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Effects of Intraspecific Competition and Larval Size on Bioconversion of Apple Pomace Inoculated with Black Soldier Fly

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Abstract: Waste from apple juice and cider industries (pomace) compares poorly against spent grains and other relatively high-nutrient wastes as a substrate for bioconversion by the black soldier fly (BSF: *Hermetia illucens*). However, global pomace production exceeds 24 million tonnes annually and novel management approaches are required to reduce waste to landfill. We examined the effects of BSF inoculation densities (intraspecific competition) and larval size categories on cohort weight gains and apple pomace waste reduction. We found that, by increasing larval densities, cohort biomass and bioconversion rates (BRs) increased; however, at very high densities (overcrowding), BRs declined and cohorts lost weight. Furthermore, larger larval size classes accelerated substrate desiccation, possibly because of greater demands for water by older larvae. Larger larvae have slower relative growth rates and BRs compared to smaller size categories and require comparatively less dry weight substrate. Our results suggest that overcrowding on low-nutrient substrates reduces BRs and could exaggerate differences between BSF relative performances in comparative studies, particularly if intraspecific interference competition for space and exploitation competition for water diminish BSF weight returns at the end of the bioconversion cycle. We make a series of recommendations for the use of BSF in pomace waste reduction.

Keywords: animal feed; black soldier fly; brewery; circular agriculture; decomposition; fertilizer; frass; orchard; recycling

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1. Introduction

Global apple production exceeds 80 million tonnes annually and has an estimated value of USD 14–15 billion [1,2]. About 25–30% of global production is used for processed products [3]. Many of these products are derived from raw apple juice, including filtered juices, apple juice concentrates, soft/sweet ciders (unfiltered apple juice), and hard ciders (fermented apple juice) [4,5]. The initial raw juice for all these products is prepared by grinding or macerating the apples and then pressing the ground apples under high pressure to extract the juice. After pressing, as much as 30% of the whole apple remains as a pomace by-product [6]. For industries that focus on apple juice or derived products, pomace management represents a considerable challenge. Over 20 million tonnes of apple pomace and 4 million tonnes of apple pomace sludge are produced each year (based on the 2008 global estimates presented by Dhillon et al. (2013) [7]). Much of this ends up as landfill [7]. However, the costs of food waste disposal and emerging legislation in many countries aimed at reducing waste to landfill [8–10] demand improved management and better utilization of apple pomace.

In the context of reducing waste to landfill, increased attention has been given to the possibilities of converting food wastes to animal feeds, proteins, and oils using decomposer insects. In particular, the black soldier fly (BSF: *Hermetia illucens*) has emerged as an efficient decomposer of wastes (including animal wastes, plant wastes, and manures) that can be easily reared at economic scales under both rustic and controlled conditions [11–14]. This tropical/subtropical fly has several advantages over other decomposers, such as mealworms or Muscidae flies: adult BSFs do not have biting or chewing mouthparts but instead have a sponge-like mouthpart [15] and, therefore, do not transmit diseases. The larvae are fast-growing and include a non-feeding, dispersing, prepupal stage that can be easily harvested (sometimes referred to as ”self-harvesting”). The late instar larvae have a high protein content (30–45% crude protein) and can be used as living or dried feed for fish, poultry, or pigs [16]. Other products, including chitins and insect oils, can also be extracted from the larvae for further industrial use [17,18], and the frass generated in production facilities can be used as an organic fertilizer [19–21] or animal feed supplement [22,23].

A number of studies have reported comparatively low bioconversion rates (i.e., substrate reduction per weight of larvae) for BSF on apples compared to other substrates [24–30]. However, the few previous studies that included apple waste reduction using BSF in comparative analyses with other relatively high-nutrient substrates did not adjust conditions (i.e., BSF densities, substrate moisture) for the different nutrient contents of the compared substrates. Some previous studies have documented the effects of intraspecific competition between BSF larvae. In general, although increasing competition reduced cohort weight gains, high densities were not associated with declines in waste reduction by BSF larvae [31–33]. However, most previous studies assessed the effects of BSF densities on high-nutrient substrates. In contrast, lower nutrient contents per standard substrate volume or standard substrate weight are expected to intensify intraspecific competition between BSF larvae [34], thereby further reducing cohort weight gains when compared to larvae on substrates with relatively high nutrient contents. Furthermore, the effects of intraspecific competition on low-nutrient substrates may be exacerbated where larvae display strong interference competition as opposed to exploitation competition because this reduces food consumption and, consequently, lowers the waste reduction efficiency of the BSF larvae.

In this study, we examined the effects of intraspecific competition between BSF larvae on apple pomace and determined optimal inoculation densities for bioconversion. In particular, we assessed whether intense competition could lower the observed waste reduction capacity of BSF larvae. To better estimate resource requirements during the bioconversion process, we also compared resource use and the bioconversion efficiency of BSF larvae as they grow and develop. To facilitate the interpretation of results and to support the development of pomace waste reduction systems, we quantified the production of pomace from three apple varieties and assessed water contents and drying rates. We also assessed larval growth and development on pomace, wheat bran, and a pomace–wheat bran mix to compare pomace against a relatively high-nutrient substrate and to facilitate comparisons with previous studies that included high-nutrient cereal-based diets [28–33]. We predicted slower BSF larval weight gains on pomace compared to bran or mixed substrates. We also predicted that intraspecific competition would reduce apple pomace waste reduction and that bioconversion efficiency would decline as larvae gained in size. To our knowledge, this is the first study to highlight the negative effects of overcrowding of BSF larvae on a low-nutrient substrate. Based on our results, we discuss the potential for the optimization of conditions for BSF use during pomace waste reduction among small and medium orchard businesses.

