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Bioconversion of organic waste using mealworms

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1 Mealworm feeding on organic waste streams

1.1 Introduction

Grape marc is a waste product from the wine-making process and presents an ongoing challenge in its disposal. A previous, small-scale study (Agnew et al. 2021) investigated the ability of nine insect species to feed on grape marc. Of these species, mealworms (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) appeared to be able to digest and develop on diets made primarily of grape marc.

To build on these trials, this year's work focused on the ability of mealworms to bioconvert various waste products. Trials this year aimed to investigate feeding larger quantities of grape marc to mealworms, along with assessment of mealworm development on this diet. Concurrently, feeding on diets comprising grape marc combined with other waste products (ground mussel shells and/or winery lees) was also assessed.

1.2 Organic waste streams provided

The organic wastes provided in these trials were sourced from the Marlborough region, from conventionally managed vineyards or the Greenshell™ mussel industry. The trialled organic wastes, including their storage, are outlined below (Table 1). The Sauvignon blanc grape marc contained more moisture than the Pinot noir grape marc. For this reason, Sauvignon blanc grape marc was spread across trays and allowed to air dry for a few hours before feeding the mealworms. None of the other wastes needed prior treatment before feeding to the mealworms. The ground mussel shell was quite odorous and had the consistency of fine sand.

Table 1. The origin and storage of the organic waste streams fed to mealworm larvae (*Tenebrio molitor*).

	Fresh grape marc	Wine lees	Dried grape marc	Ground mussel shell
Variety	Pinot noir Sauvignon blanc	Pinot noir	Sauvignon blanc	n/a
Storage	Stored at −20°C	Stored at −20°C	Stored in plastic bags at ambient temperature	Stored in plastic bags at ambient temperature

1.3 Small-scale pilot study: feeding on various organic wastes

A small-scale pilot study was conducted to investigate the ability of mealworm larvae to feed on the organic waste streams. Each substrate (Table 1) was provided to the larvae either separately or combined.

Table 2 outlines the combinations and outcomes of organic waste streams provided to mealworm larvae in this small-scale trial. Substrate/s were provided to mealworms within small, vented click-clack boxes (15.1 cm l x 10 cm w x 4.8 cm h; Figure 1). Each box held approximately 100 mealworms and between 20 and 100 g of substrate/s. Because wholemeal flour is the primary food source for the laboratory colony, flour was also provided in some small boxes to compare the larval outcome to boxes containing organic waste only.

Table 2. Outcomes of small-scale pilot studies of feeding by mealworm larvae (*Tenebrio molitor*) on different organic waste streams.

Fresh grape marc	Dried grape marc	Wine lees	Ground mussel shell	Wholemeal flour	Outcome
X		X			Mealworms healthy and active. No pupae observed so far.
X			X		Mealworms healthy and active. No pupae observed so far.
X		X	X		Some feeding; however, moisture and mould became an issue.
X		X		X	Mealworms healthy and active. Larval development into pupae observed.
	X				Mealworms healthy and active. Larval development into pupae observed.
	X			X	Mealworms healthy and active. Larval development into pupae observed.
	Ground marc				Mealworms healthy and active. Larval development into pupae observed.
	Ground seeds only				Mealworms do not appear as active as those fed on grape marc. Some death of small larvae.
		X			Mould a problem. Mealworms died. Box discarded after 12 d.
		X	X		Initially, mealworms appeared healthy and active. No mould observed. Health declined over 6 weeks, now low activity. Currently no pupal development.
		X		X	Ok, for a short time. Moisture and mould became an issue. Box discarded after 12 d.
			X		Mealworms fed but did not develop at the same rate or size as controls. No pupae observed so far.
			X	X	Mealworms healthy and active. Larval development into pupae observed.

Fresh grape marc and wine lees were from Pinot noir. Dried grape marc was Sauvignon blanc.

Figure 1. Small-scale testing of ground mussel shell consumption by mealworm larvae (*Tenebrio molitor*).

As reported in 2021, mealworms fed and developed on grape marc. This year, feeding and development on dried grape marc was also assessed. There was no noticeable difference in feeding or development on either fresh or dried grape marc alone (Table 2). Fresh grape marc was selected for further large-scale feeding assessments (see Section 1.4.2).

The addition of wine lees resulted in excess moisture and mould, leading to the death of mealworm larvae (Table 2). Of the combinations trialled with wine lees, only the fresh marc plus wine lees combination was selected to be replicated on a larger scale (see Section 1.4.2) as these larvae have remained healthy and active.

