Beet Armyworm Invasion: Can Plants Fight Back?

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Beet Armyworm Invasion: Can Plants Fight Back?

Although you might not be ready for a Beet Armyworm invasion, scientists believe that some plants will do a better job than others in defending themselves against insect feeding. Figuring out which plants do this best is the goal of this citizen science project. From there, scientists will unravel the biochemical and genetic mechanisms underlying resistance and help plant breeders develop more resistant food crops.

Project Overview

Corn, or **maize**, is the *world's* most economically important crop. The United States is the world's largest producer and exporter of corn. Every state except for Alaska grows corn! You may think of the corn kernels that we eat, but we also eat corn in its more processed forms- like corn meal and various sweeteners. Most of the corn grown is actually consumed by other animals as **animal feed**, and we in turn eat them.

Growing corn can be challenging work. Farmers have to deal with constantly changing biotic environmental conditions, such as weather and pests. One pest is the **Beet armyworm** (*Spodoptera exigua*). It can complete its **life cycle** in as few as 24 days and can destroy corn crops and transmits diseases, thereby decreasing yield. It also can feed on other crops such as beets, cabbage, corn, cotton, lettuce, onions, peanuts, peppers, potatoes, sweet potatoes, and tomatoes!

Scientists at the Boyce Thompson Institute are working with middle and high school students to evaluate **plant-insect interactions**, such as those that occur between corn and the Beet Armyworm. By identifying different corn **seed lines** that are more resistant or less resistant to **herbivory**, scientists may be able to breed corn that is more **resistant** to Beet Armyworm damage. This biodiversity may play an important role as we adapt our food supply to environmental changes associated with **climate change**.

This lesson can be taught before, during, or after students have studied topics of Genetics, Heredity, or Biodiversity. It can be used to engage students in questions around plant-insect interactions, climate change, plant defenses, and the role of citizen science.

In earlier grades this lessons can also be taught as a stand-alone scientific inquiry unit or to illustrate plant and insect growth, development and life cycles.

Grade Level

This lab is designed for students in grades 9-12 in introductory to advanced biology courses and can be modified for students in grades 7-16.

Time Frame

The activity should take a total of minutes 240 minutes of class time over 6 class periods, with an overall time frame of 4-5 weeks.

Background Information

People have been breeding plants for thousands of years. People have always wanted to grow plants that grew tastier fruits, grew faster, or had larger seeds. Over time cultures have learned to breed plants with a variety of desired traits. Thanks to Gregor Mendel and others we now have an understanding of **genetics**, and are able to intentionally breed plants for a variety of traits. Vegetables that have higher nutrient contents, flowers that bloom all summer, or plants that can withstand harsh conditions are a few examples. New breeding technologies are now used to refine and speed up the breeding process.

The plant breeder must first recognize a plant with a **variation**, and then determine if that is a desirable trait. This variation can arise spontaneously through mutations in a plant's DNA, can be induced by exposure to chemicals or radiation, or can result from plants growing in and adapting to different growing conditions.

Plants from different regions have genes that differ slightly. This genetic diversity has allowed plants to adapt to different **biotic** (insects, fungi, bacteria) or **abiotic** stresses (weather fluctuations, pollution, nutrition deficiencies.) Scientists can take advantage of this diversity, and potentially breed plants that are resistant to certain stressors. It may allow farmers to use fewer pesticides, or allow us to better cope with **climate change**.

In this experiment, you will be comparing the feeding habits of Beet Armyworm on several different varieties or **seed lines** of corn with diverse genetic backgrounds. Plants aren't passive victims of herbivores. They have a whole arsenal of physical and chemical defenses. A plant can distinguish between a mechanical injury, like the wind blowing or a tree falling, and insect damage. There are certain chemicals in insect saliva, called **elicitors**, that signal to the plant that it is under attack. In response, plants can produce chemical responses to protect themselves. These chemicals may repel harmful insects or attract beneficial insects. Each of these seed lines you will work with differs in its genetic makeup and its resistance to **herbivory.**

Plant and Insects Project

Dr. Georg Jander, a research scientist at the Boyce Thompson Institute (BTI), studies plant-insect interactions, specifically plant defenses against herbivory. *Spodoptera exigua* (Beet Armyworm) is a widespread agricultural pest and feeds readily on ***Zea mays*** (corn or maize). Your task will be to grow different seed lines of corn, and then to apply the Beet armyworm caterpillars to your crop. During this activity you will need to care for your plants and insects, collect observations and measurements, and take photos. Then you'll have the opportunity to analyze the data, draw some conclusions of your own, and then share your findings with Dr. Jander.

The objective of the project is to evaluate many diverse seed lines and identify lines with strong resistance. Plants with desirable traits may then be bred together to potentially create improved seed lines. When students or nonprofessionals contribute to research in this way, it is known as **citizen science**. This is a rare opportunity to involve students in the **research** process. Not only will students experience an authentic scientific experiment, they will have the opportunity to collaborate with a plant scientists and educators.

Learning Objectives

Students will…

- plan and carry out investigations
- observe plant growth and development from germination to young plant
- observe insect growth and development
- compare genetic variation across seed lines
- measure insect area to compare various growth rates
- formulate explanations based on their findings
- compare their findings and engage in argument from evidence
- understand the role genetic diversity may play in climate change
- share their results with BTI scientists including lab reports, and photos of any evidence of mutations

Learning Standards

Next Generation Science Standards

MS-LS-1. From Molecules to Organisms: Structures and Processes.
MS-LS-2. Interactions, Energy, and Dynamics Relationships in Ecosystems.
MS-LS-3. Heredity: Inheritance and Variation of Traits.
MS-LS-4. Biological Evolution: Unity and Diversity.
HS- LS-1. From Molecules to Organisms: Structures and Processes.
HS-LS-2. Interactions, Energy, and Dynamics
HS-LS-3. Heredity: Inheritance and Variation of Traits.
HS-LS-4. Biological Evolution: Unity and Diversity.