2. Materials and Methods

2.1. Soldier Flies

BSF eggs were obtained from a colony maintained by EcoLaVerna in Kildinan, Ireland. The colony was initiated two years prior to the experiments using ca. 1000 individuals originating from Hexafly, a commercial supplier based in Meath, Ireland. Adult flies were kept in a heated chamber (275 cm × 250 cm × 210 cm (L × W × H)) with natural lighting through a side window augmented with high-intensity indoor LED lighting. The chamber was dry heated to a temperature of 25–28 °C (40% relative humidity). Water was misted daily onto plastic sheets to provide the adults with drinking water. To obtain eggs and larvae, trays (20 cm × 20 cm × 7 cm (L × W × H)) of moistened (40% w/v) wheat bran (whole wheat (55%), wheat bran (5%)) were exposed in the chamber for 48 h. Strips of corrugated cardboard were laid across the open tops of the trays to encourage fly oviposition. The trays were placed inside screen boxes after 48 h to allow larvae to hatch and initiate feeding. As larvae grew and developed, they were transferred to larger plastic feeding boxes (42 cm × 27 cm × 35 cm (L × W × H)) and provided with food (moistened wheat bran) *ad libitum* (3 cm deep). Boxes were left open to avoid condensation and were each placed inside open skirting trays to collect any escaped larvae. The trays were checked daily, and dispersing larvae were returned to the main feeding boxes. Larvae were extracted from the feeding boxes using a soft paintbrush (early instars) or by sieving (later instars). All experiments were conducted in a second chamber (275 cm × 80 cm × 250 cm (L × W × H), 26–28 °C, 40% RH). This second chamber was kept in darkness during the experiments.

2.2. Rationale and Standardization of Experiments

Experiments were conducted to support the optimization of apple pomace waste reduction and bioconversion to BSF biomass and frass. In particular, we focused on the effects of intraspecific competition on bioconversion rates and on the effects on larval size categories on waste reduction. To further support the development of pomace-based bioconversion systems, we estimated pomace production for three apple varieties after juicing and recorded pomace water contents and drying rates in our experimental chamber. Water contents and drying rates were used to improve our estimates of bioconversion rates (see Section 2.7). Furthermore, we separated frass from larger residues (these were mainly dried apply skins) to improve our estimates of frass production during the bioconversion process. Bioconversion rates and observations related to other factors (i.e., mixing pomace with bran, substrate humidity, etc.) that improved bioconversion rates were noted during the experiments. Figure 1 indicates the relationships between the different experiments and measured parameters during the study.

All experiments were conducted using plastic recipients (17.2 cm × 9.0 cm × 4.2 cm (L × W × H) = 650 mL) that were open to avoid condensation; these were placed inside larger plastic boxes (42 cm × 27 cm × 35 cm (L × W × H)) to prevent larval escape. To avoid the effects of possible temperature and humidity gradients inside the experimental chamber, we used a block design for all experiments, with (randomized) replicated blocks each positioned at different shelf heights inside the chamber. Each experiment was replicated 5–6 times (indicated in the results) unless otherwise stated (i.e., insufficient materials were available in some cases—see below). To improve estimates of bioconversion efficiencies, we included non-inoculated pomace controls: these were used to assess water loss (wet weight loss) and possible substrate weight loss (dry weight loss) due to microbial decomposition during the experiments.

Unless otherwise stated, larvae were initially reared on a cereal-based diet until used in the experiments. All experiments were run for 7 days. At the end of each experiment, the larvae were separated from the pomace residue using a series of mechanical sieves and the larvae and residues were dried in a forced draught oven at 60 °C for 7 days. Drying the larvae and remaining substrate allowed us to estimate dry-weight bioconversion rates and, thereby, standardize for variable substrate water contents in different batches of pomace or different pomace ages. Further details of each experiment are described in Sections 2.4–2.6 below.

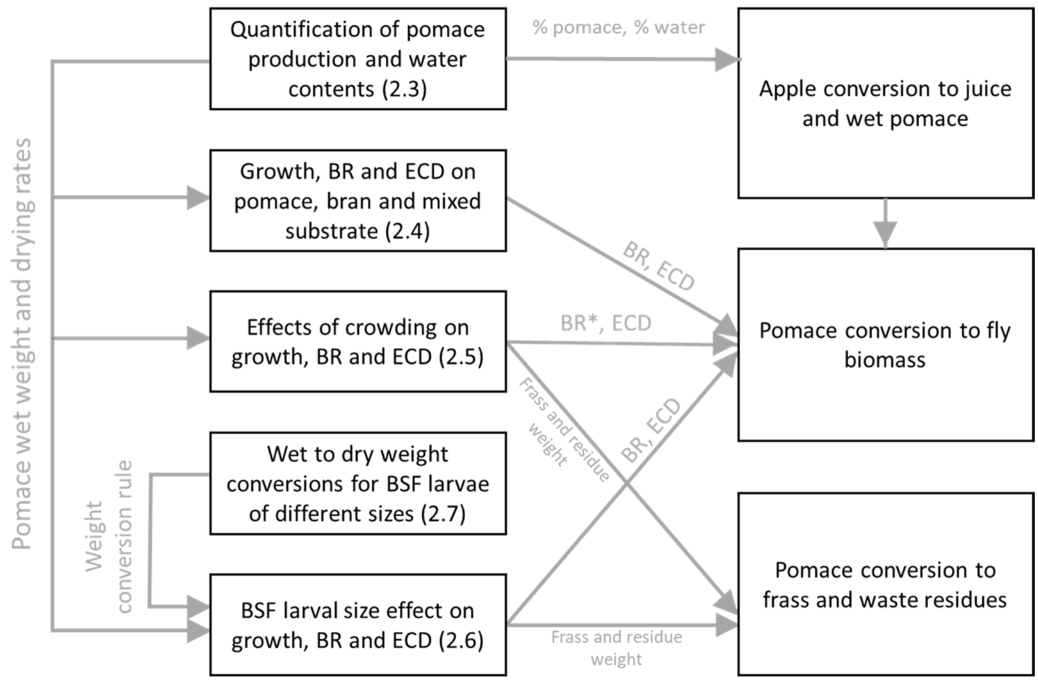

Figure 1. Connections between different experiments (indicated by section numbers in parentheses) and derived metrics (grey font) with final conversion parameters (larger rectangles) to support the design of BSF-based pomace waste reduction systems. BR = bioconversion rate, ECD = efficiency of conversion of ingested food, * = optimal values.