Ground mussel shell was fed to mealworms as an additive to other waste streams and alone. The results suggest that mealworms can feed on this substrate; however, as larval development into adults was delayed, it is unlikely to provide a complete food source for the mealworm larvae (Table 2). Microscopic visual assessment of fed vs unfed mussel shell indicated a difference (Figure 2). It is unclear whether the mealworms were obtaining nutrition from the shells or from organic contaminants (e.g. algae) that were present on the shell before it was ground.

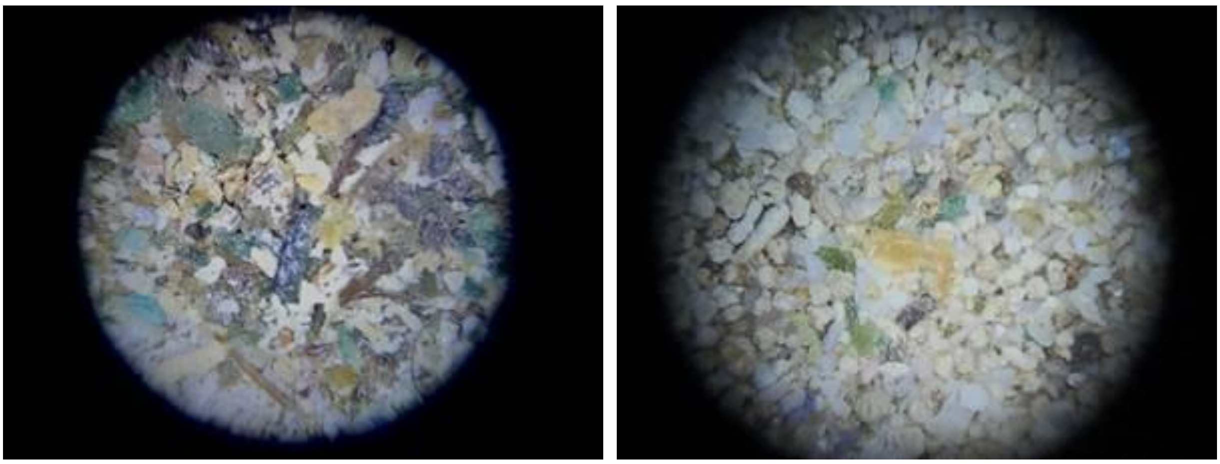

Figure 2. Visual comparison of ground mussel shell before feeding (left) compared with mussel shell that mealworm (*Tenebrio molitor*) larvae have fed on (right). The more spherical shapes of the particles post-feeding suggest these particles have been through the mealworm gut, as this is how frass looks. Chemical analysis is needed to determine whether the material has been changed via the digestive process.

Two small click-clack boxes were established to investigate if adult mealworms would lay eggs on some organic substrates using the same method outlined above (Table 3). Adults were left in the boxes for 2 weeks for oviposition before removal. Three weeks after removal of the adults, 5 mm long larvae were seen amongst the substrate/s in both boxes, indicating that egg lay and initial development on these waste streams is possible. Monitoring of larval development in these boxes is ongoing.

Table 3. Organic wastes provided to adult mealworms (*Tenebrio molitor*) for egg lay.

Dried grape marc	Wine lees	Ground mussel shell	Adults per box
45 g			15
40 g	20 g	20 g	15

Dried grape marc was Sauvignon blanc, and wine lees were from Pinot noir.

1.4 Larger-scale feeding study

1.4.1 Feeding box development

Larger-scale mealworm feeding was assessed this year to build on the 2021 trials. However, to do this required the development of appropriate feeding boxes to supply the substrates in a way that would provide the optimum environment for feeding. This required the separation of the feeding substrate from the insect frass. As part of this process, two prototype boxes were developed and are detailed below.

Prototype feeding box 1

The design of this box focused on the ability to separate insect frass from the waste substrate they were feeding on. This box consisted of a large (50 cm l x 40 cm w x 21 cm h) Perspex box with a fine mesh (1.5 mm aperture) base. Within this box sat a second false mesh base (7 mm x 5 mm aperture) with larger holes to allow the grape seeds to fall through. The box sat within a white plastic tray (51 cm l x 37 cm w x 5 cm h), raised above the base by a rubber stopper at each corner (Figure 3). Seeds within the grape marc collected between the two mesh layers, while insect frass fell through the smallest mesh and collected in the white tray beneath the box (Figure 3).

Figure 3. A large experimental feeding box (prototype 1) to monitor mealworm (*Tenebrio molitor*) feeding. The base of the box had two mesh layers. The top mesh layer allowed grape seeds to fall though and collect on top of the second layer. Insect frass was able to fall through both mesh layers into the white plastic tray. In the left image, the box has been filled with grape marc, and on the right is the separated mealworm frass in the white tray after feeding on a combination of grape marc and flour.

