Production of high value protein feeds and fertilizer from pre-consumer vegetable waste utilizing a novel black soldier fly larvae conversion process.

Dr. Beth Mason

Verschuren Center for Sustainability in Energy & Environment

Cape Breton University.

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Background & Purpose

Large amounts of food are wasted from field to fork; a dilemma that has garnered much attention across the developed world, particularly at a time when there is much concern regarding how to feed the expected increased population of 9 billion by 2050, off a finite agricultural land base. The UN and FAO (2013) in particular have expressed grave concern on how food production can possibly meet these increasing demands with limited new agricultural land, increasingly limited resources (water), and diminishing biological limits on further enhancements of crop yield. Reductions in waste are an obvious start point particularly in countries where this figure is upward of 30 to 40% of food produced. However, there is always wastage from spoilage, culls, transport loss, and natural shelf life expiry. This is particularly true of fruit and vegetables where it is difficult to increase shelf life.

The proposed research program was designed to address the issues of unused vegetable waste that currently has little value or is disposed of at a cost to the processor. Vegetable waste finds little use due to its high moisture content and rapid degradation. This lends itself to composting, yet there is little outlet for the large volumes of resulting compost produced, which pose an environmental hazard due to leachate during storage. Currently many direct farm markets, such as Masstown market near Truro, and major grocery outlets such as Sobeys and Atlantic Superstores pay to dispose of organics waste and often a viable (cost effective) use is not within a reasonable transport radius. Additionally current outlets of dumping or composting show little to no value add proposition. This project will pilot a novel bio-conversion method of processing vegetable waste volumes to add value through generation of high value marketable output commodities, exploring challenges, process optimization and potential economics. The process utilizes technology being developed by a company in BC to convert pre-consumer waste through black soldier fly larvae production into high value protein meal, oil, and fertilizer products. These have an average potential value of between $1000-$1500 per tonne in local market outlets of animal feed, aquaculture and horticulture vs compost value at $50-100/t minus freight.

Black soldier fly larvae (BSFL) have been determined to be one of the potentially viable insect candidates for commercialization, due to the fact that it is native to N. America and has a short life cycle and prolific egg production. Information on the commercial production of BSFL is very new as it has received a flurry of interest in the last couple of years within the UN and EU as a means of increasing protein production to supply the growing demand for meat consumption in the developing world, but also to provide protein to the anticipated growing human population, anticipated to increase by 30% over the next 50 years.

The European Union is funding a large scale research program “PROteINSECT” to look into commercialization of production, regulatory framework, and safety. In Europe as with one company in Canada, recently a few companies have reached commercial scale production of insects for livestock feed markets. In each of these cases Black Soldier fly have been the insect of choice as the larvae grow rapidly, and the species in endemic to many parts of the world, plus the adults do not feed or bite, during their brief lifespan. Some smaller groups are focusing on production of crickets and meal worms.

There is a large variance in the limited literature available describing what the ideal production and environmental conditions for optimal growth and reproduction of BSF are, and of course nothing published of the limited successes at the commercial scale. However on an academic level there have been a number of studies examining the use of BSFL for consumption of hog manure (ref), dairy manure and even human waste. All have shown that it is possible to grow BSFL in a variety of media, but there are obvious sensitivities of the larvae to their environment which will be reflected in growth rate, feed conversion, duration to pupae stage, and even the body composition of the larvae. In addition, based on a number of on line reports from small scale breeders (blogs), successful breeding and oviposition can be problematic, and require specific light and humidity conditions. The larvae require a warm environment, and breeding flies require specific conditions to hatch and lay eggs.

Enterra Feed Corp., in BC is one example of a successful scale up, as are Ynsect (in France) and Protix Biosystems (in Holland). At commercial scale the conversion rates are impressive, though the literature range is variable, but in general organic reductions depending on feedstock are estimated to be from 40-60% with feed conversion efficiencies in the range of 2:1, and assimilation of 40-60 % crude protein level and 40% oil in the larvae, plus assimilation of high amounts of calcium and phosphorus. The preliminary conversion numbers are also very impressive. One kg of eggs from BSF will produce 380kg of larvae in 72 hours with a 2:1 FCE achieving a 60% or greater reduction in nutrient and organic matter. In addition to being highly nutritious (40-60% Protein; up to 40% oil), they also contain chitin in the exoskeleton which is a natural antimicrobial, and have been shown to produce defense antimicrobial peptides (AMP), particularly under an environmental stress. It is possible that both feed fractions and health promoting functional nutrients can be marketable components of the production system. This would prove out to be a highly nutritious feed ingredient for most livestock and fish, potentially replacing other cereal proteins, and more importantly replacing unsustainable use of fish meal. In addition, some feeding trials have been completed (Enterra, personal communication) and reported in the literature (ref), indicating general acceptance, and safety of inclusion in feed for poultry, trout, tilapia and catfish (feedipedia). Further feeding trials are being completed in collaboration with Enterra who recently received CFIA approval of the feed for poultry in Canada, and GRASS status has been achieved in the US market.

This potential therefore to add high value marketable products, to the bioconversion of organic waste streams is a very attractive alternative to disposal and composting, provided the production parameters can be optimized and shown to be cost efficient under Atlantic Canada conditions.

Project Activities

The project comprised a sequential analysis of the optimization of growth (environmental and feeding) conditions and reproductive cycle (pupation, oviposition and hatch) of BSFL within a complete unit initially, then separately into grow-out and breeding units to better refine and scale requirements separately. BSFL larvae were initially sourced from a Canadian specialty supplier of insect feed, and larvae retained from each trial to continue the cycle for subsequent growth trials. The system was large enough to enable multiple trays of larvae fed on different organics starter materials to be run consecutively, thereby removing the influence of climate on growth performance comparisons across feedstuffs.

Initial trials utilized a combination of lighting and direct in floor electric heat to maintain temperature, and humidifiers to modify humidity. Daily temperature and humidity measurements were recorded throughout each cycle. The phases were separate in space (bottom grow-out and pupations, and top breeding and in flight mating) within the bug chamber Figure 1. Later runs of larvae growth were scaled up and separated from reproductive cycles and both housed in a larger climate controlled room.

The projects was broken down into the following specific attributes of the entire BSFL production cycle to enable complete analyses of potential for adoption and assessment of business model strategy viability:

1. Preliminary and on-going routine nutrient profiling - comparative laboratory characterization of ingredients (organic waste typical of Masstown market product) , meal and frass. Breakdown of nutrient fractions (oil & protein) and processes to separate including isolation of bio-active components.
2. Pilot production run with larvae on prescribed medium blend (typical vegetable and fruit mix). Followed by repeated side by side comparisons of variations in the feedstock (single ingredient or mixes).
3. In vitro and in vivo tests (Zebrafish model) of protein hydrolysate fractions from larvae for safety and efficacy data.
4. Plant growth analyses with frass (fertilizer) fraction to determine true value and any potential growth enhancement properties (germination and plant biomass production).
5. Development of market and feedstock source and valuation and feed into creation of possible business model incorporating mass balance data, marketing data and production data into final model, including licensing and scale up potential.

Methods & Results

Lifecycle.

The Black Soldier fly were purchased at early larval stage and grown out through a number of full lifecycles, to determine the duration and conditions required for each, which is demonstrated in the diagram below. Our trials tended to have a fairly rapid growth phase of 16-18 days, as we ran at 24-26C for most runs. Pupation duration was probably the greatest variable and most dependent on conditions, whereas oviposition is the most sensitive phase to failure as a result of many factors, still being explored and including humidity, lighting, flight area, attractants, and rearing conditions.

1. **Nutritional profiling**

Lab analyses of larval product was carried out using Elemental analysis, GC (fatty acids) and HPLC to quantify fatty acid and amino acid profiles, for larvae from each ingredient run and for comparison with larvae produced by Enterra, BC.   Frass was analyzed using the elemental analyzer. Larvae were grown out for approximately two weeks on each feedstuff, at optimal temperature and humidity, until a portion were observed to be entering the "planking" stage prior to pupation. Then all larvae were harvested and weighed. Food delivery was weighed every day along with initial base bedding mix, and frass was weighed and analysed at harvesting, along with the larvae. A portion of larvae were retained for pupation to adult flies for re-breeding each cycle. Table 2 illustrates the comparison between the first preliminary run of larvae here versus samples sent by Enterra

Figure 1. Growing and Breeding module.
For BSFL production trial.

Regardless of feedstock, larvae grew rapidly, though modifications were required in feed moisture content of feed mix as fresh veggie organics are typically too moist for early stage larvae, and can lead to restriction in ability to respire within the medium. Food waste was prepared by grinding (through a blender or juicer for comparison) and was approximately 89 % initial moisture content, and was blended in different ratios with starter bedding material to establish the early stage pupae. After a few days, larvae started to grow rapidly and consume large amounts of added feed (Figure 2.). Food delivery was provided on the top of the mix daily in incremental measured amounts (wet).

