This lesson was developed by the Khaled bin Sultan Living Oceans Foundation and Louisiana Universities Marine Consortium (LUMCON) using grant funding from the National Geographic Society. The lesson has been designed for secondary school students but can be adapted for other uses.

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About Khaled bin Sultan Living Oceans Foundation
The Khaled bin Sultan Living Oceans Foundation was incorporated in California as a 501(c)(3), public benefit, Private Operating Foundation in September 2000. The Living Oceans Foundation is dedicated to providing science-based solutions to protect and restore ocean health through research, outreach, and education. The educational goals of the Khaled bin Sultan Living Oceans Foundation and development of this are generously supported by Prince Khaled bin Sultan of Saudi Arabia.

About Louisiana Universities Marine Consortium (LUMCON)
LUMCON's vision and mission is to maintain a national and international reputation as a leading marine research and education Consortium, and stimulate, promote, facilitate, and conduct research and education collaborations among Louisiana's universities in marine and coastal sciences relevant to the sustainability of coastal and marine environments of the Gulf of Mexico.

Dear Educator,

The Khaled bin Sultan Living Oceans Foundation and Dr. Ryann Rossi have partnered once again to develop a lesson plan about the vectors of mangrove disease. The lesson provides students with the ability to participate in hands-on research through a citizen science program called Mangrove Detectives.

Around the world mangroves are in peril. Much of the decline of mangrove forests has been due to human destruction, including overexploitation, coastal development, changes in water flow, oil spills, and marine debris. In the Bahamas and greater Caribbean, there is great concern because large areas of mangroves have completely died off. As a result, scientists have been researching these areas in hope of finding out what is causing the die-off, so that damage to critical mangrove habitats can be mitigated.

Dr. Ryann Rossi first became interested in mangrove die-offs throughout the Bahamas when she was getting her doctorate at North Carolina State University. In 2013, Dr. Rossi started researching various mangrove die-off areas in the Bahamas. She found that mangroves are dying due to the combined effects of plant pathogens, herbivory, and altered abiotic factors, possibly due to humans. In 2017, Dr. Rossi and the Khaled bin Sultan Living Oceans Foundation partnered to develop a lesson plan that allowed high school students to participate in citizen science. During the lesson, students collect, process, and analyze potentially diseased leaves of mangroves. Then they determine what pathogens are present and explore what could be causing the disease. The Khaled bin Sultan Living Oceans Foundation, in collaboration with their partners at FRIENDS of the Environment, implemented these lesson plans into their Bahamas Awareness of Mangroves (B.A.M.) program. For more information about B.A.M. visit *www.lof.org/BAM*.

Dr. Ryann Rossi, the Khaled bin Sultan Living Oceans Foundation, and Friends of the Environment had a larger goal to build a program called Mangrove Detectives, which would allow them to expand the already existing curriculum and make it available to a larger audience. In 2018, they received a grant from the National Geographic Society to create Mangrove Detectives. With this funding they developed two additional lesson plans, instructional videos, and built a website to host this information and collect student data.

In this lesson, students will investigate insect grazing on the leaves of mangroves. Activities include randomly sampling mangrove leaves, categorizing the types of grazing present on the leaves, and calculating the percentage of the grazed areas on the mangrove leaves. Students will participate in citizen science by uploading their data to the Mangrove Detectives website. This information will help scientists document the types of insect grazing on mangrove leaves.

For more information, please visit the Mangrove Detectives program website at: *www.mangrovedetectives.org*.

Our Best,

Amy Heemsoth, M.Sci.
Director of Education
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Ryann Rossi, Ph.D.
Post-doctoral Research Associate
Louisiana Universities Marine Consortium (LUMCON)

Lesson 9B Teacher's Notes

Author

- Ryann Rossi, LUMCON & Amy Heemsoth, Khaled bin Sultan Living Oceans Foundation

Learning Objectives

- Record location, date, time, tide, temperature, and wind speed and direction.
- Determine the latitude and longitude of mangroves using a GPS unit or other electronic device.
- Make observations about the mangrove forest.
- Identify red mangrove trees.
- Randomly sample red mangrove tree leaves.
- Record results in data tables.
- Label and photograph mangrove leaf samples.
- Recognize and record the presence of insect grazing.
- Differentiate between the types of insect grazing.
- Categorize the type of grazing present on a mangrove leaf.
- Trace a mangrove leaf on graph paper.
- Dissect a mangrove leaf, removing the areas that were grazed on by insects.
- Calculate the total area of a mangrove leaf, the total area of grazed sections of a mangrove leaf, and the percentage of grazed areas of a mangrove leaf.
- Determine the most numerous type(s) of grazing on mangrove leaves.
- Compare and contrast the types of grazing present on mangrove leaves to other students' or groups' results, and conclude why there were similarities or differences.
- Explain the importance of calculating the total percentage of a grazed mangrove leaf.
- Conclude how insect grazing helps scientists to understand mangrove disease.
- Give your opinion why scientists should study mangrove grazers.
- Determine the most detrimental types of leaf grazers.
- Deduce the effects of insect grazing on the individual leaf and the whole plant.

Keywords

- Chewing
- Citizen Science
- Disease
- Geographic Location
- Geographic Positioning System (GPS)
- High Tide
- Host
- Insect
- Leaf Galls
- Leaf Mine
- Low Tide
- Observation
- Random Sampling
- Red Mangrove
- Skeletonization
- Stippling
- Temperature
- Trap
- Vector
- Wind Speed

Materials

- GPS or electronic device
- Scissor
- Scalpel
- White paper
- Ruler (metric)
- Camera
- **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet
- **Types of Insect Grazing Scars PowerPoint**
- **Appendix A: Instructions for Using Offline Maps**
- **Appendix B: Types of Insect Grazing Scars**
- **Appendix C: Instructions for Using IMAGEJ Software**

EXTENSIONS

- In the Bahamas, scientists have found the presence of disease in mangrove leaves. They are researching the insects that live in the mangroves because they are believed to be the vectors of disease. Students can research the presence of disease in the mangroves by completing **Lesson 8: Outbreak Investigation** or for a shorter activity, complete **Read It! Disease in the Trees**.
- Download data from *www.mangrovedetectives.org* website and compare and contrast grazed mangrove leaves from all over the world.
- Extend this lesson by using a microscope to look for leaf miners, such as mites, in the mangrove leaves that were collected during the activity.

