Title: Is predator-free the way to be?

Submitted by: Ian Grettenberger

Grade levels: 4-6

Time: Two weeks: two 45 min periods at the beginning of the experiment and then 15-20 minutes each day data is collected. The predator addition day will take longer. One hour period at the end of the experiment for data graphing and presentation and discussion.

Cost: $50-60 (primarily to order insects)

National Science Standards:

- Life cycles of organisms
- Organisms and the environment
- Populations and ecosystems
- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Entomology Literacy Element(s) and Supporting Concept(s):

- Element I: Explain how insects provide environmental services to humans.
  - Supporting Concepts: Ecosystem Functioning
- Element II: Develop ability to use insects in inquiries and provide examples of insects' investigative value.
  - Supporting Concepts: Scientific Method
- Element III. Understand and provide examples of insects' economic value. G
  - Supporting Concepts: Agriculture and Food Supply

Observations: Insect herbivores can at times reach very high population levels. However, much of the time, herbivore populations are kept in check by their natural enemies. This is very important for management of insect pests by farmers. Many insect pests never reach populations high enough to damage a crop field to a point that will cost a farmer money. This is in part due to natural enemies that eat the pests (Fig. 1). Aphids are common pests and there are many types of aphids that attack specific crops. For instance, the English grain aphid feeds on small grains like wheat and oats, the soybean aphid feeds on soybeans and the pea aphid feeds on peas, broad beans, clover and alfalfa (Fig. 2).

All types of aphids, including the pea aphid, produce many generations during a year. During the warm periods of the year, female aphids produce live offspring that are clones of themselves. They can produce many young and these young mature to a reproductive age very quickly and can soon produce offspring of their own.

Background: The pea aphid is an important pest of legume crops such as clovers, peas and alfalfa. They damage the plants by piercing the plant's phloem and ingesting the sugar-rich sap through its piercing-sucking mouthparts, or stylets. Because the sap is very high in sugars, aphids excrete excess sugars and liquids out the other end in the form of honeydew. This sticky substance is easy to observe when populations are high. High levels of honeydew on leaves promotes the growth of fungus, damaging the plant by blocking sunlight and preventing photosynthesis.

Pea aphids respond to predators by dropping from the plants. This can be observed by gently poking or breathing on the aphids. This prevents predators from catching them, but can be a costly defensive tactic because they can fall off the plant to the ground, where they no longer have food and are exposed to roving predators. Aphids also exude quick-hardening defensive secretions from their cornicles. These secretions can contain an alarm pheromone that warns other aphids.

Aphids can come in both a winged and a wingless form (Fig. 3). Young aphids begin to grow into winged aphids (alates) when the plants become stressed or the aphids become very crowded. The winged aphids are able to disperse to new hosts. Winged aphids are also produced late in the growing season.

Some species of aphids engage in a close relationship with ants. Ants tend the aphids, almost like livestock moving them to new plants if necessary and protecting them from predators. In exchange, the ants will feed upon the sugary honeydew that the aphids produce (Fig. 4).

Questions

What happens to aphid populations when they are protected from predators and provided with plenty of food?

What shape would a graph of their population growth look like over the course of two weeks?

What effect do natural enemies have on aphid populations?

<u>Hint to form hypothesis</u>: Aphids produce many offspring that very quickly begin producing offspring of their own.

If aphids are protected from predators and given plenty of food, not many of them will die before producing many offspring. Predators feed on aphids. If predators kill aphids or disturb them while feeding and prevent them from getting nutrients and reproducing, they will produce fewer offspring over their lifetime.

<u>Hypotheses</u>: Aphid populations will grow very quickly when protected from predators. The graph will look like a line that curves upward in a "J" shape. Predators will cause the population to grow less quickly or to decrease.