Table 1. Preliminary Composition of Enterra larvae and CBU Larvae on a Dry Matter basis.

Nutrient % dry matter	CBU larvae	Enterra Larvae
Protein (%)	34.2	34.38
Crude Fat (%)	28.29	35.64
Calcium (%)	3.648	1.857
Phosphorus (%)	0.564	0.653
Sodium (%)	0.121	0.30

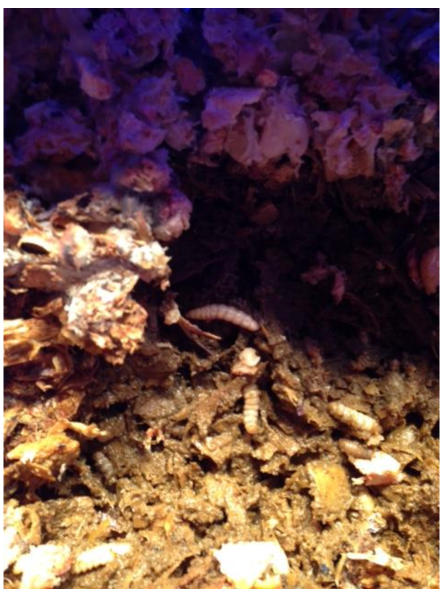


Figure 2. Larvae in feed.

Initial batch numbers demonstrated a total 161 g (at 40%DM) of larvae were produced from 660 g of dry matter organics, leaving 400 g of frass. The difference in mass balance is due to gaseous loss of CO_2 and NH_4. Larvae dry matter was low, suggesting that this batch may have grown faster than anticipated and assimilated high moisture due to the wet conditions of the mix. Dry yield percent (conversion efficiency) was almost 10% on a dry basis for larvae, whereas literature reports range from 7 to 24%, depending on feedstuff and growth conditions. This first batch of larvae grew surprisingly well considering the high moisture content of the mix, however this created quite strong odour issues when it came to separation of larvae from frass. Larvae were freeze dried (Labconoco), and frass was oven dried at 60^{0}C for 48 hours prior to any analyses. Larval protein content (CP %) was similar to that reported by Enterra at 34.3% for both samples, whereas larval fat content (%fat) was lower in the CBU larvae (28.3% vs 35.6% respectively). It is unclear why the CBU larvae contained approximately 20% less crude fat, but it is clear from the subsequent pilot runs that this is very much diet related, as described below.

2. Comparative Compositional Analysis of Black Solider Fly Larvae with Variation in feedstock

Repeat pilot runs were made to examine repeatability and the effects of varying ratios of input ingredients in the blend in quantities reflective of volumes available in industry and institutional suppliers, to mimic incorporation of the different industry volumes as they would be drawn into the system during development. The analyses performed were the same as the first run (and described below) to test repeatability of the system operating parameters and elucidate any changes in product volume and quality with changes in medium ingredient ratios. These will also occur naturally due to seasonal availability of waste products from the industry partners, fluctuations that cannot be avoided and need to be accounted for in building a robust system. The repeat runs provided additional compositional response information as well as additional mass balance data toward building the product volume expectations of a scale up system model.

Black solider fly larvae (BSFLY) grown on a mixture of fruits and vegetables (BSFLY-FV), potato skins (BSFLY-PS), fruits (BSFLY-F) and seaweeds (BSFLY-SD) were dried and pulverized in a desktop high energy vibratory ball mill (Across International, Livingston, NJ, USA). The fat in the dried, milled BSFLY was extracted with petroleum ether using Gerhardt's SOXTHERM® rapid extraction system (Königswinter, Germany) and protein content was measured in a PerkinElmer CHNS/O Elemental Analyzer (Waltham, MA, USA). The fat and protein content is shown below (Tables 2 and 3). Fat content ranges from 5.07 to 30.04%, with BSFLY grown on fruits (BSFLY-F) and seaweed (BSFLY-SD) giving the highest and least amount of fat, respectively (Table 1). The fat content recorded for BSFLY grown of seaweed (BSFLY-SD) may not be representative, due to the small amount of milled larvae available for fat extraction. Protein content ranged between 40.41 and 42.47% with one of the BSFLY grown on fruits and vegetables (BSFLY-FV) and on fruits (BSFLY-F) giving the highest and least amount of protein, respectively (Table 2).

Table 2: Fat content of Black solider fly larvae

Samples	Cup (C)	Meal (M)	Cup + Oil	Oil	%Fat
BSFLY-FV	139.1108	1.0	139.3023	0.1915	19.15
BSFLY-FV	141.1737	0.6	141.2624	0.0887	14.78
BSFLY-PS	140.2547	1.0	140.5368	0.2821	28.21
BSFLY-F	140.5344	1.0	140.8348	0.3004	30.04
BSFLY-SD	139.909	0.3	139.9242	0.0152	5.07

Table 3: Protein content of Black solider fly larvae

Samples	N_2	%Protein *6.25	AVE	SD
BSFLY-FV	6.92	43.25		
	6.67	41.69	42.47	1.10
BSFLY-FV	6.67	41.69		
	6.76	42.25	41.97	0.40
BSFLY-PS	6.72	42.00		
	6.76	42.25	42.13	0.18
BSFLY-F	6.55	40.94		
	6.38	39.88	40.41	0.75
BSFLY-SD	6.75	42.19		
	6.30	39.38	40.78	1.99

Larval protein composition remained relatively consistent across feedstock (Table 2) while oil composition was very much feedstock dependent (Table 3.). This may also have been a reflection of feedstock moisture composition, but it most likely reflective of the proportion of starch and cellulosic material, starches and sugars being more available to the larvae than cellulosic material. Further trials modifying the protein composition of the diet relative to starch, showed that 32% oil could be achieved best by more available carbohydrates than proteins. This is the guaranteed minimum that Enterra produce at, though their specs claim 40% fat in the whole larvae. Another factor impacting oil ratio is the age at which the larvae are harvested. In our studies we have tended to harves close to pupation, and likely if we bring the harvest earlier the fat content will increase further. Fatty acid composition was analyzed by GC, and figure 3 illustrates a typical chromatoghraphic output of fatty acid profile for larval oil. Despite the variance in total oil composition, the fatty acid profile appeared fairly robust in response to feedstock supply.

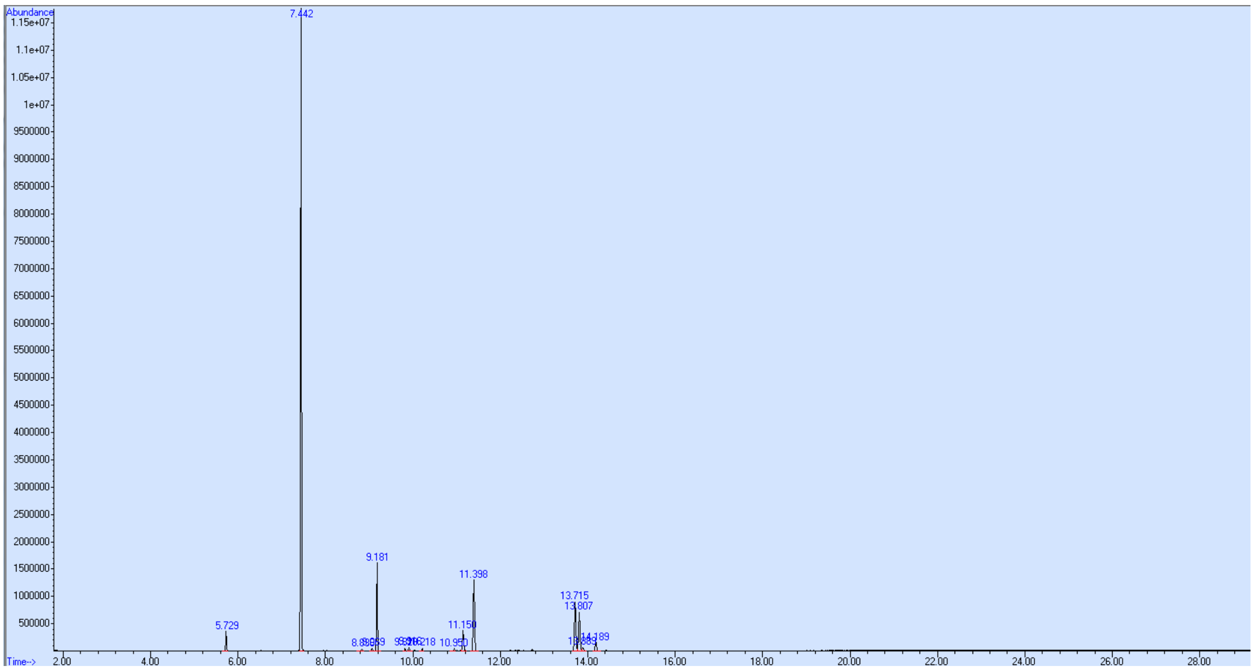


Figure 3. Typical fatty acid profile for larval oil fraction

Predominant fatty acids in the larval oil are lauric acid, palmitic acid and myristic acid (some more commonly found in coconut oils) but also oleic and linoleic. Omega 3 content is relatively low in favour of omega 6 and 9 fatty acids, and a higher saturated fatty acid content that would be found relative to fish oil. Decanoic acid derivatives, also prevalent in the larvae oil, may have potential health advantages, including anti-microbial factors while the predominant lauric acid has been shown to exhibit anti-viral properties.

