and egg mass volume ($R^2 = 0.928$) are strong predictors of egg number in female black soldier flies. The parameter estimates generated by the model are provided in Tables 4.1 and 4.2. Separate equations were generated for egg mass weight (Figure. 4.1), and egg mass volume (Figure. 4.2).

4.4.2 Validation

Egg masses from the colonies (N = 17) were randomly sampled to observe if they fell within the estimated range based on the models. For egg volume, the mean ± SE for egg number was 739 ± 100, ranging from 61 to 1590 eggs, with the mean ± SE volume of 96.28 mm^3 ± 11.03 mm^3. The egg masses randomly sampled from the population were plotted against the 99% confidence interval and were observed to fall within the established range of the model. All 17 egg masses sampled fell within the estimated range. For egg weight, the mean ± SE for egg number was 1255 ±144, ranging from 513 to 2542 eggs, with the mean ± SE weight of 0.0211 g ± 0.0022 g. The egg masses sampled from the population were plotted against the 99% confidence interval, and 16 out of 17 egg masses fell within the estimated range of the model. Figures 4.3 and 4.4 illustrate the validation of the model using the regression equations for egg mass weight and egg mass volume, with 99% confidence intervals for the individual range of egg mass weights and egg mass volumes used to calculate the regression equations.

4.5 Discussion

The black soldier fly is a strong candidate for waste reduction. It can feed on a wide variety of organic matter, including fruits and vegetables, kitchen waste, municipal waste and manure (Diener et al., 2011b; Nguyen et al., 2015; Sheppard et al., 1994). It can reduce manure by more than 50%, and at the same time, the larvae and pupae can be used as valuable feed for chicken, swine, and fish (Barragan-Fonseca et al., 2017; St-Hilaire et al., 2007). The prepupae contains 37 % to 63% protein and 7% to 39% fat, including fatty acids and essential amino acids (Barragan-Fonseca et al., 2017). Different studies have investigated different factors that can affect the waste conversion ability of the black soldier fly (Diener et al., 2009; Manurung et al., 2016; Parra Paz et al., 2015; Sheppard et al., 2002).

One of the major obstacles associated with large-scale waste reduction is optimizing the system load capacity using larval densities introduced via egg masses. To do this, industry operators must have an estimate of the number of individuals available for waste reduction. Manually counting eggs requires a lot of time (> 30 mins/egg mass) and effort (Mains et al., 2008; Rosati et al., 2015), especially when compared to the methods described here (< 2 min/egg mass) (Personal observation). Additionally, there is greater consistency and reduced human error and bias (Mains et al., 2008). This study quantified the number of eggs in the egg mass of the black soldier fly by applying ImageJ to egg mass pictures and using egg weight. Using the method described here, multiple egg masses of over 2000 eggs can be estimated in less than 2 minutes, whereas counting could take up to 1 hour (T. Tran & C demers, personal communication). While other physical measurements such as wing length or tibia and thorax lengths can be used to estimate female fecundity prior to egg deposition (VanLaerhoven & Stephen, 2003), these methods can be time-consuming and only give an estimate of the potential fecundity and not the realized fecundity. Additionally, these methods do not give industry operators a quantifiable amount. Realized fecundity is defined as the actual number of eggs oviposited and can differ significantly from the potential fecundity (Tisdale & Sappington, 2001). This study is the first to use ImageJ and egg weight to quantify egg number in the female black soldier fly. Female black soldier flies lay in clumps, and egg quantification is difficult without destroying the eggs. The mean individual egg mass weight determined by (Booth & Sheppard, 1984) was 0.0291 g from a mass with 998 eggs. This is in line with the results of our study, as we had egg mass weights of 0.025 g for masses of 1010 eggs. The egg mass number of black soldier fly egg masses range from 603 to 689 reported by Sheppard et al. (2002.), 206 to 639 by Tomberlin et al. (2002), 546 to 1505 by Booth & Sheppard (1984) and 61 to 2542 reported by this study. The egg numbers reported in the current study are greater than the number reported by previous studies (Booth & Sheppard, 1984; Sheppard et al., 2002; Tomberlin et al., 2002). However, this discrepancy could be due to differences in egg collection methods, female fecundity, or numerous females ovipositing and forming an egg mass.

Female fecundity is a major parameter in population dynamics, and the black soldier fly is semelparous (i.e., it produces offspring only once during its lifetime). Factors such as larval diet, adult size, and temperature are all factors that affect female fecundity. For example, adult *Aedes aegypti* Meigen (Diptera: Culicidae) females that were sugar-fed and then fed a blood meal weighed more and laid more eggs than females that were starved before their blood meal (Nayar & Sauerman, 1975). Understanding the different roles that abiotic factors can play in fecundity is important for a successful waste conversion system and a successful colony. The success of the colony depends on the high fecundity of females.

Furthermore, the method described here prevents the destruction of the egg masses and allows for further examination and analysis in a laboratory setting. Female fecundity research on the black soldier fly is sparse but on the rise, and a model like this could help in egg mass estimation for these studies. Studying the fecundity of the black soldier fly is necessary mainly to aid in the understanding of factors that can benefit or hinder the scaling up of a waste bioconversion facility using black soldier fly larvae. One study on the fecundity of the black soldier fly observed that female fecundity is significantly affected by temperature, especially at lower 15°C and upper 37°C temperatures; the highest fecundity was observed at 30°C (Chia et al., 2018). This trend has been documented in other insect species where increasing developmental temperatures decrease fecundity (Mehrparvar & Hatami, 2007). Studies such as these (i.e., investigating fecundity in black soldier flies) would benefit significantly from a model that predicts egg number based on egg mass volume or weight, as this significantly cuts down the time spent manually counting eggs needed for experiments or determining realized fecundity.

In an industry setting where the focus is waste conversion, establishing a model that allows egg numbers to be estimated by weight saves time and labour costs. These results of this study show strong validation of the model. A mass of approximately 2500 eggs weighs 0.053 g and takes >1 hour to count. This is a relatively small weight compared to what could be produced in a large-scale industrial setting. An egg mass of 0.5 g is 10 times the size of 0.053 g and could potentially take 10 times more time to count, and this does not account for the destruction of egg mass prior to counting. The methods described utilize free software, ImageJ and non-expensive equipment, and both are appropriate for estimating egg numbers across a broad range of egg mass weights and egg mass volumes. This makes ImageJ a valuable tool for research and digital analysis (Abràmoff et al., 2004; Mains et al., 2008; Rosati et al., 2015). This present study provides an industry scalable tool for black soldier mass rearing and waste bioconversion.

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Table 4.1. Parameter estimates generated using ANOVA to determine if egg weight can be used to predict the number of eggs in an egg mass

Parameter	Estimate	SE	*t*-value	*P*
Intercept	83.155137	36.22583	2.30	0.0276
Weight	39652.115	1894.916	20.93	<0.0001

The overall ANOVA model was significant ($F_{1,36} = 437.8774$; $P < 0.0001$)

Table 4.2. Parameter estimates generated using ANOVA to determine if egg volume can be used to predict the number of eggs in an egg mass

Parameter	Estimate	SE	*t*-value	*P*
Intercept	-84.63114	52.28189	-1.62	0.1163
Volume	10.215471	0.520157	19.64	<0.0001

The overall ANOVA model was significant ($F_{1,29} = 385.6980$ $P < 0.0001$)

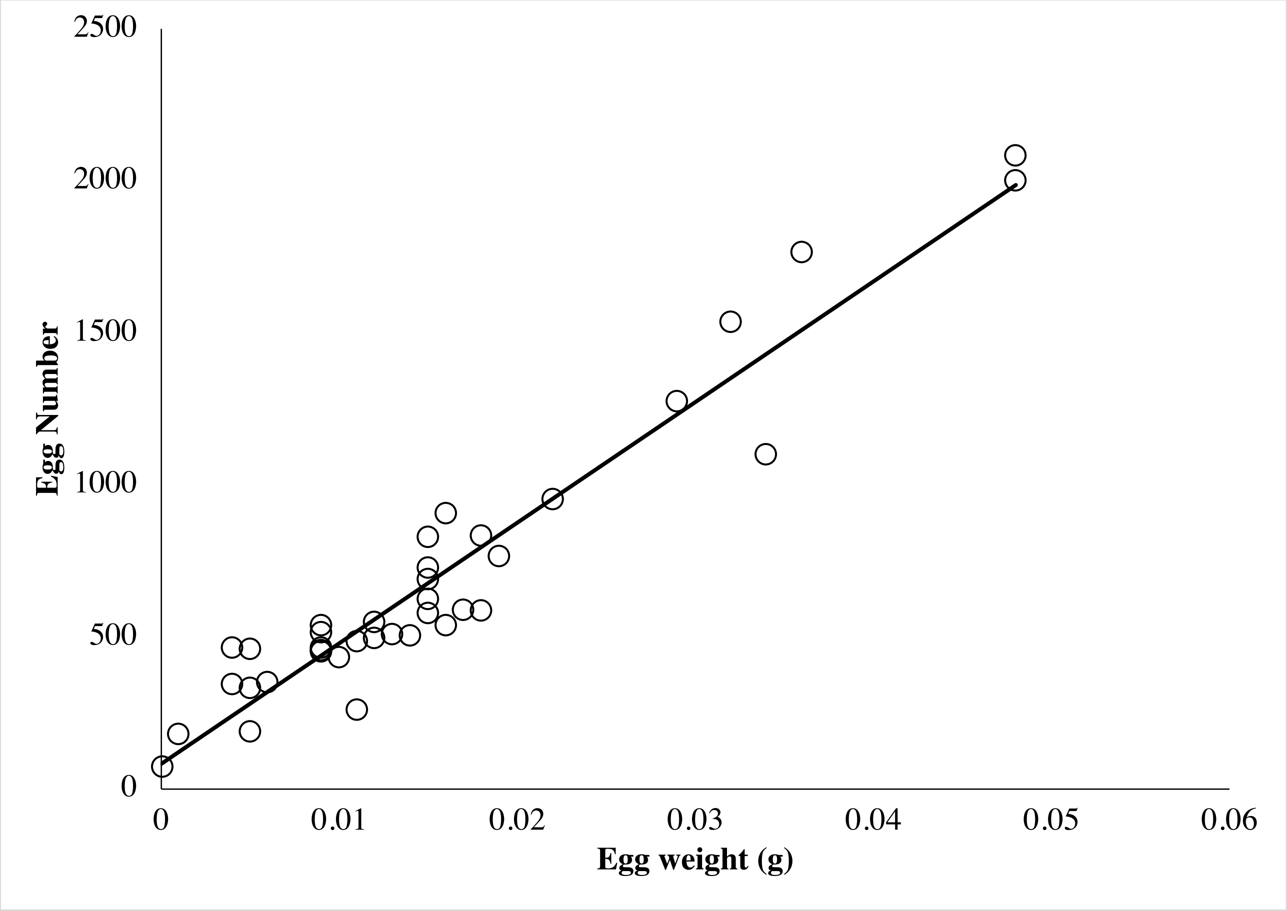

Figure 4.1. Simple linear regression equations to predict the number of eggs in an egg mass using the weight (g) of an egg mass for the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). ($y = 83.16 + 39652x$, $R^2 = 0.924$).

