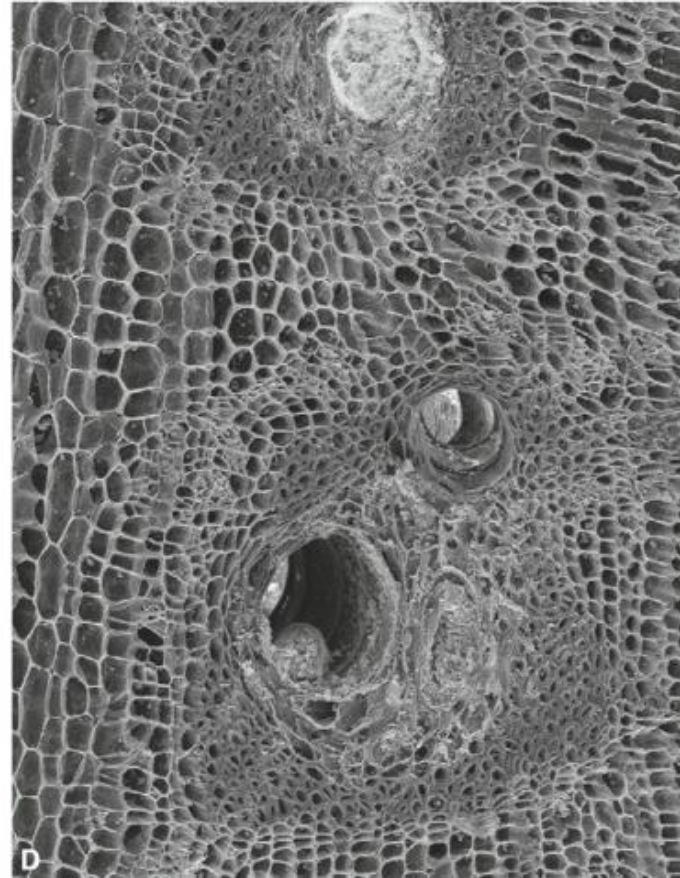
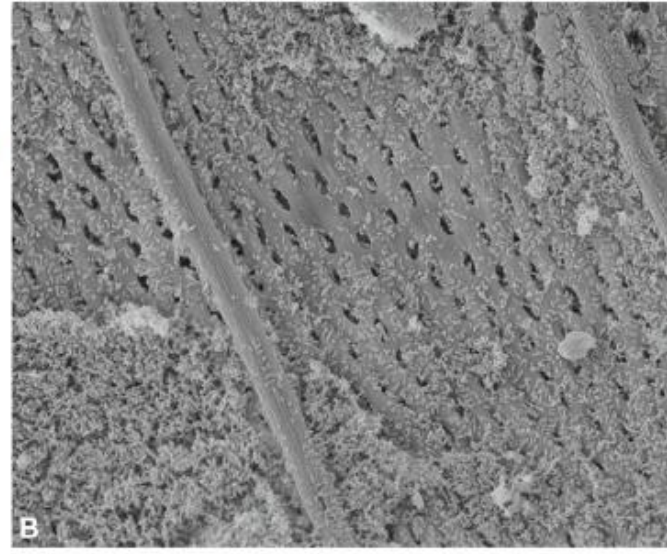


Lab 4

Symptomology

Internal

Symptoms

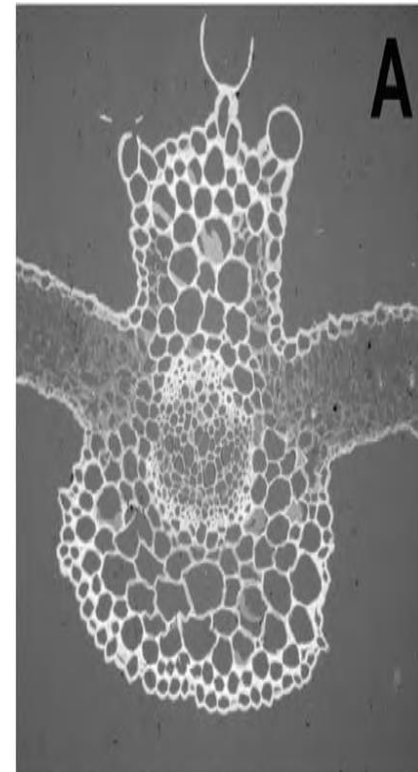


Internal symptoms(histological and cytological symptoms)

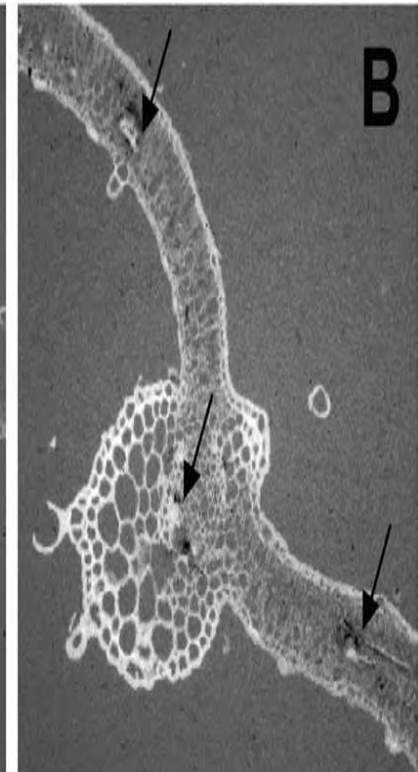
Virus infection induces changes in the infected plants.

