House Fly (*Musca domestica*) Rearing and Colony Maintenance [UC Riverside]

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Purpose:

The methods outlined in this pictorial guide are for the rearing and maintenance of large colonies of the house fly (*Musca domestica* Linn.) in a laboratory setting. This protocol is currently used by the Gerry laboratory at the University of California at Riverside and was adapted from:

> Mandeville, J. D., Mullens, B. A. & Meyer, J. A. Rearing and Host Age Suitability of *Fannia canicularis* (L.) (Diptera: Muscidae) for Parasitization by *Muscidifurax zaraptor* Kogan and Legner (Hymenoptera: Pteromalidae). *The Canadian Entomologist* **120,** 153–159 (1988).

Methods described include collection of eggs from adult flies, rearing of immature flies and maintenance of large colonies of adult house flies. This rearing protocol requires only readily obtainable products available from most hardware stores, grocery stores, or animal feed stores, in addition to some common laboratory consumables. Specific brands mentioned in this protocol can be substituted for equivalent products unless explicitly stated. Times given in the schedule are for house flies maintained and reared at 26.7 ^{o}C at 45% relative humidity.

Supplies:

Consumables (to make 2 larval rearing pans; approx. 10,000 flies):

Red Flaky Wheat Bran	16 cups (3.75L)	
Bakers Fast Rising Yeast	3 tbls (15mL)	
Nonfat Dry Milk	1 cup (240mL)	
Alfalfa Pellets	1 cup (240mL)	*Prepare the night before
Deionized Water	12.5 cups (3L)	
Evaporated milk	30 mL	
1.5 oz condiment cups		
Kimwipes		

Reusable Materials:

Dishpans, cut ~5cm in height to serve as larval rearing pans (**Figure 1**)

- We use 11.4 QT Rubbermaid dishpans (Part #2951)

Larger plastic container – 18 QT dishpan or sweater box
Sieves (US-Sieve Mesh sizes 10, 25, and 200)
15 ml centrifuge tube
Rubber bands
Adult fly cage – to make inexpensive cages, use PVC coated steel wire fencing to form cage frame; cut fencing into 36” x 9” inch sections and roll fencing to fit in dishpan; use rubber coating to cover exposed ends of cut fencing; construct mesh sock (36” x 12”) with 1 open side to slide over wire frame (**Figure 2**).

Egg Collection:

1. Make oviposition cup: Place Kimwipe into condiment dish, add ~5ml evaporated milk.
2. Place oviposition cup into cage of $\geq$ 5 day-old adult flies.
3. Leave oviposition cup in cage for 24 hours for egg deposition (**Figure 3**).

Prepare Larval Media (*use amounts indicated in consumables section above*):

1. At least 24 hours before making larval media, prepare alfalfa pellets by combining 3-parts pellets to 1-part deionized water and place in refrigerator to soak (**Figure 4A**). A large batch of alfalfa pellets can be soaked and stored in a laboratory refrigerator for up to 3 months. Alfalfa stored in this manner may become moldy, but no obvious detrimental effect has been noted for house flies reared using moldy pellets.
2. Use a large plastic container to combine yeast, powdered milk, and soaked alfalfa. Add 1L of deionized water and mix thoroughly (**Figure 4B-F**).
3. Add Bran and the remaining 2L of D.I. water (**Figure 5A-D**).
4. Mix thoroughly; media should be moist enough to clump (**Figure 5E)**.
5. Evenly divide the mixture between two larval rearing pans (**Figure 6**).

