Mealworm frass as fertilizer: changes in soil nutrient profile and growth outcomes in *Brassica rapa*

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Abstract:

As the practice of rearing insects as a food/feed source becomes increasingly widespread, the question of what to do with insect “frass” (excrement) has arisen. This study explored the potential use of mealworm frass as fertilizer for the model organism *Brassica rapa* (the Wisconsin Fast Plant). A total of 48 plants were grown according to standard practices, divided into a control and experimental group. Both groups were grown in MiracleGro potting mix, and the experimental group was treated with an additional 1/4 teaspoon of mealworm frass. After plants reached maturity at 24 days of growth, chlorophyll concentration was measured using colorimetry and dry aboveground biomass was recorded. It was determined that values for chlorophyll concentration, dry biomass, number of buds/flowers, and number of seed pods were significantly higher for the plants in the frass group than for the control group, at the alpha=.05 significance level. These results are attributed to increased macro- and micro-nutrient availability in the soil treated with frass fertilizer. These findings confirm the potential of insect frass fertilizer for benefiting plant health, offering a cost-effective and environmentally friendly alternative to commercial fertilizers. The implementation of frass fertilizers has the potential to lead to healthier and more abundant crop yields.

Introduction:

The Wisconsin Fast Plant (*Brassica rapa*), commonly known as “field mustard,” is a model organism developed for rapid growth by Paul Williams of the University of Wisconsin-Madison. The *B. rapa* species complex encompasses a wide variety of edible plants, including cabbage, turnip, mustard, radish, and broccoli. Wisconsin Fast Plants are especially useful for studying genetics and heredity due to their short life cycle of 5 weeks, allowing researchers to observe and measure transgenerational changes. They have been studied extensively and, as of 2011, their complete genome has been sequenced (Wang et al., 2011). Since many *Brassica* vegetables are commonly consumed and are known to be rich in nutrients and bioactive compounds, findings regarding their nutritional value have direct implications for human health (Favela-González et al., 2020).

In recent years, insect frass (excrement) has been considered as a potential fertilizer for crops and other plants. In order to reduce the negative environmental impacts of agriculture, rearing insects on a large scale may offer a viable alternative protein source to other meat (Houben et al., 2020). As this practice grows, waste in the form of insect frass will be generated, which could potentially serve as an organic fertilizer. The frass of herbivorous insects contains many essential nutrients for plant growth that are often found in commercial mineral and industrially-produced fertilizers, including nitrogen, phosphorus, potassium, and calcium (Houben et al., 2020). In addition, frass contains chitin, a polysaccharide found in the exoskeletons of insects and the cell walls of fungi. Previous research suggests that chitin can increase plant growth, reduce plant pathogens and pests, and increase the quantity of beneficial microbes (Sharp, 2013).

Past studies have already begun to explore the possibility of using insect frass as fertilizer, and many show promising results. One study examined the presence of cabbage moths (a common pest that feeds on *Brassica*) and the impact that they and their frass can have on the growth of *B. rapa.* Researchers measured the insect frass quality (nutrient content) of cabbage moth larvae that had been fed fertilized versus unfertilized *Brassica* leaves (Kagata and Ohgushi, 2012). The different qualities of frass were then added onto the soil of new *Brassica* plants in order to observe the plant growth response to the frass (Kagata and Ohgushi, 2012). It was concluded that the frass of insects that ate fertilized *Brassica* leaves had a higher nutrient content than those that ate unfertilized leaves (Kagata and Ohgushi, 2012). Also, *B. rapa* plant biomass increased when nitrogen-rich (high quality) cabbage moth frass was added to the soil, but biomass decreased when nitrogen-poor (low quality) frass was added (Kagata and Ohgushi, 2012). A more recent study also looked at the effects of mealworm frass as a fertilizer for barley plants (Houben et al., 2020). It was found that frass was equally effective at increasing plant biomass as an industrial NPK (nitrogen, phosphorus, potassium) fertilizer with an equivalent nutrient level as the frass, and frass also has the potential to increase soil microbial activity (Houben et al., 2020).

