PLANTS GET SICK, TOO!

A Plant Pathogen Investigation

Teacher's Guide: An Overview

Premise: Most students intuitively understand that as living organisms, plant can get sick. Some will remember that old trees sometimes become diseased and have to be cut down, or that shrubs outside where they live occasionally turn brown and die, but they have probably not been significantly impacted by the death of a plant. However, plant pathogens pose a major threat to global agriculture. Every year, plant diseases cause billions of dollars of losses to American farmers, and can lead to famine and war in the developing world. Diseases can affect plants at every stage of life, but post-harvest diseases often impact consumers when food rots before it can be eaten. Understanding where pathogens come from, how they can infect hosts, what environmental variables effect disease development, and how different hosts can defend against pathogens are important concepts in biology and the study of disease (of any organism, including animals like humans). Observing the progress of disease development on a familiar food product is a direct way to learn how hosts are impacted by pathogens. Furthermore, such knowledge is critical to understanding microbial pathogenicity and how microbes can endanger our food supply.

How *THIS* Module Works: This module consists of a lab inquiry imbedded in classroom and online discussions. The lab activity may be guided (inoculating potatoes with pre-selected variables), or inquiry-based (hypotheses developed by the students that test aspects of host susceptibility and environmental variation on disease), and is flexible enough to be easily adapted to most classroom environments. Young people are scholars of food (even if they rarely think about it), and will gain a deeper appreciation of what they eat through the course of these experiments. This lab aims to illuminate the importance of plant disease to modern agriculture

and highlight how pathogens can infect and potentially kill their hosts. Worksheets (especially those that focus on experimental design) can help students organize their thoughts and develop novel hypotheses.

Beyond the lab investigations, three other types of activities are essential to fully benefit from this module:

- **Classroom discussion:** authentic classroom dialogue before, during, or following lab activities
- **Research Blogs:** regular online contact between students and scientist mentors and peers
- **Storyboard discussion:** an extended post-lab discussion in which students share and reconcile data within and across teams

We have found that a teacher's commitment to dialogue and a focus on student ideas and reasoning emphasizing the process of science is an important aspect of building an open culture for science learning. Explanations using everyday vocabulary are valued over use of scientific vocabulary in the absence of explanations. A more detailed description of teaching and learning strategies used in the module can be found in the PlantingScience *Teacher's Handbook*.

Grade levels: Middle school and high school biology, AP biology, environmental science, AP environmental science, horticulture, botany, and other life science electives.

Class time: Students should be able to complete the thought investigations, the guided inquiry, and the open investigation over a two- to three-week period. During that time period, there will likely be time that can be used for other aspects of your curriculum. For example, when students are waiting to hear back from their mentors or are letting infected plant samples incubate, you can fill that class time with other lessons from your standard curriculum.

Computer access: Optimally, every other class session outside of the open-inquiry period and daily while designing the open inquiry; minimally, at least three times over the course of the full investigation period. Team blogs require logins, which teachers hand to students in the form of tickets.

Crosscutting Concepts, Disciplinary Core Ideas, and Practices from the Next Generation Science Standards:

Crosscutting Concepts	Scientific Practices	Disciplinary Core Ideas
• Cause and Effect • Systems and System Models • Structure and Function	• Asking Questions and Defining Problems • Planning and Carrying Out Investigations • Constructing Explanations • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	• LS1.A: Structure and Function
• Cause and Effect	• Analyzing and Interpreting Data • Constructing Explanations • Obtaining, Evaluating, and Communicating Information	• LS4.B: Natural Selection • LS4.C: Adaptation
• Cause and Effect • Stability and Change	• Developing and Using Models • Planning and Carrying Out Investigations • Constructing Explanations and Designing Solutions • Obtaining, Evaluating, and Communicating Information	• LS1.A: Structure and Function
• Cause and Effect	• Analyzing and Interpreting Data • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	• LS4.C: Adaptation

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Teacher's Guide: Planner

Suggested Schedule of Activities:

Sample topic	Pre-Lab Science Talk		Lab Activity		Post-Lab Storyboard Discussions	
Exploring the relationship between microbes and disease	What makes a microbe a pathogen?	Blogging with Scientists and Peers	Inoculating potatoes with soft rot bacteria	Blogging with Scientists and Peers	How well did the bacteria infect potatoes?	Blogging with Scientists and Peers
Exploring the relationship between hosts and disease	Why are some plants more tolerant to diseases?		Inoculating different plant products with soft rot bacteria		What plants (if any) were resistant to the soft rot pathogen?	
Exploring the relationship between the environment and disease	What environmental factors can impact plant diseases?		Incubating inoculated plant products		What environmental variables were important for disease development?	