Move Eggs to Larval Rearing Pans:

1. Collect oviposition cups from adult fly cages (**Figure 7A**).
2. Place larger #25 sieve on top of finer #200 sieve (**Figure 7B**).
3. Place oviposition cup into top sieve and gently rinse with cool tap water to remove the Kimwipe and associated fly eggs (**Figure 7C**).
4. Gently unfold Kimwipe and rinse eggs off under gentle water stream, rinsing all eggs through large sieve into finer sieve below (**Figure 8A-C**).
5. Remove top sieve and tilt bottom sieve to wash eggs towards the bottom edge of the sieve frame. Using a small transfer pipette aspirate eggs in water from sieve into a 15 ml centrifuge tube with a 1 mL mark on it (**Figure 8D-F**).
6. Let tube contents settle for 30 seconds to allow viable eggs to settle on the tube bottom. Eggs that float are not viable. Continue adding eggs until 1 ml of viable eggs are collected (**Figure 9**).
7. Gently mix the tube by inverting the tube 3-4 times to suspend viable eggs. Pour tube contents into one larval rearing pan (**Figure 10**).
8. Repeat for second larval rearing pan.
9. Cover larval rearing pans with a fine mesh sock to prevent maggot escape and protect the larval media from unwanted oviposition by other flies in your rearing room. Secure mesh sock end by knotting or using 2 rubber bands.
10. Place larval rearing pans at suitable developmental temperature (we use 26 ^{o}C).

Collecting Pupae from Larval Rearing Pans:

1. After 5 days at 26 ^{o}C, most larvae will have reached the final larval stage (L3). You will note some larvae beginning to wander on the surface of the larval rearing media. These wandering larvae are a good indication that larvae are ready to pupate. Larval media should be dark brown and loose. If sticky and moldy house flies did not develop well.
2. Place larval rearing pans into larger plastic container filled with 2-4L of dry bran (**Figure 11**). Place larval rearing pan on top of dry bran. Make sure that the sides of the larval pan do not touch the sides of the larger container (**Figure 12**)!

3. Using a spoon scrape the larval media away from the corners of each larval pan. Fill the larval pan with deionized water until the water in the corners reaches approximately half the height of the larval media (**Figure 13**). It is about 1.5L of water but it is highly dependent on initial larval density and dryness of the larval media.
4. Larvae will start to emerge from the wet media within 15-30 minutes; larvae will leave the wetted larval rearing pan by climbing the side of the pan and fall to the larger plastic container below (**Figure 14**). Larvae will then burrow into the dry bran available in the larger container where they will pupate.
5. Cover both containers with a fine mesh sock secured at the open end using 2 rubber bands to prevent maggot escape. Allow 3 days for larvae to move from the larval rearing pan to the outer container and to complete development to the pupal stage.

Sifting Pupae from the Bran:

1. Remove the larval rearing pan from the larger container with bran. Scrape the bottom of the larval pan to remove any pupae that may be stuck to the bottom of the larval rearing pan (**Figure 15A**). The wet larval media should be frozen to kill any remaining larvae or double-bagged and discarded immediately to prevent unwanted releases of flies.
2. Use #10 sieve to sift fly pupae from the bran (**Figure 15B**). The bran will pass through the sieve while the fly pupae will be retained. From 1 mL of house fly eggs, you should produce approximately 5,000 pupae (**Figure 15C**). Collect pupae from all larval rearing pans and mix together to ensure genetic similarity throughout your colony.

Adult Fly Cages:

1. Set up clean adult fly cage by placing wire cage with mesh sleeve within a dishpan to contain any spilled water or food from the cage (**Figure 16A-B**).
2. Place 250 ml of the mixed pupae (*see above*) into a container without lid to emerge into a new adult cage. This should result in about 2,000 adult flies in the adult cage. Pupae not placed into new adult cages can be placed into containers with a mesh top for air exchange and stored in a cold room at 15^{o}C (60^{o}F) for a week or more before adults begin emerging.
    - *We always keep some pupae in the cold room as a back-up in case of catastrophe in our adult fly rearing rooms!*
3. Add a water cup containing 2 paper towels and fill until water line is halfway up the paper towels. The paper towels reduce fly death due to drowning. Add a food dish containing powdered milk and table sugar (50:50 ratio) to the adult cage (**Figure 17A-B**).
4. Secure the end of the mesh sleeve with a rubber band (**Figure 17C**).
5. Check adult fly cages every Monday, Wednesday, and Friday to make sure they have adequate food and water. There is no need to come in on weekends unless you miss your flies. ☺