My research aims to answer the following question: How will the use of mealworm frass as a potential fertilizer affect overall plant health? It is hypothesized that this use of mealworm frass will positively affect the growth of *B. rapa*, increasing the height, dry biomass, chlorophyll concentration, and number of buds, flowers, and seed pods per plant; findings of previous research suggest that insect frass is high in nutrients such as nitrogen, phosphorus, calcium, potassium, and chitin, which are beneficial for plant growth (Houben et al., 2020, Lee et al., 2019, Kagata and Ohgushi, 2012). In addition, this study investigates anecdotal accounts of increased seed production when plants are raised on frass compared to other types of fertilizer (Mark Eastburn, personal communication).

Specific Aims:

Wisconsin Fast Plants will be grown according to standard practices in February 2022. Plants will be divided into a control and experimental (frass) group and grown in potting mix. Plants in the frass group will have 1/4 teaspoon of mealworm frass added and mixed into the soil. While the plants grow, quantitative measurements (number of buds/flowers and seed pods, plant height) will be taken. Half of the plants from each group will be washed and dehydrated to measure dry biomass, and the remaining plants will be used to evaluate chlorophyll concentration using colorimetry. These results using a Two-Sample t-Test.

Significance and Innovation:

The growing industry of insect rearing will generate increasing amounts of waste material in the form of frass. Insect frass is known to contain chitin and beneficial nutrients and therefore has the potential to be used as a natural fertilizer in order to grow crops more sustainably. In addition, it is important to understand how the frass of common pests affects the growth of crops. This study will explore the effects of frass on *B. rapa*, which is closely related to many edible plants such as cabbage, turnip, mustard, radish, and broccoli. If results indicate that frass may be beneficial for plant growth, farmers will be able to implement the practice of using frass as fertilizer, providing a double benefit of increased crop yields and an additional food and feed resource in the form of edible insects. This would provide a use for the excess frass from insect rearing, as well as providing a cost-effective and simple way to improve agriculture. Furthermore, the implementation of insect frass as fertilizer would help mitigate the impact of food shortages by providing healthier and more abundant crops, as well as facilitating the practice of rearing insects, which can serve as a protein source. Insect rearing is a relatively new and small-scale industry, meaning that the impacts of frass have not yet been well studied.

Methods:

A total of 48 Wisconsin Fast Plants were grown according to standard practices in small plastic pots (2.5x2.5”), with 24 plants in the control group and 24 plants in the experimental group, hereafter referred to as the frass group. The plants were grown indoors in the Wisconsin Fast Plants Plant Light House (see image below) under constant fluorescent light. Each pot was filled with 1/4 cup of MiracleGro Seed Starting Potting Mix. For plants in the frass group, 1/4 teaspoon of mealworm frass was added and mixed in evenly. It should be noted that instead of setting up a self-watering system, plants were watered daily from above with 1 tablespoon of water to ensure consistent and equal watering. On days 14-17, plants were cross-pollinated by hand with other plants within their group using a cotton swab.

On day 17, the number of flowers/buds was counted for each plant. On day 20, the number of seed pods was counted for each plant. On day 23, height of each plant was measured (from the surface of the soil to the highest point on the plant).

On day 24, twelve plants from each group were randomly selected. These plants were harvested by clipping the stems at the surface of the soil and were dried in a laboratory oven at 70°C for 48 hours. Then, the dried plants were weighed using an electronic balance to get the aboveground dry biomass.

The remaining twelve plants in each group were used to measure chlorophyll concentration. The procedure required a leaf sample of 250 mg. Due to the small size of the Wisconsin Fast Plants, some individual plants did not have sufficient mass to perform this. Therefore, plants were divided into smaller subgroups of 4 plants each within the control group or the experimental group. This resulted in three leaf samples of 250 mg from the control group and three leaf samples of 250 mg from the frass group. Each leaf sample was macerated with 10 mL of 80% acetone using a mortar and pestle. The resulting solution was pipetted into four microcentrifuge tubes of 1.5 mL each. These tubes were centrifuged at 3000 rpm for 10 minutes. The supernatant chlorophyll solution in each tube was poured into a 25 mL graduated cylinder and made up to 15 mL using 80% acetone. Next, this solution was poured into a rectangular plastic cuvette (standard size, 3.5 mL). The PASCO-CI-6747 Colorimeter (see image to the right) was calibrated using distilled water. Then, the cuvette with the chlorophyll solution was inserted into the device. All four light wavelength settings were tested, and it was determined that the blue wavelength (460nm) yielded the lowest percent transmittance for the chlorophyll solutions. The percent transmittance at the blue wavelength was recorded for all six leaf samples. Next, the percent transmittance was subtracted

from 100% to determine percent absorbance (for ease of presenting the data). Percent absorbance is indicative of chlorophyll concentration, as higher concentrations of chlorophyll would create a solution with greater color intensity and the pigments would absorb more of the light.

