Why do Plants Make Spices? Exploring Ecological Roles for Common Kitchen Ingredients

Submitted by: Jason D. Smith and Rupesh R. Kariyat, The Pennsylvania State University

Grade levels targeted: Grades 4-10 (although the language of these instructions is suited to students in grades 7-10.

National Science Education Standards:

Organisms and the environment, structure and function in living systems, diversity and adaptations of organisms, behavior of organisms and methods of scientific inquiry.

Entomology Literacy Elements:

The project demonstrates the utility of insects as model herbivores for ecological research as well as showing the potential utility of plant-derived compounds for plant protection.

Observations:

Our kitchen cupboards are filled with various spices that make our eating experiences fun and delicious. Where do these flavors come from? Mostly they come from plants! We use leaves, flowers, seeds, stems, roots and even bark from various plants to add flavor to our food. Each flavor represents a specific chemical (or a mixture of chemicals) that has been created by a plant.

Question:

Why do plants make the chemicals that we enjoy as spices? Do spices benefit the plants that make them?

Hints to Form Hypothesis:

Plants in nature are constantly under attack. They have to defend themselves against a host of pathogens (germs)—just like us! What's more, they have to defend themselves against plant-eating animals, or herbivores, most of which are insects. How do plants defend themselves? Some plants are physically tough to eat because of structures like thorns, bark, and trichomes (hairs). Other plants don't look tough, but are laced with chemical toxins that repel or poison insects, or else simply prevent them from digesting food. Is it possible that the primary purpose of spices is to defend plants from herbivores?

Some organic gardeners use mixes of garlic and cayenne pepper and soap to protect their plants from insects. Could these spices actually deter insects from feeding on their plants?

Hypothesis:

Plants make spices because they provide protection against being eaten by herbivores.

Materials:

- Caterpillar(s)
- Fresh leaves from plants the caterpillar likes to eat
- Clean container with lid (e.g., Tupperware, peanut butter jar)
- Paper towels
- Bowls or Tupperware with flat bottom approximately 4 inches across
- Plastic wrap
- Masking tape and a marker
- Scissors
- Fresh garlic clove or cayenne pepper
- Tap water
- Two paint brushes
- Clock, watch or timer

The Experiment:

To test this hypothesis, we need a spice and an herbivore. Garlic and cayenne pepper are two spices worth trying since gardeners use them to protect their plants, but you are welcome to try other spices as well. What herbivore should we use? Insects make perfect test organisms since they are small, they don't move too fast and they don't eat very much. In this experiment we recommend you use a caterpillar, but you could try using leaf-eating beetles or bugs instead. Round up these and the other materials listed here to begin.

The Experiment:

- ❖ Collect a caterpillar from your backyard or garden (or two caterpillars if you find more than one). Also collect a leaf (or two very small leaves) from a plant your caterpillar likes to eat. Place the caterpillar in a lidded container with a damp paper towel. Store the leaf in a plastic bag with a damp paper towel to prevent it from drying out. NOTE: Some furry caterpillars have barbed and/or poisonous hairs that will irritate your skin. Unless you know the caterpillar is safe to touch, do not directly touch the caterpillar with bare skin.

    - ➢ Hint 1: Look for insects that are actively feeding—this way you know two things right away: 1) they are hungry and 2) the leaves they are eating should work well for this experiment. If you find a caterpillar that is not eating, try identifying it with one of the online tools listed below under the "Resources" section. Knowing the species is key to looking up what it likes to eat, which you can also do with the resources listed below.

    - ➢ Hint 2: If you are not able to begin the experiment immediately after collecting your insects and leaves, then store the leaves in their bag in the refrigerator, and give your caterpillar an extra leaf. Also, punch holes in the lid of your container, or substitute a piece of woven cloth and a rubber band for the lid to ensure the insect has plenty of air.

    - ➢ Hint 3: Teachers interested in doing this experiment in their classroom should refer to Appendix B for more information.

- ❖ "Starve" your caterpillar by leaving it in the container without food for four to six hours (or overnight). This will heighten the caterpillar's drive to find food later in the experiment.

- ➢ Hint: Caterpillars periodically shed their exoskeleton (outer skin) to enable further growth. Caterpillars that refuse to feed in the course of this experiment may be preparing to molt. You can try again with newly-molted caterpillars a day later.

- ❖ Prepare spicy and control (non-spicy) leaves using the following instructions:

  - ➢ Garlic Experiment: Crush or finely chop one fresh garlic clove and mix thoroughly with two teaspoons of tap water. Allow mixture to set for 20 minutes. Near the end of the 20 minutes, cut the leaf into identical halves. Using a paintbrush, paint one half the leaf with garlic water until the entire top surface is wet. Pick off any garlic chunks that were transferred onto the leaf. Using a separate paintbrush, paint pure tap water on the other leaf half. Set leaves on a damp paper towel and allow them to air dry (one to two hours).