STANDARDS

Ocean Literacy Principles and Fundamental Concepts (Grades 9-12)

- Principle 5.B.11: Ocean ecosystems are connected to each other in a macro food web. Over time, organisms move from one ecosystem to another as they grow, migrate, and die. Changes in an ecosystem or an organism may have unpredictable effects on other ecosystems.
- Principle 6.E: Achieving sustainability of the diverse ecosystems and resources in the ocean depends upon collective and individual action based on scientific research and exploration.
- Principle 7.B.5: Exploring the ocean involves global participation because there is only one ocean that connects and sustains all life.

PROCEDURE

PART 1: FIELD DATA COLLECTION

A. PREPARATION:

1. In case of inclement weather, have a backup plan.
2. Make sure to follow all school or education facility policies.
3. When first discussing the field trip, be sure to cover the following safety procedures:
   a. Everyone will have a buddy. You are to stay with that buddy at all times.
   b. Do not wander from the path or away from your instructor(s).
   c. Wear comfortable, close-toed walking shoes that could possibly get dirty and/or wet.
   d. Bring water on the hike. It will be hot, and everyone needs to stay hydrated.
   e. There will probably be bugs, so bring bug spray.
   f. Wear sunscreen. Hats are allowed, as well.
   g. Do not touch anything unless your instructor asks you to.
4. Inform parents/guardians of the field trip and get all permission slips and/or liability waivers signed. Send parents a description of the field trip and a checklist of items that the students will need to bring the day of the field trip.
5. Bring a fully stocked first aid kit on the mangrove field trip.
6. In case of emergency, make sure that at least two adults have a way to communicate (such as a cell phone). Make sure to share this contact information with the school or education facility.
7. Check for wind speed and direction at *http://www.wunderground.com*.
   a. In the search field (upper right-hand corner), if conducting the activity outside of the United States, type in the name of the city and country where the mangrove field trip will take place. If conducting the activity in the United States, type in the name of the city and state.
   b. Click on the date under the 10-day weather forecast.
   c. Print out the hourly weather, which contains the wind speed and direction. **NOTE**: To be as accurate as possible, look up the information as close to the day and hour of the field trip as possible.
8. Before going to the mangrove site, make sure to check the tide tables. If the mangrove forest typically has standing water, survey when the tide is low. Tide tables can be found at *http://tidesandcurrents.noaa.gov/tide_predictions.html*.
   a. In the search field, type the name of the city and country where the mangrove walk will take place.
   b. Enter the date that you will be going on the field trip.

c. Print out the tide table.

NOTE: If you cannot find the mangrove location on the NOAA website, check tide tables at: *https://www.tide-forecast.com*.

9. Print and laminate **Appendix A: Instructions for Using Offline Maps** (optional) and **Appendix B: Types of Insect Grazing Scars**. **Appendix C: Instructions for Using IMAGEJ Software** is not required to complete this activity. If using **Appendix C: Instructions for Using IMAGEJ Software**, print and laminate.
10. If using **Appendix A: Instructions for Using Offline Maps**, make sure to charge electronic devices prior to the field trip and complete step A in *Appendix A*.
11. Decide if the students are going to collect leaves individually or in groups.
12. Find a mangrove forest that has numerous red mangrove trees.

B. FIELD TRIP:

1. Inform students to bring two pencils and their **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet on the field trip.
2. Students need to be able to identify red mangrove trees. Review the characteristics of a red mangrove tree (prop and drop roots; leaf shape, color, and texture; red tannin under bark).
3. Explain why scientists randomly sample. Tell students the number of leaves that you want each student or group to collect. Instruct students to randomly sample potentially diseased red mangrove leaves.
4. Prompt students to fill out *Table A* on **Lesson 9B: Secret Agents – Investigating Insect Vectors** *Part 1: Field Data Collection* student worksheet.

   NOTE: If your school does not have a GPS device(s), you can use a smart phone or tablet to mark the GPS location using offline maps. You do not need the internet to use these maps. One example of an app that utilizes offline maps is Google Maps. For instructions on how to use Google Maps, see **Appendix A: Instructions for Using Offline Maps**. No matter which device you choose to use, make sure to protect your device(s) from the different environmental conditions (water and heat) that are present in the mangroves by equipping your device(s) with the appropriate hardware.

PART 2: SAMPLE PROCESSING

1. Present **Types of Insect Grazing Scars PowerPoint**.
2. Hand out **Appendix B: Types of Insect Grazing Scars**.
3. In the classroom, instruct students to determine the types of grazing scars on their leaves using **Appendix B: Types of Insect Grazing Scars**.
4. Instruct the students to follow the instructions on the **Lesson 9B: Secret Agents – Investigating Insect Vectors** *Part 2: Sample Processing* student worksheet.

   NOTE: There is an alternative method to calculating the area of a grazed mangrove leaf. This information can be found in **Appendix C: Instructions for Using IMAGEJ Software**. Instead of calculating the areas of a leaf and the grazed areas of a leaf, students will use an online program called ImageJ to obtain the calculation. Students will still need to calculate the percentage of grazed areas of a mangrove leaf. ImageJ does not have this functionality. This method can be used as a substitute or additional way to calculate the area. If using both methods to figure out the percentage of a grazed mangrove leaf, ask students to compare both results and have them determine which method is more accurate.

ATTRIBUTIONS

APPENDIX B:

NOTE: Photos are listed per category and attributed in order from left to right.

