Lab Tuesday: Virus Diseases

- Quiz for Bacterial Pathogens lab (pp 67-73) and Biocontrol of Crown Gall (p. 113-117), and intro sections for Viruses (pp. 75-77) and Observation of Viral Movement in Plants (p. 119).
- Continue Koch's postulates experiment (re-inoculation step)
- Record data from Race I.D. of Wheat Stem Rust experiment
- Record data from bacterial pathogen HR assay in tobacco
- Virus lab
- Observation of Viral Movement in Plants
- Turn-in 'Disease Progress Curve' worksheet.

How much would you pay for a diseased plant?

'Tulip Mania'
The Netherlands
1630 to 1660

	Guilders
Eight fat pigs	240
Four fat oxen	480
Twelve fat sheep	120
Twenty-four tons of wheat	448
Forty-eight tons of rye	558
Two hogsheads of wine	70
Four barrels of eight-guilder beer	32
Two tons of butter	192
A thousand pounds of cheese	120
A silver drinking cup	60
A pack of clothes	80
A bed with mattress and bedding	100
A ship	500
TOTAL	3,000

Fig. 2. Value of goods equal to the price of the rare broken tulip Semper Agustus, as recorded in a pamphlet written in 1636.

Viruses

http://www.apsnet.org/education/IntroPlantPath/PathogenGroups/plantViruses/default.htm

~2500 named viruses - many more undiscovered

Obligate, intracellular parasites of virtually all cellular organisms

25% of known viruses are parasites of plants

Like other pathogen groups, some viruses infect a broad range of host, whereas others infect a single plant species only

Discovery of viruses as agents of disease:

Transmission: 1886 sap, 1904 graft, 1922 insect vectors
1892 – sap transmission after passage through bacteria proof porcelain filters
Visualization: 1940 electron micrograph
Purification: 1950
Genomics: 1980's 1st virus genome completely sequenced

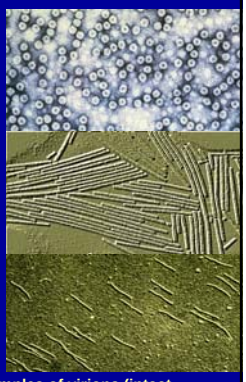
Viruses have the following properties:

Very small genome - 10^4-10^5 nucleotides – this typically codes for 4-7 proteins

Viruses contain a single type of nucleic acid, either RNA (most plant viruses) or DNA

When completely assembled (the virion), the nucleic acid is protected by a protein coat (the capsid) consisting of individual protein subunits

Viruses multiply inside living cells by hijacking the biosynthetic machinery (ribosomes) of the host



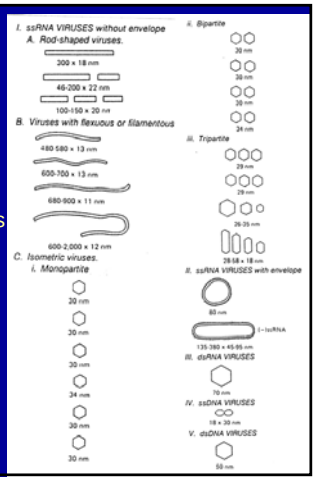
Examples of virions (intact particles) of common plant viruses

Diversity of plant viruses

Genomes of most plant viruses are comprised of 'single-stranded RNA' with the virion shaped as a sphere, a rod, or a filament.

Typically, the complete genome is contained in a single particle (monopartite), but some viruses require multiple particles (bipartite, tripartite).

The other major classifications are double-stranded RNA, single-stranded DNA, double-stranded DNA.



Plant viruses and disease

Viruses are obligate, biotrophic pathogens – unlike other pathogen groups, many viruses can infect and replicate within their hosts without causing obvious symptoms

Consequently, why symptoms (disease) develop is poorly understood. Increasingly, it appears that many viruses suppress normal recycling of messenger RNA (this is called RNA silencing). Thus, symptoms may result from over expression of the host's own genes.

In nature, viruses that cause severe symptoms in their principal host are likely to go extinct.

Ring spot symptom

Foliar symptoms of virus diseases

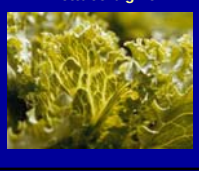
Typically, viruses cause chlorosis or color breaks in mottled or mosaic patterns

Corn mosaic

Calico mosaic of potato

Tulip break

Lettuce big vein



Bean yellows mosaic

Internal symptoms of virus diseases

Stem pitting

Net necrosis of of potato leaf roll

Black line symptom **hypersensitive reaction at the graft union**

Local lesion symptom of a virus disease

Hypersensitive reaction (HR) observed after artificial inoculation of infected plant sap onto a local lesion or 'indicator' host.

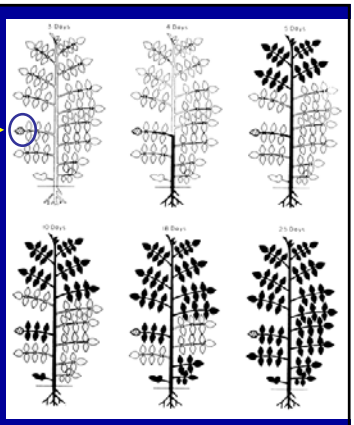
Indicator hosts were developed to aid viral disease diagnosis.

Virus movement within plants

First leaf infected →

After infection, viruses move systemically throughout a plant via the phloem transport system.

First down, then up, then throughout.



