Adv SRD Lab Notebook Template (__ / 25)

Instructions:

Below is a template to organize your lab notebook. Your project will have multiple days of experimentation with distinct goals. For each unique research goal, you must create a new notebook entry by copying and pasting the template below and filling in the required information according to the rubric. Some goals may take multiple days to accomplish especially when time is a controlled or manipulated variable. For entries that span multiple sessions, you do not need to create a new entry. Instead, you must denote which sections of the entry were completed on what date along with timestamps. For these types of entries, your Procedure Reflection/Future Endeavors should reflect up to date information about your research and data collected so far. If you would like to see an example notebook entry that satisfies the rubric requirements, follow THIS LINK.

Preparation of Mealworm Housing Units	
Date: 9/21/23	**Length of Research Period(s): 1 hour**

Goal(s)/Purpose:

The main goal for this research session will be preparing the 16 quart tubs for mealworm habitation. A total of 6 tubs must be prepared, with the most important work being required for the 3 containers containing the variable mealworm food mediums (styrofoam, BioBag, wheat bran). This process will mostly include labeling the bins and adding a random amount of food medium to each bin. Otherwise, this experimental period should be quite brief. Additionally, ventilation holes will be added to the containers via the hot glue gun, which can effectively bore through plastic.

Materials Required:

- **16 quart bins x6**
- **Masking Tape**
- **Sharpie/Permanent Marker**
- **Styrofoam Packing Peanuts**
- **BioBag Pet Waste Bags x10 units**
- **Bag of wheat bran (8qt)**
- **Scissor**
- **Hot glue gun**
- **Hot glue cartridges**

Procedure:

PREPARING HOUSING UNITS FOR FRASS PRODUCTION:

Pre-Procedure Note: To maintain the isopod cultures, adequate housing is required. For this experiment, **3 drawer plastic shelving units are used.** If these are unavailable a custom shelving system should be set up.

1. Unbox the six 16 quart plastic bins set them side by side in an accessible space.
2. Cut SIX approximately 5 cm pieces (exact size does not matter) of either duct or masking tape and place one on the lid of each of the containers.

3. Using a sharpie, label the three of the bins (writing on the tape) “Adults,” “Young” and “Pupae,” respectively.
4. Using the sharpie again, label the other three bins (writing on the tape) “Wheat” “Styrofoam” and “BioBag,” respectively.
5. Punch 6 small air holes in the sides of each of the bins using a hot glue gun to bore through the plastic, as venting is a requirement.
6. Get the bag of wheat bran and open it with scissors.
7. Fill the bins labeled Adults and Wheat with 7cm depth of wheat bran. Fill the “Pupa” and “Young” containers with just 1 cm.
8. Flatten out the substrate with hands
9. Using a binder clip, reseal the wheat bag.
10. Get the styrofoam packing peanuts and open the bag with scissors.
11. Fill the container labeled “StyroFoam” about 7cm deep with packing peanuts. Make sure they are spread evenly.
12. Reseal the styrofoam peanut bag with a binder clip.
13. Get the box of BioBags and open it. Count about 20 bags and throw them into the drawer labeled BioBag. Spread them out evenly. Close the BioBag box.
14. Once all of the containers have been filled with medium, close them all to prepare for the next section of procedure.

Data and Observations:

- As expected, no major concerns with the setup of the housing units. All containers were intact and all mediums had arrived on time, so it was easy to finish the procedure quickly. The amount of medium placed in each tub was less than half of the total depth in order to keep the mealworms away from the lid. Even though they are unable to climb smooth plastic, having a shallow substrate makes them easier to manage & observe. It will also make the goal of collecting frass significantly easier.
- Labeling the units was also fairly straightforward and not at all time consuming. Units were stored near a window in the stock room where they fit neatly & are completely out of the way.

Procedure Reflection and Future Endeavors:

- This portion of the procedure was very successful given the simplicity. Pouring materials into the plastic bins didn't require much thought and was simply a prerequisite for the mealworms which are now on their way. The effectiveness of decisions made during this entry (depth of the medium in each bin/surface area present) will be determined in later entries when the mealworms are actually added to the bins. However based on research on mealworms in unnatural habitation, ie. styrofoam substrate, I predict that the frass collection arm of the experiment will indeed be successful.

Transfer of Mealworms to Housing Units	
Date: 10/4/23	**Length of Research Period(s): 1hour and 30 minutes**

Goal(s)/Purpose:

- The main goal of this research period is to transfer live mealworms from Ccarolina Bbiological shipping containers into the three "food medium" containers. This includes the wheat bran, BioBag and styrofoam

filled containers. About 500 mealworms will be added to each of these containers, all in their larval stage. This large quantity will help to consume the medium effectively and produce a high yield of frass.

Materials Required:

- **Food medium containers (BioBag, Styrofoam, Wheat Bran)**
- **Scissors**
- **Spray bottle**
- **Mealworm Cultures**

Procedure:

UNBOXING AND CHECKING CULTURES:

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
2. Carefully remove mealworm housing containers provided by Carolina Biological from the cardboard shipping box using a scissor to cut the tape sealing the box, then set them out on a flat surface such as a countertop.
3. Open the lid of each of the shipping containers (May vary depending on company packaging changes).
    a. Note: Mealworms (Darkling beetle larvae) from Carolina Biological are shipped in plastic containers with dry medium. Mealworms should be visible within the folds of the material. **Mealworms may be shipped in many different life stages including larvae (mealworms), pupae and adult darkling beetles (see below). You should be prepared to house each. Please note that motionlessness is normal in pupae and does not equate to death. If any mealworms or adult beetles do not respond to stimuli please refer to the "Disposal" section of this procedure.**

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4. Place the lid back on each of the plastic containers to prevent escape while the rest of this "Care Info" procedure is being completed.

TRANSFERRING INDIVIDUALS TO HOUSING:

1. Empty the contents of a shipping container into a giant bin or tray with high sides so all the mealworms are together but cannot escape.
2. Sort out the individuals. Pick out adults and pupae, then place them in the containers with the matching names.
3. Once all the pupae and adults are filtered out dump all of the mealworms into (should be about 500 per shipping container) into one of the three containers: Place each of the groups into one of three containers: "Wheat," "Biobag" and "Styrofoam" .
   ***There are three groups of 500 worms, each group should go into one specific bin so that amounts of worms are equal across cohorts.**
4. Close all of the bins and return them to their place of origin.

MAINTAINING AND CULTURING

5. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
6. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
7. Check any of the containers not labeled "Adults" or "Pupae" for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the "Pupae" container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the "young" container. Any large mealworms in the "young" container should then be moved to any of the standard containers such as "Wheat," "Styrofoam" or "BioBag"

Data and Observations:

- **Mealworms all arrived healthy and active, wriggling as soon as they were removed from their respective shipping containers. They were not counted but the amount of individuals extracted from each container was roughly the same. Mealworms were simply dumped into their tubs after each container was opened, where they were then free to burrow or hide within the medium as they saw fit. In the case of the wheat bran, a substrate mimicking more natural conditions, the worms quickly buried themselves within the substrate. And interesting behavior to observe was that once the mealworms disappeared under the wheat bran, large tremors could be seen on the surface of the medium with the occasional worm peeking out.**
- **In the case of the styrofoam, which was in much larger pieces than the wheat bran, the mealworms quickly disappeared to the bottom of the container so that, from an overhead view, it appeared as if the container was empty. The worms collected at the bottom of the container en masse, which is displayed in one of the above images. They also caused the surface of the styrofoam to shake.**
- **A similar aggregation behavior was displayed in the biobag group who, because of the enormous plastic bags, were also forced to collect at the bottom. This behavior is not unnatural but instead result of substrate**

Procedure Reflection and Future Endeavors:

- This procedure went without issues as all of the worms were in shape. Hopefully, the quantity and

quality of the medium will be enough to make the worms feel comfortable and engage in their standard feeding behavior. Time will tell. In terms of actual frass collection, It's safe to say I'll have to wait a couple of weeks before I have enough to begin the growth stage of the experiment. I plan to utilize measurements from a replicate study to judge the correct amount of frass I'll need from each group.