Adult flies will begin mating soon after emergence and female flies will be ready for oviposition in approximately 5 days. We try to keep our colony on a weekly schedule so that there are specific activities that must occur on each day of the week as indicated in the example monthly schedule (**Figure 18).**

Figure 1: House fly larval rearing pans cut 5 cm high (~2 inches). Front center is a condiment cup with fly eggs (oviposition cup).

Figure 2: Supplies to construct adult house fly rearing cage. Almost any enclosed mesh container can be used if the house flies have enough room to fly. Starting from the right, dishpan, wire cage bent to fit into pan, and mesh sleeve to enclose wire cage.

Figure 3: House fly oviposition cup with house fly eggs deposited on both the Kimwipe and sides of the 1.5 oz condiment cup.

Figure 4: Mixing procedure for larval media. Combine yeast, powdered milk and 1L DI water.

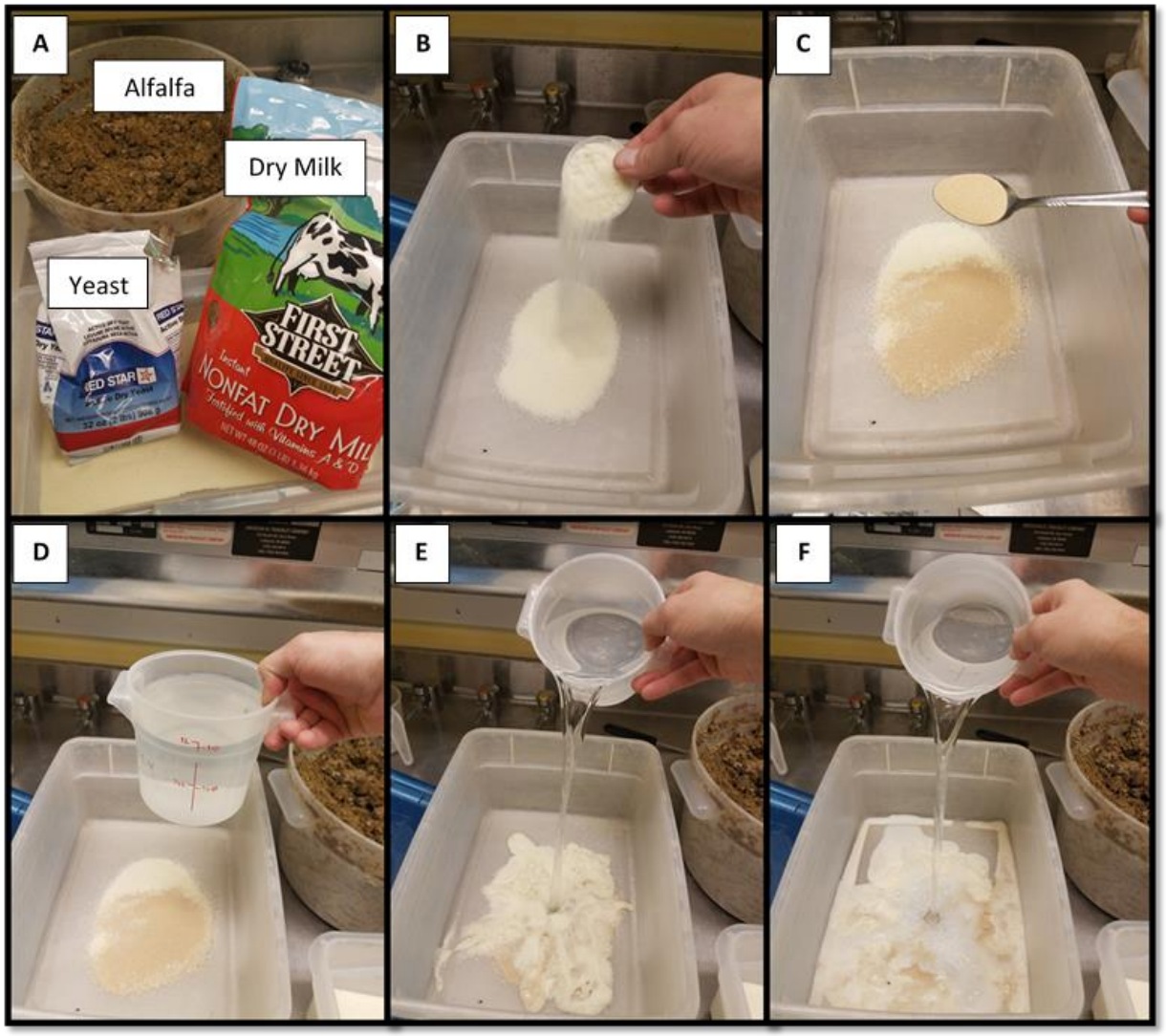

Figure 5: Add soaked alfalfa pellets, stir and add bran plus remaining water. Mix thoroughly.

Figure 6: Evenly disperse fly media between two larval rearing pans. Larval media should be about 3.8 cm deep (1.5 in).

Figure 7: Rinse all eggs out of oviposition cup and wash through top sieve (sieve with larger holes).