3. Compositional analysis of residual frass

Residual material post larvae collection from the preliminary run was pooled by feedstock and analyzed using elemental analyzer, oven dried and prepared for testing as a soil amendment for plant growth. Initial frass analysis is provided in Table 4., with comparison of values provided by Enterra, BC. Insect frass comprises cellulosic material not digested by the larvae as well as digested residues from their consumption of the material (including enzyme digest, and at pre-pupation excreted intestinal microbiota).

Table 4. Preliminary Comparison of CBU frass analysis and Enterra specifications.

Nutrient composition (%)	CBU frass	Enterra specs‡
Total Nitrogen from (Protein)	2.446	4.54
Phosphorus	0.685	1.23
Potassium	2.615	2.44
Calcium	2.471	0.64
Manganese (ppm)	200.01	13
Zinc (ppm)	149.92	49
Magnesium (%)	0.334	0.13

‡ Temple et al 2013

On a dry weight basis, frass was 60% of the weight of the original organic waste stream, giving a 40% reduction in waste volume on a dry basis. Since the frass was 24.5% dry matter, this material would be less expensive to haul on a per tonne basis vs the wet organics at 10%, hence the reduction in organic material on a wet basis (or volumetric) was from 6.6 kg to 1.6kg, or 76% reduction. Composition of CBU frass was also similar to that specified by Enterra, though slightly lower NPK than Enterra, particularly the phosphorus, though the ratio to potassium was the same. This will also be feedstock related as phosphorus in the larvae was slightly lower also, yet calcium was higher. Presumably whatever is not consumed and deposited in the larvae will remain in the frass, indicating an indirect relationship between the two.
Subsequent batches of larvae were introduced to a much drier starting material in accordance with Enterra practices, and resulted in dryer frass and improvements in bioconversion of feed materials. In order to determine market potential and value of the frass relative to such products as compost or worm castings (which comprise the opposite ends of the value spectrum) frass was tested as a soil amendment in a small barley trial to determine if it provided any incremental benefit to plant health beyond the obvious fertilizer value (see plant trial below 4.).

4. Product testing – larvae protein meal and hydrolysate

It has been demonstrated that BSFL can be fed whole (live or dried) to a range of species, however the close ratio of high protein to high oil does not suit all ration formulations, particularly those of grower and adult species of livestock. In this instance it would be more desirable to split off the oil for separate markets, as Enterra are currently working toward, leaving a high protein meal. In conjunction and separately from these trials, we are working with Enterra and Dalhousie Truro colleagues and testing the protein meal from larvae as a feed ingredient in diets of salmonids and layer hens. The results of trials on Atlantic Salmon and Laying hens fed the protein meal are reported separately elsewhere as this was completed using the NSERC Engage portion of the project and will support the Enterra application to CFI for further registration of meal vs larvae whole dried.

Early indications show that the meal is very acceptable when substituted into animal feed, at lower inclusion rates, whereas at high levels, there may be as yet unidentified factors limiting either intake or performance. This is a complex product and in order to determine some specifics of the product preliminary exploration at CBU focused on protein isolate. Larval product, produced in our trials was used to extract and separate fat and protein fractions, as it is likely these could also present different market opportunities. Oil was extracted by either ball mill and ether extract, or simply ball mill followed by enzymatic hydrolysis and decanting of oil from surface. Protein fractions were further digested by either pure enzymatic hydrolysis or a novel lactic fermentative method, then separated by UF at specific molecular weight cut off, to produce hydrolysate for testing for bioactivity markers such as anti-inflammatory attributes, by in vitro methods. The hydrolysate was tested for toxicity in a commonly used zebrafish larval trial model. This enabled optimal in vivo concentration to be identified for a small scale adult fish trial exploring bioactivity as in an oxidative stress model. Chitin, which exists in varying amounts in larval exoderm was not isolated in these trials but also presents an interesting opportunity as in high amounts it could be an antinutritional, but in low amounts may be an anti-microbial (this would require further work).

In vitro analysis of antioxidant capacity of hydrolysates from BSFL protein fraction showed significant plasma GSH enzyme activity in response to oxidative challenge but lower SH response (two typical in vitro assays utilized to demonstrate anti oxidant capacity). Hence a small in vivo trial was run with these peptide fractions.

Briefly for the oxidative stress model, adult Zebra fish (Figure 6) were fed the additive, spray coated onto feed at a delivery rate of 75ul/g against a control group on non-supplemented feed. Fish were fed this diet for one week before exposure to known oxidative stress molecules in their environment. Two compounds (FeNTA and Sodium Nitro) were used to mimic a high and low oxidative environmental stress – these would be mechanisms the fish might be exposed to in a natural environment as a result of contaminants in the water. All trials were run with CBU Animal Care committee approval. After one week exposure to the environmental oxidative stress, fish were sacrificed and a standard oxidative indicator test done on the visceral tissue. This method (TBARS), is described in the literature (ref) and is used to indicate degree of fatty acid degradation in tissue due to oxidative damage. Results of this assay for FeNTA exposure are shown in Figure 5, and seem to indicate that the BSFL peptide hydrolysate reduced the oxidative damage in visceral tissue relative to controls that did not receive the supplement. The same was true with the SNT test, indicating there may be some protective effect against oxidative damage provided by these peptides. This would need to be correlated to the typical inclusion rates being utilized and tested in the Enterra trials to determine full physiological significance and desirable marketing approach i.e. whether the benefit can be a selling point in the intact protein meal, or whether production of an isolate additive would be economically fruitful. More work would need to be carried out to elucidate the optimal peptides and their functionality within the protein meal matrix.

Figure 5. TBARS measure of oxidative damage in control exposed to oxidative stress, and apparent protective effect in fish pre-treated with BSFL peptide hydrolysate.

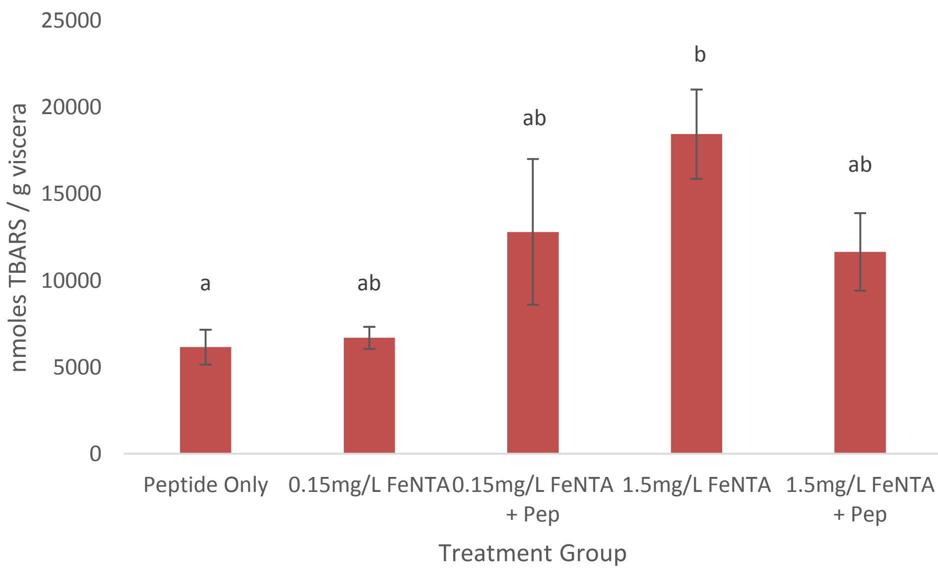
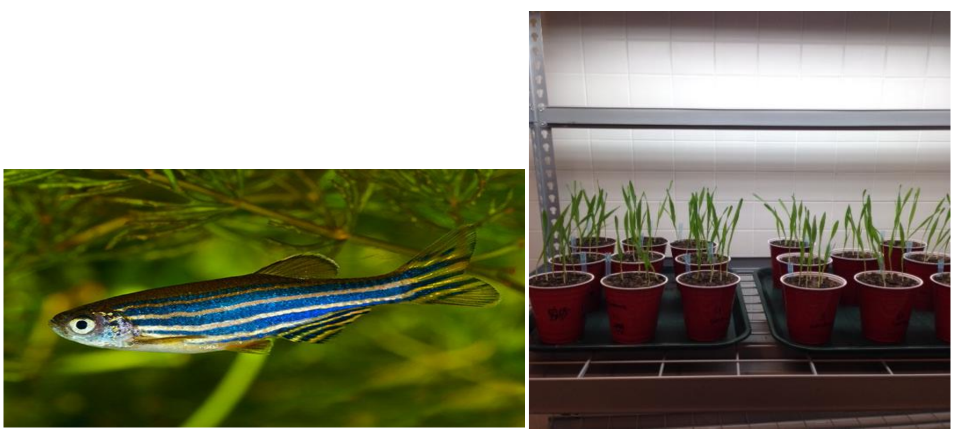


Figure 6. Zebra fish model for anti-oxidant assay, and plant growth rack for frass soil amendment evaluation.



