



FROM THE CLASSROOM TO THE FARM

PLANT PATHOLOGY

Lesson Plan

2022

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FROM THE CLASSROOM TO THE FARM


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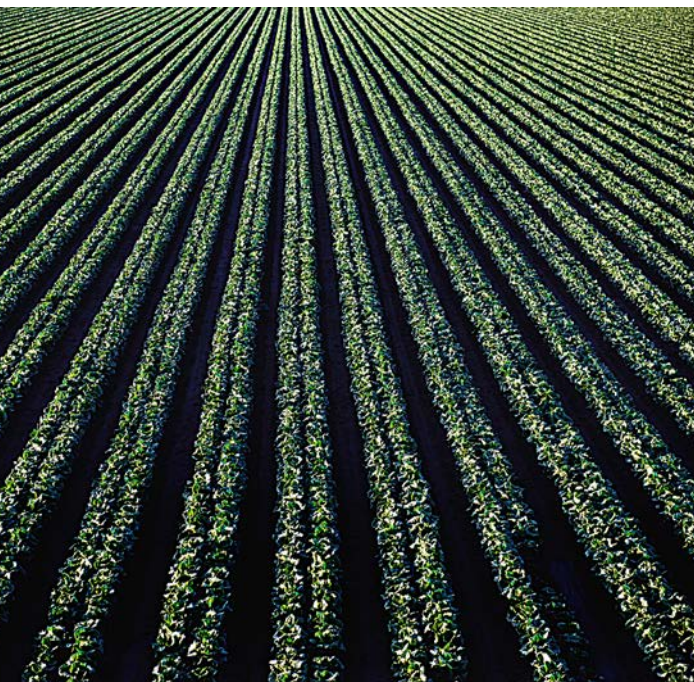
PLANT PATHOLOGY

Importance and Lesson Plan Description



Plant pathology is the study of diseases in plants caused by infectious organisms or environmental conditions. It focus on disease diagnosis and management to minimize economic losses in the agricultural field. Understanding the basics of plant pathology is vital to ensure disease reduction and management; protecting food security, and helping people worldwide (farmers, community, etc.). This plan introduces learners to the basic concepts of plant disease and control.

It will cover:

- 
- Foundations of plant pathology: disease triangle, disease agents, and disease detection
 - Characteristics and detection of plant pathogens in tropical crops
 - Control and management practices

ABOUT THE AUTHORS



Dr. Nicole Colón Carrión

completed her bachelor's degree in Natural Sciences, with a concentration in Biology at the University of Puerto Rico in Cayey. During this time, she participated in summer research internships across the United States in diverse areas, including plant development and genetics, developmental biology, microbiology, and neuroscience. She also worked in a Mycology laboratory at the University of Puerto Rico in Cayey, where she focus on identifying fungal contaminants in commercially sold rice grains in Puerto Rico. Following graduation, she joined the Post-baccalaureate Research Experience Program at Michigan State University where she worked in the area of Neurotoxicology. To continue her education, Dr. Colón Carrión completed a Ph.D. in Plant Pathology at the University of Arizona. Her research focused on understanding the effect of climate changed in plant-microbe symbioses, with a focus on fungal endophytes, and their uses for improving reforestation after natural disturbances and protecting crops from plant disease. Currently, she is a Research Investigator at Corteva AgriScience, where she evaluates the potential of chemical and natural products to control plant diseases caused by pathogenic fungi.

Dr. Sofía Macchiavelli Girón

started her higher education journey at the University of Puerto Rico-Mayagüez where she obtained her Bachelor's degree in Biology. During her undergraduate degree, she worked in a Plant Biotechnology and Genetics laboratory, where her research focused on using a technique called 'DNA Barcoding' to catalog a variety of tropical crops. To continue her education, Dr. Macchiavelli-Girón headed to the University of Wisconsin-Madison and completed a Ph.D. in Plant Pathology. Her research focused on improving the management of an economically important potato disease called silver scurf, caused by a fungus called *Helminthosporium solani*. Currently, she is an Assistant Agent of Extension at the Puerto Rican Agricultural Extension Service, which is part of the College of Agricultural Sciences at the University of Puerto Rico-Mayagüez. In her current position, she works directly with farmers and community members using non-formal education and outreach strategies to take the knowledge gained in the university directly to those who can benefit from it. This includes teaching a wide variety of courses, working in outreach events, and consulting directly with community members.



PLAN OVERVIEW

I. Learning Objectives

By the end of the plan, the goal is for all participants to be able to:

- 1- Define plant pathology and its importance.
- 2- List the major components of the disease triangle.
- 3- Distinguish between signs and symptoms of disease.
- 4- Categorize the main factors causing disease in plants.
- 5- Describe diseases in tropical crops.
- 6- Discuss basic approaches for the control of plant disease.
- 7- Apply their understanding of control practices.

II. PURPOSE

By the end of the plan, participants should be able to master the basic concepts of plant pathology and apply them to their work while fomenting collaboration with peers and Extension agents.

III. DURATION

The plan is designed to be completed in three workshops of approximately 2-3 hours each (see activity description).

III. KEYWORDS

Plant pathology, infectious agents, tropical crops, detection, control

WORKSHOP STRUCTURE

Workshop 1. Foundations of Plant Pathology

Session 1: Introduction to Plant Pathology

Session 2: Signs and symptoms of plant disease

Session 3: Factors causing disease in plants

- a. Infectious agents
 - i. Fungi
 - ii. Bacteria
 - iii. Viruses
 - iv. Nematodes
- b. Non-infectious agents

Workshop 2. Diseases in Tropical Crops

Session 1: Vegetables

- a. Cassava
- b. Sweet Potato

Session 2: Fruits

- a. Peppers
- b. Banana and Plantains

Workshop 3. Control practices

Session 1: Control Practices

- a. Chemical control
- b. Biological control
- c. Cultural control
- d. Physical control

*All presentations for the workshops are included (Github - Supplemental 8)

ACTIVITIES DESCRIPTION

K-W-L CHART

Participants will be asked to fill a K-W-L: K- what I know at this moment about the topic, W - what I want to know about the topic, L- what I learned about the topic. At the beginning of the workshop, participants will fill the K and W part of the K-W-L poll. At the end, participants will fill the last portion of the K-W-L (L - what I learned about the topic).