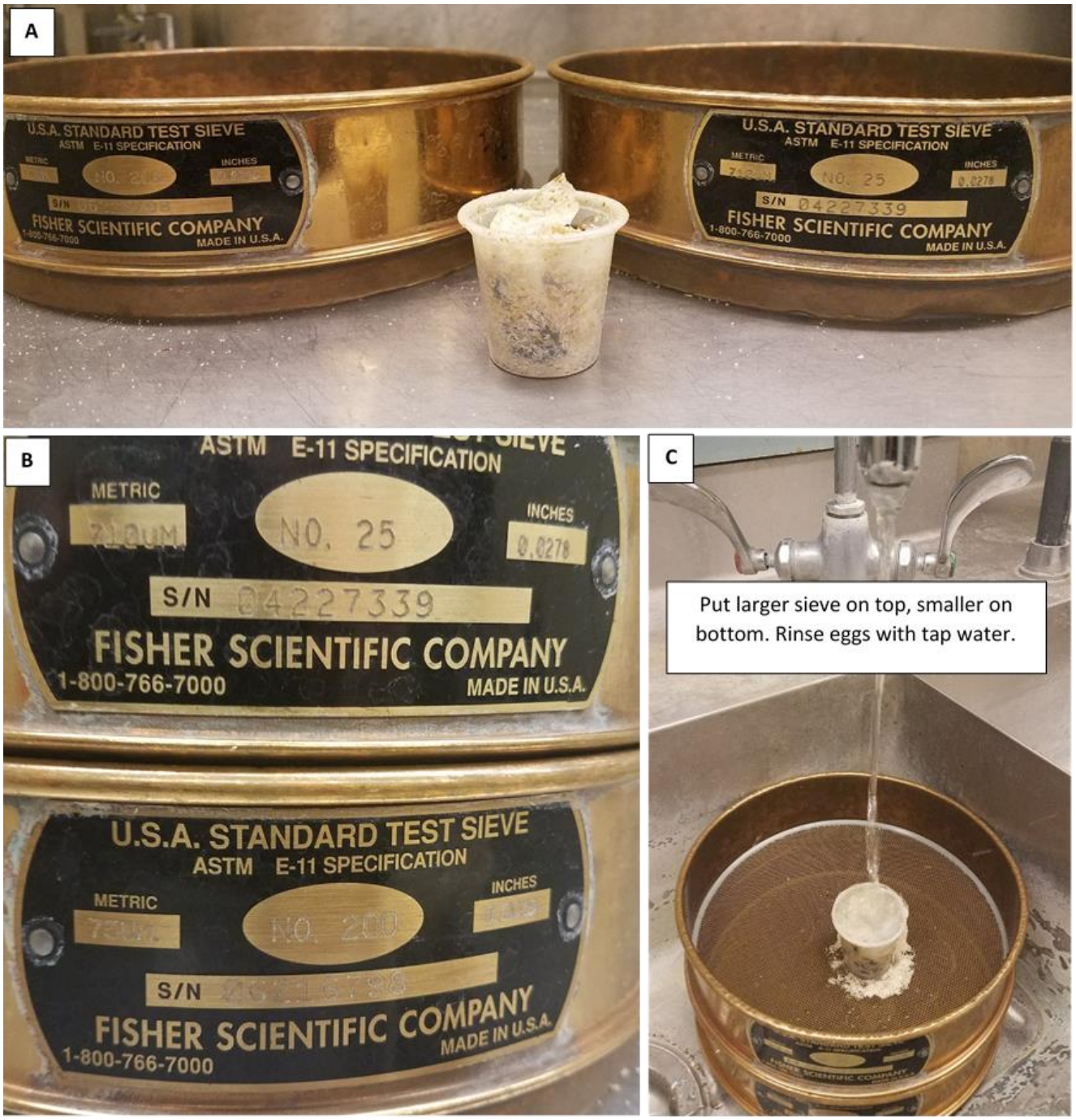

Figure 8: Rinse all the eggs off the Kimwipe and into finer sieve below. Remove the top sieve and gently rinse eggs in the fine sieve down to one side. Use pipette to collect eggs into 15mL tube.

Figure 9: Two falcon tubes with collected house fly eggs. Collect 1 mL of submerged eggs. Eggs that float are not viable, while eggs that sink are good.

