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To the Editor

Dear Editor,

We would like to present our manuscript titled "**Plastic agriculture using worms: Augmenting polystyrene consumption and using frass for plant growth towards a zero-waste circular economy**" for publication in your journal.

In this research, we studied the effects of food additives on polystyrene (PS) consumption by mealworms and superworms, as well as the use of their frass for an indoor dragon fruit cactus (*Hylocereus undatus)* that is both an ornamental and food crop plant. We found that small amounts of common condiments augmented PS consumption, potentially addressing PS food waste often contaminated with food. We found the frass of superworms fed on PS alone did not show difference from those fed on bran as determined by GC-MS, and in fact supported rooting and comparable cacti growth better than mealworm frass.

Our research here shows promising solutions to plastic pollution and urban food production in the society today. Using purely natural solutions, worm as a feasible solution to close the loop in a circular zero waste economy that is also implementable indoors. The study sheds light on the promise of worms that has been gaining a lot of attention for plastic waste management, and the safety of the frass for further agricultural uses. Our findings have significant impact on both ecological health and environmental quality. Preprint is on BioRXiv doi.org/10.1101/2020.05.29.123521

We hope this article would find a home in your journal, as we believe it is useful and of interest to the scientific community and public

Yours Faithfully,

Samuel Ken-En Gan
On behalf of the authors

Plastic agriculture using worms: Augmenting polystyrene consumption and using frass for plant growth towards a zero-waste circular economy.

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ABSTRACT

Polystyrene (PS) is one of the major plastics contributing to environmental pollution with its durability and resistance to biodegradation. Recent research has found mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) to be able to utilize PS as a carbon food source and degrade them without toxic effects. In this study, the effects of food additives on plastic consumption augmentation were studied, with small additions of sucrose and bran found to increase PS consumption. To close the plastic carbon cycle, we also evaluated the use of worm frass for dragon fruit cacti (*Hylocereus undatus*) growth and found that superworm frass supported rooting and growth better than mealworm frass and control media over a fortnight. Superworms, apart from being known fish and poultry feed, have been shown to be a suitable natural solution to the PS plastic problem that can support plant growth towards a zero-waste sustainable bioremediation cycle.

Keywords: Biodegradation, Mealworm, Superworm, Frass, Polystyrene, rooting, agricultural support, waste management

Introduction

Styrofoam is ubiquitous in life today. Widely credited to Ray McIntire of the Dow Company(Scheirs, 2003), Styrofoam or Polystyrene (PS) are light polymers which have low heat conductivity(Campbell, 2012; Scheirs, 2003) and can be synthesized to different shapes and sizes, making it a highly versatile material. From insulating material for buildings to packaging material widely used in food and beverages, PS is used worldwide, but does not have an innocuous place in marine or terrestrial environments as its resistance to chemical degradation results in accumulation and pollution(Rochman et al., 2013). While one current large-scale management of PS waste is by incineration, this leads to toxic fumes in air pollution (Elizabeth Royte, 2019; Ritchie, 2018; Verma et al., 2016), causing harm to human health (Verma et al., 2016).

Superworms (*Zophobas morio*) and mealworms (*Tenebro molitor*) belong to the darkling beetle (*Tenebrionidae*) family and are naturally voracious insect pests in agriculture, consuming dry grain stock. While in many societies, they are also food sources themselves(Sogari et al., 2019), mealworms were recently shown to be able to consume and metabolize plastics (Yang et al., 2015a, p. 1), a capability attributed to commensal gut bacteria in these worms that was confirmed with ^{13}C-carbon isotope tracing experiments among others (Yang et al., 2015b, p. 2). At the larval stage, they can be bred at high density, excreting nitrogen-rich frass waste(Kagata and Ohgushi, 2012) that can be potential fertilizers for plants. The worms are also rich in chitin (Finke, 2007; Soon et al., 2018), a chemical shown to improve the growth and the yields of plants (Egusa et al., 2015; Houben et al., 2020; Poveda et al., 2019). More recently, superworms have also been reported to consume PS at a rate higher than mealworms (Yang et al., 2020), showing promise in the use of superworms in the fight against plastic pollution.

Since food containers make the bulk of PS waste, they are often contaminated with food waste, complicating recycling methods that require clean plastic waste. In this aspect, the use of worms, and the evaluation of possible food contaminants to speed up plastic degradation may be a natural solution that has yet to be fully exploited, especially if the frass can in turn be used to support plant growth, particularly food crop agriculture production. This makes worms the key to turn plastic waste into fertilizers for food production with zero waste.

To evaluate the possibility, this study aims to investigate the role of food additives in plastic degradation by worms, and evaluate the use of worm frass to grow dragon fruit (*Hylocereus undatus*) plant, chosen as it is an easy growing indoor fruit plant with potential urban farming applications.