Activity #1

Participants will be provided images of diseased plants displaying signs or symptoms. They will be asked to classify them accordingly.

Activity #2

Participants will be provided with a diseased plant. Information about plant name, care, and environmental factors exposed will be provided. Participants will be asked to ID signs and symptoms of disease present in their plants, and take images that can help during the disease diagnosis procedure.

Activity #3

Participants will be paired with experts and Extension agents to brainstorm ideas on the best practices to manage disease in their farms.

Content	Workshop	Session	Timeline	Activity
Introduction to plant pathology	1	1	40 min*	K-W-L chart
Signs and symptoms of plant disease		2	1-hr*	Activity #1
Factors causing disease in plants		3	1-hr	No Activity
Diseases in tropical vegetables	2	1	40 min*	No Activity
Diseases in tropical fruits		2	1-hr 10 min	Activity #2
Control practices	3	1	2-hrs	Activity #3

WORKSHOP 1: INTRODUCTION TO PLANT PATHOLOGY

ACTIVITY: K-W-L CHART

CREATED BY: NICOLE COLÓN

SESSION: 1

AUDIENCE: FARMER PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the basic concepts of plant pathology: what it is and its importance in the agricultural field. It will go over the foundations and history of plant pathology. Finally, it will cover the concepts behind the disease triangle and its limitations.

LEARNING OBJECTIVES

Define plant pathology and its importance.
List the major components of the disease triangle.

KEYWORDS

plant pathology, disease, disease triangle

PURPOSE

DURATION

Expose participants to the concept of plant pathology and the disease triangle, and their importance in the field.

40 minutes

MATERIALS

K-W-L Chart, Projector, Laptop, Foundations to Plant Pathology PPT

DIRECT INSTRUCTIONS

- **Introduction:** Instructor will present a K-W-L chart to participants to evaluate prior understanding. Participants will be asked to complete the K and W portion: K- what I know at this moment about the topic, and W- what I want to know about the topic. Following, the instructor will provide a 40-minute lecture on the introduction to plant pathology and related concepts using the Foundations to Plant Pathology PPT.
- **Development:** Instructor will provide definitions of each concept, advantages and limitations, and clear examples in the field.
- **Practice:** Instructor will ask participants to reflect on what they learned and complete the last portion of the K-W-L: L- what I learned about the topic.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessment:** Instructor will assess participants' learning by analyzing the K-W-L chart.

APPLICATIONS

Participants will be asked to fill a K-W-L: K- K- what I know about the topic, W- what I want to know about the topic, L- what I learned about the topic.

ASSESSMENT

K-W-L chart will be assessed using a holistic rubric.

HOLISTIC RUBRIC FOR K-W-L CHART

Score	Description
3	Participant shows increased understanding of the concepts when comparing the K and L section. Participant develops good questions in the W section.
2	Participant shows partial understanding of the concepts when comparing the K and L section. Participant develops average questions in the W section.
1	Participant shows little understanding of the concepts when comparing the K and L section. Participant develops inadequate questions in the W section.
0	No response.

HOW TO USE THE RUBRIC

- 1- Assess each K-W-L chart individually.
 - 2- Using the K and L section of the K-W-L chart, compare responses.
 - 3- Score them using the *Holistic Rubric For K-W-L Chart*.
- *See example below.

EXAMPLE PARTICIPANT 1

K : I don't know what plant pathology is.

L: Plant Pathology is the field that studies plant diseases.

SCORE: 3

Participant shows increased understanding.

EXAMPLE PARTICIPANT 2

K : Plant Pathology is the field that studies the economics of crop production.

L: Plant Pathology studies diseases.

SCORE: 2

Participant shows partial understanding. Statement does not specify the host or specimen .

SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY.

Bargainnier, S. (2003). Fundamentals of rubrics. Pacific Crest, 1-4.

Martinez, Y. M. (2004). Does the KWL reading strategy enhance student understanding in an honors high school science classroom? (Doctoral dissertation, California State University, Fullerton).

Maulida, C. I., & Gani, S. A. (2016). KWL: Strategy on improving reading comprehension. Research in English and Education Journal, 1(1), 53-61.

Mertler, C. A. (2000). Designing scoring rubrics for your classroom. Practical assessment, research, and evaluation, 7(1), 25.

Ogle, D. M. (1986). KWL: A teaching model that develops active reading of expository text. The reading teacher, 39(6), 564-570.

Rahayuningsih, P. D. (2014). The use Of know, want to know, learned (KWL) technique to improve teaching and learning process. Jurnal Pendidikan Edutama, 1(2), 42-50.

Rahmawati, E. Y. (2018). Analysis of students' english reading comprehension through KWL (Know-Want-Learn) learning strategies. International Journal of Language Teaching and Education, 2(3), 238-247.

Sampson, M. B. (2002). Confirming a KWL: Considering the source. The Reading Teacher, 55(6), 528-532.

K-W-L CHART

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What I know

What I want to know

What I learned

WORKSHOP 1: SIGNS AND SYMPTOMS OF PLANT DISEASE

ACTIVITY: #1

CREATED BY: NICOLE COLÓN

SESSION: 2

AUDIENCE: FARMER PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the basic concepts of signs and symptoms in plant disease. It will go over the definitions of signs and symptoms, and their differences. Through the use of visuals, participants will be guided to distinguish between signs and symptoms. Finally, it will cover the importance of these concepts during disease diagnosis.

LEARNING OBJECTIVES

Distinguish between signs and symptoms of disease.

KEYWORDS

plant disease, signs, symptoms

PURPOSE

DURATION

Expose participants to the difference between signs and symptoms in diseased plants.

1 hour

MATERIALS

Activity #1 Sheets, Projector, Laptop, Foundations of Plant Pathology PPT, White cardboard, Glue, Markers

DIRECT INSTRUCTIONS

- **Introduction:** : Instructor will provide a 30-minute lecture introducing the concepts of signs and symptoms of disease using the Foundations of Plant Pathology PPT.
- **Development:** Instructor will use visuals of diseased plants to illustrate the differences between the two concepts.
- **Practice:** Instructor will ask participants complete Activity #1.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessment:** Instructor will assess participants' learning by analyzing responses provided in the Signs and Symptoms (S&S) Sheet.

