Article title: Plastic agriculture using worms: Augmenting polystyrene consumption and using frass for plant growth towards a zero-waste circular economy.
Authors: Darius Wen-Shuo Koh[1], Brennan Yi-Xing Ang[2], Joshua Yi Yeo[3], Zhenxiang Xing[4], Samuel Ken-En Gan[5]
Affiliations: A*STAR[1]
Orcid ids: 0000-0001-9936-5090[5]
Contact e-mail: samuel_gan@eddc.a-star.edu.sg

DOI: 10.14324/111.444/000048.v2
Preprint first posted online: 21 December 2020
Keywords: Biodegradation, Mealworm, Superworm, Frass, Polystyrene, rooting, agricultural support, waste management , Waste, Environmental science, Circular economy

June 24, 2020

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 their natural PS consumption, potentially addressing PS waste often contaminated with food. We found the frass of superworms fed on PS alone did not show obvious 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 are a feasible solution to close the loop in a circular zero waste economy that is also implementable even indoors. The study sheds light on the promise of worms that has been gaining a lot of attention for plastic waste management, and the potential 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.

Darius Wen-Shuo Koh[1], Brennan Yi-Xing Ang[1], Joshua Yi Yeo[1], Zhenxiang Xing[2], Samuel Ken-En Gan[1*]

Affiliation:

[1]Antibody & Product Development Lab, Agency for Science, Technology, and Research (A*STAR), Singapore

[2]Institute of Materials Research and Engineering, Agency for Science, Technology, and Research (A*STAR), Singapore

*Corresponding author: Samuel_Gan@eddc.a-star.edu.sg

A*STAR, Antibody & Product Development Lab

60 Biopolis Street, #B2 Genome

Singapore 138672

Tel: +65 6407 0584

ABSTRACT

Polystyrene (PS) is one of the major plastics contributing to environmental pollution with its durability and resistance to natural biodegradation. Recent research have found mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) to be able to utilize PS as a carbon food source and degrade them without observable toxic effects. In this study, the effects of food additives on plastic consumption augmentation were studied, where small additions of sucrose and bran found to increase PS consumption. To close the plastic carbon cycle, we further 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

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 food and beverages, PS is used worldwide, but it 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 incineration, this leads to the release of toxic fumes to cause air pollution (Elizabeth Royte, 2019; Ritchie, 2018; 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 even though they are also food sources in some cultures (Sogari et al., 2019). Recently, mealworms were shown to be able to naturally consume, metabolize and mineralize the carbon in PS (Yang et al., 2015a, p. 1), an ability attributed to the commensal gut bacteria in these worms, verified by ^{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 (Kagata and Ohgushi, 2012) and chitin rich (Finke, 2007; Soon et al., 2018) frass waste that were shown to improve growth and yields of many plants (Egusa et al., 2015; Houben et al., 2020; Poveda et al., 2019). With the potential of mealworm frass to substitute traditional NPK (Nitrogen, Phosphorus, and Potassium) based fertilizers, these worms were suggested to fuel the circular economy (Houben et al., 2020). With recent reports that superworms were able to consume PS at a rate higher than mealworms (Yang et al., 2020), there is promise in using superworms to join mealworms in the fight against plastic pollution.

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

To evaluate the possibility, this study aims to investigate 1) the efficacy of food additives to augment PS degradation by mealworms and superworms; and 2) evaluate the use of the frass of super and mealworms to support dragon fruit (*Hylocereus undatus*) cacti grown (evaluated by rooting and growth), 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 fish and bird feed sold in pet stores in the Clementi, Singapore. 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 (if any). The worms were kept in cardboard boxes with a constant humidity of ~50% and a temperature of ~25^{o}C (previously reported to be ideal for PS consumption by worms, Yang et al., 2018a) 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 in our setup were determined by rearing them separately in replicates. To control for different worm sizes, the experimental setups were based on total worm weights of between 6.22 -10.76 g and 300-390 mg of PS balls with diameters between 0.4 to 0.5 cm (Art friend, Singapore) in replicate set ups (Figure 1A & B). For food additives testing, 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. Unconsumed PS balls were collected after 4 days and weighed on an analytical balance. The final total live worm weights (Table 2) were used for calculation. Experiments were repeated in sextuplicates.

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

Frass obtained from superworms and mealworms previously reared solely on PS balls were evaluated as media for *Hylocereus undatus* cacti. Stock cacti were grown from seeds in air-conditioned office environments for more than four years prior and expanded from the same original pot. The grafting method of budding offshoots was used to expand cacti successfully multiple times on used Chinese tea leaves (termed tea leaves) throughout the four years. For this evaluation experiment, the same grafting method was used to transplant 48 selected cacti branches of as similar size as possible onto the test media and grown in cleaned plastic wineglasses in individual setups (Figure 1C) with the funnelled bottom to support upright supplanting of the cacti. Media tested included: used leaves, bran (Bob's Redmill, America), superworm frass, and mealworm frass. For each set-up, the media covered the bottom of the grafted cacti, supporting it to stay upright to form the soil line.

