

Role of Crop Genetic Diversity on Pathogen Impact: The Tale of Two Pathogens

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Abstract

Introductory genetics courses are part of the core curriculum in many different fields, including plant breeding, animal science, biology, microbiology, and natural resource management. Concepts involving genes, inheritance, evolution, and genome editing are foundational to both modern biology and agriculture. Understanding these concepts is not only important for training scientists but also for citizens who will make personal health and consumer decisions. For this learning to happen, however, we need to use evidence-based education practices to bring our teaching of the biological and agricultural sciences into the 21st century. This case study uses historical plant pathogen epidemics, such as the Irish potato famine, to guide student learning about how genes are passed from one generation to the next, the advantages and disadvantages of different farming strategies, and how the interactions between a disease-causing organism, its host, and the environment lead to epidemics. In learning about plant disease outbreaks, students also learn basic genetics and crop breeding concepts. This case study also provides teachers with instructions on how to evaluate host, microbe, and environmental data with the students and also guides student groups as they design and discuss plans to optimize yield while minimizing the risk of crop loss due to disease.

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Supporting Materials: Supporting Files S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives; S2. Tale of Two Pathogens – Interactive Lecture 1 – SCLB and PLB; S3. Tale of Two Pathogens – PLB Homework; S4. Tale of Two Pathogens – SCLB Homework; S5. Tale of Two Pathogens – Jigsaw Table Exercise; S6. Tale of Two Pathogens – Interactive Lecture 2 – Interactive Introduction to Disease Triangle; S7. Tale of Two Pathogens – Data Analysis – Environment; S8. Tale of Two Pathogens – Data Analysis – Host; S9. Tale of Two Pathogens – Data Analysis – Pathogen; and S10. Tale of Two Pathogens – Interactive Lecture 3 – What Really Happened?

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Learning Goals	Learning Objectives
Students will:	Upon completion of this activity, students will be able to:
 Understand how the disease triangle of host, pathogen, and environment determines progression of plant disease. Be able to explain how genetic diversity can decrease and its impact on host-pathogen interactions 	 Explain how crop species become monoculture. Correlate crop genetic diversity with disease resistance and/or susceptibility. Compare and contrast mitochondrial and nuclear inheritance. Compare and contrast sexual and asexual reproduction, using plants as an example.

INTRODUCTION

As Earth's population continually increases, there is a corresponding demand for safe and healthy food (1). One of the most critical challenges in food production is protecting food sources from disease, weeds, and insect pests while maintaining soil health and managing risks associated with a changing climate. An extremely valuable resource for addressing food protection needs are undergraduate students who will become future plant health professionals, providing pest management solutions through industry, extension, and public service venues. Unfortunately, there is a national shortage of people entering and persisting in both STEM and biological disciplines. The

supply of graduates in life sciences, food sciences, and plant and soil sciences is not sufficient to meet current and projected demand for skilled professionals in these fields (2). Therefore, high-quality, high accessibility undergraduate biology education resources must be developed to begin to address these critical workforce needs. One way to combat this shortage of plant health professionals is to inspire interest in these fields by exposing students to the crucial roles that plants play in our daily lives. To begin addressing this concern, we have developed an interrupted case study to help students explore the world of plant health and disease while also providing real-world examples of historically relevant plant disease epidemics and their consequences.

Potato late blight (PLB) and southern corn leaf blight (SCLB) are two disease epidemics that garnered worldwide attention and brought the issue of genetic diversity in crops to the forefront. PLB is a common and often devastating disease of Solanaceae species. During the 1800s, many people in Ireland subsisted on potatoes as their main nutritional source. Unfortunately, almost all potatoes grown in Ireland were of one specific variety, the Irish Lumper (3). While easy to grow, high yielding, and hardy even in cooler wetter seasons, this single variety of potato was grown as a monoculture, with potatoes from one season being cut into small pieces and clonally propagated the subsequent season. One danger of growing crops in a monoculture is that a plant pathogen, capable of infecting a specific variety, can cause total devastation to an entire crop relatively guickly (4). The lack of genetic diversity of the crop leaves all plants susceptible. Typically, in contrast, for a polyculture cropping system, some varieties have naturally occurring resistance genes that can block disease progression; thus, crop loss may be significant but typically not absolute.