The *Plants Get Sick, Too!* module consists of an inquiry on the nature of plant disease in which students can develop their own inoculations and undertake novel, hypotheses-driven experiments. The inquiry may be guided (inoculating potatoes) or open (investigating different hosts or environmental parameters), depending on student abilities and performance

expectations. Although distinct, this module can work in tandem with other inquiries like *Agronomy Feeds the World* or the *Celery Challenge* to illustrate important aspects of agriculture and botany.

Suggested Assessment Schema: The module is designed so that student can be assessed continuously for changes in understanding. The pre-lab ScienceTalk, teacher interaction with teams during in-class lab activities, lab journals and worksheets, and blogging with online mentors all serve as formative assessment tools. The post-lab class Storyboard discussion, a final individual reflection, and the post-experience survey serve as summative assessment tools. If desired, summative assessment in an exam format could involve written responses to question such as:

- What is the definition of disease?
- What is the definition of a pathogen?
- What are the three variables that must exist for disease to occur?
- What is the difference between infection and colonization?

Additional Resources: *Plants Get Sick, Too*: contains a bibliography of online videos, websites, and articles organized by media type. Items may relate directly to biological processes, to classroom tools and techniques for teaching about the process, or to research on effective reaching and student misconceptions.

Please let your scientist mentor(s) know

- which lessons you will be implementing;
- your expected start and end dates for interacting with students online;
- how frequently your students meet;
- the tentative dates for when students will be communicating with mentors;
- a brief summary of what students should know about plant diseases, plant physiology, and scientific inquiry;
- any special experiences or challenges that the students may have with respect to completing these activities;
- a brief description of the laboratory equipment and supplies available to your students;
- and, how often students will have computer access.

Students should work in teams of 3 to 4, and individual team members are encouraged to post online. Teams may blog from school or from home, following instructions in the *Student Guide*.

OVERVIEW (Pre-experiment examination of students' knowledge an understanding of plant diseases.)

Preparation: A minimum of one week is needed to complete an inoculation experiment, although many classes conduct the guided experiments to become familiar with the approach prior to the inquiry-based experiments. Both open and inquiry-based experiments require two weeks to undertake. However, any of the inquiries could be stand-alone lessons or simplified into a demonstration.

Materials and Methods: The following are needed for each lab group:

- At least three potatoes (or other fruits or vegetables if open inquiry)
  Suggested fruits and vegetables for open inquiry projects (You can add to table.):

Very susceptible	Moderately	Not susceptible
Potato*	Corn	Sweet Potato
Carrots	Snap Beans	Asparagus
Celery	Beets	Apples
Squash	Pears	
Cucumber		
Onions		
*(*Idaho, Russet, Red skin, etc.)*		

- Damp paper towels
- Sealable plastic bags
- Toothpicks
- Small beakers of bacterial inoculum
- A 10% bleach solution containing two drops of dish soap
- A large container for disinfesting the potatoes in 10% bleach
- Microscopy equipment (microscopes with 400X magnification, slides, coverslips, metal probes)
- Latex or nitrile gloves (soft rot bacteria are not infectious to humans, but do express a strong odor during infection).
    - Possible strategies to manage the strong odor: fan, candles, double-bag potatoes, fume hood, clothespins, handkerchiefs, etc.

Evaluations: Consider developing formative assessments that aid the students' preparation of an experimental design before the beginning of the experiments. Following inoculations and incubations, have the students evaluate how many products developed soft rot and identify symptoms and signs of disease on the inoculated plants in order to determine the associations between hosts, pathogens, and environment that result in soft rot.

Questions:

- What plant products exhibited the most symptoms? Why?
- What different environmental variables contributed to the most disease?
- What variables did you introduce into the experiment on accident that may have affected the results?
- Why do you think some plants got sick and some did not?

