TRANSMISSION ELECTRON MICROSCOPY

NEGATIVE STAINS, IMMUNO-SPECIFIC LABELING (ISEM), THIN SECTIONING OF FIXED AND EMBEDDED MATERIAL

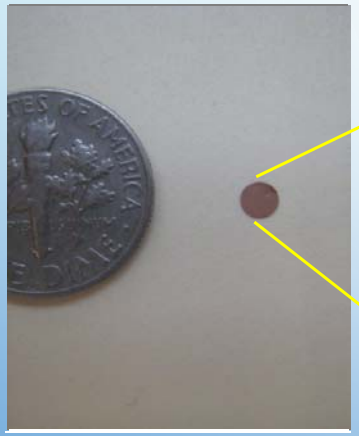
PM 7/126 (1) ELECTRON MICROSCOPY IN DIAGNOSIS OF PLANT VIRUSES

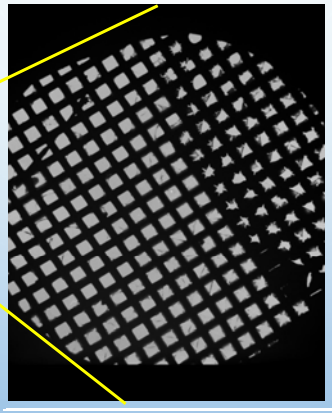
- EPPO BULLETIN (2015) 45:450-453
  - ELECTRON MICROSCOPY CAN BE USED FOR DETECTION OR IDENTIFICATION OF VIRUSES IN TISSUE EXTRACTS OF INFECTED PLANT SAMPLES
- OFTEN COMBINED WITH THE USE OF ANTISERA (IMMUNO ELECTRON MICROSCOPY)
  - COAT GRIDS WITH ANTIBODY TO 'TRAP' VIRUS PARTICLES (ISEM), OR
  - TO 'DECORATE' VIRUS PARTICLES (ESPECIALLY USEFUL IN MIXED INFECTIONS)
- DIP PREPARATION FOR FIRST SCREENING OF VIRUS PRESENCE AND PARTICLE MORPHOLOGY
- DIP PREPARATION FOR ISEM AND/OR DECORATION FOR VIRUS ENRICHMENT AND IDENTIFICATION

SAP EXTRACTS OR EPIDERMAL PEELS

- SAP EXTRACTS MAY BE PREPARED BY
  - Grinding tissue in buffer and clarifying by centrifugation or filtering through cheesecloth, Miracloth, etc.; a drop is mixed with stain
  - Chopping tissue directly in a drop of stain (e.g. K-phosphotungstate or Uranyl acetate)
  A drop of sap and stain can either be pipetted onto a grid and drawn off with filter paper, or the grid floated on the liquid
- EPIDERMAL PEELS
  - An epidermal peel made with fine-pointed forceps is drawn through a drop of stain placed on the grid, and excess liquid drawn off with filter paper
- CONCENTRATION
  Virus in sap extracts can be concentrated by low speed clarification and high speed centrifugation (note: not PEG precipitation – PEG 'boils' in the beam and obscures the sample)
- DO NOT USE 'MUSHY' SAMPLES – THEY WILL ALMOST ALWAYS YIELD POOR RESULTS !!!

