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ADULT DRW:**Starting a new DRW Cage:**

Select adult DRW that have hardened elytra (>2 days old); remove from diet cups and place in a cage with vials of flush and water cups.

Place between 150-300 adults in each cage.

Cages: use clean 24"x24"x24" metal cages with 32 mesh lumite screen.

Adults will begin feeding 10 days after being introduced to plant material and will begin laying eggs around 14 days after being placed in cages with flush.

New Cages should be started when a notable decline in egg production occurs, then start a new cage.

Maintain current cage until new cage has started producing eggs.

-See Feeding and Maintaining DRW cages section (pp. 3-4)

To terminate a cage see cage cleaning/termination section (pp. 5)

Obtaining Flush and Prepping Flush Bouquets:

Location: C. mac plants for DRW and ACP are located in GH 110.

Select stems that have fully extended leaves, but have not completely hardened.

Cut Stems of clean C. mac flush using sterile clippers. Use 10% bleach to clean clippers.

Place stems in a tub and rinse with water several times, to wash off any spray residual. Alternately place stems in a bucket of water and let soak while doing other tasks.

Note: Do not leave flush in water longer than a few hours, to reduce water absorbance and water logging.

Fill vials to the top with water and secure lids.

Vial lids should have a very small hole or an X cut in the center of the lid with a razor blade where stems can be inserted (the length of the X should only be half the diameter of the lid).

Note: Slits made by the X are better than round holes because the DRW will crawl through the holes and drown.

Make a fresh cut to each stem, strip the lower leaves and thorns from the bottom $\frac{1}{3}$ of stem and place through the lid slit of the vial containing water.

Place around 8-10 stems in each vial (more than 10 stems will split the lid).

Note: Use 8 if the stems are thick, but if they are thin, can use up to 10.

Place vials with flush in weevil cages. Gage the number of vials required by the weevil population and how voracious the weevils are.

Note: Rule of thumb: 6 vials per cage

Note: Can prep flush vials per above and leave in a tub of water overnight or up to one week. Past one week flush is no longer good. Place a plastic bag over the tub and flush to prevent evaporation.

Prepping Water Cups:

Using a razor blade place a small x in the top center of a water cup lid where a dental wick can be inserted. (Clean diet cups work well as water cups).

Take a piece of dental wick and moisten it with water, then insert into lid.

Fill water cups fill with water and secure lids containing dental wick.

Place several water cups into each cage.

Refill water cups 2x weekly

Swap out dental wicks and wash cups/lids as needed with dish detergent and rinse. (Do not wash dental wicks with detergent, because the cotton will absorb the soap and may “poison” the weevils). Rinse cotton dental wick and can lightly brush, reuse 2-3 times (or until wick turns dark and/or dirt cannot be easily brushed or rinsed off).

Note: Water cups can be prepped and filled ahead of time, then stored in 4°C. Store prepped cups in a diet cup tray and inside of a zip lock bag to prevent evaporation.

Prepping Egg Strips:

Using Paper cutter make egg strips by cutting wax paper into strips 1 -1.5” wide strips

Staple two strips together

Store in a clean dry location

Note: Can use an empty old wax paper box and label as extra egg strips.

Feeding and Maintaining DRW Cage:

Provide fresh flush to DRW, replenish water cups and swap egg strips 2x per week. *

see *A ,*B below:

Open cage door and remove the weevils on and around the door, I generally drop them in the back of the cage.

Remove old bouquets and water cups from cage, and replace with fresh ones. Remove any weevils that may be on leaves, vials etc. and place back in the cage.

***A. Partial Cage Cleaning:**

Working from one side of the cage to the other:

Remove several old bouquets

Discard any dead weevils and wipe out bottom of cage.

Replace old bequest with new ones after wiping down that section of the cage; instead of removing all the bequest at one time and then replacing. The weevils remain calmer if there is something in the cage. If it is completely empty and they don't have anything to hide in they will make a run for the door in mass.

Note: I generally take water cups out first and then place the new ones in at the end to avoid accidental spillage. If water is spilled in cage wipe up. Weevils will drown their selves.

Note: When opening and closing the door check the hinge area (between the door and cage frames. Weevils sometimes get caught in that area and are squashed by the door.

***B. Exchange Egg Strips:**

Precut egg strips (see Prepping Egg Strip Section):

Take a small piece of tape and place on stapled end of egg strip (tape dispenser in labeled box with blue lid in A121, on shelf next to cage). Temporarily tape up all 6 strips to the shelf or side of cage, so they are easy to access prior to opening the door.