1- Changes in normal tissues and cell organelles like nucleus, **chloroplasts**, mitochondria etc.

2- Formation of intracellular inclusion bodies.



Healthy



infected

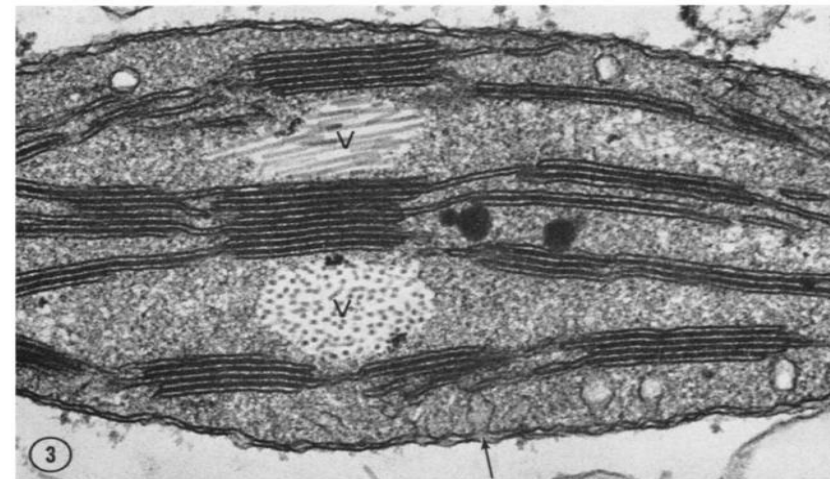
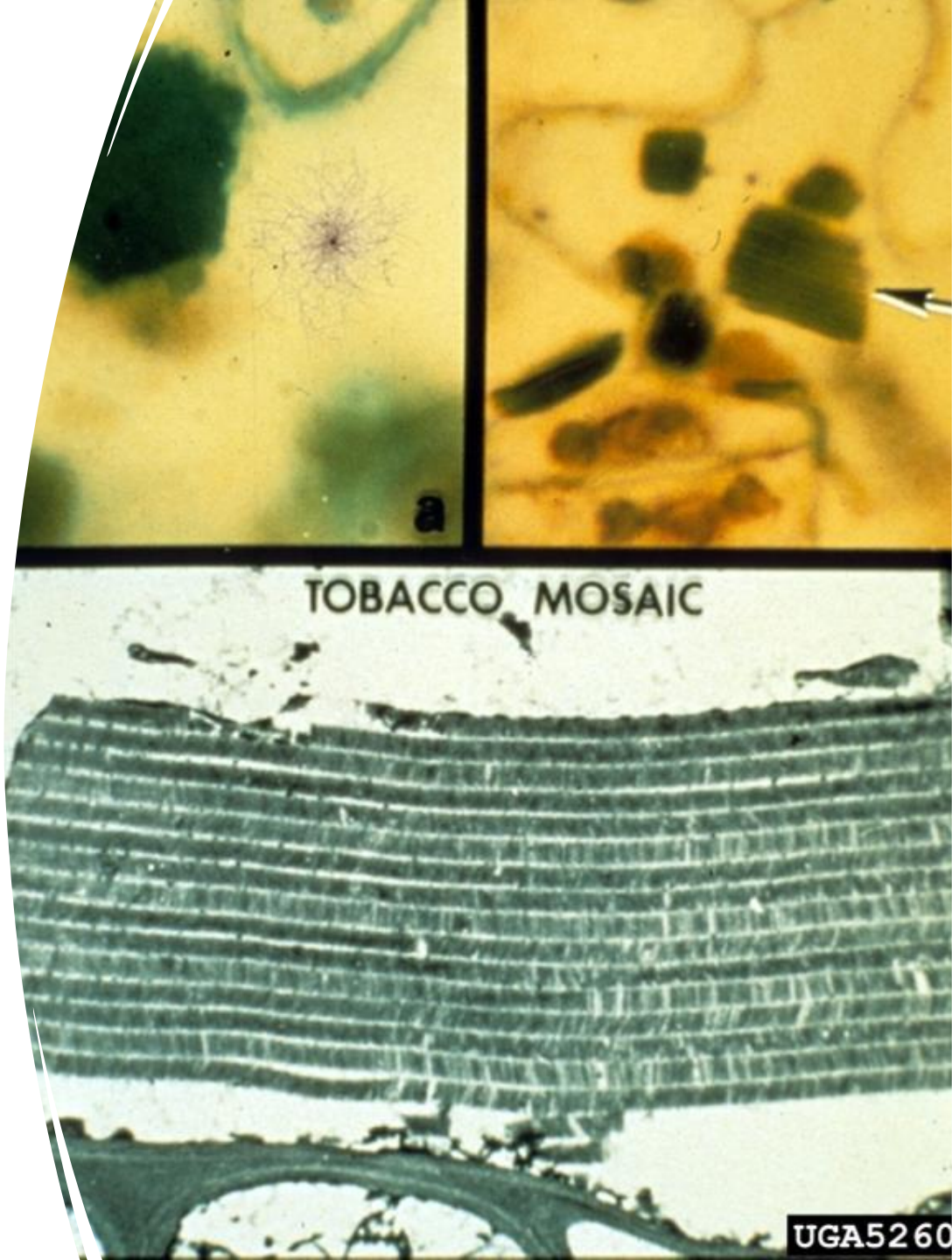


