Disease in the Squeeze

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Lesson___ Adapted from

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Additional information regarding the CATALySES project is available at https://www.cpet.ufl.edu/teachers/catalyses/.

Additional information regarding the Summer Research Experience at https://www.cpet.ufl.edu/teachers/sre/.

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Introduction

Human diseases are often discussed and represented in the media, but you don't often hear about plant illnesses. Despite the poor media coverage, it is important to know that plants get sick too! In fact, pathogens from every kingdom of life affect our crops. Humans have been battling plant diseases since the beginning of civilization and this battle is still going on today. Scientists are trying to understand, treat and prevent plant pathogens from causing disease but the average person has very little understanding of plant pathology. Industrialization and commercialization of agriculture has allowed for massive yields of crops while also allowing more people to be removed from their own food production. As a result, not only do less people understand what goes into production, but plant disease can spread rapidly through large fields and have a drastic impact on crop yields. This is the case with the Florida Citrus industry, which has been greatly impacted by diseases such as Citrus black spot, Huanglongbing (HLB), also known as citrus greening, Citrus canker and Citrus tristeza virus (CTV). Effort has been made to combat many of these diseases. Unfortunately, recent methods to prevent one disease might actually help promote another, as is the case with CTV. There has been such a focus on preventive measures for Citrus greening that the risk of Citrus tristeza virus (CTV) becoming a significant threat to the industry again is a real possibility. The diseases alone are negatively impacting the $9 billion dollar per year industry but changes in the environment are exacerbating the affects. With increases in severe weather events, yields could continue to decrease. Hurricane Irma in September of 2017 made the reduction in production from the combination of disease and extreme weather a reality.

Author's Note

In this curriculum, students will follow the story of a young girl whose life is changed by the impact of disease in the citrus industry in Florida. Students will have the opportunity to understand what is happening to the local crops, the citrus industry, and the community she lives in. Several clinical tests will be performed to diagnose what diseases are contributing to the reduction in crop yield. Using a simple model, the students will also simulate the spread of disease through a field and graph the resulting data. Finally, the students will develop a plan to combat the disease and prevent further infection.

This particular topic was chosen as a result of a grant from the National Institutes of Health Science Education Partnership Award to the UF Center for Precollegiate Education and Training. As part of a two-week fellowship, Dr. Svetlana Folimonova, an associate professor in the UF Plant Pathology Department and Emerging Pathogens Institute, has allowed teachers to intern in her lab to learn about specific plant diseases, current research, and lab technique. Dr. Folimonova has also participated in a workshop for the UFCPET teachers for the past year called "Plants Get Sick Too!" During the workshop, Dr. Folimonova discusses the diversity of plant viruses and the characteristics of several common plant viruses. Dr. Folimonova does research on Citrus Tristeza Virus (CTV) as well as Citrus Greening or Huanglongbing (HLB). Disease is impacting the citrus industry in Florida substantially; But massive weather events, such as Hurricane Irma in September of 2017, are making matters worse. Many farmers are no longer able to continue their way of life. They are having to cut jobs or important fruit. The increase in prevalence of disease and weather events affecting the citrus industry is impacting our food production, economies, and communities. Although there is still a lot of work and research to be done in the field, especially with diseases such as citrus greening, investigations are being conducted to identify ways to prevent the spread of disease. Will such advancements in prevention occur before the Florida citrus industry is devastated to a point of no return?

Tips about this Curriculum

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.

OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list.

MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.

ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.

PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.

EXTENSIONS: (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available.
STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.
TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.

Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in teacher-driven, lecture format, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

Groups: Most of the lessons are carried out in groups. While it isn't necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

Inquiry-based: The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

Technology: Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

Content: This unit provides an opportunity to synthesize discrete content facts into an authentic context. Students take concepts learned such as immune response and clinical testing procedures, and put them in the context of disease. The lessons aren't designed to teach students the intricate details of the immune system or determining an index case in an outbreak, but rather *why* these ideas are important and *how* researchers can use them.

Implementation notes: This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

Extensions: There are many opportunities to expand the lessons presented here. To help students understand the importance of vector control and mosquito biology, a concurrent activity of rearing mosquitos can help the students understand the life cycle of the vector host and its prevalence in the environment. Additionally, you may wish to expand on the idea of vaccine development, both the difficulties related to dengue and the history and current controversy over vaccination in the United States. Service projects would be a natural extension, particularly in areas with large mosquito populations.

Science Subject: Biology

Grade and ability level: 9-12 students in all levels of biology

Science concepts: virus, disease transmission, vectors, antibodies, antigens, DNA, proteins, replication, immune response, transcription, translation, ecology, Bacteria, Fungi, Protista

Lesson Summaries

Curriculum Summary: In this curriculum, students will follow the story of a young girl whose life is changed by the impact of disease in the citrus industry in Florida. Students will have the opportunity to understand what is happening to the local crops, the citrus industry, and the community she lives in. Several clinical tests will be performed to diagnose what diseases are contributing to the reduction in crop yield. Using a simple model, the students will also simulate the spread of disease through a field and graph the resulting data. Finally, the students will develop a plan to combat the disease and prevent further infection.

Lesson One: Plants Get Sick Too!

This lesson will begin with a basic overview of the different types of pathogens that cause plant disease including specific types that infect citrus crops in Florida. Students will be given summary identification cards for each disease including pictures of the pathogen and infected plant tissue. Students will then read 3 short case studies/epidemiology reports. They will diagnose each case study/report with the appropriate disease using the information on the cards.

Lesson Two: Plant Disease Epidemiology

Students will be given a "plot" game board where they will spread out different color beads. With their eyes closed, they will draw out 2 beads at a time and depending on the bead color the students will add more of a specific color bead. Using two scenarios and three different color beads, students will model the spred of disease through a field. They will then graph & analyze the data. Finally, students will study the spread of the epidemic using a network model diagram of the field.

Lesson Three: Environmental Impacts

In this lesson students will investigate how changes in the environment, like extreme weather, climate change, and other human impacts affect crop production and the spread of disease in the citrus industry. Students will be given two case studies or articles to summarize and analyze to predict further effects on the industry.

Lesson Four: Citrus tristeza virus (CTV) & The Central Dogma

Students will be gain more detailed background information on CTV and the cell processes involved in the viral life cycles (replication, transcription, translation). Then they will be given pictures and terms of replication, transcription, translation, CTV virus structure, and cell structural components involved the infection. Using these pictures and terms, students will create a concept map showing how the concepts are related and what's happening in the process.

