HOW DO CATERPILLARS CHOOSE THE RIGHT LEAVES TO EAT IN A BIG, GREEN WORLD?
Questions and experiments for curious minds.

Submitted by: Rupesh R. Kariyat, Pennsylvania State University, Department of Biology
and Jason D. Smith, Pennsylvania State University, Department of Entomology

Target group: Students in grades 4-10

Project question:

Your parents taught you not to touch poison ivy leaves, nor to eat smooth red berries (etc.) because they are poisonous. But what about small caterpillars, which do not get instructions from their parent moths or butterflies? How can they select the food that is best for them?

Observations and reflections:

In our gardens and back yards we can observe that an insect eating one kind of plant will leave other kinds of plants alone. We can also see that if we remove a caterpillar from a plant that it is eating and place it on a different plant species, the caterpillar may not eat the new plant. Why is this? Do caterpillars evaluate their food options before chowing down? Maybe eating right is important for all organisms, including small mammals and insects, just as it is for humans. We know that humans use vision, smell and touch to select good food, but what do caterpillars use?

Hypothesis-formation:
A plant that a caterpillar likes to eat is called a *host* plant for that insect. If we give a caterpillar a choice between a host plant leaf and a non-host plant leaf, which will caterpillar choose?

A hypothesis is a prediction of what you think will happen which you can test with an experiment. For example, "Caterpillars will feed on both plants." Now you make a hypothesis and write it down. What do you think will happen?

My hypothesis (write in the box):

Now let's test this hypothesis with the following experiment. There are two versions of the experiment below—one for backyard explorers and one for science classrooms (which requires lab supplies). Choose the one that is best for your situation!

EXPERIMENT VERSION I: FOR BACKYARD EXPLORERS

Materials (backyard version):

Before you begin, collect the following supplies:

- 1 to 3 Caterpillar(s). It's best to have 2 or 3 of the same kind of caterpillar.
- One fresh leaf from the plant the caterpillar was feeding on. This is the "host plant."
- One fresh leaf from a non-host plant (a different plant species from the host plant). Ideally you want the non-host plant leaves to be from a different plant family to make sure they are unrelated. If you don't have any experts or science teachers to help you, try this rule: if the host plant is a tree leaf, take a weed leaf as the non-host plant (or vice versa)
- One shallow bowl or round lid with tall edges. Should be flat and about 10 cm (4 inches) in diameter at the bottom, and tall enough on the sides that caterpillars can freely walk around on the bottom when the top is covered.
- Plastic wrap.
- Hole punch.
- Scissors
- Water
- Paper towel
- Paint brush (for moving caterpillars if they are *really* small)

Once you have all these supplies, you may follow the procedure!

Procedure (backyard version):

1. Keep caterpillars for 4-6 hours without food.
2. Cut out a paper towel circle to line the inner bottom surface of the bowl or dish.
3. After placing the papers in the dish, gently moist the plate with water (but do NOT soak it).
4. Using the hole punch, make leaf discs from leaves of the host plant and non-host plant, and place the discs in the dish on opposite sides, touching the edge of the plate (fig 1).
5. Using a paint brush, gently place one caterpillar along one of the edges of the plate away from the leaf discs.
6. Cover the dish with plastic wrap and leave the plate undisturbed for a while (at least an hour) at room temperature.
7. Check back every 10 minutes and each time write down what half of the dish the caterpillar is on and what the caterpillar is doing (examples: eating, walking, or going toward host leaf).
8. After an hour, record final caterpillar position relative to the two disks (ie, on host plant side or on non-host plant side) and make notes (table 1). Older students should also rank the amount of feeding on each leaf using a on a scale of 1 to 5.
9. If you have more than one caterpillar, repeat the experiment, but use a new piece of paper towel on the bottom of the dish and use new leaf discs. Also it's a good idea to switch the positions of the host and non-host leaf discs. OR if you have multiple dishes you can run multiple experiments at one time.
10. Try making your data into a table and a graph (see figure 2 as example)

Discussion:

Since caterpillars have very limited vision, they rely on other senses to explore the world around them. They use smell, for example, to guide them to good food, and they use taste and touch to tell evaluate the nutritional quality as well as the defenses (such as toxins and trichomes or hairs) of the plants they eat. This experiment demonstrates that caterpillars are able to locate and feed

upon good food sources (like the host plant), although you may have seen that they sometimes make mistakes. But don't jump to conclusions—some "mistakes" really just mean that your caterpillar is able to eat both kinds of leaves that you provided. Since growth, development and reproduction of all organisms is tightly linked to the quality and quantity of food ingested during early life stages, being able to locate good food is critical for these caterpillars' survival.