NGSS Cross Cutting Concepts

Patterns
Cause and Effect
Scale, Proportion, and Quantity
Systems and System Models

Structure and Function

National Common Core Standards, Dimension 3: Science:

1. Asking questions (for science) and defining problems (for engineering)
2. Developing and using models
3. Planning and carrying out investigations
4. Analyzing and interpreting data
5. Using mathematics and computational thinking
6. Constructing explanations (for science) and designing solutions (for engineering)
7. Engaging in argument from evidence
8. Obtaining, evaluating, and communicating information

New York State Living Environment Core Curriculum
Standard 1: Key Idea 1 and 3:

1.1a: Scientific explanations are built by combining evidence that can be observed with what people already know about the world.

1.3a: Scientific explanations are accepted when they are consistent with experimental and observational evidence and when they lead to accurate predictions.

1.3b: All scientific explanations are tentative and subject to change or improvement. Each new bit of evidence can create more questions than it answers. This leads to increasingly better understanding of how things work in the living world.

3.1a: Interpretation of data leads to development of additional hypotheses, the formulation of generalizations, or explanations of natural phenomena.

Standard 4: Key Idea 2:

2.1a: Genes are inherited, but their expression can be modified by interactions with the environment.

2.1b: Every organism requires a set of coded instructions for specifying its traits. For offspring to resemble their parents, there must be a reliable way to transfer information from one generation to the next. Heredity is the passage of these instructions from one generation to another.

2.2a: For thousands of years, new varieties of cultivated plants and domestic animals have resulted from selective breeding for particular traits.

2.2b: In recent years new varieties of farm plants and animals have been engineered by manipulating their genetic instructions to produce new characteristics.

Standard 4: Key Idea 3:

3.1g: Some characteristics give individuals an advantage over others in surviving and reproducing, and the advantaged offspring, in turn, are more likely than others to survive and reproduce. The proportion of individuals that have advantageous characteristics will increase.
3.1h: The variation of organisms within a species increases the likelihood that at least some members of the species will survive under changed environmental conditions.

Standard 4: Key Idea 6:
6.2b: Biodiversity also ensures the availability of a rich variety of genetic material that may lead to future agricultural or medical discoveries with significant value to humankind. As diversity is lost, potential sources of these materials may be lost with it.

Living Environment- Laboratory Checklist

- Follows safety rules in the laboratory
- Selects and uses instruments correctly
    - Uses light meter to measure light intensity
    - Uses humidity/temp meter to measure humidity and temperature
    - Uses metric ruler to measure length
- Makes observations of biological processes
- States an appropriate hypothesis
- Differentiates between the independent and dependent variables
- Collects, organizes, and analyzes data, using a computer and/or other laboratory equipment
- Organizes data through the use of data tables and graphs
- Analyzes results from observations/expressed data
- Formulates an appropriate conclusion or generalization from the results of the experiment
- Recognizes assumptions and limitations of the experiment

Materials and Resources

BTI Provided Materials

Light Rack

- This will be purchased from Carolina Biological and sent to your school address if requested.

Environmental Monitoring Meters

- Light meter for measuring light intensity in lux

- Temperature and humidity meter- measures min and max of both as well as current readings

Corn Seeds (individually labeled packets)
- 3 experimental seed lines, 25 seeds per line

Insects
- Small Beet Armyworm caterpillars on artificial diet
- These will be shipped on demand when your plants are large enough to apply the caterpillars.

Planting Materials
- 4 large watering flats or 8 small flats
- 75 3-inch pots
- 75 Plant labels, 25 each of 3 colors, one for each line of seeds

Other Materials
- 75 French Bread Bags and ties
- Paint Brushes (10)
- Soft Forceps (5)
- Toothpicks (10)
- Metric rulers (10 pack)
- 1.5 ml microcentrifuge Tubes (90)

Teacher Provided Materials

- A sunny classroom location, where temperatures are fairly constant throughout the experiment
- Three full Spectrum Florescent Light Bulbs for light rack (standard shop light size)
    - Example: Florescent light bulbs: Ex: Sylvania Octron XPS
    - 32 W 4100K and F032/841/XPS/ECO3
- Bricks or blocks to raise the light rack up (optional if light rack is adjustable)
- Potting Soil for starting seeds/vegetables
- Pencils and Markers
- Cups, glassware, or a watering can for watering
- Cameras (cell phone camera is OK)
- Camera station, stand or system to standardize camera height and angle during data collection
- White Paper without any lines or markings
- Ice, and Ice bath or bucket

Safety

- Any students with potential allergies to specific plants should notify the teacher and should avoid contact with those plants
- Beet Armyworm caterpillars do not bite or sting, and as living animals, they should be handled gently
- Any injuries while working with the laboratory materials should be reported to the teacher immediately
- Students should not manipulate the light set-up while experiment is in progress
- Corn plants have been bred via traditional means and are safe to compost.
- If the insects reach the moth stage while on the plants, it is possible that they may have laid eggs. If your plants have moths on them, the entire plant should be bagged and thrown away to avoid possible spread of eggs.
- Beet Armyworms are a pest species, so they should be killed by placing in the freezer for 1 day prior to disposal in garbage.

Laboratory Procedure

Planning Ahead

- One hour to prepare materials, assemble light rack
- Contact BTI Lab Coordinator 2 weeks before needed seed and insect requests.
- You will receive seeds from several different seed lines, each in an individually labeled packet. Students must keep accurate track of these identification numbers or the data they collect won't be of any value to the researchers.
- Watering as needed
- Make sure that students understand that the insects will all be killed as part of the experiment.

Class Time: over a span of 4-5 weeks:

- 40 min: Background information - Introduction to corn and Beet Armyworm, Project goals
- 40 min: Planting and labeling seeds
- 40 min: Apply caterpillars
- 40 min: Make observations, Remove caterpillars, Take photos
- 40 min: Measure caterpillars with ImageJ
- 40 min: Wrap up- Compile data from entire class, Draw conclusions

Teacher Wrap Up

- One hour to report data, upload photos and dispose of plants and caterpillars

Lesson Introduction (40 minutes)

Introduce some background information about corn, the Beet Armyworm, and the project goals.

Here are a few suggestions to get you started. There are more resources on the BTI website: http://bti.cornell.edu/education

- Expose students to some of the over arching concepts such as: citizen science, biodiversity, plant defenses, plant breeding, and insect life cycle.
- Introduce the idea that natural diversity is a valuable tool for plant breeders.
- This time-lapse video of Beet Armyworm eggs hatching should generate some excitement.
    - https://youtu.be/5FsS-5rGhzg
- Refer to the 2015 US Corn Update and 2015 Word Corn Report, to learn about the importance of corn globally.