- I'll also need to begin collecting pupae and adults and storing them in their respective containers to jump start the breeding process.
- Routine spraying of the housing containers will also need to be done to keep the animals hydrated, so this will be embodied in future lab notebooks

Development of Frass Collection Methods	
Date: 10/6/2	**Length of Research Period(s): 1 hour +**

Goal(s)/Purpose:

- This lab notebook will be dedicated to developing the procedure for collecting frass. High quantities of frass have been identified in the wheat bran group, meaning it's time to start filtering it out and storing it in collection containers. This process will likely be repeated when sufficient frass is detected in the other containers as well.

Materials Required:

- **Sifter (and additional screens)**
- **3 quart container x1**
- **Sifter shovel**
- **Wheat bran cohort**
- **Labeling tape**
- **Permanent marker/sharpie**
- **Spray bottle**

Procedure:

***Note.. This procedure will be used for all different mediums, however in this case it was only repeated for the wheat bran group.**

PREPARING FRASS HOLDING CONTAINERS:

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
2. Find 3 of the 3 quart containers and place them on an empty surface, such as the countertop.
3. Get the masking tape and cut three 3 cm sections of tape, then stick one on each of the 3 plastic containers. For ease of use, please arrange the tape on the lid of each container.
4. Get the sharpie and label each of the containers (writing on the tape), "Wheat Frass," "BioBag Frass," and "Styrofoam Frass" respectively.

5. Place the containers in a safe place if not immediately preceding to the next part of the procedure.

COLLECTING AND STORING FRASS:

6. Every few days, check the mealworm housing units for a powdery medium known as frass. It can be found by moving the medium in each housing container around until some powder is discovered, most often at the bottom of the container.
7. Once frass is identified, locate the sifter and choose the smallest of the three meshes. Put the selected mesh in the mesh slot.
8. Repeat the following steps for any of the following housing containers: Wheat, BioBag, and Styrofoam, whenever frass needs to be collected.
9. Select a housing tub from the three food medium tubs (Wheat Bran, BioBag, Styrofoam).
10. Get the 3 quart container with the matching name as the currently selected housing container followed by "Frass." Open it.
11. Scoop some medium from the housing container (mealworms, medium and all) using the sifter and shake it over the open 3 quart container. Frass should fall into the container below while live mealworms and food chunks remain behind in the sifter.
12. Once all the frass has been sifted out, return the mealworms and food medium back to the original housing container.
13. Repeat steps 11-12 until little to no frass remains in the housing container.
14. Return the housing container to the area of storage, closing the lid so the mealworms are contained. Additionally, return the 3 quart container (now full of frass) back to storage, ensuring the lid is on.
15. Clean out any debris from the sifter in the sink, then let it dry.

MAINTAINING AND CULTURING

8. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
9. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
10. Check any of the containers not labeled "Adults" or "Pupae" for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these

individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the “Pupae” container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the “young” container. Any large mealworms in the “young” container should then be moved to any of the standard containers such as “Wheat,” “Styrofoam” or “BioBag”

Data and Observations:

- Collected a decent amount of wheat frass as displayed in the image above. Because of the mealworm’s feeding habits, it appears that frass collects at the bottom of the substrate rather than being spread evenly throughout the substrate. This made it significantly easier to collect. Via usage of the sifter, I was easily able to separate frass from wheat bran. The smallest screen for the sifter, optimal for separating the frass, is a bit troublesome as it tends to exclude the larger frass pellets. This may come up later, but the amount produced in a short time period such as this is promising
- Mealworms were a bit frantic during the sifting process and displayed impressive boosts of speed after being separated from their substrate. All individuals are healthy, as usual, sprayed cohort containers as usual.

Procedure Reflection and Future Endeavors:

- This was overall a very successful execution of the procedure, and minimal changes were needed to make sure it went smoothly. Steps 9 and 14 were altered for clarity reasons, as the current methods does not use the previously planned drawer units (which were planned during the creation of the original procedure document.) These changes did not seriously affect execution of the procedure, which is very positive.

- Future goals will be collecting frass from other containers as well as continuing to monitor mealworm health. Transferring pupae to the pupae containers and isolating adults for breeding is also a goal for the very near future.

Routine Maintenance & Collection of Pupae and Adult Beetles	
Date: 10/13/23	**Length of Research Period(s): 30-45 mins**

Goal(s)/Purpose:

- For this entry into the lab notebook the goals are simple:
- 1) Conduct routine maintenance on the cohorts (spraying bins with water, etc)
- 2) Collecting pupae from different containers and transferring them to the pupae container so they may mature in peace. This should be a fairly straightforward and low risk research period. Will also survey for adult beetles just to be safe

Materials Required:

- **Food medium cohort containers (16 quart bins)**
- **Spray Bottle**
- **Pupae container**
- **Adult containers**

Procedure:

MAINTAINING AND CULTURING

11. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
12. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
13. Check any of the containers not labeled “Adults” or “Pupae” for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the “Pupae” container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the “young” container. Any large mealworms in the “young” container should then be moved to any of the standard containers such as “Wheat,” “Styrofoam” or “BioBag”

Data and Observations:

- The above images display some of the mealworm pupae collected during this procedure. As shown here, they vary slightly in color; this indicates their current stage of growth. Bright white pupa (right image) have just molted from their worm stage and take a while to harden. Brown pupa right, are closer to the beetle stage. They grow darker as time progresses.
- Pupae tend to wiggle violently when handled, not so much that it is an issue but rather something interesting to note. In this state they are helpless which suggests why they often pupate beneath the substrate. If not, they end up as free food for a predator. This suggests reasoning for their affinity with burrowing.
- To locate pupa in the different containers, an effort had to be made to dig through the substrate and locate worms. Not too much of an issue though. They're easy to tell apart from their larval stage.

Procedure Reflection and Future Endeavors:

- This was a relatively brief and successful procedure. It was exciting to be able to observe the worms in this transition stage, and I look forward to housing my first adult beetles. Hopefully all of the pupa will make it to adulthood as the success rate is not 100%. I plan to reference this notebook more often as the quantities of pupa begin to ramp up. This method of checking in (steps 11-13) has been quite reliable so far.

Collection of First Adult Beetles & Routine Maintenance/Observation	
Date: 10/20/23 - 10/23/23 - 10/25/23 - 10/26/23	**Length of Research Period(s): 45 mins (each during 4 different days)**

Goal(s)/Purpose:

- Another fairly simple lab notebook, the goal is to search for adult beetles in the pupae container. It has been a while since pupae were collected and some should be emerging. In addition, more pupa will be collected and check ins (spraying) will continue as normal. This all should be completed relatively quickly.

Materials Required:

- **Adult cohort container**
- **Pupae cohort container**
- **Food medium cohort containers**
- **Spray bottle**

Procedure:

MAINTAINING AND CULTURING

14. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
15. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
16. Check any of the containers not labeled “Adults” or “Pupae” for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the “Pupae” container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the “young” container. Any large mealworms in the “young” container should then be moved to any of the standard containers such as “Wheat,” “Styrofoam” or “BioBag”

Data and Observations:

- Pictured above (left) is the first adult beetle produced in the lab this year (10/20/23)! The light cream coloration indicates it recently molted, and the color will morph to a darker black as time goes on. This means its carapace is hardening which is essential to the animal's health and safety. The beetle was very active but not hard to manage, as they do not tend to fly or run too fast. This made handling straightforward and the beetle was quickly added to the adult housing container
- Pupae were also collected but the amount was not significant. All pupae in the bin seemseems to be faring well and continuing towards the adult stage.
- All food medium containers have thriving populations of worms when checked for spraying. **Mealworms are tearing into the styrofoam. Reference the image on the right, a worm has chewed its way through this packing peanut. Many other packing peanuts within the bin are displaying similar qualities (10/23/23).**

- Above (10/25/23): New pupa collected later during this experimental period. Most are early stages as indicated by their color.

- Above (10/26/23): additional beetle that was collected after the original.

Procedure Reflection and Future Endeavors:

- Seeing a beetle reach adulthood is a sign of success within this setup, and supports the reflection in the first lab notebook that we would need to wait and see whether this setup would have positive effects on mealworm health. Hopefully it will continue to push out new beetles, however time will tell.
- Next steps include continued maintenance as well as beginning to collect frass from the other food medium containers besides wheat bran.