Materials and methods

Insect rearing and frass collection

Superworms (*Zophobas morio*) and mealworms (*Tenebro molitor*) fed on bran were purchased as pet-food from various pet stores in the Clementi area, Singapore. For various experimental conditions, they were weighed and transferred to polypropylene (PP) containers (impervious to the worms) with the respective test food condiments (see Figure 1A & B for experimental setup). The collection of worm frass was performed by sifting the contents of the containers with a mesh sieve to remove uneaten PS/food and worm parts. The worms were kept in cardboard boxes with a constant humidity of ~50% and a temperature of ~25°C monitored by assembled Arduino devices (not shown).

PS consumption rate experiments

The natural rate of PS consumption (mg of PS / g of worm per day) by superworms and mealworms were determined by rearing them separately. To control for different worm sizes, experimental setups with total worm weights between 6.22 -10.76 g and 0.3-0.39 mg of similarly sized PS balls of diameters ranging between 0.4 to 0.5 cm (Art friend, Singapore) each were set up (Figure 1A & B). For experimental setup with food additives, PS balls were premixed with 25 mg of either cinnamon (Masterfoods, Australia), bran (Bob's Redmill, America), table sucrose (Lippo group) or no additive (control) in polypropylene containers. To improve adherence of food additives to PS balls, 0.9 ml of water was added to the mix. PS balls were collected after 4 days and weighed on an analytical balance to determine the unconsumed amount. Dead worms and final total live worm weights were also recorded, using only the weights of live worms for analysis. All experiments were repeated in sextuplicates.

Worm frass and Dragon fruit (*Hylocereus undatus*) experiment setups

Frass from superworms and mealworms reared solely on PS balls were used as 100 % media for *Hylocereus undatus* cacti. The stock cacti were grown from seeds in indoor office environments for more than four years. The grafting method was used to expand cacti successfully multiple times on spent Oolong leaves (termed tea leaves). For the experiment, the same grafting method was used to transplant cacti branches onto the test media and grown in cleaned plastic wineglasses in individual setups (Figure 1C). Test conditions used were spent tea leaves, bran, superworm, and mealworm frass, to cover the grafted cacti sufficiently to stand.

The grafted cacti were lined up against a window ledge and watered every 2-3 days to wet the media. As much as possible, equal conditions were applied for all the setups in triplicate batches of 3-5 technical replicates. The heights of the grafted cacti were measured before the grafting and after a period of two weeks. The cacti were straightened where necessary, measuring the height from the tip to the bottom of the stem (excluding roots). Observed rooting of the grafted cacti were recorded qualitatively with photographs.

GC-MS analysis of superworm frass

For characterisation, PS balls or frass (10 – 20 mg) from superworms reared on either polystyrene or bran were dissolved in gas chromatography grade dichloromethane solvent, diluted to the same concentrations, and incubated in Eppendorf tubes on a shaker rack for 10 minutes and subsequently centrifuged (14.8k RPM, 5 minutes using table top centrifuge) to remove undissolved solids. The solvent soluble samples were passed through a Teflon Syringe 0.45 um filter and analysed on a GC-MS system (HP 6890 gas chromatography HP-5MS column and HP 5973 mass spectrometry). The GC oven temperature was held at 50°C for 1 minute, then heated up to 250°C by ramp up rate at 10°C/minute, and then held at 250°C for 5 minutes.

Results

Effects of food additives/condiments on polystyrene consumption.

Mealworms and superworms were reared on PS with/without common food additives such as cinnamon, sucrose, and bran. Bran was used as a control as it was the food source with which the worms came purchased and was previously reported to increase the rate of PS consumption when supplemented at half the weight of PS(Yang et al., 2018a). From our results, the addition of all three food additives significantly increased the rate of PS consumption in both species of worms ($p < 0.1 - 0.05$), except for mealworms fed with cinnamon additives (Figure 2). Small amounts of sugar or bran were found to more than double the PS consumption rate, from an average of 1.035 and 1.40 mg / g of worm per day to 1.787 and 2.142 when bran was used as an additive, and 1.9 and 3.546 mg / g of worm per day when sugar was added to PS for superworms and mealworms respectively. In mealworms cofed with small amounts of sugar, the mealworms were demonstrated to significantly outperform those cofed with bran (Figure 2). When comparing the efficacy of sugar between mealworms and superworms, mealworms significantly ate more PS. No significant worm weight change (ranging from -5.43 to 1.79%) in the individual setups were observed in either the mealworms or superworms over the period of four days near the complete consumption of the provided PS (See Table S1 in the supplementary data).

Effect of Superworm and Mealworm frass on Plant growth and rooting.