Lab Day 1: Planting and labeling, watering, using meters (40 minutes)

Plant and label the different seed lines of corn.

1. Fill pots with soil, being sure to fill to the top.
2. Place pots in watering flat. Water the soil fully and evenly. *Be sure not to miss the corner pots.*
3. Using a single color label per seed line, make labels for each pot with the correct seed line and unique identifier (numbers and letters work well) *Example: (1-A, 1-B, 1-C, 2-A, 2-B, 2-C, 3-A, 3-B, 3-C etc.)* Record the planting date on the pots, or in your lab notebook.
4. After 15-20 minutes of sitting in the water, remove individual pots from the watering flat, and empty excess water from the flat.
5. Establish a consistent method that everyone will use to plant the seeds.
    - Using a pencil or marker, make a ½ inch hole in the center of each labeled pot.
    - Plant one seed per pot, cover with soil, and press lightly.
6. Label the pots as you go, so that you don't get the lines confused. **If the plants are not labeled properly, the data cannot be used by the researchers!**

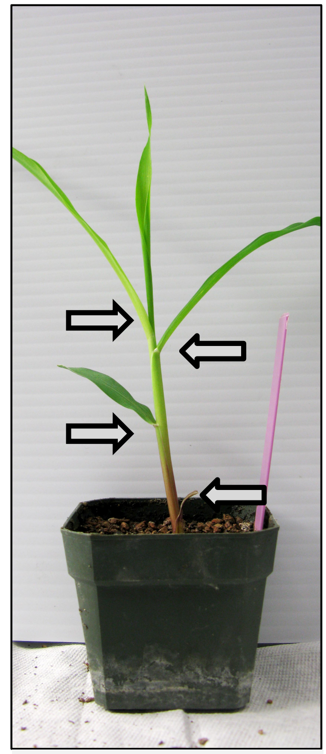
7. Return pots to the watering flats and place under the light rack in random order and location. (Randomizing the plants helps to account for any differences in light intensity, temperature, air movements, and helps scientists to be more confident in their results.) It is recommended to grow the plants using a light timer with a 12/12 or 16/8 light/dark schedule. 24 hour light is possible but not recommended.
8. There is no need to cover flats with humidity domes, as long as the soil in pots is checked daily. *Do not allowed the seeds to dry out while germinating*.
9. Set up the temperature/humidity meter and clear the memory. Record current conditions. Measure the amount of light in lux using the light meter and record the conditions.
10. Notify the BTI Teaching Lab Coordinator that you have planted your seeds, so that insects can be prepared and shipped at the right time.

Establish a consistent soil monitoring and watering plan

- **Water plants by sub-irrigation**. To do this, remove one or two pots from the watering tray. Add 1 liter of water to this opening. Replace the pots you removed. Allow the plants to absorb water 45 minutes -1 hour, then pour out any excess water.
- Do not over water or allow plants to sit in standing water for more than an hour. Doing so may induce disease and death.
- Remember that as the plants grow, they may require more water at each watering.
- Keep a watering log near the plant growing station, or ask students to keep track of their watering regimes in lab notebooks. Things to track in the log may include: date, amount of water, person who watered and removed standing water.
- Your humidity monitor will be your best resource when determining how often to water your plants. In dry conditions (30% or lower) plants may need water every day. In moist conditions (65% or higher) plants may only need water once per week. The drier the conditions, the more water your plants will need.
- It is recommended that students take turns checking on plants daily to determine if plants need water, make observations, and record the humidity/temperature/light intensity.

Lab Day 2: Select plants; Apply caterpillars (40 min)
Step 1 – Select plants
Plants will take about one week for all seeds to germinate. Approximately 2 weeks after germination (3 weeks after planting), corn plants will be large enough for the experiment. Not all plants will be used in the experiment. Some may not germinate, die or be too small to withstand continuous caterpillar feeding.

1. Sort plants into their different lines by using their colored labels as a guide.
2. Remove any plants that are much smaller or much bigger than the rest, and do not use those plants. (Researchers need the plants to be as uniform as possible so that they can be confident in their results. This is an opportunity to discuss the importance of data quality in science.)
3. Select plants with at least four visible leaves. The oldest leaf will be smaller, have a rounded tip, is closest to the soil, and may have started to die off. Be sure to include this leaf in your count. The main stem is not a leaf.


Maize plant with four visible leaf collars.

Corn development is measured in stages, based on the number of visible **leaf collars.** If you think of it like a shirt collar, it would be similar to where your collar and neck meet. When most or all of your plants have four visible leaf collars, they are ready for the caterpillars.

Step 2 – Apply caterpillars
Assemble your materials- plants, caterpillars, paintbrush, beaker of water, French bread bags & ties, white paper

**Note:*
If you want to compare the caterpillar size before and after the experiment, you should take photos of them now. See page 12 for details on taking photos.

The caterpillars move surprisingly fast, so it is helpful to work on a light colored surface, or on a piece of white paper so that you can easily see them if they escape.

1. Gather the French bread bag around your hands, like you would if you were putting on really long socks or tights. Place one of the pots on top of the bag, but don't roll it up yet.
2. Dip your paintbrush in the beaker of water. The water will help you pick up the caterpillar more easily, and without damaging it.

3. *Very gently* wipe a caterpillar with the brush to pick it up.
4. *Very gently* place the caterpillar on one of the lower leaves of the plant. It's OK if it falls onto the soil. (Sometimes the caterpillars secrete an invisible, cob-web like line, and this may be helpful in transferring them.)
5. Apply 3 caterpillars to each plant.
    a. If you don't have enough caterpillars to apply 3 to each plant, choose a smaller group of plants. It is better to have 3 caterpillars on fewer plants than fewer caterpillars on all of the plants.
6. Make sure that you choose caterpillars that are uniform in size, and alive. They should wiggle or move slightly when touched with the paintbrush. (It is easiest to remove them from the sides of the container, instead of trying to remove them from the gooey media.)
7. Quickly roll up the top of the bag, and secure it using a twisty tie. Remember that they move really fast!
8. Place the bagged plants back in the watering tray, being sure that you put them back into a random order and location.
9. During the next week when the caterpillars are feeding, make observations of your caterpillars & record in your lab notebook. Are the size and color changing? Is there an odor? Are there any surprises?

