Title: Students as Scientists: The Study of Tomato Spotted Wilt Virus in Marion County
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Forest High School

Abstract: An important objective in any science class should be to engage the students as scientists. This project takes them through the natural progression of the scientific method: questioning, studying, formulating hypotheses, experimenting, gathering data and reporting In this project the teacher provides direction by asking the students to solve the problem: "What is the prevalence of Tomato Spotted Wilt Virus in Marion County?" Students will study background information on viruses and emerging plant pathogens and make a hypothesis regarding the question. The students will gather specimens as directed by the teacher and perform a test for the virus on the specimens. Students will process the data, report their work and share what they have learned via the annual Junior Science and Humanities Symposium (through their teacher), and will also share their findings with Dr. Gallo at University of Florida, and/or the county extension service.

Mission Statement: The development of this proposed module will accomplish the following goals: 1) Students will thoroughly explore background information on viruses. 2) Students will research emerging plant pathogens focusing on the Tomato Spotted Wilt Virus. 3) Students will perform a test to detect the virus on samples they have collected. 4) Students will formally report their results to the teacher and to other scientists.

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The **teaching unit** will consists of various types of activities as follows:

- Students will perform various activities related to viruses in general. These activities will cover vocabulary, structure, types, reproduction, and specificity of viruses. Students will also explain why viruses are considered to be on the edge of life and will study viral diseases and the specific viruses that cause them.
- Students will write a short background paper on emerging plant pathogens and their impact on humans, focusing on the Tomato Spotted Wilt Virus.
- Students will perform the TSWV (Tomato Spotted Wilt Virus) ImmunoStrip assay on samples collected in the county to determine the prevalence of the virus. The students will submit a formal lab write up that includes all steps of the scientific method. The students will already be versed on the scientific method, since it is used throughout the year in the teacher's classroom.

Expected Outcomes of the Teaching Unit:

- Students will define vocabulary words and use illustrations to explain them.
- Students will draw a basic viral structure.
- Students will prepare a study guide and pass a test on the types, reproduction and specificity of viruses. The test will also include questions about the relationship of viruses to other life forms and viral diseases.
- Students will prepare a research paper on emerging plant pathogens and their impact on humans, focusing on the Tomato Spotted Wilt Virus.
- Students will perform the TSWV ImmunoStrip assay and record the data for specimens obtained, such as results, location specimen obtained, and identification of specimen.

- Students will submit a formal lab write up that includes the title, question to be answered, background research, hypothesis, materials, procedure, data table, graphic representation of data, and a conclusion.
- Students will submit their findings to other scientists and/or the county extension service.

Other ICORE objectives: The teacher will provide **in-service training to other school and district teachers** by helping to design teacher education materials (for MIP points) at the county level. The teacher will also contact administrators at her school and at the county level and report how valuable and important this training program is for science teachers. Lastly the teacher will share knowledge and skills with teachers in the science department of her school.

Expertise of the Principal Instructor:
B.S. in Medical Technology
Teaching Certification in Biology and Chemistry (9-12)
~ 6 years work experience as a Medical Technologist in the areas of Clinical Microbiology, Mycology, Parasitology, Special Bacteriology, and Blood Banking
~15 years work experience as a Teacher
ICORE training

Literature Cited:
Biology: The Dynamics of Life, Glencoe 2004 Chapter on Viruses and Bacteria
Technical sheet for Adgia ImmunoStrip™ Tests http://www.agdia.com/cgi bin/catalog.cgi/39300
Tomato Spotted Wilt Virus PowerPoint: Presented by Dr. Maria Gallo
UF IFAS Extension Sheet: http://edis.ifas.ufl.edu/PP134 http://edis.ifas.ufl.edu/PP122

Budget: $420/100 students

Budget Justification: Each student will be responsible for testing one specimen; therefore I will need one test per student. A kit has 25 tests, and costs $105.

Lesson Plan for the Investigation of the Prevalence of Tomato Spotted Wilt Virus in Marion County, Florida

Teacher: Jane Beebe of Forest High School, Ocala, Florida

Science Subject: Biology, Biotechnology, Environmental Science

Grade: 9-12 Honors Level

Science Concepts: Scientific Methods, Viruses, Biotechnology

Overall Time Estimate: 330 minutes of class for instruction and testing specimens; students will collect the specimens and write the lab paper on their own time.