We sought to determine if frass from worms solely fed on PS can be used as an alternative growth media for plants. From our results, the superworm frass supported a significantly higher proportion of rooting for the dragon fruit cacti compared to those grown on spent Oolong tea leaves or bran (Figures 3 & 4). In superworm frass media, nine cacti rooted (90%) compared to the tea leaves with five cacti rooting (45.5 % rooted, $p = .03$) ; or to those grown on bran, four cacti rooted (36.4 % rooted, $p = .01$, see Table 1). With respect to cacti height growth, plants grown on the superworm frass media gained an average height of 0.5 cm that was not significantly different from those grown on tea leaves (average gain of 0.14 cm). Mealworm frass media alone significantly impaired the growth of plants which lost an average height of 0.52 cm ($p < 0.05$). It was also observed that 5 out of a total of 11 cacti across triplicates died when grown on mealworm frass alone.

A loss of 0.43 cm was also experienced in plants grown on bran but was not significantly different. There were no significant differences between the number of rooting cacti of mealworm frass to both tea leaves and bran. Superworm frass significantly supported rooting better compared to the other media (Figure 3).

GC-MS analysis of superworm frass

To investigate the presence of PS degradation toxic by-products e.g. styrene, the superworm frass were collected and analysed using Gas chromatography–mass spectrometry (GC-MS). Frass were collected from mealworms or superworms reared on PS balls for a fortnight and dissolved in dichloromethane. The GC-MS analysis of the PS balls showed peaks corresponding to styrene and molecules containing benzyl groups, but no notable corresponding peaks were observed in the filtered frass from worms reared on PS balls (Figure 5). The frass samples had notable peaks corresponding to 9-oleamide (C18H35NO) fatty acid primary amides (FAPA) along with smaller peaks corresponding to mainly other FAPAs, short chain alkanes, alcohols and cycloalkanes (Table S3). In general, there were no notable significant differences between the GC-MS analysis of the frass of the superworms fed on PS and bran, suggesting no notable by-products present in PS-fed superworm frass.

Discussion

We set out to investigate the effects of food additives on the rate of PS consumption by mealworms and superworms, and the feasibility of their frass to support plant growth, assessed by the growth height gain and rooting of dragon fruit cacti.

Of the food additives, small additions of table sucrose (25 mg) was the most effective, conferring mealworms a significantly greater appetite to consume plastic when compared to mealworms and superworms cofed on bran and sugar. Superworms fed on sugar experienced the second highest 1.8-fold increase of PS degradation in controls. Bran, previously reported to double the rate of PS consumption when supplemented at half the PS substrate weight (Yang et al., 2018a), was also found in our study to increase the rate of PS consumption by ~1.7 and ~1.5 folds in superworms and mealworms, respectively. This was higher than when cinnamon was supplemented and in the absence of food additives. It should be noted that cinnamon elicited a significantly higher rate than no additive control for superworms, but not for mealworms, which showed no statistical difference. While this might be due to multiple factors, ranging from taste receptors to differences in metabolic/microbial processing of cinnamon, it has been shown that cinnamon did not have negative impact on mealworm consumption of PS. Given that most PS waste are food packages, this bodes good potential for the use of worms to deal with plastic food waste since both mealworms and superworms can consume organic waste and be grown in high densities with no significant weight loss over 4 days (see Table S1 of supplementary data on worm weight changes after PS consumption). Although a previous study showed a possibility of hindering mealworms life cycle on a plastic diet(Matyja et al., 2020) , we did not observe notable abnormalities during our worm breeding other than delayed stages (which is beneficial for plastic degradation) and have a second generation of superworms that is on a pure plastic diet as their parent generation was on (data not shown).

One added advantage of mealworms and superworms over other worms, is that unlike black soldier flies that are commonly used for food waste (Palma et al., 2019), the mature darkling beetles have fused wings/elytra and do not fly, making their biocontainment easier for plastic degradation setups. Thus, any large-scale setups can be performed with minimal concern for their escape.

Comparing the rate of PS consumed by weight of worms per day, there were no significant difference between mealworms and superworms (for control conditions in Figure 2), which was contrary to a recent report that showed superworms to be superior to mealworms in PS consumption(Yang et al., 2020). This was not unexpected as the study calculated and used the rate of PS consumption per individual worm as the basis of comparison. As the difference in mass of a single mealworm compared to a superworm could be as high as 20 folds, calculating by weight rather than number of worms may avoid the possible underestimation of the productivity of mealworms.

Both mealworms and superworms are known fish feed (Henry et al., 2015) and poultry (Finke, 2007), with the added advantage of being valuable plastic degraders(Yang et al., 2018a, 2018b, 2020, 2015a, 2015b) to provide cost-effective feed in food production in urban farming, the worms can contribute to addressing both plastic and food production problems. While further research is necessary to ensure that plasticizers or other plastic degradation products do not bioaccumulate and get introduced into the food chain to humans (see reviews on plasticizer accumulation in the food chain, EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016; Toussaint et al., 2019), the ability of our frass analysis showed no obvious by-product from the degradation of PS.