Lab Day 3: Make observations, remove caterpillars, image caterpillars (40 min)

One week after application to plants, it is time to remove the caterpillars and measure them.

Remove the caterpillars from your plants.

1. Label the microcentrifuge tubes in the same way that you labeled your plants.
2. Using the soft forceps, *gently* remove the caterpillars and place them into the microcentrifuge tubes that correspond to the same plant number. You can put all three caterpillars in the same tube. It is recommended to try to locate the caterpillars before opening the bag so they do not escape. **Note: Look very closely. Caterpillars may be difficult to find. They may be hiding in leaf folds, clinging to the bag, resting on the surface of the soil, or hiding under the rim of the pot. It is also possible that they have died.*
3. Place the labeled tubes into an ice bath for several minutes to slow them down. This will make it easier to make measurements.
4. Take photos of your caterpillars. It is important to take consistent, clear photos. When working in pairs, have one person position the caterpillars and one person take the photos.
   a) (Optional – this will help with consistency but is not required) Stand the metric ruler on the base of your ring stand. Measure 15cm in height, and attach your clamp at this position. This will help you to take photos from a consistent height and angle.
   b) Set the stage. Place a white, 8.5” x 11” piece of paper near the ring stand. Place the clear ruler on top of the paper. This will be your scale in the photos.
   c) Position your caterpillars. Remove your caterpillars from the ice bath and place them on your paper. Place the labeled tube next to the caterpillar. If the caterpillars are moving too fast, put them in the ice bath for a few more minutes.
   d) Take a picture. All pictures must include: ruler, caterpillars, labeled vial. Caterpillars must not be touching the ruler, or each other. If your picture is blurry, try again. *Hint: iPhone cameras can take a photo by pressing the one of the volume buttons*

Lab Day 4: Measure caterpillar photos using ImageJ (40 min)

ImageJ is imaging software developed by the National Institutes of Health (NIH.) It is free, public domain, open source software. It is used by scientists, doctors, engineers, and other professionals. It can measure the speed of spiders, the root growth of plants, the distance to the sun, and much more. You'll be using it to measure the area of caterpillars.

At BTI, very precise scales are used to measure the weight of the caterpillars. Since you don't have access to this equipment, we've come up with a different way for you to measure your caterpillars using ImageJ. You'll be measuring the area of your caterpillars. When you submit your data to BTI, your area measurements can be converted to weight measurements using a mathematical formula.

To ensure that quality data is collected, it is recommended to have students complete a trial lesson with the software. Students should practice measuring using the file "Practice Caterpillar for ImageJ." The caterpillar's area is known (0.412 cm^2) and students are deemed proficient when they are within +/-20% (0.330 – 0.494 cm^2) of that value. Students who are not proficient should be worked with to develop proficiency before proceeding.

Refer to separate ImageJ measurement protocol for specific instructions on using ImageJ and the practice image.

Lab Day 5: Wrap up, Compile data from entire class, Draw conclusions (40 min)

Develop and record results on student and teacher datasheets and report your findings back to BTI.

Compiling Class Data:
Using the provided template (Dropbox and/or BTI website) "Beet Armyworm – Maize Data Sheet" enter your results to report. Share your data, along with any interesting photos or exciting results with the BTI Team at pgrp-outreach@cornell.edu

Conclusion & Discussion:
Discuss the results of the experiment with the class. Discussion topics may include differences in caterpillar growth, differences in damage to the corn plants, problems that arose, errors that were made, and lessons that were learned.

Key Scientific Vocabulary

Animal feed- food given to domestic animals during the course of rearing them for food

Beet armyworm- see *Spodoptera exigua*

Biodiversity- a measure of the diversity of organisms present in different ecosystems

Citizen science- the practice of involving students or nonprofessionals in scientific research

Climate change- disruptions in earth's climate patterns due to human activity

Elicitor- chemicals in an insect's saliva that signal to the plant that it is under attack

Herbivory- an eating strategy employed by animals that are adapted to eating plant material

Life cycle- a series of changes an organism undergoes during the course of its life

Maize- see *Zea mays*

Monocot- a category of plants characterized by an embryo that has only one cotyledon (seed leaf); often distinguished by parallel leaf veins and flower parts in multiples of three; corn, rice, wheat, bananas, pineapples, grasses, lilies, tulips, daffodils

Mutation- a change in an organism's DNA; may result in a favorable or unfavorable change, or no change at all; can think of it as a "typo" in the DNA sequence

Seed Line- a uniform strain of seeds that is relatively pure genetically because of continued inbreeding and artificial selection

Plant defenses- mechanisms a plant uses to defend itself against attack; may be structural, or chemical in nature; thorns, hair, toxins

Plant-insect interaction- any action that occurs between a plant and insect; could be beneficial (pollination) or detrimental (herbivory)

Research- scientific study conducted to increase knowledge; may confirm, disprove, or expand on existing work

Resistance- the natural ability of an organism to withstand a specific stimuli or action

Spodoptera exigua- Beet Armyworm; a pest of numerous agricultural crops; a member of the Lepidoptera insect family; life stages include egg, larva, pupa, and adult

Variation- within a population depends on genetic and environmental factors; can result from mutations caused by environmental factors or errors in DNA replication or from chromosomes swapping sections during meiosis

Visible leaf collar- the place where the leaf blade and stem connect on a corn plant; a measure of corn development; similar to where your collar and neck meet

Zea mays- corn, maize; one of the world's most important food crops; a member of the Poaceae plant family

Extension Activities

The materials provided by this lab provide the opportunity to extend the activity in many different ways.

Natural Science

Observe the rest of the Beet Armyworm life cycle.

- Once the experiment is done, continue rearing the caterpillars. Watch them develop through the various instar stages, and see if you can keep them through adulthood when they become moths.

Observe the growth rate of corn.

- Once the experiment is done, continue growing the corn. Select a subset of the healthiest plants, remove them from the bags, and observe their growth rate over time.

Go on a schoolyard scavenger hunt, and document with photos.

- Search for other examples of monocots. How many can you find?
- Can you find examples of herbivory?
- Can you find examples of plant defenses?