Chewing

- Mangrove Skipper (*Phocides pigmalion*) (11381559594) By Bob Peterson from North Palm Beach, Florida, Planet Earth! [CC BY-SA 2.0 (*https://creativecommons.org/licenses/by-sa/2.0*)], 12 September 2013 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Mangrove_Skipper_(Phocides_pigmalion)_(11381559594).jpg*.

- Leaves with stinging nettle caterpillar feeding damage By Forest & Starr [CC BY 4.0 (*https://creativecommons.org/licenses/by/4.0/*)] 26 October 2009 via Starr Environmental. *http://www.starrenvironmental.com/images/image/?q=24619349519*.
- Leaves with native *Omiodes blackburni* moth chewing By Forest & Starr [CC BY 4.0 (*https://creativecommons.org/licenses/by/4.0/*)] 13 May 2012 via Starr Environmental. *http://www.starrenvironmental.com/images/image/?q=24774852149*.

Leaf Galls

- Leaf gall Mangroves_03977 By Vengolis [CC BY-SA 4.0 (*https://creativecommons.org/licenses/by-sa/4.0*)] 2 September 2015 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Mangroves_03977.JPG*.
- Leaf Gall (on Grey Box) (16203601706) By Patrick_K59 [CC BY 2.0 (*https://creativecommons.org/licenses/by/2.0*)] 8 January 2015 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Leaf_Gall_(on_Grey_Box)_(16203601706).jpg*.
- Mango leaf gall midge on leaf By Gutam2000 [CC BY 4.0 (*https://creativecommons.org/licenses/by/4.0*)] 17 January 2017 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Mango_leaf_gall_midge_on_leaf.jpg*.

Leaf Mining

- Leaf miner track By SKsiddhartthan [CC BY-SA 4.0 (*https://creativecommons.org/licenses/by-sa/4.0*)] 18 December 2017 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Leaf_miner_track.jpg*.
- Leaf miner on macadamia By Toby Hudson [CC BY-SA 3.0 (*https://creativecommons.org/licenses/by-sa/3.0*)] 24 March 2012 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Leaf_miner_on_macadamia.jpg*.
- Leaf miner – fern By Shyamal [CC BY-SA 3.0 (*https://creativecommons.org/licenses/by-sa/3.0*)] 19 January 2019 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Leaf_miner_-_fern.jpg*.

Skeletonization

- Palm leaf skeletonizer moth (29766989533) By Katja Schulz [CC BY 2.0 (*https://creativecommons.org/licenses/by/2.0*)] 2 October 2016 via Wikimedia Commons. *https://commons.wikimedia.org/wiki/File:Palm_Leaf_Skeletonizer_Moth_(29766989533).jpg*.
- Skeletonized Alder leaves By Forest Service photo by Tania C. Parra [Public Domain Mark 1.0 (*https://creativecommons.org/publicdomain/mark/1.0/*)] 17 September 2018 via Flickr. *https://www.flickr.com/photos/sanbernardino_nf/43897350435*.
- Skeletonized Leaf By Katja Schulz [CC BY 2.0 (*https://creativecommons.org/licenses/by/2.0*)] 18 July 2015 via Flickr. *https://www.flickr.com/photos/treegrow/23020438535.*

Stippling

- White Apple Leafhopper (Image # 5369839) By Whitney Cranshaw, Colorado State University, Bugwood.org [Creative Commons Attribution 3.0] 12 May 2008 via Forestry Images. *http://www.forestryimages.org/browse/detail.cfm?imgnum=5369839*.
- 2-s spidermite leaf stippling By Whitney Cranshaw, Colorado State University, Bugwood [CC BY 2.0] (*https://creativecommons.org/licenses/by/2.0*)] 27 July 2012 uploaded via Flickr. *https://www.flickr.com/photos/ksrecomm/7657295764*.
- Two-spotted Spider Mite (Image #5369739) By Whitney Cranshaw, Colorado State University, Bugwood.org [Creative Commons Attribution 3.0] 12 May 2008 via Forestry Images. *http://www.forestryimages.org/browse/detail.cfm?imgnum=5369739*.

Khaled bin Sultan Living Oceans Foundation

LESSON 9B SECRET AGENTS

ADDITIONAL BACKGROUND INFORMATION

Scientist Dr. Ryann Rossi has discovered the presence of diseased mangroves in the Bahamas, which may be causing large numbers of mangrove trees to die. The pathogen that causes the disease was determined to be *Pestalotiopsis*, a genus of fungus. This pathogen infects the leaves of the mangrove trees causing the leaves, and potentially the tree, to die. To make matters worse, Dr. Rossi also believes that grasshoppers may be vectors or carriers of the fungus, which cause the disease to spread. *Pestalotiopsis* spores may attach to the legs of grasshoppers and as they jump from leaf to leaf of the mangrove tree, the spores are transported, infecting more leaves. Scientists are now researching these insects and others that live in the mangroves to determine if they could be carriers of the disease and how the disease might be spreading. Today, you will be a citizen scientist participating in their research by collecting additional data about the types of insect grazing that is present on mangroves leaves.

PART 1: FIELD DATA COLLECTION

INSTRUCTIONS: Fill in the following information.
TABLE A:

Mangrove Location Name:			
Date (month/day/year):		**Time**:	
Wind Speed:		**Direction**:	
Tide			
Time Low:	**Height Low (m)**:	**Time High**:	**Height High (m)**:
Geographic Location			
Latitude (N or S):		**Longitude (E or W)**:	

Observations: Make observations about the area surrounding your trap. Remember an observation is when you draw a conclusion based off of one of the five senses: sight, sound, touch, taste, and smell.

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PART 2: SAMPLE PROCESSING

INSTRUCTIONS: Follow the instructions below.

1. For each mangrove leaf, complete the following steps and record the information in *Table B*:
   a. Record the presence (yes) or absence (no) of grazing.
   b. If grazing is present, categorize the types of grazing. **NOTE**: There may be multiple types of grazing present on one leave. List all types of grazing present.
   c. Place an individual leaf on a piece of white paper with the top side of the leaf facing upwards.
      i. Assign the leaf with a unique identification (ID) label. **NOTE**: The labels should be consecutive (e.g. ME1, ME2, ME3).
      ii. In pencil, write the ID and the word “upper side” (for upper side of the leaf) next to the leaf.
      iii. For scale, position the metric side of a ruler next to the leaf, but do not cover the ID label or word “upper side.” See the image below for an example.

