

Standard Operating Procedure for Locust Colony

December 3, 2008

1 Housing

- Animals are kept in cages measuring 18 in x 18 in x 18 in. Cages are lined on all sides with a fine mesh, except the floor which consists of a retractable aluminium sheet and a 4-mesh screen immediately below it. Both the aluminium sheet and the 4-mesh screen have three holes that need to be plugged with plastic cups. Before animals reach their reproductive stage, cups closed with a plastic lid are used. Once the animals are reproducing, the cups are filled with moist vermiculite so that the locusts can lay their eggs during their reproductive period (breeding cups).
- When starting a new cage, place a post-it note next to the cage and mark the date when hatchlings were first placed in the cage (1st instar). Inspect the cages daily. When you first detect that animals have molted to the next instar, indicate the date on the post-it note (2nd instar, 3rd, 4th, 5th and adult). Indicate also the first date of reproduction (when breeding cups filled with moist vermiculite were first placed in the cage). This will help assess the health status of the colony and determine the animals best suited for experiments.

2 Cleaning

- When a cage's population has become low (ten or less animals), empty the cage (place remaining animals in another cage of similar age) and wash it in the cage washer.
- Three times weekly (Mo, We, Fr), clean each cage floor removing fecal material, food and dead insects using a broom and a vacuum cleaner.
- Change or empty vacuum cleaner bag as needed.
- Remove old grass saucers, empty, and soak in dilute bleach overnight.
- Remove breeding cups from incubator over one and a half month old, empty, and soak in dilute bleach overnight.
- Sweep the floor daily.
- Wipe down the entire counter daily.
- Clean and thoroughly rinse cups, lids and saucers that have been left from the previous day to soak. Hang these items on the rack above the sink or place them next to the sink to dry.

3 Feeding

- Each cage gets one or two saucers of grass three times a week (on Mondays, Wednesdays and Fridays). The number of grass saucers depends on the age and the number of the animals in the cage. One saucer will be sufficient for older animals (after they started to reproduce). Locusts will eat more during their growth phase (third and fourth instar) and these cages typically will have many more animals. They should thus be provided with two saucers. If the animals completely eat the grass in one saucer place two the next time.
- Replenish oat bran as needed, approximately one table spoon in the corner of each cage or in a saucer is adequate three times a week (Mo, We and Fr). Oat bran should be sprayed with antibiotics solution 24 hours before placing it in the cages. On Fridays, Tuesdays and Thursdays, place sufficient amount of oat bran for all cages in a saucer, spray with antibiotics solution so as to lightly moisten it, mix thoroughly and let dry overnight.

4 Breeding

- When the animals reach their adult stage (fully developed wings) and/or cannot pass through the 4-mesh screen, remove the aluminium floor. This will result in fecal material falling under the cage, making the cages easier to clean and minimizing the possible spread of diseases.
- When the males start mounting females or when there are signs of eggs on the floor of a cage, the cage is ready for reproduction. Breeding cups filled with moist vermiculite should be inserted in the three holes at the bottom of the cage.
- Update the post-it note describing the cage by writing "reproduction" and the date at which reproduction started.
- Cages that have been marked for reproduction, are to be continually bred.
- Breeding cups should be filled with moist vermiculite, obtained by mixing approximately 5 parts of vermiculite for 2 parts of water (volume; each cup is about 215 ml, so about 86 ml of water). Do not fill cups up to the top to leave room for the hatchlings (approx. 1 in). There is too much water in the cup if it starts to accumulate at the bottom. Drain.
- Three times weekly (Mo, We, Fri), the breeding cups should be replaced with new ones, prepared as described above. Evaluate each cup for an egg pod. If egg pods cannot be seen, carefully scrap the surface of the cup, removing the vermiculite until you encounter the foamy material characteristic of the egg pod. If no egg pod is present discard the cup's content and soak in dilute bleach.
- If an egg pod is present, carefully scoop out the top layer of vermiculite to clean the surface of any fecal material. Spray with anti-fungicide and add a small sheet of a paper towel wetted with water to maintain moisture (too much moisture will drown the eggs). Put a lid on the cup (see next item), place a piece of tape on the cup with the date, and place the cup in the incubator.
- The lids of the cups placed in the incubator need to have a sufficiently large hole in the middle to allow good air circulation. When you prepare the lid, punch a hole in the center of the lid about one by one inch large. Seal the hole with a piece of cloth mesh using a glue heat gun.

5 New Hatchlings

- When newly hatched locusts are found, usually twenty-one days after first incubation, place either in an empty cage with the aluminium floor sheet re-inserted or in a cage with the smallest population of youngest insects. Then remove the lid. Provide food as you would normally. If the cage was empty, place a post-it note and write down the first date of hatching. Check the incubator for hatchlings daily. New hatchlings will only survive a short period of time if left in the incubator without food and light.
- New hatchlings should be placed in cages with animals of similar age to generate an homogeneous population in each cage. Therefore, a new cage should be started every 2-3 weeks, or earlier when there are many hatchlings. To free up new cages, older animals in the reproductive stage should be consolidated in fewer cages (see Cleaning above).
- Discard plastic cups that have not hatched, one and half month after the initial incubation date.