Phytophthora infestans is an oomycete water mold that resembles a fungus. *P. infestans* is also a plant pathogen and the causative agent of PLB. In the 1840s, *P. infestans* made its way to Ireland via the United States, having originated in central Mexico (5). Unfortunately, this pathogen was particularly damaging to the species of potato grown in Ireland and decimated crops for a number of consecutive years. The impact was immediate, extreme, and devastating, with Ireland's population dropping by over one-third by the 1850s, half through death and half through migration (3). Investigations as to the cause of the Irish potato famine were the start of Germ Theory in plants and the discipline of plant pathology (6).

Having not learned the lessons from the perils of potato monoculture in Ireland, corn production in the United States fell victim to similar crop devastation in 1970. Commercial corn production in the early to mid-1900s in the United States led to many lines of inbred corn that could be crossed, yielding a single generation of highly productive seed with uniform traits, termed hybrid vigor or heterosis (7). This process led to the discovery of a line of corn in Texas (Texas cytoplasmic male sterile, or cms-T) which was male-sterile, unable to produce viable pollen (8, 9). A male-sterile line of corn was exceptionally valuable to breeders, eliminating the need to pay people to de-tassel the production lines used to produce hybrid seed. Unfortunately, this led to 85% of the corn grown in the United States employing the cms-T system by 1970, another extreme example of monoculture (8). At the same time, a new physiologic race of the SCLB fungus (sexual stage termed Cochliobolus heterostrophus, asexual and in-season infectious stage termed Bipolaris maydis) had evolved that was extremely virulent on cms-T. Ideal warmer, wetter conditions in the spring and early summer of 1970 enabled this pathogen, B. maydis, to devastate corn production with average yield loss in the "Corn Belt" states of 20-30%, and some places experiencing losses as high as 50-100% (8). In terms of actual food loss, the energetic loss was considered greater than that of the PLB epidemic in Ireland, and economically, losses exceeded \$1 billion. After many years of investigation, researchers discovered that the mitochondrial genome of cms-T maize, which is maternally inherited, contains a unique gene designated T-urf13 (10-12). T-urf13 encodes a 13-kDa pore-forming protein (URF13), which is the source of the male sterility trait, and also confers sensitivity to B. maydis (13, 14).

Although the use of the cms-T system for breeding hybrid corn was a huge benefit in terms of time, cost, and labor, the resulting SCLB epidemic caused devastating losses to growers in the U.S. In Ireland, the PLB epidemic likewise led to overwhelming starvation and loss of life. This lesson was designed as an engaging three-class period exercise intended to be taught at the undergraduate level. Students began the activity by completing a short (~10 minute) pre-lesson assessment of their background knowledge related to plant disease. Students watched a short video introducing the concept of plant disease followed by the first interactive lecture with multiple "stop and ask" segments. Having set the stage for plant disease investigation, students were split into multiples of five and given information handouts to help them become experts on the host crop, pathogen, or environment to later teach their assigned group. The second-class period began with the Jigsaw exercise as the experts from each group shared their knowledge with the other team members. The directed questions and conferencing aided student familiarity with the material and ensured no students were left behind or left out. Filling out the Jigsaw Exercise Table in their small groups proved to be a valuable experience, promoting active discussion by comparing lines of reasoning (Supporting File S5. Tale of Two Pathogens – Jigsaw Table Exercise). This became the segue to the second interactive lecture, a focus on the disease triangle. Groups then decided on which variable of the disease triangle was most important for each epidemic. Homework assignments of model experimental data were then distributed based on the chosen variable; the guiding questions helped students examine the issue through the lens of the scientific process. The third class period started with students reviewing the conclusions and decisions they made for their homework and a group brainstorming session about possible strategies to reduce disease impact. Groups then posted their plans to Padlet and did a gallery walk to learn how the other groups decided on a strategy to combat the disease. The last interactive lecture revealed how things really turned out for the PLB and SCLB epidemics and spurred a thoughtful discussion about the genetic diversity in our crops today and the importance of plants in relation to human health. The post-lesson assessment was assigned as homework and suggested students made learning gains in understanding the importance of plant diseases and risks of genetic uniformity.