Lesson Five: Testing for CTV

Using "field" collections from the surrounding community or ordered samples, students will test for CTV using a lateral flow assay (ImmunoStrip test) to determine if the grove has plants that test positive with CTV.

Lesson Six: Diagnosis & Prevention

Students will read and analyze a report from samples that were "sent" to the Plant Diagnostic Center. They will use the report and the information they learned during the other lessons within the unit to write up a summary report. The summary report will contain the diagnosis from tests of their "field samples" and a plan of attack to prevent further spread of disease.

Lesson Sequencing Guide

Classrooms vary, therefore, so can the sequence of lessons and the amount of time spent on each lesson. The instructor can manipulate the pacing and sequence to best fit the needs of the students and the classroom setting. The pacing guide below can be used when planning and implementing the curriculum, assuming 50-minute class periods.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Week 1	Lesson 1 Plants Get Sick Too! (50 minutes)	Lesson 2 Plant Disease Epidemiology (50 minutes)	Lesson 3 Environmental Impacts (50 minutes)	Lesson 4 Citrus tristeza virus (CTV) & The Central Dogma Part 1 (50 minutes)	Lesson 4 Citrus tristeza virus (CTV) & The Central Dogma Part 2 (25 minutes)
Week 2	**Day 6**	**Day 7**			
	Lesson 5 Testing for CTV Part 1 (50 minutes)	Lesson 5 Testing for CTV Part 2 (25 minutes) Lesson 6: Diagnosis & Prevention (25 Minutes)			

Disease in the Squeeze Curriculum Outline

Lesson Number	Time	Overview of Lesson Learning Performances & Learning Tasks	Assessments
Lesson 1 (Start actual lessons here)	1 (50) minute class period	Plant Disease overview, types of Florida citrus diseases, history, and their characteristics summary identification cards for each disease with pictures, students then get 3 case studies/epidemiology reports and they have to diagnosis the case study/report with the disease they think it is based on the information on the cards	Check student diagnosis by walking around room and looking at or collecting student work comparing to rubric for correct diagnosis. Have students complete PPM – what do they know about Plant Pathology
Lesson 4	1.5 (50)	Detailed Specific viruses investigation –	Check student understanding

	minute class period	CTV & TMV, overview of replication, transcription, translation Students will be given background information on the viruses and the cell processes involved in their life cycles (replication, transcription, translation) Then they will be given pictures/words of replication, transcription, translation, CTV & TMV virus structure, and cell structural components involved. They will create a concept map showing how they are related and what's happening in the process and with those structures.	by walking around room and looking at student work (concept map) comparing to optional rubric
Lesson 3	1 (50 Minute) class period	Investigate how changes in the environment, like climate change, and other human impacts affect the spread of disease in the citrus industry – Students will be given 2 case studies or articles, they will summarize and predict further affects. Maybe analyze graphs/data, create a concept map with sticky notes that were written in pairs with important concepts, answer prewritten Qs about the articles?	Check student analysis and understanding by walking around room and looking at or collecting student work comparing to optional rubric
Lesson 2	1 (50 minute) class period	Model spread of disease through a field, graph data & analyze. Students will be given blue beads (representing healthy) & white beads (representing infection). They will draw out 2 beads at a time and record data. In the second scenario there will be a third type of bead added (red bead) which will	Check student understanding by walking around room and looking at or collecting student graph work and analysis comparing to rubric

		represent inoculum (alternative sources of the pathogen).	
Lesson 5	1.5 (50 minute class periods)	Field Collection & Diagnosis- collect samples from home & test for CTV (Lateral Flow assay “ImmunoStrip Test”), “send” sample to Diagnostic Center for further analysis. Students will perform the Lateral Flow Assay (ImmunoStrip Test) using citrus plant samples from their yard or community or ordered from company.	Check student understanding by looking at or collecting quiz about ELISA & Lateral Flow assay also checking student lab work comparing to expected results on rubric
Lesson 6	0.5 (50 minute class period)	What is the diagnosis and prevention plan? Students will use their experimental data/ diagnostic report data and the information they learned during the unit to write up a report of what the diagnosis was of their “field samples” and a plan of attack for the future.	Check student understanding by collecting student work and having them discuss their diagnosis and prevention plans. Also final PMM – What do they know about Plant Pathology?

Modified without background and model/practice with N. benthamiana = 7 (50 minute) class periods = 1.5 weeks

Vocabulary

Phytopathology or plant pathology: the study of plant disease.

Wilt:

Spot:

Blit:

Rot:

Canker:

Patches:

Decline:

Gall:

Stunting or dwarfing:

Mosaics or mottles:

systemic acquired resistance (SAR)

Disease: any physiological abnormality or significant disruption in the “normal” health of a plant. Disease can be caused by living (biotic) agents, including fungi and bacteria, or by environmental (abiotic) factors such as nutrient deficiency, drought, lack of oxygen, excessive temperature, ultraviolet radiation, or pollution.

microbe- or pathogen-associated molecular patterns (MAMPs) recognition of conserved molecules characteristic of many microbes.

Vein banding: occurs when there is a band of yellow tissue along the larger veins of the leaf. This symptom is observed with viral diseases and is in contrast with nutrient deficiencies which may cause a dark green band along leaf veins

hypersensitive response (HR)

plant activators

herbivory

Ring spots:

viruses

viroids,

bacteria

phytoplasmas

fungi

nematodes

protozoa

oomycetes

parasitic plants.

Cultivar: varieties of plants from selective breeding

Innate or basal resistance

papillae

Budding

Buds: Small lateral or terminal protuberance (undeveloped or embryonic shoot) on the stem of a vascular plant that may develop into a flower, leaf, or shoot. Buds arise from meristem tissue.

Superinfection exclusion or homologous interference: a phenomenon in which a preexisting viral infection prevents a secondary infection with the same or a closely related virus.

ELISA: stands for Enzyme-Linked ImmunoSorbent Assay and is a biochemical reaction based technique used to detect antibodies or antigens.

Lateral flow assay (LFA) or Immunoassay: biochemical test that measures the concentration of a substance in a biological

substance, using the reaction of an antibody to its antigen.