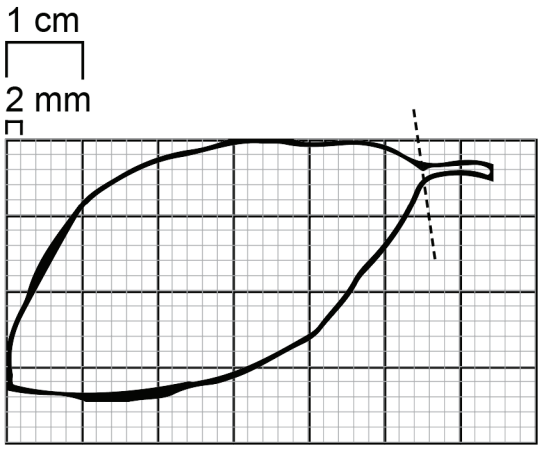
      iv. Use a camera to take a photo of the upper side of the leaf. Hold the camera approximately 3 inches above the leaf and center the camera horizontally over the leaf. Make sure that the image is not blurry and take the photo.
      v. Remove the leaf and flip the piece of paper over. Write the ID label and the word “underside” (for underside of the leaf) next to the leaf.
      vi. Flip the leaf over so that the underside of the leaf is facing upwards. For scale, position the metric side of a ruler next to the leaf, but not covering the ID label or world “underside.” See the image below for an example.

      vii. Use a camera to take a photo of the underside of the leaf. Hold the camera approximately 3 inches above the leaf and center the camera horizontally over the leaf. Make sure that the image is not blurry and take the photo.
      viii. If you have more than one leaf, repeat steps a-c.

Table B:

Leaf Sample #	Presence of Grazing (Y/N)	Types of Grazing Present (may be more than one)	Approximate Total Area of Mangrove Leaf (mm^2)	Approximate Total Area of Grazed Section(s) of Mangrove Leaf (mm^2)	Approximate Percentage of Grazed Areas of Mangrove Leaf (%)

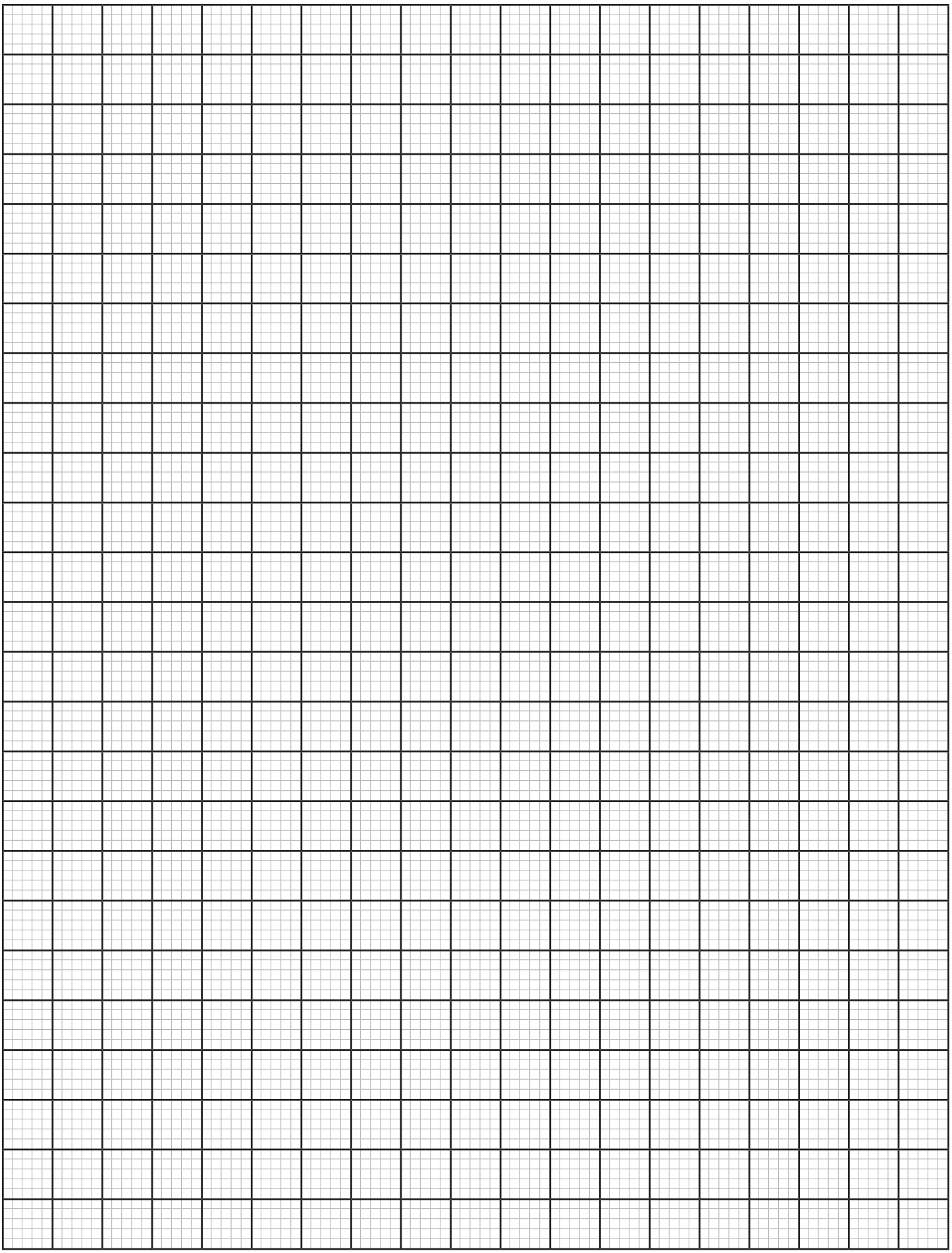
2. Determine the percentage of the mangrove leaf that has been grazed and record your results in *Table B*. Follow the instructions below to make this calculation.
   a. Estimate the total area of the leaf:
      i. Using the graph paper below or provided by your teacher, trace the outline of the mangrove leaf. **NOTE**: Do not draw the stem or petiole of the leaf. Before tracing the leaf, you can cut off the stem with a scissor. See the example below.