An area of significant concern related to biology education is the general lack of understanding of how important plants are to almost every aspect of our daily lives (15). One powerful way to combat this issue is to promote engaged learning with real-world context as to the value of plants. While <u>case studies</u> <u>related to plant disease</u> are available, they are relatively few in number. This lesson is the beginning of a series on plant diseases that we hope will offer teachers well-designed teaching tools to help open student's eyes to the importance of plants and also offer a glimpse into the many great careers that are associated with plant health.

Intended Audience

While this lesson was taught in an introductory plant pathology class and reviewed by introductory general biology students in the context of plant diversity (both courses were taught at a large R1 university), we believe this lesson is appropriate for a wide variety of biology courses. The real-world context of diseases of plants and their impact of human health provides valuable insights for students learning about the importance of genetics, breeding programs, pathogens, and their impacts on society.

Required Learning Time

This lesson consists of full three (50 minute) class periods, with student homework assigned both before the first class period and in between class 1 and class 2. There are also optional pre and post instruction assessments that can be delivered online via a course management system, such as Canvas or Blackboard.

Prerequisite Student Knowledge

Before starting this lesson, students should be able to:

- compare and contrast animal and plant reproduction
- describe the theory of evolution by natural selection

Prerequisite Teacher Knowledge

Instructors should be familiar with managing active learning in their classrooms, including

- group discussion followed by large-group debriefing
- managing a gallery walk of student ideas
- guiding students to accurately interpret data in graphs and tables

While not required, instructors should also be familiar with the concepts of the disease triangle (16) potato reproduction, and diverse vs. monoculture cropping systems. These topics are explained in the student handouts, teacher notes, and lecture materials.

SCIENTIFIC TEACHING THEMES

Active Learning

Students actively engage in two separate interrupted case studies (17). During the first class, students are sorted into groups of 5, with each person in the group responsible to read a short summary of both (SCLB in 1970 & PLB in the mid-1800s) and to become an expert in a specific aspect of each case. Using this jigsaw method(18), students are grouped by expertise at the start of the second class to compare notes within each area. For example, all of the corn breeding experts confer with each other while all of the SCLB pathogen experts confer with each other at the same time. Students then join their original groups to provide needed information to fill out a table and concisely organize the information from the two situations (Supporting File S5. Tale of Two Pathogens – Jigsaw Table Exercise). After a brief interactive lecture describing the disease triangle (Supporting File S6. Tale of Two Pathogens – Interactive Lecture 2 – Introduction to Disease Triangle), students are asked to consider how host, environment, or pathogen contributed to each devastating outcome. They leave class with experimental data that they are asked to interpret using guiding questions (Supporting Files S7-9. Tale of Two Pathogens - Data Analysis). At the start of day three, they bring their conclusions to their groups, discuss their findings and those of others. Each group then evaluates the evidence and prepares a brief Padlet post summarizing the group's conclusions to share with the entire class in a gallery walk (19).

Assessment

Prior to this lesson, students complete a multiple-choice pretest (Supporting File S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives) designed to probe student understanding of the learning objectives. After the completion of this lesson, the students take the same assessment to measure learning gains. The products of student activity (summary table and gallery walk post) are also used as formative assessment artifacts to both guide student learning and assess student readiness for summative assessment.

Inclusive Teaching

Using the Jigsaw method leverages different student abilities and perspectives, as students are given the opportunity to learn about an aspect of each story, confer with their peers, and then share this information within their groups. The "expert conference" step enables students of varying abilities to demonstrate their learning and learn from others at the same time. The data analysis exercise on day two also illustrates the value of different perspectives and uncertainty in science.

The gallery walk simulates a brief scientific poster session with students asking and answering questions of their peers. Providing students with the opportunity to practice the skills of being a scientist increases their feelings of belonging and identity as scientists (20).