APPLICATIONS

Participants will be divided into groups. Each group will receive 4 images, S&S (signs & symptoms) sheet, and a small white cardboard divided into two segments: signs or symptoms. Groups will classify each image into one of the two groups and will write the reasoning behind that classification.

ASSESSMENT

S&S Sheet will be assessed using a holistic rubric.

HOLISTIC RUBRIC FOR S&S SHEET

Score	Description
3	Participants show understanding of the concepts by classifying all images correctly.
2	Participants show understanding of the concepts by classifying some images correctly.
1	Participants do not show understanding of the concepts. All images were classified incorrectly.
0	No response.

HOW TO USE THE RUBRIC

- 1- Assess each S&S sheet per group.
 - 2- Use the S&S Answer Sheet to compare responses of each group.
 - 3- Score them using the *Holistic Rubric For S&S Sheet*.
- *See example below.

EXAMPLE GROUP 1

After comparing the S&S Answer Sheet with group 1 S&S sheet, they have 2 images classified incorrectly.

SCORE: 2

Participant shows partial understanding of concepts.

EXAMPLE GROUP 2

After comparing the S&S Answer Sheet with group 2 S&S sheet, they have all images classified incorrectly.

SCORE: 1

Participant do not show understanding of concepts.

SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY.

Bargainnier, S. (2003). Fundamentals of rubrics. Pacific Crest, 1-4.

Mertler, C. A. (2000). Designing scoring rubrics for your classroom. Practical assessment, research, and evaluation, 7(1), 25.

Riley, M.B., M.R. Williamson, & O. Maloy. (2002). Plant disease diagnosis. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-1021-01.

ACTIVITY #1: SIGNS AND SYMPTOMS OF PLANT DISEASE

INSTRUCTIONS AND HANDOUTS

I. PURPOSE

Expose participants to the difference between signs and symptoms in disease plants.

II. MATERIALS

4 images showing signs and symptoms in plants (provided below)
White cardboard
S&S Sheet
Markers
Glue

III. INSTRUCTIONS

***Instructions detailed below are for the instructor providing the activity.**

1- Divide participants into groups.

*The number of groups will depend on the amount of participants.

*For an efficient activity have no more than four participants per groups.

2- Provide each group with the S&S (signs and symptoms) sheet, 4 images provided below, and the white cardboard.

*All groups will receive the same images.

3- Ask participants to discuss with members each image and classify them as having signs or symptoms.

4- Ask participants to glue images in their respective classification in the white cardboard.

5- Ask participants to write the reasoning behind each classification in the S&S sheet.

6- Once groups are finished, reveal the classification of each image and the reasoning behind it.

*The S&S answer sheet is provided below.

7- Collect S&S sheet and whiteboard from groups.

S&S SHEET

GROUP:

DATE:

IMAGES



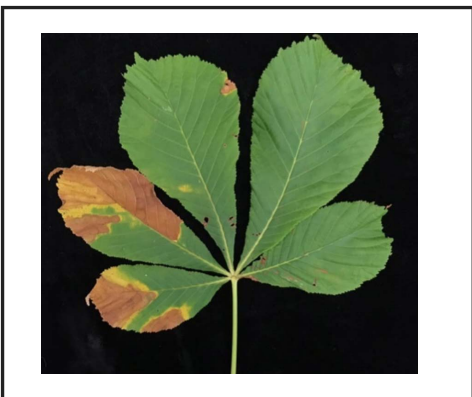
REASONING FOR CLASSIFICATION

S&S SHEET

GROUP: NA

DATE: NA

IMAGES



REASONING FOR CLASSIFICATION

THIS TOMATO EXHIBITS CIRCULAR AND FLAT LESIONS FILLED WITH BROWNISH OOZE. THAT OOZE IS A SIGN OF THE PATHOGEN. SPECIFICALLY THE PRESENCE OF A FUNGUS, COLLETOTRICHUM COCCODES. THE DISEASE IS KNOWN AS ANTHRACNOSE.

THIS JAPANESE ANEMONE LEAF EXHIBITS IRREGULAR AND BROWNISH LESIONS. THIS IS CLASSIFIED AS A SYMPTOM. THIS DISEASE IS CAUSED BY THE PRESENCE OF NEMATODES OR "ROUNDWORMS".

THIS CHESTNUT LEAF EXHIBITS IRREGULAR AND BROWNISH LESIONS. THIS IS CLASSIFIED AS A SYMPTOM. THIS DISEASE IS KNOWN AS HORSE CHESTNUT LEAF BLOTCH CAUSED BY A FUNGUS, GUIGNARDIA AESCULI.

THIS SPRUCE NEEDLE EXHIBITS BLACK SPORES. THOSE SPORES ARE A SIGN OF THE PATHOGEN. THE PATHOGEN IS RHIZOSPHAERA KALKHOFFII. THE DISEASE IS KNOWN AS RHIZOSPHAERA NEEDLE CAST.

ACTIVITY #1 IMAGES

All images are courtesy of Renata Belisário (Ph.D. student at University of Kentucky and founder of PlantPathDetective)



WORKSHOP 1: FACTORS CAUSING DISEASE IN PLANTS

ACTIVITY: NA

CREATED BY: NICOLE COLÓN

SESSION: 3

AUDIENCE: FARMER PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the concepts of agents of plant diseases. It will go over diseases caused by infectious (fungi, viruses, bacteria, and nematodes) and non-infectious agents. It will walk through each group of infectious agents and their characteristics, and describe the most common non-infectious agents. Finally, it will cover the proper ways to record data and report it to Extension agents.

LEARNING OBJECTIVES

Categorize main factors causing disease in plants.

KEYWORDS

plant disease, infectious agents, non-infectious agents

PURPOSE

Expose participants to the factors causing disease in plants.

DURATION

1 hour

MATERIALS

Projector, Laptop, Foundations of Plant Pathology PPT

DIRECT INSTRUCTIONS

- **Introduction:** Instructor will provide a 1-hour lecture introducing the concepts of agents of plant disease using the Foundations of Plant Pathology PPT.
- **Development:** Instructor will provide information on the infectious agents' characteristics and the environmental factors causing disease in plants, and how to distinguish them.
- **Practice:** No activity will be conducted.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessment:** No assessment.

APPLICATIONS

No activity will be provided for this workshop.

ASSESSMENT

No assessment.

SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY

Moskal, B. M. (2000). Scoring rubrics: What, when, and how? Practical Assessment, Research, and Evaluation, 7(1), 3.

Suskie, L. (2018). Assessing student learning: A common sense guide. John Wiley & Sons.

WORKSHOP 2: TROPICAL DISEASE: VEGETABLE

ACTIVITY: NA

CREATED BY: NICOLE COLÓN

SESSION: 1

AUDIENCE: FARMER PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the concept of tropical diseases in vegetable crops. It will go over the most common diseases in tropical vegetables with a focus on sweet potatoes and cassava. It will walk through the characteristics of the pathogens, common symptoms, and detection methods.

LEARNING OBJECTIVES

Describe diseases in tropical crops.

KEYWORDS

Plant disease, detection, diagnosis, infectious agent

PURPOSE

Expose participants to infectious agents causing disease in tropical plants.

DURATION

40 minute

MATERIALS

Projector, Laptop, Diseases in Tropical Crops PPT

DIRECT INSTRUCTIONS

- **Introduction:** Instructor will provide a 40-minute lecture introducing the concepts of agents of plant diseases in topical crops using the Diseases in Tropical Crops PPT.
- **Development:** Instructors will provide information on common infectious agents and how to detect them.
- **Practice:** Practice will take place as a whole at the end of Workshop 2 - Session 2.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessment:** : Instructors will assess participants' learning as a whole at the end of Workshop 2 - Session 2.

APPLICATIONS

Application of these concepts will take place as a whole at the end of Workshop 2 - Session 2.

ASSESSMENT

Assessment of these concepts will take place as a whole at the end of Workshop 2 - Session 2.

SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY

Moskal, B. M. (2000). Scoring rubrics: What, when, and how? Practical Assessment, Research, and Evaluation, 7(1), 3.

Suskie, L. (2018). Assessing student learning: A common sense guide. John Wiley & Sons.

WORKSHOP 2: TROPICAL DISEASE: FRUITS

ACTIVITY: #2

CREATED BY: NICOLE COLÓN

SESSION: 2

AUDIENCE: FARMER PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the concepts of plant diseases in tropical fruit crops. It will go over the most common diseases in tropical fruits with a focus on peppers and plantains. It will walk through characteristics of the pathogens, common symptoms, and detection methods.

LEARNING OBJECTIVES

Describe disease in tropical crops.

KEYWORDS

Plant disease, detection, diagnosis, infectious agent

PURPOSE

Expose participants to infectious agents causing disease in tropical plants.

DURATION

1 hour & 10 minute

MATERIALS

Activity #2 Sheets, Projector, Laptop, Diseases in Tropical Crops PPT

DIRECT INSTRUCTIONS

- **Introduction:** Instructor will provide a 40-minute lecture introducing the concepts of agents of diseases in topical crops using the Disease in Tropical Crops PPT.
- **Development:** Instructors will provide information on common infectious agents and how to detect them.
- **Practice:** Instructors will ask participants to complete Activity #2.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessment:** Instructors will assess participants' learning by analyzing the Report Sheet from Activity #2.

APPLICATIONS

Participants will be divided into groups. Each group will be provided with a diseased plant. Information about the plant taxonomy, care, and environmental factors exposed will be provided to each group. Groups will be asked to ID signs and symptoms on the plant provided. They will be asked to take images that can help Extension agents process their samples. Once groups are finished, participants will be asked to fill Activity #2 Report Sheet.

ASSESSMENT

The Report Sheet will be assessed using a checklist.

CHECKLIST FOR REPORT SHEET

Checklist number	Description	Yes =1;No =0
1	Filled all parts requested.	
2	Includes plant ID, care, and location (field).	
3	Includes farm practices and information on climatic condition.	
4	Includes signs and symptoms present in plant.	
5	Includes images of the disease plant in the field and its surrounding.	
6	Includes close up images of the signs and symptoms in the plant.	

HOW TO USE CHECKLIST

- 1- Assess each Rubric sheet per group.
- 2- Complete checklist with responses of each group in the Report sheet.
- 3- If the description is met assign a 1. If the description is not met assign a 0.
- 4- Calculate the total of descriptions met at the end (Eg. 8 out of 10 -or- 10 out of 10).

SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY

Moskal, B. M. (2000). Scoring rubrics: What, when, and how? Practical Assessment, Research, and Evaluation, 7(1), 3.

Suskie, L. (2018). Assessing student learning: A common sense guide. John Wiley & Sons.

ACTIVITY #2: FACTORS CAUSING DISEASE IN PLANTS

INSTRUCTIONS AND HANDOUTS

I. PURPOSE

Expose participants to the proper ways to record data and report it to Extension agents.

II. MATERIALS

Diseased plant or images of one
Activity #2 Checklist
Activity #2 Report Sheet
Glue
Scissors

III. INSTRUCTIONS

Part 1.

***Instructions detailed below are for the instructor providing the activity.**

1- Divide participants into groups.

*The number of groups will depend on the amount of participants.

*For an efficient activity have no more than four participants per groups.

2- Provide each group with the Activity #2 Report Sheet, Activity #2 Checklist, and a disease plant or images of one.

3- Provide each group with information about the plant taxonomy, care, and environmental factors exposed will be provided to each group.

4- Ask groups to ID signs and symptoms on the plant provided, and take images that can help Extension agents process their samples.

5- Provide participants with the Activity #2 Report Sheet and Activity #2 Checklist.

6- Ask participants to fill the Activity #2 Report Sheet with the information they collected.

7- Ask participants to use the checklist to verify all information was collected.

8- Provide participants with feedback regarding their Activity #2 Report Sheet.

9- Collect Activity #2 Report Sheets from participants for future assessment.

IV. SOURCES

Agrios, G. N. (2005). Introductory plant pathology. 5th ed. Academic Press, New York, NY

Moskal, B. M. (2000). Scoring rubrics: What, when, and how? Practical Assessment, Research, and Evaluation, 7(1), 3.

Riley, M.B., Williamson, M.R., & O. Maloy. (2002). Plant disease diagnosis. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-1021-01.

Suskie, L. (2018). Assessing student learning: A common sense guide. John Wiley & Sons.



ACTIVITY #2

CHECKLIST

☐

FILLED ALL PARTS REQUESTED.