      ii. Remove the mangrove leaf from the graph paper. Count the number of square millimeters. Estimate the number of partial squares. A good rule of thumb is to count a partial square if it is at least half covered by the leaf.
   b. Estimate the total area of the grazed section(s) of the mangrove leaf.
      i. Remove the grazed areas of the leaf with scissors and/or a scalpel.
      ii. Place the mangrove leaf over the outline that you created in Step 2ai. Color in the areas where the mangrove leaf was grazed. Do not use a dark color. Use a light color where you can still see the squares when you color over them.
      iii. Remove the mangrove leaf from the graph paper. Count the number of square millimeters where you shaded in the grazed areas. Estimate the number of partial squares. A good rule of thumb is to count a partial square if it is at least half covered by the leaf.
   c. Calculate the percentage of the grazed areas of the mangrove leaf using the following equation:

$$\%\ \text{of Grazed Mangrove Leaf} = \frac{\text{Total Area of Grazed Sections}}{\text{Total Area of the Mangrove Leaf}} \times 100\%$$

3. Upload photos and data to the project website at *https://mangrovedetectives.org/lessons/*.



INSTRUCTIONS: Answer the following questions.

1. What is the most numerous type of grazing that you found in your mangrove leaf sample(s)?

2. How did this compare to the rest of the class? Why do you think there were differences or similarities?

3. Why is it important to calculate the total percentage of the mangrove leaf that was grazed?

4. What can insect grazing help scientists to understand about mangrove disease?

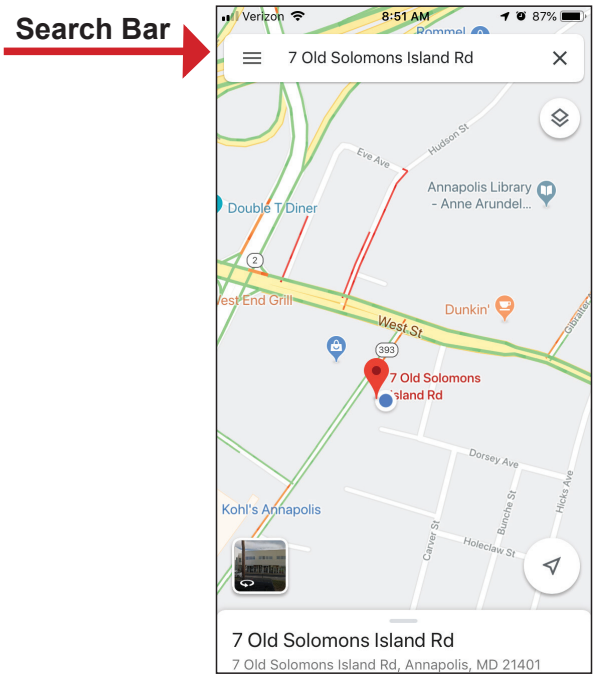
5. Which type of grazing do you think is most detrimental to leaves? Why?

6. What effects might insect grazing have on a leaf? On the whole plant?

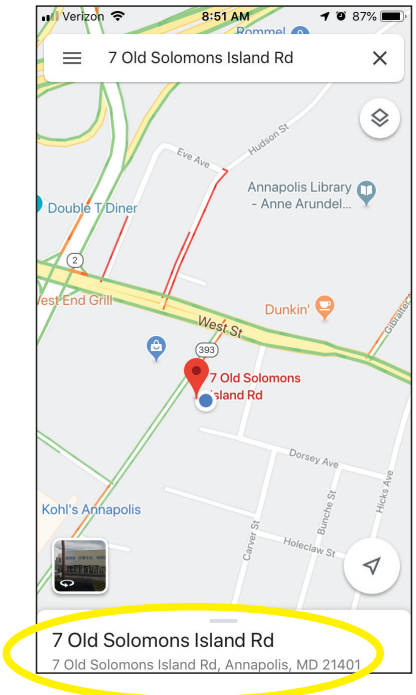
INSTRUCTIONS FOR USING OFFLINE MAPS

A. DOWNLOAD AN OFFLINE MAP:

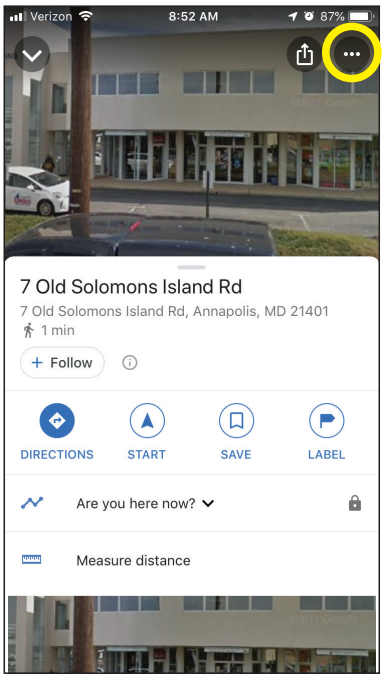
1. Establish an internet connection on your electronic device.
2. If Google Maps is not already downloaded on your device, then download the app.
3. Sign-in to Google Maps or setup login information.
4. In the search bar, enter the city or island name nearest to the location of the mangrove forest you will be visiting.