Strain: an isolate that can be distinguished from other isolates of the same genus and species by phenotypic characteristics or genotypic characteristics or both'

Next Generation Sunshine State Standards - Science

Standard	Lesson					
	1	2	3	4	5	6
SC.912.L.14.52: Explain the basic functions of the human immune system, including specific/ nonspecific immune response, vaccines, and antibiotics.				x	x	
SC.912.L.14.7 Relate the structure of each of the major plant organs and tissues to physiological processes.	x					
SC.912.L.15.6 Discuss distinguishing characteristics of the domains and kingdoms of living organisms.						
SC.912.L.15.15: Describe how mutation and genetic recombination increase genetic variation.		x				
SC.912.L.16.3: Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.		x				
SC.912.L.16.5: Explain the basic processes of transcription and translation, and how they result in the expression of genes.		x				
SC.912.L.16.7: Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology		x		x	x	
SC.912.L.16.10: Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.				x	x	
SC.912.L.16.11: Discuss the technologies associated with forensic medicine and DNA identification, including RFLP analysis.				x	x	
SC.912.L.16.12: Describe how basic DNA technology (gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).						
SC.912.L.17.4: Describe changes in ecosystems resulting from seasonal variations, climate change and succession.			x			
SC.912.L.17.5: Analyze how population size is determined by births, deaths, immigration, emigration, and limiting factors (biotic and abiotic) that determine carrying capacity.				x		
SC.912.L.17.8: Recognize the consequences of the losses of biodiversity due to catastrophic events, climate changes, human activity, and the introduction of invasive, non-native species.	x		x	x		
SC.912.L.18.1: Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.		x		x	x	
SC.912.L.18.7: Identify the reactants, products, and basic functions of photosynthesis.						
SC.912.N.1.6: Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.						x
SC.912.N.3.5: Describe the function of models in science, and identify the wide range of models used in science.				x		
SC.912.N.4.1: Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.						x
SC.912.N.4.2: Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.						x

AP Biology Curriculum Alignment: Essential Knowledge and Science Practices

Standard	Lesson					
	1	2	3	4	5	6
Essential Knowledge 1.B.1 Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today.						
Essential Knowledge 2.D.1 All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.			x			
Essential Knowledge 2.D.3 Biological systems are affected by disruptions to their dynamic homeostasis.	x					
Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis	x					
Essential Knowledge 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information		x				
Essential Knowledge 3.C.3 Viral replication results in genetic variation, and viral infection can introduce genetic variation into the hosts.		x				
Essential knowledge 4.A.1 The subcomponents of biological molecules and their sequence determine the properties of that molecule.		x		x	x	
Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes		x		x	x	
Essential knowledge 4.B.1 Interactions between molecules affect their structure and function.				x		
Essential knowledge 4.C.4 The diversity of species with in an ecosystem may influence the stability of the ecosystem.			x	x		
Science Practice 1: The student can use representations and models to communicate scientific phenomena and solve scientific problems				x		x
Science Practice 5: The student can perform data analysis and evaluation of evidence.				x	x	x
Science Practice 7: The student is able to connect and relate knowledge across various scales, concepts and presentations in and across domains.						x
LO 2.43 The student is able to connect the concept of cell communication to the functioning of the immune system.		x				

Background Information

Citrus & Plant Structures

Plants are extremely important for food and products in everyday human life. Citrus, in particular, has been significant in trade for over 2000 years. Citrus trees are angiosperms which mean they produce flowers and seeds. This genus has been important for fruit production some of which includes citron, lemon, lime, orange, and grapefruit.

Citrus trees have long been grown in groves using a technique know as grafting. Grafting is done to combine two pieces of plant so that it functions as a single growing plant. The plants are deliberately grafted together by taking a cut piece from a donor plant that will grow into the upper portion of the plant and combining it to an opening in a pre-existing original root system. The donor upper portion is called the scion and the lower root portion is called rootstock. Budding is a type of grafting where a small patch of bark is removed from the scion with its bud and then it is carefully inserted beneath the bark of the rootstock. This fusion of plants creates a compound genetic system in which both parts are genetically different from each other. This technique is one of the oldest horticultural practices and it is still used today. There are several reasons for grafting. One of which is it insures that all plants have the same desired characteristics. It can also help producers to avoid delays in crop production. When new varieties of the same crop, or cultivars, are desired, growing them can occur much faster. Replacing damaged trees can also occur much faster. Rootstocks can help control the size of trees. One of the most significant reasons for using rootstock, especially in Florida, has been environmental tolerance and abiotic and biotic disease resistance. Grafting can also be used to purposely spread pathogens or to detect if a pathogen is present.

Citrus trees in Florida have been grafted since the 1830s to increase commercial production of desired crops also protecting crops from pathogens. Common examples of grafting in citrus would be sweet orange or grapefruit on sour orange rootstocks, however because of pathogens, farmers have had to use many different varieties of rootstock. Sour orange was a preferred rootstock for a long time because it produced high quality fruit and is resistant to many other citrus diseases like citrus blight, but because it is sensitive to Citrus tristeza virus (CTV) many farmers began to use other citrus rootstocks and scions such as rough lemon and mandarins. The introduction of citrus greening or Huanglongbing (HLB) has caused growers to return to using sour orange because it is more resistant to citrus greening.

Although budding can speed up plant growth by years, citrus can be gown from seed. For a long time citrus was transported as seed preventing the spread of CTV because it can only live in phloem tissue. The seed comes straight from the desired fruit. Fruit develops from the ovary within the flower and helps disperse and protect the seed. Flowers are the reproductive structures for the plant but for fruit producing plants they are called blossoms. Surrounding the flower is a modified leaf called a sepal which protects the inner structures. Petals are inside the sepals and are much larger. There purpose is to attract pollinators for fusion of the male and female gametes (fertilization). Inside the petals are the male and female structures know as the stamen and pistil. The stamen is the male reproductive system and it consists of a filament which is a long stalk and an anther which has the pollen (contain male sex cells) on the end of the filament. The pistil is the female part of the flower that come from the modified leaves known as the carpel. It that has the stigma, the top of the pistil that collects pollen, the style, the stalk that supports the stigma and leads to the ovary, and the ovary which becomes the fruit after fertilization. In gymnosperms, the plant group that contains conifers, cones are used as the reproductive structures instead of flowers.

Seeds need plenty of oxygen, sunlight, warm soil temperatures, and moisture and they can take about two weeks to germinate. When the seed germinates it will produce a radicle which becomes the root system and cotyledons or seed leaves. Citrus plants are Dicots, so they produce two cotyledons.