GRIDS AND PREPARATION





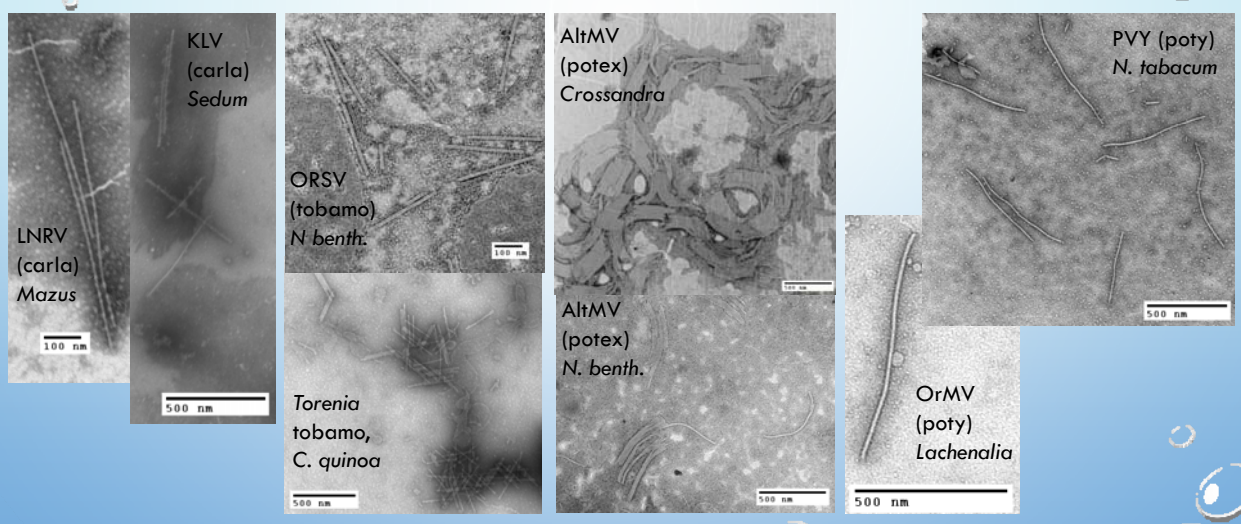
Grids are small, typically of Cu, Ni or Rh, and (at least for negative staining) have a plastic support film, which may be further coated with a carbon film to improve contrast. Different mesh sizes are used for negative stain and thin sectioned samples. Charging grids by glow discharge may improve uniform capture and spread of negatively stained virus preparations.

PARTICLE VISUALIZATION IN SAP EXTRACTS

- FLEXUOUS AND ROD-SHAPED VIRIONS
  - Typically relatively easy to 'find' even when in low concentration
  - A single particle in a field of view may be sufficient to suggest what group of virus is present
  - For flexuous particles, extract pH and divalent cation concentration (Ca, Mg) can significantly affect virion length and rigidity
  - virion fragmentation or encapsidated sgRNAs may affect estimates of average particle length
- ISOMETRIC VIRIONS
  - Often difficult to identify unless in high concentration; artefacts may resemble virions
  - May be necessary to observe multiple particles in a single field of view to have confidence that a virus is present
  - Particles may, or may not, be penetrated by stain
- Angularity or 'dimpling' of particles may provide clues to virus group
- Average sizes of multiple virions are required

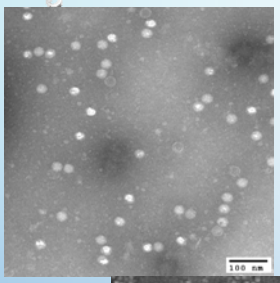
Some virus types are relatively unstable (e.g. ilarviruses) or difficult to find (e.g. tospoviruses, ophioviruses) in sap extracts. Rhabdovirus particles are often difficult to find in known infected plants, but are readily identified when observed.

EXAMPLES OF ROD/FLEXUOUS VIRIONS IN SAP EXTRACTS

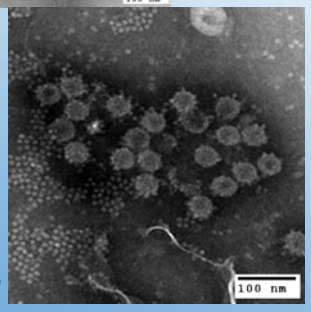


Presence of massive aggregates in leaf dips is unusual – but may occur with some virus/host combinations (see AltMV/*Crossandra*). Single particles of characteristic length and flexuousness may be sufficient to indicate the virus group (see OrMV/*Lachenalia*)