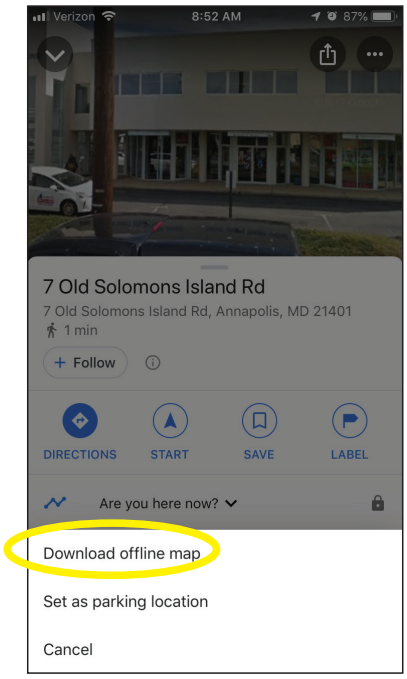
5. At the bottom of the screen, tap the name of the location that you just entered.



6. Then, tap the symbol that is circled (in yellow) in the upper right-hand corner of the screen.

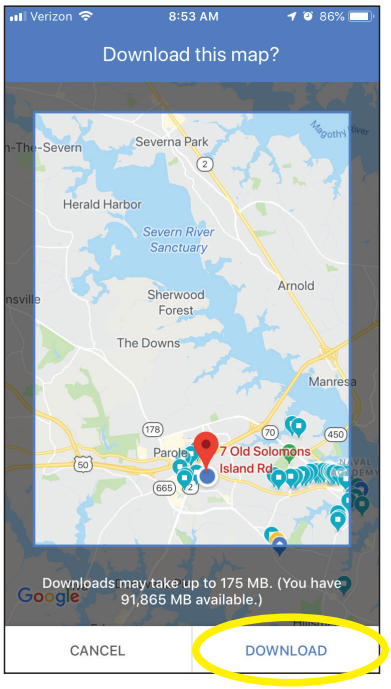


7. Select “Download offline map.”

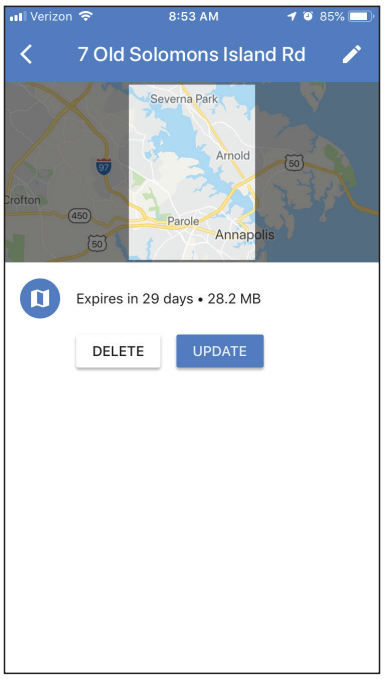


8. Using two fingers, zoom in and out to select the mangrove area. It’s better to capture a larger area that includes the area surrounding the mangroves.

9. Select “Download.”

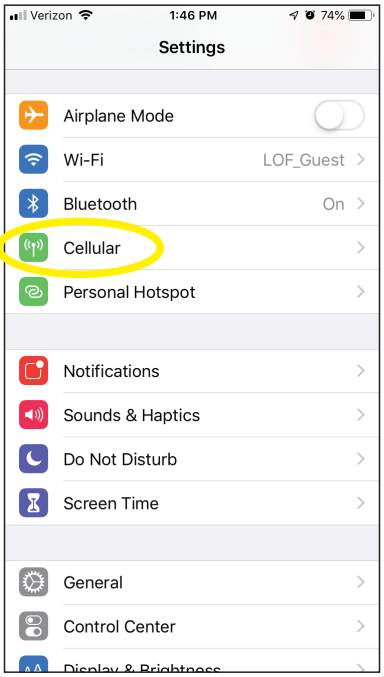


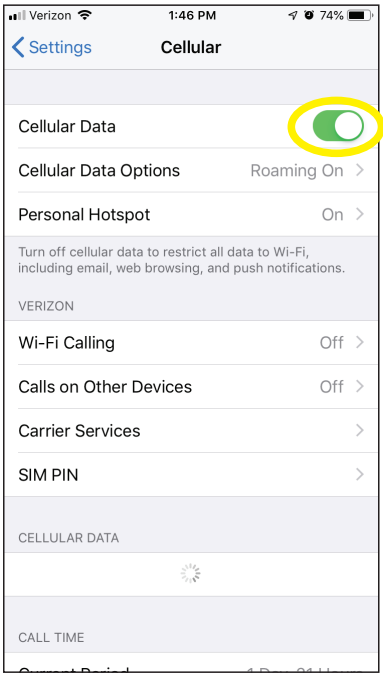
10. The map will be downloaded and saved offline for 30 days.



B. USE AN OFFLINE MAP:

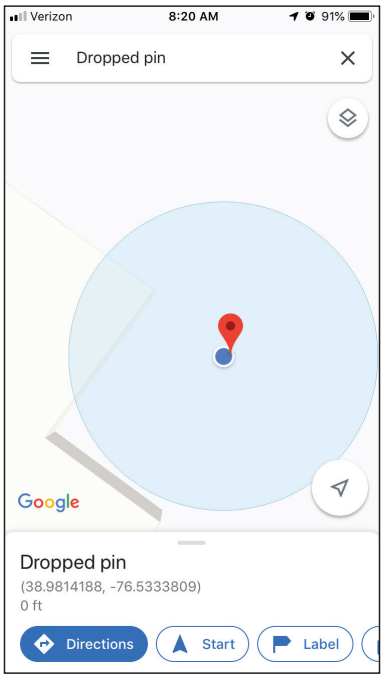
1. If applicable, turn off data on the electronic device.
   a. Go to "Settings."
   b. Select "Cellular."
   c. Turn off the "Cellular Data" by pushing the toggle (will turn grey when off).