While this feeding box worked well, there were two issues encountered: (1) the removal of the large mesh layer to access the fallen grape seeds was problematic, with grape marc and mealworms falling off the mesh during removal, and; (2) small mealworms were collecting amongst the seeds and appeared to have difficulty returning to the top layer to feed on the grape marc. Due to this, a second feeding box prototype was developed, along with testing two structures to allow any smaller mealworms to move back into the grape marc when they had fallen into the space between the mesh layers.

Prototype feeding box 2

A second feeding box was developed with the aim of overcoming the issues encountered with the first prototype. This box consisted of two stacked boxes (57 cm l x 38 cm w x 32 cm d; Figure 4). The inside box had a base made of the larger mesh used in prototype feeding box 1. The second (outer) box had a base made of the finer mesh. This sat within a plastic tray on rubber stoppers, allowing for the removal of the top (inside) box without the loss of grape marc and mealworms. Grape seeds were held within the lower box on the finer mesh, and frass fell through to the tray below.

Figure 4. A large experimental feeding box (prototype 2) to monitor mealworm (*Tenebrio molitor*) feeding. The feeding box consists of two stacked boxes. The top (inside) box has a large mesh base, allowing the grape seed to be separated. The lower box has a finer mesh, allowing frass to fall through to the tray below. The lower box sits on four rubber stoppers, one in each corner.

Both metal 'ramps' and twist tie 'climbing ladders' were trialled to provide a means of access for any small larvae that had fallen through the top mesh to return to the feeding substrate (Figure 5). Both of these methods worked; however, it appeared easier for the smaller larvae to move up the ramps. These ramps were installed in the feeding boxes between the two mesh layers.

Figure 5. Small-scale box systems trialling the use of metal ramps (left) and twist tie ladders (right) by mealworm larvae (*Tenebrio molitor*) to move from the lower level of grape seeds back up into the grape marc.

1.4.2 Feeding trials

Six large feeding boxes were established, and mealworm development was monitored to examine the ability of mealworm larvae to feed and develop on larger quantities of organic waste. Results from these boxes found that 1 kg of mealworms consumed 2 kg of grape marc every 7–10 days (Table 4). The addition of wholemeal flour did not appear to influence the rate of consumption over the trial period and did not appear to influence mealworm development (Table 4, Box 2 vs Box 5). It was observed that much of the flour appeared to fall through to the lower collection trays of the boxes, even when mixed through the substrates prior to addition to the box. Doubling the amount of grape marc in a box resulted in a similar rate of consumption (Table 4, Box 3). In the small pilot trial (Section 1.3), a mixture of grape marc and wine lees had produced promising results with larvae remaining healthy and active; however, when this was replicated in a large feeding box, it resulted in a dense, sticky substrate that the mealworms had difficulty moving through (Table 4, Box 4). This feeding box was terminated at 11 days due to mealworm death. Boxes 5 and 6 were established to test the ability of mealworms to consume grape marc and develop through their life cycle if the marc is provided in a continuous system. These continuous feed boxes are producing promising results regarding mealworm health and substrate consumption.

Table 4. Larger-scale pilot studies of feeding by mealworm larvae on different organic waste streams.

Box	Fresh grape marc	Wholemeal flour	Wine lees	Ground mussel shell	Mealworms	Outcome
1	⅔ volume	⅓ volume			Equivalent to flour measure	Test of prototype 1 feeding box. All measurements by eye. Everything apart from seeds consumed within 14 d.
2	2 kg	1 kg			1 kg	Everything apart from seeds consumed within 10 d. Much of the flour appeared to have fallen through to the collection tray.
3	4 kg				1 kg	Most grape marc consumed within 25 d. Mealworms pupated, but some were relatively small.
4	4 kg		2 kg		1 kg	Very dense sticky mixture. Mealworms unable to move around easily, and some die. Mould issues. Box terminated at 11 d.
5 (continuous feeding)	2 kg				800 g	Box fed 2 kg grape marc each week. Almost all marc consumed each week. Larvae appear healthy. Pupae developing.
6 (continuous feeding)	2 kg			1 kg	800 g	Most food consumed each week. Larvae appear healthy, and pupae developing.

Boxes 1–3 were fed Pinot noir grape marc. Boxes 4–6 were provided with Sauvignon blanc marc. Wine lees were from Pinot noir. Boxes 1–3 are prototype 1 feeding boxes, and boxes 4–6 are prototype 2 feeding boxes.