Data for all variables were averaged for the two groups, and standard deviation and standard error were calculated. Two-Sample t-Test procedures were carried out using Excel to compare the sample means of the two groups at the alpha=.05 significance level.

Results:

The data displayed in the graphs and tables below represent values for chlorophyll concentration (fig. 1), dry biomass (fig. 2), height (fig.3), number of buds/flowers (fig.4), and number of seed pods (fig. 5) for the control and experimental (frass) groups. Each group comprised 24 plants. Averages, standard deviation, and standard error were calculated. Error bars shown on the graphs represent standard error values. A Two-Sample t-Test was carried out using Excel to compare the sample means of the two groups. Data was evaluated at the alpha=.05 significance level.

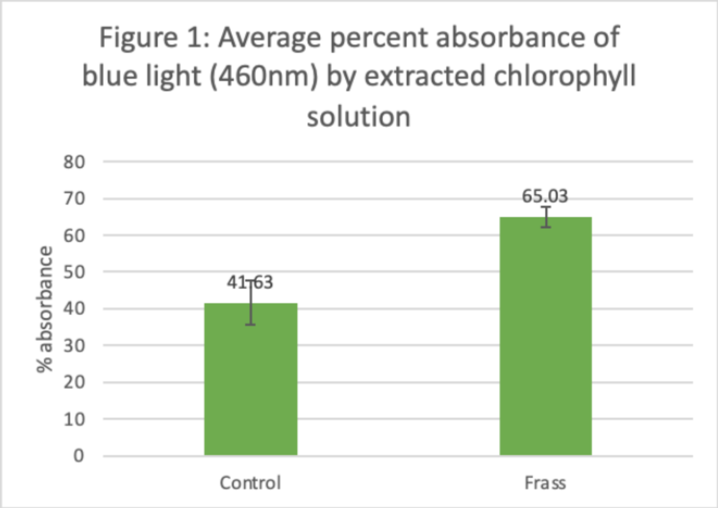

Table 1: Average percent absorbance of blue light (460nm) by extracted chlorophyll solution	Control	Frass
Average (% absorbance)	41.63	65.03
Standard Deviation	10.37	4.79
Standard Error	5.99	2.77

Two-Sample t-Test: ***P*=.02**
df = 3 t-statistic = -3.55

Annotation: Values shown represent the average of three groups of plants. The 24 plants in each group (control and frass) were randomly assigned into three groups of 4 plants. The percent absorbance of the chlorophyll solution from these three groups was evaluated using a colorimeter. The p-value of .02 *indicates that average percent absorbance of blue light for the chlorophyll solution of the frass group was significantly higher than for that of the control.*

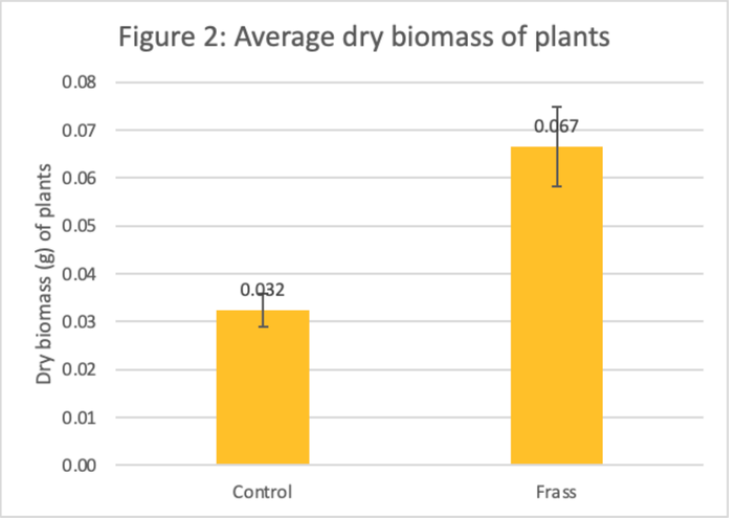
Figure 2: Average dry biomass of plants

Table 2: Average dry biomass of plants	Control	Frass
Average (grams)	0.032	0.067
Standard Deviation	0.012	0.029
Standard Error	0.004	0.008

Two-Sample t-Test: ***P*=.001**
df = 19 t-statistic = -3.56

Annotation: Values shown represent averages of aboveground dry biomass for the 24 plants in each group. The p-value of .001 indicates that average biomass for the frass group was significantly higher than for the control.