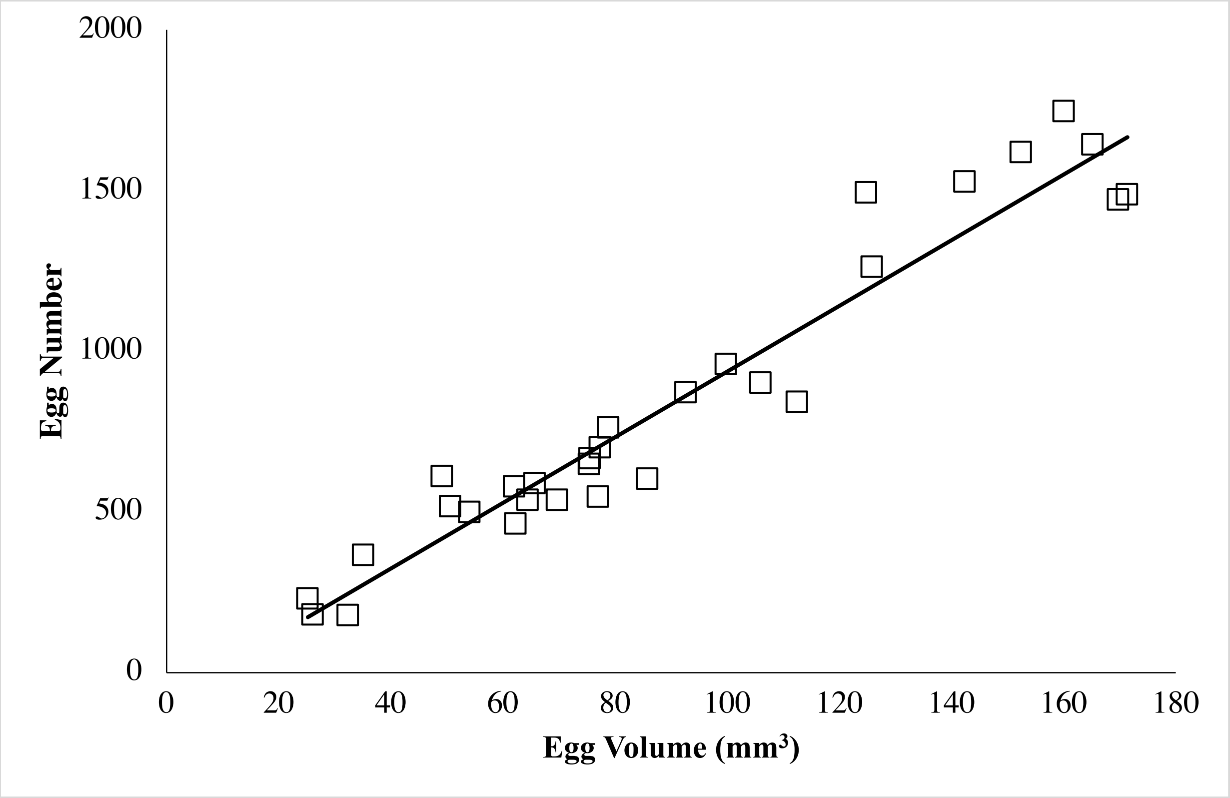

Figure 4.2. Simple linear regression equations to predict the number of eggs in an egg mass using the egg mass volume (mm^3) for the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). ($y = -84.63 + 10.22x$, $R^2 = 0.928$).

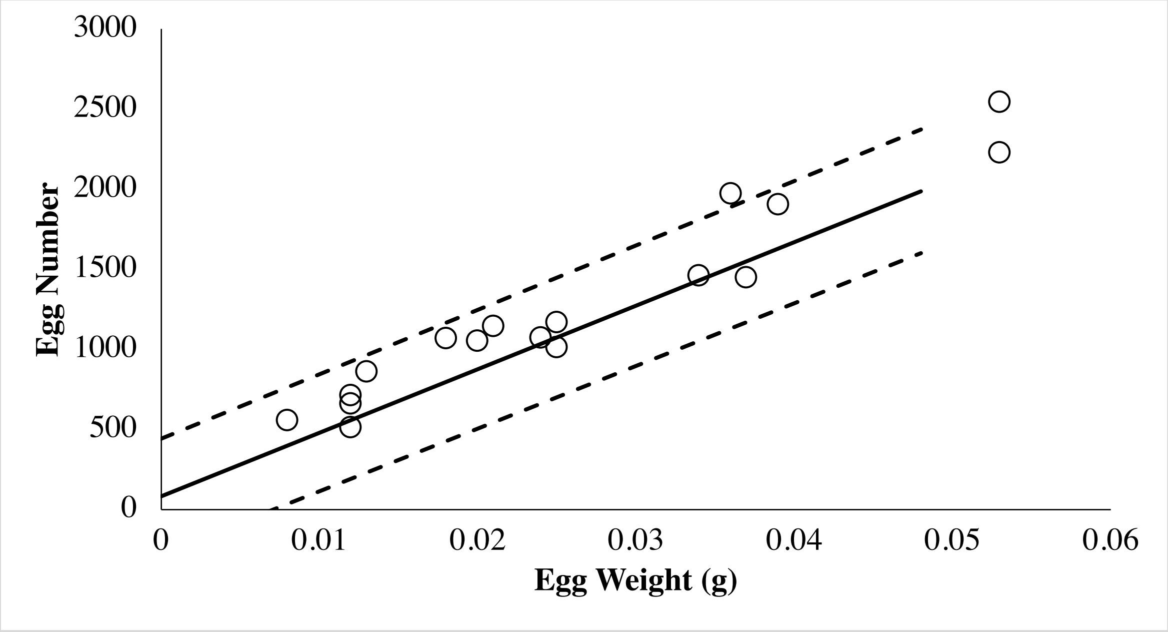

Figure. 4.3. Egg mass weight model validation with a 99% confidence interval for the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). Intervals were calculated using the linear regression equation ($y = 83.16 + 39652x$, $R^2 = 0.924$). The confidence interval was calculated for individual values used to establish the regression equation, and egg masses (N= 17) from the population were plotted to observe if they fell within the established range of the model.

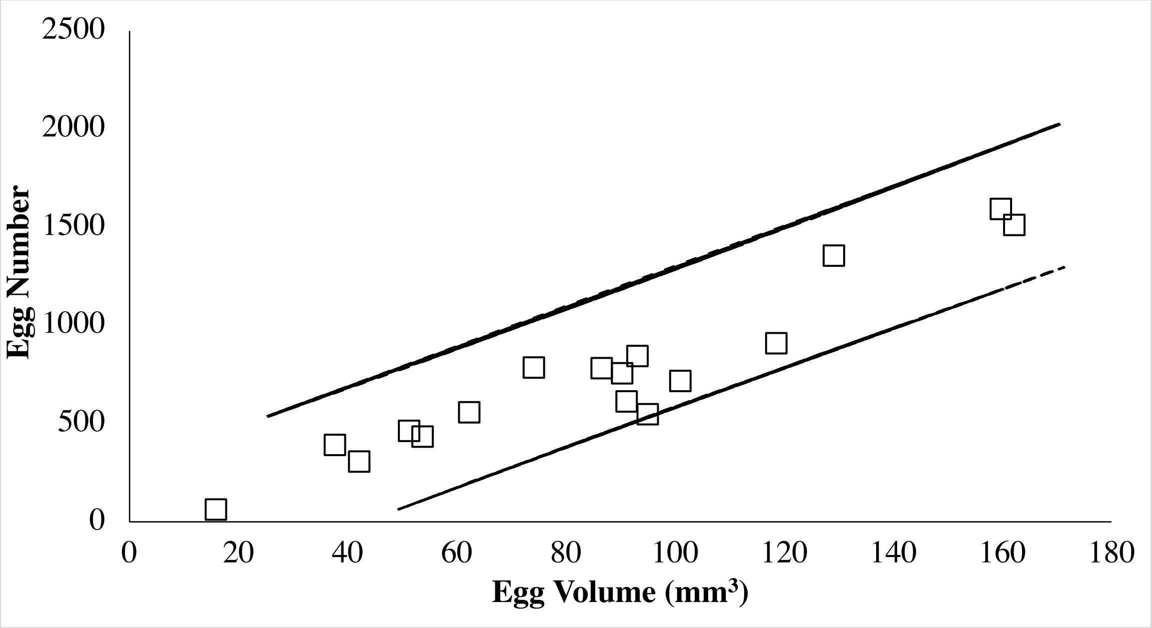

Figure 4.4. Egg mass volume model validation with a 99% confidence interval for the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). Intervals were calculated using the linear regression equation ($y = -84.63 + 10.22x$, $R^2 = 0.928$). The confidence interval was calculated for individual values used to establish the regression equation, and egg masses (N= 17) from the population were plotted to observe if they fell within the established range of the model.

Chapter 5

Is the post composting residue of the Black soldier fly a suitable soil amendment for greenhouse vegetables: A case study on cherry tomatoes.