Explore Integrated Pest Management (IPM.)

- Learn about "good bugs" versus "bad bugs." IPM is used on a commercial scale. What are some factors to think about when considering implementing IPM? How many plants do you have? How many different crops do you have? Are they growing inside a greenhouse or outside in a field?

Art

Observe a readily available organism in your schoolyard for 10 minutes. (ants, birds, dandelions, grass, etc.)

- Create a poem, song, or drawing that describes the biological process you observed. This can be repeated on different days, with different conditions, and then assembled into a book or journal.

Create a guidebook to plants of plants in your schoolyard.

- Collect plant specimens, and press them between heavy books. Use a plant ID guide to help you correctly identify them.

Create a concept for a plant-based video game- like Plants versus Zombies.

- Use real plant defenses and come up with characters, a story-board, and illustrations

Experimental Design

Create a protocol for an alternative experiment that could have been done with the corn and Beet Armyworm caterpillars.

- What other ways could we have designed the experiment? Could we have looked at the leaves, roots, or yield instead?

Math

Create graphs that illustrate the experimental temperature and humidity over the course of the experiment.

- Do you see any trends? Any surprises? What might account for these?

Find statistics on the amount of corn grown in your state, and compare it to the amount of corn grown in other states.

- How does your state compare? Does it fluctuate from year to year? What might account for this?

Explore correlations.

- We had you measure the area of your caterpillars, but in our lab we measured their weight. How is it possible for us to make valid comparisons?

Nutrition

Explore corn-based products and food additives.

- Either by reading actual product labels or researching them online, how many of your favorite foods and drinks contain corn?

Explore the many different types of corn- popcorn, dent corn, sweet corn, etc.

- What makes these types of corn different? Why does popcorn pop and other types of corn do not?

http://www.popcorn.org/ForTeachers/TeachingGuide/WhatMakesPopcornPop/tabid/88/Default.aspx

Careers

If you think of scientists as men in white lab coats that work in labs, you're not alone. Do some investigating into different types of scientists that break this stereotype.

- Horticulturist, Wildlife Biologist, Entomologist, Marine Biologist, Zoologist, Geologist, Geneticist, Aquarist, Meteorologist, Astronomer, Forensic Technician, Food Technologist

NOTES

Name____
Date______

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How does that relate to you and I?

If corn yields are dramatically impacted, you would notice, quickly. Here are some of the ways you might interact with corn today:

- If you eat cereal, Poptarts, donuts, pancakes, or bread for breakfast…you're probably eating corn!

- If you ride the bus to school, or someone drives you in a car…you're probably using gas that has ethanol in it, produced from corn!
- If you're eating a school lunch…it's possible that some of the plastic packaging is made from corn!
- If you're drinking a soda with lunch…it probably has corn syrup in it!
- If you're eating cookies with lunch…it probably has a corn-based sweetener in it!

Lab Overview

This experiment will give you the opportunity to learn about the skills a scientist uses by participating in **citizen science**. Citizen science is when researchers ask students and citizens to contribute data to a scientific research project. You will have an opportunity to:

- grow plants and insects,
- make observations and measurements,
- take photographs,
- use scientific software tools, and then
- share your data with a real scientist!

Your task will be to grow different lines of corn, and then to apply the Beet Armyworm caterpillars to your crop. You'll have the opportunity to analyze the data, draw some conclusions of your own, and then share your findings with Dr. Jander at the Boyce Thompson Institute. By doing this, you may help plant breeders to breed improved corn plants that are resistant to insect feeding. This could lead to a decreased need for pesticides in crop fields.

Materials and Resources

BTI Provided Materials

Light Rack

- This will be purchased from Carolina Biological and sent to your school address if requested.

Environmental Monitoring Meters

- Light meter for measuring light intensity in lux
- Temperature and humidity meter- measures min and max of both as well as current readings

Corn Seeds (individually labeled packets)

- 3 experimental seed lines, 25 seeds per line

Insects

- Small Beet Armyworm caterpillars on artificial diet
- These will be shipped on demand when your plants are large enough to apply the caterpillars.

Planting Materials
- 4 large watering flats or 8 small flats
- 75 3-inch pots
- 75 Plant labels, 25 each of 3 colors, one for each line of seeds

Other Materials
- 75 French Bread Bags and ties
- Paint Brushes (10)
- Soft Forceps (5)
- Toothpicks (10)
- Metric rulers (10 pack)
- 1.5 ml microcentrifuge Tubes (90)

Teacher Provided Materials
- A sunny classroom location, where temperatures are fairly constant throughout the experiment
- Three full Spectrum Florescent Light Bulbs for light rack (standard shop light size)
    - Example: Florescent light bulbs: Ex: Sylvania Octron XPS
    - 32 W 4100K and F032/841/XPS/ECO3
- Bricks or blocks to raise the light rack up (optional if light rack is adjustable)
- Potting Soil for starting seeds/vegetables
- Pencils and Markers
- Cups, glassware, or a watering can for watering
- Cameras (cell phone camera is OK)
- Camera station, stand or system to standardize camera height and angle during data collection
- White Paper without any lines or markings
- Ice, and Ice bath (optional)

Laboratory Procedure

Lab Day 1: Planting and labeling, watering, using meters (40 minutes)

Plant and label the different seed lines of corn.
1. Fill pots with soil, being sure to fill to the top.
2. Place pots in watering flat. Water the soil fully and evenly. *Be sure not to miss the corner pots.*