The grafted cacti were lined up against a window ledge and watered every 3 times a week to wet the media. As much as possible, equal conditions were applied for 11 replicates. The height of the straightened grafted cacti (from the tip to the bottom of the stem, excluding roots) were measured before grafting and after a period of two weeks. Only the rooting for living cacti below the soil line are considered (to rule out confounding existing aerial roots which occur above the soil line, and that rooting below the soil line demonstrate direct effects of the media). Observed rooting of the grafted cacti were recorded qualitatively with photographs. Dead cacti (supplementary table S1) were also recorded.

GC-MS analysis of superworm frass

For characterisation, GC-MS analysis was performed as adapted and modified from a previous report (Yang et al., 2015b). PS balls or frass (20 mg) from superworms reared on either polystyrene or bran were dissolved in a consistent manner by gram of frass to fixed volume of gas chromatography grade dichloromethane solvent for standardized comparisons of peak heights later, and incubated in 2 ml microfuge 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 filtered using a 0.45 μm teflon syringe 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 set to 50°C for 1 minute and 250°C for 5 minutes by ramp up rate at 10°C/minute.

Results

Augmentation effects of food additives/condiments on polystyrene consumption.

Mealworms and superworms were reared on PS with/without common food additives: cinnamon, sucrose, and bran. Bran was the initial food source the worms were supplied in, and thus was used as a control. It was also previously reported to increase the rate of PS consumption when supplemented at half the weight of PS (Yang et al., 2018a). From our results, addition of all three food additives significantly increased the rate of PS consumption in mealworms ($p < 0.1$) (Figure 2). Whereas only the addition of sucrose increased the rate of PS consumption in superworms. Small amounts of sucrose or bran (25 mg) 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 1.79 (not statistically significant) and 2.14 ($p < 0.05$) when bran was used as an additive, and 1.9 ($p < 0.1$) and 3.55 mg / g ($p < 0.05$) of worm per day when sucrose was added to PS for superworms and mealworms respectively. In mealworms cofed with small amounts of sucrose, the mealworms were observed to significantly consume more PS than those cofed with bran (Figure 2). Comparing the efficacy of sucrose on mealworms and superworms, mealworms significantly ate more PS. With the exception of superworms fed on PS with sucrose additives that recorded a slight increase in weight 1.79% ($p < 0.1$), no significant worm weight change were observed in either the mealworms or superworms over the period of four days on the PS diets (See Table S1 in the supplementary data).

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

We next sought to determine if frass from worms solely fed on PS can be used as an alternative growth media for plants to evaluate the zero-waste circular economy solution. From our results, the superworm frass supported a significantly higher proportion of rooting for the grafted dragon fruit cacti offshoots compared to those grown on spent 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, $p < 0.05$); or to those grown on bran, four cacti rooted (36.4 % rooted, $p < 0.05$, 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 ($p < 0.05$), due to water loss, possibly also reflecting the water holding abilities of the frass. 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 observed in plants grown on bran but was not significantly different from the other test media. 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, Figure S1).

GC-MS analysis of superworm frass

To investigate the presence of PS and possible by-products e.g., styrene, the superworm frass were collected and analysed using Gas chromatography–mass spectrometry (GC-MS). Analysis of the PS balls alone showed peaks corresponding to styrene and molecules containing benzyl groups, but no notable corresponding peaks were observed in the filtered frass from the superworms reared on PS balls (Figure 5). The frass samples had notable peaks corresponding to 9-oleamide ($C_{18}H_{35}NO$) fatty acid primary amides (FAPA) along with smaller peaks corresponding to mainly other FAPAs, short chain alkanes, alcohols, and cycloalkanes (Figure

5, Table 3). In general, there were no notable significant differences between the GC-MS analysis of the frass of the superworms fed on PS and bran.

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 alone to support plant growth, assessed by the growth height gain and rooting of grafted dragon fruit cacti offshoots.

