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1 Introduction

Over the past decade, the black soldier fly (BSF, *Hermetia illucens* (L.), Diptera: Stratiomyidae) has become one of the most commonly used insects for feed production worldwide (van Huis, 2020). This is primarily due to the wide range of substrates that the larvae can be reared on, along with the high waste-to-biomass conversion efficiency of the larvae (Gold et al., 2018). This versatility of substrate use by fly larvae has made them nature's own waste managers (Fowles and Nansen, 2020).

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http://dx.doi.org/10.19103/AS.2024.0139.02

Chapter taken from: Casillas, A. (ed.), *Insects as alternative sources of protein for food and feed*, Burleigh Dodds Science Publishing, Cambridge, UK, 2024, (ISBN: 978 1 80146 584 7; www.bdspublishing.com)

With the reported decline in terrestrial insect abundance, questions have emerged regarding the impact this will have on the different ecosystem services they provide (van Klink et al., 2020; Sánchez-Bayo and Wyckhuys, 2019). One of many ecosystem services that insects contribute to is the degradation and recycling of organic matter (Dangles and Casas, 2019) and nutrients (Woelber-Kastner et al., 2021; Yang and Gratton, 2014). The ability of detritivore insects, such as the BSF, to degrade organic matter and recycle nutrients is one of the main reasons they are of interest not only for feed production but also for waste management purposes (Čičková et al., 2015). During this process, two products are generated: a larval biomass that can be used in animal feed (Lu et al., 2022), and a treatment residue known as frass or entomocompost that can be used as an organic fertilizer or soil amendment (Lopes et al., 2022). As two valuable products are generated in the process, this waste management technology fits well within the concept of a circular economy (Ojha et al., 2020), in which the by-product/waste stream in one process becomes the resource in another (Ellen Macarthur Foundation, 2015).

2 Bio-waste streams available for rearing black soldier fly larvae

2.1 Europe

BSF larvae (BSFL) have an extraordinary ability to feed on almost any bio-waste stream, including food waste, vegetable waste, human faeces, animal manure (pig, poultry, cattle), fish and fish offal, to mention a few (Hopkins et al., 2021; Lalander et al., 2019). They can also be reared on agro- and food industry side-streams, including spent grains, bread surplus, beet molasses, banana peels and maize distillers (Hopkins et al., 2021; Chia et al., 2020; Isibika et al., 2019). However, both the process efficiency (Lalander et al., 2019) and larval composition (Barragan-Fonseca et al., 2017) are heavily influenced by the substrate the larvae are reared on. The environmental impact of insect rearing also largely depends on the substrate the insects are reared on, with residual streams such as municipal waste exerting a considerably smaller impact than generic protein feed, while beet pulp resulted in a significantly greater environmental impact (Smetana et al., 2016).

In the European Union (EU), this issue is problematic for insect breeders, as insects are considered farmed animals and thus are not allowed to be fed any animal by-products, which limits the insect sector to using primarily pre-consumer bio-waste streams, such as agro- and food industry wastes (European Commission, 2009). This regulatory shift occurred in the 1980–90s after the outbreak of bovine spongiform encephalopathy (BSE) in Europe, during which European food production went from being fairly circular to becoming almost exclusively linear (Vågsholm et al., 2020a). The main fear of European legislators is the possible occurrence of misfolded prions, which caused the BSE outbreak, in animal by-products (Lalander and Vinnerås, 2022). Unfortunately for the European insect sector, Bosch et al. (2019) demonstrated that currently legal substrates for insect breeding in the EU have a higher environmental impact than those that are not permitted. In fact, they found that on a per produced protein basis, BSFL reared on permitted substrates that could be used directly as animal feed for other farmed animals, such as brewery distiller's grains, had an even higher environmental impact than conventional protein feeds such as fishmeal and soybean meal. The use of more post-consumer residual streams, such as catering waste (bio-waste from households and restaurants), would be important not only from an environmental point of view but also in providing a sufficient feed source for the larger volumes of insects that the feed industry requires. It is in the interest of the EU to increase the local production of high-quality protein feed.

In 2015, the EU launched an action plan to facilitate the transition from a linear to a circular economy (European Commission, 2015). This action plan has been followed up by the European Green Deal, in which the emphasis lies in making Europe climate neutral (fossil free) by 2050 (European Commission, 2020a). A crucial part of this Green Deal is the Farm to Fork Strategy (European Commission, 2020b), which aims to make European food production systems fairer, healthier and more environmentally friendly. This will be accomplished by, among other things, reducing nutrient losses by 50% and fertilizer use by 30%, and ensuring stronger food security within the EU. One of the weaknesses of food security within the EU, identified by the EU Commission, is the high level of imports of feed ingredients, such as oil seed meal and soy (European Commission, 2021b). The EU currently imports 76% of oil seed meal (rapeseed and sunflower seed meal; used for both livestock feed and in food processing) and 20% of animal feed, primarily in the form of soy (European Parliamentary Research Service, 2023; European Commission, 2021b). This is not in line with the European Green Deal and jeopardizes EU food security.

Step by step, the EU has started to return to a higher level of circularity in the food industry sector, to encourage a more environmental and resilient food system. The most recent change occurred in 2021, when the EU Commission decided to allow the use of processed animal proteins (PAPs) from pigs and insects to feed poultry, and from poultry and insects to feed pigs, lifting the ban following the BSE crisis (European Commission, 2021a). However, it has not been determined if insects can be fed PAPs. In 2019, 931 million tons of food waste were generated in Europe (United Nations Environment Programme, 2021). In high-income countries in Europe, 118 kg of food waste is generated per person per year, of which 70% is mixed household, i.e. post-consumer waste (United Nations Environment Programme, 2021). However, insects can only be fed agro- and food industry waste, so-called pre-consumer waste. This

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means that approximately 30% of total food waste in Europe is available for insect production (this does not include animal manure).

2.2 Global outlook

In many parts of the world, unlike in the EU, there are no legal limitations on feeding insects with different bio-wastes. The United Nations Environment Programme (2021) found that 931 million tons of food waste were generated globally in 2019, of which the majority (~60%) was from households. In contrast to previous reports, it also found that the generation of food waste on a household level is roughly the same in all income groups: in high-income countries, the average food waste is close to 80 kg capita^{-1} year^{-1}, while in lower- to middle-income countries, it is around 90 kg capita^{-1} year^{-1} (no data for low-income countries available). While it is highly desirable to avoid food loss, there will always be a proportion of food waste that is unavoidable, such as fruit peels, inedible parts of vegetables and fruits, eggshells and bones. However, the proportion of edible to inedible fractions in food waste is very hard to define and can be the topic of debate.

Given the large amounts of available food waste, there should be an ample amount of substrate for rearing BSFL. However, when Gold et al. (2021) conducted a systematic assessment of available bio-wastes for BSFL rearing in Nairobi, Kenya, they found that most bio-waste streams in Nairobi are unavailable, as no waste segregation occurs at the source, most bio-waste streams are contaminated with inorganic material, or are already used for animal feed purposes. They recommend using a mixture of human faeces, animal manure, fruit/vegetable waste and unsegregated food waste. Purkayastha and Sarkar (2022) investigated the efficiency of rearing larvae on municipal solid waste (MSW) and investigated compositions of MSW with a 43–58% biodegradable fraction. They found that, in India, it was possible to generate US$15–43 per ton of MSW processed from the generated BSFL protein, demonstrating a potential method for rearing BSFL from MSW.

In addition to food waste, there are also large streams of bio-waste from food industries and agriculture. The total amount of agricultural bio-waste is hard to estimate but comprises harvest side-streams (inedible parts left on the field) as well as harvest and slaughter losses. The latter is a fraction that has received surprisingly little attention. WWF UK (2021) estimated that around 15% of all food produced is wasted at farm level, totalling 1174 million tons per year globally. The harvest losses include crops that are never harvested or are spoiled after harvesting due to poor storage conditions. Even though these harvest losses are an interesting potential substrate for insect rearing, from a sustainability perspective, focus should be placed on unavoidable harvest losses and food industry waste streams.