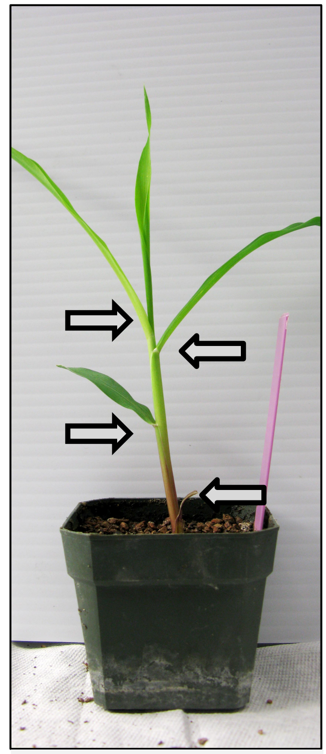
3. Using a single color label per seed line, make labels for each pot with the correct seed line and unique identifier (numbers and letters work well) *Example: (1-A, 1-B, 1-C, 2-A, 2-B, 2-C, 3-A, 3-B, 3-C etc.)* Record the planting date on the pots, or in your lab notebook.
4. After 15-20 minutes of sitting in the water, remove individual pots from the watering flat, and empty excess water from the flat.
5. Establish a consistent method that everyone will use to plant the seeds.
   - Using a pencil or marker, make a ½ inch hole in the center of each labeled pot.
   - Plant one seed per pot, cover with soil, and press lightly.
6. Label the pots as you go, so that you don't get the lines confused. **If the plants are not labeled properly, the data cannot be used by the researchers!**
7. Return pots to the watering flats and place under the light rack in random order and location. (Randomizing the plants helps to account for any differences in light intensity, temperature, air movements, and helps scientists to be more confident in their results.) It is recommended to grow the plants using a light timer with a 12/12 or 16/8 light/dark schedule. 24 hour light is possible but not recommended.
8. There is no need to cover flats with humidity domes, as long as the soil in pots is checked daily. *Do not allowed the seeds to dry out while germinating*.
9. Set up the temperature/humidity meter and clear the memory. Record current conditions. Measure the amount of light in lux using the light meter and record the conditions.
10. Notify the BTI Teaching Lab Coordinator that you have planted your seeds, so that insects can be prepared and shipped at the right time.

Establish a consistent soil monitoring and watering plan

- **Water plants by sub-irrigation**. To do this, remove one or two pots from the watering tray. Add 1 liter of water to this opening. Replace the pots you removed. Allow the plants to absorb water 45 minutes -1 hour, then pour out any excess water.
- Do not over water or allow plants to sit in standing water for more than an hour. Doing so may induce disease and death.
- Remember that as the plants grow, they may require more water at each watering.
- Keep a watering log near the plant growing station, or ask students to keep track of their watering regimes in lab notebooks. Things to track in the log may include: date, amount of water, person who watered and removed standing water.

- Your humidity monitor will be your best resource when determining how often to water your plants. In dry conditions (30% or lower) plants may need water every day. In moist conditions (65% or higher) plants may only need water once per week. The drier the conditions, the more water your plants will need.
- It is recommended that students take turns checking on plants daily to determine if plants need water, make observations, and record the humidity/temperature/light intensity.

Lab Day 2: Select plants; Apply caterpillars (40 min)
Step 1 – Select plants
Plants will take about one week for all seeds to germinate. Approximately 2 weeks after germination (3 weeks after planting), corn plants will be large enough for the experiment. Not all plants will be used in the experiment. Some may not germinate, die or be too small to withstand continuous caterpillar feeding.


Maize plant with four visible leaf collars.

1. Sort plants into their different lines by using their colored labels as a guide.
2. Remove any plants that are much smaller or much bigger than the rest, and do not use those plants. (Researchers need the plants to be as uniform as possible so that they can be confident in their results. This is an opportunity to discuss the importance of data quality in science.)
3. Select plants with at least four visible leaves. The oldest leaf will be smaller, have a rounded tip, is closest to the soil, and may have started to die off. Be sure to include this leaf in your count. The main stem is not a leaf.

Corn development is measured in stages, based on the number of visible **leaf collars.** If you think of it like a shirt collar, it would be similar to where your collar and neck meet. When most or all of your plants have four visible leaf collars, they are ready for the caterpillars.

Step 2 – Apply caterpillars
Assemble your materials- plants, caterpillars, paintbrush, beaker of water, French bread bags & ties, white paper

**Note:*
If you want to compare the caterpillar size before and after the experiment, you should take photos of them now. See page 8 for details on taking photos.

The caterpillars move surprisingly fast, so it is helpful to work on a light colored surface, or on a piece of white paper so that you can easily see them if they escape.

1. Gather the French bread bag around your hands, like you would if you were putting on really long socks or tights. Place one of the pots on top of the bag, but don't roll it up yet.
2. Dip your paintbrush in the beaker of water. The water will help you pick up the caterpillar more easily, and without damaging it.

3. *Very gently* wipe a caterpillar with the brush to pick it up.
4. *Very gently* place the caterpillar on one of the lower leaves of the plant. It's OK if it falls onto the soil. (Sometimes the caterpillars secrete an invisible, cob-web like line, and this may be helpful in transferring them.)
5. Apply 3 caterpillars to each plant.
    a. If you don't have enough caterpillars to apply 3 to each plant, choose a smaller group of plants and apply 3 to each plant.
6. Make sure that you choose caterpillars that are uniform in size, and alive. They should wiggle or move slightly when touched with the paintbrush. (It is easiest to remove them from the sides of the container, instead of trying to remove them from the gooey media.)
7. Quickly roll up the top of the bag, and secure it using a twisty tie. Remember that they move really fast!
8. Place the bagged plants back in the watering tray, being sure that you put them back into a random order and location.
9. During the next week when the caterpillars are feeding, make observations of your caterpillars & record in your lab notebook. Are the size and color changing? Is there an odor? Are there any surprises?

Lab Day 3: Make observations, remove caterpillars, image caterpillars (40 min)

One week after application to plants, it is time to remove the caterpillars and measure them.

Remove the caterpillars from your plants.