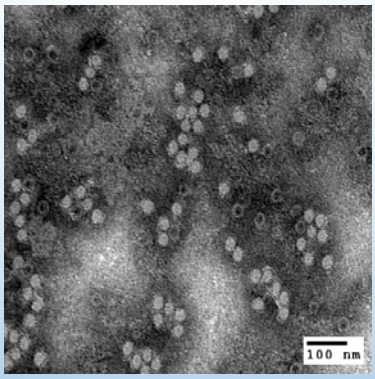
EXAMPLES OF ISOMETRIC VIRUSES IN SAP EXTRACTS



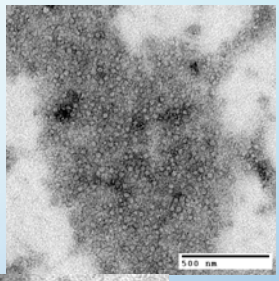
Tymovirus
Torenia



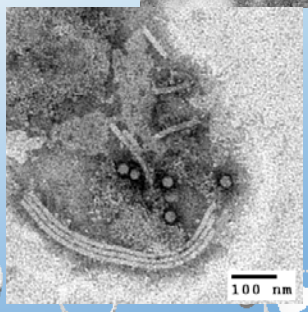
PVCV
(petu)
Petunia



AnFBV (carmo) Angelonia

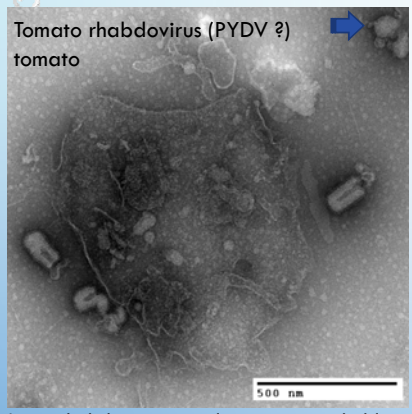


BaCV
(ilar)
Sutera



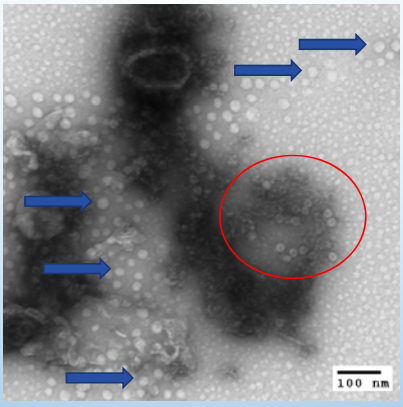
TRSV
(nepo)
Phlox
(+ AltMV)

RHABDOVIRUSES; AND ARTEFACTS POTENTIALLY CONFUSED WITH VIRIONS (ESPECIALLY ISOMETRIC VIRUSES)

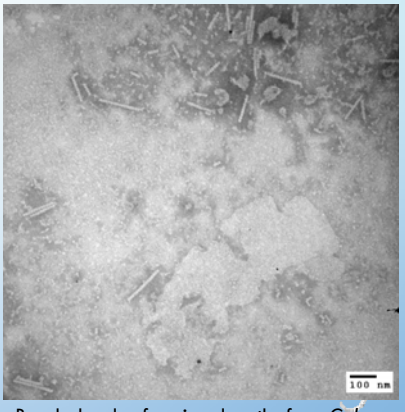


Tomato rhabdovirus (PYDV ?)
tomato

Intact rhabdovirus particles are unmistakable – but particles may be disrupted by stain and appear only as unidentifiable membranous objects (blue arrow, top right)

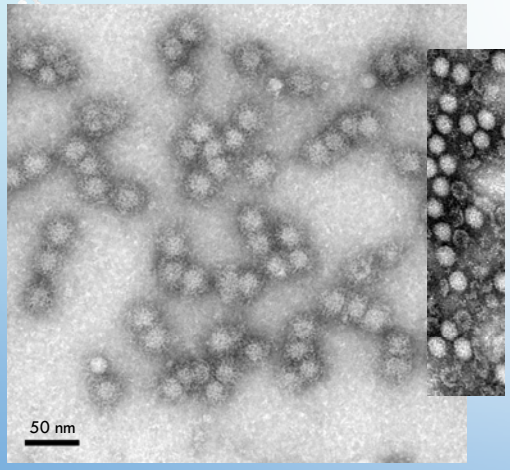


A group of isometric virions (red circle) in a field showing many artefacts of irregular size and shape (e.g. blue arrows)

