Pondering Plant Pathogens:
Exploring Tomato Spotted Wilt Virus across the Advanced Placement Environmental Science Curriculum

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Abstract: Using a case study involving Tomato Spotted Wilt Virus (TSWV) Infection, Gainesville High School students will explore the interconnectedness of several concepts across the Advanced Placement Environmental Science curriculum. They will learn through direct instruction, small group research, plant cultivation, and laboratory investigation. Initially, students will research historic and modern agricultural practices and share their findings by producing and distributing factsheets. The unit will continue with students learning specifically about TSWV and being presented with the farmer John case study (Bokor, 2009). Students will then use Immunoassay test strips to test plants they have cultivated for the presence of TSWV. Using the results of their tests, and research they have conducted, each student group will present a recommendation of what course of action should be taken by farmer John to manage his TSWV outbreak and prevent future spread of the virus amongst his crops.

Mission statement: Within my Advanced Placement Environmental Science (APES) course there is a component in which I teach my students about global food supplies, agriculture, and pest management. To fully understand how agricultural and pest management practices have evolved, students need to integrate their knowledge of history, ecology, politics, and economics. I have always felt that I would like to find a good case study to tie these ideas together in a way that would be meaningful and memorable to my students. I propose to use TSWV as a vehicle in which I will lead students into an in-depth exploration into agricultural practices, and how modern monoculture farming and globalization has facilitated the spread of this emerging plant pathogen. We will also explore the use of an enzyme-linked immunosorbant assay to detect the presence of TSWV in test plants, and finally students will learn the various high and low tech methods used to control the spread of this potentially devastating pathogen.

Description of teaching unit or module(s), including expected outcomes:
Introduction: Tomato Spotted Wilt Virus was first described in Australia in 1915 (Adkins, et al 2005). In the last 20+ years, it has spread world wide due to introductions of the thrips species that act as a vector for the virus. This has lead to monetary losses in the millions of dollars due to reductions in plant yields of vegetable and ornamental crops as high as 30 percent (Woods 2003).

TSWV and related tospoviruses pose a particular problem to farmers, because traditional pesticide spraying regimens are ineffective against the transmission of the disease. The TSWV relies on one of a few thrips species, including Western Flower Thrips (*Frankliniella occidentalis*), tobacco thrips (*F. fusca*), and onion thrips (*Thrips tabaci*) as a vector to infect host plants (Adkins, et al 2005). The ability to control the spread of TSWV is reliant on an understanding of both the life history of these thrips species, and the ecological relationship between the thrips vector and potential host plants. For example, we know that the virus is acquired by the thrips only in its larval stage, yet can be transmitted to a host plant via the saliva of larval or adult thrips who feed on the plant tissues. There are also variations in the maturation rate, and spatial mobility among the thrips species that further complicate efforts to control the spread of the TSWV (Reitz 2005). Therefore, to implement effective measures to protect plants from infection, several cultural techniques have been studied, taking into account the life history of both thrips vectors and potential target plants. Murikaima, et al demonstrated in their test plots that the genetic resistance to TSWV within a particular selectively bred cultivar was more important than planting date or row spacing in reducing rates of TSWV infection. At that time, the genetic mechanism conferring resistance was not fully understood, therefore transgenic varieties were not yet available (Murakaima, et al 2006). The prevailing wisdom among agricultural experts seems to be that an integrated pest management approach is most effective at reducing the spread of TSWV. Practices like early season planting, use of genetically resistant cultivars, introduction of natural predators (like the minute pirate bug), immune boosting treatments (Actigard), UV reflective mulch, and pesticide spraying to lower numbers of larval thrips has shown to significantly reduce crop damage due to TSWV. In some cases, using a combination of these techniques has led to a boost in crop production up to 75% over untreated crops, which may mean a monetary gain of up to $4000.00 per acre (Woods, 2003).

Teaching Unit: *This unit will require students to draw upon previous knowledge of environmental history, infectious diseases, population & community ecology, evolution, and genetics. During the course of the teaching unit, students will be introduced to the concepts of biotechnology as a means of studying and detecting emerging pathogens, modern agricultural practices and research, pest management, and plant cultivation. Because this unit requires students to have a degree of foundational knowledge it will be incorporated into the last weeks of the fall semester (November / December).*

Learning Objectives & Associated Activities: (one day is = one, 50 minute class period.)