1. Label the microcentrifuge tubes in the same way that you labeled your plants.
2. Using the soft forceps, *gently* remove the caterpillars and place them into the microcentrifuge tubes that correspond to the same plant number. You can put all three caterpillars in the same tube. It is recommended to try to locate the caterpillars before opening the bag so they do not escape. **Note: Look very closely. Caterpillars may be difficult to find. They may be hiding in leaf folds, clinging to the bag, resting on the surface of the soil, or hiding under the rim of the pot. It is also possible that they have died.*
3. Place the labeled tubes into an ice bath for several minutes to slow them down. This will make it easier to make measurements.
4. Take photos of your caterpillars. It is important to take consistent, clear photos. When working in pairs, have one person position the caterpillars and one person take the photos.
    1. (Optional – this will help with consistency but is not required) Stand the metric ruler on the base of your ring stand. Measure 15cm in height, and attach your clamp at this position. This will help you to take photos from a consistent height and angle.
    2. Set the stage. Place a white, 8.5" x 11" piece of paper near the ring stand. Place the clear ruler on top of the paper. This will be your scale in the photos.
    3. Position your caterpillars. Remove your caterpillars from the ice bath and place them on your paper. Place the labeled tube next to the caterpillar. If the caterpillars are moving too fast, put them in the ice bath for a few more minutes.
    4. Take a picture. All pictures must include: ruler, caterpillars, labeled vial. Caterpillars must not be touching the ruler, or each other. If your picture is blurry, try again. *Hint: iPhone cameras can take a photo by pressing the one of the volume buttons*

Lab Day 4: Measure caterpillar photos using ImageJ (40 min)

ImageJ is imaging software developed by the National Institutes of Health (NIH.) It is free, public domain, open source software. It is used by scientists, doctors, engineers, and other professionals. It can measure the speed of spiders, the root growth of plants, the distance to the sun, and much more. You'll be using it to measure the area of caterpillars.

At BTI, very precise scales are used to measure the weight of the caterpillars. Since you don't have access to this equipment, we've come up with a different way for you to measure your caterpillars using ImageJ. You'll be measuring the area of your caterpillars. When you submit your data to BTI, your area measurements can be converted to weight measurements using a mathematical formula.

To ensure that quality data is collected, it is recommended to have students complete a trial lesson with the software. Students should practice measuring using the file "Practice Caterpillar for ImageJ." The caterpillar's area is known (0.412 cm^2) and students are deemed proficient when they are within +/-20% (0.330 – 0.494 cm^2) of that value. Students who are not proficient should be worked with to develop proficiency before proceeding.

Refer to separate ImageJ measurement protocol for specific instructions on using ImageJ and the practice image.

Lab Day 5: Wrap up, Compile data from entire class, Draw conclusions (40 min)

Develop and record results on student and teacher datasheets and report your findings back to BTI.

Share your data with the BTI team, at pgrp-outreach@cornell.edu (Plant Genome Research Program outreach)

Key Scientific Vocabulary

Animal feed- food given to domestic animals during the course of rearing them for food

Beet armyworm- see *Spodoptera exigua*

Biodiversity- a measure of the diversity of organisms present in different ecosystems

Citizen science- the practice of involving students or nonprofessionals in scientific research

Climate change- disruptions in earth's climate patterns due to human activity

Elicitor- chemicals in an insect's saliva that signal to the plant that it is under attack

Herbivory- an eating strategy employed by animals that are adapted to eating plant material

Life cycle- a series of changes an organism undergoes during the course of its life

Maize- see *Zea mays*

Monocot- a category of plants characterized by an embryo that has only one cotyledon (seed leaf); often distinguished by parallel leaf veins and flower parts in multiples of three; corn, rice, wheat, bananas, pineapples, grasses, lilies, tulips, daffodils

Mutation- a change in an organism's DNA; may result in a favorable or unfavorable change, or no change at all; can think of it as a "typo" in the DNA sequence

Seed Line- a uniform strain of seeds that is relatively pure genetically because of continued inbreeding and artificial selection

Plant defenses- mechanisms a plant uses to defend itself against attack; may be structural, or chemical in nature; thorns, hair, toxins

Plant-insect interaction- any action that occurs between a plant and insect; could be beneficial (pollination) or detrimental (herbivory)

Research- scientific study conducted to increase knowledge; may confirm, disprove, or expand on existing work

Resistance- the natural ability of an organism to withstand a specific stimuli or action

Spodoptera exigua- Beet Armyworm; a pest of numerous agricultural crops; a member of the Lepidoptera insect family; life stages include egg, larva, pupa, and adult

Variation- within a population depends on genetic and environmental factors; can result from mutations caused by environmental factors or errors in DNA replication or from chromosomes swapping sections during meiosis

Visible leaf collar- the place where the leaf blade and stem connect on a corn plant; a measure of corn development; similar to where your collar and neck meet

Zea mays- corn, maize; one of the world's most important food crops; a member of the Poaceae plant family

Questions

1a. List the challenges you had planting the seeds, growing the plants, or making observations.

1b. How might these challenges affect your results?

2a. Did you notice any pattern to the insect feeding? Did they eat in the same place on the leaf? Did they feed on all of the different seed lines equally?

2b. What might account for this?

3a. Consider the larger picture. How might this information benefit you if you were growing a flower or vegetable?

3b. How might this information benefit other farmers that grow other crops?

Name ________________
Partner/Group ______________________________

Student Data Tables

Date seeds planted? ____________________

Watering & Growth Observations

Date	Need water? Y/N	Amount watered (L)	Any growth? Y/N	Height (cm)	Student initials

Daily Observations

Date	Humidity (%)	Temperature (C)	Light intensity (Lux)	Student initials

Date caterpillars applied? ____________________

How many plants did you apply caterpillars to? ________________

Date caterpillars removed/photographed? ____________________

Caterpillar photo log

Plant ID number	Number of caterpillars recovered	Photo number	Observations	Student initials

Date photos were measured? ____________________

Measurement log

Plant ID number	Area of caterpillar 1 (cm^2)	Area of caterpillar 2 (cm^2)	Area of caterpillar 3 (cm^2)	Average area	Student initials

ImageJ Measurement Protocol

1. Download ImageJ software for free from NIH.

http://imagej.nih.gov/ij/

- Select "Download"
- Determine your platform (Mac/Linux/Windows) then click on the appropriate link to download the program. Note that Chromebooks cannot run ImageJ without installing a new operating system. (You may also download more detailed installation instructions from this page.)
- Follow the prompts to install the program.

This is the basic process you will follow. Detailed instructions are below: *Open image→Set scale→Outline caterpillar→Measure caterpillar→Record your data*

2. Import your Images

- Import your image into the program:
  - Drag & Drop them into the program <or>
  - File, Open, and then navigate to your images.