However, there are a number of challenges in using these waste streams. They may, e.g. contain a high proportion of lignocellulosic material such as wheat straw, sugarcane bagasse, maize distillers, spent barley and spent sorghum, among others, which are not easy for fly larvae to digest and efficiently convert into biomass (Beyers et al., 2023; Hopkins et al., 2021). There may also be other issues involved with these waste streams. Isibika et al. (2023) conducted a survey on available food industry waste streams in Tanzania and found, in accordance with the findings of Gold et al. (2021), that there was competition for brewery waste (spent grains) and sunflower press cake, as these were already being used as animal feed. The food industry waste streams available for BSFL rearing included fruit peels, mango seeds, coffee husks and fish waste (comprising in this case of the fins and all internal content of the fish, including gills, liver, kidney, intestines, heart, stomach content and swim bladder). None of these sources alone have been found to be a good substrate for BSFL rearing; however, the authors recommend blending the different substrates to meet the nutritional requirements of the fly larvae.

3 Parameters affecting larval rearing efficiency: larval density

Many factors are known to affect the rearing of insects, sometimes referred to as mini-livestock, including the quantity of animals placed in a designated area, which correlates with their growth and development over time (Barragan-Fonseca et al., 2018). The most common term used for how many BSFL are placed in the rearing units is 'larval density' (Dzepe et al., 2020; Barragan-Fonseca et al., 2018), but other terms such as 'areal density' (Guillaume et al., 2023) and 'stocking densities' (Delvendahl et al., 2022) have also been used. Regardless of the terminology, these terms refer to the number of individuals present per unit area, which is most commonly expressed as larvae per square centimetre (larvae cm^{-2}). We have opted to use the term larval density.

In general, it is assumed that the higher the larval density, the lower the final weight of each individual, as larvae will compete for the available resources with more individuals, restricting growth (Barragan-Fonseca et al., 2018). However, when it comes to insect larvae, which are commonly reared in containers into which their feed substrate is placed, density alone does not account for the impact on process efficiency. Other parameters that are interconnected and impacted by larval density include nutrient availability per larva, larval feeding does/rate, substrate depth and moisture (Lopes et al., 2023). In addition, a key issue to consider is the type of container used to rear the larvae, as going from small-scale set-ups often used in research, to more industrial set-ups, is typically not linear (Yang and Tomberlin, 2020). Many studies have been carried out in quite small-scale set-ups, and it is therefore necessary to bear in mind the need for validating previously reported results on a larger scale.

Parra Paz et al. (2015) investigated the impact of larval density (2, 4 or 6 larvae cm^{-2}) on growth on a very small scale (using 49 cm^2 rearing boxes) in a factorial design with three feeding rates (60, 130 and 200 mg of feed per larvae per day). It was demonstrated that density was the most influential variable in that study, with the authors concluding that high densities and feeding rates would be counterproductive as the process efficiency would probably be reduced. Barragan-Fonseca et al. (2018) confirmed that increasing larval density reduced individual larval growth. They evaluated four densities (0.31, 0.62, 1.23 and 2.47 larvae cm^{-2}) in 155 cm^2 containers and obtained a linear response for reduced larval growth when density increased. Nevertheless, the total yield per container increased linearly with density, highlighting that even though individual larvae grew less, the total output can be higher when increasing larval density. The same trend was reported by Dzepe et al. (2020), who assessed the impact of density and substrate moisture on larval growth and development. The authors evaluated densities of 1–10 larvae cm^{-2} and verified lower individual weight and body thickness (in mm, measured using a calliper) in larvae placed at higher densities (6–10 larvae cm^{-2}), conducting the experiments in very small cylindrical containers with an area of 15.05 cm^2. They reported significantly negative correlations between substrate reduction and survival rate with increasing larval density. A negative impact of increasing densities (from 1 larvae cm^{-2} to 10 larvae cm^{-2}) on survival was also reported by Opare et al. (2022) when rearing BSFL in round containers with a total area of 86 cm^2, even when feeding the larvae *ad libitum*. Survival rates decreased by approximately 10–12% when density was increased from 1 larvae cm^{-2} to 5 larvae cm^{-2} and from 5 larvae cm^{-2} to 10 larvae cm^{-2}.

Using slightly larger containers for larval rearing (502 cm^2), Jiang et al. (2022) tested densities based on the wet weight of the waste stream being treated, in this case, swine manure. The evaluated densities were represented by mass fraction based on pig manure and larval weight initially, with the amount of added larvae being 0.08%, 0.24% and 0.40% of the manure's mass in each treatment, with the exact number of larvae not being disclosed. The authors found similar trends in comparison to the studies mentioned earlier, with lower individual weight and higher yield at higher densities. In a more realistic set-up, Miranda et al. (2020) used BSFL for bio-digesting manure (swine, dairy and poultry) in experimental units of 2289 cm^2, at a density of 4.37 larvae cm^{-2}, resulting in individual larval weights above 150 mg, comparable to their control reared on the Gainesville diet. Extreme values of individual weight can be obtained when either low or high densities are used. For instance, Lalander et al. (2019) reared BSFL using multiple substrates at a density of 0.6 larvae cm^{-2} and registered individuals weighing more than 300 mg after 10–12 days when fed abattoir waste and human faeces, while Opare et al. (2022) obtained larvae that did not reach 80 mg when reared at a density of 10 larvae cm^{-2}.

Opare et al. (2022) suggested that there is a need to find a balance between the trade-offs (biological and economical) when rearing insects under varied densities and conditions. Lopes et al. (2023) reached a similar conclusion when investigating the individual and combined effects of substrate properties and rearing conditions on process efficiency in BSFL rearing across various substrates. They examined the influence of varying feed doses (0.1–0.5 g volatile solids per larvae) and densities (3.03–8.33 larvae per cm^2), resulting in distinct depths within the rearing boxes (1.0–6.5 cm) and assessed their impact both individually and in combination. While a treatment with a medium depth (around 3 cm) and a medium larval feed dose (approximately 0.2 g of volatile solids per larvae) was suggested as a good starting point when initiating BSFL rearing on a new substrate, these factors may need adjustment to account for the specific characteristics of the substrate. In addition, Yakti et al. (2022) demonstrated significant differences in larval composition when using rearing boxes of varying size (2060, pp. 964 cm^2, 466 cm^2 and 194 cm^2), despite maintaining consistent process parameters across the scales. These differences were attributed to the elevated temperatures reached in the largest boxes, which exceeded 39°C, while it did not surpass 35°C in the other sized boxes.

Density is often discussed as a matter of welfare in livestock production. According to Dossey et al. (2016), the adoption of high larval densities is not problematic when it comes to fly larvae, as insects generally 'live in large groups in small amounts of space' in nature. However, this argument is not sufficient to justify rearing the insects without considering their behaviour and performance. It is known that under certain conditions (e.g. poor ventilation, uncontrolled moisture content and blends of substrates), temperatures inside rearing units can easily surpass 55°C (personal communication), which can result in reduced process efficiency. In addition, Vogel et al. (2022) suggested that high densities might increase the risk and spread of infectious diseases, as observed for other insect species (e.g. *Drosophila melanogaster*), even though the authors did not present direct evidence for BSFL.

4 Parameters affecting larval rearing efficiency: substrate depth

Some of the studies cited earlier failed to provide crucial information on the experimental set-up such as substrate moisture level, properties of the substrate at the end of the bioconversion process (whether it could be sieved or not), physical traits such as bulk density and the presence of resistant fibres, and substrate pH at the beginning and during the trials. A key factor that is known to interfere with larvae performance over time is the depth to which the substrate is placed in the treatment boxes. Industrial rearing of BSFL typically involves 1–3 feedings provided throughout the bioconversion process, and the feed substrate depth changes over time with such feeding regimes (personal communication).