Learning Styles: Visual and kinesthetic

Lesson Summary: Students will have already covered the Nature and Methods of Science Unit. In this lesson, they will apply scientific methods to answer a question. This question is, What is the prevalence of Tomato Spotted Wilt Virus (TSWV) in Marion County, Florida? Students will collect specimens (either agricultural specimens or weeds). The specimens will be labeled with the name of the student, the name of the specimen and the location and date collected. They will also note the general appearance of the plant. Students will then perform an immunoassay to test the leaf material for the presence of TSWV. Results will be compiled, and a map will be used to identify areas where plants were collected that tested positive and negative.

Expected Outcomes of the Teaching Unit:

- Students will define vocabulary words and use illustrations to explain them.
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- Students will perform the TSWV ImmunoStrip assay and record the data for specimens obtained, such as results, location specimen obtained, and identification of specimen.
- Students will submit a formal lab write up that includes the title, question to be answered, background research, hypothesis, materials, procedure, data table, graphic representation of data, and a conclusion.
- Students will submit their findings to other scientists and/or the county extension service.

Florida Benchmarks: (the new ones) SC.912.L.14.7, SC.912.N.1.1, SC.912.L.14.2

Materials:
textbook, world wide web, computer, paper, pencil
large Ziploc bags with labels
Agdia Immunostrip™ Tests and directions, 1 per student
scissors, 1 per group
collected Specimens (weeds or agricultural plants as shown below)
map of Marion County

Table 1. Partial host range of tomato spotted wilt virus.*

ORNAMENTALS			
African Violets	Columbine	Gaillardia	Poppy
Amaryllis	Cosmos	Gladiolus	Primrose
Anemone	Cyclamen	Gloxinia	Ranunculus
Aster	Dahlia	Impatiens	Salvia
Begonia	Delphinium	Larkspur	Snapdragon
Calendula	Dusty Miller	Marigold	Stock
Calla	Exacum	Nasturtium	Statice
Chrysanthemum	Fushia	Peony	Verbena
Cineraria	Geranium	Petunia	Zinnia
VEGETABLES			
Bean	Celery	Lettuce	Potato
Broccoli	Cucumber	Pea	Spinach
Cabbage	Eggplant	Peanut	Tomato
Cauliflower	Kale	Pepper	
WEEDS			
Burdock	Curly Dock	Lambsquarter	Pigweed
Buttercup	Field Bindweed	Morningglory	Shepherdspurse
Chickweed	Jimsonweed	Nightshade	Wild Tobacco
Clover			
MISCELLANEOUS			
Grape	Pineapple	Tobacco	

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*Table modified from Putnam and Dutky, *Tomato Spotted Wilt Virus*, Maryland Department of Agriculture.

Background Information: In this lesson plan students will read this information, but they must also incorporate background information on viruses, in general into their research papers.

Tomato Spotted Wilt Virus

Guide H-242

Natalie P. Goldberg, Extension Plant Pathologist

College of Agriculture, Consumer and Environmental Sciences New Mexico State University

This Publication is scheduled to be updated and reissued 4/05.

Diagnosis at a Glance

Caused by

Tospovirus--a ssRNA virus

Common hosts

Tomatoes, peppers, celery, eggplant, peanuts, lettuce, pineapple, many legumes, many ornamentals, and weeds such as field bindweed and curly dock

Symptoms

- Young leaves turn bronze in color.
- Leaves develop numerous small, dark spots.
- Plants appear wilted.
- Tips dieback.
- Dark streaking of the terminal stems
- Stunting
- Chlorotic ringspots and raised bumps on fruit
- Fruit are deformed.
- Reduced fruit quality and yield

Transmitted by

Thrips, in a persistent manner

Disease conditions Warm temperatures and high thrips populatio

Disease management

Cultural practices:

- Remove all infected plants.
- Weed and insect control
- Crop rotation
- Use reflective mulches.

Check seed sources for "new" tolerant cultivars

Tomato spotted wilt virus (TSWV) is an important disease of many different crops grown in temperate and subtropical regions of the world. TSWV is a unique virus in a virus class by itself. The virus has a wide host range, but some of the more common hosts are tomatoes, peppers, celery, lettuce, eggplant, peanuts, pineapple, many legumes, many ornamentals, and many weeds such as field bindweed and curly dock (table 1). This disease is especially damaging in the ornamental and vegetable greenhouse industry.

Symptoms of TSWV are numerous and varied. However, there are two fairly common symptoms for which this disease was named. First, the young leaves turn bronze and subsequently develop numerous small, dark spots. Second, the leaves often droop on the plant, creating a wilt-like appearance. Other symptoms include die-back of the growing tips and dark streaking of the terminal stems. Affected plants may develop a one-sided growth habit or may be stunted completely. Plants that are affected early in the growing season often do not produce any fruit, while those infected after fruit-set produce diseased fruit with striking symptoms, including chlorotic ringspots, raised bumps, uneven ripening, and deformation. Infected plants produce poor quality fruit and reduced yield.