☐

INCLUDES PLANT ID, CARE, AND LOCATION (FIELD).

☐

INCLUDES FARM PRACTICES AND INFORMATION ON CLIMATIC CONDITION.

☐

INCLUDES SIGNS AND SYMPTOMS PRESENT IN PLANT.

☐

INCLUDES IMAGES - DISEASE PLANT IN THE FIELD AND ITS SURROUNDING.

☐

INCLUDES CLOSE UP IMAGES OF THE SIGNS AND SYMPTOMS IN THE PLANT.

ACTIVITY - REPORT SHEET

INFORMATION ON FIELD AND FIELD PRACTICES

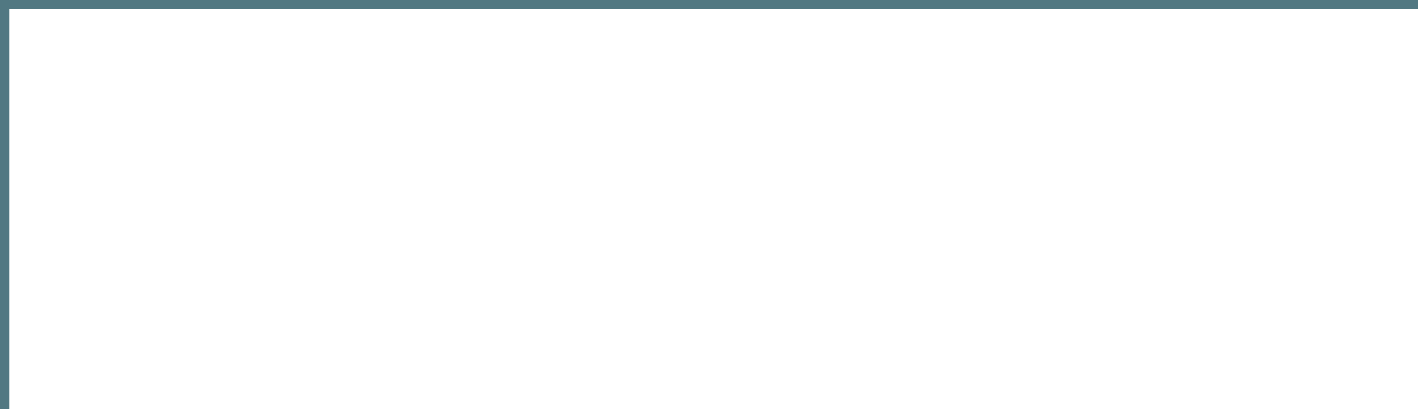
INFORMATION OF PLANT ID, CARE, CLIMATIC CONDITIONS

SIGNS AND SYMPTOMS

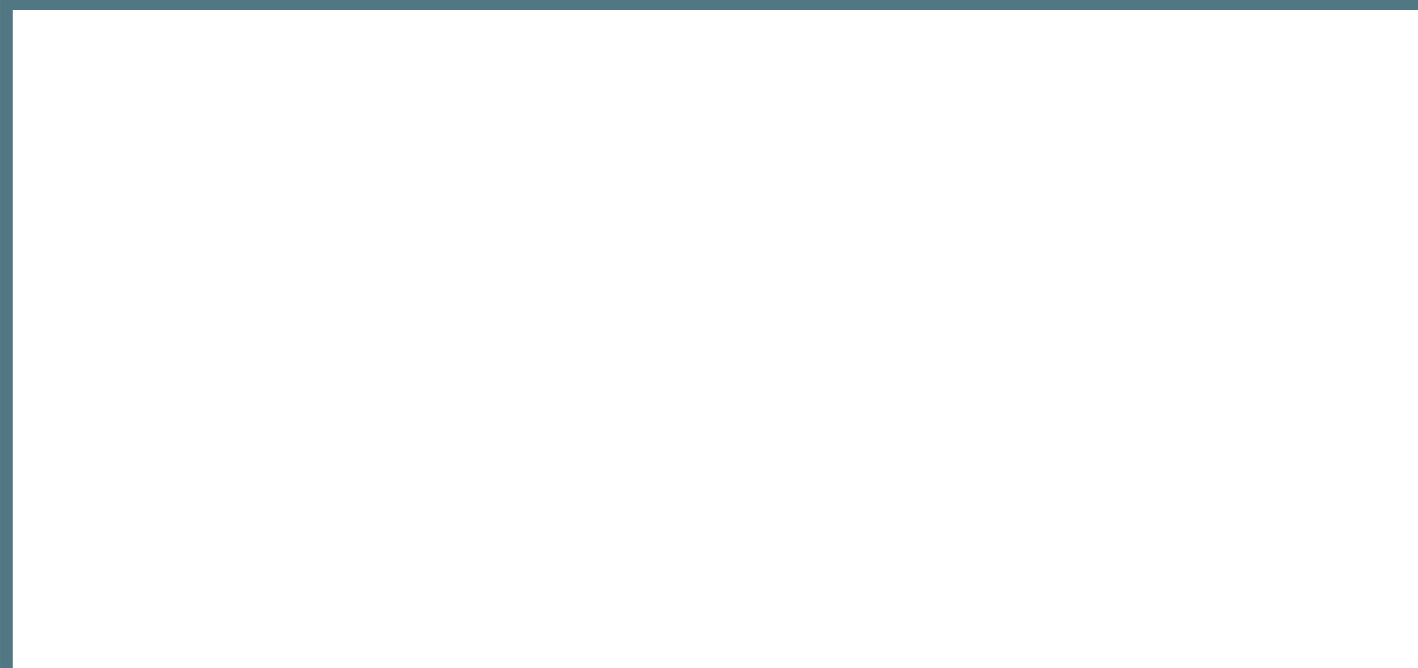
IMAGES OF WHOLE PLANT IN FIELD



IMAGES OF SURROUNDINGS



CLOSE UP IMAGES



ALTERNATIVE ACTIVITY #2: CHARACTERIZATION OF DISEASE

EXPERIMENT DETAIL, PROTOCOLS, AND HANDOUTS

I. PURPOSE

Expose participants to hand-on practice centered around the application of concepts explained in previous lectures.

II. ADDITIONAL INFORMATION

- For the purpose of this activity, we will only focus on morphological bacterial and fungal detection.
- Lab safety training will be provided by instructors to participants.
- Participants will receive a Report Checklist Sheet to guide them.