Communicating with Your Mentor:

Allow time for students to communicate with their scientist mentor online. If not already acquainted with the mentor, students should introduce themselves and their teammates and ask some general questions. If they have already sent their mentor a message, ask them to check to see if they have heard back from their mentors.

Additional Resources:

Dr. Lou Hirsch's website – contains access to the NABT paper (Hirsch, Miller, and Halterman, 2018) and tutorial videos: https://www.drlouhirsch.com/an-inquiry-based-investigation-of-b

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Teacher's Guide: An Overview of Setup

Time required to establish communication with mentors and set up inoculations and experimental designs:

Timeline	Activity
Day 1	Set up inoculum cultures
Days 2-3	Create teams' set up team ResearchBlog and make first entry; Sciencetalk
Day 3	Develop Research Question* and experimental design; ResearchBlog
Day 5	Refine experimental design; surface sterilize fruits or vegetable host tissues; ResearchBlog
Day 7	Inoculate and incubate plants
Day 14	Observe the plant samples for disease
*(Open inquiry)	

We recommend that students first inoculate potatoes to observe soft rot infections and identify signs and symptoms of disease. This would be Week 1. In Week 2, students should work with their teams and mentors to develop a testable research question with the materials provided.

Proposed Schedule of Experiment:

Days to inoculation	Activity
7 days before	Set up culture to acquire inocula from a cut and soaked potato.
1 day before	Disinfest potatoes by soaking in a 10% bleach solution.
Lab day 1	Inoculate* and incubate potatoes.
Lab day 7	Observe the macro and microscopic signs and symptoms of bacterial soft rot.
Lab day 14	Brainstorm variables for the inquiry-based protocol. Inoculate and incubate plant samples.
Lab day 21	Observe and measure the macro and microscopic signs and symptoms of bacterial soft rot.
**Keep in mind that fresh inoculum is always best to use.*	

Learning Goals

- Develop models for plant disease
- Design and carry out an experiment
- Observe the development of disease symptoms over time
- Observe signs of the pathogen in diseased tissue
- Interpret data from multiple experiments.

Common Misconceptions and Student Biases

- The difference between **pathogens** and **diseases**: Pathogens are organisms that cause disease, and diseases are disruptions in normal cellular processes. In this experiment, the bacteria are the pathogens and soft rot is the disease.
- The difference between **infection** and **infestation**: Infection is the initiation of a parasitic relationship with a host, whereas infestation is to reside on the outside of a host. In this

experiment, the pathogenic bacteria infest the exterior of the potato from the field, and infect when the environment becomes conducive for the development of disease.

- The difference between **infection** and **colonization:** Infection is the initiation of a parasitic relationship with a host, while colonization follows infection, when the pathogen begins to spread throughout the host often leading to symptom development.
- The difference between **signs** and **symptoms**: In plant pathology, *signs* of an infection include the actual pathogen – e.g., bacterial streaming or ooze. *Symptoms* are host responses to pathogens, and include discoloration, a pungent odor, and soft rot.
- Students often do not make the connection between plant diseases and human health. Even though plants do not possess an immune system, they can mount defense responses, and are useful analogs for important human diseases.

	Plant Pathology and disease	**Human Pathology and disease**
Signs	Positive identification of a pathogen (either by eye or with a microscope)	Observed externally or with a microscope, or indirectly with different diagnostic tests.
Symptoms	Lesions, discoloration, soft rot, odor observed externally.	Inflammation, fever, cough, runny nose, upset stomach.

Preparations

- Review the student and teacher procedures for the lesson.
- Gather a range of materials that students may use for their investigations.
- Students will post to their blog and receive feedback from their scientist mentors on their investigations at several points during this activity. Communicate with the mentors ahead of time to let them know when to expect blog posts and to find out how quickly they will be able to provide feedback. This information will help you schedule class time for completing the open-inquiry investigations.
- **Procedure Note to Teachers:** The procedure that follows provides a framework for using this lesson in the classroom. However, you should feel free to modify it based on your students' prior experiences, knowledge, and abilities. The step numbers listed in

these procedural steps match those in the student pages.