Observations on Styrofoam Frass & Additional Pupa collection	
Date: 10/31/23	**Length of Research Period(s): 1 day**

Goal(s)/Purpose:

- The main goal of this research period is to complete check ins as normal, which includes collecting pupae and adults and storing them in bins. I also need to check in on the different containers to see the progress on the feeding behavior, especially in the styrofoam container. This will allow me to decide

when I'll begin collecting frass from these containers.

Materials Required:

- **Adult cohort container**
- **Pupae cohort container**
- **Food medium cohort containers**
- **Spray bottle**

Procedure:

MAINTAINING AND CULTURING

17. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
18. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
19. Check any of the containers not labeled "Adults" or "Pupae" for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the "Pupae" container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the "young" container. Any large mealworms in the "young" container should then be moved to any of the standard containers such as "Wheat," "Styrofoam" or "BioBag"

Data and Observations:

- Good news! Mealworms are tearing through the styrofoam. As shown in the image to the left, the white powder collecting near the worms is their frass, the result of their feeding behavior. Squeezing the packing peanuts allowed frass powder to fall out, yielding even more. This allows me to hypothesize that there's quite a high quantity of frass to be collected.
- Pupae continue to mature and morph into beetles, and the pupae container is always receiving new members (see right)

Procedure Reflection and Future Endeavors:

- The quantity of frass identified is promising. No changes were needed for this check in procedure. The most important thing now is to get started with frass collection and mixing of frass/soil mixture. This will most likely be completed in the following lab notebook. The procedure may have to be adjusted to cater specifically to the styrofoam peanuts.

Collection of Styrofoam Frass & Preparation of Planting Units	
Date: 11/2/23 & 11/6/23	**Length of Research Period(s): 50-70 mins (two separate days)**

Goal(s)/Purpose:

- There is much to accomplish over this research period. The first goal is to continue check-ins as normal to separate adults and hydrate the cohorts. Next, frass must be collected from the styrofoam cohort, as it has been determined that sufficient amounts are present. Tweaks will need to be made to the original procedure to accommodate the styrofoam medium. Finally, methods for preparing the frass based soil

must be determined and executed so that it can be potted and prepared for planting. The goal is to also plant the seeds at the end of this research period so that growth can begin as planned.

Materials Required:

- Cohort Tubs
- Sifter (all screens)
- Scale
- Black gold soil
- Shovel
- Gloves
- 3 quart container
- Large bowl
- Labeling tape
- Permanent marker
- Seed germination kits
- Weigh Boat
- Scoopula
- Graduated Cylinder

Procedure:

COLLECTING AND STORING FRASS:

16. Every few days, check the mealworm housing units for a powdery medium known as frass. It can be found by moving the medium in each housing container around until some powder is discovered, most often at the bottom of the container.
17. Once frass is identified, locate the sifter and choose the smallest of the three meshes. Put the selected mesh in the mesh slot.
18. Repeat the following steps for any of the following housing containers: Wheat, BioBag, and Styrofoam, whenever frass needs to be collected.
19. Select a housing tub from the three food medium tubs (Wheat Bran, BioBag, Styrofoam).
20. Get the 3 quart container with the matching name as the currently selected housing container followed by “Frass.” Open it.
21. Scoop some medium from the housing container (mealworms, medium and all) using the sifter and shake it over the open 3 quart container. Frass should fall into the container below while live mealworms and food chunks remain behind in the sifter. *Note: If collecting styrofoam frass, it’s essential to crack the packing peanuts to release the frass within. Mealworms tend to burrow within the packing peanut, trapping the frass within as they go. Simply cracking the peanuts with gloved hands allows for easy and fast collection.
22. Once all the frass has been sifted out, return the mealworms and food medium back to the original housing container.
23. Repeat steps 11-12 until little to no frass remains in the housing container.

24. Return the housing container to the area of storage, closing the lid so the mealworms are contained. Additionally, return the 3 quart container (now full of frass) back to storage, ensuring the lid is on.
25. Clean out any debris from the sifter in the sink, then let it dry.

Procedure for Creating Fertilized "Frass Soil" Blends:

********This procedure may be repeated for any type of frass made in the Frass Production procedure.*

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
2. Get three large zip-lock bags and some masking tape.
3. Get the sharpie and label the bags "Wheat Frass Soil," "Styrofoam Frass Soil," and "Biobag Frass Soil" respectively.
4. Once there is a sufficient amount of frass collected in one of the 3 qt containers for any frass type, select that container and put it on the countertop.
5. Get the zip-lock bag with the matching frass name followed by "soil."
6. Get the bag of Black Gold Soil and open it, then get the large bowl.
7. Take out the scale, power it on and place the large bowl on top of it.
8. TARE the scale.
9. Pour soil into the bowl until the scale reads about 193 grams.
10. Remove the bowl from the scale
11. Reseal the Black Gold bag with binder clips, then store it away.
12. Select a fresh weigh boat and place it on the scale, then TARE the scale again.
13. Pour frass from the selected container until the scale reads about 1 gram.
14. Remove the weigh boat from the scale and dump the frass into the same bowl where the soil currently is.
15. Using the scoopula, mix the frass into the soil unit it is evenly distributed. It should be mixed for a minimum of 30 seconds.
16. If storing the frass soil mixture for later, dump the mixed soil from the bowl into the zip-lock bag with the matching name, then clean out the bowl in the sink. Proceed to the following step. If not, move on to the next section of the procedure.
17. Reseal the bag and return it to an area of storage for later procedures. Return the selected 3 qt container to the same location.

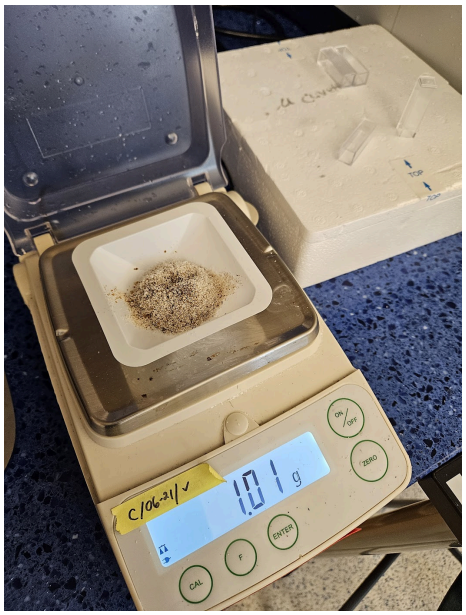
(Repeated for Wheat and Styrofoam soil mixtures as well)
Negative Control- Growing Plants in Normal Topsoil:

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.

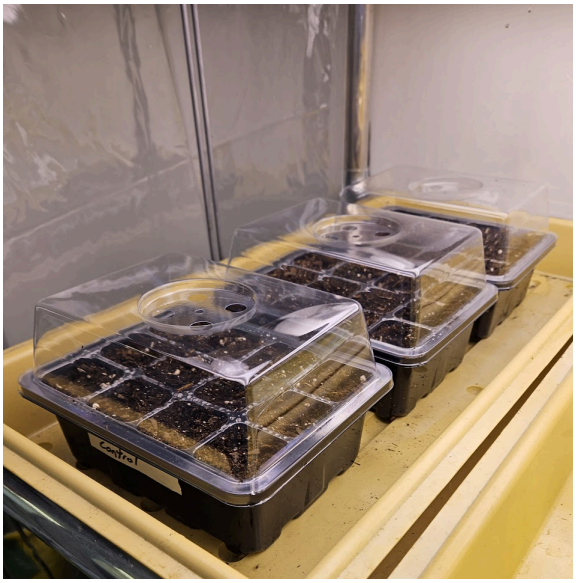
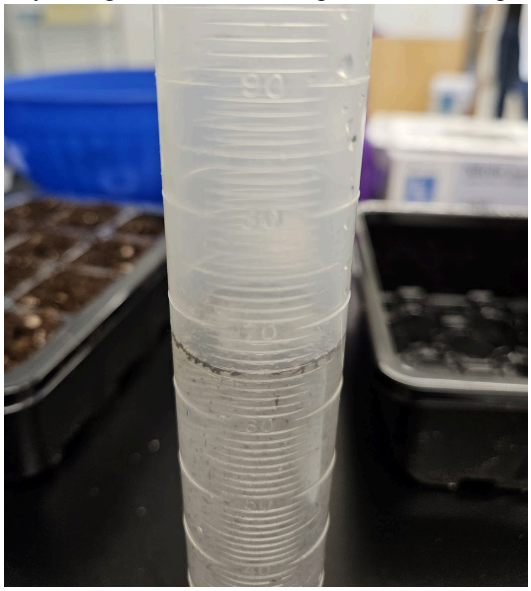
2. Get one of the seed starter pots from the pack of 5 and set it, lid and all, out on the countertop.
3. Get the masking tape and cut a 3 cm piece of tape. Stick the tape to the side of the starter pot bottom.
4. Get the sharpie and label the tape "Neg Control."
5. Take the lid off of the starter pot and set it aside on the countertop.
6. Get the bag of topsoil and cut it open with a scissor.
7. Using a scoopula, scoop topsoil out of the bag/bowl and fill all of the "sub-pots" within the starter pot to the brim.
8. Reseal the topsoil bag with the binder clips.
9. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
10. Using the glass stir rod poke a small hole in the surface of each sub pot.
11. Place a single seed in each of the sub pot holes, pushing it slightly under the surface of the soil and covering it with the remaining soil.
12. Spray all of the pots and close the lid, then place the starter pot in an area that receives high light for most of the day (window, artificial, grow cart)
13. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray. **If plants are too tall, remove the lid completely.**