Food additives

Of the food additives, small additions of table sucrose (25 mg) were found to be the most effective, conferring mealworms greater consumption of the PS balls when compared to mealworms and superworms cofed on bran and sucrose respectively. Mealworms and superworms fed with sucrose additives experienced the highest increase in PS degradation, ~2.5 ($p < 0.05$) and ~ 1.8-fold ($p < 0.1$) respectively. 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 (not statistically significant) and ~1.5 ($p < 0.05$) folds in superworms and mealworms, respectively. This was higher than cinnamon- supplemented PS, 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. While this might be due to multiple factors, ranging from different taste receptors to metabolic/microbial processing of cinnamon, cinnamon did not have negative impact on mealworm consumption of PS. Given that most PS waste are food packages, the findings here bode well since both mealworms and superworms can consume organic waste and be reared in high densities. There was no significant weight loss over the 4 days of experimentation (see Table 2). Although a previous study showed possible hindering of 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) given that the larvae ate more than adult beetles. In fact, our superworm beetles laid eggs to give rise to a second generation of superworms growing on a pure plastic diet as their parent generation did (data not shown).

Comparison of superworms to mealworms

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 have normalized the underestimation of mealworm productivity. Given that in future real-life application, actual counting of worms is not feasible to deal with the tonnes of PS waste generated daily, we adopted weight as the measurement for future scalability purposes.

Zero-waste circular economy

Both mealworms and superworms are known fish (Henry et al., 2015) and poultry (Finke, 2007) feed, and now with the added advantage of being valuable plastic degraders (Yang et al., 2018a, 2018b, 2020, 2015a, 2015b)**.** As a potential cost-effective feed source for urban poultry and fish farming, the worms could already 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), there is great promise in the use of the worms themselves as a solution to plastic waste. In fact, one added advantage of mealworms and superworms over other beetles larvae, 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 significantly easier for plastic degradation setups. Thus, any large-scale setups can be performed with minimal concern for their escape, leaving their frass to be addressed in a zero-waste circular economy.

Frass analysis

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). Through GC-MS analysis, we did not detect styrene in the frass of the PS fed superworms despite it being detected in the PS ball control analysis, nor were there any major notable additional degradation products in the filtered frass of superworms fed on PS compared to those fed on bran. Further evaluation using more sensitive methods to rule out possible bioaccumulation, including testing a wider range of PS products such as coloured or other PS products with additives should be performed before implementation in real-life settings. As was found in previous reports (Yang et al., 2018a, 2018b, 2020, 2015a, 2015b), microplastics can be present in the frass and these can indeed pose a problem as they accumulate. However, it is possible that with repeated re-digestion of the frass by the worms, this problem can be mitigated. A possible industrial set up is to have a multi-layered process where frass is then fed to another chamber of worms and the process repeated until complete removal of microplastics. It is possible that both mealworms and superworms can be utilized in such a setup as our other experiments (not shown) showed that apart from occasional cross-eating, they can be bred together.

For ease of operation, the dragon fruit cacti (*Hylocereus undatus*), an easy to grow indoor plant that is both an ornamental and food crop was chosen for the evaluation of frass for urban farming. The superworm frass alone was found to be better at supporting rooting (90% rooting compared to 45.4% in used tea leaves) and was at least as effective as spent tea leaves (the media used in the last four years to expand the cacti) 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 (likely due to its observed poorer ability to retain moisture), while bran media resulted in poor height and rooting support, with shrinkage due to water loss in some replicates. It is possible that short chain growth promoting alkene semiochemicals which were detected from GC-MS analysis (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 smelling mealworm frass, providing more reasons 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 though unlikely, the difference in frass from mealworms that are fed purely on PS. It may be possible to further evaluate mealworm frass for other food crops.

Further research

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 here to be the better candidate 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, addressing even microplastics. Since many 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 urgently increasingly pressing problem of plastic waste made worse by lockdown measures during the COVID19 pandemic. 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 (Ma et al., 2018) 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.

Acknowledgements

This work was supported by A*STAR core funds. The authors thank Ghin-Ray Goh, Benjamin Jun-Jie Aw, Elizabeth Yun-Ling Tay, Wen-Jie Tan, Xiaoyun Guan, Wai-Heng Lua for assistance rendered in the husbandry of the worms and in parts of writing of the manuscript.

We also thank Alfred Huan for access to the GC-MS.

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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. Rooting was counted only if they appeared below the soil line as the cacti often had pre-existing aerial roots.