Note: Do not use a long piece of tape. Tape so there is none exposed between strip and frame as weevils can get "stuckup" on exposed tape. Use clear scotch tape.

Remove the old egg strips. Replace with the new egg strips. Tape onto the interior of the top metal cage frame. 2 on each side and 2 on back = 6 total strips/cg

Note: Females will sandwich egg clusters in between the two wax paper strips.

Place all old egg strips (fold over) in a zip-lock bag. Push out air (try not to smash eggs!) and seal (to prevent desiccation) then place in plastic shoe box and label with date. EC: date (abbreviation for egg collection date).

Note: Do not leave egg strips with eggs in cage longer than 4 days because they will desiccate.

Place box of collected eggs into Incubator #4 in Insectary hallway (14:10 L/D cycle).

Note: Healthy, fertilized eggs are a perfect oval and are an opaque color. Unhealthy, unfertilized eggs are irregular and misshapen and discolored and/or darkish in color.

***C Cage Feeding and Maintenance Checklist:**

Double check cage and surroundings:

- a. Fresh Flush in cage
- b. Fresh Water cups in cage
- c. New Egg strips in cage
- d. No standing water in bottom of cage.
- e. Check around cage and under shelf for any escapees and place back in cage if found.
- f. Check that tape and marker has been returned to storage box and the lid is closed
- g. Lid is on trash can.

*At end of week take trash bag of trash out, tie off and put into large trash can in the head house area, or take to steamer.

- h. Place a new trash bag in small can in A121.
- i. Door is closed securely

Cleanup of Vials and Used Material:

Flush removed from cage: Dispose of in trash bags and take to the steamer, or freeze prior to putting in dumpster.

Collect dirty vials, water cups, etc. and take to A105.

Wash vials and water cups and lids with dawn dish detergent and scrub with bottle brushes

OR rinse and put in a dishpan with detergent to soak; then wash later.

Water Wicks: Prior to washing water cups, remove cotton wicks, do not get detergent on water wicks, can rinse with clean water, and gently brush with small bottle brush.

Note: Can reuse until they become soiled to the point that the above cleaning methods are no longer effective.

Cage Cleaning/Termination:

Remove everything from cage.

Insects (including adults, egg strips, etc.). and plant material should be frozen for more than 24 hours or steamed.

Empty cages of all insects and other contents, take to benches out behind insectary to wash.

Wash cages: Take hose and spray whole cage (screen and metal frame), can get all loose dirt off by spraying with hose. Use some dawn dish detergent and scrub with scotch bright scrubber thoroughly, then rinse well (with hose) and allowed to dry. *Do not use bleach on the metal cages as it will rust them.

Diet Prep:

See Diet recipe (PP. 12) regarding how to make diet.

Prep for diet pour in a sterile environment; wearing gloves: Place clean cups from packages into clean cup holding trays. If cups fall to floor etc. do not use for diet cup (save for water cup, or other purpose).

Place trays of cups under UV for 20 minutes (Single row in laminar flow hood)

Sterilize lunch trays by wiping down with 75% EtoH

Place UV'd trays of cups onto a sterile lunch tray, 2 stacks of 6 cup trays side by side and tie off bag.

Cup holding trays should be wiped clean of any diet, rinsed and left to soak in a tub of detergent and bleach.

After soaking for several hours trays should be checked for any dirt, diet etc. and scrubbed

Rinse cup holding trays after soaking and scrubbing and allow to dry completely before stacking.

To clean lunch trays wash diet with dish detergent and a dilute solution of bleach. Rinse thoroughly.

After pouring the diet package the trays containing filled diet cups back onto lunch tray in a bag and tie off.

Diet trays containing diet can be stored in 4° C up to 3 months prior to use.

Diet trays containing diet are taken out of 4° C and placed under Laminar Flow Hood for 1-3 hours prior to using. This allows diet to warm up to room temp and the accumulated condensation to evaporate from the surface. Time is dependent on how wet the diet is. Don't let the diet dry out to much.

USDA, ARS, USHRL Insectary Rearing Protocol

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Anna Sara Hill

LARVAE PRODUCTION:

*Infesting and Transferring should be conducted in a laminar flow hood or other sterile environment. Prior to and upon completion of infesting and transferring, sterilize the hood, utensils and transfer trays with 75% EtoH

*Prior to disposing larvae in dumpster kill by freezing for a couple of days (more than 24 hours), or steaming/autoclaving.