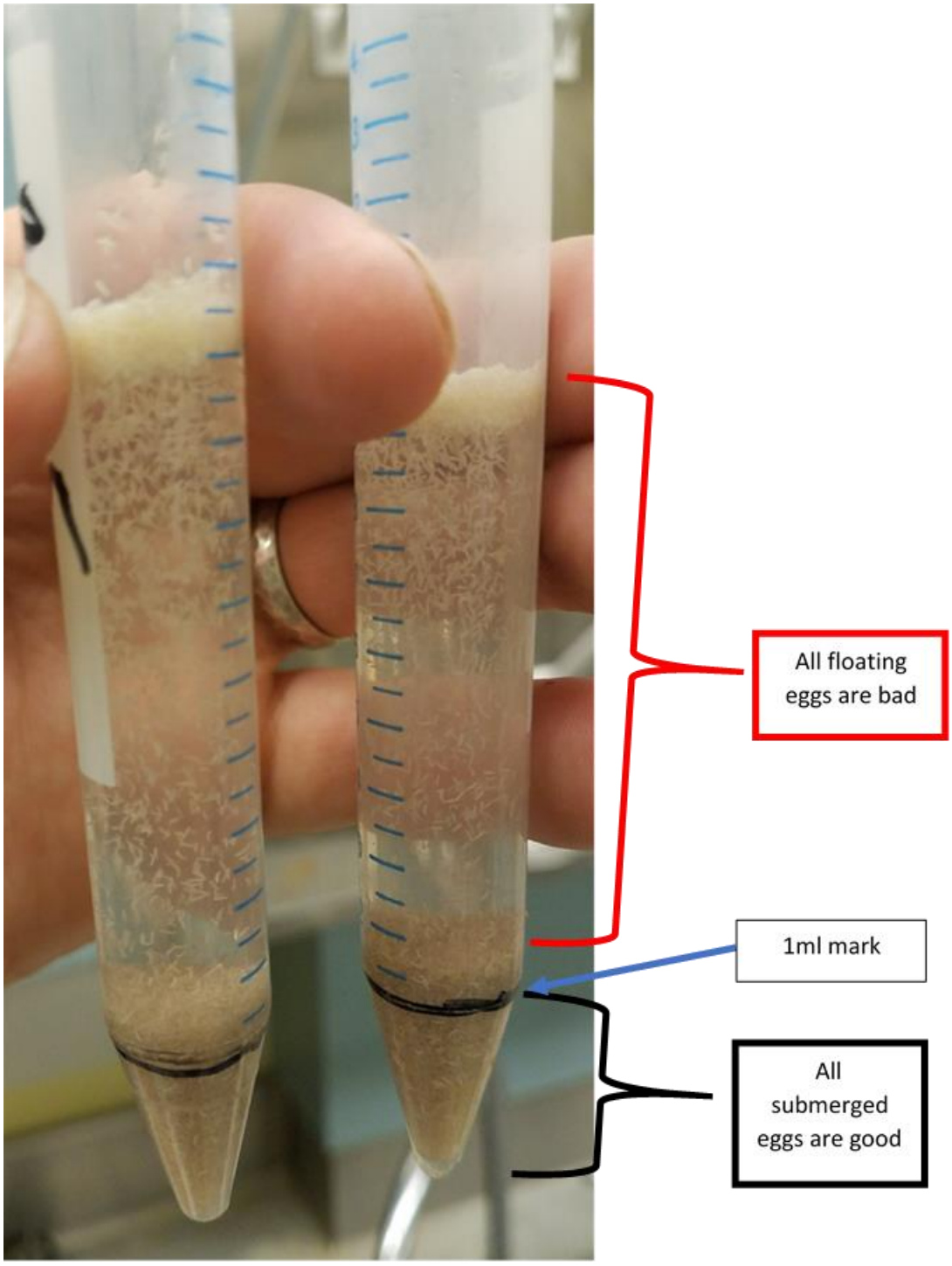

Figure 10: Pour the 1mL of eggs into a one of the newly created larval pans. Add second 1mL of eggs to the second larval pan.

Figure 11: Pupation container filled with 2-4L dry bran (ca. 4cm depth). Container should be large enough to hold the larval rearing pan inside without touching the sides of the container.

Figure 12: Larval rearing pan placed into pupation container filled with bran.

Figure 13: Flood larval pans with enough water to fill the pan to half of the height of the larval media. If overfilled, mortality can be high. If underfilled, larval will not leave media to pupate.

Figure 14: L3 larvae beginning to wander from a wetted larval pan.

Figure 15: Pupa in the bottom of the pupal collection container. Sift pupae from dry bran using a #10 sieve.

Figure 16: Supplies necessary to set up adult house fly cage. A 11.4 qt dishpan, wire cage and mesh sock.