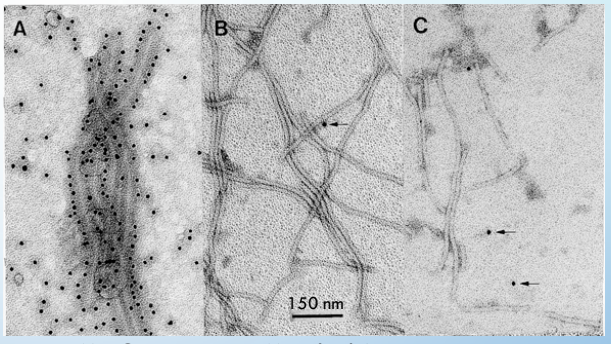


Beaded rods of various lengths from *Coleus*; objects of similar appearance have been observed in various species without yet being associated with any phytopathogen.

IMMUNOSPECIFIC ELECTRON MICROSCOPY (ISEM)



AnFBV particles decorated with AnFBV-specific antibody – note fuzzy 'halo' of antibody (inset: purified virus for comparison)



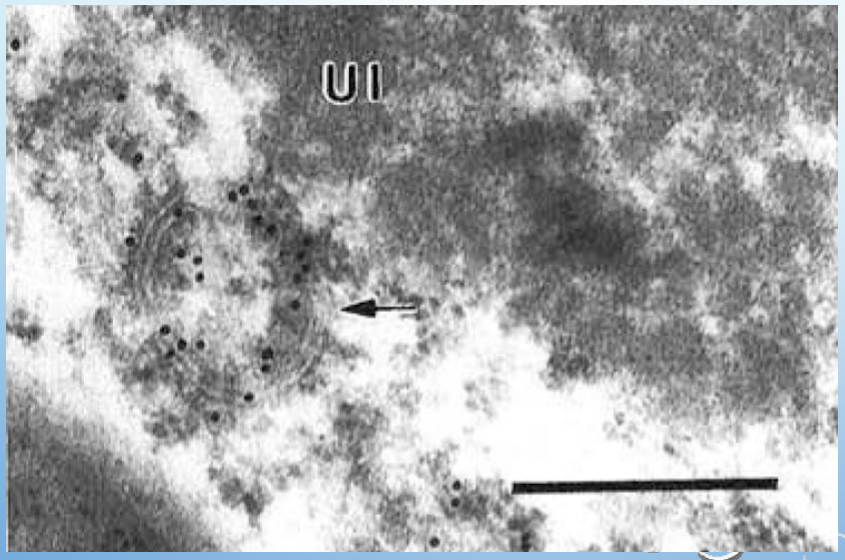
BYMV-GDD BYMV-Ideal A PVY

Potyvirus preparations labeled with monoclonal antibody PTY 43 and goat antimouse antibody conjugated to 10 nm gold particles. MAb PTY 43 is specific for BYMV-GDD. Note 'fuzzy' appearance of the MAb-coated virions and close association of gold particles; only background gold particles are seen on BYMV-Ideal A or PVY preparations (arrows).