5.1 Introduction

One of the most critical challenges for agriculture is sustainable food production for a continuously growing population (Tilman et al., 2011). The growing population has put increasing pressure on natural resources, and as a result, new ways of farming sustainably are emerging. Conventional soil-based agriculture has drawbacks such as large land requirements, extensive use of fertilizers and water, and soil degradation (Killebrew & Wolff, 2014; Lambin et al., 2013; Pradhan & Deo, 2019). Hence, the use of soilless systems is rising in popularity due to the increase in world population, the reduction in land available for soil-based farming, and the high demand for large quantities of food (Pradhan and Deo, 2019). Soilless agriculture is a promising method for improving the growing of cash crops due to its advantages: it saves over 85% of irrigation water, has almost low environmental pollution, can be used in areas unfavourable for ordinary farming, and can be used for farming year-round. Soilless mediums require fertilizer for plant nutrition as most crops are grown on inert media such as coconut fiber, rock pieces, rockwool, perlite, vermiculite, and others (Pradhan & Deo, 2019; Setti et al., 2019). Most soilless systems cannot ensure proper plant growth and yield because they contain insufficient nutrients. Hence, plants are fed with nutrient solutions containing the minerals and nutrients required for growth and yield. These nutrients include nitrogen, phosphorus, potassium, magnesium, zinc, calcium, iron, copper, manganese, and others (Pradhan & Deo, 2019). Crucial parameters to soilless farming include these nutrient solution components which play a big part in the growth and yield of the plant, and must be managed appropriately. The pH level of the nutrient solution should remain between 5.8 and 6.5, and finally, the electrical conductivity of the nutrient solution should stay with ideal concentrations of 1.5 and 2.5 dS/m (Pradhan & Deo, 2019).

Due to the inert nature of the growing mediums used, soilless farming relies extensively on mineral fertilizers, leading to an increase in nutrient-rich wastewater contributing to eutrophication (Arcas-Pilz et al., 2021; Sanjuan-Delmás et al., 2018). The addition of nutrients to soilless systems can be costly due to the production and extraction process, such as the high energetic cost of nitrogen production and depleting sources of phosphate rock for phosphorus extraction (Rufí-Salís et al., 2020; Cherkasov et al., 2015). These issues necessitate the identification of alternative fertilizers that improve plant growth while supporting sustainable agriculture within the framework of a circular economy. A circular economy is a zero-waste model that encourages the reuse, recycling, and reintegration of outputs back into the system (Romero-Hernández & Romero, 2018). Studies have evaluated municipal solid waste (Herrera et al., 2008), food substrates (Gao et al., 2015), and manure (Bustamante et al., 2008) as alternative fertilizers to improve plant yield. Most studies suggest that alternative fertilizers improve plant growth and align with sustainable farming.

The black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae), is rising in popularity due to its ability to quickly reduce large amounts of food waste, municipal solid waste, sewage sludge, feces, manure, and coffee pulp (Banks et al., 2014; Diener et al., 2009, 2011; Lardé, 1990; Nguyen et al., 2015) Additionally, its larvae can be utilized as a protein substitute for fish, poultry, and pigs (Maurer et al., 2016; Schiavone et al., 2017; Spranghers et al., 2018; Wang & Shelomi, 2017). Depending on the fish species, black soldier fly larvae can substitute up to 100% of the fish diet (Belghit et al., 2019; St-Hilaire et al., 2007). Insect production leaves a small ecological footprint, and black soldier fly prepupae are good sources of minerals such as calcium, iron, potassium, magnesium, phosphorus and zinc, as well as vitamins such as B12, thiamine, and riboflavin, and essential amino acids such as lysine, isoleucine, threonine, valine, and methionine (Spranghers et al., 2017).

The second by-product of black soldier fly farming is the black soldier fly processing residue (BSFPR), consisting of insect frass, chitin, and waste residue (Gärttling et al., 2020). Together, these have the potential to be an effective soil amendment (Gärttling et al., 2020; Setti et al., 2019), but this requires more study to assess effects on plant growth, root health, nutrient composition, and microbial activity, particularly in soilless/ hydroponic systems. It is particularly important to evaluate the fertilizing potential of insect frass. As the insect production industry continues to grow, frass will become a widely available by-product of insect farming. It currently represents approximately 30% of the total output of an insect production system (Gärttling et al., 2020). Yet the purchase of fertilizer represents one of the most expensive crop inputs, particularly since one of the most important challenges for agriculture is to provide sustainable food while satisfying the increasing population of consumers (Brunelle et al., 2015). This daunting prospect presents concerns for agricultural sustainability given current climate impacts and soil management practices (Brunelle et al., 2015; Ray et al., 2013). These concerns about sustainable agriculture suggest the need to discover new novel technologies to increase crop production.

However, research on BSFPR as a fertilizer has provided varying results. Setti et al. (2019b) observed better growth rates for baby leaf lettuce, basil, and tomatoes when peat growth media was supplemented with 10-20% BSFPR. Additionally, improved crop yield of chilli peppers and shallots was observed in field-scale experiments when the soil was amended with black soldier fly frass collected from brewery wastes (Quilliam et al., 2020). Quilliam et al. (2020) also observed improved plant health due to the presence of chitin in the BSFPR. Sudan grass and basil grew well on BSFPR from pig slurry (Newton et al. 2005). However, Choi et al., (2009) compared BSFPR to commercial fertilizer and observed no differences in growth on cabbage plots. Additionally, high nitrogen, phosphorus and potassium (NPK) values were observed in BSFPR in comparison to organic poultry fertilizer and worm castings, however high BSFPR application rates to crops, such as lettuce, bok choi, potatoes and beans, decreased profitable yields (Temple et al., 2013).

Black soldier fly processing residue presents an opportunity to participate in the circular economy model and while the benefits of BSFPR are emerging, the results are variable and require more research. The goal of the current study was to compare cherry tomato plant growth on three levels of BSFPR to that grown with slow-release commercial fertilizer in a hydroponic coconut coir growth matrix within rhizoboxes. In this study, coconut coir was amended with 10, 20, or 30% BSFPR compared to coconut coir with commercial fertilizer or coconut coir on its own. As previous research has suggested BSFPR is comparable to commercial fertilizers, it is expected that plant growth parameters such as time to flowering, root system weight, shoot weight, and plant height will be similar in commercial fertilizer and BSFPR treatments.

5.2 Methods

5.2.1 Colony maintenance and Residue production

The compost residue used for these experiments was derived from laboratory colonies of *Hermetia illucens* (Linnaeus) established in 2018 and maintained at the University of Windsor, Windsor, Ontario, Canada. The colony was supplemented with commercially acquired larvae yearly (wormlady.myshopify.com, Ontario, Canada). Adults were held in a black mesh (1.5 mm) cage constructed with polyvinyl chloride (PVC) pipes (1.8 m x 1.8 m x 1.8 m). The enclosure was provided with golden pothos plants as a lekking site for males (Tomberlin and Sheppard 2001). Lighting was provided using 150-W high-pressure LED lights (Model: BSF-4C-200-3030, Eco Conversion Systems LLC, Texas, USA) to maintain a photoperiod of 16:8 (L:D) cycle. Colonies were maintained at approximately 26.7°C ±0.9 and relative humidity (RH) of at least >20%. A water misting system (set for 30-sec intervals twice a day) maintained humidity in the enclosure and provided water droplets for adult consumption. An oviposition site was provided by using corrugated cardboard taped to the side of a Tupperware container (24.43cm x 16.81cm x 8.55cm) (Snaptite, ID: 10-1001012). The Tupperware was filled with poultry feed saturated with water, which served as an oviposition attractant (Purina Gold'N start & grow crumbles, product number: 6040, Mississauga, Ontario). Females were allowed to oviposit for 24 h, and then the Tupperware container with the corrugated cardboard now filled with eggs is moved into a growth chamber set at 27°C, 70% RH, and 16L: 8D and monitored daily until eclosion. After eclosion, larvae were transferred into a Rubbermaid bin (82 cm x 51.8 cm x 42.4 cm) and fed food waste *ad libitum* until they reached the prepupae stage at which point they stopped feeding. After feeding ceased, feeding bins were moved into the colony cages for adult emergence. After the adult emerged, the leftover residue and pupal casings were stored at room temperature 19°C-21°C in Rubbermaid bins (82 cm x 51.8 cm x 42.4 cm) until ready for use.

5.2.2 Experimental design

To assess the suitability of the BSFPR as an amendment, five different fertilizer treatments were composed, all with coconut coir as the growth media, as follows: 1) (CF) Commercial fertilizer with 20-5-10 slow-release solid fertilizer (Nutricote 20-5-10 type 100, Plantproducts.ca, using 140 g/box); 2) (BSF10) 10% BSFPR using 0.25 kg/box; 3) (BSF20) 20% BSFPR using 0.45 kg/box; 4) (BSF30) 30% BSFPR using 0.7 kg/box; and 5) (CC) 100% Coconut coir with no fertilizer added. These ratios were chosen based on previous studies that have investigated BSFPR as an amendment (Setti et al, 2019). The slow-release fertilizer was used to mimic the one-time addition of BSFPR to the growth media. Coconut coir (CANNA Coco, www.canna.ca) is manufactured from coconuts grown in Sri Lanka, is 100% organic product with a homogenous structure and without chemical additives. In the CF treatment, the slow-release solid fertilizer was added once before sowing to mimic the single application of BSFPR in the other treatments. The black soldier fly processing residue (BSFPR) was sent for analysis to the Agriculture and Food Laboratory (AFL) in Guelph ON and the total nitrogen, phosphorus and potassium content was measured. These are summarized in Table 5.1. All growth media containing BSFPR showed a neutral pH range of 7.3 – 7.5 with a cation exchange capacity (CEC) (meq/100g) range of 25.4 – 47.9. CF had an acidic pH (5.7) with an CEC (meq/100g) of 16.4, while CC had a slightly acidic pH (6.2) with a CEC (meq/100g) of 12.7. The total chemical composition of BSFPR is comparable to other studies by other authors (Setti et al., 2019).

The experiment was carried out in a room with a constant temperature of 25ºC, relative humidity ranging from 50% - 60%, and a photoperiod with 16 h of light (intensity 400-500 umole/m^2/ s) and 8 h dark. Tomato (*Solanum lycopersicum* L., var. *cerasiforme* (cherry falls)) were sown manually with 3 seeds per rhizobox. After germination and the appearance of true leaves the rhizoxes were pruned, and the two weakest plants were removed leaving only one plant per Rhizobox. After three weeks, plants that did not germinate were resown. Rhizoboxes measured 71 x 41 x 1.3 cm and were filled with the different growth media. Each rhizobox was placed on a metal stand and arranged in a completely randomized design with six replicates.

5.3 Statistical analyses

All analyses were completed in JMP (version 16.1.0). Normality was tested using the Shapiro-Wilk test. Homogeneity of variance was tested using Levene's test. All variables did not meet the assumptions of normality and were analyzed using the Kruskal wallis test. The effect of growing medium on tomato (*Solanum lycopersicum* L., var. *cerasiforme* ('Cherry Falls')) leaf area, root depth, shoot dry weight, root dry weight, number of compound leaves, number of flowering trusses, shoot length and root to shoot ratio were analyzed using the Kruskal-Wallis test. There was no germination at the two highest BSFPR treatments (BSF20 and BSF30) and hence they were excluded from the analysis.