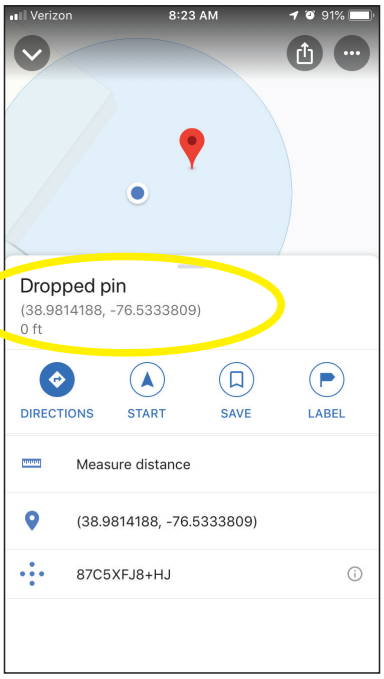
2. Open Google Maps app.
3. Use Google Maps as you normally would.
4. When you need to find your GPS coordinates, use two fingers to zoom in to your location, which is represented by a blue dot. Make sure to scroll in close to the blue dot.
5. Drop a pin over the blue dot. To do so, hold your finger over the blue dot to drop a pin.

Latitude, Longitude

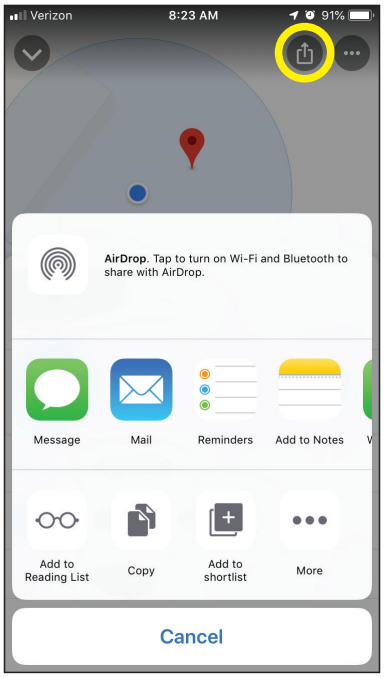


6. Record the GPS location (latitude and longitude) in *Table A* on your **Lesson 9A: Secret Agents – Investigating Insect Vectors** student worksheet.

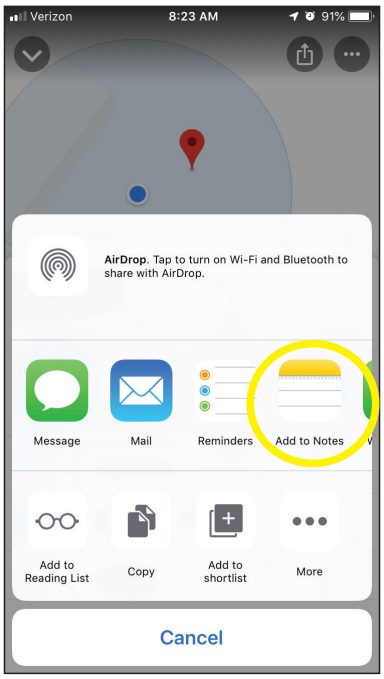
7. To save a pin:
   a. For Apple devices:
      i. At the bottom of your screen, use your fingers to tap the GPS location.



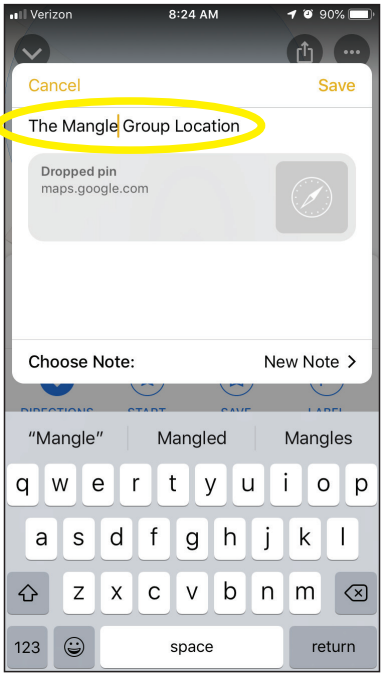
      ii. Select the Share button circled in yellow.



iii. Select “Add to Notes.”



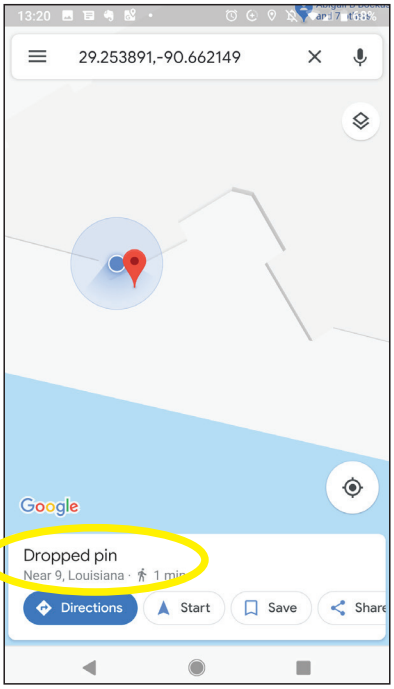
iv. Label the pin with your group information or your name.



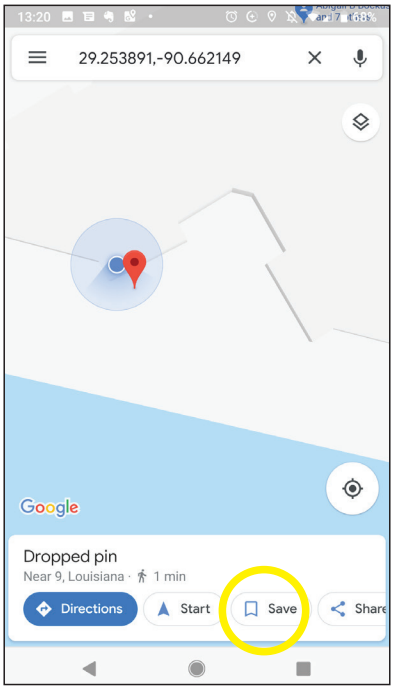
v. Select “Save.”