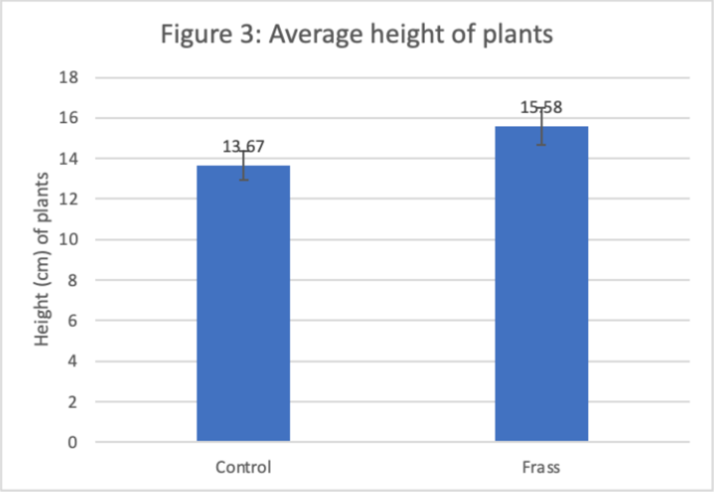
Figure 3: Average height of plants

Table 3: Average height of plants	Control	Frass
Average (cm)	13.67	15.58
Standard Deviation	3.46	4.49
Standard Error	0.71	0.92

Two-Sample t-Test: ***P*=.052**
df = 43 t-statistic = -1.66

Annotation: Values shown represent averages of the 24 plants in each group. The p-value of .052 indicates that there is no statistically significant difference in average height between the two groups.

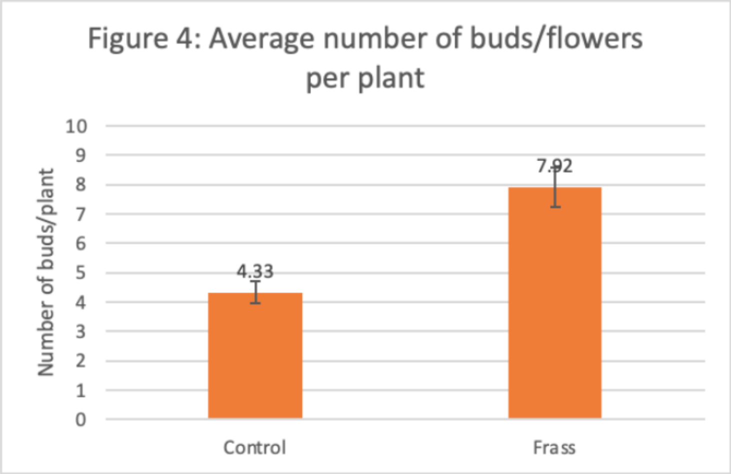
Figure 4: Average number of buds/flowers per plant

Table 4: Average number of buds/flowers per plant	Control	Frass
Average (number of buds/ flowers)	4.33	7.92
Standard Deviation	1.75	3.27
Standard Error	0.36	0.67

Two-Sample t-Test: ***P*<.001**
df = 38 t-statistic = -4.61

Annotation: Values shown represent averages of the 24 plants in each group. The p-value of <.001 indicates that the average number of buds/flowers for the frass group was significantly higher than for the control.

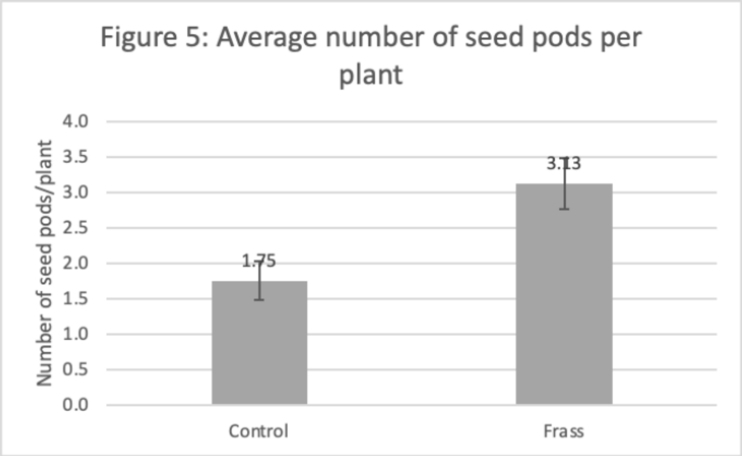
Figure 5: Average number of seed pods per plant