2.3. Pomace Preparation and Quantification

Golden Delicious, Granny Smith, and Bramley apples were acquired at local supermarkets. Golden Delicious and Granny Smith are sweet and sour apples, respectively, often consumed as cut slices, whereas Bramley apples are very sour and mainly used in cooking [35–37]. To prepare pomace, the apples were inspected to ensure there were no signs of spoilage, washed, and cut into quarters. Apples of each variety were bulked to 500 g batches (including skins and seeds) (N = 10). Before juicing, ca. 25 g of whole apple (as a narrow slice), for each batch of apples, was maintained to estimate the water content by drying it in a forced draught oven at 60 °C for 7 days and then reweighing it. The 500 g batches were processed using a household juicer (EK3454 600w Power Juicer, Salter, Manchester, UK) that ground and pressed the apples through a fine sieve to extract the juice and conveyed the pomace to a separate recipient. After juicing, the volume of juice derived from each 500 g batch was measured in a graduated cylinder and the weights of the juice and pomace were recorded using a digital balance. A 20 g sample of pomace was taken from each processed batch to estimate the pomace water content by drying it for 7 days in a forced draught oven at 60 °C and then reweighing it. The pomace produced from each batch of fresh apples was placed in plastic bags and immediately frozen at −20 °C until required for experiments. All juicer components were thoroughly cleaned after processing each 500 g batch and before processing any different varieties of apples. Materials trapped in the juicer after processing each batch were collected, dried, and weighed. To compare the drying rates of pomace from the three apple varieties and, thereby, facilitate the interpretation of results, 50 g of fresh pomace was place in 650 mL containers (3 varieties × 10 replicates = 30 containers) and exposed in the experimental chamber at 28 °C for 7 days. We used differential drying rates to select the most suitable variety (i.e., with the slowest drying rate) for experiments with larger larvae, which, compared to smaller larvae, were associated with higher rates of pomace water loss (see Section 2.6 below).

2.4. Effects of Mixed Pomace–Wheat Bran on Growth and Conversion Efficiency

To compare pomace as a substrate against relatively high-nutrient diets, three substrates were prepared for exposure to BSF larvae: (1) pressed pomace derived from Golden Delicious apples, (2) pressed pomace mixed with equal amounts of moistened wheat bran (2:1, bran:water), and (3) moistened wheat bran (2.5:1, bran:water). The mixed substrate was thoroughly homogenized. Portions of each substrate (50 g) were placed in the plastic 650 mL recipients and each was infested with 10 BSF larvae (5–7 days old). Pomace was misted with water each day after day 2 (5 mL day^{-1}).

2.5. Effects of Inoculation Density on Growth and Conversion Efficiency

Pomace was prepared from Golden Delicious and Granny Smith apples. The pomace was separated into 120 g portions (using approx. 500 g of fresh apples for each portion) and placed in plastic 650 mL recipients. Recipients were infested with 8–10 day old BSF larvae to achieve starting densities of 0.05, 0.1, 0.2, 0.3, and 0.4 g BSF per g pomace. The larvae were weighed on a digital balance to the required inoculation weights. Densities were replicated five times (except 0.4 g per g of Golden Delicious pomace, which had no replicate (N = 1)). Pomace was misted with water each day after day 2 (5 mL day^{-1}).

2.6. Conversion Efficiency Based on Larval Size Categories

The BSF larvae were collected from stock colonies of different ages and were separated by size. All larvae (irrespective of initial size) were initially reared on the cereal-based substrate for 3–5 days before switching larvae to a pomace substrate. This was done to ensure that differences in growth rates or bioconversion efficiencies were due to size categories and not because of differences in the time spent on the cereal-based substrate. Larvae were separated into four categories (<3 mm (8–10 days old), 3–8 mm (8–10 days old), 8–12 mm (14–16 days), and 12–15 mm (14–16 days)) using mechanical sieves. The BSF larvae were portioned into 1 g quantities and the numbers of larvae per g were counted. Due to the relatively rapid pomace desiccation when exposed to larger larval size categories, we used Bramley apple pomace in the experiment (because this dries more slowly—see below) and supplemented the pomace with fresh pomace at intervals throughout the experiment. At the start of the experiment, 50 g of pomace was placed in each 650 mL recipient and 1 g of fly larvae added. After 3 days and 5 days, a further 20 g of fresh pomace (Bramley) was added to each recipient and to the controls (total wet weight of pomace supplied = 90 g).

2.7. Calculating Conversion Efficiencies

We used the bioconversion rate (BR) and efficiency of conversion of digested food (ECD) to assess BSF larval performance on substrates. The BR is a measure of the proportional weight of larval biomass per substrate weight. The ECD is the gain in larval weight per reduction in substrate weight. To calculate the BR and ECD, we modified the formulas presented by Ribeiro et al. (2022) [30]. For our calculations, we used the dry weights of the starting and final substrates (i.e., before and after exposure to BSF, respectively). This was to standardize for differences between the moisture levels of different diets, as explained above. Furthermore, because we sieved residues to remove frass, we improved the accuracy of our estimated final-residue dry weights. To estimate biomass gained by BSF larvae in the experiments, we estimated the dry weights of the BSF larvae at the start of each experiment and dried and weighed the larvae at the end. This was undertaken to limit growth rates to within the time of the experiments and because, in the size category experiment, relatively large larvae were used in some treatments. To estimate larval dry weights from initial wet weights, we determined a conversion rule by measuring the wet and dry weights of larvae selected across a range of larval sizes from stock colonies reared on Bramley pomace.

2.8. Statistical Analyses

The production of pomace from different apple varieties and the effects of pomace or bran substrates and larval size classes on BSF weight gain and bioconversion were analysed using univariate general linear models (GLMs). For analysis of the effects of size classes, we initially included colony age (8–10 days or 14–16 days) as a blocking factor; however, this was removed because it had no effect on any of the measured parameters. Comparisons of growth rates and conversion parameters for apple varieties under a range of BSF starting densities were analysed using two-way GLMs. As frass wet weights and residue weights were not independent, when these waste components were measured, we used MANOVA to analyse treatment effects. Prior to analyses, percentages were arcsine-transformed and weights were log(x + 1)-transformed. Following analyses, residuals were plotted to check for homogeneity and normality. To further explore density effects on BSF weight gains and substrate reductions and to derive a formula for converting wet to dry larval weights, we used best-fit models.

3. Results

3.1. Apple Pomace Produced during Juicing

Between 26.1 and 30.6% of the wet weight (41.8 to 56.8% of the dry weight) of whole apples remained as pomace after juicing (Table 1). Although similar quantities of juice were extracted from the three apple varieties, juice weights were higher and pomace weights (% wet and dry weight of whole apples) were lower for Granny Smith apples than for the other varieties (Table 1). Bramley apples had higher water content than the other varieties and, although not statistically significant, resulted in wetter pomace after juicing. Bramley apple pomace maintained higher moisture content in the insect-rearing chamber (i.e., the water content of the exposed pomace after 7 days at 28 °C, Table 1).