Black represents areas of the plant where the virus can be found

Virus transmission between plants

Vegetative propagation
Grafting
} **High rates of transmission for nearly all viruses**

Mechanical transfer of sap (rubbing, cutting, handling, etc. - relatively uncommon in plant production but important in the lab)

Seed ~100 viruses – efficiency (% of seed with virus) usually low

Pollen 9 viruses – usually a fruit crop (blueberry, caneberry, cherry)

Mite, Nematode & Fungal vectors – a few dozen viruses

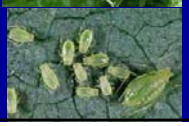
INSECTS !! aphids, leafhoppers, whiteflies, others – this is the most common and economically important means of virus transmission

Important insect vectors of plant viruses

Green peach aphid

Alate (winged) adult

Apterous (wingless) adult

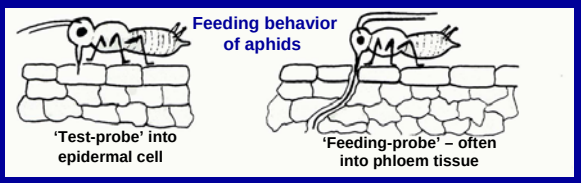


Mother with children

Aster leafhopper

Silver leaf whitefly

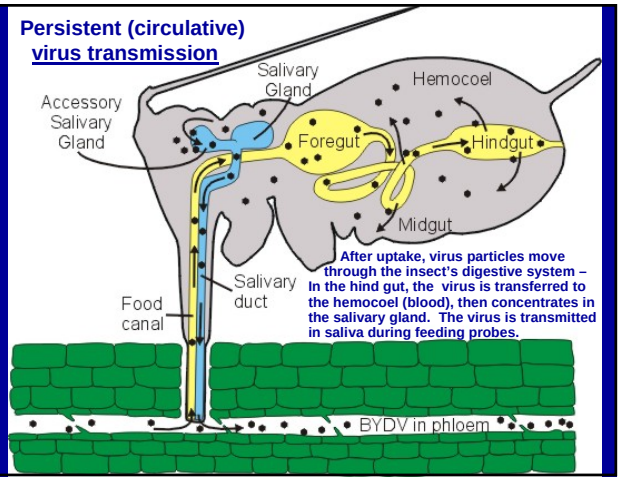
Non-persistent and Persistent types of vector transmission



This type of feeding behavior is associated with 'non-persistent' or 'stylet-born' virus transmission (~230 viruses)
Test-probes are frequent and of short duration (seconds)

This type of feeding behavior is associated with 'persistent' or 'circulative' virus transmission. (~80 viruses)
Feeding-probes are infrequent and of long duration (min to hr)

Persistent (circulative) virus transmission

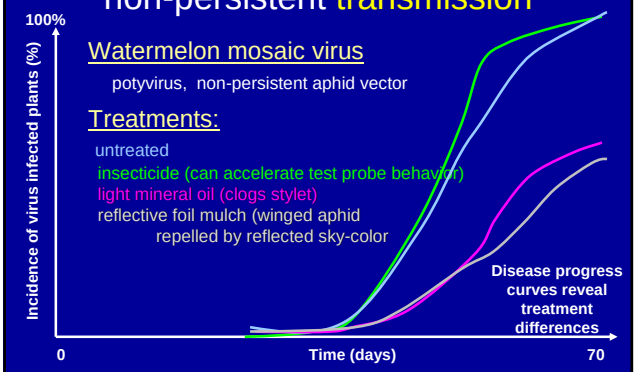


After uptake, virus particles move through the insect's digestive system – In the hind gut, the virus is transferred to the hemocoel (blood), then concentrates in the salivary gland. The virus is transmitted in saliva during feeding probes.

Characterization of types of vectored transmission

Persistent: (circulative / propagative)		Non-Persistent: (stylet-born)
Minutes to hours	Acquisition time	seconds
yes	Incubation period in insect	no
yes	Insect infective after molt	no
Minutes to hours	Transmission time	seconds
no	Virus inoculated mechanically	yes
yes	Disease spread reduced with insecticides	no

Vector control and non-persistent transmission



Watermelon mosaic virus

potyvirus, non-persistent aphid vector

Treatments:

untreated

insecticide (can accelerate test probe behavior)

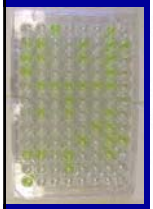
light mineral oil (clogs stylet)

reflective foil mulch (winged aphid repelled by reflected sky-color

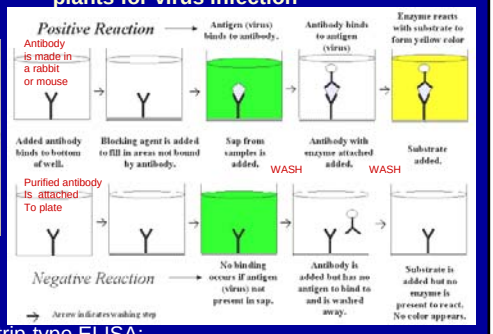
Viral diagnostics

- Pathogenicity -- Bioassays using **indicator hosts**
- Transmissibility – **Vector transmission assays**
- Architecture of the virus particle -- **Electron microscopy**
- Antigenic properties of the viral protein coat
  - **ELISA** (**enzyme-linked immunosorbent assay**)
- Characterization of viral nucleic acid –
  - **Polymerase chain reaction** amplification (PCR) and agarose gel electrophoresis
  - **Detection of double-stranded RNA in host tissue**
  - **Genome sequencing**

Enzyme-linked immunosorbent assay (ELISA) is the most practical method for testing large numbers of plants for virus infection



ELISA plate – colored wells are positives



Cartoon of strip-type ELISA:
http://www.whfreeman.com/kuby/content/anm/preg-testv03.swf