We did not focus our frass analysis on the mealworm frass as they did not support cacti growth, and the literature on plastic degrading mealworms was already quite extensive (Houben et al., 2020; Yang et al., 2018b). Styrene was not detected in the frass of the PS fed superworms despite it being detected in the PS ball control analysis, neither was there any major notable additional degradation products in the filtered frass of superworms fed on PS compared to those fed on bran. Further evaluation to a wider range of PS products, including coloured or other PS products with additives should be performed before implementation in real-life settings.

For ease of operation, the dragon fruit cacti (*Hylocereus undatus*) was chosen as it is an easy to grow indoor plant that is both an ornamental and food crop for the evaluation of frass for urban farming. The superworm frass alone was better at supporting rooting (90% rooting compared to 45.4% in used tea leaves) and was at least as effective as spent tea leaves in supporting growth as determined by cacti height gain over a fortnight. Mealworm frass on the other hand, resulted in a lot of failed grafts, while bran media resulted in poor height and rooting support. It is possible that short chain growth promoting alkene semiochemicals (e.g. Heptacosane, Nonadecane and Octadecane, Jishma et al., 2017), as well as chitin in the superworm frass may have augmented rooting (chitin was previously reported to support rooting, Winkler et al., 2017), or that there was some other auxin like compound present that would require further analysis. It should also be noted that the superworm frass was less pungent than the ammonia-tinted mealworm frass, giving more support beyond rooting and comparable cacti growth for the use of superworm frass.

The lack of support of mealworm frass on dragon fruit cacti growth is unexpected given a previous report (Houben et al., 2020; Poveda et al., 2019) **.** This difference may be due to the usage of 100% frass for our evaluation or due to the different nutritional requirements of the dragon fruit cacti, or the difference in frass from mealworms that are fed purely on PS. It may be possible to tailor this to looking in other plants.

Given that there was no known benefit of mealworm frass in the dragon fruit cacti in our setups, and that consumption of PS by both mealworms and superworms showed no difference, the use of superworms over mealworms is proposed in closing the loop from plastic to fish/poultry feed to frass-supported agriculture. Much remains to be studied on the possible accumulation of plasticizers or other plastic-derived chemicals as well as the frass on a variety of other food crops, but current results are promising with previous studies showing about 40 to 50% degradation of polystyrene monomers in mealworms(Yang et al., 2015a) and superworms(Yang et al., 2020), respectively in the span of a fortnight as determined by respirometry experiments. With further incubations of the waste frass supplemented with food additives and even tailored microbial assimilation of the PS polymers, total degradation could be made more complete if necessary. Since plants are able to clear up toxins from the environment (Cristina Negri and Hinchman, 1996), it is possible that any potential toxic substances arising from other plastics, could be combined with phytoremediation (Cristina Negri and Hinchman, 1996; Negri and Hinchman, 1996).

There are exciting research based on enzymes isolated from bacteria present in plastic eating worms (Austin et al., 2018; Danso et al., 2019; Palm et al., 2019; Yoshida et al., 2016), but the implementation of these processes towards complete degradation into harmless substances at industrial scale will require further research and engineering in the face of an increasingly pressing problem of plastic waste. In the meantime, the natural solution of worms can be investigated further for more immediate implementation, especially their simultaneous roles for urban farming in both fish/poultry feed and their frass for food crops. Worms are naturally more resistant to environmental factors compared to pure enzymes and can overcome obstacles for enzymes in plastic crystallinity or accessibility of the polymer chains, such that while protein engineering of such enzymes [31] are promising, there is still much to optimize before large scale implementation compared to worms.

The setups of both PS consumption by worms and frass-supported cacti growth were all performed indoors, demonstrating the possibility of worms to be an environmentally friendly urban solution to plastic waste and food sustainability that can be implemented widely, even within homes.

Conclusion

In conclusion, with evidence that food additives augment rather than antagonize PS degradation, and that the frass can be used to support food crop growth while the worms are themselves sources of poultry and fish feed, the answer in the worms is a very fitting scalable solution to both the plastic pollution and food (aquaculture and agriculture) production problems.

Declarations and Conflict of Interests

The authors declare no conflict of interest with this work.

Author Contributions

DWSK, BYXA, JYY, SKEG performed the worm culturing and plant growth experiments. ZX performed the GCMS experiments. DWSK, JYY, SKEG, analysed the results and wrote the manuscript. SKEG designed and supervised all aspect of the study. All authors read and approved the manuscript.