LESSON PLAN

Preparation for Class

Pre-test Assessment of Student Prior Knowledge

The short pre-test (Supporting File S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives) can be used to assess the prior knowledge of students in the classroom. If administered by a learning management system (LMS), such as Canvas or Blackboard, faculty can quickly review results to identify strong and weak points in student understanding prior to starting the first class session. Key reminders are the difference between sexual and asexual reproduction as well as transmission genetics. This is also a good time to set up a Padlet board for the gallery walk held during the third class session.

Class Session 1 Introducing the Problem

Interactive Lecture 1

The first class session begins with a short video (Plants get sick) and specific details about the Irish Potato famine and *Phytophora infestans*, the causative agent of PLB. This is followed by a more modern example, the SCLB (*Cochliobolus heterostrophus*, asexual stage: *Bipolaris maydis*) (21) which devastated corn harvests in 1970.

Throughout class, the instructor asks the class to compare and contrast the two epidemics in terms of host (potatoes vs corn), pathogen (fungus (SCLB) vs oomycte (PLB)), and environment. This interactive lecture in Supporting File S2. Tale of Two Pathogens – Interactive Lecture 1 – SCLB and PLB.

Setting up Expert Groups & Assigning Take-Home Work

In a class of less than 50, students count off using the following formula (total # of students/5 = number of to count). There should be 5 students in each group. Each group discusses the findings from the mini lecture and decides on roles for each group member. Each group should have a potato expert, corn expert, *Phytophora infestans* expert, *Bipolaris maydis* expert, and an expert studying the environment of the United States in 1970 vs Ireland in the mid-1800s. The entire class then picks up and pre-reads their handouts (Supporting Files S3. Tale of Two Pathogens – PLB Homework and S4. Tale of Two Pathogens – SCLB Homework) describing both the epidemics before leaving for the day.

Class Session 2 Leveraging Expertise and Introducing Plant Pathology Concepts

Expert Group Conference

Using a Jigsaw method, the instructor sets up 5 areas for each expert group to meet. All of the corn experts compare notes, as do all of the potato experts and so on. The experts compare notes and prepare to inform their original groups. Leading questions at this stage are "What two things do all of you think are important?" and "What one thing did you think was relevant that the rest of the group missed?" Using these directed questions encourages focused discussion of the findings from the assigned reading. Conferring with fellow experts helps all students gain confidence and check their responses with others. During this time, faculty and learning assistants can circulate throughout the room (or breakout rooms if this is done online), directing students to share their expertise.

Gathering Groups and Jigsaw Assembly

Students return to their original groups of 5 and confer with each other to complete the graphic organizer found in Supporting File S5. Tale of Two Pathogens – Jigsaw Table Exercise. By filling out the table, students can compare and contrast details about the pathogen, host, and environment in both the SCLB and PLB epidemics.

Interactive Lecture and Class Discussion

The instructor reviews the table (Supporting File S5. Tale of Two Pathogens – Jigsaw Table Exercise) as an introduction to the second interactive lecture (Supporting File S6. Tale of Two Pathogens - Interactive Lecture 2 - Introduction to Disease Triangle). The idea that disease is caused by the intersection of vulnerable host, virulent pathogen, and a favorable environment is a foundational concept of both plant pathology, the study of plant diseases, and crop management. Most formal crop management plans are designed to interrupt one or more of these factors. This exercise is designed to introduce these concepts. At the close of this mini-lecture each group decides which of the three variables was most important for these two epidemics. The groups are then given one of three homework assignments. Each homework provides details of experiments and experimental data that support one of the three sides of the disease triangle (host, pathogen, environment). These assignments are found in Supporting Files S7-S9. Tale of Two Pathogens – Data Analysis. Students use the data given to answer guiding questions and draw evidence-based conclusions about the importance of each variable. If there is time, students can work through their data together or as homework.