Table 5: Average number of seed pods per plant	Control	Frass
Average (number of seed pods)	1.75	3.13
Standard Deviation	1.36	1.75
Standard Error	0.28	0.36

Two-Sample t-Test: ***P*=.002**
df = 43 t-statistic = -3.04

Annotation: Values shown represent averages of the 24 plants in each group. The p-value of .002 indicates that the average number of seed pods for the frass group was significantly higher than for the control.

Summary: Average values for chlorophyll concentration (fig. 1), dry biomass (fig. 2), number of buds/flowers (fig.4), and number of seed pods (fig. 5) were all significantly higher for the frass group than for the control group, as confirmed by the p-values of less than .05. However, there was no statistically significant difference in average height (fig. 3) between the two groups.

Discussion:

It was hypothesized that this use of mealworm frass will positively affect the growth of *B. rapa,* increasing the height, dry biomass, chlorophyll concentration, and number of buds, flowers, and seed pods per plant; findings of previous research suggest that insect frass is high in nutrients such as nitrogen, phosphorus, calcium, potassium, and chitin, which are beneficial for plant growth (Houben et al., 2020, Lee et al., 2019, Kagata and Ohgushi, 2012). This hypothesis was supported by the findings of the study. Average values for chlorophyll concentration, dry biomass, number of buds/flowers, and number of seed pods were all significantly higher for the frass group than for the control group. However, there was no statistically significant difference in average height between the two groups. Statistical significance was evaluated at the alpha=.05 level using a Two-Sample t-Test.

As shown in figure 1, the average percent absorbance of blue light (460nm) for the chlorophyll solutions from leaf samples from the frass group was 65.03±2.77%, which is significantly higher than the average percent absorbance for the control, which was 41.63±5.99%. The statistical significance is confirmed by the p-value of .02. Percent absorbance is indicative of chlorophyll concentration, as higher concentrations of chlorophyll would create a solution with greater color intensity and more light would be absorbed by the pigments.

One study conducted an analysis of the nutrient levels present in different types of insect frass, concluding that frass had adequate concentrations and contents of macronutrients (NPK), secondary nutrients (Ca, Mg, and S), and micro-nutrients (Mn, Cu, Fe, Zn, B, and Na) for optimal plant growth (Beesigamukama et. al, 2022). It is therefore likely that the soil for the frass plants had greater nutrient availability while the control plants were nutrient deficient. Specifically, potassium and iron deficiency can lead to yellowing leaves (chlorosis) and reduced photosynthetic output (Morgan and Connolly, 2013). Chlorosis would be caused by lower concentrations of chlorophyll in the leaves. This accounts for the less intense green color of the leaves and chlorophyll solution and the lower percent absorbance observed in the samples from the control group.

As shown in figure 2, the average dry biomass for the frass group was 0.067±0.008 g, which is significantly higher than the average dry biomass for the control, which was 0.032±0.004 g. The statistical significance is confirmed by the p-value of .001. Once again, previous research indicates that a reduction in biomass can be attributed to nitrogen deficiency (Morgan and Connolly, 2013). In addition, one study indicated that nutrient shortage can alter biomass allocation, causing plants to allocate a greater proportion of their biomass to the root system. This is consistent with my findings, especially since only above-ground biomass was measured (Hermans et. al, 2006).

There was no significant difference between the average heights of the two groups of plants. As shown in figure 3, the average height for the frass group was 15.58±0.92 cm, while the average height for the control was 13.67±0.71 cm. Since the p-value of .052 was greater than alpha=.05, the difference in height is not statistically significant. It is possible that plant height was not necessarily reflective of overall plant health in this case.