5. <u>Product testing – Frass fertilizer value from barley plant growth trial</u>

The residual organic "frass" is considered to be an ideal fertilizer (Enterra), and was therefore utilized in a small barley growth trial in the plant growth room at VCSEE, kept under constant temperature and a 14:8 light regime.

A plant growth trial was conducted with the residual growth medium (frass) from the larvae using a standard barley germination test developed at the VCSEE to examine the true (analysed vs available) nutrient quality as compared to commercial fertilizer of equivalent ratio and a commercial growth medium of similar composition (peat:sand mix).

The proposed inclusion rate according to Enterra is 1-2% of potting mix. Control potting mix used was half peat and half sand. BSFL frass was incorporated to replace peat at a rate of 0%, 4% and 8% of potting mix by volume. Barley seedlings were planted six per cup, with 15 cups per control or treatment, allocated randomly within one shelf. Seedlings were maintained under plastic until germination (seven days) and germination rates measured at that time. All pots were watered once per day following germination, having been soaked thoroughly at seeding. Control pots received a commercial fertilizer (20:20:20) at planting, while treated plants had an equivalent amount of frass incorporated so as to be calculated to deliver an equivalent nitrogen level plus or minus a margin, that created equivalence in either phosphorus of potassium as these ratios were slightly dissimilar in the HA frass to the Enterra specifications.

At germination, two plants from each pot were harvested to measure root development (WinRhizo), and chlorophyll content. Remaining seedlings were grown with no further additions of fertilizer or frass, only watered every second day, until eight weeks, at which point all plants were harvested and monitored for dry matter yield, root to shoot ratio, chlorophyll content (a & b) and plant carbon and nitrogen content.

Trial layout is indicated in Table 5 below, and treatment data is provided at germination and harvest in Table 6. Frass inclusion improved germination rate and plant biomass development. N:P:K delivery was calculated for each treatment for assessment of nutrient level provided. Measurements included germination rate, plant biomass production, root to shoot growth and chlorophyll and plant sugars analysis.

Plant treatment protocol

Table 5: Detailed information about the insect frass used in the barley growth trials.

Stock Material	Treatment	Mix	Number of Reps
fruit/veg pulp + poultry litter	Control	50:50 sand peat (v/v)	9
fruit/veg pulp + poultry litter	4%	50:50 sand peat (v/v) with 4% frass (w/w)	9
fruit/veg pulp + poultry litter	8%	50:50 sand peat (v/v) with 8% frass (w/w)	8

Frass trial results

Inclusion rates were selected on the basis of literature for worm castings and private communications with Enterra regarding a field trial in BC with vegetables.

Table 6. Comparison of treatments at germination for shoot and root development.

	Germination	Brix %	Root vol	Surface area (cm²	Root diameter	Shoot length	
Control	87%	5.5	0.018	2.89	0.245	19.978	
4% frass	94.5%	6.7	0.023	2.9	0.310	21.706	
8%frass	97.5%	6.0	0.036	3.66	0.388	19.919	

Barley Seed Germination

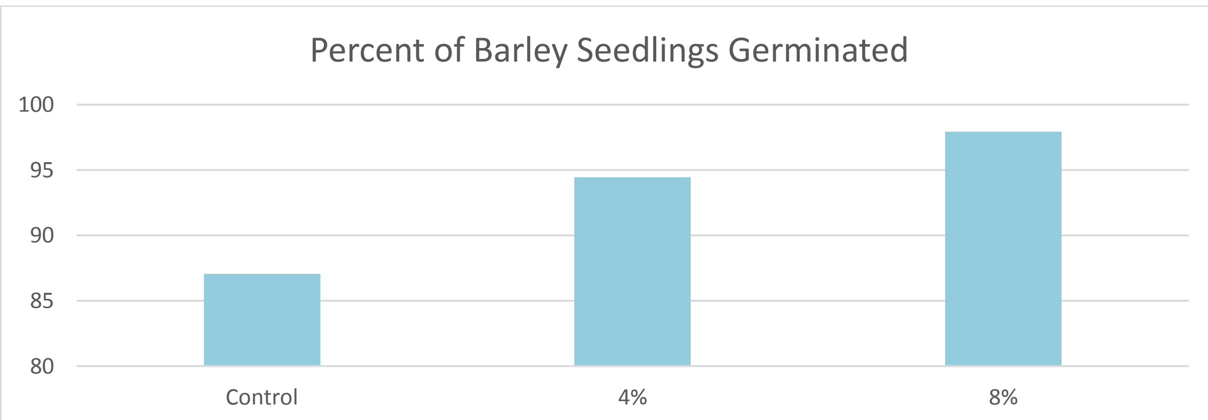


Figure 7: Percentage of barley seedlings germinated during growth trial using a 50:50 sand and peat mix amended with 4% and 8% insect frass.

The pots amended with 8% insect frass showed the highest number of germinated seeds with almost 100% of seeds germinating. Control had the lowest number of barley plants germinated per pot

Plant Growth Analysis

Plant growth performance was assessed at germination and day 44 post planting (approximately one month post germination) by measurement of biomass yield (dry and wet yield of material), chlorophyll content and brix (plant sugars by refractometer), as well as root analysis (length, surface area, tap root length) using Win Rhizo software.

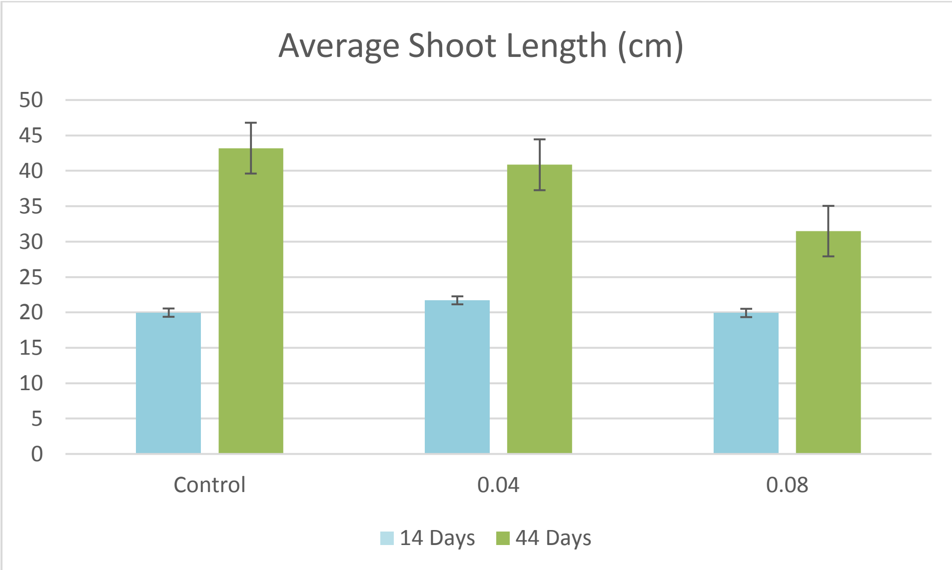


Figure 8: Average shoot length of barley plants measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

Shoot lengths for each treatment were similar at 14 days after planting, however, the control and 4% had a significantly greater increase in shoot length when compared to the 8% treatment.