III. MATERIALS

Disposable Petri dishes containing Malt Extract Agar (MAE) + KCAT
Disposable Petri dishes containing Tryptic Soy Agar (TSA)
Sample of disease plants
Inoculation loop
Sterile scalpel
Light Microscope
Lactophenol Cotton Blue
Crystal violet
Iodine
Safranin
70% EtOH
Parafilm
Sharpies (to label)
Microscope slides
Cover slips
Bunsen burner
Report Sheet
Pictorial Atlas of Soilborne Fungal Plant Pathogens and Diseases
Phytopathogenic Bacteria and Plant Diseases Book
Incubator

IV. PROTOCOL

Groups will receive a sample of a diseased plant. Information about plant name, care, location, environmental factors exposed, signs and symptoms, and type of pathogens will be provided. Using this information, groups will brainstorm detection practices and design a plan to isolate the pathogen focused on morphological methods. Groups will discuss their plan with instructors prior to moving to next steps. They will modify their plan based on the instructor's feedback. Following, they will move on to the practice. Each member of the group will wear a lab coat, eye protection, and gloves. This activity will take place over multiple workshop sessions (plan accordingly).

Isolation Procedure:

- 1- Wipe down counter with 70% EtOH to ensure clean working surface.
- 2- Turn on the Bunsen burner.
- 3- Remove sterile scalpel from foil packet.
*Keep the packet closed when not in use (help decrease contamination).
- 4- Remove a piece of disease tissue from the bag and cut it into little pieces.
- 5- Put two pieces in MEA or TSA media.
*Keep the plate closed when not in use (help decrease contamination).
*Media will depend on the type of isolate. MAE: fungi; TSA: bacteria.
- 5- Once pieces are placed in media, Parafilm plate 2 times.
- 6- Store plate
 - a- For bacterial pathogens: plates will be stored at 37°C, in the dark, and upside down.
 - b- For fungal pathogens: plates will be stored at 25°C, in the dark, and upside down.
- 7- After a week, groups will return for morphological characterization.

Morphological Classification Procedure:

- 1- Wipe down counter with 70% EtOH to ensure clean working surface.
 - 2- Turn on the Bunsen burner.
 - 3- Take Parafilm off plate.
 - 4- If your isolate is a fungi move to step 5. If your isolate is a bacteria move to step 6.
 - 5- Fungal isolation (SEE FUNGAL STAINING PROTOCOL GRAPHIC BELOW)
 - a- Open lid of plate.
 - *When opening the plate, keep it close to the flame (help decrease contamination).
 - b- Take a microscope slide and add a drop of Lactophenol Cotton Blue.
 - c- Flame the tip of the inoculation loop and let it cool.
 - d- Remove a small amount of mycelium with cooled loop.
 - e- Place loop with mycelium in the drop of Lactophenol Cotton Blue on the microscope slide.
 - f- Place a cover slip on top and clean edges of residue.
 - g-Take slide to microscope and record the morphology and characteristics.
 - 6- Bacterial isolation (SEE GRAM STAINING PROTOCOL GRAPHIC BELOW)
 - a- Open lid of plate.
 - b-Take a microscope slide and add a drop of water.
 - c- Flame the tip of the inoculation loop and let it cool.
 - d- Swab isolate.
 - e- Place swab in drop of water on microscope slide and swab slide.
 - f- Heat fix the sample to the slide by carefully passing the slide through a Bunsen burner three times.
 - g- Add the crystal violet to the slide and incubate for 1 min.
 - h- Rinse slide for 3 seconds to remove unbound crystal violet.
 - i- Add Gram's iodine for 1 min.
 - j- Rinse slide with alcohol for 10 sec.
 - k- Rinse slide with water.
 - l- Add safranin to the slide and incubate for 1 min.
 - m- Wash with water for 3 sec.
 - n- Put slide under the microscope and characterize isolate, and record morphology.
- *When using microscope, add immersion oil to the 100X lenses. After each use, clean every lenses, put lenses to the 5X, and bring platform down.
- 7- Record all information into the group's report sheet. Include images and necessary information of the isolate. Use atlas to ID the isolate based on all the information provided and recorded.

V. PREPARING FOR ALTERNATIVE ACTIVITY #2 - For instructors only

1- To prepare crops for Activity #2 instructors have two options:

a- Contact an Extension agent and ask for samples of diseased crops.

*Make sure all the information necessary is collected from the Extension agent, including but not limited to the pathogen, host type, and conditions exposed.

b- Select a sample of a diseased plant from the environment.

*If this is selected, it is crucial to keep in mind that you may isolate multiple microorganisms and not be the causal agent.

VI. SOURCES

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Ryan, P. (unknown). Keeping a lab notebook: Basic principles and best practices. NIH, Office of Intramural Training and Education.
[https://www.training.nih.gov/assets/Lab_Notebook_508_\(new\).pdf](https://www.training.nih.gov/assets/Lab_Notebook_508_(new).pdf)

Smith, A.C., & Hussey, M.A. (2005). Gram stain protocols. American Society for Microbiology, 1-9.

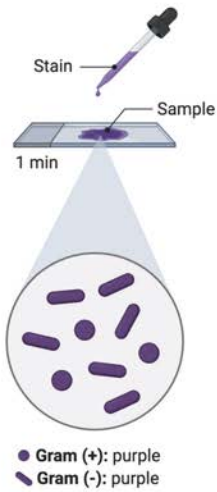
Watanabe, T. (2018). Pictorial atlas of soilborne fungal plant pathogens and diseases. CRC Press.

GRAM STAINING PROTOCOL

Step 1

Crystal violet

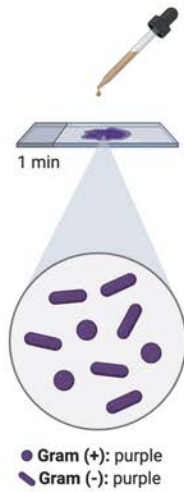
Primary stain added to specimen smear.



Step 2

Iodine

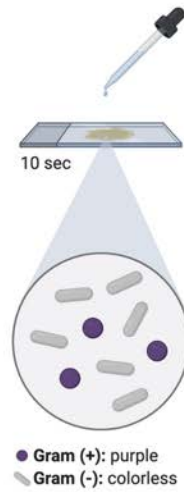
Mordant makes dye less soluble so it adheres to cell walls.