Infest Diet with Neonates:

Neonates can be placed on diet up to 3-4 days after hatching from eggs

Check neonates under microscope to ensure that they are alive.

Note: Healthy neonates are a whitish yellow color, and move when exposed to light. Desiccated and dead neonates are a dark orange and brownish color. Do not use neonates if the bag has mold.

Under a laminar flow hood or other sterile environment (wearing gloves):

- Sterilize all work surfaces and items used with 75% EtoH
- Place a piece of filter paper into a Buchner funnel in an Erlenmyer flask that is connected to a vacuum pump.
- Wet filter paper with 5% bleach and turn on vacuum to ensure a good suction
- With vacuum running pour neonates onto filter paper
- With a pair of forceps carefully remove any small debris and egg casings.
- Wash neonates with 5% bleach and allow excess bleach to be pulled through the funnel until filter paper is dry.
- Rinse neonates with DI water and allow all the filter paper to dry.

Note: If there is moisture on neonates or paper the neonates will clump together and die.

- When paper is completely dry pour cleaned neonates into small vial that has a small holes in the lid
- Using a sterile spatula score diet in cups (generally make an x in diet, this aids neonates burrowing into the diet).
- Shake neonates from the vial into diet cups (using a “salt shaker” technique). (This is not quantitative.)
- Secure lids on diet cups

Label trays with Egg Collection date (EC), and Infestation date (I).

Store in incubator at 27°C. Dark Incubator (0:24 L/D).

Clean up work area by sterilizing with 70% EtoH.

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Transfer Healthy Larvae to Individual Diet Cups:

3-4 weeks after infesting diet trays with neonates transfer healthy larvae into single diet cups.

Surface sterilize hood, forceps, spatula, trays, and other supplies with 70% EtoH prior to beginning.

Wear gloves and work under a sterile laminar flow hood:

- Take diet cups out of 4°C and set under laminar flow hood a minimum of 1 hour prior to using to allow condensation to dry from cups.

Note: Gage length of time needed to dry cups. If diet is very wet, or is soft, leave under hood longer prior to beginning.

- Using a sterile spatula score diet in cups (generally make an x in diet, this aids neonates burrowing into the diet)
- Dump diet cups to be transferred into a container (a few cups at a time to keep it manageable).

Note: Shallow white Nalgene trays are reserved for this purpose and are kept in the Drawer marked Transfer Supplies.

- Select healthy larvae that do not have scarring and are the largest
- With a pair of sterile forceps transfer selected individuals to a single diet cup (1 larvae/each cup).
- Dump used diet and larvae that are not selected into a trash bag
- Repeat until all the larvae in that batch have been checked.
- Secure lids on diet cups

Label trays with EC, I, and Transfer date (T).

Note: I peel the old label with EC and I date off and place on new tray and add the T date.

Store in a 27° C. Dark Incubator (0:24 L/D).

Larvae will undergo molts, pupate and emerge as adults in the diet cup.

Cleanup after Infest and Transfer:

Surface sterilize hood with 70% EtoH.

Wash forceps, spatula, trays, etc. with solution of dawn dish detergent, and rinse well with water.

Store transfer supplies in drawer marked transfer supplies

Tie off and take trash bag with used diet and larvae to larger trash can destined for steamer or directly to steamer.

Put away unused diet cups containing diet (store in large ziplock bags (2 trays stacked on top of each other fit well. Seal bags, label and place back in 4°C and use next time.

Sorting and Culling:

At regular intervals (~ once a month) sort through the larvae in the trays of cups in the incubator and dispose of the following:

- Cups that have dried died

- Cups that have mold

- Larvae that are dead

- Larvae, Pupae, or Adults that are deformed

Separate adults into separate trays so they are ready for use when needed

Separate pupae if they are being used, or if you are tracking when adults emerge.

Place the “good” cups/trays back in the incubator

If mold spores get into incubator clean it with a dilute solution of bleach. Rinse and air out prior to putting larvae back in.