EXPERIMENT VERSION II: FOR SCIENCE CLASSROOMS

Materials (classroom version):

- Tobacco Hornworm (*Manduca sexta)* caterpillars (one per replicate of the experiment; see information at bottom for ordering). Ideally do 5 or more replicates. If several students are participating in the experiment, allow each student to be in charge of one or two replicates. If caterpillars are purchased as eggs, larva can be fed on artificial diet (see references) or leaves from any Solanaceae plant.
- Fresh leaves from any Solanaceae plant (common examples include Tomato, Potato, Peppers, Horsenettle, Egg plant).
- Fresh leaves from a non-Solanaceae plant (any plant from a different family). If you are not sure what to use, try leaves from maple or oak trees.
- Petri plates and lids (one per replicate of the experiment)
- Hole punch (ideally one that punches holes about 2cm in diameter)
- Paper towel
- Scissors
- Water (use distilled, if available)
- Paint brush (for handling really small caterpillars)

NOTE: *Refer to the back-yard version for easy-to-find substitutions to many of these supplies.*

Procedure (classroom version):

1. Starve the caterpillars by removing their food for a period of 4-6 hours. Preferably use second instar caterpillars—which means they have molted once after hatching. For instar identification, refer to Manduca lifecycle information at bottom.
2. Cut out paper towel circles to line the inner bottom surface of the petri dishes, one circle per plate (Filter paper circles also work well for this).
3. After placing the filter papers in the plate, gently moist the plate with water (but do NOT soak it).
4. Using the hole punch, make a leaf discs from leaves of the host plant and non-host plant. Place one of each kind of disc in the petri dishes on opposite sides, touching the edge of the plate (see figure 1).
5. Using a paint brush, gently place one caterpillar near the edge of the petri plate equidistant from the leaf discs.
6. Repeat this setup with 5 or more separate plates, but alternate the position of host and non-host plant (for example, if host plant is on right side in plate one, put it on the left side in plate 2, etc) and also alternate the position of caterpillar relative to yourself (near versus far from you; see figure 1).
7. Close the lid and leave the plate undisturbed at room temperature.

8. After 5-10 minutes check for movement of the caterpillars. Replace any caterpillars that do not show signs of movement with new caterpillars, and record the new starting time of the experiment.
9. Check back every 10 minutes and record what half of the dish the caterpillar is on and what the caterpillar is doing (examples: eating, walking, or going toward host leaf).
10. After an hour, record final caterpillar position relative to the two disks (ie, on host plant side or on non-host plant side) and make notes (table 1). Older students should also rank the amount of feeding on each leaf using a on a scale of 1 to 5.
11. Compile the data into a table and a graph (see figure 2)

Expected Result:

Most Tobacco Hornworm caterpillars should successfully locate and feed on their host plant.

Discussion:

Since caterpillars have very limited vision, they rely on other senses to explore the world around them. They use smell, for example, to guide them to good food, and they use taste and touch to tell evaluate the nutritional quality as well as the defenses (such as toxins and trichomes or hairs) of the plants they eat. This experiment demonstrates that caterpillars are able to locate and feed upon good food sources (like the host plant), although you may have seen that they sometimes make mistakes. But don't jump to conclusions—some "mistakes" really just mean that your caterpillar is able to eat both kinds of leaves that you provided. Since growth, development and reproduction of all organisms is tightly linked to the quality and quantity of food ingested during early life stages, being able to locate good food is critical for these caterpillars' survival.

Additional Resources (classroom version):

Where to purchase petri plates:
http://www.coleparmer.com/catalog/product_view.asp?sku=1400520&pfx
Petri dishes: 50 plates for $22.00 (2010 prices), can usually be ordered in smaller quantities. The petri plates are reusable after washing with water and soap.

Where to purchase Tobacco Hornworm *(Manduca sexta)* eggs and artificial diet:
http://www.carolina.com/
Manduca Eggs: $45.00 plus shipping for batch of 100 eggs (2010 prices). *Manduca* typically have a very high hatching rate (close to 100%), so 100 eggs can give enough caterpillars for a lot of experiment runs.

Manduca sexta (Tobacco hornworm) life cycle:
http://manduca.entomology.wisc.edu/about/lifecycle.html

Manduca sexta care sheets:
http://www.carolina.com/category/teacher+resources/care-guides/hornworms.do

Further Suggestions for Teachers (classroom version):

1. Use newly-molted caterpillars that have not yet eaten. They will have a good appetite.
2. Moisture levels and temperature levels in the petri dish can affect the experiment. Try to maintain equal conditions in every plate. If you rear out the caterpillars after the experiment, make sure they have a high-humidity environment so they don't dry out.

3. The experiment can be made more objective by keeping the identity of the host plant and non-host plant unknown to the students until the end.
4. Depending on the grade levels and scientific curiosity of your students, you can use this experiment to demonstrate how bigger and more complex experiments in sciences are being carried out.
5. Discussions with older students should also highlight the steps of the scientific process (ask a question, form a hypothesis, design an experiment, analyze the results, decide if the hypothesis is true or false).
6. Point out that replicating the experiment several times is important because the caterpillars vary in their behavior. If there was only one replicate, and that caterpillar happened to eat the non-host leaf, this would lead you to the wrong conclusion! For this reason it is always important to conduct multiple trials of the experiment.