Dortmans et al. (2017) suggested that substrate depth should not exceed 5 cm, in order to guarantee the larvae will reach the bottom of the treatment unit and process all provided material. Brits (2017) evaluated substrate depths of 5–20 cm and demonstrated that BSFL did not access the substrates in the bottom of cylindrical containers of 11.2 cm diameter and 28.2 cm height. Larvae survival was highly affected when depth increased, decreasing from around 95% (5 cm depth) to about 50% (20 cm depth), and consequently, the waste reduction and bioconversion of the feed substrate were reduced, demonstrating considerable loss of efficiency.

Abduh et al. (2018) evaluated the conversion of Philippine tung seed by BSFL at varying depths of 4, 6, 8 and 10 cm, in small-scale experimental units (416 cm^2). The authors reported that up to 6 cm depth, larval productivity was good (increasing by 56% from 4 cm to 6 cm depth); however, once the depth increased to 8 cm, it was significantly reduced by around 16%. When the substrate depth increases, larvae tend to place their head part into the material to allow their posterior spiracles to stick out of the material to improve their breathing, limiting their movement to the substrate surface, resulting in the material at the bottom of the container being left undigested, as reported by Lalander et al. (2020). In addition, greater depths (>5 cm) can result in anaerobic conditions, especially at the bottom of the container, which not only causes undesirable odours due to possible anaerobic decomposition but also affects the bioconversion process as a whole (Barrett et al., 2023).

Achieving an adequate substrate depth is challenging, especially when considering that the composition of feed substrates may change over time and that multiple feeding occasions increase the cost of treatment. An interesting observation was made by Bekker et al. (2021) when treating chicken feed at varying moisture contents (45–85%) with BSFL. At 45% moisture, the chicken feed would form lumps at the surface of the boxes, resulting in the larvae crawling to the bottom half; on the other hand, at 55–65% moisture, the chicken feed formed a crust at the surface and larvae were found at all depths. Conversely, at 75% moisture, a thin water film was observed on the surface of the chicken feed and larvae were predominantly in the upper half of the box. The specific moisture content at which these observations become apparent may vary in different feed substrates, but the general trend is likely to be similar.

5 Parameters affecting larval rearing efficiency: substrate moisture content

The initial moisture content of the feed substrate greatly influences the efficiency of the process. It not only impacts upon efficiency but also the possibility of harvesting the larvae at the end of treatment. At a high initial substrate moisture content, the treatment residue at the end of the treatment may be too wet to use a dry separation technique (Lalander et al., 2020; Cheng et al., 2017; Dortmans et al., 2017). In addition, it must be noted that using high-moisture waste streams may result in the transportation of water (contained in the waste) to the treatment plant, as well as the need to evaporate significant amounts of water throughout the process, which places higher demands on the ventilation system (Lalander et al., 2020).

There is no 'ideal' initial moisture content of the feed substrate, but values ranging from 45% to 80% are considered adequate for good process efficiency. Generally, in a substrate with an initial moisture content over 80%, larvae development and survival might be hampered, and the processed (or not so well processed) substrate could end up being very humid, preventing dry separation (Cheng et al., 2017). However, once effective ventilation is adopted, it becomes possible to treat substrates with water content exceeding 90%, even though it may sometimes not be feasible to do so, thus necessitating dewatering of the substrate (Lalander et al., 2020), or alternatives. It has been demonstrated that challenging wastes, such as faecal sludge from pit latrines with 90% moisture, can be bio-converted (material reduction above 60%) by BSFL without any dewatering step (Tokwaro et al., 2023). However, in that specific case, the authors did not mention the bioconversion efficiency or the sievability of the substrate at the end of the trial.

Varying substrate moisture contents between 45% and 75%, Bekker et al. (2021) demonstrated that BSFL growth and development remained unaffected, even though other process parameters were impacted. For instance, low moisture content promoted microbial degradation of the substrate and resulted in shorter larval growth time and small prepupae compared to those in higher moisture conditions. Similarly, Dzepe et al. (2020) found that higher moisture content (40–80%) led to longer BSFL development time (12.2–18.8 days) and a final larval weight of 0.12–0.19 g larva^{-1}. In addition, Liu et al. (2023) demonstrated that larvae pupation depended on both substrate type and moisture content, with increased pupation being observed in substrates with a higher moisture content (>60%).

Substrate moisture content also correlates closely with the capacity for treating varying volumes of waste within the same space. This is particularly important in upscaling; e.g. treating 200 kg of waste per day within a given area differs significantly from treating 500 kg in the same space. As demonstrated by

Lalander et al. (2020), the higher the initial moisture content, the higher the water evaporated from the substrate, with up to 840 g of water having to be removed from every kilogram of the substrate during the bioconversion process of very wet substrates. This means that more powerful ventilation systems are required for upscaling processes, or effective dewatering processes are needed, which both will depend on evaluating the economic and technical feasibility of the process as a whole.

6 Nutritional composition of feed substrates: protein, carbohydrate and lipid content

BSFL have been reported to thrive on a wide range of feed substrates, from feed mixtures (e.g. chicken feed and grain mixtures) to waste streams with varied and heterogeneous composition. BSFL can digest almost any organic material due to their high plasticity in terms of nutrient utilization and developmental time. As a result, most organic waste streams can serve as feed substrate for BSFL, regardless of how challenging they are in terms of bioconversion efficiency.

Nutritional balance is required for all livestock production – including insects. As extensively discussed by Seyedalmoosavi et al. (2022), both the quantity and quality of a specific dietary component are crucial in larval development and bioconversion efficiency, as well as the availability of nutrients in the larval diet. Even though BSFL are highly plastic in converting feed substrates of varying composition (Barragan-Fonseca et al., 2021), there are a number of known thresholds affecting how much of each dietary compound (e.g. proteins, carbohydrates and lipids) should be provided to maintain good process performance.

Considerable research has been directed to understand the protein (Pr) and carbohydrate (CHO) requirements of BSFL. Barragan-Fonseca et al. (2019) investigated multiple diets containing 10–24% Pr and 35–55% CHO and various combinations. In another study, the same authors evaluated 25 artificial diets with varying Pr+CHO and Pr:CHO proportions (Barragan-Fonseca et al., 2021). Both studies emphasized that the adequate dietary balance of Pr and CHO enabled better larval performance compared to diets based on Pr:CHO ratios. This means that by separately respecting the limits for both Pr and CHO, an efficient BSFL-rearing process – in terms of bioconversion efficiency, yields, larval growth and survival – can be achieved. The authors also highlighted the great plasticity that BSFL have when consuming feed substrates, as they thrive on diets containing Pr levels ranging from 10% to 15% and CHO levels from 10% to 60%. Fuso et al. (2021) demonstrated that once the minimal critical amount of Pr in the larval diet is reached, there are no advantages to increasing the Pr in the larval diet, as the process performance will not increase while its related costs might.

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Barragan-Fonseca et al. (2018, 2019) showed that the higher the Pr content in the larval diet, the higher the protein and fat accumulation in the larvae. Lopes et al. (2020b) also reported that even small inclusions of fish to bread waste resulted in higher Pr accumulation in the BSFL, while (Ewald et al., 2020) observed that larvae accumulated more fat when fed diets with higher Pr content. Gold et al. (2020) found that larvae reared on vegetable canteen waste containing $12\%_{DM}$ Pr had a Pr content of $25\%_{DM}$, while the Pr content of BSFL reared on mixed canteen waste with a Pr content of $32\%_{DM}$ was $36\%_{DM}$. However, the biomass conversion efficiency when rearing the larvae on vegetable canteen waste was considerably higher than when rearing them on mixed canteen waste: $23\%_{DM}$ compared with $15\%_{DM}$. This could be explained by the observation made by Lalander et al. (2019): when it comes to larval development time, both the daily feeding rate of Pr and CHO are important, while only the CHO daily feeding rate matters when it comes to the final larval weight. The larvae reared on a CHO-rich and Pr-poor substrate (vegetable waste) grew quickly but took longer to turn into prepupae, probably because they had to accumulate sufficient Pr. Maximizing biomass conversion may thus not always yield the highest value, if the BSFL produced has a low Pr content.