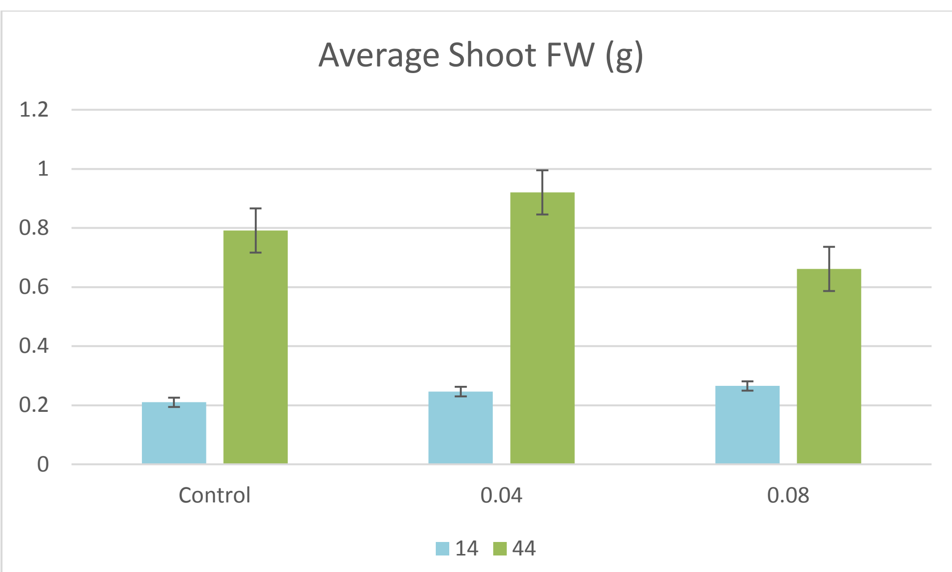


Figure 9: Average fresh weight (g) of barley shoots measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

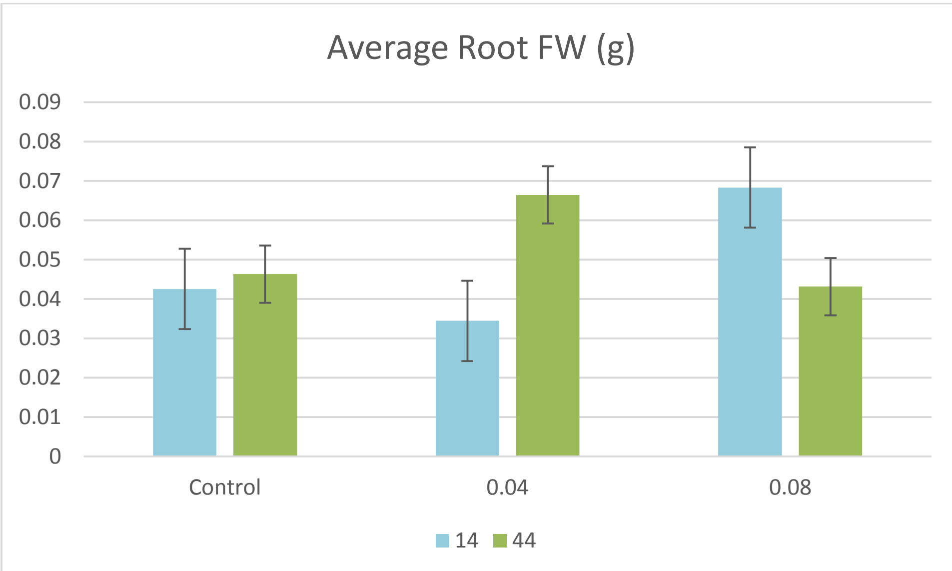


Figure 10: Average fresh weight (g) of barley roots measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

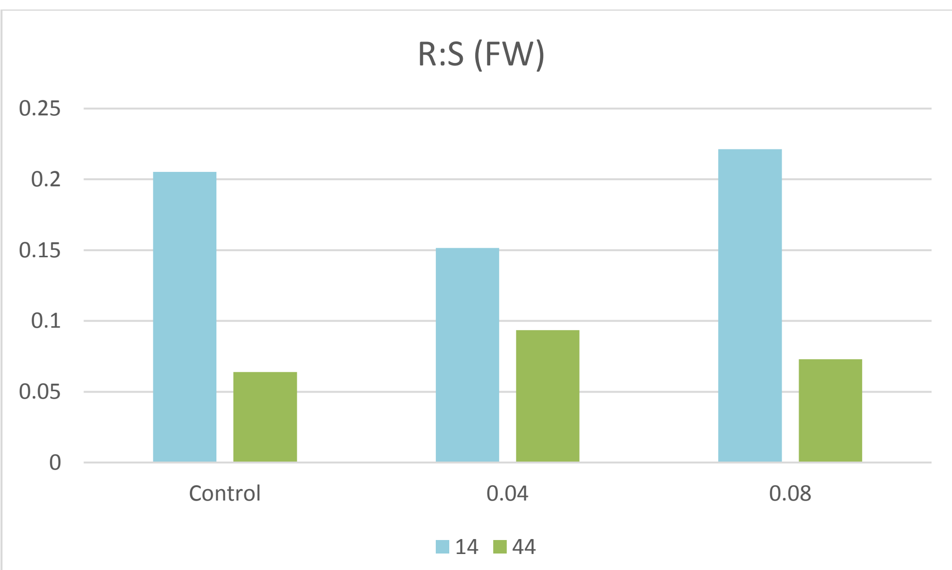


Figure 11: Root to shoot ratio of barley plants (fresh weight) measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

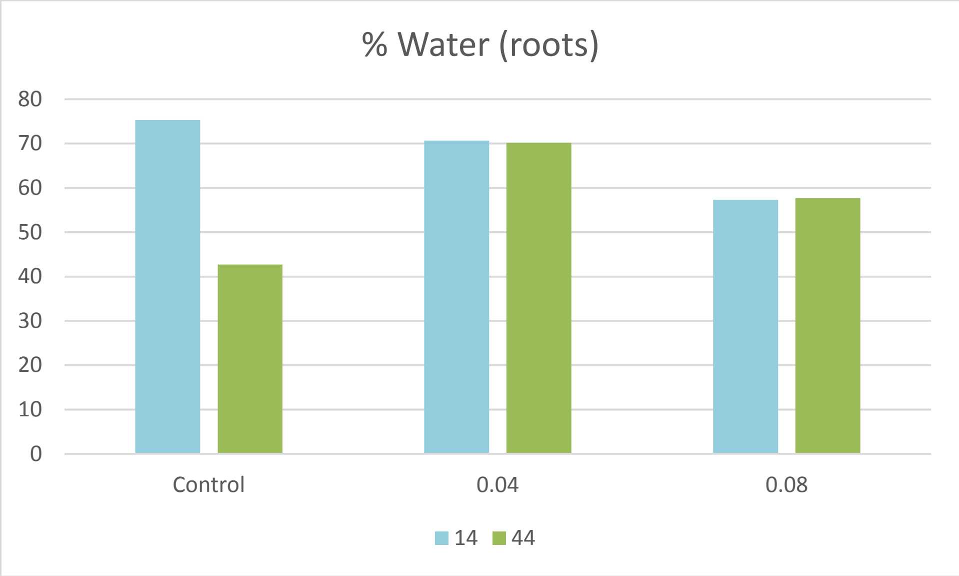


Figure 12: Average percent water content from barley roots measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

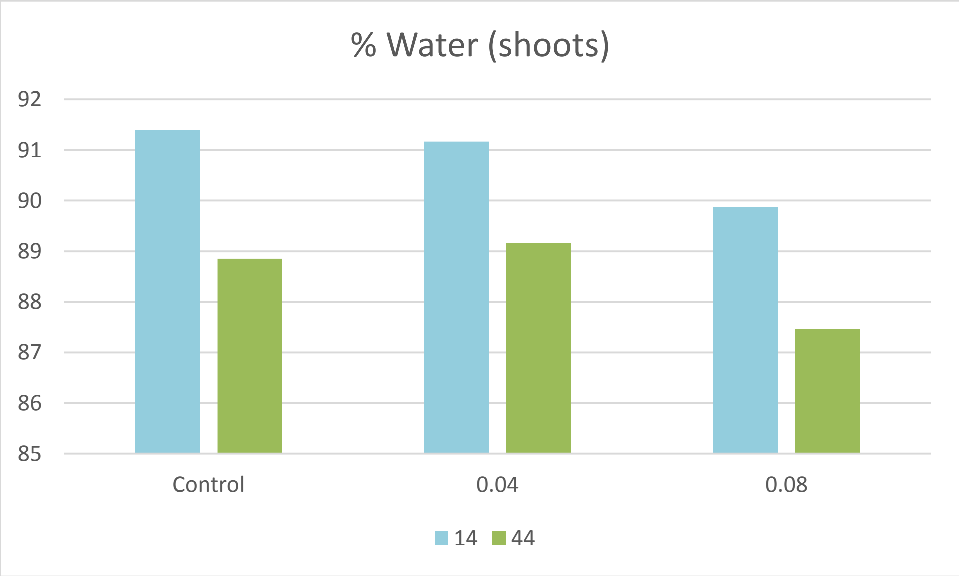


Figure 13: Average percent water content from barley shoots measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

Root development (root length, surface area, tap root length) was greater in control versus treated plants and yet shoot development was greater, hence root to shoot ratio was lower, which may be indicative of higher nutrient concentration in the root zone in frass treated plants.