6 Planting

- Grass saucers should be prepared one week in advance, one or two per cage (see above). Typically 23-25 are necessary to place two saucers in cages with young animals (15 cages total). Prepare saucers and set wheat to sprout on Mondays, Tuesdays and Thursdays.
- For each saucer mix 1 cup of topsoil and 1 cup of sphagnum peat moss. Moisten thoroughly and fill the saucer.

- For each saucer, place 1/4 of a cup of hard winter wheat in a sprouting jar. Rinse three times with water and then fill the jar with water. Leave in water for 12 hours (until the evening). In the evening, rinse three times with water and let the wheat sprout in the jar at an angle of forty-five degrees overnight.
- On the next morning (Tuesdays, Wednesdays and Fridays) spread the wheat grass seeds evenly over the soil in the saucers prepared the preceding day. Water generously and cover with a saucer for 48 hours (except on Fridays, 72 hours).
- On Thursdays, Fridays and Mondays uncover the wheat planted on Tuesdays, Wednesdays and Fridays, respectively.
- Water liberally and place directly under light.
- Water all other saucers in the room as well.

7 Temperature Control

- On Mondays, change the temperature chart. Press on the "chart" button. At the prompt: "Change chart?" select the "yes" button and press "enter". Unscrew the central piece holding the chart, take the chart out and place a new one. At the prompt "Start new chart?" select "yes" and press enter.
- Verify daily that the temperature record is appropriate (12 hours at 78 deg Fahrenheit during the night and 12 hours at 90 degrees Fahrenheit during the day). If there are any irregularities, immediately notify the supervisor.
- Change the marker pens when necessary.

8 Stocking

- Make sure there are clean saucers and cups for the following day, on a daily basis.
- Make sure there are plenty of vacuum bags, oat bran, topsoil, peat moss and wheat seeds; enough vermiculite, temperature charts, replacement pens, glue for the heat gun and cloth mesh. Notify the supervisor when these items are getting low.
- Notify a lab member when running low on antibiotics solution or antifungicide for a refill.

9 Essential Duties for Mondays

- Feed each cage one or two saucers of fresh grass and a table spoon of oat brand.
- Wipe the bottom of each cage clean with a broom and vacuum.
- Check all breeding cups for egg pods.
- Check incubator for hatchlings.
- Replace all breeding cups with fresh ones.
- Prepare lids for cups that go in the incubator as needed.
- Clean cups and trays that have soaked overnight.
- Put empty saucers and used breeding cups to soak.
- Fill new saucers with soil and set wheat to sprout.
- Uncover the wheat saucers planted on Friday.
- Water generously all uncovered wheat saucers.

10 Essential Duties for Tuesdays

- Clean cups and trays that have soaked overnight.
- Plant the wheat that was set to sprout on Monday, cover with a saucer.
- Fill new saucers with soil and set wheat to sprout.
- Water generously all uncovered wheat saucers.
- Prepare oat bran for feeding by spraying with antibiotics.

11 Essential Duties for Wednesdays

- Feed each cage one or two saucers of fresh grass and a table spoon of oat brand.
- Wipe the bottom of each cage clean with a broom and vacuum.
- Check all breeding cups for egg pods.
- Check incubator for hatchlings.
- Replace all breeding cups with fresh ones.
- Prepare lids for cups that go in the incubator as needed.
- Put empty saucers and breeding cups to soak.
- Plant the wheat that was set to sprout on Tuesday, cover with a saucer.
- Water generously all uncovered wheat saucers.

12 Essential Duties for Thursdays

- Clean cups and trays that have soaked overnight.
- Fill new saucers with soil and set wheat to sprout.
- Uncover the wheat saucers planted on Tuesday.
- Water generously all uncovered wheat saucers.
- Prepare oat bran for feeding by spraying with antibiotics.

13 Essential Duties for Fridays

- Feed each cage one or two saucers of fresh grass and a table spoon of oat brand.
- Wipe the bottom of each cage clean with a broom and vacuum.
- Check all breeding cups for egg pods.
- Check incubator for hatchlings.
- Replace all breeding cups with fresh ones.
- Prepare lids for cups that go in the incubator as needed.
- Put empty saucers and breeding cups without egg pods to soak.
- Plant the wheat that was set to sprout on Thursday, cover with a saucer.

- Uncover the wheat saucers planted on Wednesday.
- Water generously all uncovered wheat saucers.
- Prepare oat bran for feeding by spraying with antibiotics.

14 Material

- The seed for planting is "hard winter wheat" and can be found at Whole Foods Market (item number 5907, .49/lb) .
- The oat bran should be fine (coarsened) and organic (Whole Foods Market, item number 5871, .99/lb).
- Both the sphagnum peat moss and the topsoil should be organic (compost mix for top soil and no fertilizers in both topsoil and sphagnum peat moss).

15 Work hours

- Mo, We, Fri: 4 hours
- Tue, Thur: 2 hour.
- Total: 16 hours/week.