Data and Observations:

- As shown here, plenty of styrofoam frass was collected during this procedure, almost equivalent to the initial amount of wheat bran frass collected; this will allow for preparation of the soil mixtures which will be prepared shortly. Cracking the styrofoam packing peanuts provided high amounts of frass. In addition, many pupae have morphed into beetles ass shown above, which will hopefully help to jump start the breeding process



- Soil measurements and frass measurements are displayed above. Soil measurement was developed on the spot based on the amount needed to fill all potting units within each starter pot. The frass measurement was more based off of the paper mentioned in my background, but was adapted for the lab space as well.

- Three seed kits were filled with soil and seeded, each with one fast plant seed. 65ml of water was added to the bottom tray on each in addition to spraying the surface of each pot. This should provide sufficient hydration for the plants over weekend periods or simply in between days.

Procedure Reflection and Future Endeavors:

- There was much to cover over this research period, however small changes to my procedure allowed things to run smoothly. Because of the cracking method for the styrofoam packing peanuts, I was able to maximize the amount of styrofoam frass collected (Step 21). Unfortunately, the amount of BioBag frass is still insufficient, so the BioBag trial will begin later than these other trials.
- Goals for the next research period include monitoring plant health across the different cohorts and developing a procedure for data collection after plants =

TITLE	
Date: 11/8/23-11/1323	**Length of Research Period(s): 50 mins, repeated over two days**

Goal(s)/Purpose:

The main purpose of this lab notebook is to check on the progress of plant growth as well as make any changes necessary to accommodate the plants in question. This includes activities such as replacing the 65 mLl of water in each tray and spraying the surface of each growing unit.

Additional note: Because of the state of some of the plants (strange growth, leaning) I'll also be installing toothpick supports within each pot unit to support plant growth. The procedure will have to be adapted to accommodate this.

Materials Required:

- Toothpicks
- Starter pot units
- Graduated cylinder

Procedure:

(Repeated for Wheat and Styrofoam soil mixtures as well)
Negative Control- Growing Plants in Normal Topsoil:

14. Stick two toothpicks in each of the sub pots, preferably on the sides to provide support for the sprouting plants
15. Spray all of the pots and close the lid, then place the starter pot in an area that receives high light for most of the day (window, artificial, grow cart)
16. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray. **If plants are too tall, remove the lid completely.**

Data and Observations:

- Plants have sprouted in all of the containers as of 11/8/23, with a few exceptions in each container. Since that day the plants have continued to grow, however they've grown rather crooked. To combat this, toothpicks were used. About two were placed in each port to provide an x for the plants to grow off of. This should facilitate stronger growth, as I'm uncertain why they are growing this way. This behavior was observed on 11/13/23.

Procedure Reflection and Future Endeavors:

- I'm pleased to see that the plants are growing well and have all sprouted for the most part. However, there is no real observable difference between each of the groups of plants as they most likely are still growing off of their sprout energy. However, step 14, the addition of toothpicks, should provide necessary support for the plants. This should be sufficient.
- For future goals, continued observation of the plants and maintenance on the worms is most of what I have planned. I'm still researching for a way to actively judge plant health, so this will be determined later on.

Maintenance of Fast Plants	
Date: 11/20/23	**Length of Research Period(s): 20 mins**

Goal(s)/Purpose:

- The purpose of this lab notebook entry is to conduct maintenance as normal and survey the health of the fast plants. This includes spraying the plants to keep them hydrated and filling their water tray if it has begun to dry out. Once complete, plants will be returned to the grow cart to continue their growth as planned.

Materials Required:

- Spray Bottle
- Fresh water
- Graduated Cylinder
- Fast Plant Pots

Procedure:

1. For a total of 25 days, spray the plants each day using the spray bottle, doing three short bursts over each pot. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray using the graduated cylinder and only refill when the tray is completely empty. **If plants are too tall, remove the lid completely.**

Data and Observations:

- Plants have continued to grow steadily since the last check in and are continuing to sprout new leaves. Unfortunately, they continue to display a crooked growth pattern, growing straight up for a while before becoming "leggy" and slumping over. This is something happening in all of the different soil substrates, so this could indicate some issues with planted depth or environment that need to be further investigated.
- At this point, as shown in the image above, none of the plants are displaying any flowers yet. Budding should begin shortly.

Procedure Reflection and Future Endeavors:

- This procedure was simple and minor changes were made to the single step. The addition of the line "only refill when the tray is empty" clarifies to not continuously add 65 ml of water every day, as the pots would fill and the plants would become overwhelmed very quickly. Also, the need for the graduated cylinder to measure is clarified, as this was not mentioned beforehand.
- The day count was also updated to match a more realistic time frame (or at least the frame I've been following) and the amount of sprays done was clarified to allow for a more efficient execution of the procedure.
- Future plans include continuing to observe general plant health and ensuring that the growing environment remains consistent.

Maintenance of Fast Plants and Collection of Frass	
Date: 11/21/23	**Length of Research Period(s): 45 mins**

Goal(s)/Purpose:

- Very similar to the previous entry, should be combined however there are slight differences between the goals. Here, plant health and progress of growth will be observed. In addition, maintenance will continue as usual (spraying plants and, if necessary, refilling water trays).
- Finally, additional styrofoam frass will be collected as a high quantity has been located in one of the mealworm housing units. No major changes should be needed to this procedure given its past success.

Materials Required:

- **Sieve**
- **Spray Bottle**
- **Graduated Cylinder**
- **Fast plant pots**
- **Styrofoam mealworm cohort**
- **Styrofoam frass storage container**
- **Fresh water**

Procedure:

2. For a total of 25 days, spray the plants each day using the spray bottle, doing three short bursts over each pot. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray using the graduated cylinder and only refill when the tray is completely empty. **If plants are too tall, remove the lid completely.**

COLLECTING AND STORING FRASS:

1. Every few days, check the mealworm housing units for a powdery medium known as frass. It can be found by moving the medium in each housing container around until some powder is discovered, most often at the bottom of the container.
2. Once frass is identified, locate the sifter and choose the smallest of the three meshes. Put the selected mesh in the mesh slot.
3. Repeat the following steps for any of the following housing containers: Wheat, BioBag, and Styrofoam, whenever frass needs to be collected.
4. Select a housing tub from the three food medium tubs (Wheat Bran, BioBag, Styrofoam).
5. Get the 3 quart container with the matching name as the currently selected housing container followed by “Frass.” Open it.
6. Scoop some medium from the housing container (mealworms, medium and all) using the sifter and shake it over the open 3 quart container. Frass should fall into the container below while live mealworms and food chunks remain behind in the sifter. ***Note: If collecting styrofoam frass, it’s essential to crack the packing peanuts to release the frass within. Mealworms tend to burrow within the packing peanut,**

trapping the frass within as they go. Simply cracking the peanuts with gloved hands allows for easy and fast collection.