Table 1. Details of water, juice, and pomace components of three apple varieties.

Processing Parameters	Bramley Apple (11) [2]	Golden Delicious (14) [2]	Granny Smith (13) [2]	F-Variety [3]
Water content of whole apple (%)	84.36 ± 0.41 [b]	82.48 ± 0.53 [a]	81.71 ± 0.18 [a]	10.300 ***
Juice volume (mL 1000 g^{-1})	599.64 ± 4.11	587.46 ± 10.73	595.25 ± 6.41	0.578
Juice weight (wet g 1000 g^{-1}) [1]	679.22 ± 5.20 [a]	698.27 ± 5.19 [a]	735.25 ± 6.14 [b]	25.230 ***
Pomace weight (wet g 1000 g^{-1}) [1]	306.26 ± 6.34 [b]	295.34 ± 5.57 [b]	261.73 ± 5.96 [a]	14.972 ***
Pomace water content (%)	71.20 ± 0.50	70.05 ± 0.55	70.83 ± 0.48	1.280
Weight lost in processing (wet g 1000 g^{-1})	50.81 ± 8.73 [b]	23.73 ± 7.08 [a]	12.56 ± 4.99 [a]	7.433 ***
Juice (% of wet weight of whole apple)	67.92 ± 0.52 [a]	69.83 ± 0.52 [a]	73.52 ± 0.61 [b]	25.232 ***
Pomace (% of wet weight of whole apple)	30.63 ± 0.63 [b]	29.53 ± 0.56 [b]	26.17 ± 0.60 [a]	14.963 ***
Processing waste with juicer (%)	5.08 ± 0.87 [b]	2.37 ± 0.71 [a]	1.26 ± 0.50 [a]	7.437 ***
Dry weight of whole apple (dry g/1000 wet g)	156.36 ± 4.11 [a]	175.21 ± 5.28 [b]	182.93 ± 1.80 [b]	10.299 ***
Dry weight of pomace (dry g/1000 wet g)	88.36 ± 2.93 [b]	88.71 ± 2.88 [b]	76.34 ± 2.16 [a]	7.014 ***
Pomace (% of dry weight of whole apple)	56.83 ± 2.20 [b]	51.27 ± 2.32 [b]	41.81 ± 1.33 [a]	13.627 ***
Water content of exposed pomace after 7 days at 28 °C (% of pomace) [4]	51.78 ± 0.30 [b]	45.19 ± 1.46 [a]	45.96 ± 0.44 [a]	16.884 ***

[1]: Corrected for processing losses based on proportions of juice to pomace weights; pomace indicated here was used in the experiments to assess larval density and size category effects on bioconversion; [2]: numbers are means ± SEM (numbers in parentheses are sample sizes; each sample consisted of 500 g of whole apple); lowercase letters indicate homogenous groups; [3]: DF = 2,38; *** = $p < 0.001$; [4]: pomace was exposed in the rearing chamber without BSF larvae.

3.2. Effects of Mixed Substrate on Growth and Conversion Efficiency

BSF weight gains were higher on the mixed pomace and bran substrate than on the other substrates (Table 2). Bioconversion rates were highest on substrates that contained pomace, with the bran-only substrate resulting in high residue weights (Table 2).

3.3. Effects of Inoculation Density on Growth and Conversion Efficiency

The absolute ($F_{1,36}$ = 13.185, $p < 0.001$) and proportional ($F_{1,36}$ = 10.326, $p < 0.001$) weight changes in BSF cohorts were greater on Golden Delicious pomace (Figure 2A,B; see growth rates in Table 3). Absolute weight changes (Figure 2A: $F_{4,46}$ = 8.107, $p < 0.001$) and proportional weight changes (Figure 2B: $F_{4,46} = 29.542$, $p < 0.001$) declined significantly at densities above 0.3 g g^{-1} (see best curve fits presented in Figure S1). At the end of the experiment, the reduction in pomace dry weight was similar for both varieties ($F_{1,36} = 1.703$, $p = 0.200$) and highest at densities of 0.2 and 0.3 g g^{-1} ($F_{4,36} = 4.662$, $p = 0.004$: Figure 2C). Residues consisted of larger particles where larvae were infested at the lowest density ($F_{4,36} = 17.736$, $p < 0.001$), irrespective of variety (Figure 2D: $F_{1,36} = 0.053$, $p = 0.820$). The weight of frass and particles < 1 mm increased linearly as BSF densities increased (Figure 2E: $F_{4,36} = 42.085$, $p < 0.001$). There was a significant variety × density interaction ($F_{4,36} = 10.466$, $p < 0.001$) because of higher production of frass/small particles on Granny Smith pomace by larvae at the two lowest densities, but similar production on pomace of both varieties at higher densities (Figure 2E).