The production of leaves is very important for the plant to start photosynthesizing and producing its own nutrients. Until the production of leaves, the seed must rely on the endosperm where carbohydrates and other nutrients are stored as a temporary food source. Photosynthesis is essential for the function of the plant where light energy along with water and carbon dioxide is converted into sugars to be used and stored in the plant for cellular processes. The light for photosynthesis is absorbed into the leaf tissue. Dicots leaves have a large flat blade and petiole where the leaf attaches to base and stalk. Special tissues, called the mesophyll, in the leaf contain the palisade cells which is where there is an abundance of chloroplasts with chlorophyll. The chloroplasts are the specialized organelles that perform photosynthesis for the plant. Below this layer, the gases from photosynthesis and cellular respiration collect in what's called the spongy parenchyma. The spaces in the spongy parenchyma are connected to the stomata, which are openings in the underside of the leaf to allow exchange of carbon dioxide, oxygen, and water vapor. The movement of water vapor through these opening is essential for transpiration to move water and nutrients from the roots to the leaves in the vascular tissue. However, especially during warm periods, the plants can lose too much water so specialized guard cells surrounding the stomata help open and close it as need.

CTV can cause what is known as seedling yellows where the leaves turn yellow in color (chlorosis). This happens because there is a lack of minerals like iron which are necessary to produce chlorophyll, the photosynthesizing pigment that gives the plant a green color.

The radicle in the developing seed eventually becomes the tap root which consists of a root cap and an area called the meristematic region. The root cap protects the growing root and the meristematic region produces new cells that eventually differentiate into specialized tissues. Root hairs extend out from the root to increase water absorption and xylem cells develop and connect to transport water and nutrients to the rest of the plant. Sugars are transported by phloem cells which develop from live sieve tubes that are controlled by adjacent companion cells. Xylem and phloem make up the vascular tissue of the cell. Tree decline can result from death and blockage of phloem tissue. Since phloem is important for the movement of sugars produced from photosynthesis, the blockage prevents the roots from getting enough nutrients eventually causing death of the tree.

Stems develop from buds, or undeveloped shoots, and they help expose leaves to sunlight and transport essential compounds and nutrients. In older woody plant stems a cork cambium separates the vascular tissue and creates new phloem and xylem cells. Tree rings develop from the cambium pushing new phloem cells out while xylem is produced toward the inside. CTV causes a condition known as stem-pitting where pits form in the wood under the bark. This can affect the tree growth and production of the fruit because it causes the phloem tissue to grow irregularly.

Structural Defense

The outer coverings of the plant are significant as the first line of defense for preventing pathogens from entering. This protective tissue consists of the epidermis which is found in outside leaves, reproductive structures, fruits, seeds, stems, and roots. Special epidermal cells called trichomes, can prevent pest from feeding or repel them. The outside is also covered in a cuticle which helps prevent water loss and accumulation of water on the surface of the leaf. Guard cells are found in the outside surface of the leaf and they help open and close the openings for gas exchange called stomata. Along with changes in humidity, this opening can close in response to chemicals and molecules that are common in pathogens (MAMPs) but pathogens can get into the plant through stomata if they can evade that response.

Inside the plant tissue, cell walls provide a structural barrier around the cells to prevent infection. They consist mostly of long chains of cellulose and branched cross-linking glycans and pectins which help control the water content of the wall. These pectins are often the target of pathogens causing fruits to become mushy. The chemical lignin is another polymer in the cell wall, especially in wood, that makes it rigid and protects the plant from pathogens. The cell wall plays an important roll in the basal defense response of the plant by detecting and reacting to pathogens. An example of this is when chemicals, called papillae, are deposited at the site of infection to prevent the pathogen from getting into the cell.

Other Plant Defenses

Much like humans and animals, plants have developed defenses against pathogens. Plant immune systems, however, are not comparable to animals. What is similar is that there are innate and passive responses as well as acquired and active responses. Passive defenses consist of preventing production of some of the proteins the viruses need to use in the host for reproduction or movement. Active defenses consist of recognizing and responding to pathogens. A response could be the production of chemicals, pathogen-degrading enzymes, protein inhibitors, or destruction of virus-infected cells. Plant active defense methods have a high energy cost, so they often wait until the pathogens are detected inside the cell before initiating a chemical response.

Innate or basal resistance includes recognition of all pathogens using microbe-associated molecular patterns (MAMPs). This includes recognizing certain molecules that are common in microbes such as specific proteins, lipopolysaccharides, and cell wall components; however, sometimes even non-pathogen particles elicit a response. If a pathogen is able to suppress this response in the plant, then the more specific hypersensitive response (HR) will cause plant cell death at the site to prevent further infection. Although the plant is harmed because its own cells are attacked, the HR can be beneficial because plant tissues will then experience systemic acquired resistance (SAR). In SAR the plant is in a “heightened state of readiness” in which the tissues are very resistant to an extensive range of pathogens. This response pathway is actually being used to artificially initiate SAR because its much less toxic then spraying chemicals like fungicides and its effects can last much longer.

The plant defense system also includes RNA silencing, where viral RNAs are attacked. This process is similar to the immune system in humans, except instead of the pathogen’s proteins, the pathogen’s RNA is attacked. Another aspect of the plant defense system that is like the human immune system is the ability of the plant to “remember” a viral pathogen by keeping a template of the broken down viral genome for rapid response to a future attack.

Plants have developed defense mechanisms specific for herbivory. Toxic chemicals, sharp crystals, stinging cells, rough texture, or bitter-tasting chemicals are methods used by plants to deter organisms that feed on them. Many plants also produce chemical compounds that emit a fragrance which are used in essential oils. Although humans use these essential oils in products like seasonings and perfumes, they are insecticides and insect neurotoxins.

Finally, some plants have developed symbiotic relationships that help protect them against pathogens. One example of this is ants (*Pseudomyrmex ferrugineus*) and acacia (*Acacia hindsii)* plants. The plants provide resources such as food and shelter and the ants protect against herbivores and pathogens.

Plant Epidemiology

Since plants can be infected by pathogens this means plant crops can also experience an epidemic. Plant epidemiology is the study of the spread and development of disease in plant populations. The need for epidemiology has increased with the rise of shipping, globalization, large-scale commercial citrus production, and adoption of monocultures in which there are large fields of genetically and phenotypically uniform crops. The combination of these changes in crop production has led to many of the CTV epidemics including those that have impacted Florida. Epidemics are defined as the wide spread development and increase in incidence of the disease over time.

Plant Diseases

Devastating economic loss, extinction of species, starvation and mass migration of humans have all been the result of plant disease. The types of organisms or agents that cause disease, the mechanisms of disease, how disease is spread, movement of disease, and methods for prevention are the focus of plant pathology. The agents of plant disease come from almost every kingdom of life. There are plant diseases caused by viruses and viroids, bacteria and phytoplasmas, fungi, nematodes, protozoa, oomycetes, and even parasitic plants. Many other nonliving factors also cause or contribute to plant disease such as changes in the environment from pollution and or weather events.

