12-14-23
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TMH

Cabbage White Butterfly Rearing Protocol

This protocol covers everything you need to know for rearing Cabbage White Butterflies.

Supplies needed for rearing:

Freshly rinsed Brassica Leaves
Young Brassica Plants for oviposition
Nectar wicks
Fresh nectar (made in the last 3 days)
Yellow laminated flowers
Butterfly cages
10 ml beaker
Large Petri dishes
Sponge cubes
Lab tapes
Sharpie
Butterfly net

1. Field Collection & Transport

Catch *Pieris rapae* in the field and transport them on ice carefully back to the lab.

Procedure

- Catch butterflies in the field using a net.
- Place each butterfly in a glassine envelope.
- Store envelopes in a cooler with an ice pack.
- Transport cooler back to the lab immediately.

⚠ **Aim to return to the lab before 2:00 PM. Setting up butterflies in the greenhouse in full sun encourages egg-laying and has led to greater success.**

2. Oviposition Cage Setup

Once butterflies are back at the lab, set up cages promptly.

Step-by-Step Setup

1. Prepare 2-3 collard plants per cage:
    - Check the undersides of all leaves for pests; remove any aphids.
    - Ensure soil is moist – water individual plants before placing in cages if needed.
2. Set up nectar in cage:
    - Place a 100 ml beaker with a 10 ml flat-bottomed plastic vial filled with fresh nextar inside each cage.
    - Thread which through rubber top and yellow laminated flower.
    - Dip both ends of the wick and laminated flower in nectar to thoroughly saturate the wick and place wick in vial.
3. Release butterflies into cages:
    - Open the glassine envelope and use a downward-sweeping motion to transfer the butterfly into the cage
    - Alternatively, use flat forceps to place the butterfly directly on the nectar wick – be careful to not crush the abdomen if you choose to do this!
    - Keep a net nearby in case a butterfly escapes.

⚠ **If a butterfly escapes and is not caught, notify Taylor immediately. Unknown mother lines must be tracked — uncaptured individuals could confound experiment results.**

3. Butterfly Cull & Freezer Storage

Butterflies are kept in cages for 48 hours to standardize caterpillar development timing

At the 48-Hour Mark:

1. Remove the butterfly from the cage,
2. Label a glassine envelope with:
    - Mom number
    - Date caught
    - Date placed in envelope
    - Collection location (e.g., Picardo, Magnuson, Carnation)
    - Whether it laid eggs or not
    - Dead or alive when frozen
3. Place labeled envelope in the -80°C upstairs on the 4th floor in the corresponding white freezer box.

Note: *If a butterfly dies before the 48-hour mark, note the date of death and move immediately to a labeled glassine envelope for storage in the -80°C freezer.*

4. Daily Monitoring & Egg Counts

Daily Checks

- Monitor butterflies and plants daily.
- Check if plants need watering.

Egg Counting (within 2–3 days of cage setup)

1. Carefully remove plants from the cage.
2. Count each individual egg on the undersides of leaves.
3. Record in your lab notebook: mom number, egg count, collection location, date of collection.

Note: *Assign mom numbers only to individuals that laid eggs. IDs must be unique — start at ♀1 at the beginning of the season and count up. Consult greenhouse cages or fellow lab members to confirm the next available number.*

6. Caterpillar Rearing

Egg Hatching & Transfer

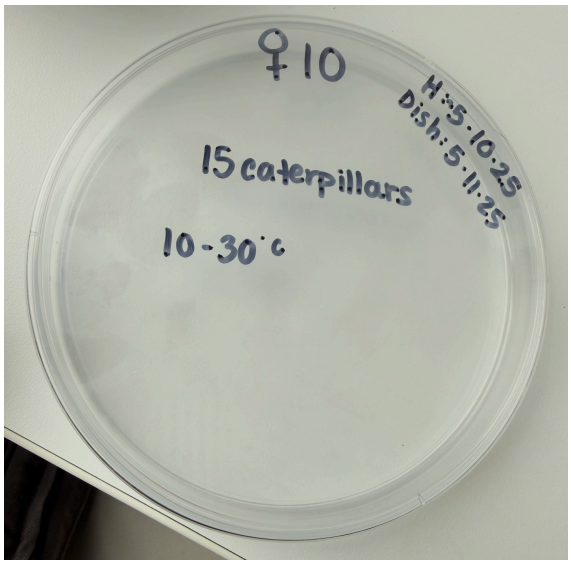
- Observe plants every day and record the date eggs hatch.
- Once the majority of eggs on a leaf have hatched, transfer caterpillars based on experiment type:
  - Leaf experiment: cut the leaf at the stem (taking care not to crush wandering caterpillars) and place on a freshly rinsed and dried, similar-sized leaf in a petri dish with a wet sponge at the stem end.