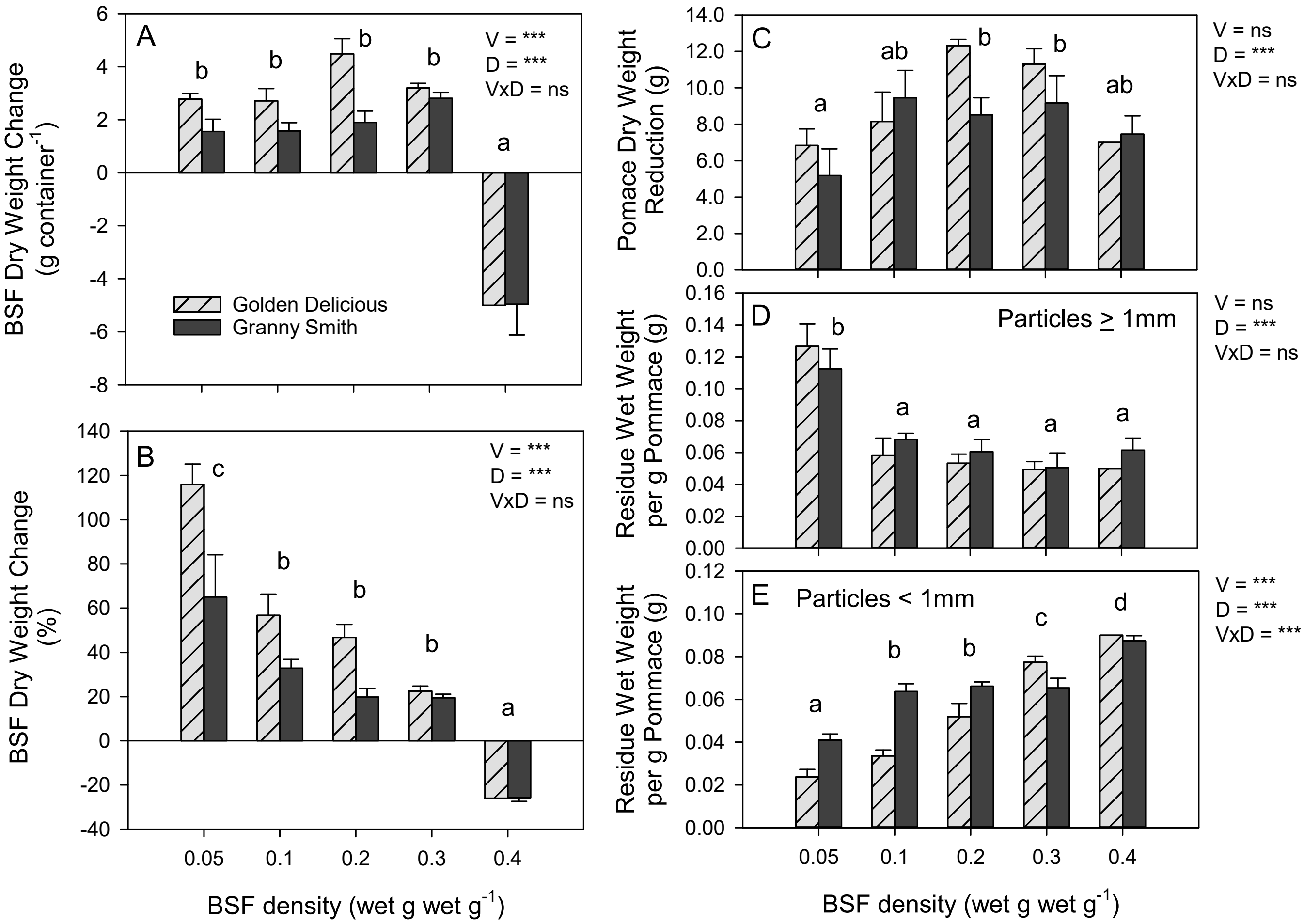

Figure 2. Absolute (**A**) and proportional (**B**) weight changes in BSF larval cohorts inoculated at 5 densities with 120 g of apple pomace (Golden Delicious = hatched, Granny Smith = solid). The experiment was initiated with 8–10 day old larvae. The estimated waste reduction (**C**) is presented with the make-up in residue as (**D**) large (≥1 mm) and (**E**) small (<1 mm) particles. The main results of the univariate GLMs are indicated, with V = variety, D = density, ns = no significant effect, and *** = $p < 0.001$. Lowercase letters indicate homogenous density groups. Standard errors are indicated (N = 5, except Golden Delicious at 0.4 g g^{-1}, where N = 1) (see also Figure S1 and Table 3).

Table 2. Survival and weight gain of BSF larvae and bioconversion efficiencies on pure and mixed apple and bran substrates.

Parameters	Apple Pomace [3]	Pomace + Bran [3]	Bran [3]	F-Values [4]
Water content (% of substrate) [1]	73.34	57.18	32.74	
Substrate weight lost in controls (% of substrate)	3.26 ± 0.32	6.93 ± 0.22	5.10 ± 1.13	3.294
BSF survival (%)	88.33 ± 5.43	98.33 ± 1.67	98.33 ± 1.67	2.857
BSF weight gain (dry mg container^{-1})	22.50 ± 3.68 [a]	59.88 ± 10.79 [b]	40.50 ± 3.94 [ab]	7.213 **
Average BSF weight gain (dry mg larva^{-1})	2.53 ± 0.34 [a]	5.93 ± 1.03 [b]	4.10 ± 0.36 [ab]	6.670 **
Larval growth rate (dry mg larva^{-1} day^{-1}) [2]	0.36 ± 0.05 [a]	0.85 ± 0.15 [b]	0.59 ± 0.05 [ab]	6.712 **
Residue dry weight (dry g container^{-1})	7.90 ± 0.04 [a]	18.99 ± 0.19 [b]	30.76 ± 0.26 [c]	3703.810 ***
Efficiency of conversion of ingested food (%) [2]	5.52 ± 0.95	2.41 ± 0.36	1.46 ± 0.19	2.455
Bioconversion rate (%) [2]	0.27 ± 0.04 [b]	0.28 ± 0.05 [b]	0.12 ± 0.01 [a]	5.176 *

[1]: Water content of substrate after exposure; [2]: see Section 2.7 for details of calculations; [3]: numbers are means ± SEM (N = 6), lowercase letters indicate homogenous substrate groups; [4]: DF = 2,15; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 3. Residue dry weight and bioconversion efficiencies of BSF larvae at varying densities on pomace from two apple varieties.

Variety	Starting BSF Density (wet g g^{-1}) [1]	Residue (Dry g) [2]	Larval Growth Rate (Dry mg Larva^{-1} Day^{-1}) [2,3]	Efficiency of Conversion of Digested Food (ECD) (%) [2,3]	Bioconversion Rate (BR) (%) [2,3]
Golden Delicious	0.05	13.30 ± 1.25 [b]	0.40 ± 0.03 [b]	11.23 ± 0.37	7.33 ± 0.59 [a]
	0.10	7.7 ± 1.11 [a]	0.39 ± 0.07 [b]	9.54 ± 1.53	7.43 ± 1.10 [a]
	0.20	9.32 ± 0.52 [a]	0.64 ± 0.08 [b]	15.61 ± 2.09	10.17 ± 2.13 [a]
	0.30	10.85 ± 0.41 [ab]	0.46 ± 0.03 [b]	12.37 ± 0.56	8.70 ± 0.36 [a]
	0.40	10.48 [ab]	−0.71 [a]	−18.51	−13.33 [b]
Granny Smith	0.05	12.18 ± 0.28 [b]	0.22 ± 0.07 [b]	6.61 ± 1.72	4.33 ± 1.17 [a]
	0.10	10.08 ± 0.65 [a]	0.23 ± 0.04 [b]	7.07 ± 1.47	4.93 ± 1.10 [a]
	0.20	10.36 ± 0.64 [a]	0.27 ± 0.06 [b]	7.55 ± 1.52	4.33 ± 1.17 [a]
	0.30	10.53 ± 0.31 [ab]	0.40 ± 0.03 [b]	9.87 ± 0.76	7.19 ± 0.54 [a]
	0.40	10.36 ± 0.14 [ab]	−0.71 ± 0.17 [a]	−27.28 ± 6.62	−17.37 ± 4.15 [b]
F-variety (V) [4]		0.519	7.244 **		7.787 **
F-density (D) [4]		8.531 ***	33.492 ***		28.939 ***
VxD [4]		1.911	1.418	3.942 **	0.603