b. For Android devices:

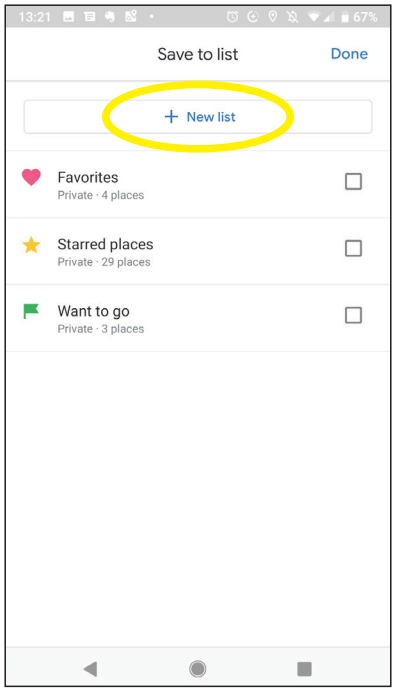
i. Tap the location of the place.



ii. Tap “Save.”



iii. Click on “New List.”



iv. Type your group information or your name into the text field.

v. Hit “Create.”

TYPES INSECT GRAZING SCARS

CHEWING

Part of tissue and veins are absent on the leaf. This is caused by insects that have mandibles or jaws to bite, tear, and chew food.



LEAF GALLS

Abnormal growth or swelling on the external tissue of the leaf. This is caused by insects or mites feeding or laying eggs.



LEAF MINE

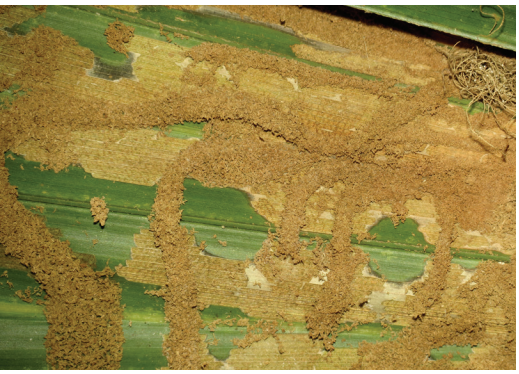
Absence of leaf tissue often forming linear patterns on the leaf. This is due to larval insects that bore into the leaf tissue and feed on it.



TYPES INSECT GRAZING SCARS

SKELETONIZATION

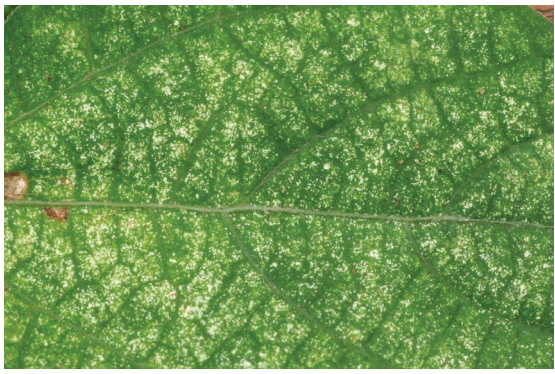
Absence of leaf tissue but the veins of the leaf remain. This is caused by insect feeding.



STIPPLING

Small whitish colored dots or specs caused by insects sucking out the leaf's sap using their piercing mouthpart.





INSTRUCTIONS FOR USING IMAGEJ SOFTWARE

1. Download the photos of the mangrove leaves that you took during *Part 2: Sample Processing* **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet and save them to your computer.
2. Go to the following website: *http://imagej.nih.gov/ij/.*
3. Choose the "*Download*" tab at the top of the web page.
   a. If using a Mac computer, click on "*Instructions*" under "Mac OS X*"* and follow the steps to download the software.
   b. If using a Windows computer, click on "*Instructions*" under "Windows*"* and follow the steps to download the software.
4. Open the ImageJ software on your computer.
5. In the ImageJ software, click on "*File"* and then "*Open*." Select one of the mangrove leaf photos that you downloaded and select "Open." **NOTE**: The upper side and underside of the leaves are photographed. Use the upper side of the photos.
6. Set the scale in ImageJ by clicking on "*Analyze*" and then "*Set Scale*."
   a. A window will open. Fill in the following information:
      i. Distance in pixels: 144
      ii. Known distance: 6
      iii. Pixel aspect ratio: 1.0
      iv. Unit of length: millimeters (mm).

   NOTE: After you have set the scale, you should not need to reset it, unless you shut down the ImageJ software.
7. You are now going to trace the entire leaf including the lesions.
   a. Select the "*Freehand Selection Tool*." 
   b. Trace the leaf by holding down the mouse and dragging it around the leaf. **NOTE**: Do not include the stem or petiole of the leaf.
   c. Click "*Analyze*" and then "*Measure*."
   d. A window with the measurements will appear. This measurement is the area of the leaf. Record the following information in *Table B* of your **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet:
      i. Leaf Sample #
      ii. Presence of Grazing (Y/N)
      iii. Approximate Total Area of the Mangrove Leaf (Recall that we set the scale to millimeters, so the area is measured in mm^2)
8. You are now going to trace the lesion(s) on the leaf
   a. Select the "*Freehand Selection Tool*."
   b. Trace the lesion by holding down on the mouse and dragging in around the lesion.
   c. Click "*Analyze*" and then "*Measure*."
   d. A window with the measurements will appear. This measurement is the area of the lesion. Record the following information in *Table B* of your **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet:
      i. Approximate Total Area of Grazed Sections(s) of the Mangrove Leaf (Recall that we set the scale to millimeters, so the area is measured in mm^2)
   e. Repeat *steps 8a-d* for each lesion on the leaf. If you have more than one lesion on your leaf, then you will need to add the areas together.
9. Calculate the percentage of grazed areas of the mangrove leaf. Follow *step 2c* instructions on *Part 2: Sample Processing* **Lesson 9B: Secret Agents – Investigating Insect Vectors** student worksheet.