Materials needed

- Pea aphids (available from Berkshire Biological, http://www.berkshirebio.com/Insects.html)
- Fava bean seeds (garden store or online)

- Materials for pots and cage (per group)
  - 4 2L clear soda bottles
  - Organza or tulle (mesh) fabric
  - Hot glue gun
  - Scissors/box cutter
  - Plates or large yogurt or deli container lids
  - Drill (optional)
- Potting soil
- Very fine paint brushes (groups can share)
- Dissecting microscopes and/or hand lenses (optional)
- Fluorescent light (if well-lit windowsills are not available)
- Lady beetles – wild-caught or ordered from an online supplier, e.g.:

http://www.arbico-organics.com/product/live-ladybugs/80

http://www.gardensalive.com/product.asp?pn=5065&sid=0159337&utm_medium=cpc&gclid=CKm8872Ms7kCFcOh4Aod7zQAqA

http://www.gurneys.com/product/stahome__lady_beetles_&p=0533398&utm_medium=cpc&utm_source=google?gclid=CK-opImNs7kCFdGd4AodGDEA0g

http://www.berkshirebio.com/Insects.html

- Data sheets
- Computers + projector (optional)

Methods

Constructing the habitats

Remove the labels from the bottles. Each habitat will use two bottles. Cut one bottle approximately 12 cm from the bottom (Fig. 5). This will serve as the pot. Drill or cut drainage holes in the bottom. Cut the bottom and top off the other bottle, cutting as far down as you can while keeping the shape a cylinder with an equal diameter throughout. Cut several 5-6 cm vertical slits (or as long as needed) at the bottom of the bottle. This will allow the top to slide into the bottom. Glue a piece of organza to the top of the bottle. The mesh will improve airflow, but still restrict the insects to the habitat. The plates or plastic lids will catch any water that drain out of the bottom. Add pre-moistened (damp, but not soggy) soil to the habitat to within about 2 cm of the rim.

Growing fava bean plants

A fluorescent light will be necessary if the plants cannot be grown on a well-lit windowsill that does not get very hot or very cold. Hang the light about 2-3 feet above the plants. To test if you may need a light, try growing fava bean plants where you are hoping to put the experimental plants. If they grow well and are a vibrant green after several weeks, they should be fine. It is also good to test how long the plants will take to reach 8-10 cm at which point they will be ready to use for experiments. This should take 2-3 weeks depending on environmental conditions. A practice run growing plants will also show you how big they will get during the experiment. It is best to avoid having the plants touch the cage (it is easier to not disturb the aphids).

Plant seeds in holes 2.5 cm deep. You can mark pencils or fingers with a line to obtain the correct depth. Water plants when the top of the soil has begun to dry out, but be careful not to overwater. Seeds will take about 6-7 days to sprout (Fig. 6).

Starting an aphid colony

You will likely need to order pea aphids from an online supplier. Collecting aphids is difficult because many feed on a small number of hosts (but see notes below about wild pea aphids and "Extensions or adaptations for this experiment" for information on rearing other species of aphids below). Make sure you have plants ready for when the aphids arrive. Plants should be this size for the experiment (see below), but larger plants can be used for the colony since they do not need to fit as well within the cage. Colony plants should be caged if near aphid-free plants because aphids will infest those plants. If possible, separate the colony from the experimental set-up. Plant the first colony plants 2-3 weeks before the aphids will arrive to guarantee they will have food. Plant a few more plants one week before they arrive and then continue to plant seeds every week or two to maintain a constant supply of fresh plants.

Once the aphids arrive, gently shake them onto the plant. Use the paintbrush to move any stray aphids to the plant. Lightly dampening the paintbrush will make this easier. If the aphid colony gets very large, they will begin to kill plants quickly. At this point, freeze the old, dying plant and most of the aphids and only transfer a small batch of the aphids to the new plant.

You may be able to find wild aphids on field peas, garden sweet peas or in an alfalfa field. If the aphids are on one of these legume hosts and closely match the description of pea aphids, there is a good chance they are pea aphids. Try putting some on fava bean plants and see what happens.

Make sure to always have a few extra plants. You don't want to lose your colony!