1. Preparation (1-2 days): We will begin several weeks before the teaching unit begins by planting the seeds from a variety of common crop plants, including (if possible) at least two cultivars of peanut with varying TSVW resistance, tomatoes, cucumbers, peppers, lettuce, and beans.
2. Developing Background Knowledge (3-4 days): Through lecture and group research, students will learn about ancient and modern agricultural practices. We will explore a variety of cultivation methods including, but not limited to, soil amendment techniques (organic & inorganic), tillage, intercropping – vs monoculture, and pest management strategies. Each student group will be responsible for preparing a factsheet on their assigned agricultural practice which will be shared with their classmates.
3. Presentation of TSWV Case Study (1-2 days): Students will be introduced to the idea that emerging pathogens occur in plants as well as animals. Ideally, I would love to have a guest speaker introduce students to the Tomato Spotted Wilt Virus (Dr. Maria Gallo) and the methods used to diagnose the virus both in the field and in lab. They will then be introduced to the case study involving Farmer John and his crop plants (Bokor, 2009).
4. Students are the scientists detecting the TSWV in test plants and weed species (~2 days): Students will collect tissue (leaf, stem & root samples) from previously cultivated plants as well as suspected naturally growing weed species found on campus. They will perform a visual assessment of plant morphology, predict the TSWV status of each sample, then use Agida Immunostrip ™ tests to verify whether plant samples are infected with TSWV (Bokor, 2009, Lesson 1).
5. Students research best practices for control of TSWV in crops and present action proposals(~3-4 days): Students will work in groups of 2-3 to search existing literature to learn what methods exist for managing an outbreak of TSWV and preventing further

spread of the disease. Integrating their research with previous knowledge, each group will prepare and present a proposal outlining their recommended course of action with regards to farmer John's crops.

6. Extension of knowledge and skills: If time and finances allow, we will conclude this unit with a field trip to the University of Florida in which students will have the opportunity to further investigate TSWV by extracting DNA from peanut seeds, amplifying a selected DNA segment using PCR, and visualizing that DNA using Gel Electrophoresis (Bokor, 2009 Lessons 2-4).

Assessment: (ongoing throughout the teaching unit)
Students will demonstrate their learning throughout this unit of study. Group products (agricultural factsheets and presentations of best practices / recommendations) will be assessed by a pre-determined rubric which will be shared with the students from the outset of the assignment. Laboratory procedures and data collection will be assessed both through observations of students while they are working, and evaluation of a final written lab report.

Gayle Nelson Evans is the sole contributor and PI for this proposal. She participated in the ICORE institute at the University of Florida in June, 2009 and has direct laboratory experience performing immunoassay testing of peanut plants to determine the presence of TSWV. Ms. Evans also has experience in other biotechnology procedures including, but not limited to performing gel electrophoresis, ELISA assays, and PCR; which will be used in this teaching unit.

Ms. Evans has been a science teacher at Gainesville High School, Alachua County, Fl. since 1998. During this time, she has taught Physical Science, Anatomy & Physiology (Honors), Pre-AP Biology, Advanced Placement Environmental Science, Advanced Placement Biology and AICE Biology (AS and A Level), and Biology I. She earned her BA in Biology (minor in Environmental Studies) from Mount Holyoke College in 1994, and a M.Ed. in Secondary Science Education from University of Florida (PROTEACH) in 2000. Ms. Evans is a National Board Certified Teacher (Adolescent & Young Adult, Biological Sciences, 2002) and is Florida certified in middle grades general science and high school biological sciences.

In addition to working as a teacher, Ms. Evans has extensive field data collection experience, including work with endangered sea turtles in Florida, Central America, and the Caribbean islands; as well as nesting songbirds in the Ouichita Mountains of Arkansas, and Spectacled Flying Foxes in North Queensland, Australia.

Resources:
Bokor, Julie. 2009. "Investigating Tomato Spotted Wilt Virus: Can We Stop It?" Laboratory Protocol developed for the ICORE Program.
Agida Immunostrip ™ tests: Item ISK 39300/0025 www.agida.com
*Tomato Spotted Wilt Virus PowerPoint: Dr. Maria Gallo
*Peanut plants provided by Dr. Maria Gallo, University of Florida

** Pending approval by Dr. Maria Gallo*

Literature cited:
Adkins, Scott; Zitter, Tom, and Momol Tim. 2005 (reviewed 2009). Tospoviruses (Family *Bunyaviridae*, Genus *Tospovirus*). Fact Sheet PP-212. Plant Pathology Department,

Florida Cooperative Extension Services, Institute of Food and Agricultural Sciences, University of Florida.