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Table 1. Effect of different medias on number and proportion of rooting cacti. n=44, df=1

Media	Control	Proportion of Cacti rooted	Observed Total Number of Cactus Rooted	Observed Total Number of Cactus Not Rooted	Total	Pearson's χ^2 *P*-value	Pearson's χ^2 totals
Tea leaves	Bran	45.5%	5	6	11	.66	0.2
Bran	Tea leaves	36.4%	4	7	11	.66	0.2
Mealworm frass ^	Tea leaves	15%	2	4	6	.49	0.5
	Bran					.63	0.2
Superworm frass ^	Tea leaves	90%	9	1	10	.03*	4.7
	Bran					.01*	6.4

Note. * denotes $P < .05$. ^ Five out of eleven cactus plants with mealworm frass died. One out of eleven cactus plants with superworm frass died

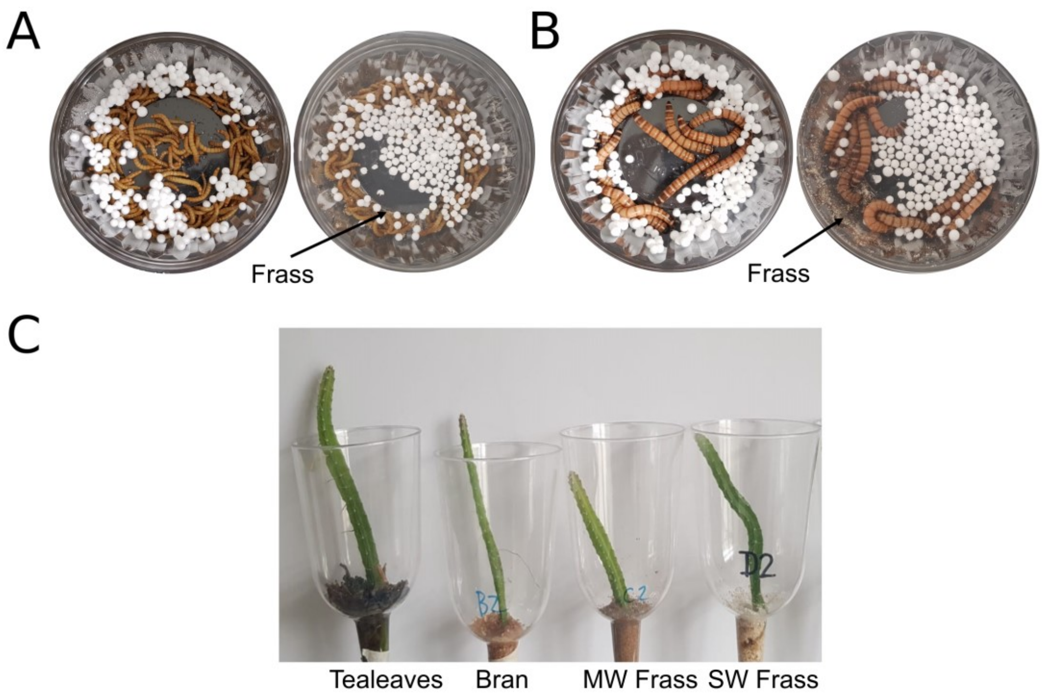

Figure 1: Representative images of the setups for testing PS consumption rates by A) mealworms; B) Superworms. For both A and B, the left are the initial setups, and the right showed the setup after four days where frass was produced from the PS consumption. (C) Setup of the dragon fruit cacti grafted onto the test media of tea leaves, bran, MW, and SW frass.