It is important to note that yields may vary depending on the quality of the feed substrate and other compounds in the substrate. It is possible to produce BSFL with a 'tailored composition', as demonstrated by Barroso et al. (2019). The authors replaced a control (chicken feed-based) diet with fish waste over varying periods of time, meaning that some larvae consumed fish for 1 day, some for 4 days and others for the entire process period of 12 days. It was observed that the treatments with most fish waste resulted in lower Pr accumulation in the larvae, while treatment with lower inclusions (i.e. providing fish waste to larvae for shorter times (1–4 days)) resulted in higher Pr accumulation. The authors did not report the bioconversion efficiency or the sieving performance for larval separation, which can sometimes be affected by an unbalanced addition of substrates such as fish waste (Lopes et al., 2020b).

While most studies on BSFL nutrition focus on Pr and CHO and the interaction between these two factors, a very limited number of studies have focused on lipids. It is known that larval development can be shortened by providing lipid-rich diets to BSFL (Nguyen et al., 2013) and that lower-lipid diets can reduce fly emergence rates (Bellezza Oddon et al., 2022a). A thorough evaluation of lipid requirements by BSFL was conducted by Bellezza Oddon et al. (2022b), in which inclusion levels of dietary lipids were varied from 1% to 4.5% (dry basis). The authors found that a 1% level of lipids on a dry matter basis had a negative impact on growth performance resulting in the smallest larval size and longer development times, while a 4.5% level yielded better growth performance. However, the authors recommend further investigation of lipid levels exceeding 4.5%.

While balancing nutrients can be a strategic approach for producing BSFL of tailored composition, treating wastes of varying composition over time can be quite challenging. Nonetheless, there are ways of tackling such challenges. Gold et al. (2020) evaluated several waste streams (mill by-products, canteen waste, human faeces, poultry slaughterhouse waste, cow manure and vegetable waste) as feed substrate, either alone or in combination, with combinations achieving a Pr:CHO ratio of approximately 1:1. They found positive correlations between waste reduction and bioconversion efficiency with dietary lipids and negative correlations between the same process parameters and fibre dietary content. Their general conclusion was that blending different waste streams achieves higher bioconversion than treating materials alone and that a simplistic approach, such as balancing nutrients and other dietary compounds, can significantly increase the predictability of the bioconversion process, which is the target of every enterprise working with insect production. Similar findings were made by Isibika et al. (2021), who found that the biomass conversion of a banana and orange peel mixture could be more than doubled by including 25% fish waste. No improvement was found when altering the Pr:CHO ratio from 0.4 (25% fish waste inclusion) to 0.9 (50% fish waste inclusion), supporting the findings of Fuso et al. (2021).

7 How to deal with 'poor' substrates

Many suboptimal waste substrates for rearing BSFL have been explored as feed substrates. The reason why the substrates are not ideal for BSFL rearing can be substrates characterized by high lignocellulosic content (e.g. wheat straw and cattle manure) and/or are low in protein (e.g. fruit peels and vegetable wastes). Bioconversion efficiencies are quite low for these sort of waste streams, $<5\%_{DM}$ for brewery waste, wheat straw, sugar cane bagasse and orange and banana peels (Theron, 2022; Isibika et al., 2021; Liu et al., 2018), compared with more promising substrates, such as food waste, with conversion efficiencies $>20\%_{VS}$ (Lindberg et al., 2022a). Lignocellulosic substrates have a high lignin, hemicellulose and crystalline cellulose content that are difficult to degrade, which means larvae are unable to assimilate nutrients and convert them into their own biomass (Peguero et al., 2022).

In order to improve the bioconversion of these waste streams, different physical, chemical and biological pre-treatments have been assessed, aiming at degrading lignin and hemicellulose and reducing the crystalline structure of cellulose. Physical pre-treatments include heat treatments, with and without pressure, and mechanical pre-treatments, such as grinding, which is used to reduce particle size and increase porosity. Grinding is a widely used pre-treatment that is generally incorporated in most BSFL treatments, even for more suitable substrates (Dortmans et al., 2017).

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Heat treatments have not shown promising results for BSFL applications. Isibika et al. (2019) found that the biomass conversion efficiency of banana peels was reduced by almost 20% when heating banana peels to 120°C under 2 bar pressure for 1 h compared with the control and stipulated that it was due to the release of tannins. Theron et al. (2023) did not observe any reduction in conversion efficiency when steam pre-treating wheat straw and sugar cane bagasse at different temperatures, ranging from 140°C to 185°C, but also found no improvement. At 200°C and 215°C however, conversion efficiency was greatly reduced, probably due to increased levels of furfural acids in the pre-treated material. Lower temperature pre-treatments (around 50–60°C) may, however, be suitable prior to BSFL rearing.

When it comes to chemical pre-treatments, mostly alkaline pre-treatments have been assessed prior to BSFL rearing. The addition of $1\%_{WW}$ ammonia to banana peels under 1 week did not result in any significant improvement in conversion efficiency (Isibika et al., 2019). At an addition of $5\%_{DM}$ for 3 days, Peguero et al. (2023) demonstrated that the conversion efficiency of cow manure was halved, even though the fibre content was reduced. They concluded that ammonia pre-treatment was not suitable for BSFL rearing due to the toxic nature of ammonia at elevated concentrations.

Microbial pre-treatments have been shown to be more efficient prior to BSFL rearing. Isibika et al. (2019) demonstrated a great increase in the conversion efficiency of banana peels for both fungal (*Trichoderma reesei* and *Rhizopus oligosporus*) and microbial (isolated BSF gut bacteria) pre-treatments, but only for pre-treatments that lasted 14 days, with no improvement for the substrates pre-treated for only 7 days. In accordance with this, Mazza et al. (2020) demonstrated a 28.6% increase in BSFL weight gain and high material reduction rates when rearing BSFL on chicken manure enriched with a $1\%_{WW}$ solution of companion bacteria isolated from the BSF egg surface (*Kocuria marina*, *Micrococcus luteus, Enterococcus faecalis, Lysinibacillus boronitolerans, Gordonia sihwensis* and *Proteus mirabilis*) and BSFL gut (*Bacillus subtilis*). Somroo et al. (2019) reported a considerable increase in material reduction and biomass conversion, as well as crude protein content of larvae, when co-digesting soybean curd with *Lactobacillus buchneri*. Microbial pre-treatments thus appear promising for achieving increased efficiencies when rearing BSFL on more difficult substrates. However, one has to consider the increased complexity of the treatment, as well as prolonged treatment times in some cases.

Another interesting route could be the use of enzymes, a commonly used pre-treatment for the hydrolysis of cellulose and hemicellulose in the lignocellulosic feedstock used in second-generation bio-ethanol production (Alvira et al., 2010). To date, little work has focused on enzyme treatment prior to BSFL rearing. Lindberg et al. (2022b) found that it was better to add enzymes directly to vegetable waste than an enzymatic pre-treatment prior to the addition of BSFL. With the direct addition of a cellulase enzyme cocktail (SAE0020 Sigma-Aldrich, 1%$_{WW}$), a 22% increase in conversion efficiency was achieved, while no improvements were found for 2–4 days pre-treatment. This could be an advantage, as no additional step would be necessary, lowering the complexity of the pre-treatment. However, the authors conducted the enzyme pre-treatment at 28°C, which appeared to favour microbial degradation of the material prior to BSFL addition. An enzyme pre-treatment step conducted closer to the optimal temperature of enzymatic hydrolysis of the selected enzyme could yield different results. Theron et al. (2023) conducted enzyme hydrolysis using a cellulolytic enzyme cocktail (Cellic® CTec3, 2 FPU g^{-1} DM) of steam-pre-treated wheat straw and sugar cane bagasse at 40°C for 21 h and found a great improvement in conversion efficiency. Despite these promising results, Theron (2022) concluded that the use of wheat straw and sugar cane bagasse as a feedstock for BSFL is not economically viable due to the high cost associated with required pre-treatments.