Plants infected by pathogens show an array of symptoms and characteristics. Leaves can show wilting or spots. Fruit can show blight or rot and sometimes cankers. Roots can also show rot and stems, or woody tissue can show cankers and galls. Grasses can show patches of decline. Other overdevelopmental issues can also occur such as witches' brooms, and profuse flowering. In viral diseases especially, stunting or dwarfing can occur, mosaics or mottles or vein banding could appear, and ring spots could be seen. When identifying if plants could be infected with a pathogen, it is important to note what healthy tissues of that species or cultivar normally looks like. Comparisons can then be made between healthy characteristics and the characteristics of the plant in question. Even with such comparisons, it can be very hard to identify whether a plant is infected with a pathogen just by visual observation. Symptoms can also change over time showing primary and secondary symptoms. Later symptoms can often make it hard to identify the original symptoms and therefore disguise the source of the problem. There may also be variation in symptoms between the same type of plants. This could be caused by a number of factors such as infection by multiple types of pathogens. Environmental conditions and the combination of pathogens to host can cause a range of symptoms from asymptomatic to severe.

More accurate identification of plant pathogens can be done with laboratory test. Tissue samples are collected that could possibly contain the pathogen. The plant material may be incubated, for example, in a moist chamber, to isolate and identify the pathogen. Once a pathogen is identified, it may be used to inoculate healthy plants. The purpose of this step is to see if the pathogen is causing the symptoms seen in the field or if something else is the source of the symptoms. Sometimes it can be very difficult to replicate the symptoms, but this process is important for research, especially if the pathogen is novel in some way.

Certain plant pathogens can be difficult to grow in artificial media. Viruses, some fungi, and phytoplasmas all require a host, so these pathogens are identified using live cultures, microscopy, or diagnostic tests such as ELISA (enzyme-linked immunosorbent assay), lateral flow assays (LFAs), or polymerase chain reaction (PCR).

The disease of interest in the curriculum, Citrus tristeza virus (CTV), can produce symptoms very similar to two other endemic citrus diseases in Florida known as citrus blight and citrus greening or Huanglongbing (HLB). As a result, grafting, syringe injection tests, or clinical tests like ELISA should be done to correctly identify the pathogen causing disease.

Diagnostic Tests

ELISA and lateral flow assays are commonly used to detect the antigens of specific pathogens. There are several different kinds of ELISAs and LFAs, but the ones used in this lesson are compound or sandwich ELISAs and LFAs. The Sandwich ELISA involves the attachment of a capture antibody at the bottom of a 96 -well polystyrene plate. This type of plate is used because it will allow antibodies and proteins to bind while allowing excess nonbound material to be easily washed away. After antibodies have bound, samples that would contain the pathogen antigen are added to the well. A detection antibody with a substrate specific enzyme conjugate is added and once the substrate is introduced into the well the positive reaction will cause a color change. A microplate reader can read the amount of light absorbance to determine the strength of the reaction and therefore the amount of antigen present.

Lateral flow assays are very similar to ELISAs; however, they can be less expensive and much faster. LFAs are paper-based tests and they are used in many fields. One of the most common LFAs on the market are pregnancy tests. With plant tissue samples, soft tissue such as the leaf or petiole is mashed with buffer to create a liquid sample. The liquid is absorbed through the sample pad then travels through capillary action to the conjugate release pad. This area contains antigen specific antibodies conjugated to colored particles. The liquid, now with antigens joined to the conjugated antibodies, migrates to the detection zone where it will react with immobilized antibodies. A colored test line will appear if the sample is positive. There should also be the appearance of a control line which shows the sample has properly traveled through the test strip.

Plant Viruses & CTV

Tobacco mosaic virus (TMV) was the first plant virus to be identified but today there are about 1,000 plant viruses recognized and they are causing significant damage to crop yields. Just like animal viruses, plant viruses are microscopic pieces of nucleic acid covered in protein. Viruses are obligate parasites, meaning they need a host to replicate and therefore they are considered by some to be nonliving. They also lack the ability to produce energy and rely on hosts or the environment to spread. Most plant viruses contain RNA instead of DNA and the RNA is most often positive-sense single stranded.

The virus life cycle can vary greatly depending on the type of virus but most start by getting into the cell. The virus cannot pass through the tough structures of the plant cuticle or the cell wall, so instead they pass into plant tissue through wounds. Once inside the cell, the protein coat is removed, and the cell's transcription and translation enzymes are used to produce more viral particles. These proteins, along with the cell's proteins, manufacture copies of the viral genetic material. The genetic material combines with proteins to reform new viruses. For this process to occur and the virus to move to another cell, it takes up to several hours. Viruses normally travel passively through the vascular system of the plant through phloem sieve elements. The virus moves to surrounding cells from the phloem. Once the virus spreads to another plant it completes the virus life cycle. Most plant viruses are spread from plant to plant through vectors such as arthropods or nematodes. Aphids and whiteflies are known for transmitting the largest number of virus species, but viruses can also be spread through vegetative propagation processes like grafting.

Viruses are usually grouped based on their genome. Citrus tristeza virus (CTV) is a positive-sense single-stranded (ss) RNA virus in the *Closteroviridae* family. For positive ssRNA viruses, like Citrus tristeza virus (CTV), translation can happen without having to perform transcription. This is because the positive single-stranded RNA is in the correct orientation so that it can serve as messenger RNA. Translation can therefore happen directly using the host cell's ribosomes.

CTV is significant because it has caused devastating loss to the citrus industry and has been responsible for the loss of over 100 million trees. CTV infects the phloem of a narrow range of species in the Rutaceae family and *Citrus* genus. It was identified in the 1920s and 30s but the first confirmed report of CTV in Florida was in 1952. The virus is helical in shape. Its long and narrow at about 2000nm in length and 10 – 11 nm in diameter. The CTV genome is quite large at about 20 kilobases. There are also several strains of the virus which are quite different genetically from each other. Some strains cause very severe symptoms, where trees can decline in a matter of weeks, while others cause no symptoms at all. It is important to note, however, almost all trees infected with CTV in Florida contain a population of two or three strains.

The most common strains in Florida are T30, T36, and VT. T30 is recognized as mostly a mild strain while T36 is identified as a strain that causes severe decline in sour orange. The mild strains and severe strains share less than 79% of their nucleotide sequences which shows significant diversity between the different CTV strains.