TSWV is transmitted from infected plants to healthy plants by at least nine species of thrips. Thrips are tiny (approximately 1/16th of an inch) winged insects that feed on plants through sucking mouthparts. Thrips transmit the virus in a persistent manner, which means that once the insect has picked up the virus, it is able to transmit the virus for the remainder of its life. The virus is not passed on from adult to egg; however, progeny that develop on infected plants will quickly pick up the virus and be effective disease vectors.

Controlling this disease is difficult. The wide host range, which includes many perennial ornamentals and weeds, enables the virus to successfully overseason from one crop to the next. Additionally, efforts to control the insect vectors in agricultural fields has had little effect on TSWV. This is likely due to the fact that large populations of thrips may fly or be blown into treated fields from non-treated areas nearby.

Controlling thrips is somewhat more effective in greenhouse situations. In greenhouses, however, growers should take care to avoid repeated sprays of similar insecticides, as thrips are able to build up resistance to commonly used insecticides in a relatively short time. Rotating the insecticide class is the best approach to insect control. Control of thrips may be obtained with pyrethroids, carbamates, chlorinated hydrocarbons, organophosphates, and soaps. Insecticides are most effective when applied in the morning, when the thrips are most active and the chance for plant damage is reduced. Pesticide regulations change frequently, so check with your local county extension service for information on available insecticides.

While elimination of disease may not be possible, the incidence and severity of the disease may be reduced by using cultural practices such as starting with virus-free plant material, removing all infected plants (once virused, there is no cure for the diseased plant), controlling weeds, and rotating crops. Some studies also have shown that the use of reflective mulches under plants may help to reduce infection. In greenhouses, it may be possible to greatly reduce the number of thrips entering the greenhouse by covering doors and air intakes with a fine mesh (400 mesh) cloth. Efforts are underway to breed cultivars with good horticultural charasteristics that also exhibit tolerance to the virus.

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Advanced Preparation:
Order Adgia Immunostrip™ Test Kits at www.agdia.com (Item ISK 39300/0025)
Obtain positive and negative control plants from Dr. Maria Gallo, University of Florida
Instruct students on how/when to collect plant, emphasizing that if they do not follow through with agreed upon collection procedures, they will not participate in the lab.

Lesson Plan Procedure:
1. Lecture on Viruses, focusing on TSWV
2. Students will complete a virus study packet and take a test on viruses in general.
3. Students will complete a pre-lab activity that focuses on the scientific method as it applies to their research paper.
4. Students will be given one week to collect plants – students will be encouraged to obtain plants from random locations in the county. A map will be used to make sure our sampling in widespread.
5. Students will perform the immunoassay on collected plans. Each student will record their data on a form and then we will put all of the data on a map to be distributed to all student.
6. Students will submit a formal lab write up that details our experiment. This will focus on the TSWV and viruses as pathogens.
7. Teacher and students will share data via a presentation and email.

Vocabulary Exercise: Define the words listed below. Use an illustration to show your understanding on the words that are marked with a *. Some will be defined in lecture.

*bacteriophage	*capsid	host cell	lysogenic cycle
lytic cycle	prion	*provirus	retrovirus
*reverse transcriptase	viroid	virus	immunoassay
thrips	TSWV	tospoviruses	vector
reservoir host	antigen	antibody	

Additional Activities
Draw the 4 viral structures shown on page 477 of your textbook (papilloma, tobacco mosaic, HIV and T4 bacteriophage); be sure to label the structures shown.

Draw, label and describe the lysogenic and lytic cycles of viruses.

Read section 18.1 of Biology The Dynamics of Life by Glencoe (2006)

Take the test on viruses in general. This is the study guide that goes with section 18.1 of the Glencoe book.

Pre-lab Activity on page that follows test.

Directions for the formal lab write-up: Use the steps of the scientific method that we have covered in class (problem, background research, hypothesis, materials, procedure, data table, graph and conclusion) to submit a **typed** paper. The title will be the same as the problem statement. The data tables and graph should be typed also. The background research should be at least one page single spaced, and a bibliography will also be necessary (normally, I do not require this for lab write ups).
Procedure for Adgia Immunostrip™ Test: Please see separate attachment for the user guide.

Name **Date** **Class**

Viruses Test

For each item in Column A, write the letter of the matching item in Column B.