DIAPREPES ARTIFICIAL DIET RECIPE:

The following amounts are used in 50 L Steam Jacketed Kettle:

725 g. of Agar

1 box weevil premix #F9855 (9.2kg) Frontier Scientific

Approximately 40 liters of water

Directions

1. Weigh out Agar and add enough water to make a 5% gel.
2. Heat Agar and Water to boil – mix constantly (set steam jacketed kettle to 4.5). Takes approximately 45 min.
3. When agar has reached boiling point (85C or 185F) and is smooth and opaque add diet and water (pour diet in slowly and simultaneously add small amounts of water. *make sure agar is a rolling boil.
4. Finish filling kettle with water to 45 L mark. With a whisk break up any clumps of diet, stir until smooth.
5. Increase temperature as needed to bring diet to boil. Stir continuously (best if kettle has a paddle).
6. Cook until diet is near boiling and starts thickening. (Make sure diet is not too thin or too thick), if it is too thin it will not set up properly and larvae will flounder. If diet is too thick it is difficult to pour. If diet is too thin add more diet or cook longer. If diet is too thick add some water. Add small amounts of water because if the agar/water ratio is messed up the diet will not gel. Diet consistency should be like a thick pudding, but viscous enough to pour.

Note: Can also add Agar, but must be dissolved in water and at boiling temp (can do this in microwave). This is tricky because Agar will cool and solidify on top

Caution: Agar sometimes will separate and solidify on top of diet. This is not conducive to good larvae growth as diet is inconsistent and will stay liquid and soft underneath, agar layer.

7. Pour the diet. Fill the cups approximately half full.
8. Keep Diet hot while pouring, it cools rapidly and starts solidifying. Allow diet trays to cool in laminar flow hood. When diet starts pulling away from sides of cup it is ready for use. Do not let diet dry out completely. If diet starts cracking on top, it is too dry.
9. Double bag diet trays and store in a 4°C refrigerator

*Makes 100-120 trays of diet (30 cups/tray)

SHIPPING:**Larvae and Pupae:**

Larvae and Pupae can be shipped in diet cups.

Place cups with diet and larvae in cup holder trays and place 2 trays in a large ziplock bag

Put bagged trays together in a larger bag or trash bag.

Put in appropriate size box, fill space with bubble wrap or air bags, etc.

Include copy of shipping permit

Tape box well, reinforce bottom of box with tape.

Label this side up

Ship overnight.

Adults:

Adults that are teneral and have not been removed from diet cups yet can be shipped in diet cup as per larvae

Adults that have been actively feeding in cages can be shipped in an empty clean diet cup with a wet water wick.

If sending adults that are not in cups with diet ship with a cool pack.

Overnight

Eggs:

Eggs can be shipped, but are more fragile. Eggs that are close to hatch do not survive well.

If shipping eggs, ship freshly laid eggs.

Double bag in small to medium ziplock bags with air compressed from bag to prevent desiccation, be careful not to squash eggs when removing air. Ensure both ziplocks are sealed well.

Then place in another large ziplock bag that has air, to help protect eggs from damage.

Place in small box with plenty of bubble wrap or air bags.

Ship overnight.

Note: Ship on the senders account number.

WEEVIL STAGES AND DEVELOPMENT TIMELINE:

Egg: Collected 3-4 days after being laid

Neonates: 7-10 days to hatch from egg

Larvae:

Pre-transfer: 4-6 weeks after infestation

Post-transfer Larvae: 3-4 months to pupation.

Pupae: emerge as adults in 3-4 weeks.

Adults:

Tenerals: 24-48 hours to harden elytra

“Hardened” Adults: Live up to 3 months (max 4 months) in cage

Feeding and Egg Production: 7-10 days after placing on flush in cages

Peak Egg production: 6 weeks – 10 weeks after placing in cages

DIAPREPES REARING SUPPLIES AND SOURCES:

Product	Product #	Source	Link
Dental Wick	200404	Richmond Dental	Wrapped Rolls Richmond Dental
24"x24"x24" Lumite Cage	1450DS	BioQuip	Search (bioquip.com)
1 oz (30 ml) clear portion containers (cups) Dart/Conex	100pc	Webstaurant Store	https://www.webstaurantstore.com/dart-conex-complements-100pc-1-oz-translucent-plastic-souffle-portion-cup-case/301100PC.html
clear plastic lids for 100 PC cups	PL100N	Webstaurant Store	https://www.webstaurantstore.com/dart-solo-pl100n-small-clear-plastic-souffle-cup-lid-case/301PL100N.html
Pre-mix for Diaprepes Root Weevil	F9855	Frontier Scientific Services	sales@insectrearing.com +1 302-533-3540 *Special order by request
Agar	7060	Frontier Scientific Services	https://insectrearing.com/product/product-7060-agar/
Cup Trays – 30 well	9040	Frontier Scientific Services	Cup Tray-30 Wells (9040) - Frontier Scientific Services Agriculture (insectrearing.com)