Trees that show symptoms have been associated with certain types of rootstocks. This is especially true with trees grafted on sour orange (C. *aurantium*) root stock, where rapid wilting, honeycombing, root damage, and stunting of growth are symptoms. Stem-pitting is often seen in grapefruit (C. *paradisi*) and sweet orange (C. *sinensis*) and dieback has devasted limes (C. *aurantifolia*). Leaves can also develop yellow flecks along the veins. In the Folimonova lab alemow (C. *macrophylla*) was used because it reacts to most strains of the virus and is good for maintaining cultures.

Sour orange was a preferred rootstock for crop production because it produced high quality fruit and is resistant to many other citrus diseases like citrus blight, however, because of CTV, sour orange rootstock was on the decline. The introduction of citrus greening or Huanglongbing (HLB) has caused growers to return to using sour orange because it is more resistant to citrus greening. They believe that with the continual use of insecticide to protect against pests that spread disease there will not be substantial issues with CTV. However, commercial groves are likely to have difficulty controlling aphid transmission with this method because acquisition of the virus usually happens before the aphids are harmed by the insecticide.

Although CTV has been in Florida for a long time, outbreaks were on the decline after the 1970s and 1980s until the introduction of the brown citrus aphid (Toxoptera citricida) in 1995. CTV is transmitted most efficiently by the vector organisms in the aphid species *Toxoptera citricida* and *Aphis gossypii (melon aphid),* but the brown citrus aphid can transmit strains of CTV up to 25 times more efficiently than the melon aphid. The aphids can pick up and spread the virus within minutes of feeding on infected plant tissue and then they can continue to transmit it for up to 48 hours after acquisition.

Disease Control, Management, & Prevention

Plant disease management consists of prevention and treatment. Prevention may include quarantines before infection spreads and treatment may include chemical treatments after infection occurs. Attempts have also been made to eradicate or eliminate pathogens as is with Citrus canker. In this case massive amounts of citrus trees were removed and burned. Initially the eradication program seemed to have worked, but recently the disease has reappeared. Crop rotation can be used as well to reduce the amount of pathogen.

Treating and controlling CTV can be very difficult. Unlikely in humans, there are really no antiviral compounds available to cure plants of viral diseases, however there has been development of resistance methods. Natural or engineered resistance genes can be introduced into the plant. Plants can also be given small fragments of viral genetic material to build up the plant's RNA silencing defense system. However, like most other plant pathogens, methods are mainly focused on traditional control or prevention. Some of these methods include using certified-pathogen free seed or vegetative rootstocks, using resistant rootstocks, changing harvesting and planting methods, eradicating virus reservoirs in the surrounding environment, and controlling the vector through insecticides or fungicide. For some pathogens, chemicals called plant activators can be sprayed on the plants to artificially trigger the plants to have a heightened state of readiness for an attack (SAR).

There has been quiet a bit of effort and research put into finding a mild strain of CTV that could protect against severe strain CTV by preventing superinfection; however, this has not been shown to be very effective because the strains are so different from each other genetically. Current research is being done to identify specific genes to modify mild strains to protect more effectively against severe strains.

Once crops have severe decline the only option is to remove the diseased trees and replace them with certified tress or tolerant rootstocks.

Information about citrus plants, plant pathology, and CTV is not limited to the information above. Images, detailed explanations, and other print resources for students can be found by viewing the original sources. There are many other great sources of information, but the content provided here was selected from the sources below.

The American Phytopathological Society (APS)

https://www.apsnet.org/careers/careersinplantpathology/Pages/default.aspx

https://www.apsnet.org/edcenter/intropp/topics/Pages/PlantDiseaseDiagnosis.aspx

https://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/PlantViruses.aspx

https://www.apsnet.org/edcenter/intropp/topics/Documents/PlantDiseaseManagement.aspx https://www.apsnet.org/edcenter/intropp/topics/Pages/OverviewOfPlantDiseases.aspx

https://www.apsnet.org/edcenter/advanced/topics/EpidemiologyTemporal/Pages/default.aspx

UF CPET

http://history.cpet.ufl.edu/lm/elisa/types01.html

NIH

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3653117/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4986465/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2812332/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3632782/

https://www.ncbi.nlm.nih.gov/books/NBK8174/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3764332/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3014543/

Association of Applied Biologists

http://www.dpvweb.net/dpv/showdpv.php?dpvno=353

UF IFAS

http://edis.ifas.ufl.edu/pdffiles/pp/pp26300.pdf

http://www.crec.ifas.ufl.edu/extension/trade_journals/2015/2015_April_ctv.pdf

http://edis.ifas.ufl.edu/cg039

http://edis.ifas.ufl.edu/hs242

https://plantpath.ifas.ufl.edu/plant-virus-profiles/ssrnaplus/Citrus%20tristeza%20virus/index.html

http://edis.ifas.ufl.edu/in133

http://edis.ifas.ufl.edu/ch159

http://edis.ifas.ufl.edu/fe915

http://ufdc.ufl.edu/IR00002686/00001

http://www.crec.ifas.ufl.edu/extension/trade_journals/2006/Oct%202006%20citrus%20tristeza.pdf

University of Illinois Extension

http://extension.illinois.edu/focus/index.cfm?problem=chlorosis

Purdue

https://hort.purdue.edu/newcrop/janick-papers/c09.pdf

Citrus Resource Warehouse

https://www.citrusresourcewarehouse.org.za/home/document-home/learning-aids-and-resources/citrus-academy-learning-programmes/citrus-secondary-programme/1103-citrus-secondary-programme-module-08-plant-structures-functions/file

Background Narrative

A young girls life is changed by the impact of disease in the Florida citrus industry….The daughter of farm workers plays soccer in the fields near the grove. She notices over several months that some of the trees are starting to look very different – they look sickly. During this time her parents mention to her that they may have to move because there just isn't enough crop production to maintain their jobs on the farm. She is upset because she likes where she lives, and she wonders if anything can be done to help figure out what's happening to the field.

Q- What is happening to the grove and can it be saved?

Lesson One: Plants Get Sick Too!

This lesson will begin with a basic overview of the different types of pathogens that cause plant disease including specific types that infect citrus crops in Florida. Students will be given summary identification cards for each disease including pictures of the pathogen and infected plant tissue. Students will then read 3 short case studies/epidemiology reports. They will diagnose each case study/report with the appropriate disease using the information on the cards.