Column A

_____________ **1.** Genetic material of a virus

_____________ **2.** Where a virus attaches to a host cell

_____________ **3.** Nonliving particle that replicates inside a living cell

_____________ **4.** A virus's protein coat

_____________ **5.** Interlocks with a molecular shape in a host cell's plasma membrane

_____________ **6.** Layer that surrounds the capsid of some viruses

_____________ **7.** A virus that infects *E. coli* bacteria

_____________ **8.** A cell in which a virus replicates

Column B

a. virus

b. T4 phage

c. DNA or RNA

d. capsid

e. receptor site

f. envelope

g. host

h. attachment protein

Complete the table by checking the correct column for each statement.

Statement	**Lytic Cycle**	**Lysogenic Cycle**
9. Viral genes are expressed immediately after the virus infects the host cell.		
10. Many new viruses are assembled.		
11. This cycle is preceded by a virus entering a host cell.		
12. Viral DNA is integrated into the host cell's chromosome.		
13. Viruses are released from the host cell by lysis or exocytosis.		
14. Reverse transcriptase is used to make DNA from the RNA of a retrovirus.		
15. A provirus is replicated along with the host cell's chromosome.		

Use each of the terms below just once to complete the passage.

DNA	**white blood cells**	**lysogenic**
lytic	**AIDS**	**proviruses**

Many disease-causing viruses have both lytic and **(16)** __________________ cycles. For example, when HIVs infect **(17)** ______________________ , the viruses enter a lysogenic cycle. Their genetic material becomes incorporated into the **(18)** __________________ of the white blood cells, forming **(19)** __________________ . When this happens, the white blood cells still function normally, and the person may not appear ill. Eventually, the proviruses enter a **(20)** __________________ cycle, killing the white blood cells. As a result, the person loses the ability to fight diseases and develops **(21)** __________________ .

In your textbook, read about viruses and cancer, plant viruses, and the origin of viruses.

If the statement is true, write *true*. If it is not, rewrite the italicized part to make it true.

____________________ **22.** Some viruses can change normal cells to *tumor* cells.

____________________ **23.** Retroviruses and the papilloma virus, which causes *hepatitis B*, are examples of tumor viruses.

____________________ **24.** *All* plant viruses cause diseases in plants.

____________________ **25.** The first virus ever identified was the plant virus called *tobacco mosaic virus*.

____________________ **26.** The patterns of color in some flowers are caused by *tumor* viruses.

____________________ **27.** Tumor viruses contain genes that are found in *normal* cells.

____________________ **28.** Scientists think viruses originated from *their host cells*.

Pre-Lab Activity

1. What is the title of this lab?

2. What is the problem to be solved in this lab? Identify the dependent and independent variables in this experiment.

3. What kind of information will you need to include in your background research paper on viruses and TSWV?

4. What is your hypothesis regarding the outcome of this lab? Specifically what kind of plants will have the virus, and where will these plants be found. WHY DO YOU THINK THIS?

5. What materials do you need for this lab?

6. What is the procedure for collecting the specimens and performing the test?

7. How will you record individual and group results?

8. How will you graphically represent this data? A map will be used, but what kind of graph should you set up? What will be the title of the graph? How will the axes be labeled? Will you need a key? What is the scale? What are the units for each axis?

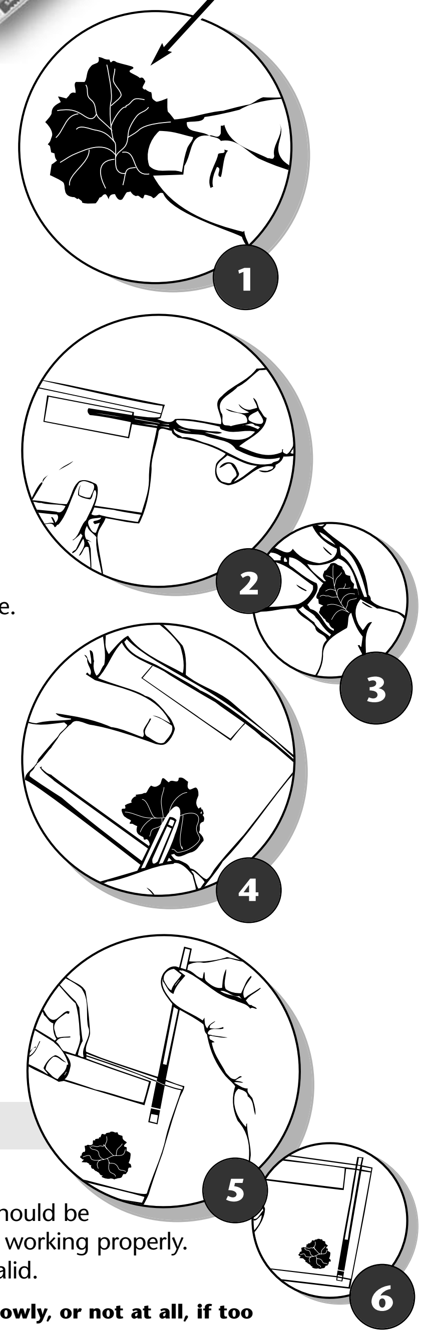
9. How will you write your conclusion?