Follow-up Steps:

1. Ask students to share what they have learned from the previous lessons. If the class has been completing the storyboard, take time to review the progress. Introduce students to the idea that this time they will be developing their own research question and then planning and conducting their own investigation to answer the question.
   Allow time for students to read through all the procedural steps in their student guide for this investigation.

2. Introduce students to the *Student Roadmap Through an Investigation* resource (https://plantingscience.org/studentdoingscience) on the PlantingScience website. Students should refer to this resource at various times during their investigations for guidance.

3. Students should begin thinking about what interests them about plant diseases. Instruct students to read through the information found at the *Explore Your Topic* link in the *Student Roadmap*. Students can spend some time individually thinking about their interests. If, during previous investigations, they wrote ideas for future investigations, they can refer back to those now. After working individually, teams can start discussing their ideas together.

4. During these steps, students read information about testable questions in their procedure and in the *Research Question* section of the *Student Roadmap*.
   a. If helpful, hold a brief discussion about testable questions before students move on to the next step.

5. Allow time for teams to work together to come up with 2–3 potential research questions to investigate. For each of their ideas, they should write answers to the questions listed in this step.
   a. The questions should help students think more about their research questions and the possible investigations they would do. Because students will share this with their mentors, the questions also provide a framework for presenting ideas to the mentors.
   b. As teams are working on their research questions, they may have questions and need

more information. Include links to this information here.

c. One consideration related to potential research questions is relevance. Will this research question investigate something that has a real-world connection? For example, a common idea for student investigations is to see what happens to disease development if they soak their plant product in water. Such a research question doesn't have a strong connection to typical shipment and storage conditions for most store-bought produce. Another problem with such investigations is that there are other contaminating microbes that can easily consume plant material when incubated in submerged environments. In these cases, most produce submerged and incubated over long periods of time nearly always become rotten, and cannot be meaningfully analyzed. As the teacher, you can watch out for questions such as this, and the mentors will also provide feedback if students present a research question that lacks sufficient depth.

6. Teams should take pictures of their notebook pages with information about their brainstormed research questions and their responses to the Step 5 questions. Teams can upload these photos to their project page on the website for their mentor to view.
    a. Each team should work together to reach a consensus about which information should be presented to the scientist mentor.

7. After receiving feedback from their scientist mentor, each team should narrow their focus and decide on a single research question that they will investigate. Teams should add information and suggestions from their mentors to their notes from Step 6.
    a. The feedback that teams get from their mentors can help identify potential problems or provide suggestions for how teams can strengthen their research questions.

8. Allow time for students to read the *Planning Your Study* section in the *Student Roadmap*. This information provides guidance on matching the experimental method to the research question as well as developing research and data collection plans.

9. Allow time for teams to work on their experimental design for their chosen research

question. The questions listed in the procedure should help teams think through details about their experiment. Encourage students to check with you about materials or supplies that they may want to use. If certain supplies are not available, they may need to modify their design.

10. After teams agree on their experimental design, they should post pictures of their notebook pages with details of their design for their mentors. Allow time for mentors to review the experimental designs and provide feedback before moving forward with the investigations.

11. Allow as many class periods as possible (45-minute periods—or perhaps 1 block schedule period) for students to conduct their investigations (after incorporating feedback from mentors). Be clear with students as to how much time they have to complete their investigations (five to seven days after inoculation is usually sufficient to observe disease development). Teams may need guidance about fitting their investigation into the available class time. For example, they may need to figure out whether they can complete their investigation in 1 day or whether they need to think about how they could do part on one day and the rest on a future date when they expect disease symptoms to be present. Or, alternatively, consider advising the students to observe their inoculated products daily during breaks in between classes. If necessary, help students identify appropriate stopping points in their procedure. Teams may need to spend some time testing parts of their procedure before beginning their real investigation. Encourage them to ask questions of their mentor if they run into problems.

12. Allow time for teams to work on the analysis of their data. Encourage teams to think about the best ways to summarize their data. Sometimes, a photograph works well. In other cases, they may need a graph or diagram. Often, they may want to use multiple formats.

13. Teams should work together to make sense of their results. The questions listed with Step 12 should help guide students' thinking and help them present their results to their mentors. When teams are ready, they can take pictures of their notebook pages and upload them for mentors to review and discuss. Optimally, you will allow time for mentors to view blog posts from students and give their feedback. Teams may want to discuss the feedback with the mentors before moving on with the rest of the investigation.