The next afternoon the girl goes over to her friend's house and mentions that she is worried about her parents losing their jobs. Her friend's dad, who works at the UF/IFAS Citrus Research and Education Center, over hears the conversation, and suggests that its possible the crops are sick with a pathogen that causes plant disease.

Q- Could the citrus plants be sick with an infectious disease?

-Background on plant diseases – summary ID cards (like the UF/IFAS cards)

-3 case study examples – the neighbor's dad presents as evidence as why he thinks they might be sick.

Lesson Two: Plant Disease Epidemiology

Students will be given a "plot" game board where they will spread out different color beads. With their eyes closed, they will draw out 2 beads at a time and depending on the bead color the students will add more of a specific color bead. Using two scenarios and three different color beads, students will model the spred of disease through a field. They will then graph & analyze the data. Finally, students will study the spread of the epidemic using a network model diagram of the field.

The next day the girl decides to get a better look at the citrus grove. She starts mapping out where the "sick" looking trees are in the field and what characteristics make them look unhealthy.

Q- If they are sick with an infectious disease how could it spread and how quickly?

- Background on tracking spread of disease
- Students see her map and use it to predict how the disease will spread using concepts about modeling epidemic networks
- Students will then do the modeling epidemic activity with the beads to see how quickly it could spread and how much time they could have until the field is completely infected.

Lesson Three: Environmental Impacts

In this lesson students will investigate how changes in the environment, like extreme weather, climate change, and other human impacts affect crop production and the spread of disease in the citrus industry. Students will be given two case studies or articles to summarize and analyze to predict further effects on the industry.

A few days later she shares her detailed map with her neighbor's dad. He mentions that the information she collected lead him to hypothesis the trees maybe sick with a disease called Citrus greening or possibly CTV. He also notes that there are many factors that could affect how quickly disease can spread in a grove. He suggests she read a couple articles that show examples of the environment contributing to crop decline.

Q- How could the environment affect the grove?

- Students read and analyze case studies/articles
- Maybe include about of decline after hurricane/ have students analyze look at graphs.
- Answer "What could impact the groves in the future?"

Lesson Four: Citrus tristeza virus (CTV) & The Central Dogma

Students will be gain more detailed background information on CTV and the cell processes involved in the viral life cycles (replication, transcription, translation). Then they will be given pictures and terms of replication, transcription, translation, CTV virus structure, and cell structural components involved the infection. Using these pictures and terms, students will create a concept map showing how the concepts are related and what's happening in the process.

She quickly realizes that she is running out of time as changes in the environment continue to cause crop decline. She mentions to her friend's dad that she wants to learn more about the diseases like CTV. He hands her some info cards he had from when he did community outreach.

Q- Could the "sick" trees be infected with CTV?

- Students gain background info on CTV & central Dogma
- Students arrange cards to create a concept map

Lesson Five: Testing for CTV

Using "field" collections from the surrounding community or ordered samples, students will test for CTV using a lateral flow assay (ImmunoStrip test) to determine if the grove has plants that test positive with CTV.

Based on what she learned from the cards she is certain that the grove could have trees infected with CTV. Her friend's dad mentions that samples can be analyzed quickly and test positive or negative for the

CTV antibodies using ImmunoStrip Test. He says they can also be sent to the UF/IFAS Plant Diagnostic Center for a more detailed analysis.

Q- Could the trees have severe strains of CTV?

- Students test samples using ImmunoStrip Test / lateral flow assay – they test positive and so she decides to send samples off to the UF/IFAS Plant Diagnostic Center

Lesson Six: Diagnosis & Prevention

Students will read and analyze a report from samples that were "sent" to the Plant Diagnostic Center. They will use the report and the information they learned during the other lessons within the unit to write up a summary report. The summary report will contain the diagnosis from tests of their "field samples" and a plan of attack to prevent further spread of disease.

After a week she receives a report in the mail from the Plant Diagnostic Lab. It states the samples are positive for CTV and some samples have the severe strain while others have the mild strain. The report also suggests information on what to do with the new information on the report.

Q- What is the final analysis of the results and what can be done?

- Students read and analyze the Plant Diagnostic Report – they then write and summary report and decide what the best way to go forward/ a recommendation for a prevention plan.

APPENDIX: OVERALL CURRICULUM SUMMARY

Curriculum Summary: In this curriculum, students will follow the story of a young girl whose life is changed by the impact of disease in the citrus industry in Florida. Students will have the opportunity to understand what is happening to the local crops, the citrus industry, and the community she lives in. Several clinical tests will be performed to diagnose what diseases are contributing to the reduction in crop yield. Using a simple model, the students will also simulate the spread of disease through a field and graph the resulting data. Finally, the students will develop a plan to combat the disease and prevent further infection.

Standards & Learning Goals as well as Learning Performances and Learning Tasks are in the table below:

Disease in the Squeeze Curriculum Outline

Lesson Number	Next Generation Sunshine State Standards for Science Biology Standards	AP Biology Curriculum Alignment	Time	Overview of Lesson Learning Performances & Learning Tasks	Assessments
Lesson 0.1 Background	SC.912.L.15.6 Discuss distinguishing characteristics of the domains and kingdoms of living organisms. SC.912.L.14.1 Describe the scientific theory of cells (cell theory) and relate the history of its discovery to the process of science. SC.912.L.14.3 Compare and contrast the general structures of plant and animal cells. Compare and contrast the general structures of prokaryotic and eukaryotic cells. SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological	Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes. Essential Knowledge 1.B.1 Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today.	1 (50 minute class periods)	Diversity of life, Characteristics of life, differences between viruses, bacteria, plants, animals, fungi, plants, and protists – summary identification cards for each, students then get list of organisms or characteristics and they have to identify if living or not, and/or what organisms it would be using the info on the cards.	Check student identification of organisms/ characteristic by walking around room and looking at student work comparing to rubric