IMMUNOSPECIFIC ELECTRON MICROSCOPY ON THIN SECTIONS OF EMBEDDED MATERIAL

- ANTIBODY LABELING CAN ALSO BE USED ON EMBEDDED AND THIN-SECTIONED MATERIAL
  - Best results are obtained in special types of embedding resins (typically hydrophilic resins such as LR White)
- Either gold-conjugated virus-specific antibodies, or virus-specific antibody followed by gold-conjugated antibody (e.g. goat anti-mouse or goat anti-rabbit antibody conjugated to gold)
- Different antibodies can be conjugated to different-sized gold particles (e.g. 5 nm, 10 nm, or 15 nm gold particles) to differentiate various target antigens such as viral coat proteins and non-structural proteins

IMMUNO-GOLD-LABELED THIN SECTION

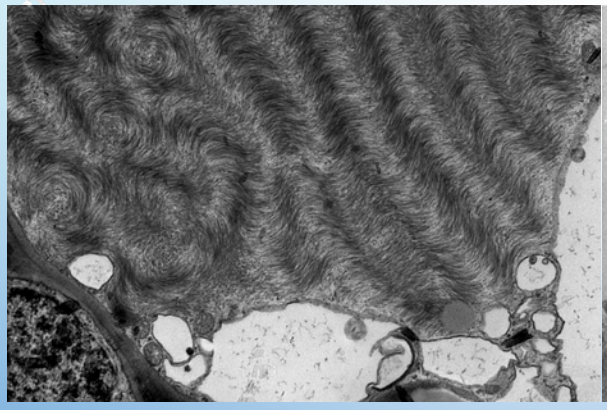


'HT-1-specific N-protein rabbit polyclonal antibody followed by 10 nm-gold-labeled goat anti-rabbit conjugate, revealing N protein labeling around membrane ring near unidentified inclusions (UI) in tissue of Impatiens.

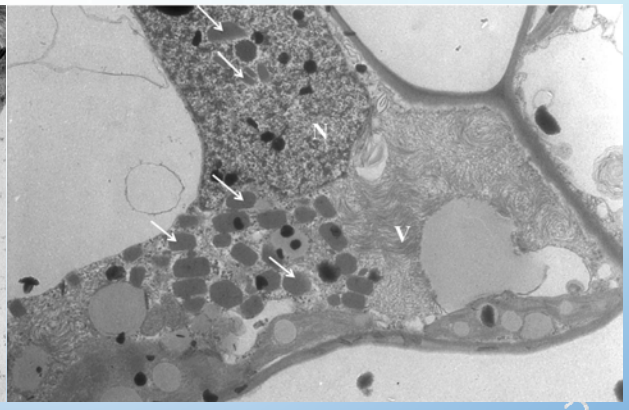
Image from Lawson et al., 1996, Phytopathology 86:650-661

Bar = 250 nm

CYTOPATHIC EFFECTS - POTEXVIRUSES

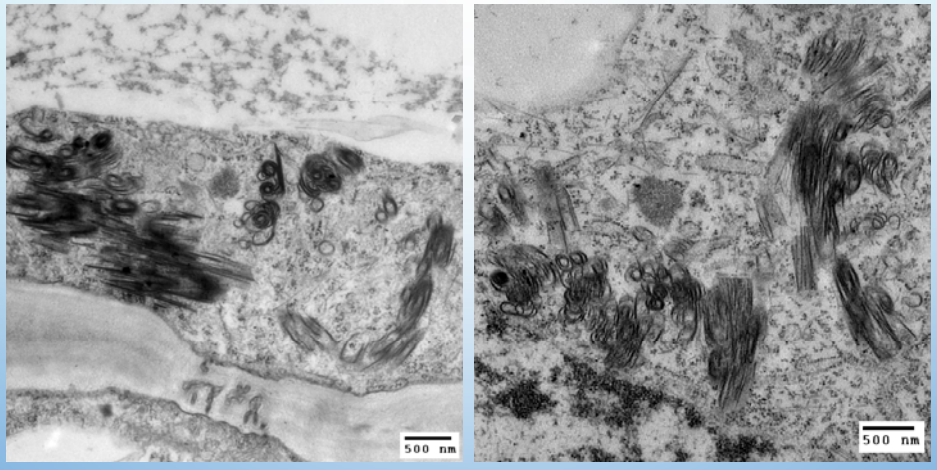


Banded inclusion of AltMV virus particles in infected *Nicotiana benthamiana* (an unusually densely-filled cell)