Figure 1: Petri plate set up for the choice test

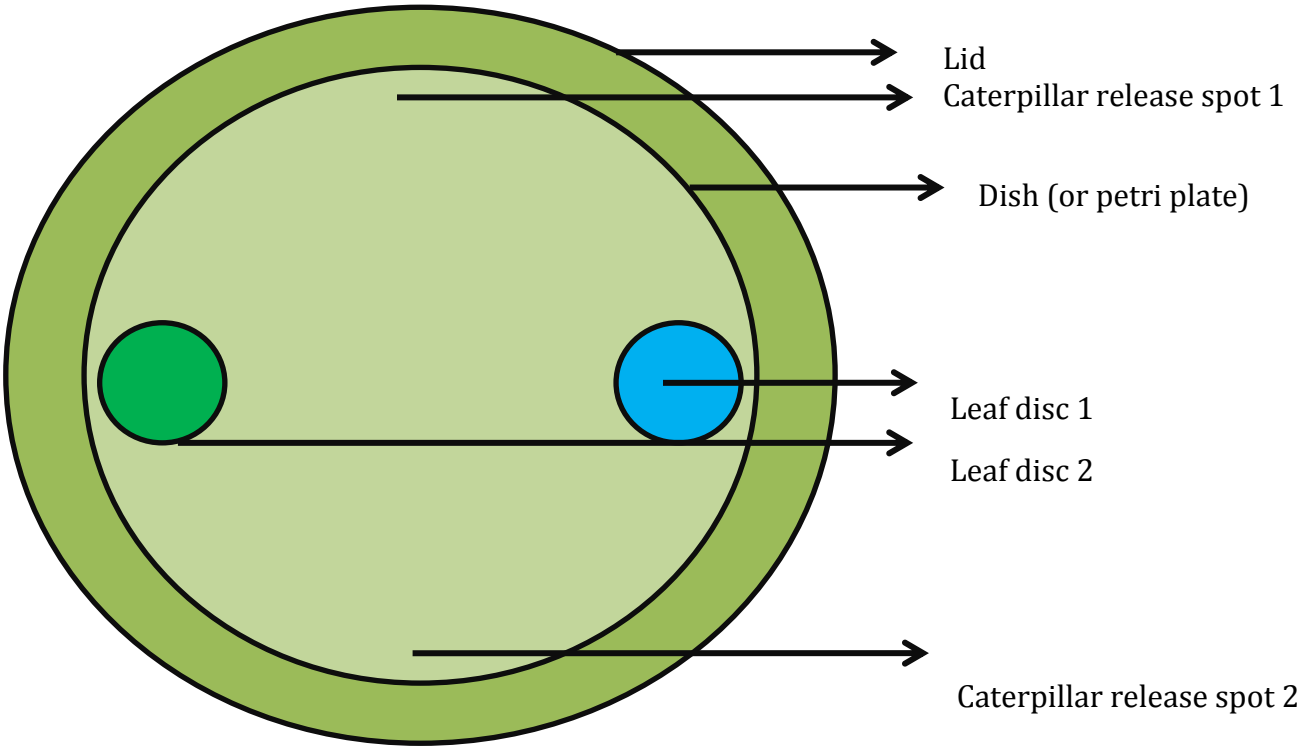
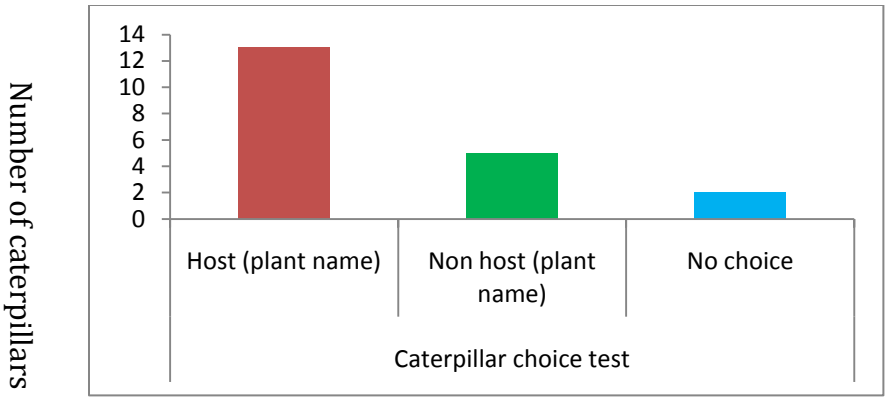


Table 1: Data sheet template for recording observations:

Plate number	Student name	Start time	End time	Feeding on host plant	Feeding on non host plant	No choice
1						
2						

Figure 2: Example for graphical representation of the results for 20 replicates. This graph shows that 13 caterpillars chose the host plant, 5 chose the non-host plant and 2 made no choice at all.



Contact: Please feel free to contact us for further information regarding this experiment or related subjects. We are more than willing to help!

Rupesh R. Kariyat (ruk157@psu.edu)
The Pennsylvania State University
Department of Biology

and

Jason D. Smith (jds517@psu.edu)
The Pennsylvania State University
Department of Entomology