8 Sustainability issues in substrate use

As discussed, it is evident that BSFL are highly efficient at converting a wide range of organic waste streams, even though the process efficiency is influenced by multiple interconnected parameters that must be taken into account when aiming for a productive process that yields high-quality products (biomass and fertilizer). It is well known that the sustainability of insect production depends on the substrate on which the larvae are reared (Smetana et al., 2016). As discussed in the introduction, many organic waste streams are not yet permitted as feed substrates for insects. Due to legislative constraints, the insect sector in the EU only has access to approximately 30% of the food waste generated within the EU, with a great share of these streams already having ‘traditional’ destinations (e.g. direct use as animal feed, such as brewery spent grains). This competition for legal waste streams complicates their use by the insect industry and may lead to an added cost for utilizing more promising substrates.

Many insect producers come from an animal feed production perspective, viewing the insects as mini-livestock, with the aim of attaining a high-quality product of predictable composition. They accomplish this by using substrates that can be used directly as food or animal feed (more heterogeneous waste materials) with the complementary use of by-products of added value, such as commodities (e.g. wheat bran or soybean bran) (Rumbos et al., 2020), to balance diets and ensure an efficient bioconversion process (personal communication). This means that insect breeders currently have considerable control over the process, but the environmental sustainability of the process is low (Fig. 1).

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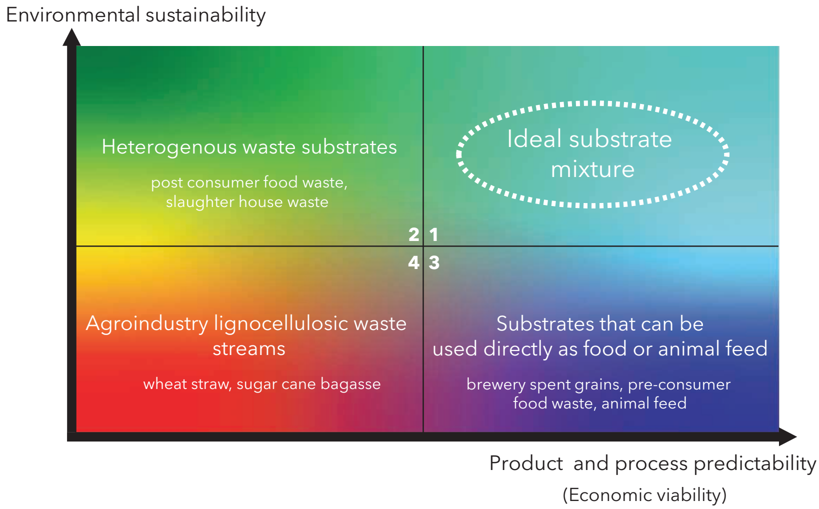

Figure 1 Representation of the equilibrium between environmental sustainability and predictability of the production of BSFL larvae with organic waste streams. Currently, the insect sector is mostly placed in quadrant '3', as only pre-consumer food waste and other high-grade substrates that could be directly used as animal feed are being utilized, resulting in the insect production process being less sustainable but highly predictable in terms of product quality and process performance. The streams represented in quadrant '2', such as post-consumer food waste, have more heterogeneous composition but its use represents lower environmental impact and higher sustainability, while quadrant '4' has streams that are difficult to handle with BSFL due to the high fibre content, which leads to low conversion efficiency. Direct gains in environmental sustainability might represent losses in predictability; however, by mixing feed substrates of varying composition and carrying out the bioconversion process considering all pertinent factors could transfer the industry to higher degrees of sustainability, while maintaining good predictability, represented by quadrant '1'.

Bosch et al. (2019) compared the global warming potential (GWP) of BSFL reared on feed, food or waste substrates and highlighted that when larvae are reared on feeds such as the Gainesville diet (combining water, wheat bran, maize flour and alfalfa), chicken feed or processed wheat, the GWP of larvae meal can be even higher than that of fish and soybean meal. However, the authors assumed low land use values for the latter two and did not consider biodiversity losses in their sustainability assessment, even though both the use of fishmeal and monoculture soy production contributes considerably to biodiversity loss (Green et al., 2019; Ortuño Crespo and Dunn, 2017). Nonetheless, Bosch et al. (2019) highlighted that when using residual streams such as food waste, manures or other animal-derived substrates, the GWP of BSFL production is significantly reduced. In summary, the use of varied waste streams that are currently unauthorized could significantly increase the sustainability of insect production in Europe, even though product and process predictability could be slightly reduced (Fig. 1). Based on the quantities of available food waste in the EU, the insect industry will only thrive, in the sense of having competitive volumes of products, if the use of currently unauthorized wastes is revised (Lalander and Vinnerås, 2022).

9 Safety issues in substrate use

It is noteworthy that the existing barriers to using animal by-products as feed for BSFL were established to reduce the risks of recycling pathogens and other contaminants when recirculating such materials within the food chain (Boqvist et al., 2018). These barriers became even stricter because of the risks associated with these wastes containing prions related to prion disease, which, in turn, have negatively affected the possibility for recirculating food waste into the food chain (Vågsholm et al., 2020b). It is critical to ensure good hygiene in circular systems such as the BSFL rearing systems to avoid recirculating contaminants with substrates. Many studies have assessed the safety potential of rearing BSFL using substrates contaminated with bacteria, fungi, toxins and even pharmaceuticals and pesticides.

BSFL are able to inactivate several microorganisms, such as *Salmonella* spp., *Escherichia coli* (Lopes et al., 2020a; Lalander et al., 2015) and bacteriophages (Lalander et al., 2013), as well as reduce the concentration of some pharmaceuticals and pesticides (Purschke et al., 2017; Lalander et al., 2016) and mycotoxins (Bosch et al., 2017; Purschke et al., 2017) in contaminated feed substrates. No studies on the impact on spore-forming bacteria have been conducted, but Van Looveren et al. (2022) found that *Clostridium perfringens* levels in the BSFL were below the detection limit, even though it was present in their feed substrate. When it comes to more stable biological contaminants, such as helminth eggs, it has been demonstrated that BSFL treatment has no impact on their survival (Lalander et al., 2013, 2015a). Thus, even though BSFL treatment can improve the hygienic quality of the treated substrate, the adoption of post-treatments of final products (larval biomass and frass) is necessary to guarantee the safety of use, as is the case for many other feed products and fertilizers available on the market, including PAPs and manures.

When it comes to more persistent contaminants, such as heavy metals, it has been demonstrated that cadmium and lead are bio-accumulated in BSFL (Purschke et al., 2017), while mercury, for instance, can be bioaccumulated by BSFL but rapidly eliminated from the larvae after a short fasting period (Cardoso et al., 2023). van Der Fels-Klerxet al. (2020) investigated the fate of various chemical contaminants found in bio-wastes of European food waste and found that some of these chemicals were bio-accumulated in the BSFL. However, none accumulated to a level that exceeded the legal thresholds in the EU. The only way to guarantee the quality of products in a circular system is by guaranteeing the quality of the substrate flowing into the system. Substrates with excessive levels of persistent contaminants, such as heavy metals, are not suitable for insect rearing. In this sense, up-stream work is crucial for ensuring sufficiently high quality of the food waste substrates. When it comes to persistent biological contaminants, no studies to date have been conducted on the fate of prions during BSFL rearing. This research is crucial in order for insects to truly unlock the potential of insect-assisted nutrient recycling in Europe. The limitations imposed by the EU and other countries around the world, preventing the use of post-consumer food waste and other animal-containing waste streams as feed substrate for BSFL, are hampering the growth of the insect industry and its competition with less sustainable feed sources, such as fishmeal and soybean meal. Once these substrates are allowed to be used by insect breeders, it will be possible to substantially increase the environmental sustainability of the process, ensuring the provision of high-quality products for the consumer market.