As shown in figure 4, the average number of buds/flowers on day 17 for the frass group was 7.92±0.67, which is significantly higher than the average number of buds/flowers for the control, which was 4.33±0.36. The statistical significance is confirmed by the p-value of <.001. As shown in figure 5, the average number of seed pods on day 20 for the frass group was 3.13±0.336, which is significantly higher than the average number of seed pods for the control, which was 1.75±0.28. The statistical significance is confirmed by the p-value of .002. The lower number of buds/flowers and seed pods in the control plants can once again be explained by reduced nutrient availability. Potassium deficiency can lead to reduced growth and fertility (Morgan and Connolly, 2013). Additionally, nitrogen plays a key role in plant reproductive growth by initiating meiosis—the production of gametes in plant pollen and ovules (Rye et. al, 2016). Nitrogen deficiency can lead to underdeveloped flowers, which explains the reduced number of buds/flowers and seed pods (Yang et. al, 2022). Seed pods are also considered a major nitrogen sink during development, which would make them also affected by reduced nitrogen availability (Mechthild and Masclaux-Daubresse, 2017).

Interestingly, one study also brought up the possibility of phytotoxicity in frass. For some insect species including mealworms, the use of frass as fertilizer led to lower germination rates (Beesigamukama et. al, 2022). This is attributed to high salt (cation) concentration and high electrical conductivity (Beesigamukama et. al, 2022). However, reduced germination was not observed in either group of my study. It is possible that phytotoxic effects might be observed at higher concentrations of frass in the soil.

There were several limitations of this study. One shortcoming is the relatively small sample size. The study was restricted by time and access to resources, meaning that only 48 plants could be grown in a small indoor lightbox. One possible source of error could have been the distribution of light within the lightbox. Continuous lighting was provided by two fluorescent bulbs and the reflective interior of the light box was intended to ensure that light reached all plants in a roughly equal way. However, it is still likely that plants in the center (some frass plants, some control) received light of greater intensity. To remedy this, the individual plants could be rotated daily or tube lights could be used rather than bulbs to ensure a more equal distribution of light. Second, there was one outlier plant in each group that germinated late and did not grow nearly as large as the other plants. The outlier in the control group grew to be 4 cm tall and the outlier in the frass group grew to be 3 cm, which contributed to the high standard error for heights. It is unknown why these two plants did not exhibit typical growth, but it can likely be attributed to the seed itself rather than the growing conditions, since other plants grew normally. Finally, more accurate data on percent light absorbance could have been obtained if spectrometry was used rather than colorimetry. Spectrometry would be able to account for specific wavelengths of light for each chlorophyll pigment, whereas the colorimeter was limited to four set wavelengths.

There is still much to explore regarding insect frass fertilizer. One potential avenue to investigate is the potential for increased disease resistance. Frass contains chitin, a polysaccharide found also in fungal cell walls. Therefore, exposure to frass could potentially ward off disease and pests, making it important to measure chitinase expression in plants exposed to insect frass. It is also important to measure the impact of frass fertilizer on nutrient and bioactive compound content in *B. rapa* and other vegetables to see whether crops can be made more nutritious. One issue that arose in earlier iterations of the study was finding an ideal amount of frass to incorporate into the soil without causing mold growth. A study could potentially be conducted with different concentrations of frass to find an optimal amount and compare it to different commercial chemical and organic fertilizers. It would also be beneficial to explore the logistics of implementing frass fertilizer on a larger/industrial scale. This could be done through economic modeling and by contacting farmers, calculating prices and travel costs, etc.

The findings of this study indicate that frass is indeed an effective fertilizer compared to potting mix alone. Plants grown with frass added to the soil were healthier overall, likely due to higher nutrient availability of nitrogen, phosphorus, potassium, and calcium. This has major implications for the growing insect agriculture industry. Insects are already being raised for animal feed, as well as for human consumption and the production of insect oil. Insect agriculture offers a more space-effective and environmentally friendly alternative to traditional agriculture and oil industries. The implementation of insect frass as fertilizer could produce healthier and more abundant crops in addition to the benefits of insects themselves as a protein source. This would help mitigate food shortages in the case of bad crop yields or increased extreme weather events due to climate change. The confirmation that frass is an effective fertilizer offers a way to repurpose frass in a productive way by using it to enrich soil and produce healthier crops. This will allow insect farmers and crop farmers to work in tandem by simultaneously raising insects and growing crops, a practice that will lower land use and carbon emissions.

In summary, this study found that the use of mealworm frass as fertilizer had benefits for plant health and growth, including increased chlorophyll concentration, dry biomass, number of buds/flowers, and number of seed pods compared to a control group grown in potting mix without any fertilizer. Insect frass fertilizers can provide a cost-effective and environmentally friendly alternative to commercial fertilizers.

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