Obtaining predators

Lady beetles are the best predator to use for this experiment because they are voracious aphid predators. They can be ordered online from garden supply companies. They can be stored in a refrigerator until they will be used (do not freeze them). It is best to only store them for about a week or two.

Lady beetles can also be collected and used in the experiment. Similar to lady beetles that are bought, they can be stored for short periods in the refrigerator.

The experiment

Aphid observations

Gently remove leaves with aphids on them from the colony plants and put them in petri dishes or on plates. Have students make observations about the aphids after viewing them under a microscope or with a hand lens. Do any of the aphids have wings? How many different sizes of aphids are there? Are any aphids giving birth? Are some aphids still feeding on the leaf? Are there any light-colored exoskeletons left over from when the aphids molted?

Have students practice gently picking up aphids with the paintbrush and then putting them back into the petri dish. Lightly tapping the brush should dislodge the aphid. If not, they can be gently "wiped" off the paintbrush onto the leaf or allowed to walk off on their own.

Population growth

For the experiment, assign students to small groups. Each group will have two plants, one that will remain predator-free and one that will receive a predator during the experiment. On day 0, each group should add two aphids to each plant. They should use the same size of aphids on both plants. If they do not control for aphids size and one plant receives two large aphids while the other receives two small ones, their populations will likely be different after a week. Have several extra plants to infest with aphids just in case something happens to one of the group's plants. During the first part of the experiment, the plants do not need to have the cages on them.

Students will collect data every day or every other day. Students should count their aphids and record their population numbers on their data sheet. They should also record observations about their aphids either on the data sheet or on a separate observations sheet. Prompts for observations can be similar to when they observed the aphids in the petri dish. They can also make observations about how far the aphids seem to have moved, where they are located on the plant and if it looks like one aphid is surrounded by many of its offspring. Care should be taken when manipulating the plants because of the dropping behavior of the aphids.

After one week, students will add one or two lady beetles to one of their habitats. At this point, cages should be added to both plants. The predator-free habitat receives a cage to control for any potential effects of the cage on plant growth or the aphid population. Have students make observations about their lady beetles. Are they feeding on the aphids? What is the response of the aphids to the predator? On their data sheets, students should put an arrow and record what day they added the predator. Students should continue to collect data for another week. The experiment can be extended another week as well. If the lady beetle is not found during the data collection period, a new one should be added. The lady beetle should be restricted to the top cage or to a cup while counting aphids. If aphid populations are still low after one week, allow them to grow a bit more before adding the predator.

Results

Students should graph their results, with day on the x-axis and number of aphids on the y-axis. It will likely be easiest for them to graph and observe patterns if they draw two separate graphs. Excel can be used to enter data and it is easiest to provide them with a template graph into which they enter their data. Have them observe the shape of the graphs. What shape do they take? What does this tell them about the growth of their aphid populations? Are their graphs the same for the first week? Are they the same after the predator is added? If not, how are they different? The predator-free graph should roughly follow a "J" shape that is characteristic of exponential growth. The predator graph should be approximately the same after the first week, but the rate of change should decrease once the predator is added and the population may even decrease. Groups can also compare their graphs. Their results may vary depending on how carefully they collected data and cared for their plants and aphids.

In addition, the class data should be graphed on one graph for each treatment (or one for both) with the means taken for the whole class. In this case, each group is a replicate for each treatment. This is a good time to stress the importance of replication in scientific work. For instance, one group's results do not necessarily tell you what the true pattern is. However, if we combine all groups' results and average them, the true underlying pattern should emerge.

Another way to describe the importance of replication is to describe an experiment in which you flip a coin. An individual coin flip is a replicate. If you attempt to generalize the results from one or two coin flips, you may mistakenly generalize from your results that every time you flipped the coin it lands on heads. In reality, on average, half the time you flip the coin it will be heads and half the time it will be tails. If your experiment included enough replicates, for instance 50 coin flips, you would able to identify the true pattern.

