Diagnosis of Diseases Caused by Viruses and Viroids

This presentation:

1. The importance of correct and rapid diagnosis/identification
2. The steps used to diagnose a disease caused by a virus
3. How Koch's Postulates are used for the identification of a virus as the causal agent of a disease

Full Definition of DIAGNOSIS:

plural **di·ag·no·ses** \-ˌsēz\

1a: the art or act of identifying a disease from its signs and symptoms

1b: the decision reached by diagnosis

2: a concise technical description of a taxon

3a: investigation or analysis of the cause or nature of a condition, situation, or problem <*diagnosis* of engine trouble>

3b : a statement or conclusion from such an analysis

Rapid and Accurate Diagnosis is Important -

Results can have far ranging consequences

In 1995 *Tomato yellow leaf curl virus* (TYLCV) was identified as the cause of a disease in tomatoes in the Dominican Republic

Within months the Florida Division of Plant Industry instituted a new law which prevented the importation of all vegetable transplants from countries outside the U.S.

Norway, March 2001: *Pepino mosaic virus* (PeMV) found in one glasshouse producing tomato fruit.

Expensive eradication measures were implemented –

- Destruction of all plant material & growing media
- Disinfection of all surfaces in the glasshouses.
- Surveys were carried out in 19 other tomato production sites in the area…. The outbreak was considered successfully eradicated in April 2001.

Discovery of *Cucumber green mottle mosaic virus* (*Virgaviridae, Tobamovirus*) in Australia in 2014 resulted in quarantines and a lot of expensive testing of plants in order to eradicate the virus

Cucumber green mottle mosaic virus

ABC Rural

Anxious times for Northern Territory horticultural sector as most Top End crops are tested for Cucumber Green Mottle Mosaic Virus

ABC Rural By Matt Brann

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The Northern Territory's department of primary industry has started testing all crops in the Top End which could be susceptible to the recently discovered Cucumber Green Mottle Mosaic virus (CGMMV).

The virus has been found in several watermelon farms near Katherine and one melon farm near Darwin.

Yesterday, in Katherine, quarantine officers began testing other farms which grow cucurbits susceptible to the virus such as pumpkins and zucchinis.

Hundreds of farms in the Darwin rural area will also be tested over the next three to four weeks.

PHOTO: The NT Government has started testing a range of crops for Cucumber Green Mottle Mosaic Virus. (Daniel Fitzgerald)

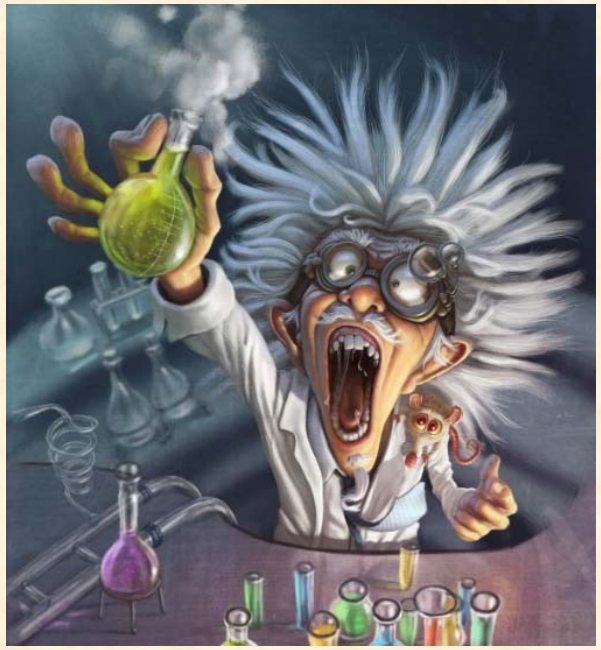
Steps In Diagnosing Plant Virus Diseases:

1. Determine plant species and cultivar infected;
2. Record the symptoms and collect samples
3. Look for potential virus vectors on the infected plants
4. Assess circumstances, distribution pattern, and incidence of the disease
5. Go to the literature
6. Compare the facts with viruses already known in the area
7. Select one or more assays and use them to test samples

- This list of steps is what I use when I am called out to diagnose a problem that might be caused by a virus. It's a useful set of steps.

- However, I know from experience its human nature to want to skip to step 7: "**Select one or more assays and use them to test samples".** Everyone wants to start using cool assays!

- Keep in mind that there are approx. 1600 plant virus species with many more awaiting discovery. Step 7 should only be attempted once you have done steps 1-6 to narrow down your possible assays. Otherwise you get very frustrated and go bankrupt using assay after assay.



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- Does the cultivar have resistance to any viruses?
- Are there any known problems with this cultivar?
- Knowing the crop species is essential to a literature search on known diseases

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- Describe the symptoms in words
- Record how much of the plants show symptoms (ie how many leaves on the plant show symptoms). You can estimate when the infection occurred based on how fast the plant is growing and how many leaves on the plant show symptoms
- Take pictures!