Marshall, David W. 2002. Tomato Spotted Wilt Virus Strikes Again. file:///C|/Documents%20and%20Settings/User.000/Desktop/co-op/news/2002/042502a.htm (1 of 2)4/4/2007 10:18:41 AM

Murakamia, M., Gallo-Meaghera, M., Gorbet, D.W., Meagher R.L. 2006. Utilizing immunoassays to determine systemic tomato spotted wilt virus infection for elucidating field resistance in peanut. Crop Protection 25: 235–243.

Reitz, Stuart R. 2005. Biology and Ecology of Flower Thrips in Relation to *Tomato Spotted Wilt Virus*. Proc. 1st IS on Tomato Diseases. Eds. M.T. Momol, P.J. and J.B. Jones. Acta Hort. ISHS. 695: 75-84.

Woods, Chuck. 2003. Vanquishing a Virus. IMPACT. Summer: 26-29

Budget and budget justification:

Item Description	Unit Cost	Qty	Total
Agida Immunostrip ™ tests: Item ISK 39300/0025 www.agida.com	$105.00 /25 tests	2	210.00

Viral Encounters of the Botanical Kind:
Learning how to manage virus outbreaks in crop plants

SCIENCE SUBJECT: Advanced Placement Environmental Science

GRADE AND ABILITY LEVEL: College bound students in grades 10-12 with a strong science background including previous courses in Biology and Chemistry.

SCIENCE CONCEPTS: Infectious Diseases: transmission and management, Plant biology, Interspecies Interactions, Pest Management Practices, Environmental Decision Making

OVERALL TIME ESTIMATE: Approximately five 50 minute class periods, plus time at home to research and prepare presentations.

LESSON SUMMARY: This lesson is the culmination of a larger unit in which students will have previously grown various genetic strains of crop plants such as tomato and peanut- each with a varying level of resistance to Tomato Spotted Wilt Virus (TSWV). They will then have tested their plants for the presence of TSWV. In addition students will have done background research and presented information about a variety of ancient and modern agricultural practices. Students will work in groups of 2-3 to search existing literature to learn what methods exist for managing an outbreak of TSWV and preventing further spread of the disease. Integrating their research with previous knowledge, each group will prepare and present a proposal outlining their recommended course of action with regards to farmer John's crops.

STUDENT LEARNING OBJECTIVES WITH STANDARDS: *At the conclusion of this lesson each student will be able to...*

1. Describe TSWV, its effects on plants, mode of transmission, and infection cycle (SC.912.L.14.6).
2. Apply previous knowledge of plant transport systems to describe the physiological effects of TSWV on an infected plant (SC.912.L.14.2).
3. Explain the relationship between thrips species and affected crop plants in the transmission of vector-bourne viral diseases such as TSVW (SC.912.L.17.6).
4. Summarize a body of research relating to agricultural practices used to contain and control disease outbreaks in crop plants (SC.912.N.1.1, SC.912.N.1.4).
5. Using information gathered during research and data collected in lab, synthesize and present an effective plan of action to be used to best manage a virus outbreak (SC 912.L.16.10, SC.912.17.13, SC.912.N.4.1, SC.912.N.4.2).

MATERIALS: Each group of students needs access to the following:
For research:

- Computer with internet access
- Library collection of periodicals
- Data from TSWV tests on their plants

For presentations:

- Each group will arrange a plan of presentation with the instructor previous to the due date to ensure that all required materials are available.

BACKGROUND INFORMATION:
Tomato Spotted Wilt Virus was first described in Australia in 1915 (Adkins, et al 2005). In the last 20+ years, it has spread world wide due to introductions of the thrips species that act as a vector for the virus. This has lead to monetary losses in the millions of dollars due to reductions in plant yields of vegetable and ornamental crops as high as 30 percent (Woods 2003).

TSWV and related tospoviruses pose a particular problem to farmers, because traditional pesticide spraying regimens are ineffective against the transmission of the disease. The TSWV relies on one of a few thrips species, including Western Flower Thrips (*Frankliniella occidentalis*), tobacco thrips (*F. fusca*), and onion thrips (*Thrips tabaci*) as a vector to infect host plants. (Adkins, et al 2005). The ability to control the spread of TSWV is reliant on an understanding of both the life history of these thrips species, and the ecological relationship between the thrips vector and potential host plants. For example, we know that the virus is acquired by the thrips only in its larval stage, yet can be transmitted to a host plant via the saliva of larval or adult thrips who feed on the plant tissues. There are also variations in the maturation rate, and spatial mobility among the thrips species that further complicate efforts to control the spread of the TSWV (Reitz 2005). Therefore, to implement effective measures to protect plants from infection, several cultural techniques have been studied, taking into account the life history of both thrips vectors and potential target plants. Murikaima, et al demonstrated in their test plots that the genetic resistance to TSWV within a particular selectively bred cultivar was more important than planting date or row spacing in reducing rates of TSWV infection. At that time, the genetic mechanism conferring resistance was not fully understood, therefore transgenic varieties were not yet available. (Murakaima, et al 2006). The prevailing wisdom among agricultural experts seems to be that an integrated pest management approach is most effective at reducing the spread of TSWV. Practices like early season planting, use of genetically resistant cultivars, introduction of natural predators (like the minute pirate bug), immune boosting treatments (Actigard), UV reflective mulch, and pesticide spraying to lower numbers of larval thrips has shown to significantly reduce crop damage due to TSWV. In some cases, using a combination of these techniques has led to a boost in crop production up to 75% over untreated crops, which may mean a monetary gain of up to $4000.00 per acre (Woods, 2003).