14. Ask teams to create a presentation about their investigation and the results. Explain your preferences regarding presentation format and time.
    For example, do you want students to prepare posters or PowerPoint presentations?

15. Have each team present its research question and experimental results with the class. As teams present, discuss how these results add to the storyboard that the class has been developing and how they are consistent (or inconsistent) with the results of other teams. Discussing how different investigations adds to students' knowledge of plant pathogenesis helps students fit their experimental results into a larger conceptual understanding. Because different teams will investigate different aspects of plant disease development, this discussion can help students see how the results of their investigations relate to others. Do the results of their experiment make sense when thinking about other teams' experimental findings?
    a. **When conducting the discussion and adding to the storyboard**, consider the following:
        i. How do these results relate to the previous investigations performed in the module
           (thought investigations and guided investigations)?
        ii. What is the quality of the evidence and reasoning for the explanation given?
        iii. Are there weaknesses in reasoning that become apparent? (If this happens while
           presenting, assure students it is fine to reconsider their explanation.)
        iv. How do the explanations developed by different teams reconcile with data from other teams' investigations?

16. If time permits, conduct another open-inquiry investigation. This may be something from the original set of interesting research questions or a new idea that arose during the investigation.
    a. Often, one investigation will spark ideas for new investigations. If possible, allow time for students to experience the excitement of science by continuing with a new research question and continuing to interact with their mentors.

Storyboard Discussion

What is the purpose of a storyboard?

Storyboarding consolidates "the evidence" so students can consider how their data fit into their models of plant disease. By **sharing** their stories and allowing other students to **question** their conclusions in light of data, observations, experiments, or everyday and school science experiences, students learn to **reconcile** evidence as scientists do. This process is sometimes referred to as **scientific thinking**.

Preparing storyboards

A storyboard consists of the following elements:

- Research question
- How students investigated the question
- How students know the experiment was technically successful (i.e., the method worked)
- Data summaries
- What the data mean
- A model of how plant diseases occur, including how the data fit the model
- Data from class or other teams' experiments that are or are not consistent with their explanations and model

During this guided inquiry, students may make team storyboards if there is time. Each team can present their "story" to the whole class, then answer questions from the class. Alternatively, the teacher may diagram the processes described by students as their ideas about the plant disease triangle emerge during discussion. As the teacher models how a storyboard is constructed, students can see how to make their own team storyboards after their open- inquiry experiment(s).

Integrating writing and discussion

Students should be encouraged to diagram on paper to develop and supplement their explanations. Diagramming can be a good shorthand for students to get their working ideas on paper—scientists often use this approach, too! If students are not used to sketching out their thoughts in pictures, teachers can model this by drawing pictures as the class develops a working model.

The questions that students answer in their science notebooks during this investigation are only a guide. If students think other information should be recorded or if a drawing or diagram would help, they should be encouraged to add these items.

If poster-sized whiteboards are available for each team, a large erasable working surface can be helpful as students work through their thinking as a team. Their final "story" can then be presented from the whiteboard.

Juicy questions

(EXAMPLE) What are the optimal conditions for soft rot development?

Teams should be able to use their observations from the guided inquiry to help answer this question.

(EXAMPLE) Why was it important to 'inoculate' potatoes with blank toothpicks?

Storyboard Discussion (continued)	
What is the purpose of a storyboard? Storyboarding consolidates "the evidence" so students can consider how their data fits into their models of plant disease. By **sharing** their stories and allowing other students to **question** their conclusions in light of data, observations, experiments, or everyday and school science experiences, students learn to **reconcile** evidence as scientists do. This process is sometimes referred to as **scientific thinking**.	
The goals of the guided inquiry lab are: • Establish proper inoculation methods • Establish best incubation methods • Understand the importance of positive controls • Understand the importance of negative controls	This may be a good time to remind students of the importance of controls when doing a scientific investigation. **Key questions** • What constitutes evidence? How do the group's data fit into its own model of the disease triangle? • In what ways is the method limited? • Can the method be improved?
Product At the end of the discussion, the class should arrive at a consensus model for optimal growth conditions for soft rot bacteria in potato slices.	