5.4 Results

Effects of fertilizer type on crop production

Type of fertilizer influenced leaf area ($X^2 = 26.7898$; df = 2; $p < 0.001$). The leaf area was higher with black soldier fly processing residue (BSF10) than commercial slow-release fertilizer, or in the absence of fertilizer (Figure 5.1a). The number of compound leaves present was influenced by the fertilizer type ($X^2 = 11.3353$; df = 2; $p = 0.0035$), with the highest for tomato plants grown in BSF10, twice as many as compared to commercial fertilizer, or absence of fertilizer (Figure 5.1b). The number of flowering trusses was also influenced by fertilizer type ($X^2 = 11.3353$; df = 2; $p = 0.0035$), again with the greatest number on tomato plants grown in BSF10 compared to commercial fertilizer or absence of fertilizer (Figure 5.1c). Additionally, the time to first flowering truss was influenced by fertilizer type ($X^2 = 5.7431$; df = 1; $p = 0.0165$), the earliest flowering truss was observed on tomato plants grown in BSF10 compared to commercial fertilizer (Figure 5.1d). No true leaves were observed in the absence of fertilizer and hence was excluded from this analysis.

Shoot dry weight ($X^2 = 10.8$; df = 2; $p = 0.045$) and shoot length ($X^2 = 10.6813$; df = 2; $p = 0.0048$) were both affected by fertilizer type, with the greatest weight and length for tomatoes grown in BSF10 compared to commercial fertilizer or no fertilizer (Figure 5.2a-b). In contrast, shoot-to-root ratio was also impacted by fertilizer type ($X^2 = 10.2149$; df = 2; $p = 0.0061$), but only in the sense that the lack of fertilizer resulted in the greatest ratio, with no difference between tomato plants grown on BSF10 or commercial fertilizer (Figure 5.2c).

Root dry weight ($X^2 = 10.7107$; df = 2; $p = 0.0017$) and root depth ($X^2 = 8.189$; df = 2; $p = 0.0167$) were impacted by fertilizer type with greater weight and deeper roots for tomato plants grown in BSF10 than commercial fertilizer or in the absence of fertilizer (Figure 5.3a-b). In actuality, tomato plants grown on commercial fertilizer or without fertilizer did not differ in root dry weight or root depth. The root system architecture (RSA) was more extensive in tomato plants grown in BSF10, and root density was higher compared to commercial fertilizer or in the absence of fertilizer (Figure 5.4).

5.5 Discussion

One of the most critical challenges for agriculture is sustainable food production that satisfies the needs of the consumers. Greenhouses have improved their production of crops in and out of season due to the rising populations using soilless systems. Soilless systems are a method of growing crops without soil (Putra & Yuliando, 2015). Soilless systems are used to avoid fluctuations in the water and nutrient in the soil. Soilless systems typically use inorganic growth media such as rockwool, sand, perlite, vermiculite, pumice, or coco coir (Setti et al., 2019). The use of these growth media alone is not sufficient to ensure the good growth of crops because they do not supply enough nutrients. Hence, fertilizers are dissolved in the irrigation water, and the solution is known as the “nutrient solution.” Soilless systems rely heavily on mineral fertilizer which adds to nitrates and phosphate discharged into the wastewater (Sanjuan-Delmás et al., 2018). The widespread use of fertilizers is also unsustainable due to the high costs associated with production and extraction, for example in nitrogen fertilizers and phosphorus rock extraction (Arcas-Pilz et al., 2021). Soilless systems rely entirely on these nutrients, making them unsustainable in the long run. These concerns require researchers to identify and assess new growth media capable of increasing crop yields while preserving agricultural sustainability. Many strategies have been described in recent years, and studies have evaluated the use of composted residues (sewage sludge, livestock manure) as alternatives that embrace a circular economy framework. However, some of these alternatives result in lower crop yield. Hence our study evaluated the potential for black soldier fly processing residue (BSFPR) as a fertilizer, compared to a conventional slow-release fertilizer.

A major component of insect processing residue is insect exuviae which are made up of chitin, which is a high molecular weight polysaccharide also present in fungal cell walls and the exoskeleton of many crustaceans (Barragán-Fonseca et al., 2022; Roer et al., 2015). In our study, growth media containing as little as 10% BSFPR resulted in larger plant biomass when compared to growth media with control-release fertilizer. The addition of insect residue to soil has been shown to provide nutrients to plants and lead to an increase in biomass and other nutritional contents (Poveda, 2021). Some studies suggest that the nutrients present in insect residue can be easily assimilated by plant roots (Poveda, 2021). The addition of both chitin and insect frass as amendments may impact the microbiome of the soil, which may be an essential factor in promoting plant growth (Barragán-Fonseca et al., 2022).

Our studies show that cherry tomatoes successfully germinate and develop to the flowering stage when grown in growth media containing BSFPR however, this depends on the percent composition of BSFPR in the growth media. Seedlings sown in growth media with greater than 10% BSFPR did not germinate. This result is consistent with other studies showing stunted growth or no growth at high BSFPR concentrations (Newton et al., 2005; Setti et al., 2019). This result could be due to nutrient stress (high nutrient concentrations) and salt stress. The salt and overall nutrient concentrations in 20% BSFPR and 30% BSFPR were higher, and studies have reported stunted plant growth and lack of germination when plants were under nutrient stress such as ammonia toxicity (Saunkaew et al., 2011). At high concentrations, plants had shorter and fewer roots, suppressed shoot production, and had a low shoot-to-root ratio (Saunkaew et al., 2011).

Salinity is a severe concern when soluble salts occur in excessive concentrations, and it imposes osmotic and ionic stress on plants which can lead to morphological and physiological changes (S Puvanitha & Sivaguru, 2017). When soluble salt concentrations are high, it leads to a decrease in the development of the xylem, a reduction in the fresh and dry weights of the shoot system, and a decrease in the dry matter content (S Puvanitha & Sivaguru, 2017; Taffouo et al., 2010). Studies have also shown that potassium (K^+) uptake and water content values are negatively affected by salinity, i.e., increased salinity results in lower water content and reduced potassium (K^+) uptake (Taffouo et al., 2010). Salinity is responsible for decreasing the economic yield and productivity of crops, it reduces the availability of water to the roots due to osmotic stress and affects the absorption of other minerals leading to suppressed growth (Taffouo et al., 2010). It is possible that due to the high soluble salts present in 20% BSFPR and 30% BSFPR the nutrients available to the seeds were restricted, and the seeds had to spend more energy to sustain themselves. In addition, osmotic stress on the seeds due to salinity may have caused water to move out of the seeds into the surrounding soilless culture resulting in the lack of germination.

In the present study, plants grown on 10% BSFPR had significantly larger root biomass (deeper roots and heavier roots) than plants grown on synthetic fertilizer treatments. Deep roots are advantageous for water uptake, nutrient uptake and higher crop yields (Maeght et al., 2013). They also give back to the soil because the deeper and broader they go, the more benefits they provide for soil fertility and stable carbon storage in soils (Maeght et al., 2013). The root system architecture (RSA), i.e the spatial distribution and morphology of the roots, play a vital role in the ability of plants to access nutrients. Deeper roots are essential for the uptake of nutrients such as potassium (K) and nitrogen (N), and deep roots can expand the soil volume accessible for uptake and thus increase the uptake fraction (Maeght et al., 2013). A positive relationship is observed between root density, deep roots and nutrient uptake (Maeght et al., 2013). This difference observed could be due to the nature of the control-release fertilizer being temperature dependent and the experimental room experiencing temperature fluctuations during the course of the experiments. Temperature is a major abiotic factor that can influence the growth, development and yield of a plant (Walne & Reddy, 2022). Under normal circumstances, the control-release fertilizers would provide crops with sustained and consistent nutrients over a specified time period compared to conventional forms of fertilizer which have immediately available forms of nutrient release. The gradual release from control-release fertilizer acts to minimize nutrient leaching and improve the overall fertilizer use efficiency (Morgan et al., 2009). Control-release fertilizers vary in their mechanism of nutrient release, and in this study, we chose a formula that steadily releases nutrients at 25°C. This means that at 25°C , there is a steady and constant release of nutrients, and at temperatures lower than that, nutrient release decreases. We suspect there were temperature fluctuations in the experimental room as a temperature of 25°C was achieved using a space heater, and the room had no temperature control. These fluctuations in temperature may partially explain the decreased biomass observed in the fertilizer treatment.

Another explanation of the greater growth in 10% BSFPR compared to commercial fertilizer could be the cation exchange capacity and the growth media pH. The Cation exchange capacity (CEC) measures a soil's ability to hold water and nutrients (Manrique et al., 1991). The CEC in the commercial fertilizer treatment was lower when compared to BSFPR treatment, suggesting nutrients were lost in the fertilizer treatment during watering due to leaching. Cation exchange capacity is a fundamental soil property that influences soil structure stability, nutrient availability, and soil's reaction to fertilizers (Manrique et al., 1991; McCauley et al., 2009). The fertilizer treatment had a slightly acidic pH, and acidic soils tend to have lower CEC values which could mean that in treatments with acidic pH, such as the synthetic fertilizer treatment, nutrients are either absorbed by the plant or lost through leaching. Soil pH also affects nutrient availability, macronutrients such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), (Magnesium) Mg and Sulphur (S) are most available within a pH range of 6.5 to 8; outside of this optimal range, nutrients are less available to plants (McCauley et al., 2009). Although growth media pH values overall ranged between 5.7 and 7.5, which were suitable for producing cherry falls tomatoes (Setti et al., 2019), the fertilizer treatment was at the lowest end (pH of 5.7), which is just under the pH at which various macronutrients are available, plants grown in the fertilizer treatment have less access to macronutrients. While not tested in this study, it is also possible that BSFPR contained beneficial microbes such as plant growth-promoting rhizobacteria. For example, during decomposition, mealworm exuviae were shown to have a high diversity of chitinolytic bacteria, and these bacteria are capable of promoting plant growth (Poveda, 2021; Poveda et al., 2019). These plant growth-promoting rhizobacteria function to fix nitrogen, solubilize phosphates and promote zinc absorption in plants (Poveda et al., 2019).