7. Once all the frass has been sifted out, return the mealworms and food medium back to the original housing container.
8. Repeat steps 11-12 until little to no frass remains in the housing container.
9. Return the housing container to the area of storage, closing the lid so the mealworms are contained. Additionally, return the 3 quart container (now full of frass) back to storage, ensuring the lid is on.
10. Clean out any debris from the sifter in the sink, then let it dry.

Data and Observations:

- Plants have begun to bud! Buds can be seen as little green “balls” at the top of each plant. It doesn’t seem like any have begun to open up into flowers, however it does appear that some are much closer to others. Buds are not present on all of the plants, but instead the vast majority, including those that are rather crooked.
- Plants have continued to grow in that strange, leggy manner, however they still seem to be bright green and healthy.
- The tray had dried up for the most part so around 65 ml of water was added.

- A large amount of styrofoam frass was collected via the procedure, as made clear in the picture above. Mealworms are clearly consuming large amounts of styrofoam, which is continuing to solidify data from the various experiments I've researched.

Procedure Reflection and Future Endeavors:

- These two procedures have continued to be successful and no problems occurred. The only actual change to the procedure was making the note on step 6 bold so that it was clear how important it was to differentiate the styrofoam collection from the standard collection. This could go a long way for anyone who would benefit from replicating this procedure.
- For the future entries, I hope to observe flowering fast plants as this will be a significant step forward and mark a key point in the plant's growth

Maintenance of Mealworm Beetles and Fast Plants	
Date: 11/27/23 & 12/4/23	**Length of Research Period(s): 1 hour**

Goal(s)/Purpose:

- The goal of this research period is to check on both the plants as well as the mealworm colonies. Checking on plants is simple and will continue as usual; spraying, refilling the tray and making notes about their general welfare. For the mealworms, I will check for any significant deaths in the colonies and transfer any adult beetles identified in the different containers to the adult housing container..
- No significant changes should be required for the procedure here, given how similar this is to previous entries.

Materials Required:

- Graduated Cylinder
- Spray bottle
- Fast plant pots
- Mealworm colony containers
- Fresh water

Procedure:

3. For a total of 25 days, spray the plants each day using the spray bottle, doing three short bursts over each pot. Replace the lid if plants are short enough. In addition, pour

65 ml of water into the bottom of each tray using the graduated cylinder and only refill when the tray is completely empty. **If plants are too tall, remove the lid completely.**

MAINTAINING AND CULTURING

1. Place the habitat in an area that receives indirect light and maintains a temperature of 20 to 22 °C (68 to 72 °F) such as a storage cabinet or closet with a closing door and shelves.
2. Mist the habitat with 9-10 short bursts of water using the spray bottle once every 1-2 days with water to provide the necessary water for the animals. **Do not allow the habitat to dry out for too long as mealworms/ beetles will be unable to drink**. **If access to lab space is limited on weekends, add a moistened paper towel to each container to maintain a damp environment along with the standard misting with the spray bottle.**
3. Check any of the containers not labeled "Adults" or "Pupae" for adults and pupae daily, or whenever possible. If any are found, record the number and transfer these individuals to either the pupae or adult containers depending on what they actually are. Additionally, move any adults from the "Pupae" container and dispose of any dead adult beetles. Finally, check the adult container for baby mealworms and move them to the "young" container. Any large mealworms in the "young" container should then be moved to any of the standard containers such as "Wheat," "Styrofoam" or "BioBag"

Data and Observations:

- Fast plants have finally flowered! All plants seem to display flowers at varying levels of intensity. Interestingly, there is no general trend and no specific substrate seems to be growing a higher amount of flowers. Plants have also continued to develop healthily and don't seem to be having any issues with leaf rot, parasites or burns. Leaves are quite large on some plants, and almost all are still leggy for the most part. However, many of the plants displaying flowers have parts that are tall and straight, however they may bend over soon given the tendency of this to occur throughout the experimental period
- Plants were sprayed and the tray was filled with 65 ml of water as usual.
- Adult beetles continue to appear in many of the different cohort bins at surprising rates. All beetles found were moved to the adult containers, which has become densely populated. Whenever the paper towel above the substrate is sprayed, (for hydration) beetles come to the surface almost all at once and make it clear just how numerous they really are.

&
12/4/23

- On a later date, beetles were observed again, and it is once again clear just how many there actually are. This picture is just one corner of the adult group, but there are just as many across the entire bin.

Procedure Reflection and Future Endeavors:

- This procedure continues to be successful, a good indicator of this is the general welfare of both the plants as well as the beetles. The flowering is, as mentioned before, a key sign of successful plants. While the continued leggy growth is strange, it doesn't seem to be having any seriously negative effects on the plants as they grow.
- The mealworm beetles seem comfortable and I haven't noticed any deaths, so I believe the maintenance outlined in Steps 1-3 has been helpful. The amount of water has been just enough.
- Because plants are nearing a very important step in their growth, I hope to remove them from the soil in the next entry and prepare them for data collection.

Extraction and Dehydration of Fast Plants	
Date: 12/8/23-12/16/23	**Length of Research Period(s): 45 mins on 12/8/23, then <15 mins over different days**

Goal(s)/Purpose:

- This entry marks the end of the growth period for the fast plants. Plants will be removed from the starter pots, delicately and by hand, and then placed in the dehydrator to prepare for data collection. Because this method was not outlined in my original procedure, I'll have to create a new procedure on collecting and dehydrating mature plants. This shouldn't be too difficult and, if executed properly, will allow for smoother trials in the future.

Materials Required:

- **Dehydrator**
- **Fast Plant pots**
- **Weigh Boats**
- **Paper towels**
- **Small Plastic tub x3**

Procedure:

COLLECTION AND DEHYDRATION OF MATURE FAST PLANTS

After the end of the indicated growth period, follow this procedure to prepare fast plants for data collection.

1. Set out the starter pots containing your different groups of fast plants out on a workspace or countertop.
2. Get paper towels and use them to cover the planned work area.
3. Select a starter pot to start working with.
4. Remove the plastic "pot" section from the water tray and place it on a paper towel.
5. One by one, push fast plants out of the pots gently using bare hands. Squeezing the plastic pots themselves works very effectively for this.
6. After all plants are removed, remove soil chunks from the lower end of the plant, taking care not to cause damage to the roots.
7. Get 12 weigh boats and set them out in the workspace.
8. Place each plant in an empty weigh boat until all weigh boats have been filled.
9. Place the 12 weigh boats in a plastic tub, stacking them and labeling the tub with the same name of the substrate the plants where grown in.
10. Repeat steps 3-9 for all the remaining starter pot groups.

Dehydration

1. Select a tub of plants created in step 10 of the previous procedure and set them out on a workspace.
2. Get the dehydrator and plug it in.
3. One by one, remove weigh boats (filled with plants) from the tub and place them on the

different shelves of the dehydrator.
4. Close the dehydrator and set the temp to 100°F.
5. Set the time to 4 hours and click start.
6. After 4 hours have passed, return the weigh boats, now containing dry plants, to their plastic tub of origin.
7. Repeat steps 1-6 for any other groups of plants that need to be dehydrated.

Data and Observations:

- Plants were easily removed and dehydrated. The result of dehydration is shown in the image above, plants shrivel up as a result of severe water loss. 100 degrees is the perfect temperature to dehydrate the plants without damaging their structure and causing burns, which is inevitable at higher temperatures.

Procedure Reflection and Future Endeavors:

- This new procedure was straightforward and effective. As indicated in the data collection, the 100 degrees outlined in step 4 of the dehydration procedure is perfect for preserving the plants while also removing water. This will help to accurately quantify biomass in the future. Soil may have to be removed more effectively from the roots as chunks are still visible in the weigh boats.
- The plan for the future is to use the highly specialized scale to quantify the plant biomass and observe other features such as leaf count and flower presence on individual plants. Hopefully, this data collection will influence some positive changes to the procedure.