Discussion

Students should reflect on their results. Are their results different than what they were expecting? Are they similar or different to other groups? If they are different do they know why? What sort of pattern emerged from the data from the whole class? If they continued their experiment even longer, what would have happened in the predator-free habitat? What if they used a larger cage and continued to add new plants? Is this likely to happen in the wild? Have them subtract the final aphid number for the predator habitat from the predator-free habitat. Is this difference the number of aphids eaten by the lady beetle? Why or why not? Why would predators in a field benefit a farmer? What other experiments might they conduct with aphids? What other factors might affect aphid population growth?

Extensions or adaptations for this experiment

- There are many species of aphids. One option is to find wild aphids on wild plants or on garden plants. Aphids can be found on milkweed, goldenrod, wild legumes and many other species. In the garden, aphids can be found on roses, cabbage, lettuce and others. New plants can be grown from seed and then infested with aphids or small plant can be transplanted and moved. Aphids can be reared for short periods on cuttings of some plants kept in water.

- Many predators eat aphids and vary in their ability to suppress aphid populations. If you can obtain several different species of lady beetles or another type of aphid predator such as damsel bugs or lacewing larvae (the latter of which are commercially available), the class can compare how well each type of predator suppresses aphid populations.

- Follow up on suggestions for further experiments and start a new project using the pea aphids.

References

Aphid background:
http://www.biokids.umich.edu/critters/Aphididae/
http://en.wikipedia.org/wiki/Aphid
Pea aphid background: http://en.wikipedia.org/wiki/Acyrthosiphon_pisum
Pea aphid background (pest fact sheet):
http://extension.entm.purdue.edu/fieldcropsipm/insects/pea-aphid.php
Article about different colors of pea aphids:
http://www.npr.org/templates/story/story.php?storyId=126312333
Information about "secondary outbreaks" of pea aphids after spraying insecticides in fields: http://extension.usu.edu/htm/publications/file=5342

Videos:

Lady beetle(s) eating aphids:
http://www.youtube.com/watch?v=zaDTlVwKgck
http://www.youtube.com/watch?v=t8ghplqDAak

Parasitoids (another type of natural enemy of aphids):
http://video.nationalgeographic.com/video/animals/bugs-animals/bees-and-wasps/wasp_parasitic/

Contact

For assistance on this project or with any additional experiments based on this lesson plan, please contact Ian Grettenberger at img103@psu.edu.

Images

Fig. 1 – Seven-spotted lady beetle eating an aphid

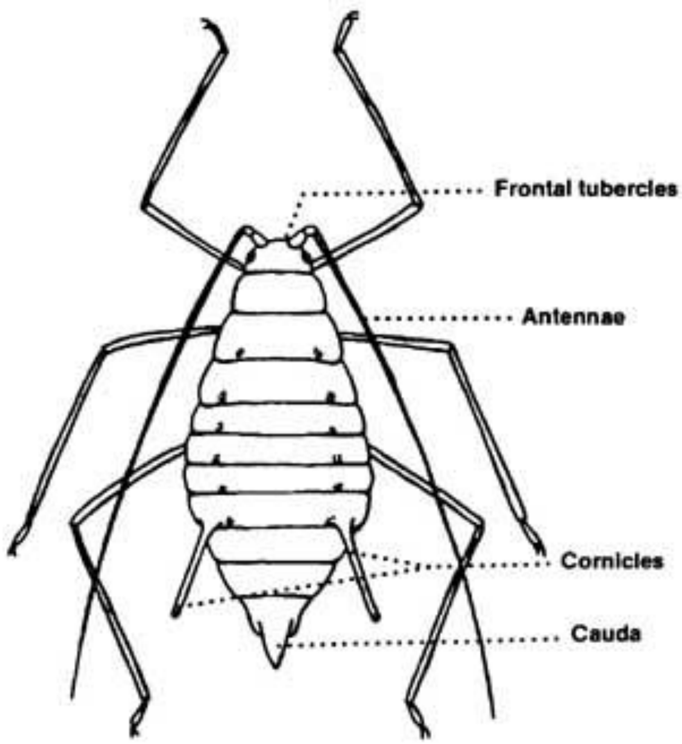
http://www.californiaorganicgardening.com/ladybugs-and-aphids-organic-pest-contro/