10 Future research trends

The effective bioconversion of different organic waste streams can be achieved by overcoming challenges such as choosing the right feed substrate and understanding how it should be provided to the larvae, as well as adjusting relevant variables within the process (density, feeding rate, substrate nutritional composition, among others). It is worth noting that such variables are always interconnected, making it nearly impossible to separate one from another. Many waste streams are not suitable for larvae alone (e.g. lignocellulosic wastes such as plant parts or brewery spent grains) but can be bioconverted by BSFL if blended with other waste streams or if pre-treatments are carried out, even though economic challenges can arise from the adoption of pre-treatments. Conversely, other waste streams that are more easily degraded by the larvae and are not yet legal as feed substrate in some countries (e.g. post-consumer food waste and slaughterhouse waste), despite their heterogeneity, could benefit the insect sector's environmental sustainability, in particular, if blended with other substrates to ensure product quality (reaching quadrant 1 in Fig. 1). In essence, the holy grail of insect production is to be able to produce insects from waste streams and in that way valorize the waste to improve waste management (Singh and Kumari, 2019), which in turn guarantees that the insect production is sustainable (Parodi et al., 2022), while at the same time producing a high-quality commodity (Lu et al., 2022) that can contribute to improved food security.

11 Conclusion

In this chapter, we have explored available substrates for BSFL rearing and outlined current understanding of how substrates impact both process efficiency and larval composition. Additionally, we have delved into the impact of other critical process parameters, including larval density, larval feed dosage and the depth at which the substrate is provided, on process efficiency and larval composition.

We presented possible strategies for handling nutritionally unbalanced substrates, including blending various waste streams or implementing pre-treatments to enhance nutrient availability for the larvae. Furthermore, we emphasized that substrate properties not only influence process efficiency and larval composition but also have a significant impact on the overall sustainability of the process.

Unfortunately for the European insect sector, currently permitted substrates result in larval biomass with a higher environmental impact compared to BSFL reared on substrates that are not allowed. However, true waste substrates (i.e. post-consumer waste streams) pose challenges due to their heterogeneity, which can lead to unpredictable product quality, and the risk of containing different pollutants.

The current challenge faced by the insect sector is to find ways to safely rear sufficient volumes of larval biomass of predictable quality and with low environmental impact.

12 Where to look for further information

International Platform of Insects for Food and Feed (IPIFF):

- https://ipiff.org/.

13 References

Abduh, M. Y., Nadia, M. H., Syaripudin, S., Manurung, R. and Putra, R. E. 2018. Factors affecting the bioconversion of Philippine Tung seed by black soldier fly larvae for the production of protein and oil-rich biomass. *Journal of Asia-Pacific Entomology* 21(3), 836–842.

Alvira, P., Tomás-Pejó, E., Ballesteros, M. and Negro, M. J. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology* 101(13), 4851–4861.

Barragan-Fonseca, K. B., Dicke, M. and Van Loon, J. J. A. V. 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - a review. *Journal of Insects as Food and Feed* 3(2), 105–120.

Barragan-Fonseca, K. B., Dicke, M. and Van Loon, J. J. A. V. 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (Hermetia illucens). *Entomologia Experimentalis et Applicata* 166(9), 761–770.

Barragan-Fonseca, K. B., Gort, G., Dicke, M. and Van Loon, J. J. A. 2019. Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly Hermetia illucens. *Physiological Entomology* 44(2), 148–159.

Barragan-Fonseca, K. B., Gort, G., Dicke, M. and Van Loon, J. J. A. 2021. Nutritional plasticity of the black soldier fly (Hermetia illucens) in response to artificial diets varying in protein and carbohydrate concentrations. *Journal of Insects as Food and Feed* 7(1), 51–61.

Barrett, M., Chia, S. Y., Fischer, B. and Tomberlin, J. K. 2023. Welfare considerations for farming black soldier flies, Hermetia illucens (Diptera: Stratiomyidae): a model for the insects as food and feed industry. *Journal of Insects as Food and Feed* 9(2), 119–148.

Barroso, F. G., Sánchez-Muros, M. J., Rincón, M. Á., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. and Guil-Guerrero, J. L. 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. *Journal of Food Composition and Analysis* 82, 103237.

Bekker, N. S., Heidelbach, S., Vestergaard, S. Z., Nielsen, M. E., Riisgaard-Jensen, M., Zeuner, E. J., Bahrndorff, S. and Eriksen, N. T. 2021. Impact of substrate moisture content on growth and metabolic performance of black soldier fly larvae. *Waste Management* 127, 73–79.

Bellezza Oddon, S., Biasato, I. and Gasco, L. 2022a. Isoenergetic-practical and semi-purified diets for protein requirement determination in Hermetia illucens larvae: consequences on life history traits. *Journal of Animal Science and Biotechnology* 13(1), 17.

Bellezza Oddon, S., Biasato, I., Resconi, A. and Gasco, L. 2022b. Determination of lipid requirements in black soldier fly through semi-purified diets. *Scientific Reports* 12(1), 10922.

Beyers, M., Coudron, C., Ravi, R., Meers, E. and Bruun, S. 2023. Black soldier fly larvae as an alternative feed source and agro-waste disposal route – A life cycle perspective. *Resources, Conservation and Recycling* 192, 106917.

Boqvist, S., Söderqvist, K. and Vågsholm, I. 2018. Food safety challenges and One Health within Europe. *Acta Veterinaria Scandinavica* 60(1), 1.

Bosch, G., Fels-Klerx, H. J. V. D., Rijk, T. C. D. and Oonincx, D. G. A. B. 2017. Aflatoxin b1 Tolerance and Accumulation in Black Soldier Fly Larvae (Hermetia illucens) and Yellow Mealworms (Tenebrio molitor). *Toxins* 9(6), 185.

Bosch, G., Van Zanten, H. H. E., Zamprogna, A., Veenenbos, M., Meijer, N. P., Van Der Fels-Klerx, H. J. and Van Loon, J. J. A. 2019. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. *Journal of Cleaner Production* 222, 355–363.

Brits, D. 2017. Improving feeding efficiencies of black soldier fly larvae, Hermetia illucens (L., 1758) (Diptera: Stratiomyidae: Hermetiinae) through manipulation of feeding conditions for industrial mass rearing. Ph.D. Diss. Stellenbosch University, Stellenbosch.

Cardoso, D. N., Silva, A. R. R., Morgado, R. G., Mostafaie, A., Pereira, A., Pinto, J., Lopes, I. G., Murta, D., Soares, A. M. V. M., Brooks, B. W. and Loureiro, S. 2023. Improving Product Safety for Edible Insects: toxicokinetics of Hg in Tenebrio molitor and Hermetia illucens. *ACS Food Science and Technology* 3(4), 790–798.

Cheng, J. Y. K., Chiu, S. L. H. and Lo, I. M. C. 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Management* 67, 315–323.

Chia, S. Y., Tanga, C. M., Osuga, I. M., Cheseto, X., Ekesi, S., Dicke, M. and Van Loon, J. J. A. 2020. Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products. *Entomologia Experimentalis et Applicata* 168(6–7), 472–481.

Čičková, H., Newton, G. L., Lacy, R. C. and Kozánek, M. 2015. The use of fly larvae for organic waste treatment. *Waste Management* 35, 68–80.

Dangles, O. and Casas, J. 2019. Ecosystem services provided by insects for achieving sustainable development goals. *Ecosystem Services* 35, 109–115.

Delvendahl, N., Rumpold, B. A. and Langen, N. 2022. Edible insects as food - Insect welfare and ethical aspects from a consumer perspective. *Insects*, 13(2), 121.

Dortmans, B., Egger, J., Diener, S., Verstappen, B. and Zurbrügg, C. 2021. Black soldier fly biowaste processing - A step-by-step guide, 2nd Edition Dübendorf: Eawag – Swiss Federal Institute of Aquatic Science and Technology Department of Sanitation, Water and Solid Waste for Development (Sandec).