Above-ground plant biomass is an important factor in the study of plant biology and growth analysis. It is a key consideration in many allometric relations, and biomass measurements are the basis for calculating net primary production and growth rates. The overall shoot biomass (shoot length and shoot dry weight) was highest in 10% BSFPR compared to synthetic fertilizer and 100% coconut coir. These results are broadly consistent with other studies that reported that growth media amended with black soldier fly processing residue affected the height and biomass of tomato, basil, leaf lettuce and bush green bean (S. Choi & Hassanzadeh, 2019; Quilliam et al., 2020; Setti et al., 2019). Other important traits linked to plant biomass measured by this study include leaf area, number of compound leaves, number of flowering trusses and root-to-shoot ratio. All except the root-to-shoot ratio were significantly larger/higher in 10% BSFPR when compared to control-release fertilizer and 100% coconut coir. These results are consistent with studies that have shown that the use of BSFPR as an ingredient in growth media preparation positively affected the number of leaves in baby leaf lettuce, basil and tomato potted plants compared to synthetic fertilizers (Setti et al., 2019). The number of leaves was positively linked to leaf area, directly correlated with photosynthetic ability. The leaf area determines light interception for photosynthesis and is an important measurement of plant productivity (Weraduwage et al., 2015). The greater the leaf area, the greater the photosynthetic ability of the plant. This means more carbon allocation to plant productivity metrics and the bigger the plant will be able to grow. The leaf area in tomatoes grown in 10% BSFPR was significantly higher than in tomatoes grown on control-release fertilizer, 100% coconut coir had no true leaves emerge, and hence leaf area was equal to zero.

The root-to-shoot ratio measures the amount of supportive plant tissues (roots) compared to the amount of growth plant tissue (shoot). In this study, root to shoot ratio was not significantly different between the 10% BSFPR and the control-release fertilizer treatments despite the difference in other plant growth metrics measured by this study. This was an unexpected observation and could mean that plant growth resources were allocated similarly in both treatments. In both treatments, shoot dry weight was higher than root dry weight, meaning that resources were allocated more to shoot growth than root growth. This results in a trend of smaller root-to-shoot measurements in bigger plants, as the highest root-to-shoot ratio was observed in the 100% coconut coir treatment. Resource allocation in the 100% coconut coir treatment is understandable as coconut coir is an inert growth media with little to no nutrients available. Hence, roots were thin but deep due to the search for nutrients compared to the shoot biomass with no true leaves developing in any 100% coconut coir tomato plants.

Future directions could involve testing BSFPR on other vegetable plants to better understand the tolerances of those plants, additionally testing even lower concentrations of BSFPR on tomato plants could yield positive results and this could greatly improve productivity as a little of the BSFPR can go a long way. The potential microbial community within the BSFPR that could benefit plant growth needs to be explored and better understood. Ultimately, the use of BSFPR was successful as a soil amendment and can allow for the replacement of synthetic fertilizers in soilless systems, provided care is taken to ensure amounts are within the salt tolerances of the plants to be grown. Black soldier fly processing residue seems to provide nutrients in forms acceptable for plant uptake and this may be supplemented by the potential presence of microorganisms capable of stimulating plant growth. Additionally, the use of BSFPR as a soil amendment fulfills the requirements of a circular economy where processing residue produced from a waste conversion system using the black soldier fly can be reintegrated back into the ecosystem as fertilizer that supports plant growth.

5.6 Reference

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Table 5.1. Growth media analysis report from Agriculture and Food Laboratory (AFL) in Guelph ON

Sample number	**CF (fertilizer)**	**BSF10**	**BSF20**	**BSF30**	**CC**	**100% BSFPR**
pH	5.7	7.3	7.5	7.4	6.2	6.7
Lime index	6.4	6.7	6.7	6.7	6.3	6.4
Total Organic matter %	67.5	68.4	68.6	64.4	80.3	59.9
Phosphorous Bicarb ppm	373	159	194	419	10	879
Phosphorus P ppm	1473	608	774	1646	15	3495
Potassium K ppm	2934	1764	2301	3640	213	5737
Magnesium Mg ppm	196	330	394	710	165	1121
Calcium Ca ppm	1220	2806	3123	5002	1860	5569
Aluminum Al ppm	47	38	53	60	49	186
Sulfur S ppm	2158	245	306	668	13	1748
Zinc Zn ppm	4.1	8.6	11.5	22	2.4	48.4
Manganese Mn ppm	6	9	10	18	7	26
Iron Fe ppm	76	81	68	93	107	83
Copper Cu ppm	0.8	1.9	2.9	5.3	0.6	10.3
Boron B ppm	1.4	1.3	1.4	2.1	1	3.5
Sodium Na ppm	86	944	1174	1780	63	2735
Nitrate-N NO3-N ppm	1854	4	2	4	1	19
Soluble salts ms/cm	16.60	2.93	4.43	6.87	0.14	14.24
CEC meq/100g	16.4	25.4	29.9	47.9	12.7	64.5

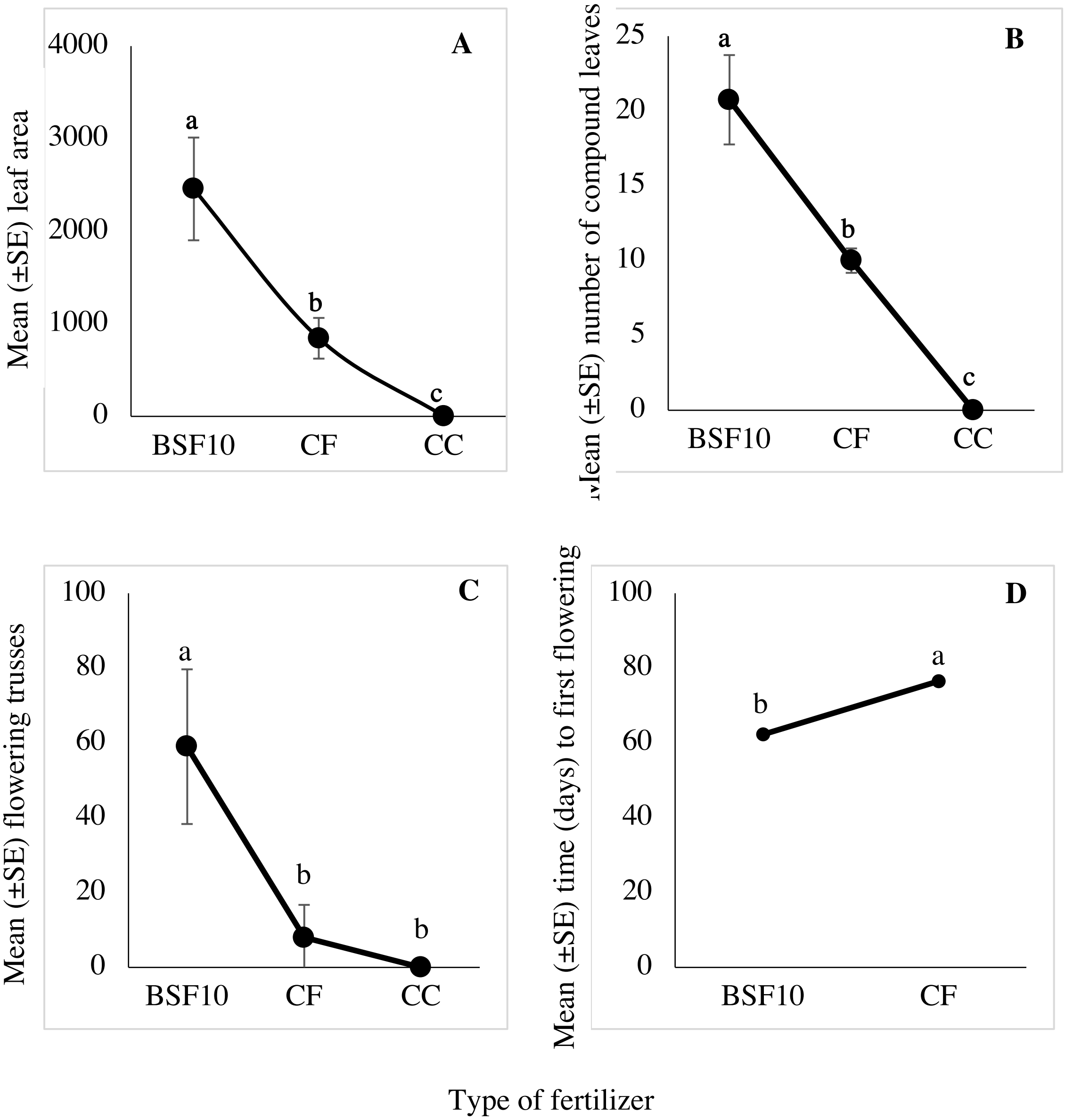

Figure 5.1. Tomato (*Solanum lycopersicum*) var. Cherry Falls grown in coconut coir with: 1) 10% black soldier fly processing residue (BSF10); 2) Commercial slow release fertilizer (CF); or 3) No fertilizer (CC). A – Mean (±SE) leaf area (cm^2). B - Mean (±SE) number of compound leaves. C - Mean (±SE) flowering trusses. D – Mean (±SE) time (days) to first flowering. Means with the same letters within the same graph do not differ (p >0.05).