[1]: 0.05 = 200–250; 0.1 = 400–450; 0.2 = 800–850; 0.3 = 1200–1300; 0.4 = 1700–1800 larvae per container; [2]: numbers are means ± SEM (N = 5, except Golden Delicious at 0.4 g g^{-1} where N = 1)), lowercase letters indicate homogenous density groups; [3]: see Section 2.7 for details of calculations; [4]: DF—variety = 1,46, density = 4,46, interaction = 4,46; ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Bioconversion rates were higher for Golden Delicious pomace and declined significantly at the highest larval densities (Table 3). There was a significant variety × density interaction between estimates of ECD because of similarly high efficiencies at 0.3 g g^{-1} and similarly low efficiencies at 0.4 g g^{-1} for both varieties, but there were lower ECDs for Granny Smith pomace at lower densities (Table 3).

3.4. Effect of Starting BSF Larval Size on Conversion Efficiency

The relation between the wet and dry weights of larvae across different size classes was best described by a quadratic function (Figure 3), indicating that larger larvae had proportionally higher water contents.

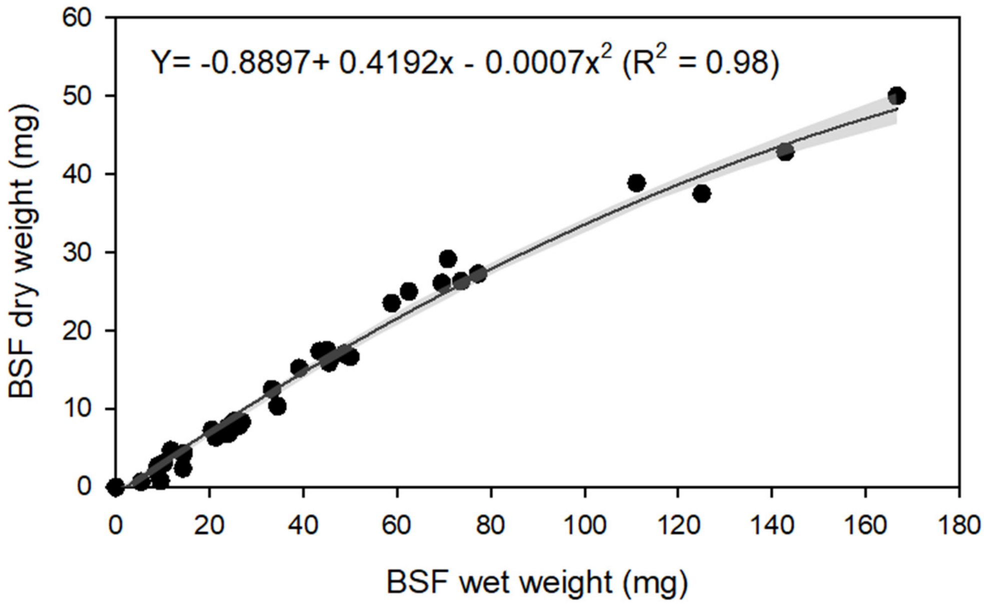

Figure 3. Relation between the wet and dry weights of BSF larvae. The equation was used to estimate the dry weights of starting BSF cohorts in the experiments. All larvae were reared on Bramley apple pomace. The conversion formula is presented. The shaded band indicates 95% confidence intervals.

Survival of larvae from the smallest size category was lower than those from the remaining categories (Table 4); however, inoculation with the smallest larvae resulted in the highest final BSF weights per container, the highest weight gains, and the fastest individual growth rates; in each case, the results were significantly higher than for the largest size category (Table 4). Consequently, the proportional weight gain, ECD, and BR estimates were significantly higher for small larvae compared to the large larvae (Table 4). Dry residue weights were similar across larval size categories; however, larger quantities of frass together with smaller waste particles were associated with the smallest larvae (Table 4). Although not significant, residue moisture content tended to decline as the average size of starting cohorts increased (Table 4).

Table 4. Growth and bioconversion rates for BSF cohorts of different larval sizes reared on apple pomace.

Parameter	**BSF Size [1] Category**				**F-Values [2]**
	Small	**Medium**	**Medium Large**	**Large**	
Number of larvae per wet g	370.33 ± 18.93	123.00 ± 9.55	44.38 ± 1.99	23.5 ± 0.72	
Size range (number per wet g)	300–460	100–160	40–60	20–30	
Survival (%)	88.02 ± 3.36 ^{a}	95.74 ± 1.89 ^{ab}	98.86 ± 1.40 ^{b}	96.44 ± 1.74 ^{ab}	4.448 *
Weight of survivors (dry g container^{-1})	0.86 ± 0.03 ^{b}	0.73 ± 0.12 ^{ab}	0.58 ± 0.08 ^{ab}	0.52 ± 0.06 ^{a}	3.767 *
Larval growth rate (g day^{-1})	0.12 ± 0.01 ^{b}	0.10 ± 0.02 ^{ab}	0.08 ± 0.01 ^{ab}	0.07 ± 0.01 ^{a}	4.132 *
Dry weight gain (dry g container^{-1})	0.86 ± 0.03 ^{b}	0.73 ± 0.12 ^{ab}	0.57 ± 0.08 ^{ab}	0.50 ± 0.06 ^{a}	4.017 *
Percentage weight gain (conainer^{-1})	180.00 ± 9.51 ^{c}	166.67 ± 11.16 ^{b}	91.67 ± 7.49 ^{a}	58.33 ± 6.01 ^{a}	48.480 ***
Weight of frass (wet g container^{-1})	3.88 ± 0.08 ^{b}	3.00 ± 0.45 ^{ab}	2.67 ± 0.42 ^{ab}	2.17 ± 0.17 ^{a}	5.061 **
Weight of larger (>1 mm) residue particles (wet g container^{-1})	11.38 ± 0.49	10.67 ± 0.71	12.33 ± 0.56	12.33 ± 0.42	2.116
Residue moisture content (% water)	35.29 ± 4.85	42.18 ± 7.10	21.87 ± 5.04	26.16 ± 4.16	2.85
Residue weight (dry g container^{-1})	10.22 ± 1.02	8.15 ± 1.35	11.89 ± 1.35	10.79 ± 0.90	1.78
Efficiency of conversion of ingested food (%) [3]	5.23 ± 0.41 ^{b}	4.27 ± 0.45 ^{ab}	3.69 ± 0.33 ^{ab}	3.07 ± 0.28 ^{a}	5.087 **
Bioconversion rate (%) [3]	3.17 ± 0.12 ^{b}	2.69 ± 0.45 ^{ab}	2.10 ± 0.28 ^{ab}	1.86 ± 0.22 ^{a}	4.061 *