Step 3

Alcohol

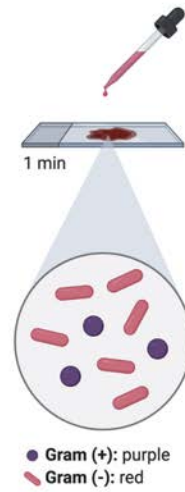
Decolorizer washes away stain from gram (-) cell walls.



Step 4

Safranin

Counterstain allows dye adherence to gram (-) cell walls.

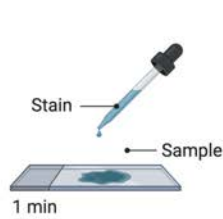


FUNGAL STAINING PROTOCOL

Step 1

Lactophenol

Add Lactophenol Cotton Blue



Step 2

Take isolate

Take mycelial fragments



Step 3

Add isolate

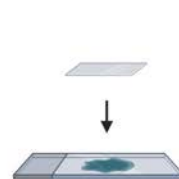
Add fragments to slide



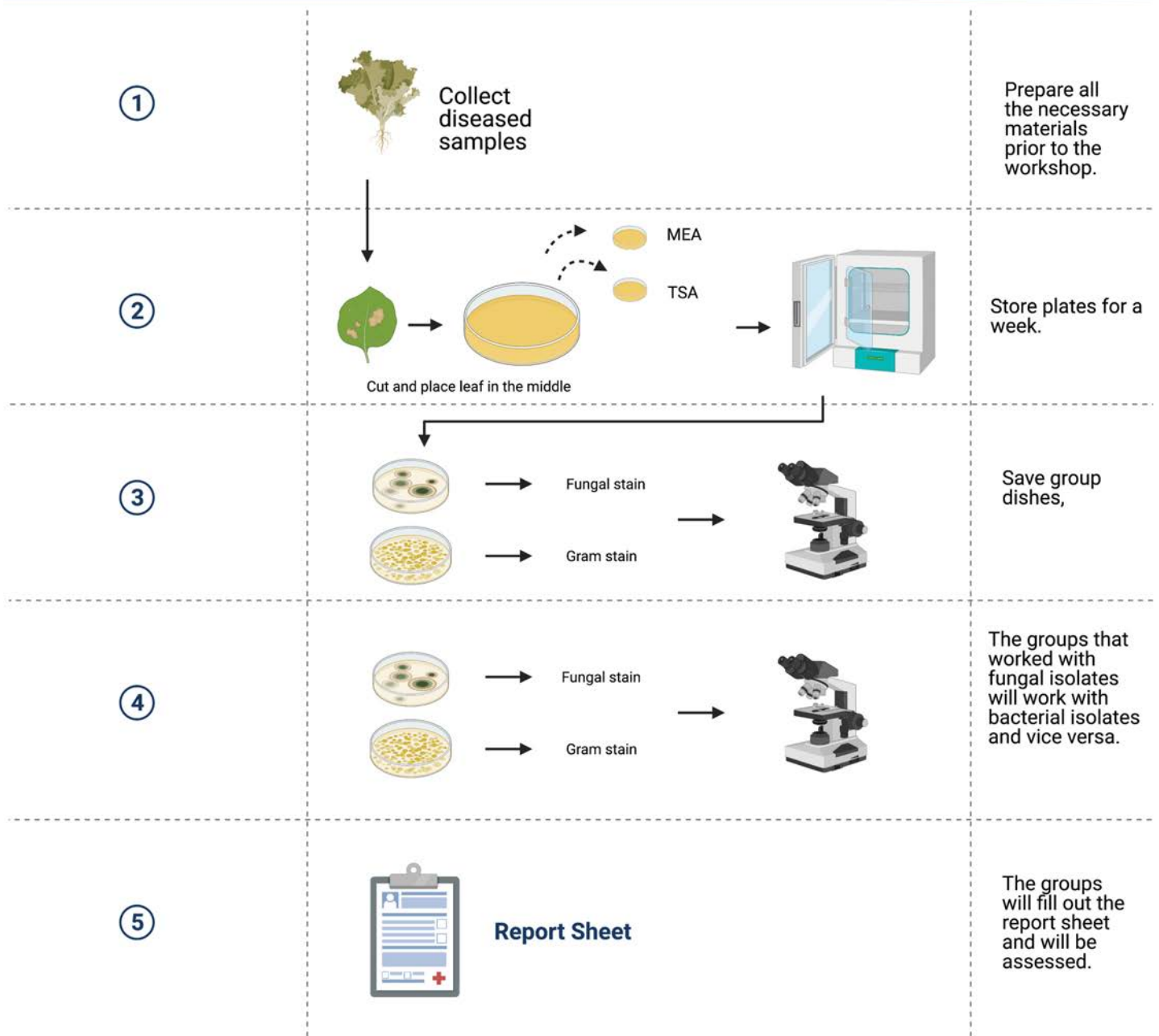
Step 4

Cover isolate

Cover sample with a cover slip



Overview of alternative activity



***INSTRUCTORS WILL DISPOSE OF PLATES AND REMAINING TISSUE.**

***PLATES WILL BE CLOSED AND PARAFILMED 3 TIMES.**

***PARAFILM PLATES WILL BE PUT IN ZIP-LOCK BAGS.**

***DISEASED TISSUE WILL BE KEPT IN ZIP-LOCK BAGS.**

***ZIP-LOCK BAGS WILL BE DISPOSED IN THE BIOHAZARD WASTE.**

WORKSHOP 3: CONTROL PRACTICES

ACTIVITY: #3

INSTRUCTOR: N. COLÓN

SESSION: 1

AUDIENCE: FARMERS PARTICIPANTS

INTRODUCTION/MODULE DESCRIPTION

This module introduces the concepts of control practices for plant disease. It will go over the different types of control practices, including biological control, chemical control, cultural control, and physical control. It will discuss their differences, uses, advantages, and limitations.

LEARNING OBJECTIVES

Discuss basic approaches for the control of plant disease.
Apply concepts learned.

KEYWORDS

plant disease, control, management

PURPOSE

Expose participants to control practices used to prevent or manage plant diseases in the field.