Sugar Content in Shoots

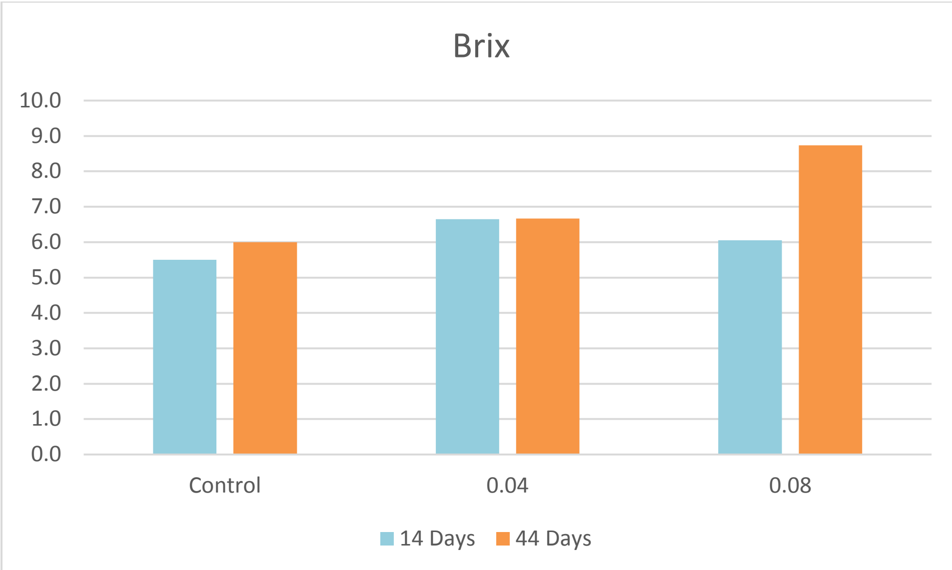


Figure 18: Average sugar content of barley shoots measured at 14 and 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

Chlorophyll Analysis

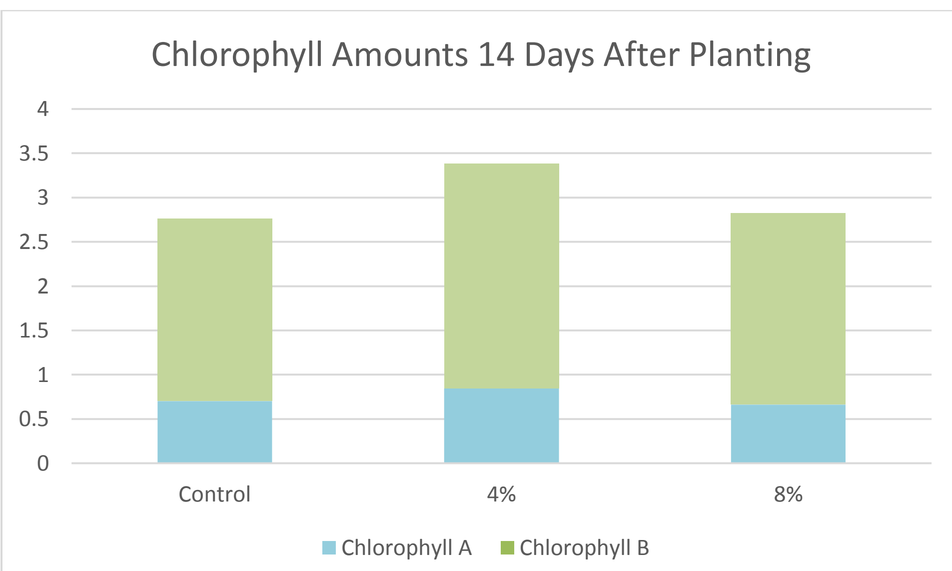


Figure 19: Average chlorophyll amounts from barley shoots measured at 14 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

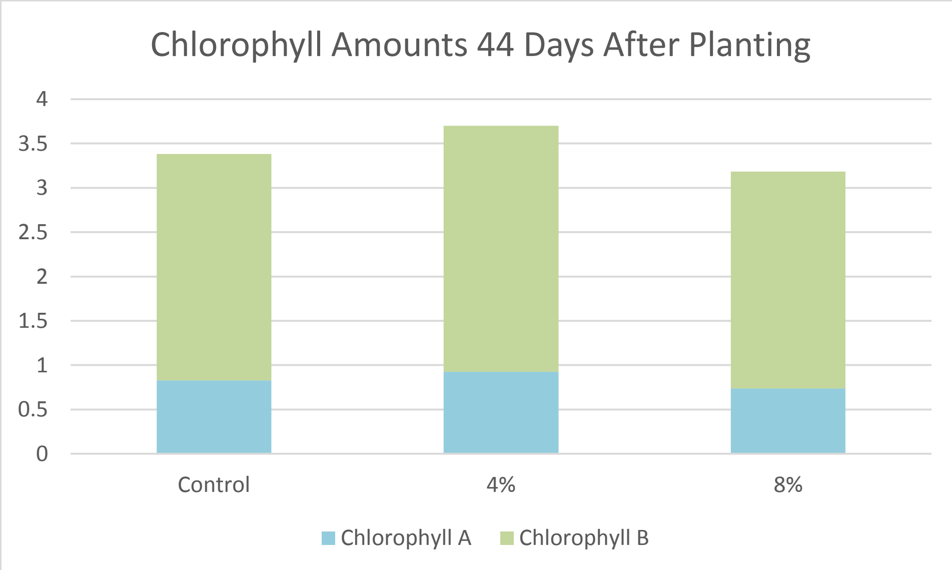


Figure 20: Average chlorophyll amounts from barley shoots measured at 44 days after planting. Plants were grown in a 50:50 peat and sand mix and amended with 4% and 8% insect frass.

Overall there was a curvilinear response to frass addition, and the lower inclusion – only 4% of potting mix achieved the best results in terms of above ground biomass, chlorophyll (indicative of respiratory capacity) and brix (indicative of plant health). There were definite advantages to the inclusion of 4% frass in the potting mix, even though the fertilizer in all plots was adjusted for nutrient composition. This may mean that nutrient is more readily available from the frass versus inorganic sources or that there are attributes in the frass that promote nutrient uptake by the roots and induce plant protective changes. This would comply with suggestions made by Enterra that there are perhaps patentable plant defense promoting properties within the frass.

6. Mass balance and market data.

Literature values for bioconversion vary widely, and our data from different runs and different feedstock also show quite a large variance, hence the importance of standardizing feedstock delivery and environmental conditions. Average values have been taken from the multiple runs of larvae production made in the trials to put together an approximate mass balance on which to base an estimate of production economics. Comparing these with Enterra and other literature values for bioconversion with BSFL, we achieved a mid point range (table 7), similar to what Enterra are actually achieving at commercial scale currently. Using their model of 100t wet organics per day, and running 5 days per week, 2500t per month is used in the bioconversion model below Figure 21. For comparison, a small vegetable market like Masstown, or average size commercial greenhouse produces about 2-5t/week, so this model would require at least ten customers of their size. This provides an example of estimated total biomass conversion and volume of final product when calculated for a system built to handle these volumes. Market prices are based on current commodity prices competing with the BSFL meal and oil, assuming fractionation, and corrected for relative protein content. This would give a price equivalent, and so to ensure uptake of product, there may need to be a differential against existing protein and oil commodities. This discounted price is not used here, as it is assumed that uptake would be driven by the need for more sustainable fish meal replacements and the reduced availability of such (not reflected in the price structure. In addition there is a freight advantage to locally produced ingredients as most competing protein meals originate outside the Province and are brought in by rail car, adding to the price used here.

These assumptions would have to be tested in the market place, and would depend on the location of the BSFL facility. The modular unit, was not set up to separately assess heating and lighting cost in this first phase, so cost of production is not accounted for in the value calculation of the mass balance presented in figure 6, nor is capital cost.

> Assumptions; Typical organics mix is similar to that tested – comprising potato, vegetable and fruit waste. Conversions match those achieved at scale, and waste volumes are able to be collated within a small radius.

Notes:

1. Bio-Conversion of DM to larvae range in literature 7-24% ; 15% used in example. Dry matter of larvae 28-37%; 28% used.
2. Reduction on wet basis used is 75% for wet feedstock i.e. 25% of original wet weight as frass. Dry matter of frass ranges 29-38%; 30% used.
3. Losses in DM, occur through gaseous exchange and evaporation.
4. Conversion to meal and oil by screw press.
5. Larvae composition and yield influenced by moisture and nutrient in medium; assumed optimal 40% CP, 35% oil.