Class Session 3 Solving the Problem (Gallery Walk)

Group Report & Brainstorming

At the start of the third class session, students review their homework in their groups, discussing the experiments, conclusions, and the answers to the guiding questions. Students then brainstorm ideas to prevent or lessen the impact of each plant disease. They design a strategy to reduce the impact of plant disease and provide evidence and reasoning to justify their choices. Key concepts for *Phytophora infestans* are that there is variation between the different isolates, the oomycete thrives in moist, cool climates, and that potatoes are grown by clonal propagation, which means the genetic variety in the potato fields was minimal in the 1840s. Key concepts for the *Bipolaris maydis* fungus are the T toxin it produces, that affects the mitochondria of corn, the warm, wet summer in 1970, and the genetic uniformity of the corn host plants. Corn in the 1960s and up to 1970 had been bred using T-cytoplasm as female parents, and thus, did not have functional pollen, so the crop breeders could control mating between corn plants and achieve designed traits (yield, height, resistance to drought, etc.). Unfortunately, this corn all had the same cytoplasmic traits and mitochondria, which were especially susceptible to the *B. maydis* T toxin.

Padlet Gallery Walk

Once each group has created their plan, they post it on <u>Padlet</u>. Students from the class read over the posted ideas and vote (via Padlet) on the ideas they think would work. This gallery walk simulates a short poster session, with students reviewing and being reviewed by their peers. This collaborative process is a key element to the nature of science (22).

Interactive Lecture #3 Instructor Debrief: What really happened

Once students have voted for the strategy they think would work best, the instructor reviews what happened in the years after each plant disease epidemic (Supporting File S10. Tale of Two Pathogens – Interactive Lecture 3 – What Really Happened?). Anton deBary's experiment using the same potatoes, same environment, but exposing the experimental group to infected plant tissue and not exposing the control group demonstrated that PLB was caused by an unseen pathogen. In modern times, fungicide is used to control this pathogen. This is very important because this pathogen can now reproduce sexually, which may lead to resistance to fungicides if the pathogens are allowed to grow unchecked. Since the T-cytoplasm and male-sterile corn plants were especially susceptible to disease, plant breeders stopped using these breeding lines to prevent the devastation of the 1970 SCLB epidemic. Detasseling was again needed, to create hybrids and prevent crops from self-fertilizing. Uniformity in both corn and potatoes remains a problem in the 21st century.

Post Lesson Assessment

The day after the third class session, instructors can assign the multiple-choice post test (Supporting File S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives) to determine student learning gains.

TEACHING DISCUSSION

This lesson was first employed early-semester in an undergraduate plant pathology course to help students more fully appreciate why an understanding of plant health and plant disease management is a vital field of study, relevant to all. After the lesson was completed, students provided written feedback and comments about; what was most effective, what did they learn from this lesson, what they would have liked to see more of, and what they felt was not effective. This valuable and constructive feedback was incorporated into the final version of the lesson. Structurally, the three class period arrangement proved to be a reasonable time commitment and was used in place of two traditional lectures that introduced plant disease and the disease triangle, respectively. The initial video, "Plants get sick" could also be assigned as pre-lesson homework if the class size is particularly large and organizational time for building and organizing groups is perceived to be a potential concern.

Student reflections on the lesson generally suggested some background knowledge of plant health but a much shallower understanding of monoculture and genetic diversity in our crops. The Jigsaw exercise and the Padlet galley walk both were well received and considered useful. While the group activities were a bit slow to get students actively engaged, they quickly began to discuss a variety of on-topic and peripheral discussions. For a larger classroom setting, it would be valuable to have a couple teaching assistants to walk around to answer group questions and, perhaps more importantly, keep group conversations on topic.

Taken together, the deployment of this lesson was cohesive, concise, and fit the level of complexity for an undergraduate course. The interactive lectures and group work utilized both lower and higher order levels of Bloom's taxonomy and suggested an appropriate blending of delivery and learning styles. The lesson was also informative in gauging student background knowledge and general interest in the field, an unanticipated benefit that aided planning the remainder of the semester's course content.