ADVANCE PREPARATION: Before students begin research, the instructor should do a literature search on the Internet and a periodical search to ensure adequate information is available. Arrange a library visit for the class with the media specialists at least 2 weeks in advance.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:
1. ***Brief overview of the lesson (5-10 minutes):***
"For our final lesson, we will continue working with our research groups. You have taught each other about various agricultural methods, learned about TSWV, grown your own plants and tested them for TSWV, and now it is time to pull all the pieces together. For this assignment you will be using all of your previous knowledge, plus what you learn in a literature search to formulate a recommended plan of action for Farmer John (FJ) to follow to best manage his crops. For this assignment, you need to consider all the things that FJ cares about: cost, labor, environmental impacts, effectiveness, etc. Once you have come up with a plan, you will need to present it in a compelling and convincing manner. You will be competing with your peers, since FJ will only ultimately choose the recommendation that he feels best meets his needs. Questions about logistics?

2. ***Students break into groups and discuss possible options and plan course of research (20 minutes)***
   - Review previous knowledge about agriculture
   - Brainstorm ideas for what might work best

3. ***Students visit school library to conduct research (50 minutes + outside time):***
   - Brief orientation by media specialist about reliability of info on Internet, how to conduct a periodical search, and proper citation / avoiding plagarism.
   - Students use library resources and internet to collect and evaluate information.

4. ***Student Groups assemble information and plan their presentation (50 minutes + outside time):***
   - Decide how to involve all students in presentation
   - Determine best presentation method to catch attention and stand out
   - Discuss plans with / gain approval from instructor
   - Put presentation together

5. ***Group presentations (1-2 class periods)***
6. ***Evaluations & Winner chosen (On-going throughout presentations)***
   - Instructor will use a rubric to evaluate each group
   - Winner will be chosen either by guest teacher or class vote

ASSESSMENT SUGGESTIONS:
Presentations will be graded according to the following guidelines:

Objectives: CONTENT **How well did this group...**	**Points possible**	**Points earned**	**Comments**
1. Describe TSWV, its effects on plants, mode of transmission, and infection cycle (SC.912.L.14.6).	10		
2. Apply previous knowledge of plant transport systems to describe the physiological effects of TSWV on an infected plant (SC.912.L.14.2).	10		
3. Explain the relationship between thrips species and affected crop plants in the transmission of vector-bourne viral diseases such as TSVW (SC.912.L.17.6).	10		
4. Summarize a body of research relating to agricultural practices used to contain and control disease outbreaks in crop plants (SC.912.N.1.1, SC.912.N.1.4).	10		
5. Using information gathered during research and data collected in lab, synthesize and present an effective plan of action to be used to best manage a virus outbreak (SC 912.L.16.10, SC.912.17.13, SC.912.N.4.1, SC.912.N.4.2).	10		

Objectives: PRESENTATON How well did this group...	**Points possible**	**Points earned**	**Comments**
1. Hold the attention of the class throughout the presentation	10		
2. Demonstrate or describe a plan that was feasible, cost effective and environmentally sound	10		
3. Show that time and care was taken in the preparation of this presentation	10		
4. Involve ALL members of the group in the research AND presentation.			
5. Demonstrate creative thinking and problem solving in the formulation of their plan.			
BONUS: The winner gets 20 points extra!			

RESOURCES/REFERENCES:

Adkins, Scott; Zitter, Tom, and Momol Tim. 2005 (reviewed 2009). Tospoviruses (Family *Bunyaviridae*, Genus *Tospovirus*). Fact Sheet PP-212. Plant Pathology Department, Florida Cooperative Extension Services, Institute of Food and Agricultural Sciences, University of Florida.

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