2 Nutritional analysis of mealworm larvae

Preliminary analysis to assess the viability of using mealworms as a component of fish food has been conducted within the Growing Futures Aquafeeds Programme funded by The New Zealand Institute for Plant and Food Research Limited (PFR). The nutritional content, including minerals, fatty acids and amino acids, are being measured. Data for amino acid content is not available at this time; however, we report on mineral and fatty acid content below.

To determine if the method of preparation influences fatty acid oxidation and therefore, availability, samples of mealworm larvae were prepared using various combinations of the following four treatments: larvae were culled by either blanching in boiling water or freezing at −20°C and then either freeze-dried or oven-dried. These larvae were collected from a laboratory colony reared on flour and vegetables. Results from these samples do not show significant differences in available minerals, dependent on the method of larval preparation (Table 5, Alayon unpub. data). Mealworms were high in iron and zinc, had almost 50% crude protein and 40% fat (Table 5). Fatty acid analyses of these samples are not yet available.

For comparison, however, mealworms fed on grape marc and flour for 6 weeks were collected at the conclusion of the 2021 trial (Agnew et al. 2021) and these were analysed for fatty acid content and compared with mealworms reared on flour only (full results not presented here). Results indicated that, regardless of food source, the mealworm larvae were high in palmitic (C16:0) and oleic (C18:1n9c) acids, these two making up about 30% each of the total fatty acid content (Afsar unpub. data). Both the omega-3, alpha-linolenic acid (C18:3c9, 12, 15), and the omega-6 linoleic acid (C18:2n6c), represented 0.25% and 15% of the total fatty acids, respectively (Afsar unpub. data). Both of these are essential fatty acids that cannot be produced by the body of most animals, including fish, and so need to be provided within their diet (Di Pasquale 2009, Ruess & Müller-Navarra 2019).

Table 5. Mineral content (calculation dry basis) of mealworm larvae (*Tenebrio molitor*) reared on wholemeal flour, cabbage and carrot (Alayon, unpub. data).

Culled by: Preparation:	Blanched Freeze-dried	Blanched Oven-dried	Frozen Freeze-dried	Frozen Oven-dried
Total nitrogen g/100g	7.5	7.8	7.6	7.7
Crude protein g/100g	46.8	48.4	47.6	47.9
Total fat g/100g	40.7	41.2	41.4	40.6
Calcium g/100 g	0.0	0.0	0.0	0.0
Magnesium g/100 g	0.3	0.3	0.3	0.3
Potassium g/100 g	0.8	0.8	0.8	0.8
Sodium g/100 g	0.1	0.1	0.1	0.1
Phosphorus g/100 g	0.7	0.8	0.8	0.7
Sulphur g/100 g	0.3	0.3	0.3	0.3
Iron mg/kg	41.8	45.4	42.4	44.8
Boron mg/kg	0.9	1.3	0.8	1.1
Copper mg/kg	13.1	14.2	13.1	14.7
Manganese mg/kg	11.8	12.4	12.0	11.8
Zinc mg/kg	111.0	119.6	110.7	114.6

Mealworms were culled either by blanching in boiling water or freezing at −20°C. These samples were then freeze-dried or oven-dried prior to analysis.

3 Conclusions

Trials this year have indicated that mealworm larvae can feed and survive on grape marc and, to some extent, on ground mussel shells. Investigation of larger-scale feeding determined mealworm larvae consumed twice their weight in food, including grape marc, per week. Wine lees were a problematic feeding substrate, and further investigation into pre-treatments would be advantageous.

Future work could focus on:

- Nutritional analysis of mealworms fed on waste streams over multiple generations.
- Analysis of mealworms fed on ground grape seeds, either exclusively or combined with other waste streams. Does incorporating these into the diet influence the nutritional content of the larvae?
- Analysis of mealworms fed on ground mussel shell: Does adding mussel shell to grape marc improve the frass as a fertiliser? Is the nutritional content of these mealworms any different from those fed on the standard flour diet?
- Pre-treatments of wine lees, potentially by fermentation. Yeast is often added to insect diets to improve colony health. Fermented lees may provide additional supplements when included in an insect diet and make the lees less moist.

- Mass-rearing systems for optimum conversion of organic waste streams into useable insect biomass. This includes:
    - Optimising feeding cages – how big can we go?
    - Optimising larval density per feeding box
    - Testing mixing systems to ensure mixing of diet and heat throughout the feeding box.
- Measuring biomass production from mealworms fed on different waste stream food sources to assess the best options for commercial mealworm production.

4 Acknowledgements

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5 References

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