SUPPORTING MATERIALS

- S1. Tale of Two Pathogens PrePost Test MC Assessment of Learning Objectives
- S2. Tale of Two Pathogens Interactive Lecture 1 SCLB and PLB
- S3. Tale of Two Pathogens PLB Homework
- S4. Tale of Two Pathogens SCLB Homework
- S5. Tale of Two Pathogens Jigsaw Table Exercise
- S6. Tale of Two Pathogens Interactive Lecture 2 Introduction to Disease Triangle
- S7. Tale of Two Pathogens Data Analysis Environment
- S8. Tale of Two Pathogens Data Analysis Host
- S9. Tale of Two Pathogens Data Analysis Pathogen
- S10. Tale of Two Pathogens Interactive Lecture 3 What Really Happened?

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Table 1. Lesson Timeline. The lesson best spans three class sessions as well as outside of class time for preparation and independent student activity.

Activity	Description	Estimated Time	Notes
Students take short pre-test	Pre-Test (on LMS) (Supporting File S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives)	20 minutes	Students take the Pre-test at least 1 day before starting this lesson.
Class Session 1	• •	·	
Interactive Lecture and short video	Overview of Potato late blight and Southern Corn Leaf Blight (S2. Tale of Two Pathogens – Interactive Lecture 1 – SCLB and PLB)	25 minutes	Iowa PBS – "Plants get Sick"
Set up Expert Groups	Organize class into groups of 5	15 minutes	Each group should identify a potato, corn, <i>Phytophora infestans, Bipolaris maydis</i> , and environment (1970s & 1840s) expert.
Hand out Homework	Brief summary of the Potato late blight (PLB) and Southern Corn Leaf Blight (SCLB) Epidemics with leading questions (S3. Tale of Two Pathogens – PLB Homework and S4. Tale of Two Pathogens – SCLB Homework)	10 minutes	Students read the entire document, while taking note of their area of expertise, as this is what they will be reporting on.
Class Session 2			
Expert Conferencing	Set up the classroom into 5 groups of experts, so all of the experts of the same type are taking together	10 minutes	Students have an opportunity to share their findings and learn from others of the same expert group.
Jigsaw Assembly	Students return to their groups of 5, confer with each other to fill out Table from S5. Tale of Two Pathogens – Jigsaw Table Exercise	15 minutes	Students collaborate and share their expertise with the group.
Jigsaw Debrief	Instructor leads discussion of Jigsaw Table Exercise	5 minutes	Students from all groups contribute to completing the worksheet as a class.
Interactive Lecture	Introduce the Disease triangle, using the SCLB and PLB epidemics as examples (S6. Tale of Two Pathogens – Interactive Lecture 2 – Introduction to Disease Triangle)	10 minutes	Think-Pair-Share groups answer questions about the disease triangle in the context of SCLB, PLB
Set-up for Class #3	Class Discussion: Students evaluate the relative importance of host, pathogen, and environment	5 minutes	Ask the class two Questions: Which aspect of the disease triangle was most important for these epidemics? What experiment(s) would support your hypothesis?
Homework	Provide students with evidence for each of the 3 aspects of the disease triangle being important	5 minutes	Different homework assignments (S7-9. Tale of Two Pathogens – Data Analysis) are passed out. Each one supports a different aspect of the disease triangle with leading questions to guide student interpretation of the graph or table provided.
Class Session 3			
Group Discussion	Students summarize the data from their homework and share it within their groups	15 minutes	Quick summary of the key findings for each variable: host, pathogen, environment
Group Brainstorming	After discussing the data, students design a strategy to reduce the impact of SCLB and PLB and post it on Padlet for a Gallery Walk	20 minutes	Students post their ideas on Padlet or similar whiteboard system. Each group reviews and comments on the other group's posts, voting (with likes) on which plan they like the best.
Instructor Debrief: What really happened?	Interactive lecture describing how SCLB and PLB were eventually controlled. (S10. Tale of Two Pathogens – Interactive Lecture 3 – What Really Happened)	15 minutes	Since these are both true stories, students can compare their ideas to expert conclusions and discuss genetic diversity of crops in modern times.
Post Lesson Asse	ssment		
Students take short Post-test	Post-Test (on LMS) (S1. Tale of Two Pathogens – PrePost Test - MC Assessment of Learning Objectives)	20 minutes	Students take the Post-test 1-2 days after completing the lesson