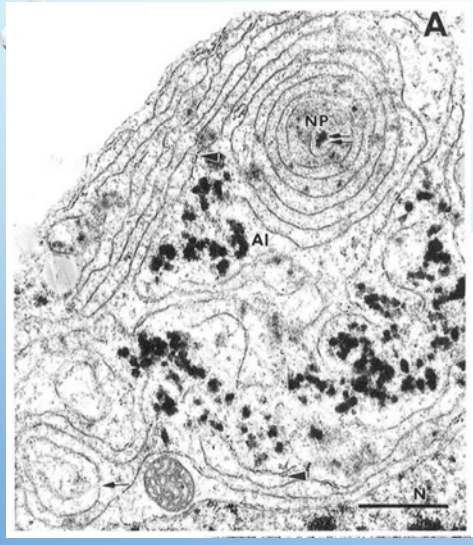
AltMV cytoplasmic and nuclear inclusions (arrows) assumed to be TGBp1, in *N. benthamiana*, plus banded inclusion of virus particles (V). N = nucleus

CYTOPLASMIC INCLUSIONS - POTYVIRUSES



Pinwheels and scrolls (different planes of sectioning)

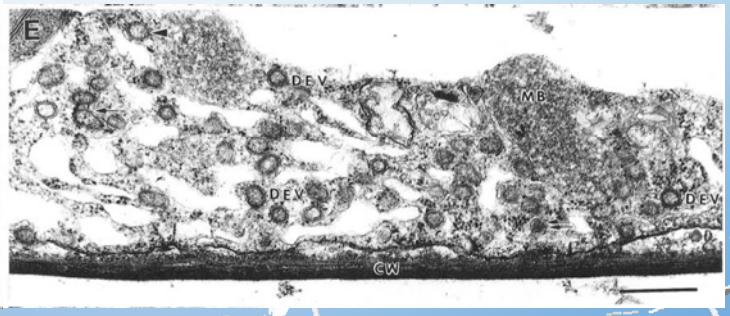
CYTOPATHIC EFFECTS - TOSPOVIRUSES



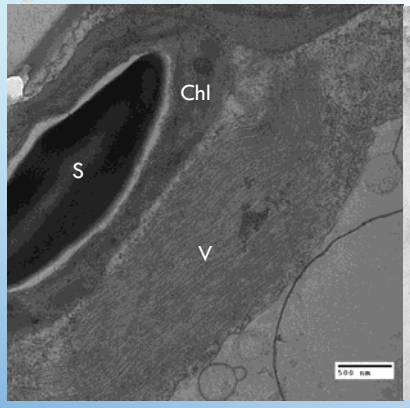
A. Extensive networks of double-membranes, with amorphous inclusions (AI) and nucleoprotein (NP); N = nucleus, bar = 1.2µm

Images from Lawson et al., 1996, Phytopathology 86:650-661

E. Double-enveloped virions (DEV) and single-enveloped virions (double arrow, lower right); CW = cell wall; MB = microbody; bar = 420 nm

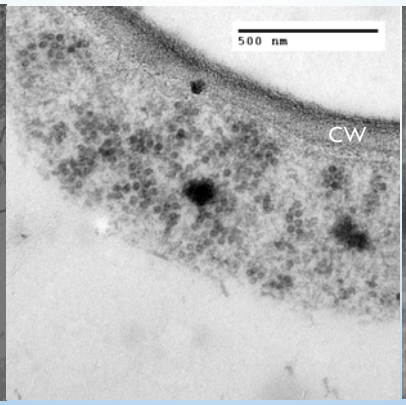


CYTOPATHIC EFFECTS – VIRUS AGGREGATES



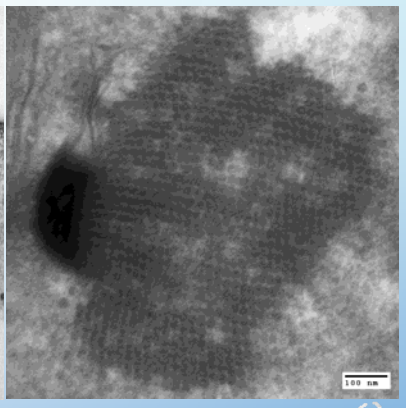
LoLV (lola)