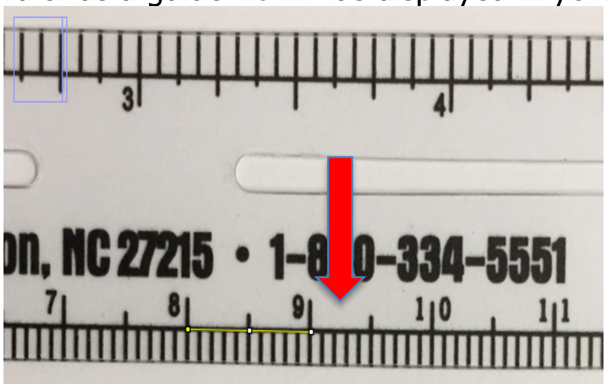
**Tips:*

  - *You may rotate the image for a better view. (Image, Transform, Rotate)*
  - *You may zoom in or out for a better view. (Image, Zoom)*
  - *You may adjust the view by selecting the Scrolling tool, and dragging left or right on the image.*

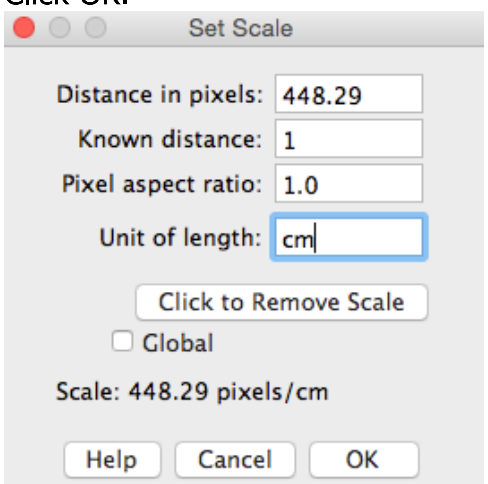
3. Set Scale

- Select the Straight Line tool (HIGHLIGHTED BELOW)

- Use the crosshairs to trace a line that runs the length of 1cm using your ruler as a guide. It will be displayed in yellow and can be hard to see.



  - From the Top menu select Analyze, then Set Scale.
  - Enter the Known distance to "1," and the Unit of length to "cm."
  - Distance in pixels and Pixel aspect ratio will pre-populate.
  - Click OK.



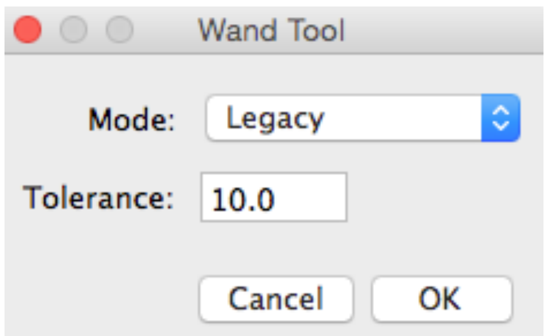
***You will need to reset the scale EACH TIME you open a new image*.**

4. Outline the Caterpillar

- To select/**outline the caterpillar** there are two options: Wand and Freehand. You should try both to see which method you prefer. The Wand method is explained first, then the Freehand.

    - Select the **Wand tool** (HIGHLIGHTED BELOW)

    - Double click the wand tool and set the Tolerance to "10."
    - Click OK.

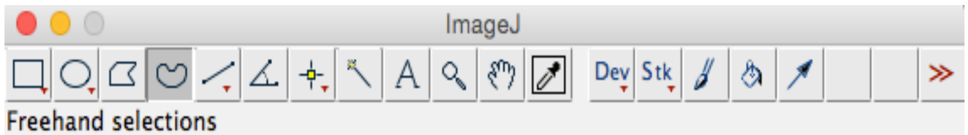


    - Click on the inside of the caterpillar to make a rough selection. It will be outlined in yellow.

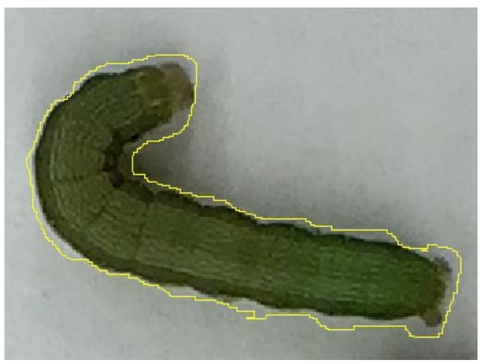
- Refine your selection to be more accurate. Clicking while holding down Shift will allow you to add areas, Clicking while holding down Alt-Option will allow you to remove areas.
- Most of the caterpillar should be outlined in yellow. Be as accurate as you can, but limit yourself to 4 or 5 clicks. It won't be perfect.
- If your tool gets stuck and you cannot remove the yellow outline, click outside of the caterpillar to start over.

<or>

Select the **Freehand tool** (HIGHLIGHTED BELOW)



- Click and drag to outline the caterpillars' body. It will be outlined in yellow.
- Most of the caterpillar should be outlined in yellow. Be as accurate as you can. It won't be perfect, as shown below.
- If your selection tool gets stuck and you cannot remove the yellow outline, try clicking on the outline to remove it.



5. **Measure the caterpillar**
   - Select Analyze from the top menu, then Measure
   - The Results dialogue box will show you the area, mean, min and max. We are focusing on the Area, so record this measurement. The units are in cm^2.

     **Note: Measurements should be less 1. If you end up with numbers in the thousands, you need to reset your scale.*
6. **Record** your data in your data table.
7. **Repeat** this process until all the caterpillars in your image have been measured.
8. **Close** your image and Results dialogue box. Do not save changes to either of these.
9. **Repeat** this entire process with each of your images.

ImageJ Practice

Learning new skills takes time. This lesson will give you practice using the ImageJ software. The goal is to ensure that you are collecting quality data that is accurate and usable.

Practice using both the **Wand tool** and **Freehand tool** to measure the caterpillar in the file "Practice Caterpillar for ImageJ". When you begin to feel comfortable with the software, record your measurements below.

Wand tool	Freehand tool
1.__________ cm^2	1.__________ cm^2
2.__________cm^2	2.__________ cm^2
3.__________cm^2	3.__________ cm^2

Now average all these together and record here. __________ cm^2

The result should be 0.412 cm^2. If your results are within the range:
0.330 – 0.494 cm^2 then you have successfully demonstrated proficiency using ImageJ. If your results are outside of that range, keep practicing until you become proficient.

You may notice that you have a preference for either the Wand tool and Freehand tool. You should use which ever is more accurate for you.

NOTES

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