DURATION

2 hours

MATERIALS

Activity #3 Sheets, Projector, Laptop, Control Practices PPT

DIRECT INSTRUCTIONS

- **Introduction:** Instructors will provide a 1 hour lecture introducing the best practices for the control and management of plant diseases using the Control Practices PPT.
- **Development:** Instructors will provide definitions of each concept, advantages and limitations, mode of action, and examples of their use in the field.
- **Practice:** Instructor will ask participants to complete Activity #3.
- **Closure:** Instructor will wrap up concepts learned by providing a graphical summary.
- **Assessments:** Instructor will assess participant's learning by analyzing the concept maps.

APPLICATIONS

Participants will be asked to develop a concept map of one challenge they had experienced in their farms, related to plant diseases, and what practices they could implement to control it. Because the development of concept maps can be daunting for some individuals, we will incorporate a "Fill-in-the-map" template. Once the concept maps are finished, they will be paired with an Extension agent and will discuss the concept maps while receiving feedback.

ASSESSMENT

Concept maps will be assessed through a cross link scoring rubric.

CROSS LINK SCORING RUBRIC FOR CONCEPT MAPS

Rating level	Score	Description
Invalid	0	Cross link or connection between terms is incorrect.
Average	2	Cross link or connection between concepts is valid, but not meaningful for solving the problem.
Good	4	Cross link or connection between concepts is correct.

HOW TO USE RUBRIC

- 1- Assess each concept individually.
- 2- The concept maps are composed of 8 interconnections (4: can be resolved using; 4: because). When assessing focus on these.
- 3- Analyze each interconnection answering two questions:
 - a. Are participants linking concepts learned in the lectures to their applications in the field?
 - b. Are the links among concepts correct?
- 4- Score each interconnection using the cross link scoring rubric.
- 5- Calculate the total score.

***SEE CONCEPT MAP EXAMPLE FOR SCORING**

***SCORES ARE IN RED; REASONING FOR SCORE ARE IN YELLOW; TOTAL SCORE IN PURPLE**

SOURCES

Agrios, G. N. (2005). *Introductory plant pathology*. 5th ed. Academic Press, New York, NY.

Croasdell, D. T., Freeman, L. A., & Urbaczewski, A. (2003). Concept maps for teaching and assessment. *Communications of the Association for Information Systems*, 12(1), 24.

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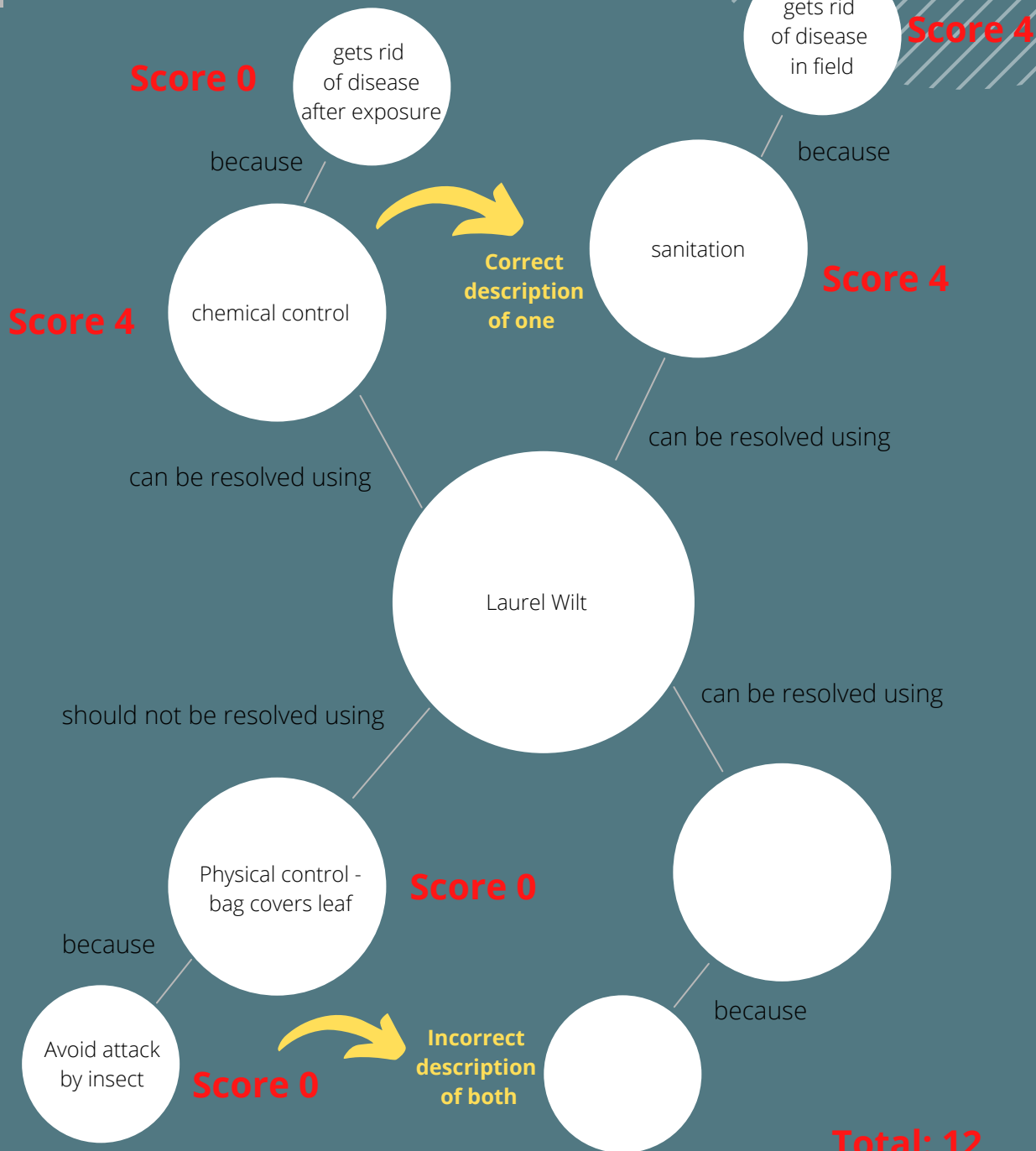
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Concept Map Example

"Fill-in-the-map"

Correct description of both



Concept Map Example

"Fill-in-the-map"