Fig. 2 – Pea aphid

http://en.wikipedia.org/wiki/Acyrthosiphon_pisum

Fig. 3 – Aphid anatomy

http://www.ext.colostate.edu/pubs/insect/05531.html
http://www.ipm.ucdavis.edu/TOOLS/KEYAPHIDGRAIN/



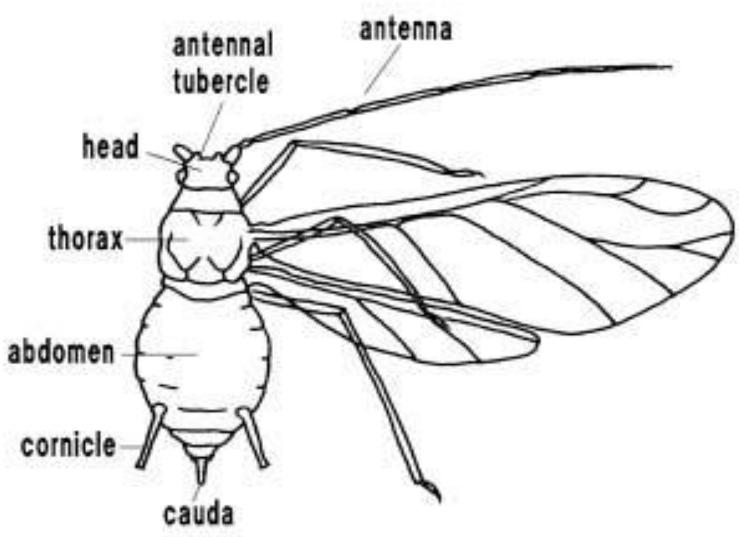


Fig. 4 – An ant feeding on the honeydew produced by an aphid

http://theaphidroom.wordpress.com/category/symbiosis/

Fig. 5 – Aphid habitat

Adapted from:
http://sketchup.google.com/3dwarehouse/details?mid=defc45107217afb846564a8a219239b

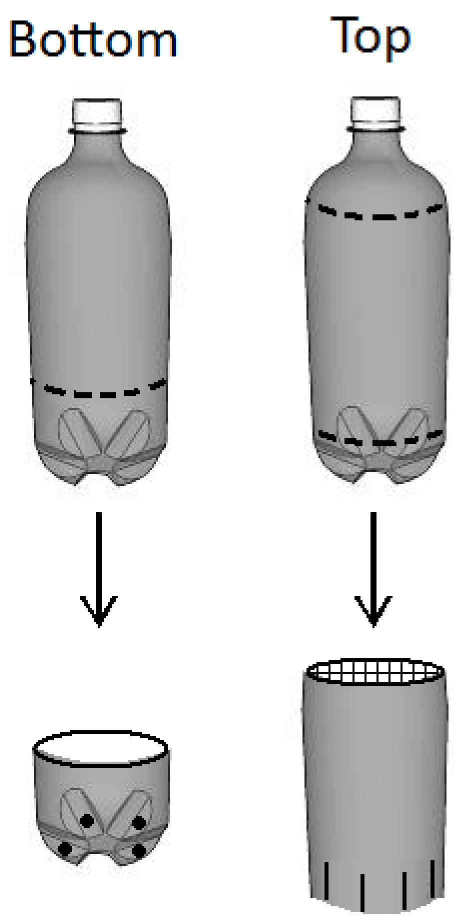


Fig. 6 – Fava bean seedlings

http://mastergardenertompkins.blogspot.com/2013/03/growing-fava-beans-in-upstate-new-york.html

Data Sheet (two per student, adapt as needed)

Predator or Predator-free (circle)

Day	**Aphids (total)**	**Winged aphids**	**Observations**
0			