  - ➢ Cayenne Pepper Experiment: Thoroughly mix ¼ teaspoon cayenne pepper powder with two teaspoons of water. Allow mixture to set for 20 minutes. Near the end of the 20 minutes, cut the leaf into identical halves. Using a paintbrush, paint one half the leaf with pepper water until the entire top surface is wet. Using a separate paintbrush, paint pure tap water on the other leaf half. Set leaves on a damp paper towel and allow them to air dry (one to two hours).

    - ▪ Hint 1: To ensure the halves are identical, cut along both sides of the center vein of the leaf and discard the center vein.

    - ▪ Hint 2: If you are working with leaves smaller than a quarter, it might be better to use two similar leaves.

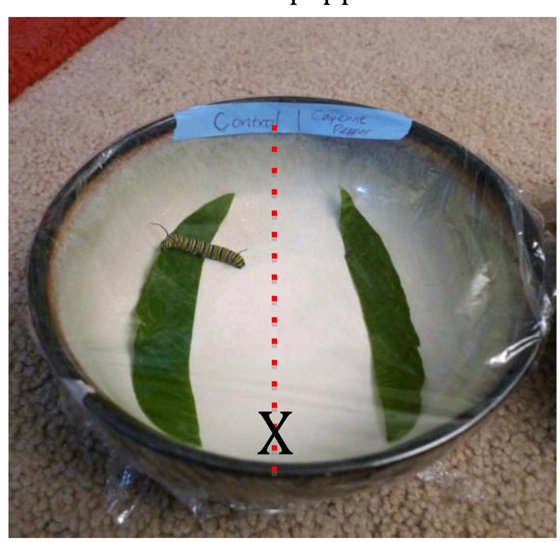
- ❖ Once the caterpillar is "starved" place the spicy and control leaves opposite each other along the outer edge of the bowl (or tupperware) allowing 2.5 to 3 inches between the leaves if possible. Use the marker and tape to clearly indicate which side of the bowl contains which leaf. See photo 1 on page 4.

- ❖ Place caterpillar along the center axis of the bowl so that it has to turn right or left in order to find one of the leaves. Cover the bowl with plastic wrap to prevent caterpillars from escaping.

- ❖ When the caterpillar begins walking (sometimes right away), begin timing the experiment and use the tables provided in Appendix A to record data on insect choice and plant consumption.

  - ➢ Hint 1: If caterpillars do not begin eating after 15 minutes, use another caterpillar if you have an extra one.

Photo 1: Monarch caterpillar choosing between control and pepper-coated leaf.



- Hint 2: If time allows, extend your data tables out to 24 hours. Why not see what happens?

Results:

Which leaf did the caterpillar contact first? Which leaf did the caterpillar eat more of?

Based on these results, would you accept or reject the hypothesis that "Plants make spices because they provide protection against being eaten by herbivores"?

If this experiment is done repeatedly (reasons to do this are explained in the discussion), results should be plotted as shown in Appendix A.

Discussion:

If your caterpillar preferred the control leaf over the spicy leaf, this suggests that the spice indeed protects its plant against herbivores. How does it do this? Possibly by creating a repulsive smell. If your caterpillar contacted the control leaf first, it could have been repelled by to the odor of the spice. But the spice could also protect the plant by making the leaf taste bad. This may be true of your spice if you observed the caterpillar feed only briefly on the spicy leaf before wandering off.

If your caterpillar preferred the spicy leaf or if it ate equally from both leaves, it is possible that your spice does not affect insects, or that your insect is immune to the spice, or that it is unable to sense the spice. Remember that plants are not only defending themselves against insects, but diseases—and your spice could primarily exist to fight off bacteria or fungi.

Another important consideration is that your results are simply unusual. Maybe most caterpillars dislike garlic but you happened to find the odd caterpillar that actually likes it. This could happen, just like you may have a friend who likes to eat anchovy peanut butter sandwiches even though most humans would never do that. To be certain of your findings, therefore, you would need to repeat this experiment with many sets of caterpillars and leaves. Repeating an experiment in this way is called replication, and it is a critical aspect of scientific research.

Hopefully you observed the ability of garlic or pepper to repel insects. If so, you'll realize why plant compounds are frequently used in organic farming and gardening to protect crop plants from insect herbivores. Although synthetic pesticides have been helpful to growers, we must remember that nature has an entire arsenal of anti-herbivore compounds stored up in plants!