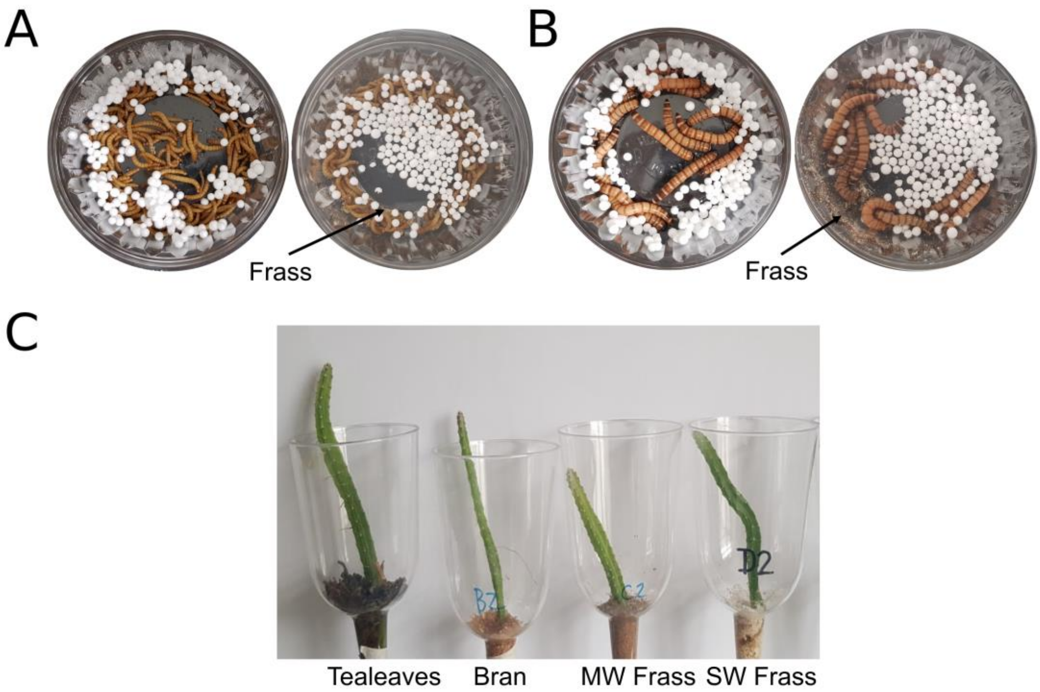

Figure 1: Representative images of the setups for testing PS consumption rates by (A) mealworms; (B) Superworms. For both A and B, the left are the initial setups, and the right showed the setup after four days where frass was produced from the PS consumption. (C) Setup of the dragon fruit cacti grafted onto the test media of tea leaves, bran, MW, and SW frass in reused plastic wineglasses. The funnel end allowed the grafted cacti to be covered by less frass and also stay upright.

