

# DETECTION, IDENTIFICATION, AND DIAGNOSIS OF PLANT VIRUSES



## Objectives:

1. Know the steps used to diagnose a disease caused by a virus
2. Understand how to apply Koch's Postulates for the identification of a virus that is the causal agent of a disease

## 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

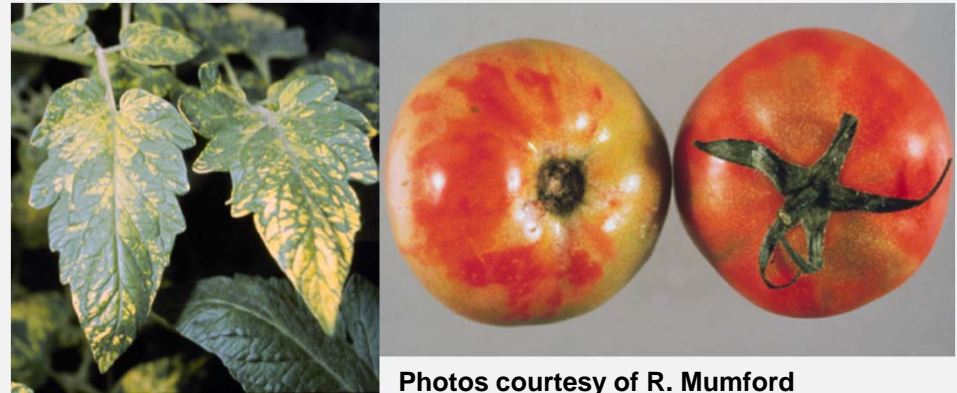


Florida Division of Plant Industry instituted a new law which to prevented importation of all vegetable transplants from countries outside the U.S.

## Rapid and Accurate Diagnosis is Important

### Results can have far ranging consequences

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



- ➔ 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.

## Steps In Diagnosing Plant Virus Diseases:

1. Determine plant species and cultivar infected
2. Record the symptoms associated with the disease
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 proceed



## 5. Go to the literature: Some internet sources for viruses:



<http://ictvonline.org/> -- lists all the approved species of viruses as of 2015

Viral Zone <http://viralzone.expasy.org/>



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

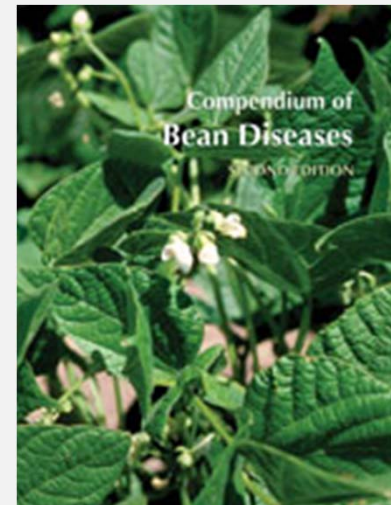
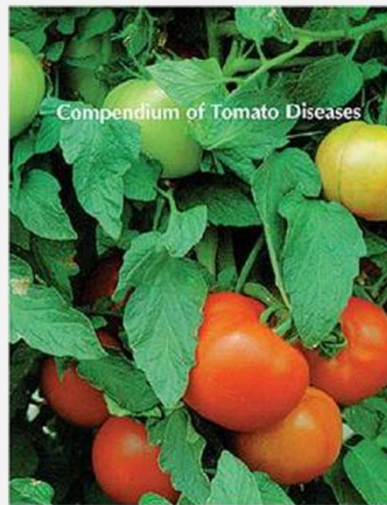


The American Phytopathological Society.  
A wide range of information on plant pathology,  
courtesy of the APS. <http://www.apsnet.org/>

## APS Crop Compendia:

**Less good but sometimes  
useful:**

University and 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...

## Steps In Diagnosing Plant Virus Diseases:

1. Determine plant species and cultivar infected;
2. Record the symptoms
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





## 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 (Rolling circle amplification)

RPA (Recombinase polymerase amplification)

Viral genome sequence

Microarray

Deep sequencing (Next Gen Sequencing)

## Summary- Available Diagnostic/Detection Assays

### C. Viral protein assays

Inclusion body visualization

Electron microscopy

Virus purification

Serological assays (many)

Microarray

## Summary- 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.)

## 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

# Diagnosis of Diseases Caused by Viruses

## 1. Known disease

## 2. Known disease but new symptoms or new location or new cultivar

## 3. New Disease

**Caused by a known virus in a new host**

**Caused by a undescribed virus**

Must fulfill Koch's Postulates to determine that the virus is the cause of the disease

# Diagnosis of Diseases Caused by Viruses

## 1. Known Disease

known virus, host and etiology, previously reported in same location (Koch's post. 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)**

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

## **2. Known disease, but new symptoms or new location or new cultivar**

**Positive diagnosis should always:**

**1) Use two or more different techniques**

**1. Biological assays**

**2. Viral protein assays**

**3. Genomic nucleic acid assays**

**2) Use an asymptomatic 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!**
- **This has been done for every disease where we know the cause**
- **For non-culturable pathogens: viruses, viroids, satellites:**
  - 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.
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 in the host plants (inoculated in step 3) showing the characteristic symptoms.