Dossey, A. T., Tatum, J. T. and Mcgill, W. L. 2016. Chapter 5. Modern insect-based food industry: current status, insect processing technology, and recommendations moving forward. In: Dossey, A. T., Morales-Ramos, J. A. and Rojas, M. G. (Eds), *Insects as Sustainable Food Ingredients*. San Diego: Academic Press.

Dzepe, D., Nana, P., Fotso, A., Tchuinkam, T. and Djouaka, R. 2020. Influence of larval density, substrate moisture content and feedstock ratio on life history traits of black soldier fly larvae. *Journal of Insects as Food and Feed* 6(2), 133–140.

Ellen Macarthur Foundation 2015. Growth within: a circular economy vision for a competitive Europe. In: Gurewitsch, S. (Ed.). https://ellenmacarthurfoundation.org/growth-within-a-circular-economy-vision-for-a-competitive-europe: Ellen MacArthur Foundation, the McKinsey Center for Business and Environment (sponsered by Stiftungsfonds für Umweltökonomie).

European Commission 2009. Regulation (EC) No 1069/2009 of the European parliament and of the council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-Products Regulation). https://eur-lex.europa.eu/eli/reg/2009/1069/oj.

European Commission 2015. Closing the loop - an EU action plan for the circular economy. https://eur-lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-01aa75ed71a1.0012.02/DOC_1&format=PDF.

European Commission 2020a. Circular economy action plan - the European green deal. https://ec.europa.eu/environment/circular-economy/pdf/new_circular_economy_action_plan.pdf.

European Commission 2020b. Farm to fork strategy - for a fair, healthy and environmentally-friendly food system. https://food.ec.europa.eu/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf.

European Commission 2021a. Commission regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021R1372.

European Commission 2021b. Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions contingency plan for ensuring food supply and food

Published by Burleigh Dodds Science Publishing Limited, 2024.

security in times of crisis. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM:2021:689:FIN.

European Parliamentary Research Service 2023. EU feed autonomy - Closing the gaps in European food security. https://www.europarl.europa.eu/thinktank/en/document/EPRS_BRI(2023)739328.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. and Lalander, C. 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – possibilities and limitations for modification through diet. *Waste Management* 102, 40–47.

Fowles, T. M. and Nansen, C. 2020. Insect-based bioconversion: value from food waste. In: Närvänen, E., Mesiranta, N., Mattila, M. and Heikkinen, A. (Eds), *Food Waste Management: Solving the Wicked Problem*. Cham: Springer International Publishing.

Fuso, A., Barbi, S., Macavei, L. I., Luparelli, A. V., Maistrello, L., Montorsi, M., Sforza, S. and Caligiani, A. 2021. Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. *Foods*, 10(8).

Gold, M., Cassar, C. M., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S. and Mathys, A. 2020. Biowaste treatment with black soldier fly larvae: increasing performance through the formulation of biowastes based on protein and carbohydrates. *Waste Management* 102, 319–329.

Gold, M., Ireri, D., Zurbrugg, C., Fowles, T. and Mathys, A. 2021. Efficient and safe substrates for black soldier fly biowaste treatment along circular economy principles. *Detritus* 16, 31–40.

Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C. and Mathys, A. 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. *Waste Management* 82, 302–318.

Green, J. M. H., Croft, S. A., Durán, A. P., Balmford, A. P., Burgess, N. D., Fick, S., Gardner, T. A., Godar, J., Suavet, C., Virah-Sawmy, M., Young, L. E. and West, C. D. 2019. Linking global drivers of agricultural trade to on-the-ground impacts on biodiversity. *Proceedings of the National Academy of Sciences of the United States of America* 116(46), 23202–23208.

Guillaume, J. B., Mezdour, S., Marion-Poll, F., Terrol, C. and Schmidely, P. 2023. Asymptotic estimated digestibility, a new indicator of black soldier fly (Hermetia illucens) conversion efficiency in relation to larval density. *Journal of Insects as Food and Feed* 9(7), 893–906.

Hopkins, I., Newman, L. P., Gill, H. and danaher, J. 2021. The influence of food waste rearing substrates on black soldier fly larvae protein composition: a systematic review. *Insects* 12(7), 608.

Isibika, A., Simha, P., Vinnerås, B., Zurbrügg, C., Kibazohi, O. and Lalander, C. 2023. Food industry waste - an opportunity for black soldier fly larvae protein production in Tanzania. *Science of the Total Environment* 858(3), 159985.

Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C. and Lalander, C. 2019. Pre-treatment of banana peel to improve composting by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. *Waste Management* 100, 151–160.

Isibika, A., Vinnerås, B., Kibazohi, O., Zurbrügg, C. and Lalander, C. 2021. Co-composting of banana peel and orange peel waste with fish waste to improve conversion by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. *Journal of Cleaner Production* 318, 128570.

Jiang, D., Jiang, K., Li, R., Zhao, L., Liu, Z., Xiong, B., Jin, D., Hao, X., Zhu, L., Kang, B. and Bai, L. 2022. Influence of different inoculation densities of black soldier fly larvae

(Hermetia illucens) on heavy metal immobilization in swine manure. *Environmental Science and Pollution Research International* 29(36), 54378–54390.

Lalander, C. H., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A. and Vinnerås, B. 2013. Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens) – from a hygiene aspect. *Science of the Total Environment* 458–460, 312–318.

Lalander, C. H., Diener, S., Zurbrügg, C. and Vinnerås, B. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). *Journal of Cleaner Production* 208, 211–219.

Lalander, C. H., Ermolaev, E., Wiklicky, V. and Vinnerås, B. 2020. Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content. *Science of the Total Environment* 729, 138968.

Lalander, C. H., Fidjeland, J., Diener, S., Eriksson, S. and Vinnerås, B. 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development* 35(1), 261–271.

Lalander, C. H., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K. and Vinnerås, B. 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of the Total Environment* 565, 279–286.

Lalander, C. H. and Vinnerås, B. 2022. Actions needed before insects can contribute to a real closed-loop circular economy in the EU. *Journal of Insects as Food and Feed* 8(4), 337–342.

Lindberg, L., Ermolaev, E., Vinnerås, B. and Lalander, C. 2022a. Process efficiency and greenhouse gas emissions in black soldier fly larvae composting of fruit and vegetable waste with and without pre-treatment. *Journal of Cleaner Production* 338, 130552.

Lindberg, L., Vinnerås, B. and Lalander, C. 2022b. Process efficiency in relation to enzyme pre-treatment duration in black soldier fly larvae composting. *Waste Management* 137, 121–127.

Liu, Z., Minor, M., Morel, P. C. H. and Najar-Rodriguez, A. J. 2018. Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology* 47(6), 1609–1617.

Liu, Z., Morel, P. C. H. and Minor, M. A. 2023. Substrate and moisture content effects on pupation of the black soldier fly (Diptera: Stratiomyidae). *Journal of Insects as Food and Feed* 9(4), 415–425.

Lopes, I. G., Lalander, C., Vidotti, R. M. and Vinnerås, B. 2020a. Reduction of bacteria in relation to feeding regimes when treating aquaculture waste in fly larvae composting. *Frontiers in Microbiology* 11, 1616.

Lopes, I. G., Lalander, C., Vidotti, R. M. and Vinnerås, B. 2020b. Using *Hermetia illucens* larvae to process biowaste from aquaculture production. *Journal of Cleaner Production* 251, 119753.

Lopes, I. G., Wiklicky, V., Ermolaev, E. and Lalander, C. 2023. Dynamics of black soldier fly larvae composting – impact of substrate properties and rearing conditions on process efficiency. *Waste Management* 172, 25–32.

Lopes, I. G., Yong, J. W. H. and Lalander, C. 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. *Waste Management* 142, 65–76.

Lu, S., Taethaisong, N., Meethip, W., Surakhunthod, J., Sinpru, B., Sroichak, T., Archa, P., Thongpea, S., Paengkoum, S., Purba, R. A. P. and Paengkoum, P. 2022. Nutritional composition of black soldier fly larvae (*Hermetia illucens* L.) and its potential uses as alternative protein sources in animal diets: a review. *Insects* 13(9), 831.