10. How will the data be shared with other scientists?

Instructions for Agdia ImmunoStrip™ Test

Strip tests for the detection of plant pathogens

Approximate sample size



SAMPLE PREPARATION

1. Samples should be taken from plant tissues that are showing symptoms of virus infection and then be ground in buffer. Agdia sample extract bags make grinding easy. Each bag contains 3 ml of sample extract buffer. We recommend making a 1:20 dilution, which would require about a 0.15 g sample (leaf area about 3 to 5 cm^2 or 1 $inch^2$).

   NOTE: The ImmunoStrip will not perform properly if too much plant tissue is used.

2. Cut off the top of the sample extract bag, being careful not to spill the buffer.

3. Place sample *between* the mesh linings of the bag.

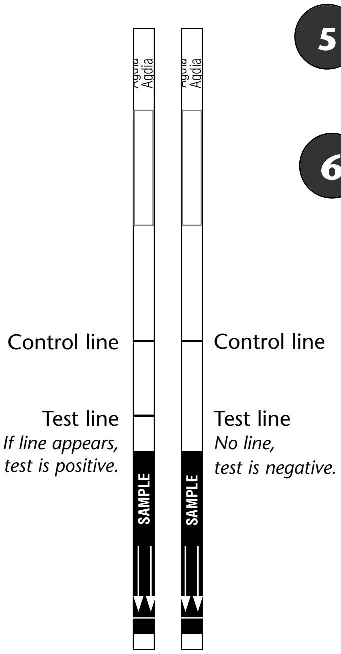
4. Rub the bag with a pen or blunt object to completely crush sample. Use only one sample per bag and be sure to label each bag.

INSTRUCTIONS FOR USE

Remove one strip from the packaging. When handling the strip, always grasp the top of the strip marked with the test name. Do not remove protective covering.

5. Keeping the strip in a vertical position, insert the end of the strip marked "sample" into the extract.

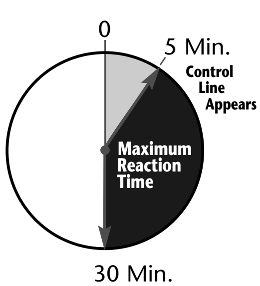
6. Do not allow much more than 0.5 cm or 1/4 inch of the end of the strip to be submerged in the extract. Be sure the strip remains in the extract during the test. You can simultaneously test for other pathogens by inserting a second ImmunoStrip into the same extract bag.



RESULTS

RESULTS

The control line will usually appear in 3 to 5 minutes. Maximum reaction occurs in 30 minutes, at which time the ImmunoStrip should be removed from the buffer. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.



NOTE: The ImmunoStrip will develop slowly, or not at all, if too much plant tissue is used.

If the sample is positive, the test line will also appear. If the sample is negative, the test line will not appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary.

If you wish to keep the strips as permanent records, cut off the sample pads (green ends marked "sample") and discard. Then blot the ImmunoStrips between paper toweling. This prevents any liquid still in the sample pads from interfering with results.

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LIMITATIONS

The following is a description of factors that could limit test performance or interfere with proper test results.

- Sample Dilution: Strip performance is very dependent on the proper sample dilution. It is best to use 0.15 g of plant tissue. Strips will not properly absorb sample extracts containing large amounts of tissue.
- Submerging the Strip: Test strips must not be submerged more than 0.5 cm or 1/4 inch. If too much of the strip is submerged, certain components of the strip are released into the sample instead of being wicked upward by the strip. This most often results in a failed test in which no control line forms.
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. If the ImmunoStrip package is left open too long, the strips may absorb moisture. This may affect test results.
- Expiration: Test should be used within one year of purchase.
- Temperature: Optimal test results will occur when the test is run in an environment where the temperature is between 60° and 95° F (15° and 35° C).
- Some plant tissues may cause what appears to be a green test line. This may be due to the tissue type or to samples containing too much tissue. If no red to purple color is present, a green line should be interpreted as a negative result.
- Pigments from red, orange, or purple fruits may result in what appears to be a positive test line. It is recommended that you call Agdia for guidance when testing fruits.

TECHNICAL SERVICE

If you have any questions about using this test, contact Agdia by phone (**1-800-622-4342** or **1-574-264-2014**) or by email (info@agdia.com).