Resources:

- Basic key to insect orders: http://www.sci.sdsu.edu/classes/bio462/easykey.html
- For caterpillar identification: http://www.discoverlife.org/20/q?guide=Caterpillars
- For identification help plus information on lifecycle and diet: http://bugguide.net

- Hint: You can submit photos of your insects to bugguide.net to request help from volunteer naturalists to identify them.

Estimated time: 6 hours, with breaks (teachers could easily adapt experiment to be completed in one lab period or over course of two days).

Estimated cost: $0.00 or $1.00 if your spice cabinet is empty.

Further Experiments:

- Try other spices! Cinnamon, ginger, red pepper, black pepper and more are likely to have effects upon insect behavior.
- Try other insects! Leaf beetles and leaf bugs, for instance, have to evaluate their food based on smells and tastes—so

Contact Information:

- The authors of this experiment invite you to send us your questions and feedback via email. We are glad to help you explore nature!
- Jason D. Smith (jds517@psu.edu), The Pennsylvania State University, Department of Entomology and Rupesh R. Kariyat (ruk157@psu.edu), The Pennsylvania State University, Department of Biology

Appendix A: Data Tables and Chart

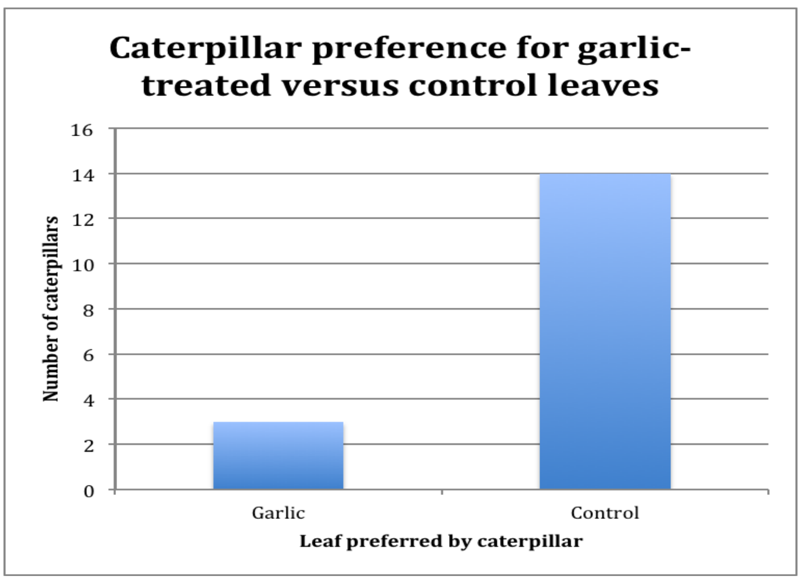
Table 1: Insect Choice Data
Record position of caterpillar at each time point

Initial Choice: (first leaf contacted)	spicy leaf	control leaf	---
5 minutes	spicy leaf	control leaf	no leaf contact
10 minutes	spicy leaf	control leaf	no leaf contact
20 minutes	spicy leaf	control leaf	no leaf contact
30 minutes	spicy leaf	control leaf	no leaf contact
45 minutes	spicy leaf	control leaf	no leaf contact
60 minutes	spicy leaf	control leaf	no leaf contact
Final Choice: (final leaf contacted)	spicy leaf	control leaf	---

Table 2: Plant Consumption Data *Record which leaf has been damaged more at each time point.*				
5 minutes	no damage	equal damage	spicy leaf	control leaf
10 minutes	no damage	equal damage	spicy leaf	control leaf
20 minutes	no damage	equal damage	spicy leaf	control leaf
30 minutes	no damage	equal damage	spicy leaf	control leaf
45 minutes	no damage	equal damage	spicy leaf	control leaf
60 minutes	no damage	equal damage	spicy leaf	control leaf

Appendix A, cont.

Chart 1:



This graph demonstrates how data can be plotted if this experiment is replicated. In this example, the experiment was replicated 17 times, and in 14 of these replicates the caterpillar ate more of the control leaf than of the garlic leaf.

Appendix B: Information for Teachers

To scale this experiment up for a classroom of students, you can do this experiment with Tobacco Hornworm caterpillars (*Manduca sexta)*, tomato plant leaves and petri plates.

- Use newly-molted caterpillars that have not yet eaten. They will have a good appetite.
- Moisture levels and temperature levels in the petri dish can affect the experiment. Try to maintain equal conditions in every plate by lining the plates with damp paper towel. If you

rear out the caterpillars after the experiment, make sure they have a high-humidity environment so they don't dry out.

- Discussions with older students should 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).
- Point out that replicating the experiment several times is important because the caterpillars vary in their behavior.
- Pool data from all students to create a graph.

Resources

- 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
  - 

Gives "control freak" a new meaning, doesn't it?