[1]: Numbers are means ± SEM (N = 6), lowercase letters indicate homogenous size categories; [2]: DF = 3,24; * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$; [3]: see Section 2.7 for details of calculations.

4. Discussion

Compared to wheat bran, pomace was a poor substrate for developing BSF larvae in our experiments. However, mixing equal weights of pomace with bran resulted in the highest weight gains and growth rates in BSF larvae (Table 2). Therefore, incorporating apple pomace into mixed feeding streams with other, higher-nutrient substrates is potentially effective in reducing pomace waste and achieving relatively high production of insect biomass. Nevertheless, where options to mix feeds are not available—for example, in small orchards or integrated farms—our results suggest that pomace waste can be significantly reduced through exposure to BSF larvae and that waste reduction efficiency can be improved by avoiding overcrowding. We discuss the effects of intraspecific competition and overcrowding, as well as the effects of larval size categories, on pomace waste reduction and BSF weight gain in the following two sections. Finally, based on our quantification of pomace generated during juicing (Table 1), and considering conditions to achieve optimal bioconversion rates (Tables 2–4), we discuss the potential for orchard industries to employ BSF-based pomace waste reduction for insect biomass and frass production.

4.1. BSF Intraspecific Competition on Apple Pomace

Intraspecific competition significantly affected BSF weight gains and bioconversion rates (Figures 2 and S1). In our density experiment, larval weight gains were highest at the lowest densities and declined with increasing densities of conspecifics (Figure 2), as has been demonstrated in several previous studies [31–34]. However, despite the impact of crowding on individual larval weights, inoculating the pomace at intermediate densities was optimal because it resulted in the greatest gains in cohort biomass and the highest bioconversion rates (Table 3). For 8–10 day old larvae, the optimal density was about 0.2 g per g of substrate for 7 days (Figure 2). Such moderate crowding of larvae has further possible benefits; for example, at higher densities, substrate spoilage is reduced [38] and larvae accumulate less crude fat [33,34]. Furthermore, high densities may be associated with improved interspecific competition that reduces contamination of substrates with other decomposer flies. For example, a small number of studies suggest that BSF larvae reduce house fly (*Musca domestica*) oviposition and development on waste substrates [39–41]; however, this may be affected by the sequence of colonization [42].

Unlike some previous studies [31,32,34], our results indicated strong negative effects on bioconversion rates from overcrowding BSF larvae. We observed cohort weight losses and low bioconversion rates at the highest inoculation densities in our density experiment (Figure 2A). To the best of our knowledge, such weight declines have not been reported previously. However, many previous studies that compared BSF density effects on bioconversion used high-nutrient substrates, including chicken and dog feeds, or grain-based diets [31,32,43], and weight reductions are probably more prevalent where larvae are overcrowded on relatively low-quality substrates, such as apple pomace. This idea is supported by the results of Barragan-Fonseca et al. (2018) [34]. These authors compared the effects of crowding on substrates that varied from low to high nutrient contents (prepared by diluting chicken feed with varying amounts of cellulose). With bulk feeding (as in our experiments), cohort weight gains increased at increasing larval densities; however, when the larvae were fed based on visually assessed food requirements, the authors observed a significant interaction between density and food quality. At higher densities, cohort weight gains with a relatively poor diet were markedly reduced [34]. Our results extend these observations by indicating that overcrowding on a low-quality diet can also cause cohort weight losses, thereby producing stagnation in production gains and reducing bioconversion or waste reduction efficiency (Figure 2). These results suggest that comparative studies that do not adjust for the effects of overcrowding on poor-quality diets will underestimate the relative potential for BSF bioconversion of low-nutrient wastes. This may be exacerbated in studies that use sliced apples instead of pressed pomace [28,29] because lower surface area to volume ratios potentially increase negative intraspecific interactions.

Using a mixed fruit and vegetable substrate, Parra Paz et al. (2015) [38] also observed slightly better relative growth rates for BSF larvae at intermediate densities (although waste conversion was greatest at the highest density used in their experiments; i.e., 1 larva per 60 mg of substrate). We observed similarly improved growth rates in our density experiment (Table 3). Better growth at intermediate densities could be due to intraspecific facilitation during feeding. For example, when large numbers of BSF feed together, they generate heat, which can enhance growth performance [38]. However, the observations by Parra Paz et al. (2015) [38] could also be due to weight losses during periods of the bioconversion process when food availability declined (e.g., before adding the substrate sequentially or close to the end of the bioconversion process; see also Barragan-Fonseca et al. (2018) regarding visually assessed substrate addition [34]). This is further suggested by the declining cohort weight gains in our density experiments during overcrowding and further supports the idea that high larval densities should be avoided on relatively poor-quality substrates.

4.2. Growth Rates and Bioconversion Efficiencies as a Function of BSF Larval Size

Daily larval growth rates and cohort weight gains declined linearly with increasing larval size (Table 4). As pomace waste reductions were relatively similar across larval size categories, the higher weight gains of small larvae resulted in significantly higher BRs and ECDs when compared against larger larvae (Table 4). The greater conversion of food to insect biomass by smaller larvae also resulted in significantly greater frass (particles < 1 mm) production. Frass can be used as a rich organic fertilizer [19] or as a nutrient supplement for catfish [22,23].