  One person's mottle is another person's mosaic

  Pictures can be shared with other experts

- Collect plenty of samples and process for longer term storage. You never know how long the crop will be there. Even if the grower destroys his crop, he will still need to know the cause of the problem that made him plow down his crop.

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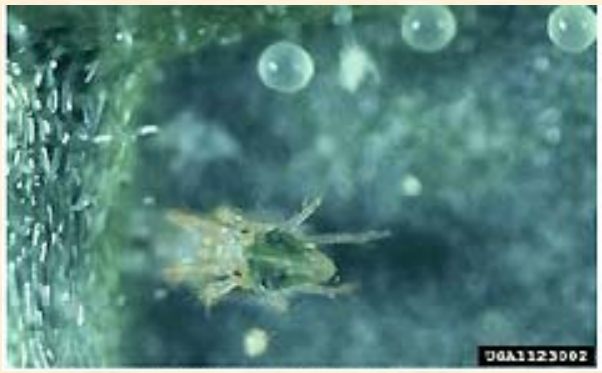
- What vectors are or were present can help in the diagnosis
- Keep in mind that some vectors pass through the field and can only be detected with traps



Detection: Look for signs of potential vectors

Thrips feeding damage

Leafhopper feeding damage

Spider mite feeding damage

Sooty mold
(can indicate feeding by aphids, whiteflies)

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- Virus spread in a field follows certain patterns depending upon the vector or means of spread

- Pesticide damage has its own patterns, that can be similar or different than virus-infected plants

- Did the grower or his neighbor use herbicides lately? Look for dead plants.

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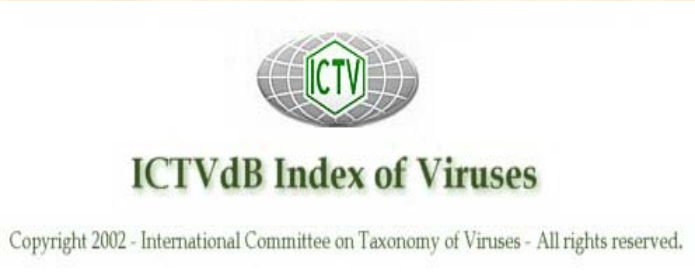
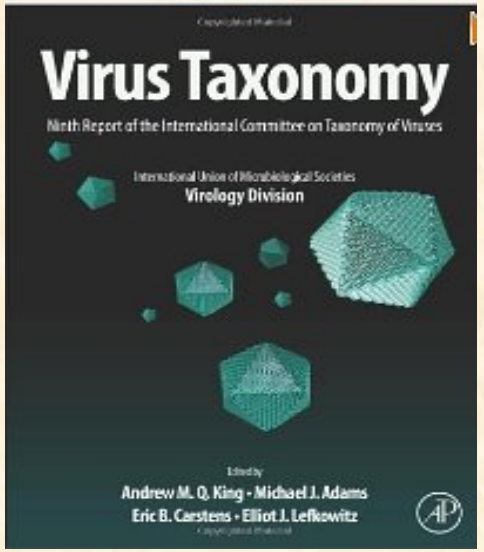
5. Go to the literature

- This is a very important step and can save you a lot of money and time
- What diseases are known in this crop?
- What do the symptoms of these diseases look like?
- Have these symptoms been seen before in the region? Diagnostic labs that keep records (with pictures) of what they identify can be very helpful and save much time and expense.
- In the US plant diagnostic labs have formed networks with neighbors – they share their records (with pictures) of what they diagnosis. This has saved a lot of time and money.
- Contact experts familiar with viruses of this crop (send pictures)

Some internet sources for viruses:

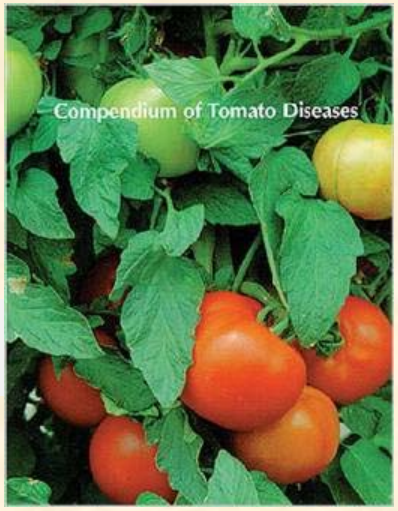


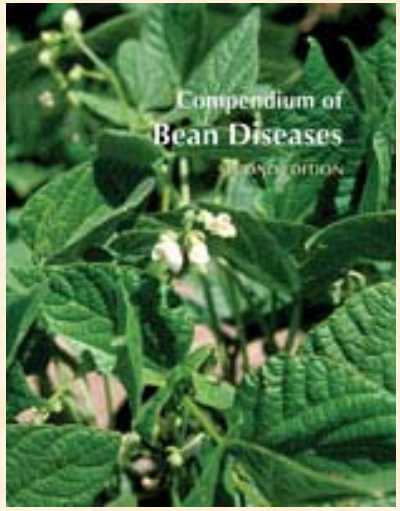
A compendium of Web sites dealing with virology,
http://www.virology.wisc.edu/links.html



The American Phytopathological Society.
A wide range of information on plant pathology, courtesy of the APS.
http://www.apsnet.org/

APS Crop Compendia





Less good but sometimes useful:

University, State and Federal Extension Publications:

Examples from Florida:

http://edis.ifas.ufl.edu/ (EDIS Publications)

- Plant Pathology Fact sheets & Circulars
- Plant Protection Pointers

– Crop Management Guide:
- Vegetable, Ornamental, etc…

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A database of viruses known to be present is very useful. This is assembled over time by diagnostic labs or diagnosticians.