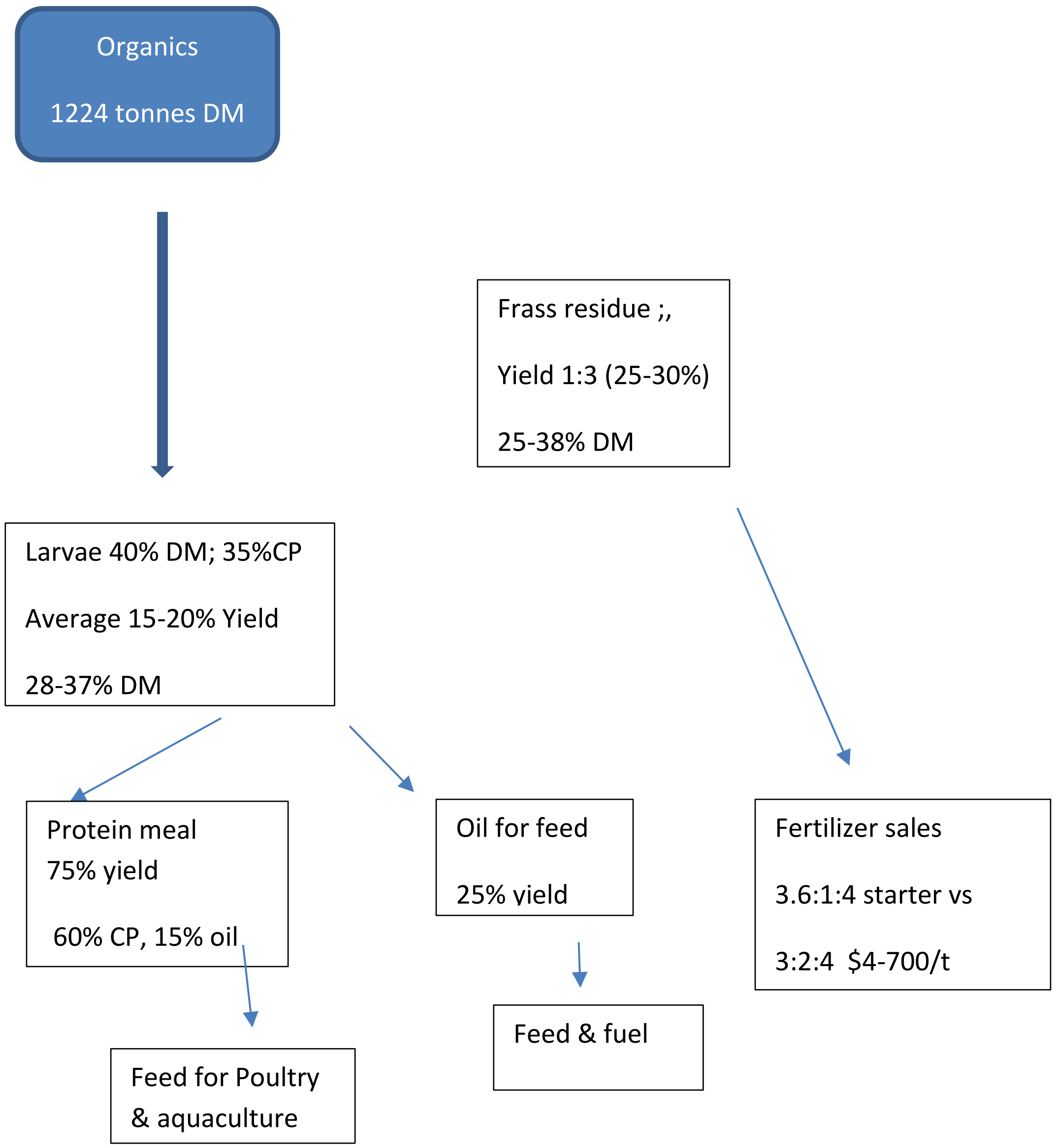
Table 7. Example run of larvae growth in two week period, with five different feeds all provided at the same daily rate. Run 2. Approx. 480g feed delivered overall.

	Larvae weight before feeding (g)	Count after feeding	Larvae weight after feeding (g)	Change in weight from before to after feeding (g)	Weight of larvae sent to the oven (g)	Dry weight (g)
A	12.80	112	29.76	16.96	28.26	4.185
B	11.95	88	23.53	11.58	21.87	2.517
C	13.87	104	29.41	15.54	27.44	4.231
D	10.10	80	25.27	15.17	24.16	3.250
E	13.20	112	28.82	15.62	26.38	4.400

Table 8. Range in monthly revenue on 100 tonne per day organics bioconversion

Gross Margin calculation BSFL wet Tonnage		Larvae Tons	DM & tons larvae meal	Frass Tons	DM & tons Fertilizer	minm Price meal $/t	max Price meal/t	min Price frass/t	max Price frass/t
Tons per day, wet	100	17	0.28	25	0.3				
tons DM			4.76		7.5	$800	$1,600	$400	$700
Tons per month	2500	340	95.2	500	150	$76,160	$152,320	$60,000	$105,000
			Tonne/mo meal		Tonne/mo frass				
Revenue/month	minm					$136,160			
	maxim					$257,320			

Figure 21. Mass balance flow chart



7.Value of Outputs & Markets

Based on the comparative experimental and projected yields in this study the preliminary economics are described below, and the approximate range in sales figures based on price range for each product are shown in Table 8. The biomass conversion shows that the meal can also be separated into protein meal and oil for sale into separate markets, to try to maximize revenue from the dried larvae. Currently only dry larvae (whole) are approved under CFI, but meal and oil will follow shortly. It is debatable that the combined value of the parts minus the cost of separation equipment yields more revenue, but what it does provide is market differentiation and diversity, as not all livestock species require this level of oil in the diet, reducing overall inclusion of whole larvae if not separated.

Market for Insect meals.

As discussed, the dried whole larvae recently received CFI approval for sale as poultry feed, specific to larvae grown on pre-consumer organics. Registration is underway for the separated larvae protein meal. In the US, the product has GRASS status (generally recognized as safe) and can already be marketed. The EU is expected to approve larvae meal under EFSA this coming year, and the separated oil can already be marketed under feed oil regulations. All this bodes well for the acceptance as larvae as animal feed in North America and Europe. This has all been achieved within the last year, so countries seem willing to move quickly on acceptance, though considerable research within the EU (PROteInsect)is being undertaken to study biosafety, transmission of contaminants from feedstock to larvae, pathogen kill of drying methods, and other sensitive aspects of the food chain. For this reason it is expected that larvae produced from manure based feedstock will not be approved for some time until this research is complete.

Potential markets for both larvae and protein meal are broiler feed, starter feeds for hogs and aquaculture feeds, as insects would be a natural food source for these species, though other markets include pet feed. A number of rations in these primary species have been explored between published literature accounts and privately funded trials by Enterra. There has been sufficient to register the ingredient for poultry feed, and between the recent trial at Dal Truro, and previous trials on Trout it is anticipated that registration for aquaculture will be achieved shortly. The most interesting of these is aquaculture, as monogastric species such as poultry and hogs can consume cereal based proteins readily and only early life stage diets tend to be formulated to include any high price protein meals such as plasma or fishmeal (and whey). These are low volume rations but generally high margin, and present a good base entry point. Aquaculture however, has come under fire for the non sustainable use of ocean catch fishmeal and despite ingenious efforts to raise salmonids and other species on cereal based protein, there are drawbacks including lower omega 3 oil content of the flesh, and hyperallergenic intestinal reactions in the fish. Hence this industry desperately needs a viable replacement for fishmeal.

Data to date show an acceptable inclusion of larvae in the diets of salmonids up to 30% without reduction in growth performance, and of the meal to replace up to 30% of fishmeal. This could represent a considerable tonnage of ingredient, and saving on fishmeal required. If it is assumed that feed conversion rates for salmon are around 1.2 to 1, and in Atlantic Canada 60,000 tonnes of salmon were raised in 2015, this represents 72,000 tonnes of feed. If the inclusion of larvae meal is 15 % only, then this would equate to 10,800 tonnes of dry meal. In the example of the average Enterra size operation above, only 1,142 tons of dry meal are produced per year; one tenth of the market. On any new feed ingredient success in 10% of the marketplace would be considered a reasonable easy target. Although production of salmon is dispersed over a large number of farms, many source feed from a small number of specialty fish feed manufacturers (e.g. Ewos –Nutreco, Skretting), hence the customer base would be small relative to the volume throughput for the market.

On this basis, the aquaculture industry is a very attractive customer for the larvae meal ingredient and is awaiting a high quality consistent and safe product. Pricing in Table 8., is based on the range in price over a ten year cycle for the competing ingredient replaced by the larvae meal, which would be fishmeal. Prior to the last decade fishmeal ran at around $1000-1200/tonne, but with diminishing ocean stocks and increasing commodity prices, the price has trended ever higher, hence it would be safe to assume revenues from the upper price range of $1200-1800/t for larvae protein meal. If separation of products is practiced, the oil portion would replace expensive fish oils (and some high omega 3 cereal oils), which would also run in a similar price range, so for the purposes of the business model, the product is treated as whole meal.

Potential Frass market & reduction in organics volumes.