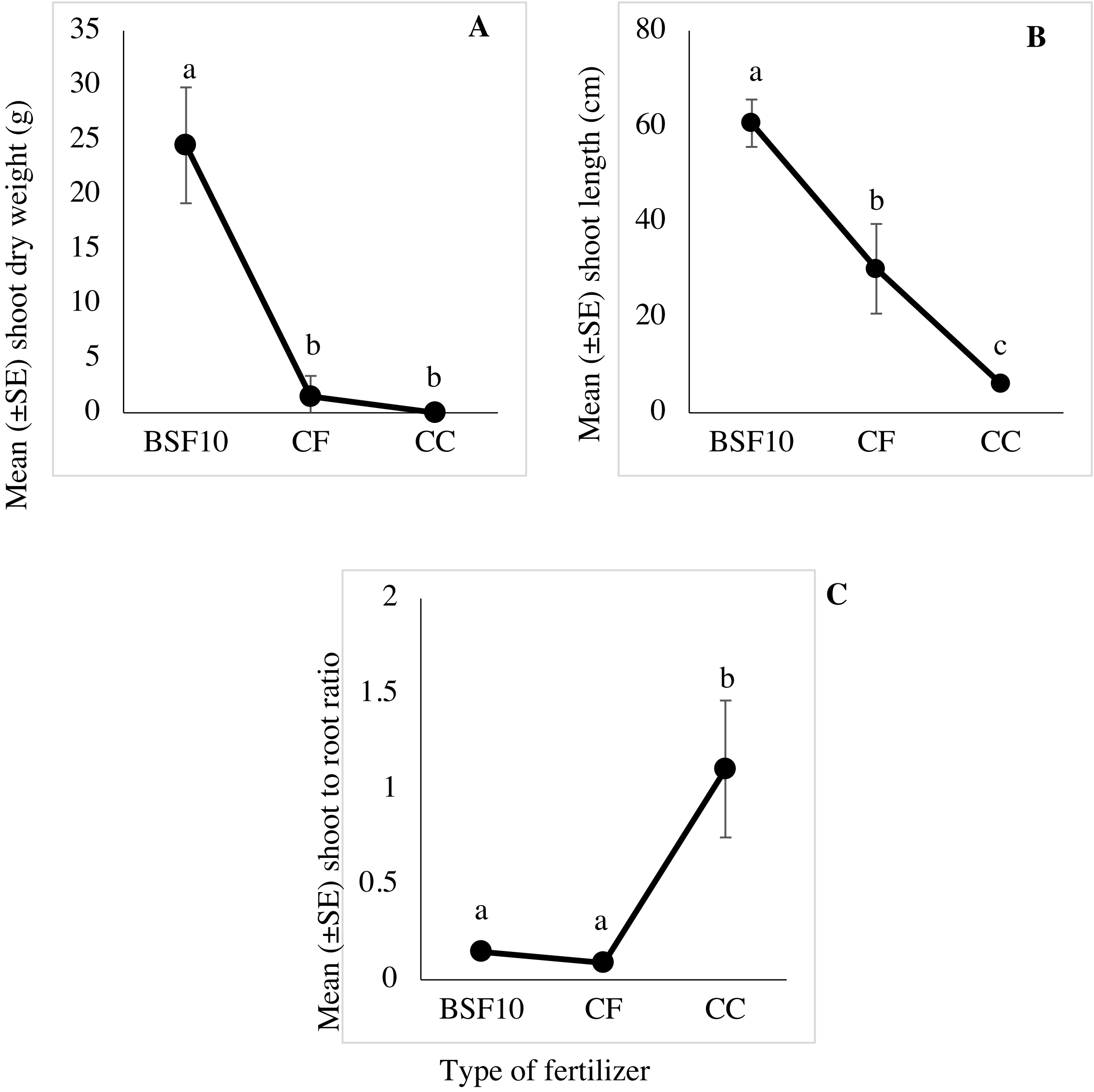

Figure 5.2. Tomato (*Solanum lycopersicum*) var. Cherry Falls grown in coconut coir with: 1) 10% black soldier fly processing residue (BSF10); 2) Commercial slow release fertilizer (CF); or 3) No fertilizer (CC). A – Mean (±SE) shoot dry weight (g). B - Mean (±SE) shoot length (cm). C - Mean (±SE) shoot to root ratio. Means with the same letters within the same graph do not differ (*p* >0.05).

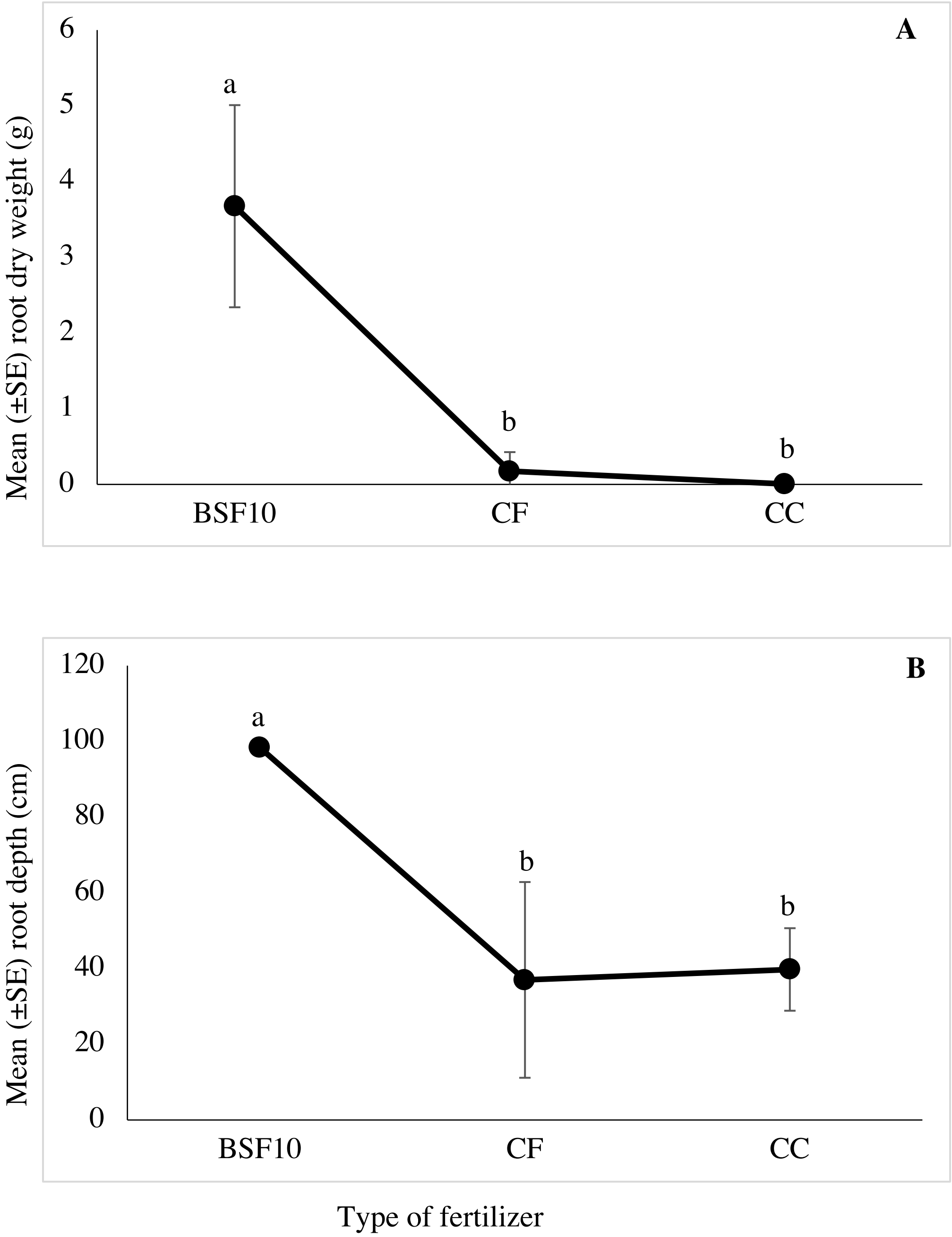

Figure 5.3. Tomato (*Solanum lycopersicum*) var. Cherry Falls grown in coconut coir with: 1) 10% black soldier fly processing residue (BSF10); 2) Commercial slow release fertilizer (CF); or 3) No fertilizer (CC). A – Mean (±SE) Root dry weight (g). B - Mean (±SE) root depth (cm). Means with the same letters within the same graph do not differ (p >0.05).

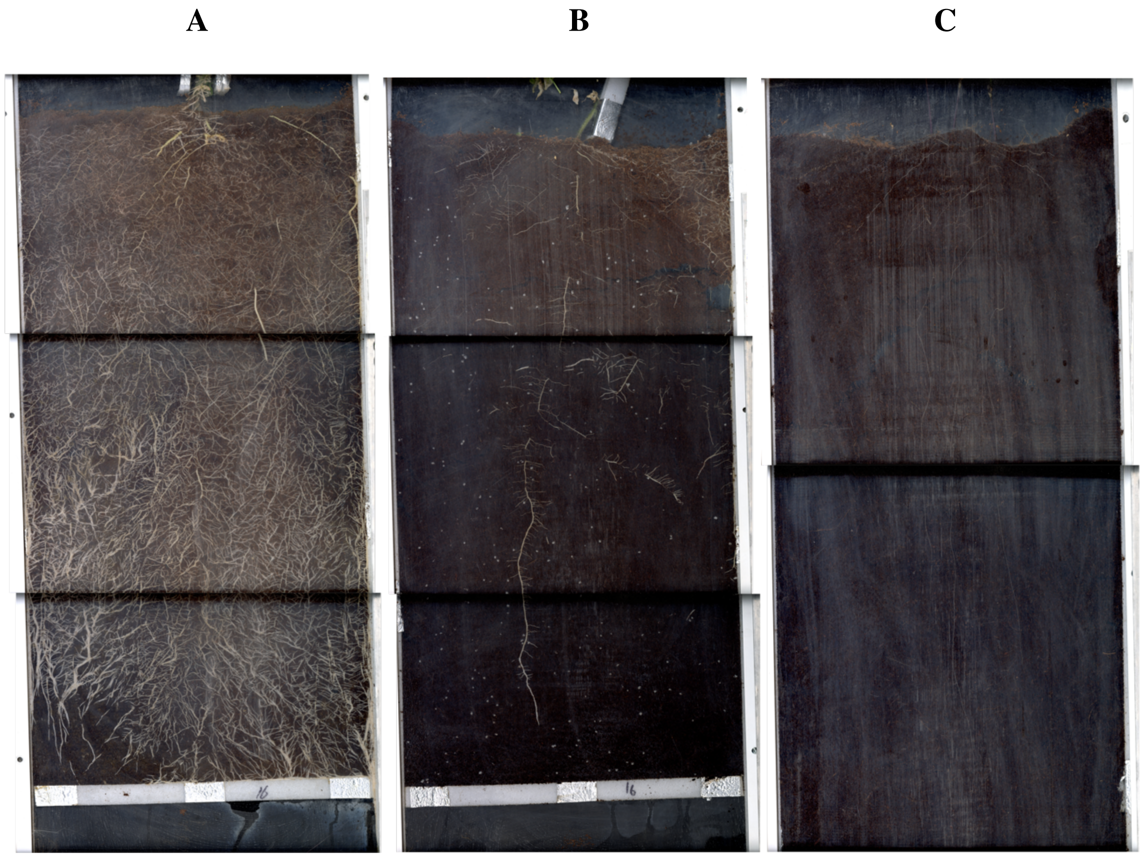

Figure 5.4. Rhizobox root scans of tomato (*Solanum lycopersicum*) var. Cherry Falls plants at 12 weeks post germination A = BSF10 (10% black soldier fly processing residue), B = CF (slow-release commercial fertilizer), and C = CC (100% coconut coir with no fertilizer).