This database could include:

- known hosts
- when and where it was found
- pictures of symptoms in hosts
- available or appropriate assays

- Keep analyzing what you find and explore different hypotheses. At some point, the facts should start to fit one hypothesis better than the others

- If you are lucky it's a disease already reported, and your guess can be confirmed with some simple tests

- If the facts aren't fitting any of your guesses, then it can get complicated as a lot more work may be required.

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Summary- Available Diagnostic/Detection Assays

A. Biological assays

Host range
Symptoms
Methods of transmission

B. Viral nucleic acid assays

Inclusion body visualization
dsRNA visualization
Nucleic acid hybridization
PCR, RT-PCR, RCA, RPA
Viral genome sequence
Microarray
Ecogenomics

C. Viral protein assays

Inclusion body visualization
Electron microscopy
Virus purification
Serological assays (many)
Microarray

Assays for diagnosis, detection and identification of plant viruses:

- No one assay is usually definitive – often have to use multiple assays
- Use your understanding of the assays and logic to determine which assays and in which order to use them
- No assay is perfect for every situation - each assay has its strengths and limitations

Selection of Diagnostic Assays

Factors to consider in choosing a diagnostic technique:

- Goal – what is your objective?
- Time required for diagnosis
- Sensitivity of diagnosis
- Cost of diagnosis
- Facilities/equipment available for diagnosis
- Number samples to be tested
- Properties of the virus

  (phenotypic and genomic characters that allow the discrimination among members of different species or different genera.)

- If you are lucky your diagnostic sample is a disease already reported, and your guess can be confirmed with a simple test
- If the facts aren't consistent then it gets complicated.
    - You many have a new disease for the area.
    - You may have a new disease in that crop.
    - You may have a new virus or new strain of a known virus
- What do you do with your results?
- How many tests do you need to conduct to be sure of what you have?

Diagnosis of Diseases Caused by Viruses

3 possible scenarios:

1. **Known Disease in the Region**

2. **Known disease but new to your region, or in new cultivar**

3. **New Disease**

   Caused by a known virus in a new host
   Caused by an undescribed virus

Diagnosis of Diseases Caused by Viruses

1. Known Disease in the Region

known virus, host and etiology, previously reported in same location (Koch's postulates previously fulfilled)

If the disease is a well-recognized one, can use one virus-specific assay or combination of appropriate assays (symptoms on the host plus at least one laboratory assay), assuming that you use a positive control.

If the assay results are clearly positive, then nothing more is needed.

2. Known disease, but causal agent is new to your region OR uncharacteristic symptoms (due to cultivar or environmental differences)

Positive diagnosis should always:

1) Use two or more different detection assays
    1. Biological assays
    2. Viral protein assays
    3. Genomic nucleic acid assays

2) Use an asymptomatic control as well as a positive control

3) Test more than one symptomatic sample

3. New Disease

Caused by a known virus but in a new host
Caused by a undescribed virus

- Must complete Koch's postulates!
  - Detection of a virus in a diseased plant when it's a new disease is not sufficient
  - Its possible to have mixed infections where only one of the viruses is causing the disease
  - Some diseases are caused by a mixture of viruses

3. New Disease

Caused by a known virus but in a new host
Caused by a undescribed virus

- Koch's Postulates have been done for every disease for which we know the cause

- For non-culturable pathogens such as viruses:
  Complete a modified version of Koch's postulates

Modified Koch's Postulates:

1. The virus must always be associated with a set of symptoms in the diseased host.

2. The virus must be cloned/purified from the diseased plant, multiplied in a propagative host, and properties of the virions determined (usually clone the genome and obtain its sequence, but could include virus purification and identifying the shape, size of the virion as well as the genome sequence).

3. The purified virus must reproduce the disease when inoculated into healthy host plants of the same species (and cultivar whenever possible) on which the initial disease appeared.

4. The same virus must be demonstrated to occur (detected) in the host plants (inoculated in step 3) which show the characteristic symptoms (as those in step 1).

- Fulfilling Koch's postulates is a lot of work and expense. It is beyond the capacity of a diagnostic laboratory.
- It usually requires a well equipped virology laboratory. Diagnosticians who find evidence of a new virus must work with or send their samples to virologists who are willing to spend the time and money required.
- However an understanding of Koch's postulates is important for diagnosticians because they must read the literature and sort out solid facts from assumptions as to whether a particular virus is the cause of a disease.