Petri Dish Labeling

Label each petri dish with:

- *Mom number*
- *Number of caterpillars*
- *Approximate hatch date*
- *Date moved to the dish*
- *Rearing treatment (e.g., 10-30°C or 11-35°C)*

Note: *Aim for 10–15 caterpillars per dish to avoid overcrowding.*



Split Sibling Design

All caterpillars must be split into a split-sibling design between the two rearing treatments (10–30°C and 11–35°C):

- Divide caterpillars as evenly as possible between treatments.
- Example: 30 caterpillars from ♀2 → 15 in 11-35°C and 15 in 10-30°C.
- Example: 100 caterpillars from a mom → 50 in each treatment.
- Try to transfer caterpillars the day they hatch so they experience the full rearing treatment from the start.

Chamber Placement

- Bring petri dishes to the B2 chamber.
- Place dishes on a white tray in the appropriate rearing chamber: B226-3 or B226-1.

7. Daily Feeding & Dish Maintenance

Check caterpillars daily

Daily Check Procedure - more in depth protocol here

1. Open the dish and carefully move leaves from the bottom half to the top half.
2. Move any caterpillars not on leaves using soft-touch forceps.
3. Wipe out frass from the now-empty half with a clean paper towel and discard.
4. Replace leaves and move caterpillars back to the cleaned side.
5. Ensure the sponge remains damp.

⚠ **If any dish shows signs of poor health (dead caterpillars, discoloration, visible sores), disinfect the soft-touch forceps with 70% isopropyl alcohol and dry with a clean paper towel between each dish.**

8. Caterpillar Development Timeline

Pieris rapae has 5 larval instars before pupation. Experiments focus on the 4th and 5th instars. Monitor daily and track head capsule size to identify instar stage.

Identifying instar stage: If the head is large relative to the body, the caterpillar is early in its instar. A small, clear or green pinhead indicates an upcoming molt.

Instar	Duration	Body Length	Key Features
1st	~2.5 days	~3 mm	Newly hatched
2nd	1.5–2 days	~5.5 mm	Yellowish green body
3rd	~2 days	~9 mm	Similar to 2nd; faint yellowish dorsal band appears
4th ★	~2 days	~15.5 mm	Abundant fine white setae; begin temp treatment within first 24 hrs of molting
5th ★	2.5–3 days	Up to 25 mm	Similar to 4th; yellow band more prominent; pupation follows

★ = *Instars of interest for this project*

Weekly Overview

- Week 1: Eggs hatch within 1–3 days of being laid.
- Week 2: Larvae molt 2–3 times (instars 2–4).
- Week 3: Growth into final stage; larvae pupate around days 18–20 from laying.

Experiment Planning by Instar

Use collection date to estimate when caterpillars will be ready for experiments:

- 4th instar caterpillars: ready approximately 11–13 days after catch date.
- 5th instar caterpillars: ready approximately 13–15 days after catch date.

9. Cleaning & Disinfection

After Each Experiment

All items used in experiments must be decontaminated after use. Bring items to the weighing room on the 4th floor.

- Soak in a 10% bleach bath.
- Rinse thoroughly in a clean water bath.

Items requiring disinfection:

- Petri dishes
- Oviposition wicks
- Yellow laminated flowers
- Petri dish sponges

- Environmental chambers should also be disinfected between experiments using a 10% bleach solution.

⚠ **Use only 10% bleach or the alkaline detergent located next to the sink. Do NOT use dish soap.**

10. Related Protocols & Notes

Active Protocols

- Caterpillar Feeding Protocol
- Greenhouse Planting Protocol
- Butterfly Cage Cleanup Protocol

Protocols No Longer Applicable (post-2025)

- CUH Field Check Protocol
- Garden Experiment Setup Protocol
- Field Selection Study

Pending Items

- Pot Washing Protocol — to be added
- Catching Protocol — to be added once finished

Other protocols pertaining to butterfly experiments can be found at the below links:
Caterpillar Feeding Protocol

Greenhouse Planting Protocol

****** Need to add pot washing protocol ******

CUH Field Check Protocol - not applicable after 2025

Garden Expt Setup Protocol - not applicable after 2025

Field selection study- https://docs.google.com/document/d/1JaJPiU_4wUAJaHMjzXQ4pswxRbC2R453CXtmgkHKjyI/edit - not applicable after 2025

Butterfly Cage Cleanup Protocol

**include catching protocol once it is finished

This protocol was written by Taylor Hatcher, edited by Leo Rupp, edited again by Taylor and then Taylor used Claude to clean up the format and organize the steps better.