Chapter 6

Solving the Wicked Problem with a Circular Economy Approach

Food waste has been described as a “relentless and wicked problem” because while one third of all foods produced are wasted globally, one in nine people are globally suffering from food insecurity (Guo et al., 2020; Gustavsson et al., 2011; Hoornweg & Bhada-Tata, 2012; Närvänen et al., 2020). Within Canada, food waste is on the rise and occurs when food is thrown away during the production, handling, processing, or storage stages in the food manufacturing chain (Hanson & Ahmadi, 2022). Food waste results in the wastage of resources used during food production and the wastage of environmental resources because the food sector is responsible for 22% of the world's greenhouse gas emissions and 30% of the world's total energy consumption (Hanson & Ahmadi, 2022). As urbanization increases, so will the rate of food waste due to the increase in the consumption of goods and services (Hoornweg & Bhada-Tata, 2012; Ma & John Taylor, 2020; Ozbay et al., 2021). Food waste in Canada is a systematic issue due to food waste occurring at every level of the food supply chain, a lack of redistribution of excess food, and overproduction (Hanson & Ahmadi, 2022). Since 1961, food waste in Canada has risen to 40% as of 2009 and continues to increase due to an increase in the demand for health-conscious foods, slow population growth, and concerns for quality and freshness from Canadian consumers (Hanson & Ahmadi, 2022).

Additionally, the COVID-19 pandemic resulted in a change in food purchasing behaviours across the country (Music et al., 2021). Many Canadian consumers created food reserves to ensure food safety during an unprecedented time. However, this purchase panic exceeded the normal consumption levels in households and led to an overall increase in food waste (Music et al., 2021). Consumers cited stress and health concerns as bigger priorities over the environmental impacts of food waste (Music et al., 2021). One estimate suggests Canadians generate 12.6 million tonnes of organic waste sent to landfills for disposal (von Massow et al., 2019). Food waste also has a devastating effect on the environment as landfilling is one of the most popular waste management strategies and impacts the environment by taking up landfill space, producing leachate, and releasing greenhouse gases. Landfills account for 18% of anthropogenic greenhouse gas emissions and are the third largest emitters after agriculture and transportation (Sun et al., 2019). Some of the common by-products of anaerobic landfill decomposition include methane (CH_4), carbon dioxide (CO_2), ozone (O_3), nitrous oxide (N_2O), and other hazardous gases (Johnson et al., 2007). Carbon dioxide has the greatest climate warming potential (57%), while CH_4 and N_2O account for 27% and 16%, respectively (Johnson et al., 2007). About 60 million tonnes of greenhouse gasses are generated by anaerobic waste decomposition in landfills due to inadequate gas collection systems at landfill sites (Humer & Lechner, 1999). Leachate production is another disadvantage of landfilling as a waste management strategy. Leachate is rainwater or moisture from organic waste that percolates and becomes contaminated as it filters through the waste (Renou et al., 2008). Leachate contains polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (PFCs); organic pollutants which have been detected in landfills all over Canada and persist through the leachate treatment process (B. Li et al., 2012). Due to their structural similarities to thyroid hormones, they can affect the nervous, endocrine, and immune systems, and exposure to even small amounts can cause nervous system or reproductive system damage (B. Li et al., 2012).

A circular economy is one with a zero-waste model that focuses on designing out waste from the system and is a sustainable alternative to the take-make-waste model typically found in the economy. The main objective of a circular economy is to keep products and materials in use throughout a product's life cycle and close the waste loop (Salmenperä et al., 2021). A 53% decrease in the consumption of new materials by 2050 is possible if the circular economy model is followed (Esposito et al., 2017). Waste management practices that move away from landfilling and towards ways that view food waste as a resource will help transition the economy into a circular model where designing out waste and regenerating and repurposing biological materials are key principles. Currently, there are a number of ways that food waste is dealt with within the economy. Some methods are preventative, while others are reactive. One preventative method to avoid food waste is educational tools that teach about the dangers of food waste and provide measures that encourage the donation of excess foods (Närvänen et al., 2020). Other, more reactive methods, focus on what happens after food is wasted, such as compositing, landfilling, and more valorization by insect larvae.

Valorization by insect larvae is a novel technique and involves insect larvae feeding on decomposing organic material such as food waste. The black soldier fly is a large wasp-like fly native to the Americas and approximately 13 mm to 20 mm in length (Sheppard et al., 2002; Tomberlin & Sheppard, 2001). Adults can survive in temperatures ranging from 24°C to 36°C with five larval stages (Sheppard et al., 2002). Black soldier fly larvae can feed on a wide range of diets (Diener et al., 2009, 2011; Nguyen et al., 2013, 2015; Oonincx et al., 2015). Eggs hatch in 4 days, and the larval stages last 22 to 215 days, depending on temperature and diet (Oonincx et al., 2015; Tomberlin et al., 2009). During the wandering stages, maggots move away from their food source to seek a pupation site which can last weeks to months, depending on the developmental temperature and pupation medium (Holmes et al., 2013; Tomberlin et al., 2009). Adults survive 8-10 days when provided with water and are non-feeding (Tomberlin et al., 2002). This means they do not require any additional nutrition to mate and reproduce, and instead survive on the energy in the fat body accumulated during their larval stages. Mating occurs two days after emergence and is influenced by the time of day, light intensity, and humidity (Tomberlin & Sheppard, 2001). More than 85% of mating occurs in the mornings, while the number of mating pairs observed decreases throughout the day (Zhang et al., 2010).

The black soldier fly can consume a wide variety of decomposing organic material ranging from an all-vegetable to an all-meat diet, including human wastes (Diener et al., 2011; Nguyen et al., 2015). This makes it a potential strategy for Canada's food waste problem. In 2002, Canadians produced 30.4 million tons of food waste (Cameron et al., 2005), and most of this waste was landfilled. This waste can take up to 30 years to decompose in landfills, and landfills are reaching their capacity (Cameron et al., 2005). The black soldier fly is non-feeding, adaptable to mass rearing, not considered a pest, and can reduce a wide variety of waste in a relatively short time period at optimum temperatures. Therefore, the focus of this dissertation was the black soldier fly and its ability to reduce food waste during its larval stage, as well as ways to optimize its waste reduction and keep with a circular economy. In my dissertation, I developed a baseline for black soldier fly waste reduction on food waste within Windsor-Essex (Chapter 2). I assessed the possibility of optimization by adding a microbial inoculant to the waste prior to the addition of black soldier fly larvae (Chapter 3). I developed a mathematical scalable formula for establishing reliable initial densities required for mass-rearing the black soldier fly and introducing known densities for effective bioconversion of wastes (Chapter 4). Finally, I tested the post-processing residue and its potential suitability as a soil amendment (Chapter 5).

The baseline for BSF was reduction was investigated by determining the waste reduction efficiency of the BSF, including the effects of the larval diet on the life history traits of the fly. This was achieved using chicken feed as a control diet and food waste from a composting farm. Overall, the results showed that BSF was capable of reducing food waste by almost 70% over a period of 19 days compared to 15 days when fed with chicken feed. These results are broadly consistent with other studies that have found 50-70% of waste reduction when waste was inoculated with BSF larvae (Diener et al., 2009; Sheppard et al., 1994). Survival was higher, and development time was shorter for larvae fed with chicken feed compared to larvae fed with food waste. This is to be expected as chicken feed is a chemically balanced diet for young poultry birds to ensure proper development, while food waste is heterogenous and mostly composed of scraps and leftovers. The waste reduction percentage was similar between diets. However, the waste reduction index, which is waste reduction percentage as a function of larval development time, significantly differed between the two diets. The waste reduction index was higher in chicken feed due to larvae developing faster and having a relatively shorter development time. Surprisingly, although larvae had shorter development, higher survival and higher waste reduction index (waste reduction efficiency), there were larger adults and high bioconversion efficiency on the food waste treatments compared with the control. Bioconversion efficiency measures how well larvae turned diet into body weight and could be a proxy for body size. Body size and bioconversion efficiency were higher when larvae were fed with food waste, and this could mean higher fecundity of adults that emerged from these treatments. Larger body size is an indicator of fitness in insects, as larger adults generally have higher longevity and fecundity compared to smaller individuals. Overall, these experiments showed that the heterogenous nature of food waste might be beneficial to black soldier fly development since larger body size and higher bioconversion efficiency were observed when larvae were fed a variable food diet. These results lend support to black soldier fly being a resourceful alternative to landfilling and a waste reduction alternative. It shows that black soldier fly is a viable strategy to landfilling within the Windsor-Essex region. However, the system requires optimization to ensure high waste reduction efficiency.

One strategy for optimization of the BSF system was investigated using microbes. Food waste conversion tends to be lower due to the heterogeneous nature of food waste. This can be due to the presence of lignin and hemicellulose -rich content that is inaccessible to the larvae and potentially low protein content (Gold et al., 2020; Lalander et al., 2015; Nguyen et al., 2013). It is impossible to ensure high-quality diets for larvae when dealing with food waste, and one way to combat this is the pre-digestion of food wastes with microbes in order to improve process efficiency. In this study, diets were pre-digested at different time periods at different microbial levels to improve the waste reduction capability of the black soldier fly. The role of microbes in nutrient breakdown is well-documented in nature (Dilly et al., 2001; Lauber et al., 2014). The treatment with microbes resulted in variable results that were complicated to explain. In most cases, fitness (survival) decreased as the pre-digestion time increased and development time decreased as well. However, metrics such as waste reduction index and relative growth rate were highest at the 2-day pre-fermentation time. Interestingly, all metrics measured were negatively impacted at 7-days of pre-digestion, suggesting the black soldier fly larvae are negatively influenced by old microbial-laden waste. Future directions should look into pre-digestion using enzymes as opposed to adding microbes that might compete with the black soldier fly larvae. Another future direction might consider continuous feeding over single feeding events (i.e., the continuous addition of fresh waste for larvae during waste bioconversion). This could be useful in improving waste conversion as fresh waste will be high in nutrients required for black soldier fly growth compared to the old waste, and the microbes present in the old waste will help break down complex polymers that might be present in the new waste stream. Co-digestion could also be a solution. Co-digestion involves digesting a highly nutritious waste source with less nutritious waste to improve bioconversion by insect larvae (X. Li et al., 2021). Co-digestion can improve the waste conversion of less nutritious waste as larvae are feeding on a combination of highly nutritious waste and less nutritious waste concurrently, and this may create a better nutrient balance for the larvae.