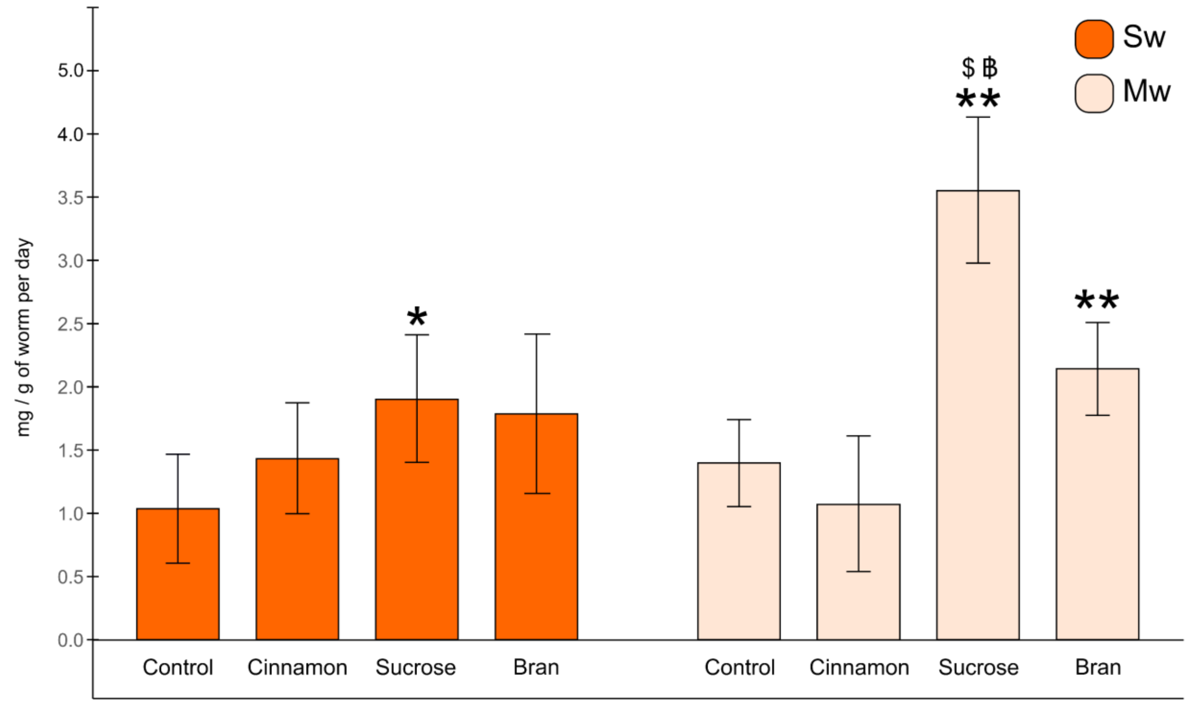

Figure 2 Average rate of PS consumption (mg / g of worm per day) by superworms (Sw) and mealworms (Mw) with and without food additives (cinnamon, sucrose and bran). Additives were mixed with PS balls and sprayed with DI water to allow the additives to adhere to the styrofoam balls. The residual PS were weighed after four days. Results are reported as standard error of means from 6 replicates, statistical analysis were performed with two tailed 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 (co-feeding bran had been previously been reported to boost PS consumption); $ $p < 0.05$ versus corresponding setup of a different worm species.

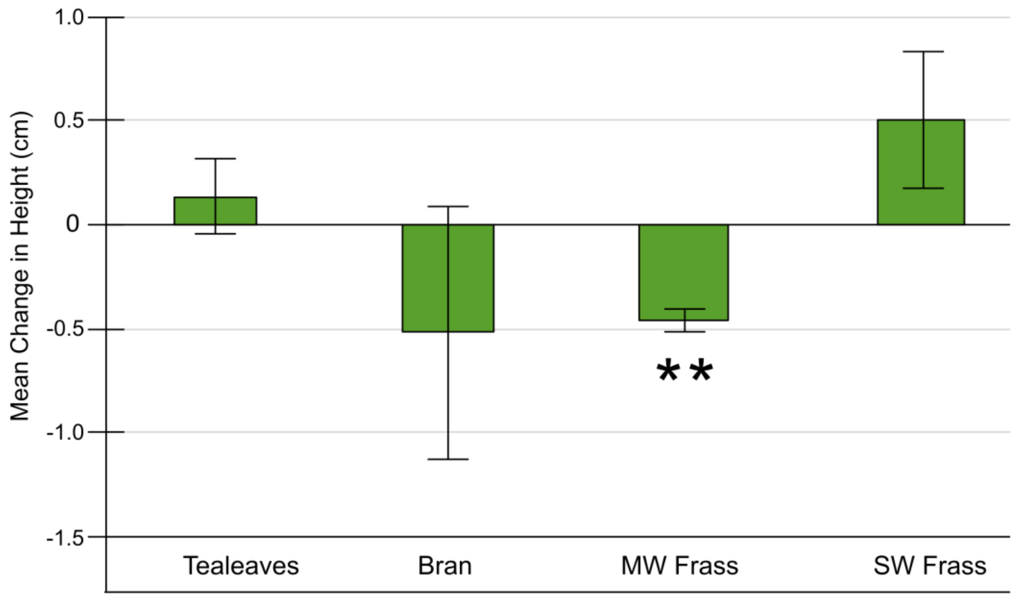

Figure 3: Mean cacti height differences grown on the respective media over a fortnight with standard error from 11 replicates. ** = significant changes in cacti height compared to tea leaves control ($p < 0.05$, two-tailed student's T-test). MW = mealworm frass, SW = superworm frass.

Figure 4: Dragon fruit cacti grown on frass, bran and tea leaves after a fortnight. (A) Representative pictures of cacti grown on tea leaves (a, b), Bran (c, d), Mealworm frass (e, f) and Superworm frass (g, h). (B) Dead cacti from mealworm frass setups. Aerial roots occurring above the soil line were not counted, as many of them pre-existed prior to the start of the experiment.