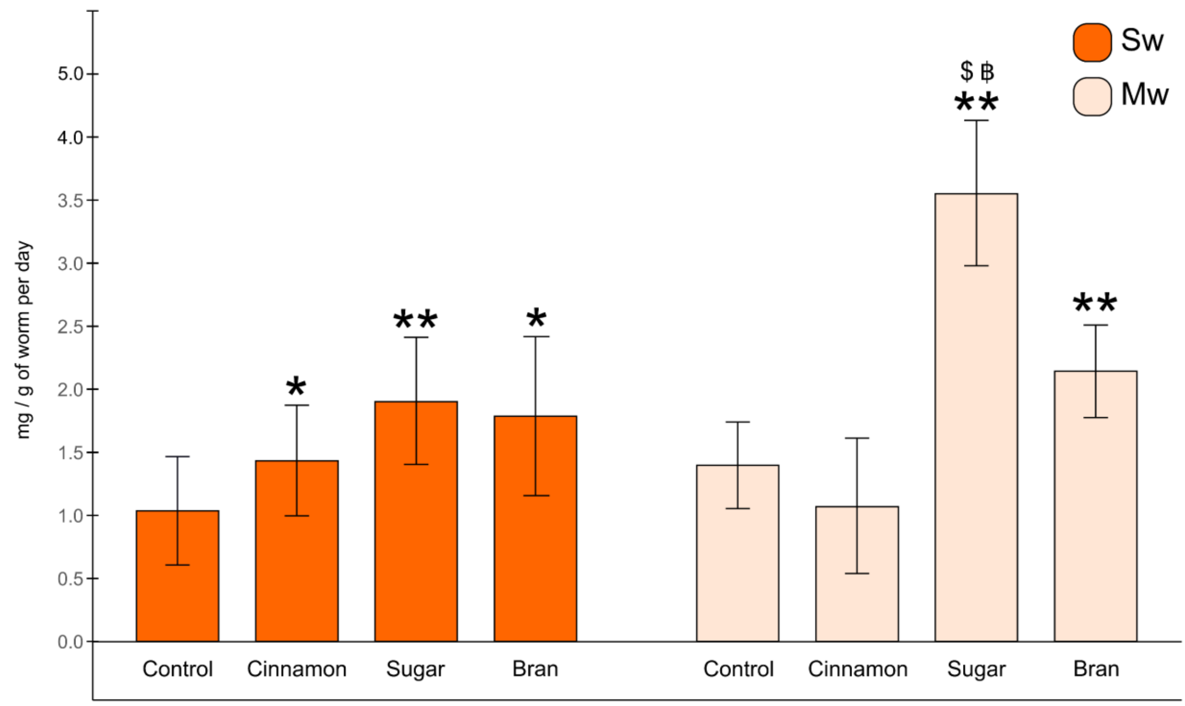

Figure 2 Average rate of PS consumption (mg / g of worm per day) by superworms (Sw) and mealworms (Mw) with and without food additives (cinnamon, sugar and bran). Additives were mixed with PS balls and sprayed with DI water to allow the additives to adhere to the Styrofoam balls. The residual PS were weighed after four days. Results are reported as standard error of means from 6 replicates, statistical analysis were performed with one tail Student's T-test. * = $p < 0.1$, **= $p < 0.05$ versus corresponding controls of the same worm species; ฿ $p < 0.05$ versus Bran of the same worm species (cofeeding bran had been previously been reported to boost PS consumption); $ $p < 0.05$ versus corresponding setup of a different worm species.

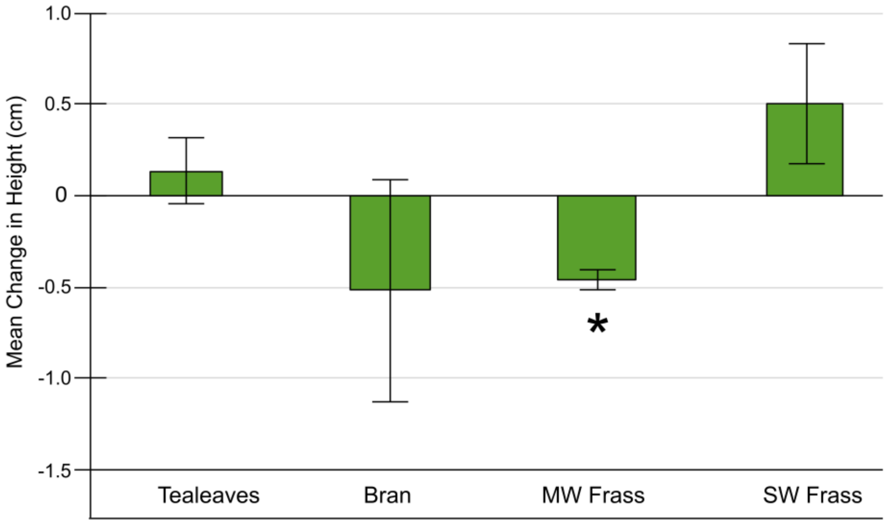

Figure 3: Mean cacti height differences grown on the respective media over a fortnight with standard error from three sets of 3-5 technical replicates each. * = a significant change in cacti height compared to tea leaves control ($p < 0.05$, two-tailed student's T-test). MW = mealworm frass, SW = superworm frass.