The frass product left after screening of larvae from the food medium is like a fine soil and ideal either as a potting mix (for greenhouse seedling development) or for sale externally into already existing retail fields, particularly targeting Co-op type store sales of bagged products. The product at Enterra is dried, pelleted and sold currently as fertilizer, but they also have a patent on its capacity as a plant defense product against wire worm. Our trials have demonstrated that the frass has a desirable nutrient ratio as a fertilizer, but also in small inclusion rates in potting mix, appears to have some health benefits beyond its nutrient content. This moves the product into the equivalent market to worm casting products, which are highly prized, high margin. The volume of product is considerable, 1800 tonnes per year in the example above and would easily find a home on the potting mix market where product is bagged and sold in 12 to 20 kg bags. Before bagging cost, these composts typically sell for $4-8/bag, which would yield the lower end of the price range utilized. However, it would be worth exploring the worm casting market, as this product seems to be sold at retail in 1-2 kg bags at much higher price, generating considerably more revenue. Customers for potting mix would likely be commercial greenhouse, whereas customer for worm castings tend to be the large retailers such as Rona and Home Depot, as well as local nurseries. Castings demand higher market price due to claims on enhancing soil health, improved nutrient availability, improved crop yield and plant and soil health, demonstrating that the frass contributes considerable revenue to the business model.

Although the final fertilizer has desirable values for the balance of nutrients (N,P,K), it is a concentrated form of the residuals from the original organic waste, after the larvae have extracted significant proportions of the nutrients for growth. Initial figures indicate a potential reduction in total organic volume of up to 70% and of nutrient load by 45-65%, as illustrated in the example conversion Table 9. Hence it is important to consider the role of these bioconversions in reducing the overall organics waste issue, as well as the considerable value addition to each bio-product.

Table 9. Nutrient reduction loading calculated from manure to frass.

Initial feedstock % composition	1 t contains kg	Compost comp%	0.3 t contains	% reduction
N: 3.28	32.8	4.0	12.0	***N=63.4***
P: 1.80	10.8	2.0	6.0	***P=44.4***
K: 1.52	15.2	2.5	7.5	***K=50.6***

8. **Business Model**

The Enterra model is only one way to look at the commercialization of BSFL production, which is centralized large scale collection of organics waste and bioconversion under one roof. This model has been utilized by other companies in Europe in proximity to large urban centers (Ynsect, Kreca, Protix, Biosystems and AgriProetin). Investment in infrastructure and transportation logisctics to accompany this scale of production will be large. Enterra reports an expenditure of approximately $10 million for its first unit in BC. However this would be considerably less if they were to build from scratch and not incur all the conversion costs from taking over a large greenhouse/warehouse facility. However other facilities in Europe look to have invested even more in large scale growing, breeding and milling operations.

The site at Enterra however, is approximately one hectare (107,640 sqft), and commonly reported building costs for greenhouse or steel frame buildings, including insulation and heating infrastructure for Canada are in the range of $15-30/sqft. This would equate to an investment of $1.6 to $3.2 million for the size of operation in our example.

Annualized sales for this example are between $1.633 million for the minimum product price range to $3.087 million for the maximum price range, hence on sales alone, an ROI of only one year would be possible.

However, there will be considerable operating costs, likely similar to those required for greenhouse minus paid inputs, which, based on a 2013 survey of greenhouse production in Alberta is estimated to be about $5/sqft. This would mean an expense of about $544,600 per year for this operation and would not include transportation/logistics costs or capital costs. Since these would relate entirely to distance from source of organics, and distance to market outlets, these are not included here. Similarly in our scale up operation at CBU, we did not have separate heat and light meters to confirm these estimates. It might be assumed that freight cost of organics to the plant will be borne by the producer (retail outlet) and therefore the organic feedstock has a delivered cost of zero, however transport of product to market on the other end may be substantial. If large volumes can be warehoused, and then full flatbed loads shipped out, to markets within a 100km radius, total product would be 250 t/month, which is 6-8 loads (tridem to B-train size), at approximately $750 per load in this radius would be an annual cost of over $70,000. Additional bagging, handling and marketing costs may also be incurred.

Even considering these costs at the minimum product price, there is a gross margin of approximately $1million per year, giving a three year ROI on the higher cost building.

One of the problems with this business model is that it is highly dependent on being in close proximity to both organics supply and market outlets, which typifies some large urban centers. For this reason companies like Enterra and Ynsect (France) do not appear to be looking to joint venture in smaller urban or more rural centers like the Atlantic Provinces. Instead the CEO of Enterra is currently looking for JV's in Toronto and California.

Looking at the diverse population in Atlantic Canada, a more spatially integrated model may be more appropriate, whereby the most cost intensive, high risk aspects of the production cycle are centralized and the less difficult production/grow out operations are located in proximity to feedstock, on a smaller scale. Then bio-products can either be shipped back for centralized marketing, or sold direct on a smaller licensing basis to local markets. The economics of this model would be a little more complex, but worthwhile delineating.

9. Benefits of the Business Plan.

The potential financial benefits of this program are many, and can be locally or widely distributed. Primarily there are environmental benefits to valorizing the large volume of organics currently underutilized. In addition, the aquaculture and livestock industries can benefit by locally sourced cost competitive alternate protein meals. Greenhouse and vegetable production industries can achieve a double benefit, through an outlet for organics waste and return of high quality growing medium to enhance plant growth and health. The high value market products both lend themselves to pelleting, which enables economic delivery to a wider market, should production exceed local market demand, which is unlikely within Atlantic Canada.

Additionally, potential health benefits may be derived from what would be a natural feed ingredient for free range poultry and fish, from the ideal nature of the amino acids, possible antimicrobial peptides and natural chitins. This is significant in light of the legislated move away from prophylactic probiotic use, and the lack of viable alternatives, and particularly in the aftermath of the disease issues arising in Canada and the US from use of meat meals and porcine plasma as starter ingredients.

In terms of further sustainability of the agriculture industry, vegetable wastes left in fields represent leachate and contamination of the environment and so must be hauled away to disposal sites, and it has proved very difficult to find alternative methods of nutrient management. Anaerobic digestion has been used in Europe to incorporate some source separated vegetable waste into methane production but can only comprise a small portion of feedstock and requires very large capital investment. Similarly to composting this leaves large problematic volumes of liquid waste for disposal post digestion. The larval growth model provides for many of these issues providing nutrient recycling, reduction, and value add, all significant contributions to the economic viability, competitiveness and strategic development of the horticulture and greenhouse industries.

An over-riding benefit of this project to the Province would be the potential development of a new "value add" industry to service existing bio-product markets, allowing enhanced competitiveness and potential expansion of other primary industries. All these benefits revolve around the core benefit of removing organic wastes from the landfill and disposal systems, freeing up space, potentially saving large amounts of costs on transportation, while providing much higher value add end products.

Conclusions

- BSFL grew well on a mix of organics singularly or in mixed ground and fermented forms provided the moisture content is kept to around 65-80% in the growth medium. The lower dry matter enables easier separation of larvae from frass.
- Composition of larvae produced was not dissimilar to that from a commercial supplier and mass balance was also similar, both being influenced by feedstock supply and form.
- Frass produced showed positive impacts on plant growth when incorporated in potting medium and could be marketed for horticulture and greenhouse use, or can be used as bulk fertilizer for sale.
- Reduction of nutrients in the remaining frass as well as volumetric reduction resulted in a 50-60% reduction in nutrient loading and up to 70% volumetric reduction.
- Larval meal incorporated into salmon and layer rations at 15-30% inclusion rate supported equivalent growth performance to fishmeal.
- Separation of larvae into protein meal and oil provides additional marketing flexibility and diversity, and hydrolysis products showed anti-oxidant capacity in vitro as well as an in vivo fish model. This opens the prospect of enhanced value for meal beyond the high quality protein aspects.
- Fatty acid profile of separated oils is moderately desirable, though lower in omega 3 fatty acids than fish oil, it has other fatty acids with potential antimicrobial and anti viral properties. These would be desirable in aquaculture and livestock nutrition.
- Some further research is required to better refine ideal conditions required for hatching, breeding and oviposition in the adult BSF, in order to maintain a continuous system of organics reduction.
- Estimated growth margins (prior to capital servicing) of a centralized production facility appear to provide a very good return on investment.
- There may be potential for a modified integrated production system model for smaller population centers.

Future work required to refine the model

To enhance the robustness of the model, further information and testing is required in specific aspects including some of the following;

- Narrow down equipment and technologies for heating, humidity, drying and extraction to determine the costs of production and processing for addition into the model
- Refine total lifecycle performance, particularly breeding and oviposition parameters to provide better guarantee of performance
- Expand market appetite for sales, and narrow down customer base and market size and proximity.
- Refine volumes, locations and characterization of organic wastes.
- Examine enzyme and microbial dynamics of larvae bioconversion to look at methods of enhanced feed safety and increased efficiency of bioconversion.
- Test for carryover of contaminants from feedstock to feed relative to process conditions.
- Further animal feed testing to expand market for fractions of larvae, and further plant trials to examine growth enhancing properties of frass, both of which will increase market price.

References/Links