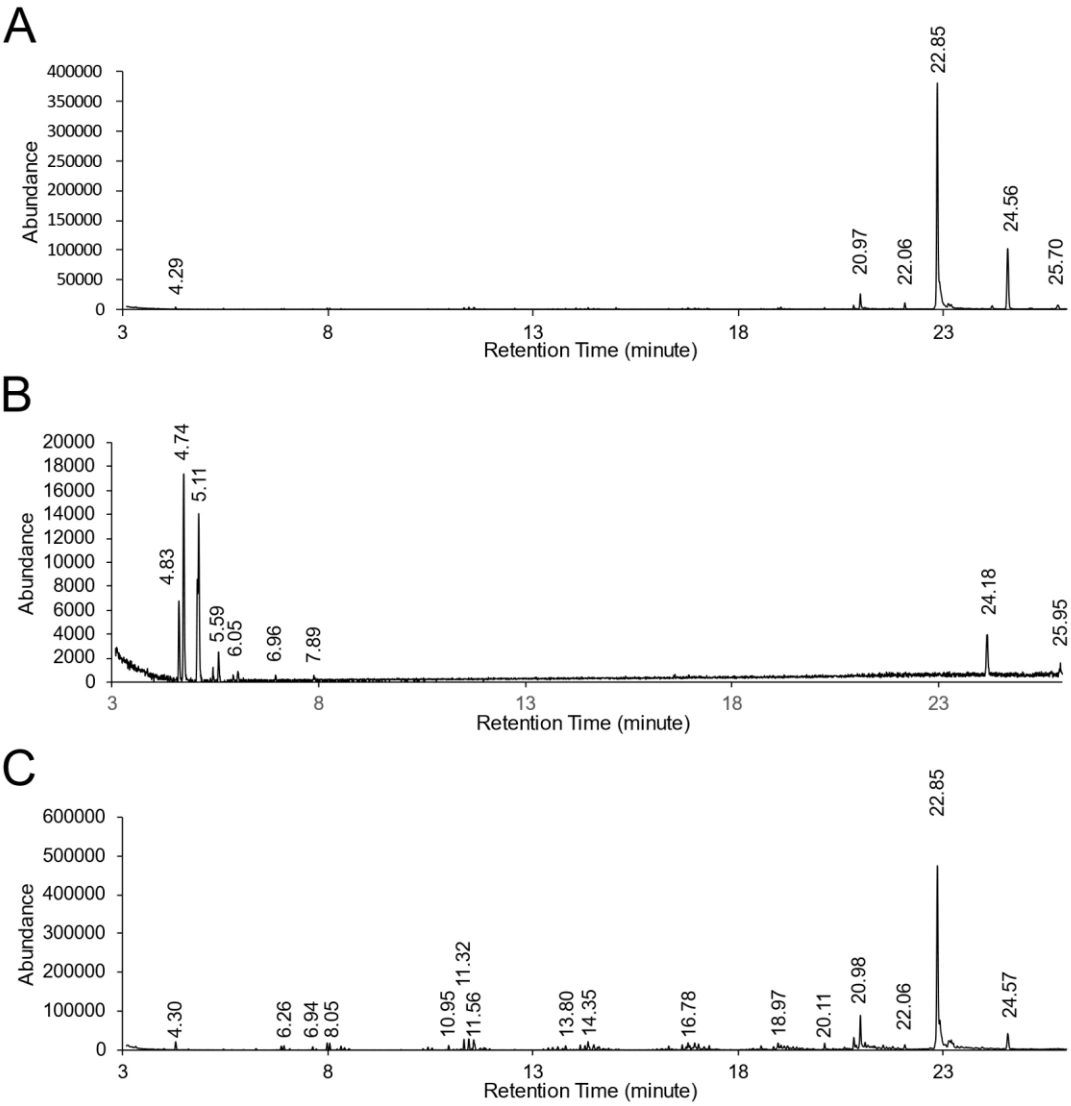

Figure 5: Representative GC-MS graphs from (A) frass of PS fed superworms, (B) PS balls control, and (C) frass from superworms fed on bran. A table of proposed chemicals corresponding to the identities of the different peaks are provided in Table 3

Table 2. Change in worm weight after four days with different additives. Each set-up was performed in sextuplicate. Statistical analyses were performed using two-tailed Student's T-test * = $p < 0.1$, with the average change in worm weight calculated in (g) and (%).

Set-Up		Initial Worm Weight (g)	Final Worm Weight (g)	Change in worm weight (g)	Average change in worm weight (g)	SEM	Average Change in worm weight (%)	t-test P value (two-Tailed)
Superworms		6.95	7.19	0.24				
		7.42	7.46	0.04				
	Control	7.46	7.55	0.09	<-0.01	0.08	-0.04	0.97
		10.40	10.38	-0.02				
		10.70	10.67	-0.03				
		10.33	9.99	-0.34				
		6.98	6.93	-0.05				
		7.08	7.11	0.03				
	Cinnamon	6.77	6.97	0.20	-0.11	0.09	-1.24	0.27
		10.66	10.48	-0.18				
		10.40	10.14	-0.26				
		10.33	9.94	-0.39				
		7.01	7.30	0.29				
		7.40	7.73	0.33				
	Sucrose	6.62	6.90	0.28	0.16	0.07	1.79	0.06*
		10.55	10.53	-0.02				
		10.31	10.36	0.05				
		10.71	10.72	0.01				
		6.57	6.67	0.10				
		7.26	7.13	-0.13				
	Bran	7.05	7.30	0.25	-0.06	0.08	-0.69	0.50
		10.10	9.99	-0.11				
		10.48	10.15	-0.33				
		10.39	10.25	-0.14				
Mealworms		6.79	6.36	-0.43				
		6.29	5.37	-0.92				
	Control	6.44	5.65	-0.79	-0.24	0.22	-2.85	0.32
		10.49	10.58	0.09				