Our results suggest that the reduction in bioconversion efficiency associated with increasing larval size may have been partly due to a greater loss in substrate moisture when pomace was exposed to large larvae. The optimal substrate humidity for developing BSF larvae is suggested to be about 52–70% [16]. Pomace has a water content of about 70% (Table 1), which is about the maximum substrate moisture content for rearing BSF larvae [16]. We found that, when exposed for 7 days in the heated chamber (without BSF larvae), the water content of pomace declined to about 45–52% (Table 1); when pomace was infested in trays with early instar larvae, moisture declined to 35–42%, but with older instars, it declined to as little as 21–25% (Table 4). This suggests that the larger larvae consumed more water. Indeed, we found a quadratic relation between the dry and wet weights of larvae as larval size increased (Figure 3), with larvae of >100 wet mg having relatively high moisture contents. It is possible that water and not nutrients represented a greater limiting factor for larger larvae in our experiments. The movements of larger larvae (interference competition for space) may also have exposed more of the pomace surface area to drying in the chamber, thereby further reducing the substrate moisture content.

Previous studies have manipulated the moisture contents of dryer substrates, such as grain-based or pelleted diets, by adding water [30,34]; however, manipulating moisture contents is more difficult when substrates are not homogenized or powdered or when they tend to clump, as occurs with apple pomace. To avoid high substrate water contents, we suggest that pomace could be aged for up to 72 h before exposure to BSF larvae. Furthermore, based on our results, to avoid excessive drying of the pomace substrate, we recommend continuous feeding systems as opposed to bulk feeding—in particular, for larger larvae or later instars. Adding the substrate sequentially based on apparent needs can reduce larval growth rates by increasing intraspecific competition [34]; however, by estimating relative cohort weight gains and bioconversion efficiencies over time (i.e., using the information in Table 4) and noting the changing food requirements of different larval instars (e.g., moisture requirements), periods of intense competition could be reduced (see below). Due to competition for water, particularly among older larvae, requirements for supplementary water should be assessed; in particular, toward the end of the bioconversion process.

4.3. Potential of BSF-Based Waste Reduction in Apple Orchards

Strong interference competition leading to cohort weight losses during overcrowding and declining conversion efficiencies as larvae age partly explain why low-quality substrates, such as pomace, have not been recommended for bioconversion to insect protein [28–30]. As indicated in this study, growth rates can be improved by adding high-nutrient substrates, such as wheat bran. However, for smaller-scale facilities, BSF larvae can still be effectively used to reduce waste volumes and convert pomace to frass and moderate amounts of insect biomass (Figure S2); in particular, if inoculation densities and size-specific nutrient and water requirements are optimized. Our density experiment suggested an optimum of 1 larva per 5 g (0.2 g mg^{-1}) of dry pomace for 8–10 day old larvae, which is considerably lower than the recommended BSF inoculation densities for relatively nutrient-rich substrates [16,32,34,43,44]. Although we did not examine the nutrient contents of the apples or the developing larvae, our results suggested that the amounts and combinations of sugars in sweet apples (i.e., Golden Delicious apples are sweeter than Granny Smith apples but have similar moisture contents (Table 1) [35–37]) improve BSF growth rates (Figure 2, Table 3). As the pomace from sour apples reduced larval weight gains and bioconversion rates in our density experiment, to further improve bioconversion, we recommend that pomace from sour apples should be mixed with sweet apples where possible.

Based on estimates of dry apple converted to dry insect biomass, we achieved bioconversion rates of over 10% at optimal larval densities, and the larvae produced a homogenous frass (Figure 2, Table 3). For comparison, Ribiero et al. (2022) [30] achieved bioconversion rates of between 2.5 and 4% for BSF apple pomace (based on wet weights). Based on estimated juice and pomace production during processing (Table 1) and estimated bioconversion rates at optimal densities (Table 3), we suggest that juice and cider industries could produce over 21 kg of BSF biomass and over 37 kg of frass per tonne of fresh apple (Figure S2). Importantly, pomace waste could be reduced by nearly 90% or 267 kg of wet waste per tonne of fresh apple (Figure S2). Correcting conservatively for moisture contents, we suggest that the apple bioconversion rates attained by Scala et al. (2020) [28] using industry-sized cohorts could be further improved by up to 50%. Yang and Tomberlin (2020) [44] suggest that benchtop studies such as ours are prone to underestimating biomass production by as much as 24% while overestimating bioconversion rates by about 3%; therefore we expect that even higher BSF larvae dry weights could be attained in larger-scale converter systems.

For industries that are highly specialized or where orchards produce their own juices or ciders [45], we suggest that BSF bioconversion of pomace could represent an effective waste management strategy and provide useful by-products (Figure S2). Further research is required to determine optimal blends of pomace with high-nutrient substrates, such as cereals, or optimal times at which cereal substrates could be added (e.g., during late instar stages) to reduce the costs of insect biomass production on predominantly low-nutrient substrates, such as apple pomace. Furthermore, larger-scale studies that approximate industrial facilities are required to assess the practicality and possible benefits of apple pomace-only bioconversion systems for small- and medium-scale orchard industries.

5. Conclusions

Our results suggest that comparative studies that do not adjust for the effects of overcrowding on poor-quality diets will underestimate the relative potential for BSF bioconversion of low-nutrient wastes, such as apple pomace. Unlike BSF larvae on high-nutrient substrates, the overcrowding of BSF larvae on apple pomace can lead to losses in cohort weight and a decline in waste reduction efficiency. Based on our results, we recommend that fresh pomace should be dried before exposure, particularly for early instar BSF larvae (to allow partial decomposition and reduce excessive moisture contents); that pomace should be added sequentially (to maintain substrate moisture above 40%); and that very high densities of larvae should be avoided. Under optimal conditions, BSF bioconversion of apple pomace could improve the efficiency of orchards or integrated farms by reducing waste via the production of BSF biomass and frass.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture13020452/s1, Figure S1. Absolute weight change for BSF larvae reared on apple pomace at five starting densities with the proportional changes in BSF weight. Figure S2. Schematic of the juicing process and pomace production with bioconversion and final BSF products as outcomes.

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