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Materials List (by relation):

Mealworm Housing/Care

- Carolina Mealworms (pack of 500)
- 3 stack clear plastic drawers x2
- Spray bottle x1
- Wheat Bran 5qt (bedding and food source)
- Three screen sifter for frass sorting
- Plastic 3 quart containers (Pack of 6)

Plant Housing/Care

- Coco Fiber Brick (Pack of 2)
- Wisconsin Fast Plants seeds pack of 50
- Seed starter pots w/ lid (pack of 5)

Styrofoam and Plastic Sourcing

- BioBag Premium Pet Waste Bags Pack x1
- Packing Peanuts (Polystyrene)

General Lab Materials/Standard Utilities

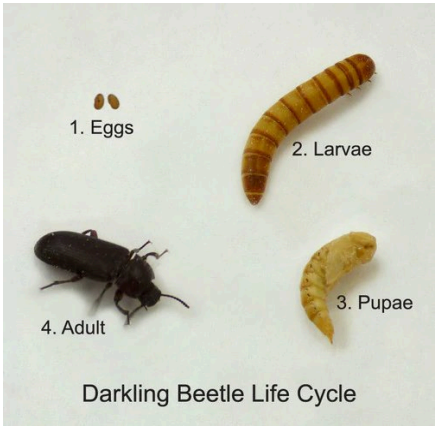
- PPE:
    - Blue Nitrile Disposable Gloves
    - Lab Coat
    - Plastic Safety Goggles
- Scissors
- Binder Clips (1 inch)
- Plastic Cup
- Zip-Lock Bags (large)
- Plastic Spoon
- CGOLDENWALL Precision Lab Scale Digital Analytical Balance Laboratory Balance Jewelry Scale Scientific Scale 0.01g Accuracy 110V (5000g, 0.01g)
- Labeling tape

- Fine-tipped Sharpie
- Masking Tape
- Glue Gun (No specificity or cartridges required)
- Ruler
- Glass beakers (250 mL, 500 mL, 600 mL)
- Glass stir rod
- Flinn Scientific Electronic Balance, 410 x 0.01-g | (OB2142)
- 2 Pieces Rubber Bent Tip Tweezers PVC Rubber Coated Soft Non Marring Curved Tweezers Lab Industrial Hobby Craft Jewelry Hobby Coin Stamp Tweezers Tools (Silver, Black) | Honoson (Honoson-Tweezers-S7884)
- Lab Scoop Spatula (Pick Scoop), Stainless Steel, 6-piece | EISCO (CHO635A)
- 35 x 25 cm metal baking tray | Kitchen Craft (KCMCHB23)

Care Info (Adapted From Carolina Biological et al.)

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.

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.

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”

FEEDING

These detritivores get all the food they need from their substrate and all the water they need from periodic spraying. Please refrain from additional feeding for the sake of experimental simplicity.

DISPOSAL

8. At the end of the investigation, dispose of unwanted bugs by either:
    a. Donating them to another individual is recommended. Mealworms can be a nutritious food source for small amphibians and reptiles.
    b. If the above option is out of the question with the resources or time available, place unwanted organisms in a sealed container in a freezer for 48 hours. Dead mealworms or those purposely terminated by cold exposure can be disposed of in the regular solid waste.

Procedure for Frass Production and Collection:

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:

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.

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 5 grams.
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.

—>> Experimental Trials: *Recommended to begin only when sufficient frass mixture has been collected. Can all be conducted at the same time to get quicker results. Procedures are simple and repetitive.*

Negative Control- Growing Plants in Plain Coco Fiber:

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.**

Exp (1) - Growing Plants with Wheat Frass Blend:

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 top of the starter pot lid (clear plastic).
4. Get the sharpie and label the tape “Exp (1).”
5. Take the lid off of the starter pot and set it aside on the countertop.

6. Get the bag of **wheat frass soil** (Created in ***Procedure for Creating Fertilized "Frass Soil"*** *Blends)* and open it.
7. Using the plastic spoon, scoop soil out of the bag and fill 9 of the "sub-pots" within the starter pot to the brim.
8. Reseal the bag with the zip-lock mechanism.
9. Select 9 labels (included with the starter pot kit) and using the sharpie label them with numbers 1-9.
10. Stick one label in each sub pot. There should now be 9 pots filled with soil and labeled 1-9.
11. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
12. Place a single seed in each of the 9 labeled containers, pushing it slightly under the surface of the soil.
13. 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)
14. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. **If too tall, remove the lid completely.**
15. After 15 days have elapsed, set the starter pot on the countertop and remove the lid.

Exp (2) - Growing Plants with Styrofoam Frass Blend:

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 top of the starter pot lid (clear plastic).
4. Get the sharpie and label the tape "Exp (2)."
5. Take the lid off of the starter pot and set it aside on the countertop.
6. Get the bag of **styrofoam frass soil** (Created in ***Procedure for Creating Fertilized "Frass Soil"*** *Blends)* and open it.
7. Using the plastic spoon, scoop soil out of the bag and fill 9 of the "sub-pots" within the starter pot to the brim.
8. Reseal the bag with the zip-lock mechanism.
9. Select 9 labels (included with the starter pot kit) and using the sharpie label them with numbers 1-9.
10. Stick one label in each sub pot. There should now be 9 pots filled with soil and labeled 1-9.
11. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
12. Place a single seed in each of the 9 labeled containers, pushing it slightly under the surface of the soil.

13. 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)
14. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. **If too tall, remove the lid completely.**
15. After 15 days have elapsed, set the starter pot on the countertop and remove the lid.

Exp (3) - Growing Plants with BioBag Frass Blend:

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 top of the starter pot lid (clear plastic).
4. Get the sharpie and label the tape "Exp (3)."
5. Take the lid off of the starter pot and set it aside on the countertop.
6. Get the bag of **BioBag frass soil** (Created in ***Procedure for Creating Fertilized "Frass Soil"*** *Blends)* and open it.
7. Using the plastic spoon, scoop soil out of the bag and fill 9 of the "sub-pots" within the starter pot to the brim.
8. Reseal the bag with the zip-lock mechanism.
9. Select 9 labels (included with the starter pot kit) and using the sharpie label them with numbers 1-9.
10. Stick one label in each sub pot. There should now be 9 pots filled with soil and labeled 1-9.
11. Get the bag of Wisconsin fast plant seeds and cut it open using the scissor.
12. Place a single seed in each of the 9 labeled containers, pushing it slightly under the surface of the soil.
13. 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)
14. For a total of 15 days, spray the plants each day using the spray bottle. Replace the lid if plants are short enough. **If too tall, remove the lid completely.**
15. After 15 days have elapsed, set the starter pot on the countertop and remove the lid.

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.