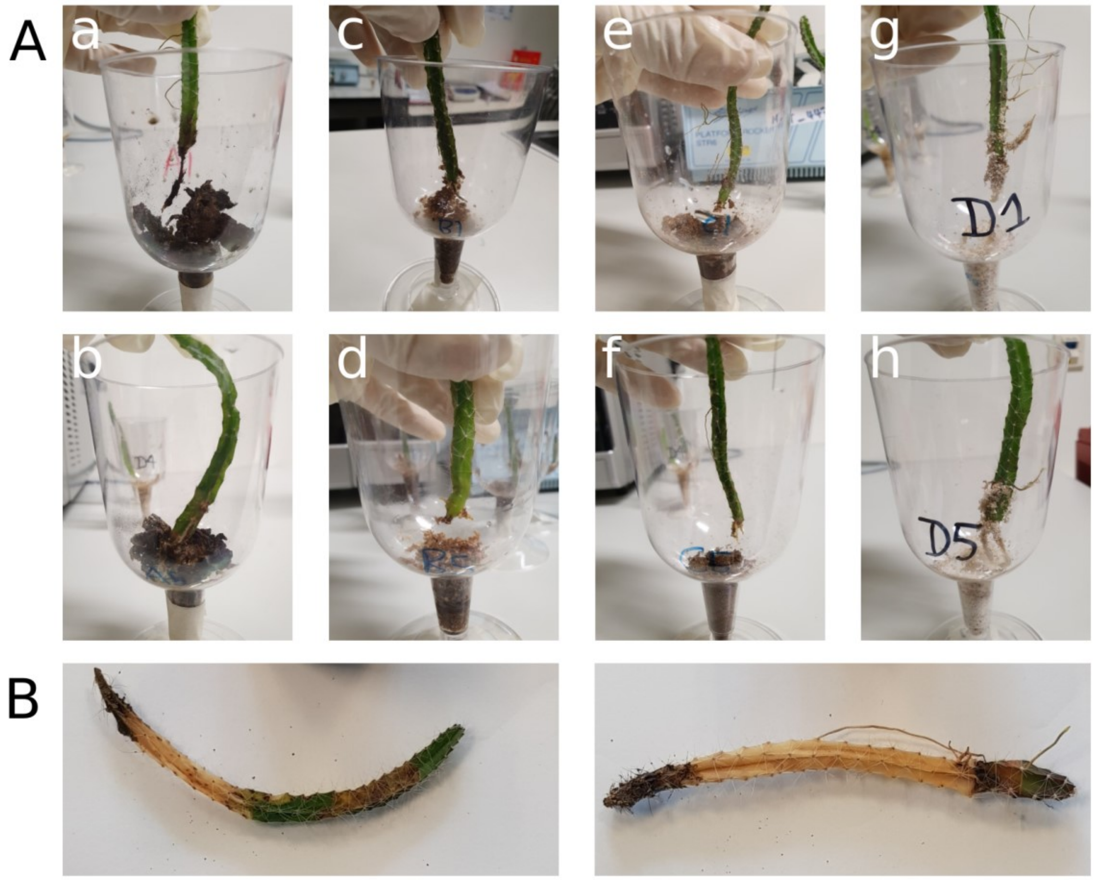

Figure 4: Dragon fruit cacti grown on frass, bran and tea leaves after a fortnight. (A) Two technical replicates of cacti grown on tea leaves (a,b), Bran (b,f), Mealworm frass (c,g) and Superworm frass(d,h). (B) Dead cacti from mealworm frass setups.