	10.46	10.70	0.24				
	10.76	11.11	0.35				
Cinnamon	6.22	5.74	-0.48	-0.20	0.16	-2.36	0.27
	6.23	5.89	-0.34				
	6.45	5.89	-0.56				
	10.54	10.63	0.09				
	10.44	10.90	0.46				
	10.45	10.09	-0.36				
Sucrose	6.36	5.57	-0.79	-0.46	0.29	-5.43	0.18
	6.45	4.77	-1.68				
	6.34	5.87	-0.47				
	10.56	10.34	-0.22				
	10.59	10.71	0.12				
	10.52	10.80	0.28				
Bran	6.40	5.84	-0.56	-0.17	0.12	-1.98	0.23
	6.11	5.76	-0.35				
	6.59	6.20	-0.39				
	10.69	10.84	0.15				
	10.75	10.89	0.14				
	10.50	10.50	0.00				

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Table 3 List of individual chemicals from a mass search of the peaks detected in GC-MS.

	PK	RT (mins)	Library search results
Frass from Superworms reared on PS	1	4.29	2,4-Dimethyl-1-heptene
	2	11.44	1-Octadecanol
	3	19.04	Pentanamide, 4-methyl-
	4	20.81	Cyclopentylacetone 2-Propanone
	5	20.97	Hexadecanamide
	6	22.06	Hexacosane
	7	22.85	9-Octadecenamide, (Z)-
	8	23.12	9-Octadecenamide, (Z)-
	9	23.18	1-Heptadecanamine
	10	24.19	Benzonitrile, m-phenethyl-
	11	24.56	Pentacosane
	12	25.79	Tricosane, 2-methyl-
PS Balls	1	4.63	Benzene, ethyl
	3	5.07	1,3,5,7-Cyclooctatetraene
	4	5.1	3-Hexene, (E)-
	7	6.05	Benzene, propyl-
	9	25.95	Vanadium, (η7-cycloheptatrienylium)(η5-2,4-cyclopentadien-1-yl)-
Frass from Superworms reared on Bran	1	4.3	2,4-Dimethyl-1-heptene
	2	7.99	Heptane, 4-methylene-
	3	8.05	1-Hexadecanol
	4	11.32	1,1-dimethyl-2-propylcyclohexane
	5	11.44	4-Decene, 3-methyl-
	6	11.56	Cyclohexane, 1,2,3-trimethyl-
	7	14.35	Heneicosane
	8	14.48	Ethanone, 1-cyclopentyl-
	9	16.64	2-Undecene, 4,5-dimethyl
	10	16.78	Pentacosane
	11	16.94	Trifluoroacetyl-3,7-dimethyloctano
	12	17.04	Cyclohexane, 1,2,4-trimethyl-
	13	18.98	Heneicosane
	14	20.11	cyano-8-pentadecene
	15	20.82	9-Octadecenamide
	16	20.98	Hexadecanamide
	17	21.09	Nonahexacontanoic acid
	18	22.86	9-Octadecenamide,
	19	22.91	9-Octadecenamide,
	20	23.13	9-Octadecenamide,
	21	23.19	Tetracosane