Virions (V) in close proximity to chloroplast (Chl) containing starch grain (S)



AnFBV (carmo)

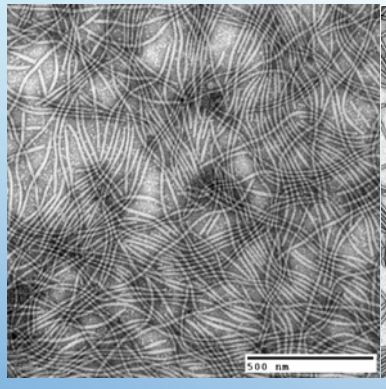
Aggregates of virions of AnFBV in association with the cell wall (CW)



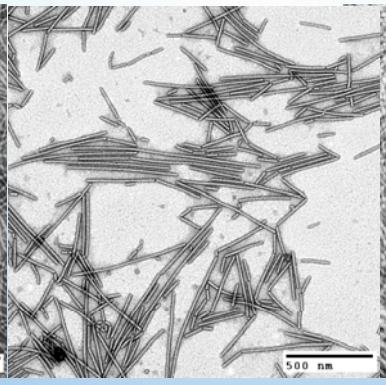
Bermudagrass isometric

Quasicrystalline aggregates of virions in cytoplasm of infected cell

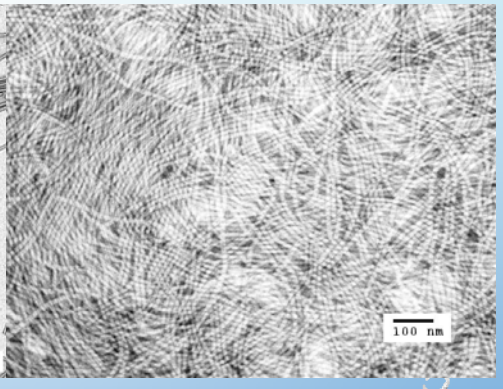
PURIFIED VIRUS PREPARATIONS



PVY (poty)



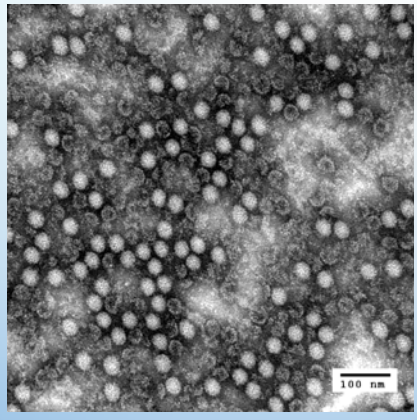
PhlVS (carla)



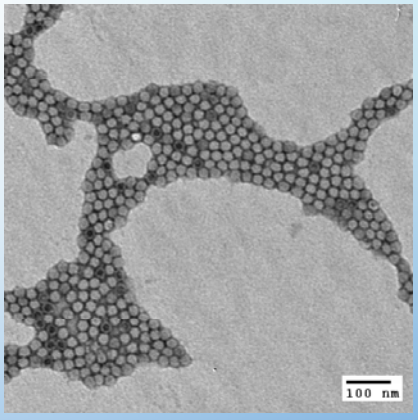
AltMV (potex)

PURIFIED VIRUS

AnFBV (carmovirus) CsCl gradient, single band



AnFBV (carmo)



TRSV (nepo)

Note that both AnFBV and TRSV preparations have some particles penetrated by stain, but both originated from single bands on CsCl gradients, and all particles are predicted to contain RNA. Some viruses (such as tymoviruses) produce 'top' component particles with little or no RNA which are readily penetrated by stain.

WHAT IS YOUR DIAGNOSIS ?