Mazza, L., Xiao, X., Ur Rehman, K., Cai, M., Zhang, D., Fasulo, S., Tomberlin, J. K., Zheng, L., Soomro, A. A., Yu, Z. and Zhang, J. 2020. Management of chicken manure using black soldier fly (Diptera: Stratiomyidae) larvae assisted by companion bacteria. *Waste Management* 102, 312–318.

Miranda, C. D., Cammack, J. A. and Tomberlin, J. K. 2020. Mass production of the black soldier fly, Hermetia illucens (L.), (Diptera: Stratiomyidae) reared on three manure types. *Animals* 10(7).,1243.

Nguyen, T. T. X., Tomberlin, J. K. and Vanlaerhoven, S. 2013. Influence of resources on Hermetia illucens (Diptera: Stratiomyidae) larval development. *Journal of Medical Entomology* 50(4), 898–906.

Ojha, S., Bußler, S. and Schlüter, O. K. 2020. Food waste valorisation and circular economy concepts in insect production and processing. *Waste Management* 118, 600–609.

Opare, L. O., Holm, S. and Esperk, T. 2022. Temperature-modified density effects in the black soldier fly: low larval density leads to large size, short development time and high fat content. *Journal of Insects as Food and Feed* 8(7), 783–802.

Ortuño Crespo, G. and Dunn, D. C. 2017. A review of the impacts of fisheries on open-ocean ecosystems. *ICES Journal of Marine Science* 74(9), 2283–2297.

Parodi, A., Ipema, A. F., Van Zanten, H. H. E., Bolhuis, J. E., Van Loon, J. J. A. and De Boer, I. J. M. 2022. Principles for the responsible use of farmed insects as livestock feed. *Nature Food* 3(12), 972–974.

Parra Paz, A. S., Carrejo, N. S. and Gómez Rodríguez, C. H. 2015. Effects of larval density and feeding rates on the bioconversion of vegetable waste using black soldier fly larvae *Hermetia illucens* (L.), (Diptera: Stratiomyidae). *Waste and Biomass Valorization* 6(6), 1059–1065.

Peguero, D. A., Gold, M., Endara, A., Niu, M., Zurbrügg, C. and Mathys, A. 2023. Evaluation of ammonia pretreatment of four fibrous biowastes and its effect on black soldier fly larvae rearing performance. *Waste Management* 160, 123–134.

Peguero, D. A., Gold, M., Vandeweyer, D., Zurbrügg, C. and Mathys, A. 2022. A review of pretreatment methods to improve Agri-food waste bioconversion by black soldier fly larvae. *Frontiers in Sustainable Food Systems* 5.

Purkayastha, D. and Sarkar, S. 2022. Black soldier fly larvae for treatment and segregation of commingled municipal solid waste at different environmental conditions. *Journal of Environmental Management* 302(A), 114060.

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A. and Jäger, H. 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (Hermetia illucens) for use in the feed and food value chain. *Food Additives and Contaminants: Part A* 34(8), 1410–1420.

Rumbos, C. I., Karapanagiotidis, I. T., Mente, E., Psofakis, P. and Athanassiou, C. G. 2020. Evaluation of various commodities for the development of the yellow mealworm, Tenebrio molitor. *Scientific Reports* 10(1), 11224.

Sánchez-Bayo, F. and Wyckhuys, K. A. G. 2019. Worldwide decline of the entomofauna: a review of its drivers. *Biological Conservation* 232, 8–27.

Seyedalmoosavi, M. M., Mielenz, M., Veldkamp, T., Daş, G. and Metges, C. C. 2022. Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (Hermetia illucens) larvae compared to monogastric livestock species: a review. *Journal of Animal Science and Biotechnology* 13(1), 31.

Singh, A. and Kumari, K. 2019. An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: a review. *Journal of Environmental Management* 251, 109569.

Smetana, S., Palanisamy, M., Mathys, A. and Heinz, V. 2016. Sustainability of insect use for feed and food: life cycle assessment perspective. *Journal of Cleaner Production* 137, 741–751.

Somroo, A. A., Ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. and Zhang, J. 2019. Influence of Lactobacillus buchneri on soybean curd residue co-conversion by black soldier fly larvae (Hermetia illucens) for food and feedstock production. *Waste Management* 86, 114–122.

Theron, W. 2022. Steam pretreatment of wheat straw and sugarcane bagasse to produce feed for black soldier fly larvae. Master of Engineering. Stellenbosch University, Stellenbosch.

Theron, W., Teke, G. M., Diedericks, D., Van Rensburg, E. and Görgens, J. F. 2023. Steam pre-treatment of sugarcane bagasse and wheat straw as a cleaner feedstock for black soldier fly larvae rearing. *Journal of Insects as Food and Feed* 1–15.

Tokwaro, R., Semiyaga, S., Niwagaba, C. B., Nakagiri, A., Sempewo, J. I., Muoghalu, C. C. and Manga, M. 2023. Application of black soldier fly larvae in decentralized treatment of faecal sludge from pit latrines in informal settlements in Kampala city. *Frontiers in Environmental Science* 11.

United Nations Environment Programme 2021. *Food Waste Index Report 2021*. Nairobi, Kenya.

Vågsholm, I., Arzoomand, N. S. and Boqvist, S. 2020. Food security, safety, and sustainability—getting the trade-offs right. *Frontiers in Sustainable Food Systems* 4.

van Der Fels-Klerx, H. J., Meijer, N., Nijkamp, M. M., Schmitt, E. and Van Loon, J. J. A. 2020. Chemical food safety of using former foodstuffs for rearing black soldier fly larvae (Hermetia illucens) for feed and food use. *Journal of Insects as Food and Feed* 6(5), 475–488.

Van Huis, A. 2020. Insects as food and feed, a new emerging agricultural sector: a review. *Journal of Insects as Food and Feed* 6(1), 27–44.

Van Klink, R., Bowler, D. E., Gongalsky, K. B., Swengel, A. B., Gentile, A. and Chase, J. M. 2020. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* 368(6489), 417–420.

Van Looveren, N., Vandeweyer, D., Van Schelt, J. and Van Campenhout, L. 2022. Occurrence of *Clostridium perfringens* vegetative cells and spores throughout an industrial production process of black soldier fly larvae (*Hermetia illucens*). *Journal of Insects as Food and Feed* 8(4), 399–407.

Vogel, M., Shah, P. N., Voulgari-Kokota, A., Maistrou, S., Aartsma, Y., Beukeboom, L. W., Salles, J. F., Van Loon, J. J. A., Dicke, M. and Wertheim, B. 2022. Health of the black soldier fly and house fly under mass-rearing conditions: innate immunity and the role of the microbiome. *Journal of Insects as Food and Feed* 8(8), 857–878.

Woelber-Kastner, B. K., Frey, S. D., Howard, D. R. and Hall, C. L. 2021. Insect reproductive behaviors are important mediators of carrion nutrient release into soil. *Scientific Reports* 11(1), 3616.

WWF UK 2021. *Driven to Waste: The Global Impact of Food Loss and Waste on Farms*. WWF-UK.

Yakti, W., Schulz, S., Marten, V., Mewis, I., Padmanabha, M., Hempel, A.-J., Kobelski, A., Streif, S. and Ulrichs, C. 2022. The effect of rearing scale and density on the growth and nutrient composition of Hermetia illucens (L.) (Diptera: Stratiomyidae) larvae. *Sustainability* 14(3), 1772.

Yang, F. and Tomberlin, J. K. 2020. Comparing selected life-history traits of black soldier fly (Diptera: Stratiomyidae) larvae produced in industrial and bench-top-sized containers. *Journal of Insect Science* 20(5).

Yang, L. H. and Gratton, C. 2014. Insects as drivers of ecosystem processes. *Current Opinion in Insect Science* 2, 26–32.