Preparing Coco Fiber Units	
Date: 1/11-12/24	**Length of Research Period(s): 1 hour per day**

Goal(s)/Purpose:

- **Setting up Coconut Fiber Planters:** After discovering that there is too much fertilizer within the black gold soil used in previous trials, this research period will focus on the use of a new substrate, coconut fiber. Coconut fiber differs from conventional soil in that it lacks much of the added fertilizers that normally boost plant growth. This will allow me to accurately judge how the presence of frass within the soil actually impacts plant growth. Seeds must be planted by the end of this trial.
- **Determining new measurements for coco fiber - frass ratios:** Because of the difference in mass between coco fiber and the black gold soil, new measurements regarding mass of coco fiber within each planter will be recorded.

Materials Required:

- **Coco Fiber Brick x1**
- **2100 ml of water**
- **Beaker (500 ml preferably)**
- **Gloves (1 pair)**
- **Starter Pots x3**
- **Large plastic bowl (x1)**
- **Spray Bottle (x1)**
- **Scale x1**
- **Scoopula x1**

Procedure:

Procedure for Creating Fertilized "Frass Soil" Blends:

***This procedure may be repeated for any type of frass made in the Frass Production procedure.*

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
2. Get three large zip-lock bags and some masking tape.
3. Get the sharpie and label the bags "Wheat Frass Soil," "Styrofoam Frass Soil," and "Biobag Frass Soil" respectively.
4. Once there is a sufficient amount of frass collected in one of the 3 qt containers for any frass type, select that container and put it on the countertop.
5. Get the zip-lock bag with the matching frass name followed by "soil."
6. Get a coconut fiber brick, then get the large bowl and place the brick within it.
7. Por a total of 2100 ml of water into the bowl so that it dilutes the brick and allows it to separate. Use gloved hands to break up the hydrated brick and mix it into a substrate form.
8. Take out the scale, power it on and place the large bowl on top of it.
9. TARE the scale.
10. Pour coco fiber into the bowl until the scale reads about 83.67 grams.
11. Remove the bowl from the scale
12. Store the remaining coco fiber somewhere safe.

13. Select a fresh weigh boat and place it on the scale, then TARE the scale again.
14. Pour frass from the selected container until the scale reads about 1 gram.
15. Remove the weigh boat from the scale and dump the frass into the same bowl where the coco fiber currently is.
16. Using the scoopula, mix the frass into the coco fiber unit it is evenly distributed. It should be mixed for a minimum of 30 seconds.
17. If storing the frass soil mixture for later, dump the mixture from the bowl into the zip-lock bag with the matching name, then clean out the bowl in the sink. Proceed to the following step. If not, move on to the next section of the procedure.
18. Reseal the bag and return it to an area of storage for later procedures. Return the selected 3 qt container to the same location.

(Repeated for Wheat and Styrofoam coco fiber mixtures as well)
Negative Control- Growing Plants in Normal Topsoil:

1. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
2. Get one of the seed starter pots from the pack of 5 and set it, lid and all, out on the countertop.
3. Get the masking tape and cut a 3 cm piece of tape. Stick the tape to the side of the starter pot bottom.
4. Get the sharpie and label the tape "Neg Control."
5. Take the lid off of the starter pot and set it aside on the countertop.
6. Get the coco fiber bowl and set it on the countertop.
7. Using a scoopula, scoop coco fiber out of the bag/bowl and fill all of the "sub-pots" within the starter pot to the brim.
8. Return the coco fiber to its area of storage.
9. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
10. Using the glass stir rod poke a small hole in the surface of each sub-pot.
11. Place a single seed in each of the sub-pot holes, pushing it slightly under the surface of the substrate and covering it with the remaining substrate.
12. Spray all of the pots and close the lid, then place the starter pot in an area that receives high light for most of the day (window, artificial, grow cart)
13. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray. **If plants are too tall, remove the lid completely.**

Data and Observations:

- Coco fiber is a highly absorbent material that seems to demand a ridiculous amount of water to fully break down into a usable substrate. It is extremely coarse and dense when dry but slowly takes on a texture almost identical to the previously used soil after hydration.

- Mass of the coco fiber used to fill the starter pots. This was recorded after the dispersed fibers had dried slightly, allowing them to be effectively used to fill in the starter pots. This mass differs greatly from that of the black gold soil, which was often measured when wet.
- Planted seeds within coco fiber for the three main groups (control, wheat bran and styrofoam). There were no issues during planting.

Procedure Reflection and Future Endeavors:

- Overall a relatively quick and straightforward procedure. While some properties of the coco fiber were unexpected, such as the amount of water required for hydration, a sufficient solution was determined for each. This allowed for a relatively simple procedure that didn't deviate much from the original idea and only demanded a few extra steps, such as step 7 of the preparation procedure which details how to water the coco fiber. In addition, the word "soil" was replaced with the word "coconut fiber" or "substrate" in several cases.
- The plan for the future is to see how well the coco fiber actually performs. It would be ideal to start collecting meaningful data and this trial could be the first example of that. The next research period will most likely focus on observing the growth rates of the plants and conducting the watering protocols as usual,

Observing Growth Stages of Fast Plants over Multiple Days	
Date (s): 1/16, 1/22, 1/25	**Length of Research Period(s): approx 1 hour each day**

Goal(s)/Purpose:

Note: This lab notebook is a consolidation of multiple lab notebooks over the course of a few weeks. The reason for this is because each of these research periods were identical, and the only changes were the conditions of the plants. This means that the procedure applied for each date and that the data/observations as well as the reflection will contain the most relevant information.

- **Watering and observing plants:** On each date, plants will be observed, photos will be taken, and water will be added to each tray to encourage growth. Plants will also be sprayed regularly to keep the environment as moist as possible.

Materials Required:

- **Control (no frass), Wheat frassgrass, and Styrofoam frass starter pots**
- **Graduated Cylinder x1**
- **Water (tap or distilled)**
- **Spray bottle (x1)**

Procedure:

(Repeated for Wheat and Styrofoam coco fiber mixtures as well)
Negative Control- Growing Plants in Normal Topsoil:

14. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
15. Get one of the seed starter pots from the pack of 5 and set it, lid and all, out on the countertop.
16. Get the masking tape and cut a 3 cm piece of tape. Stick the tape to the side of the starter pot bottom.
17. Get the sharpie and label the tape "Neg Control."
18. Take the lid off of the starter pot and set it aside on the countertop.

19. Get the coco fiber bowl and set it on the countertop.
20. Using a scoopula, scoop coco fiber out of the bag/bowl and fill all of the “sub-pots” within the starter pot to the brim.
21. Return the coco fiber to its area of storage.
22. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
23. Using the glass stir rod poke a small hole in the surface of each sub-pot.
24. Place a single seed in each of the sub-pot holes, pushing it slightly under the surface of the substrate and covering it with the remaining substrate.
25. Spray all of the pots and close the lid, then place the starter pot in an area that receives high light for most of the day (window, artificial, grow cart)
26. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray. **If plants are too tall, remove the lid completely.**

Data and Observations:

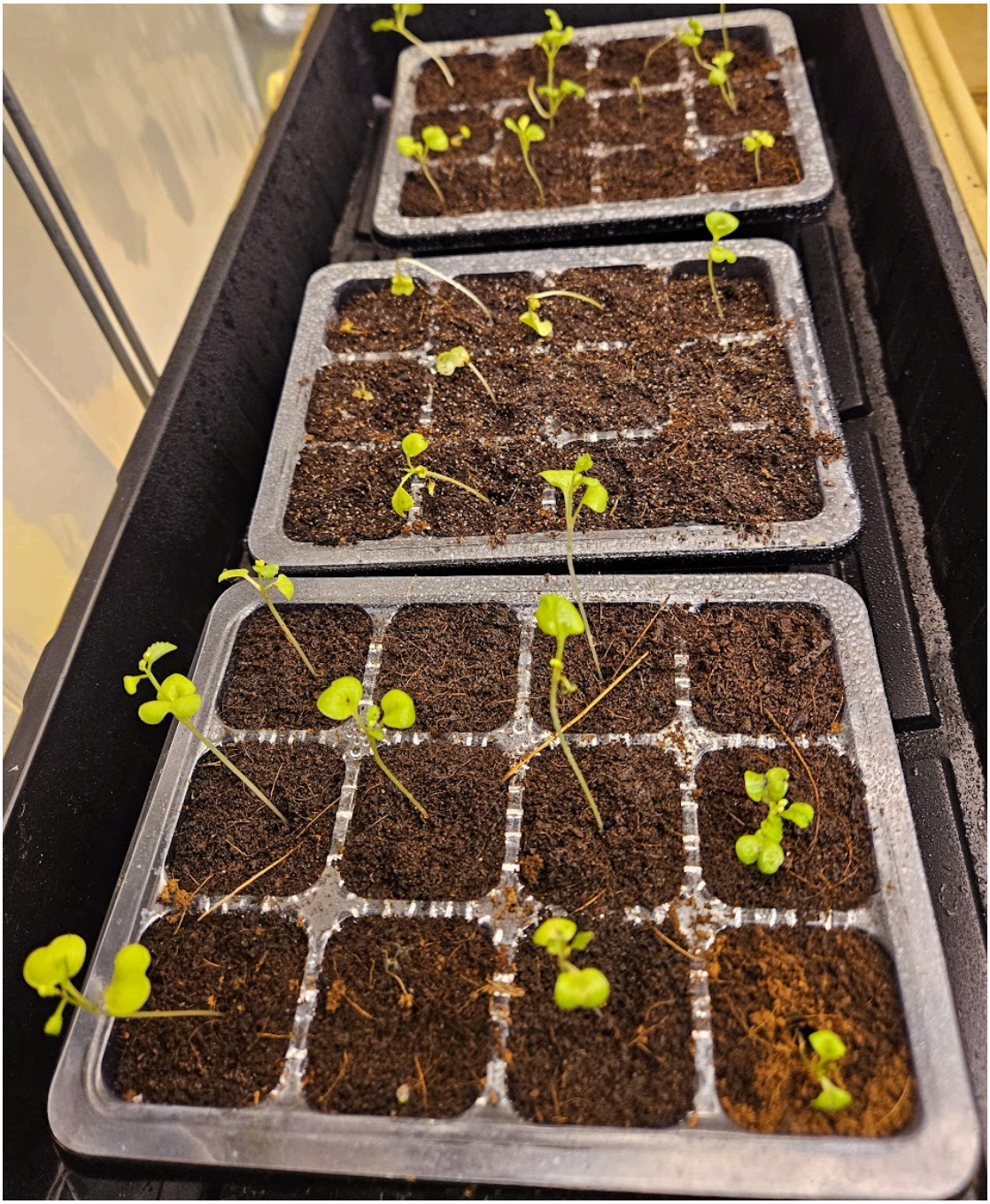
- ^^^^^Jan 16th: On this date, sprouts were observed within each of the starter pots. There were no significant differences between the groups at this time. The most important fact was that the majority of the plants successfully sprouted through the coconut fiber.

(Wheat Frass)

(Control Group)

(Styrofoam)

- ^^^^^Jan 22: See the above images, labeled based on the plant group they were a part of. After several days of growth, trends have begun to emerge. The Wheat Frass plants can be regarded as “most successful” given their superior height as well as their larger leaf count and size. The control group is significantly shorter, but still displays healthy plants. The styrofoam group is significantly shorter, but two of the plants have also wilted away which is worrying. This is to be closely monitored in the next check in.



- ^^^Jan 25: Wheat frass continues to push forward as dominant, and the control group is growing steadily. Unfortunately, the styrofoam group continues to appear weaker and weaker as the days go on. This is good data, and supports the fact that the waste is not completely pure of styrofoam toxins and still contains that one flame retardant.

Procedure Reflection and Future Endeavors:

- Step 26 of the watering procedure continues to be successful and effective as I move through this experiment. While the success of the wheat frass is positive, the loss of many of the styrofoam plants was significantly less exciting and something to take note of. Lack of nutrients shouldn't be the problem

given how the control plant was able to successfully grow within the coco fiber. These results may instead point to the fact that the frass is still somewhat toxic because of the flame retardant and would need to be broken down further by some other microorganism or chemical. Observing the properties of the frass could be an interesting project of the future.
- For the next notebook, I'll most likely be collecting data about the plants and setting up a new trial with a slightly higher concentration of each frass fertilizer to see if it has any impact on plant growth. Looking forward to positive results!

Secondary Shortened Trial (Increased Frass Concentrations)	
Date: 1/29, 1/30, 2/1-7	**Length of Research Period(s): Less than 30 mins**

Goal(s)/Purpose:
- Like the previous entry, this entry will encompass multiple lab notebooks over a short period of time. Procedures were quite similar and the most notable changes occurred towards the end of the growth period.
- **Setting up shortened trial:** This notebook entry focuses on setting up and monitoring a shortened trial measuring the growth rates of plants with increased concentrations of the indicated frass variants (wheat, styrofoam). This includes mixing new soil, planting seeds and making temporary adjustments to the procedure.
- **Continuing to maintain previous plants:** Plants from the ongoing trial which began in the first entry of this document still need routine maintenance, so they will be hydrated as usual. See the procedure.

Materials Required:
- **Coconut Fiber bowl**
- **Styrofoam Frass ContainerContainner**
- **Water**
- **Spray Bottle x1**
- **Graduated Cylinder x1**
- **Starter pots x2**
- **Mixing bowlx1**
- **Scalex1**
- **Scoopula x1**

Procedure:

Procedure for Creating Fertilized "Frass Soil" Blends:

********This procedure may be repeated for any type of frass made in the Frass Production procedure.*

19. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
20. Get three large zip-lock bags and some masking tape.
21. Get the sharpie and label the bags "Wheat Frass Soil," "Styrofoam Frass Soil," and "Biobag Frass Soil" respectively.

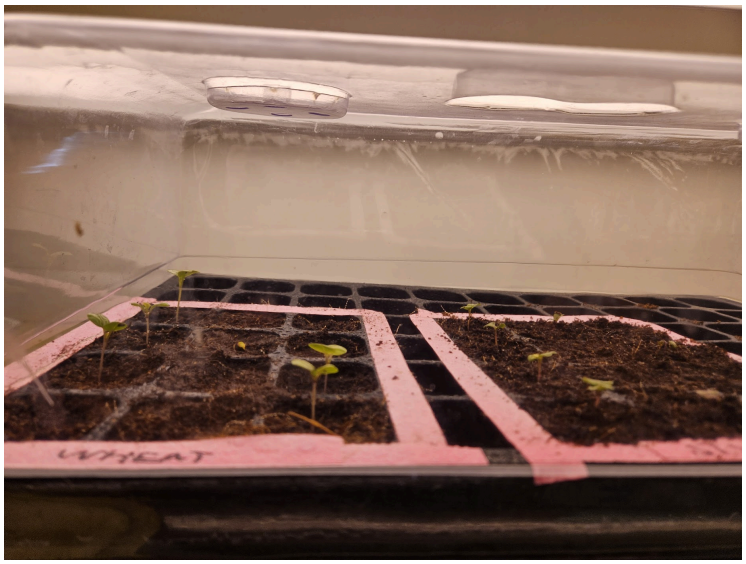
22. Once there is a sufficient amount of frass collected in one of the 3 qt containers for any frass type, select that container and put it on the countertop.
23. Get the zip-lock bag with the matching frass name followed by “soil.”
24. Get a coconut fiber brick, then get the large bowl and place the brick within it.
25. Por a total of 2100 ml of water into the bowl so that it dilutes the brick and allows it to separate. Use gloved hands to break up the hydrated brick and mix it into a substrate form.
26. Take out the scale, power it on and place the large bowl on top of it.
27. TARE the scale.
28. Pour coco fiber into the bowl until the scale reads about 83.67 grams.
29. Remove the bowl from the scale
30. Store the remaining coco fiber somewhere safe.
31. Select a fresh weigh boat and place it on the scale, then TARE the scale again.
32. Pour frass from the selected container until the scale reads about 5 grams.
33. Remove the weigh boat from the scale and dump the frass into the same bowl where the coco fiber currently is.
34. Using the scoopula, mix the frass into the coco fiber unit it is evenly distributed. It should be mixed for a minimum of 30 seconds.
35. If storing the frass soil mixture for later, dump the mixture from the bowl into the zip-lock bag with the matching name, then clean out the bowl in the sink. Proceed to the following step. If not, move on to the next section of the procedure.
36. Reseal the bag and return it to an area of storage for later procedures. Return the selected 3 qt container to the same location.