	macromolecules.				
Lesson 0.2 background	SC.912.L.14.7 Relate the structure of each of the major plant organs and tissues to physiological processes. SC.912.L.18.7 Identify the reactants, products, and basic functions of photosynthesis.	Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes. Essential knowledge 4.A.4 Organisms exhibit complex properties due to interactions between their constituent parts. Essential knowledge 2.A.2 Organisms capture and store free energy for use in biological processes. Essential knowledge 2.A.3 Organisms must exchange matter with the environment to grow, reproduce and maintain organization.	1 (50) minute class periods	Plant structures and function for photosynthesis, reproduction & fruit production, homeostasis, identification of structures for collection of samples and understanding what parts of the plant the disease affects. summary identification cards for each structure with function and picture, students then get list of structures, pictures, or functions and they have to match them with the appropriate function, structure, or picture.	Check student understanding by walking around room and looking at student work comparing to rubric
Lesson 1 (Start actual lessons here)	SC.912.L.14.7 Relate the structure of each of the major plant organs and tissues to physiological processes. SC.912.L.17.8 Recognize the consequences of the losses of biodiversity	Essential knowledge 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis	1 (50) minute class period	Plant Disease overview, types of Florida citrus diseases, history, and their characteristics summary identification cards for each disease with pictures, students then get 3 case studies/epidemiology reports and they have to diagnosis the case	Check student diagnosis by walking around room and looking at or collecting student work comparing to rubric for correct diagnosis. Have students complete PPM –

	due to catastrophic events, climate changes, human activity, and the introduction of invasive, non-native species.	Essential Knowledge 2.D.3 Biological systems are affected by disruptions to their dynamic homeostasis.		study/report with the disease they think it is based on the information on the cards	what do they know about Plant Pathology
Lesson 2	SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules. SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology. SC.912.L.16.3: Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information. SC.912.L.15.15 Describe how mutation and genetic recombination increase genetic variation. SC.912.L.16.5: Explain the basic processes of transcription and translation, and how they result in the	LO 2.43 The student is able to connect the concept of cell communication to the functioning of the immune system. Essential knowledge 4.A.1 The subcomponents of biological molecules and their sequence determine the properties of that molecule. Essential Knowledge 3.A.1 DNA, and in some cases RNA, is the primary source of heritable information Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes. Essential Knowledge 3.C.3	1.5 (50) minute class period	Detailed Specific viruses investigation – CTV & TMV, overview of replication, transcription, translation Students will be given background information on the viruses and the cell processes involved in their life cycles (replication, transcription, translation) Then they will be given pictures/words of replication, transcription, translation, CTV & TMV virus structure, and cell structural components involved. They will create a meaning map/concept map showing how they are related and what's happening in the process and with those structures.	Check student understanding by walking around room and looking at student work (concept/meaning map) comparing to optional rubric

	expression of genes. SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.	Viral replication results in genetic variation, and viral infection can introduce genetic variation into the hosts.			
Lesson 3	SC.912.L.17.4 Describe changes in ecosystems resulting from seasonal variations, climate change and succession. SC.912.L.17.8 Recognize the consequences of the losses of biodiversity due to catastrophic events, climate changes, human activity, and the introduction of invasive, non-native species.	Essential Knowledge 2.D.1 All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy. Essential knowledge 4.C.4 The diversity of species with in an ecosystem may influence the stability of the ecosystem.	0.5 (50 Minute) class period	Investigate how changes in the environment, like climate change, and other human impacts affect the spread of disease in the citrus industry – Students will be given 2 case studies or articles, they will summarize and predict further affects.	Check student analysis and understanding by walking around room and looking at or collecting student work comparing to optional rubric
Lesson 4	SC.912.L.17.5 Analyze how population size is determined by births, deaths, immigration, emigration, and limiting factors (biotic and abiotic) that determine carrying capacity. SC.912.L.17.8 Recognize the consequences of the losses of biodiversity	Essential knowledge 4.C.4 The diversity of species with in an ecosystem may influence the stability of the ecosystem. Science Practice 1: The student can use representations and models to communicate	1 (50 minute) class period	Model spred of disease through a field, graph data & analyze. Students will be given a brown lunch baggy with blue beads (representing healthy) & white beads (representing infection). They will draw out 2 beads at a time and record data. In the second scenario there will be a third type of bead added (red bead) which will represent inoculum (alternative sources of the	Check student understanding by walking around room and looking at or collecting student graph work and analysis comparing to rubric

	due to catastrophic events, climate changes, human activity, and the introduction of invasive, non-native species. SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.	scientific phenomena and solve scientific problems		pathogen).	
Lesson 4.5 (could skip)	SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules. SC.912.L.14.52 Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics. SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology. SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.	Essential knowledge 4.A.1 The subcomponents of biological molecules and their sequence determine the properties of that molecule. Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes. Essential knowledge 4.B.1 Interactions between molecules affect their structure and function. Science Practice 1: The student can use representations and models to	2 (50 minute) class periods	Intro & Practice diagnosis tests (ELISA, Lateral Flow assay) using model organism – N. benthamiana Students will learn about and practice the ELISA and Lateral Flow Assay using samples from N. benthamiana	Check student understanding by walking around room and looking at or collecting quiz about ELISA & Lateral Flow assay also checking student lab work comparing to expected results on rubric

	SC.912.L.16.11 Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.	communicate scientific phenomena and solve scientific problems Science Practice 5: The student can perform data analysis and evaluation of evidence.			
Lesson 5	SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules. SC.912.L.14.52 Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics. SC.912.L.16.7 Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology. SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues. SC.912.L.16.11	Essential knowledge 4.A.1 The subcomponents of biological molecules and their sequence determine the properties of that molecule. Essential knowledge 4.A.2: The structure and function of subcellular components, and their interactions, provide essential cellular processes. Science Practice 5: The student can perform data analysis and evaluation of evidence.	2 (50 minute class periods)	Field Collection & Diagnosis- collect samples from home & test for CTV (ELISA, Lateral Flow assay) Students will perform the ELISA and Lateral Flow Assay using citrus plant samples from their yard or community.	Check student understanding by looking at or collecting quiz about ELISA & Lateral Flow assay also checking student lab work comparing to expected results on rubric

	Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.				
Lesson 6	SC.912.N.4.1 Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making. SC.912.N.4.2 Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental. SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied. SC.912.N.4.2 Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.	Science Practice 1: The student can use representations and models to communicate scientific phenomena and solve scientific problems Science Practice 5: The student can perform data analysis and evaluation of evidence. Science Practice 7: The student is able to connect and relate knowledge across various scales, concepts and presentations in and across domains.	0.5 (50 minute class period)	What is the diagnosis and prevention plan? Students will use their experiment data and the information they learned during the unit to write up a report of what the diagnosis was of their "field samples" and a plan of attack for the future.	Check student understanding by collecting student work and having them discuss their diagnosis and prevention plans. Also final PMM – What do they know about Plant Pathology?

Total Time for Unit = 525 minutes = 10.5 (50 minute) class periods = about 2 weeks.	**Modified without background and model/practice with N. benthamiana = 6.5 (50 minute) class periods = 1.5 weeks**

A