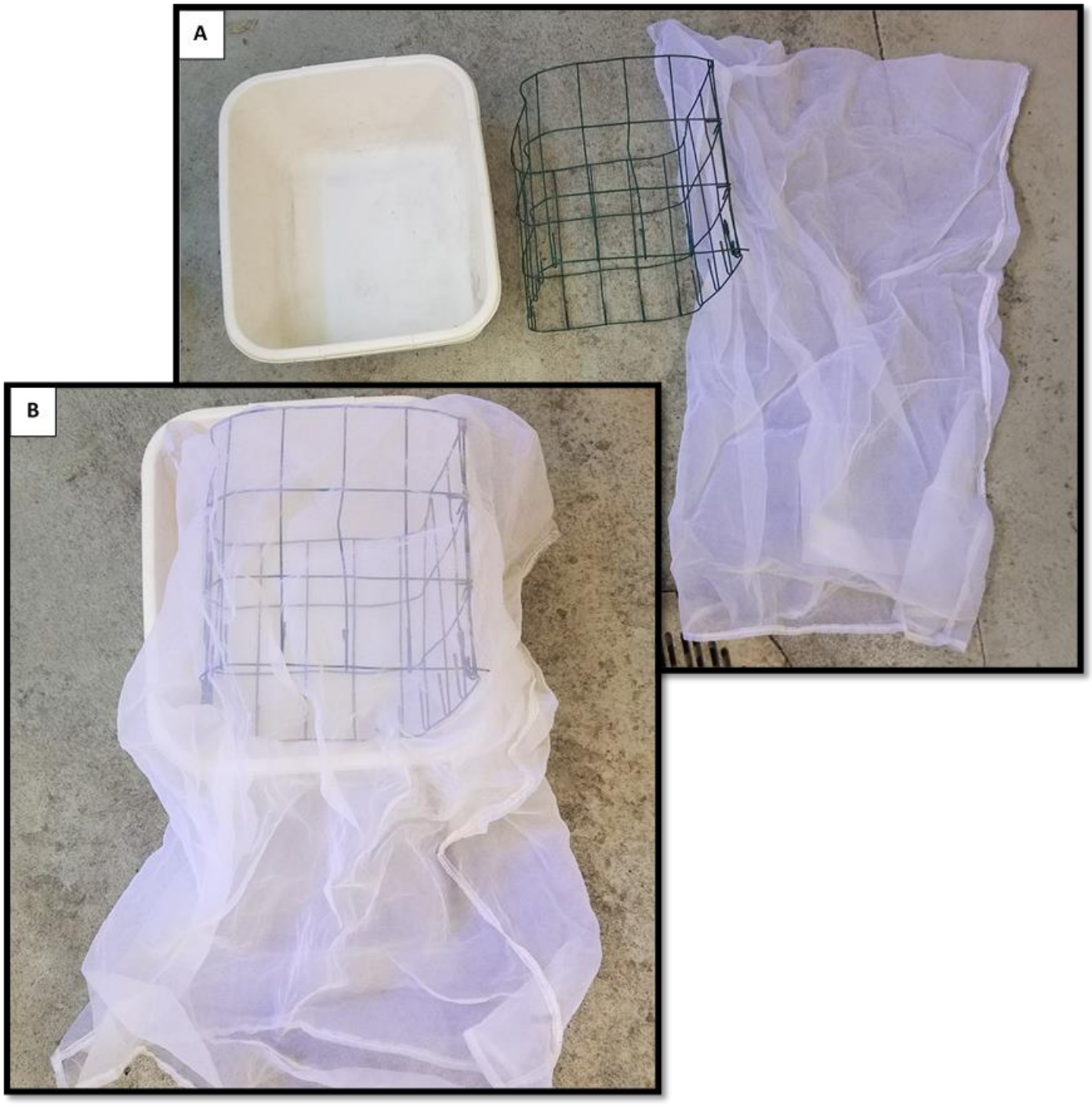

Figure 17: Consumables used to maintain adult house flies.