	22	24.57	Pentacosane

Supplementary Data

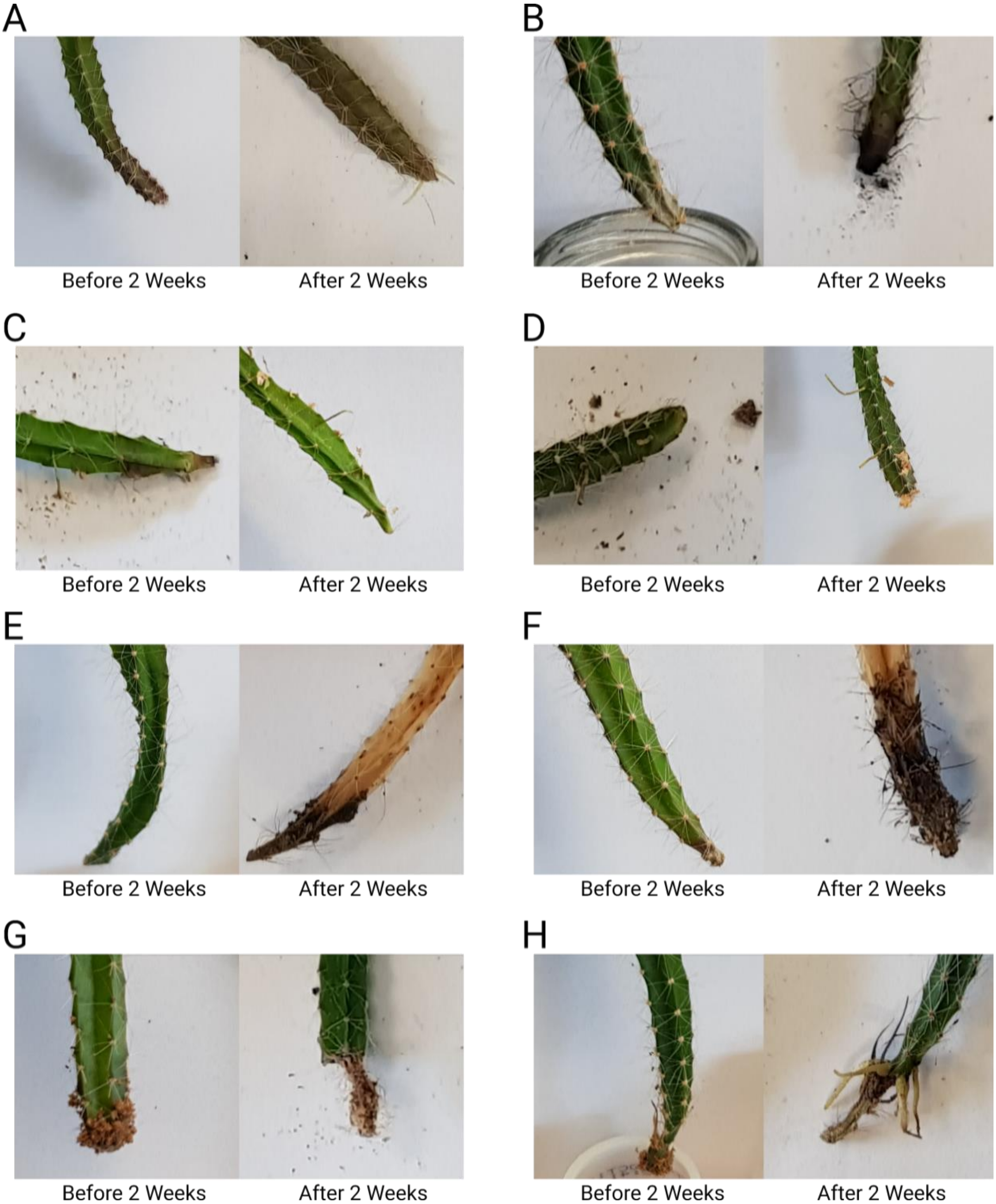

Figure S1: Representative pictures of cacti before and after 2 weeks of growth on tea leaves (A, B), Bran (C, D), Mealworm frass (E, F) and Superworm frass (G, H) based media. “Before 2 weeks” refer to the start of the experiment, and “After 2 weeks” refers to the end of the experiment.

Table S1. Effect of different media on mean change in height of the cacti. Each set-up was performed with eleven replicates. Statistical analyses were performed using the two-tailed Student's T-test ** = $p < 0.05$.

Plant Media	Initial Height (cm)	Final Height (cm)	Change in Height (cm)	Mean Change in Height (cm)	SEM	Control	t-test *P* value (2-tailed)
	9.4	9.5	0.1				
	13.0	13.5	0.5				
	10.4	10.6	0.2				
	7.9	6.7	0.8				
	12.6	11.6	-1.0				
Tea leaves	15.8	15.5	-0.3	0.14	0.18	Bran	.32
	10.0	10.0	0.0				
	10.9	10.6	-0.3				
	8.2	8.0	-0.2				
	9.0	10.1	1.1				
	20.1	20.7	0.6				
	7.6	8.0	0.4				
	10.9	11.5	0.6				
	6.9	6.7	-0.2				
	10.3	10.8	0.5				
	6.1	5.8	-0.3				
Bran	10.1	10.1	0.0	-0.52	0.60	Tea leaves	.32
	8.5	8.5	0.0				
	12.3	12.2	-0.1				
	7.0	6.9	-0.1				
	8.1	8.1	0.0				
	17.2	10.7	-6.5				
Mealworm Frass	8.1	7.6	-0.5	-0.43	0.06	Tea leaves	.01**
	7.5	7.3	-0.2				

	6.1	5.4	-0.7				
	7.7	7.1	-0.6				
	9.9	9.6	-0.3				
	11.9	11.6	-0.3				
	13.1	Dead					
	12.1	Dead					
	7.1	Dead				Bran	.89
	9.5	Dead					
	6.2	Dead					
	10.4	12.5	2.1				
	11.2	13.0	1.8				
	12.3	13.0	0.7			Tea leaves	.36
	7.6	7.7	0.1				
	9.1	10.5	1.4				
Superworm Frass	11	9.4	-1.6	0.50	0.33		
	18.1	18.7	0.6				
	10.8	10.8	0.0				
	5.9	Dead				Bran	.16
	8.2	7.9	-0.3				
	7.3	7.5	0.2				

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