Various factors can affect the bioconversion process, and knowledge and understanding of these factors are very important, especially for a species of economic value such as black soldier fly. The bioconversion process depends on population size, which are introduced via egg masses. Therefore, another step in optimization is determining system load capacity by identifying how many individuals are present within an egg mass for waste reduction. It is important to develop a methodology that can reliably predict the number of eggs within an egg mass without affecting the integrity of the mass. The perfect combination of parameters such as type of food waste, quantity and environmental factors such as temperatures could improve the waste reduction process. A linear model using egg mass volume and egg mass weight was developed to predict the number of eggs within an egg mass. These models were validated, and they reliably predict the number of eggs within an egg mass. Manually counting eggs requires a lot of time and effort and an increased incidence of human error and bias (Mains et al., 2008; Rosati et al., 2015). Using the model developed, several egg masses of over 1000 eggs were estimated in less than 2 minutes. Using egg mass weight, egg masses are simply weighed, and the weight is inputted into the model to predict the number of eggs within that mass. Using egg mass volume requires using a license-free digital analysis program known as ImageJ. Egg mass surface area needs to be calculated using ImageJ, and depth measurements are taken to determine egg mass volume, which can then be used in the model to predict egg mass number. These methods described are scalable to industry operators who must have an idea of how many individuals are available for quick and efficient waste reduction. This experiment is the first of its kind to develop a model for predicting egg masses in black soldier fly.

A by-product of waste conversion using the black soldier fly will be an abundance of black soldier fly prepupae which can be utilized as an alternative protein for livestock, poultry and commercially raised fish (St-Hilaire et al., 2007). The prepupae contain approximately 63%-37% protein and 40%-20% fats (Newton et al., 2005). They are also a good source of vitamins such as B12, thiamine, riboflavin and various essential amino acids (Spranghers et al., 2017). The fatty acids contained in the prepupae can also be mechanically pressed and biotransformed into biodiesel (Surendra et al., 2016). Analysis of the fatty acids revealed that lauric, palmitic and linoleic acids were the most common fatty acids derived from the black soldier fly prepupae and these fatty acids were more dominant within the black soldier fly prepupae than in crops commonly used for biodiesel production. A circular economy encourages the reuse and reintegration of waste products back into the economy. A part of this dissertation investigated this idea by testing black soldier fly post-processing residue (BSFPR) as a fertilizer soil amendment for the growth of tomatoes. Sustainable food production for a continuously growing population is one of agriculture’s critical challenges. Most greenhouse growers opt to use soilless systems for their advantages, such as low environmental pollution and saving 85% of irrigation water (Pradhan & Deo, 2019). However, soilless systems require fertilizer for plant nutrition, as most soilless are inert media and do not provide enough nutrition for plant growth. Five different growth media were tested, three of which were composed of the black soldier fly processing residue (BSF10, BSF20, BSF30) at varying percentages and a positive (Slow-release fertilizer) and negative control (no fertilizer). The growth media comprised of five different growth media were composed as follows: coconut coir (CC) 100% + 20-5-10 slow-release solid fertilizer (Nutricote 20-5-10 type 100, Plantproducts.ca, using 140g/box) (CC+Fert) ; CC 90% +BSFPR10% (CC+BSF10); CC80% + BSFPR 20% (CC+BSF20); CC70% + BSFPR 30% (CC+BSF30); CC 100% (CC). The surprising results were that growth media with high concentrations of BSFPR did not support the growth of cherry tomato plants. This could be due to high nutrient and salt stress in media with greater than 10% BSFPR, and this explanation is consistent with studies that have shown stunted and lack of germination when plants were under nutrient and salt stress (Saunkaew et al., 2011). Another surprising result is that plants grown with 10% BSFPR had greater overall plant development compared to plants grown in slow-release fertilizer. Tomatoes grown on 10% BSFPR had greater root and shoot biomass, larger leaf areas and higher flowering trusses. This could be because plants in the fertilizer treatment could have lost nutrients due to leaching after being watered. The cation exchange ratio in the BSFPR treatments was higher than the fertilizer, which means the BSFPR was less prone to leaching due to watering. Additionally, the fertilizer was applied once in the fertilizer treatment compared to greenhouses that apply fertilizer through the irrigation water every time the plants are watered to counter the potential leaching risks. The slow-release fertilizer used in this experiment released nutrients at a steady rate at 25°C, and it is possible the environmental temperature fluctuated and affected the rate of nutrient release.

The results of this experiment are broadly consistent with other studies that have grown different crops with BSFPR as a soil amendment (Choi & Hassanzadeh, 2019; Quilliam et al., 2020; Setti et al., 2019) with high concentrations of BSFPR resulting in no growth or stunted growth and low concentrations showing the greatest developments. This effect of stunted growth/no growth at high concentrations of BSFPR might be crop-dependent, and less sensitive plants might respond well to high concentrations of BSFPR.

Landfills are rapidly reaching their capacity, and due to the continuous increase in waste production, more landfills will need to be open as older landfills close. There might also be challenges associated with siting locations for new landfills because landfills take up land space that can be utilized for other economic developments and must be far enough away from residential, commercial and city centers. Based on the current economic trends, more landfills will be required in Ontario if more effort is not made to divert organic waste away from landfills. Using landfills as a waste management system is a poor choice as landfills take up land space that could be utilized for other economic developments and produce toxic chemicals such as greenhouse gases and leachate, which contribute to climate change. The positive results of this study mean that the black soldier fly and its uses both as a soil amendment and as a waste management strategy fit into the framework of a circular economy. Remember that circular economy encourages and supports the reuse and reintegration of otherwise wasted products (i.e., food waste) back into the economy as resource (i.e., compost) after bioconversion by the black soldier fly.

This study also provides immediate ready-to-use mathematical models for large-scale black soldier fly rearing and waste bioconversion. These models can be applied on a lab scale for black soldier female oviposition choice studies or at an industrial scale to determine the larval density required for an efficient waste reduction on a large scale. Future directions for this research could involve testing microbial pre-treatment of waste for the black soldier fly but instead of a one-time feeding event, incorporate continuous feeding to counteract the effects of depleted nutrients that occurs during fermentation. Another step would be to pre-treat the waste with enzymes instead of microbes, effectively cutting out the middleman (i.e., microbes) to avoid the antagonistic role the microbes later play in waste reduction. More directions for this research could also involve testing the BSFPR on different types of common vegetables at varying concentrations. In the present study, no germination of tomatoes was observed at high concentrations of BSFPR, but this could simply be plant-specific effects opposed to a common standard. The future of black soldier fly research is vast and can incorporate some of the suggestions outlined here. However, despite more research necessary to continue to understand the black soldier fly and its role in waste management and optimization, we believe this technology is ready to be adopted at least at the municipal level, to divert municipal food waste away from landfills. Black soldier fly in this study reduced more than 60% of the waste provided to them, and this could mean a 60% reduction in municipal food waste and a significant reduction in the amount of organics that are sent to landfills. It is important to recognize that at a municipal level, waste is usually picked up once a week and if pre-digestion is considered, the organic waste might be sufficiently broken-down or require a fermentation time less than what is presented in the current study. Additionally, there might be seasonal variations based on food availability by season that could impact the heterogenous nature of the waste. At the municipal level, green boxes can be provided to households for organic waste, which can be picked up and transported to a black soldier fly waste conversion facility. To roll out this program, pamphlets can educate people on the dangers of food waste and landfilling and encourage the use of government-provided green bins for organic waste disposal.

Overall, this study provides useful information for understanding how the black soldier fly can be applied as a waste management strategy in Windsor -Essex. It provides an industry scalable model for the use of black soldier fly for waste reduction at an industrial scale, and it provides uses for black soldier fly processing waste after the waste reduction process.

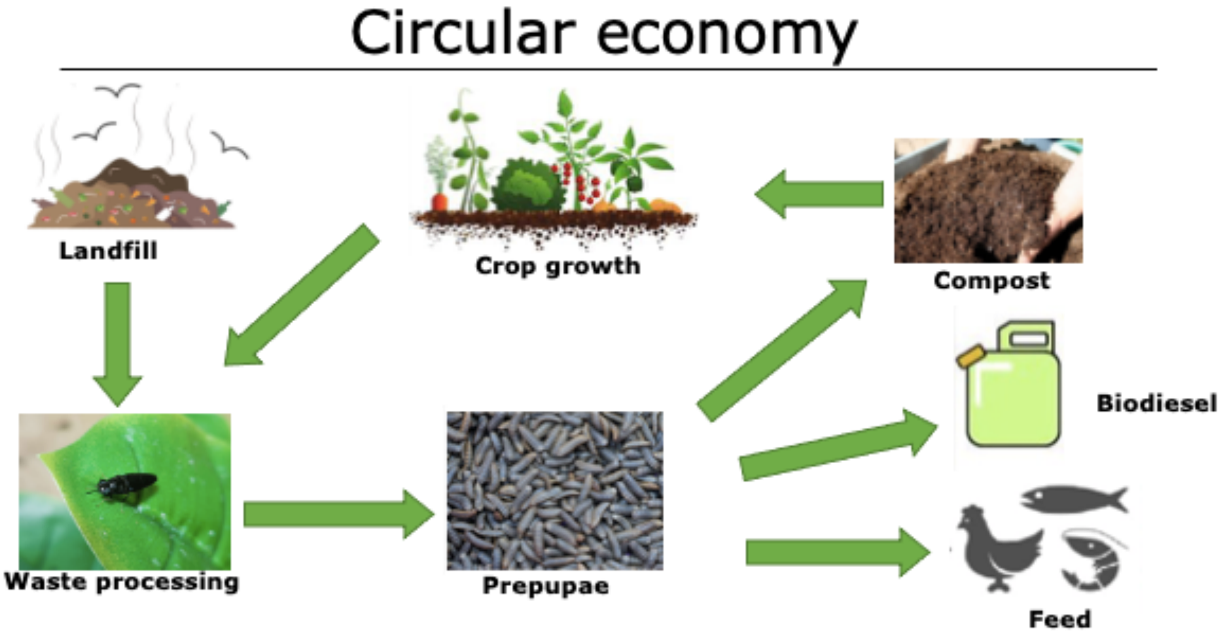

Figure 6.1. Schematic diagram showing the black soldier fly waste processing integrated into a circular economy. Waste can be diverted away from landfills towards a black soldier fly waste processing facility, prepupae can be utilized as feed or processed as biodiesel and compost can be used as a soil amendment.

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