Repeated for Wheat and Styrofoam coco fiber mixtures as well)
Negative Control- Growing Plants in Normal Topsoil:

27. Equip PPE, including the following: Lab coat, blue nitrile gloves and safety goggles.
28. Get one of the seed starter pots from the pack of 5 and set it, lid and all, out on the countertop.
29. Get the masking tape and cut a 3 cm piece of tape. Stick the tape to the side of the starter pot bottom.
30. Get the sharpie and label the tape “Neg Control.”
31. Take the lid off of the starter pot and set it aside on the countertop.
32. Get the coco fiber bowl and set it on the countertop.
33. Using a scoopula, scoop coco fiber out of the bag/bowl and fill all of the “sub-pots” within the starter pot to the brim.
34. Return the coco fiber to its area of storage.

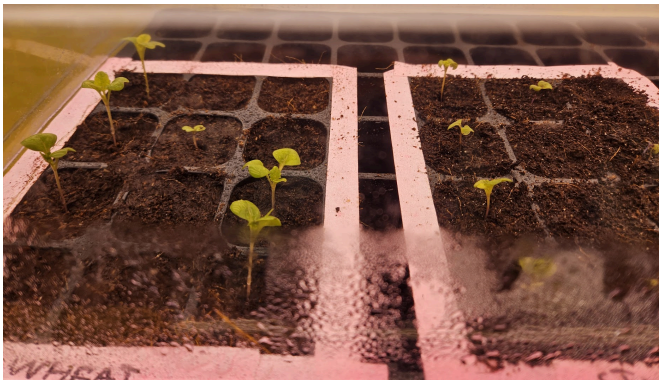
35. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
36. Using the glass stir rod poke a small hole in the surface of each sub-pot.
37. Place a single seed in each of the sub-pot holes, pushing it slightly under the surface of the substrate and covering it with the remaining substrate.
38. Spray all of the pots and close the lid, then place the starter pot in an area that receives high light for most of the day (window, artificial, grow cart)
39. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. In addition, pour 65 ml of water into the bottom of each tray. **If plants are too tall, remove the lid completely**

Data and Observations:



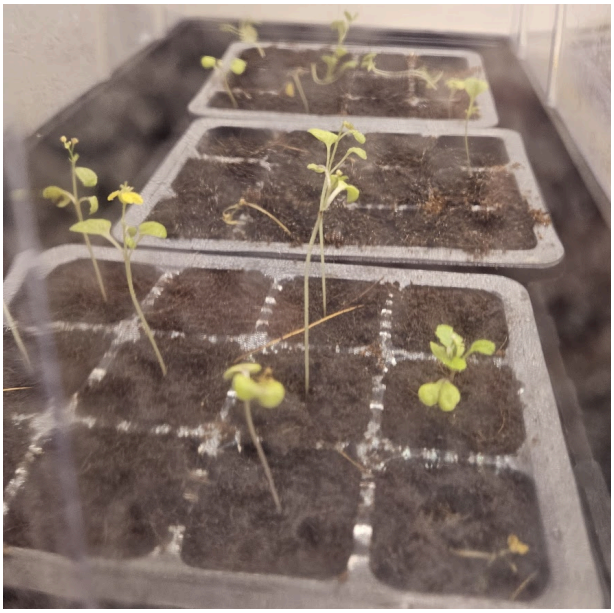
Jan 29- Jan 30:

- Here are images from the plants in the new trial from jan 29 (left) to jan 30 (right). On the first date, the plants were approximately the same height due to sprouting. However by the second date the differences became quite clear. The wheat group was, again, outperforming the styrofoam group, growing taller and exhibiting fuller leaves. This is consistent with what was shown in the previous experiment. It also seemed like, in comparison to the previous trial, the wheat sprouts here had grown to a greater height in a much shorter period of time.



Feb 1:

- An image displaying the stages of growth the plants were at by Feb 1. The difference in growth between the wheat and styrofoam groups is clear. The wheat group boasts heights double the size of their styrofoam neighbors, and the styrofoam group even displays some droopy looking leaves. Presence of styrofoam within the soil, therefore, seems to only be having a negative effect on the sprouts.



- Progress of the plants from the previous trial. Wheat frass group is still growing strong and will continue to be monitored. The styrofoam group has completely died off, which was expected.

Procedure Reflection and Future Endeavors:

- Overall, this trial was very successful in determining the true effects that styrofoam frass had on the growth of the fast plants. The most significant change is definitely to 32 of the mixing procedure, where the amount of frass (mass) was changed to 5 grams. This allowed for more significant and accelerated growth in the wheat frass group and may be the best concentration to see a true difference.
- In the next entry, I will most certainly be collecting data from the trials and stop all growing. This data will be compiled at a later date.

Forming Procedure For Data Collection	
Date: 2/8	**Length of Research Period(s): less than 30 mins**

Goal(s)/Purpose:

- **Creating a Table:** This lab notebook will be focused on creating a table which is used to store the data from these experiments. This will most likely be a sheets file like I usually do.
- **Compiling Data:** The second goal, which may occur first, focuses on actually collecting data about certain aspects of the plants, chiefly plant height and leaf count/size.

Materials Required:

- **Wheat, Styrofoam and Control Plant Pots**
- **Gloves (1 pair)**
- **Ruler x1**

Procedure:

COLLECTION OF MATURE FAST PLANTS

After the end of the indicated growth period, follow this procedure to prepare fast plants for data collection.

1. Set out the starter pots containing your different groups of fast plants out on a workspace or countertop.
2. Get paper towels and use them to cover the planned work area.
3. Select a starter pot to start working with.
4. Remove the plastic "pot" section from the water tray and place it on a paper towel.
5. One by one, push fast plants out of the pots gently using bare hands. Squeezing the plastic pots themselves works very effectively for this.
6. After all plants are removed, remove soil chunks from the lower end of the plant, taking care not to cause damage to the roots.
7. Get 12 weigh boats and set them out in the workspace.
8. Place each plant in an empty weigh boat until all weigh boats have been filled.
9. Place the 12 weigh boats in a plastic tub, stacking them and labeling the tub with the same name of the substrate the plants where grown in.
10. Repeat steps 3-9 for all the remaining starter pot groups.

Procedure for Compiling and Storing Data:

1. Create a copy of the following Google Sheets File: Frass effects on Plant Growth
2. Starting from the first group of plants harvested in the previous procedure, use the ruler to measure the height of each plant in centimeters and count the number of leaves present. Record this information in the cell that falls under the plant's group of origin. For example, if a plant was from the wheat frass group, record its results of height and leaf count under the column labeled "WHEAT FRASS"
3. Mark any deceased or plants that failed to germinate with an x.
4. In the event of a secondary trial, duplicate the table to fill with new data.

Data and Observations:

Heights (cm) and Leaf Counts For Plants Grown in Varying Substrates

Control (plain coco fiber)		Wheat Frass		Styrofoam Frass	
Height (cm)	Leaf Count	Height (cm)	Leaf Count	Height (cm)	Leaf Count
5.7	3	8.5	4	x	x
6	3	7.2	3	x	x
5.9	4	7	4	x	x
6.2	4	7	3	x	x
4.9	2	6.7	4	x	x
6.1	4	6.8	3	x	x
5.5	2	7.1	4	x	x
5	2	6.6	2	x	x
5.1	3	6.7	3	x	x

- Above is the data collected from the trial. While leaf count is a bit more on the random side, height shows a clear trend among the groups with wheat frass proving its dominance as expected. Styrofoam groups were represented with x's given that they all died before collection.

Procedure Reflection and Future Endeavors:

- Overall a pretty successful procedure. The table format is simple and allows for an easy understanding of the data, and the collection process was quick and easy. If I conduct future trials, I hope that I will not need to make many changes to this procedure. Steps 1-4 of the data compiling procedure are fairly straightforward and easy to understand/follow. I also believe the "Collection of Mature fast plants" section of the procedure has helped to keep me organized mainly due to the weigh boats in steps 7-10
- For future plans, I think one particularly smart one would be closely analyzing the styrofoam frass groups to see what properties are negatively impacting the plants. I'm not sure how I'll go about it at the moment, but I'd like to look into some easy to execute options.