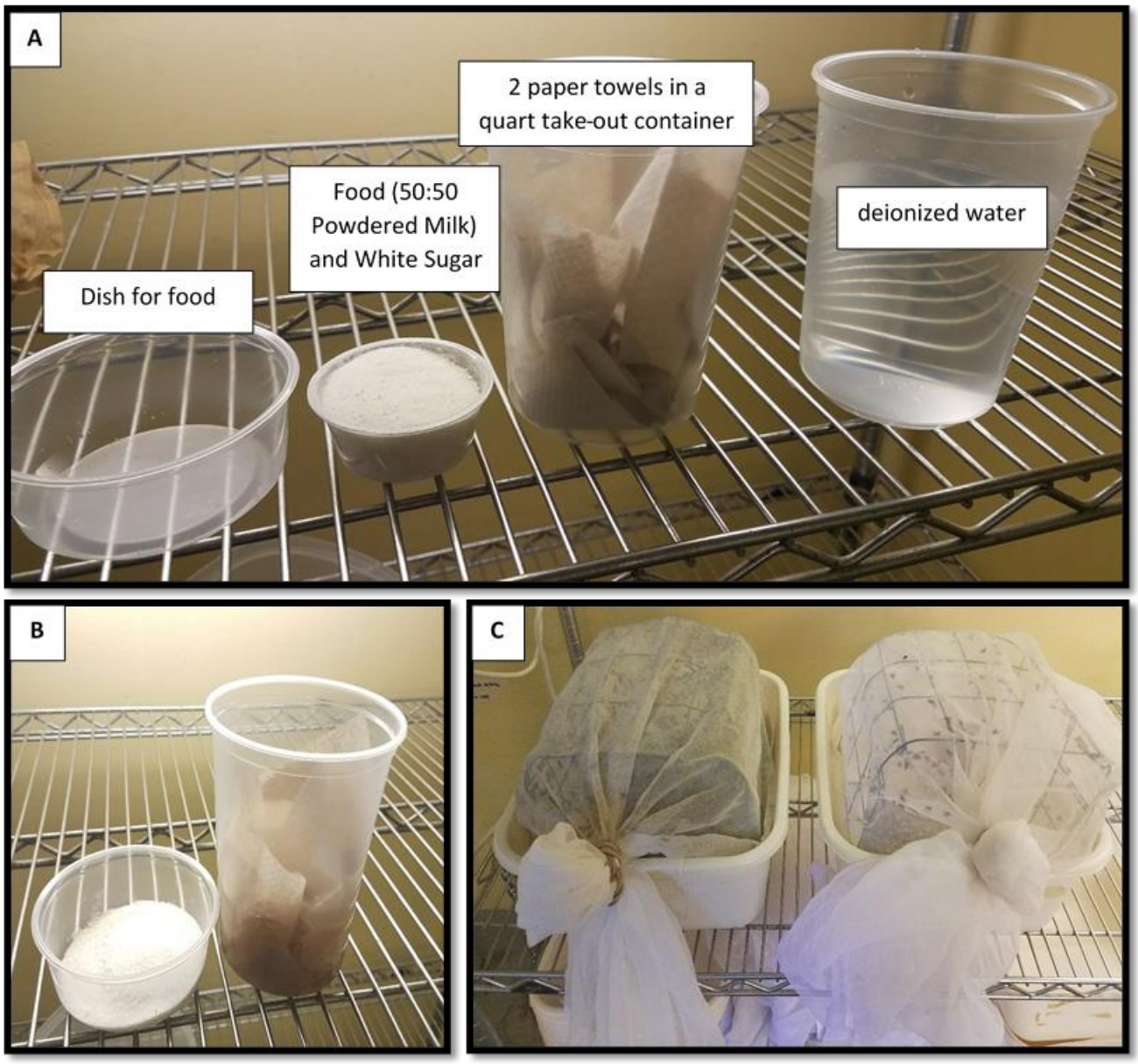

Figure 18: Example fly rearing schedule over a month-long period. Colors indicate steps associated with a single filial generation of flies (**F1, F2, F3, F4**), with multiple filial generations reared in succession. Using this method personnel do not need to come in on weekends to manage house flies. Rearing rate can be doubled by making additional larval pans on Friday of each week while maintaining the same day to day intervals for other rearing events. Food and water should be checked in adult cages 3x per week on Mondays, Wednesdays, and Fridays (not indicated on chart).

June 2018						July ▶
Sun	Mon	Tue	Wed	Thu	Fri	Sat
					1	2
3	4	5 Insert Oviposition Cup	6 Make Larval Pans	7	8	9
10	11 Flood Larval Pans	12 Insert Oviposition Cup	13 Sift Pupae Make Larval Pans	14	15	16
17	18 Flood Larval Pans	19 Insert Oviposition Cup	20 Sift Pupae Make Larval Pans	21	22	23
24	25 Flood Larval Pans	26 Insert Oviposition Cup	27 Sift Pupae Make Larval Pans	28	29	30