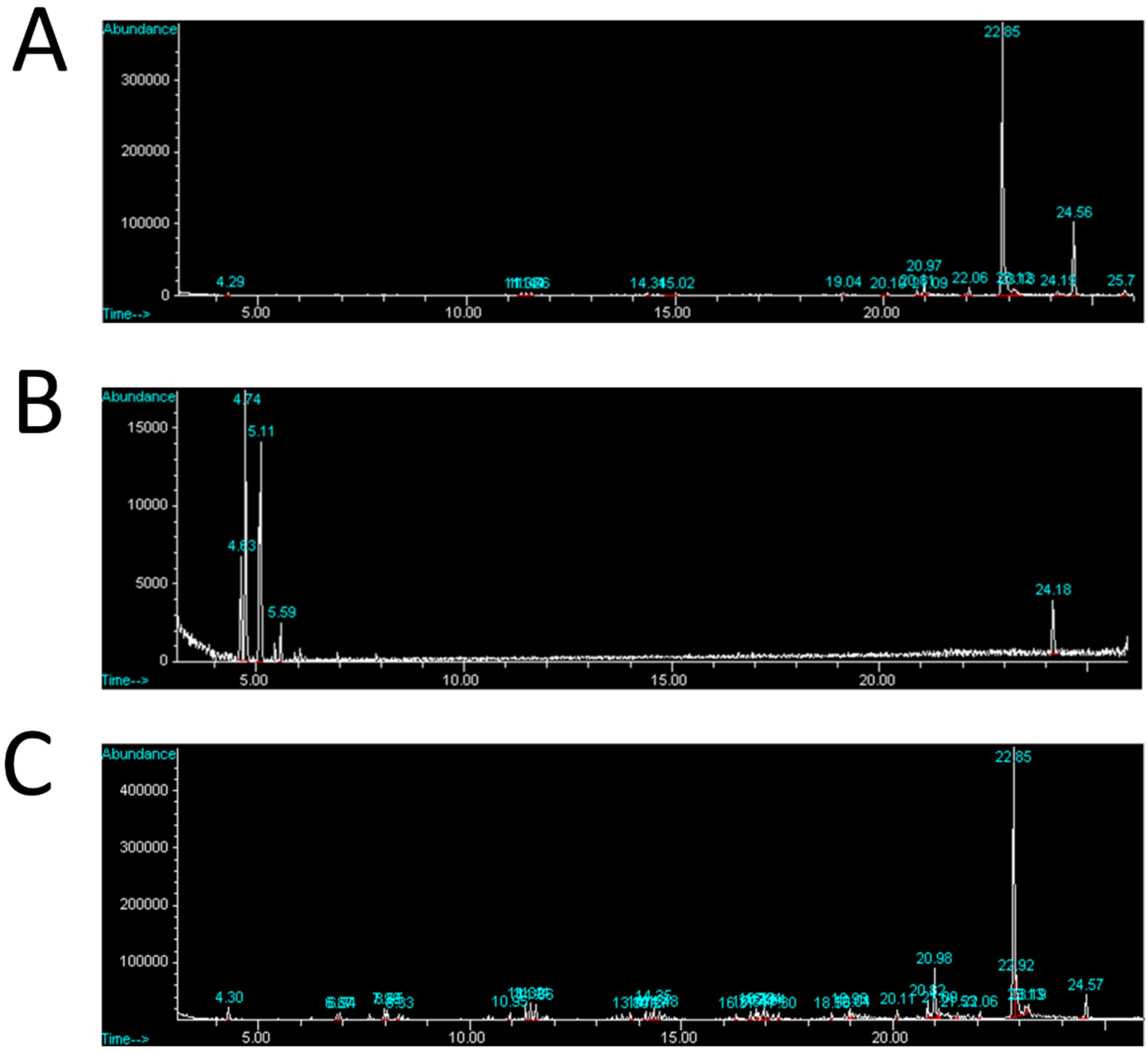

Figure 5: Representative GC-MS graphs from (A) frass of PS fed superworms, (B) PS balls control, and (C) frass from superworms fed on bran. A table of proposed chemicals corresponding to the identities of the different peaks are provided in the supplementary table S3.

Supplementary Data

Table S1. Change in worm weight after four days with different additives. Statistical analyses were performed using two-tailed Student's T-test.

Sample	Average change in worm weight (g)	SEM	Average Change in worm weight (%)	t-test *P* value (two-Tailed)
Control SW	0.00	0.08	-0.04	0.97
Cinnamon SW	-0.11	0.09	-1.24	0.27
Sugar SW	0.16	0.07	1.79	0.06
Bran SW	-0.06	0.08	-0.69	0.50
Control MW	-0.24	0.22	-2.85	0.32
Cinnamon MW	-0.20	0.16	-2.36	0.27
Sugar MW	-0.46	0.29	-5.43	0.18
Bran MW	-0.17	0.12	-1.98	0.23

Table S2. Effect of different media on mean change in height of Cactus plant. statistical analyses were performed using two-tailed Student's T-test.

Media	**Control**	**t-test *P* value(two - tailed)**	**Mean Change in Height (cm)**	**SEM**
Tea leaves	Bran	.32	0.14	0.18
Bran	Tea leaves	.32	-0.52	0.60
Mealworm frass	Tea leaves	.01*	-0.43	0.06
	Bran	.89	-0.43	0.06
Superworm frass	Tea leaves	.36	0.50	0.33
	Bran	.16	0.50	0.33

Note. * denotes $P < .05$. 5 out of 11 cactus plant replicates with mealworm frass died. 1 out of 11 cactus plant replicates with superworm frass died

Table S3. List of chemicals from a mass search of the peaks detected in replicates of GC-MS in the runs.

Peak	Retention Time (minutes)	Potentially proposed chemical
1	4.63	Benzene, ethyl-
2	4.74	Benzene, 1,4-dimethyl-
3	5.11	Styrene
4	5.59	Benzene, (1-methylethyl)-
5	8.05	Cyclopentane, (2-methylbutyl)-
6	11.32	1-Methyl-3-propyl-cyclooctane
7	11.44	Cyclohexane, 1,2,3-trimethyl-, (1.alpha.,2.beta.,3.alpha.)-
8	14.15	Octadecane, 1-(ethenyloxy)-
9	14.48	Octadecane, 1-chloro-
10	16.78	Heptacosane
11	16.94	1-Dodecanol, 3,7,11-trimethyl-
12	18.98	Nonadecane
13	19.05	N-tetradecanoic acid amide
14	20.11	2-ethylthio-N-allyl-N-methylaniline
15	20.82	9-Octadecenamide, (Z)- or OLEOAMIDE
16	22.86	9-Octadecenamide, (Z)- or OLEOAMIDE
17	23.13	9-Octadecenamide, (Z)- (CAS) or OLEOAMIDE
18	24.18	Benzenepropanamine, N-methyl-, hydrochloride
19	24.57	Pentacosane