FIGURE 3 *Nicotiana tabacum*. Chloroplast containing virus particles (V) in two aggregations. In one of these the particles are seen in their longitudinal extent, in the other they were sectioned transversely. One of the vesicles near the periphery of the chloroplast is seen as a continuation of the inner membrane of the chloroplast envelope (arrow). $\times 72,000$.

Inclusion Bodies

- One of the most important internal symptoms inside the host cells. serve as diagnostic tools because their distinct shapes are characteristic of specific viruses. Not all viruses produce them, and some functions remain unknown.



Inclusion Bodies

These are intracellular structures formed due to the virus infection.

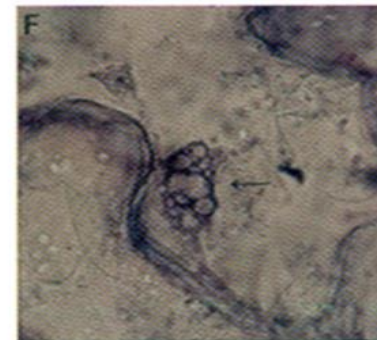
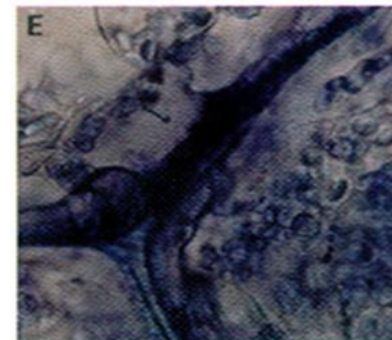
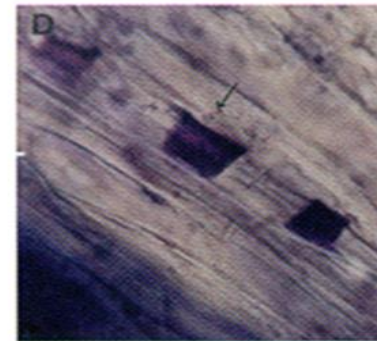
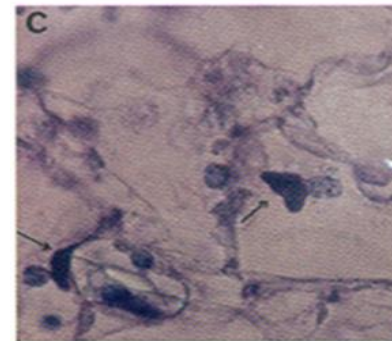
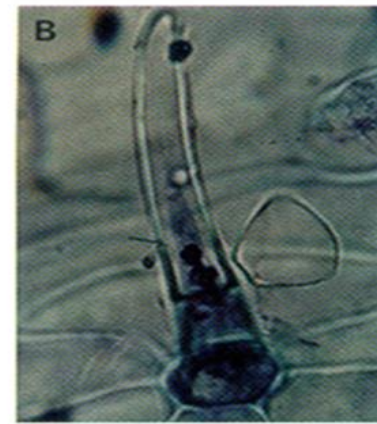
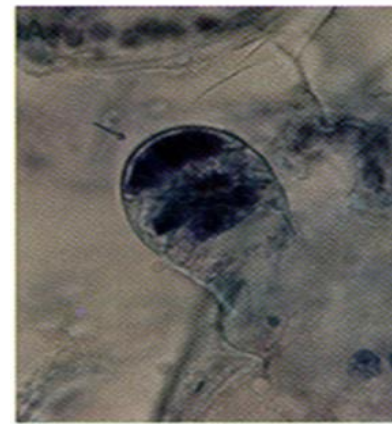
These are divided into two types:

1-amorphous inclusions:

- are known as X bodies.
- Appear elliptical almost entirely composed of dense granules varying in size.

2-Crystalline

- appear as crystal , no granular

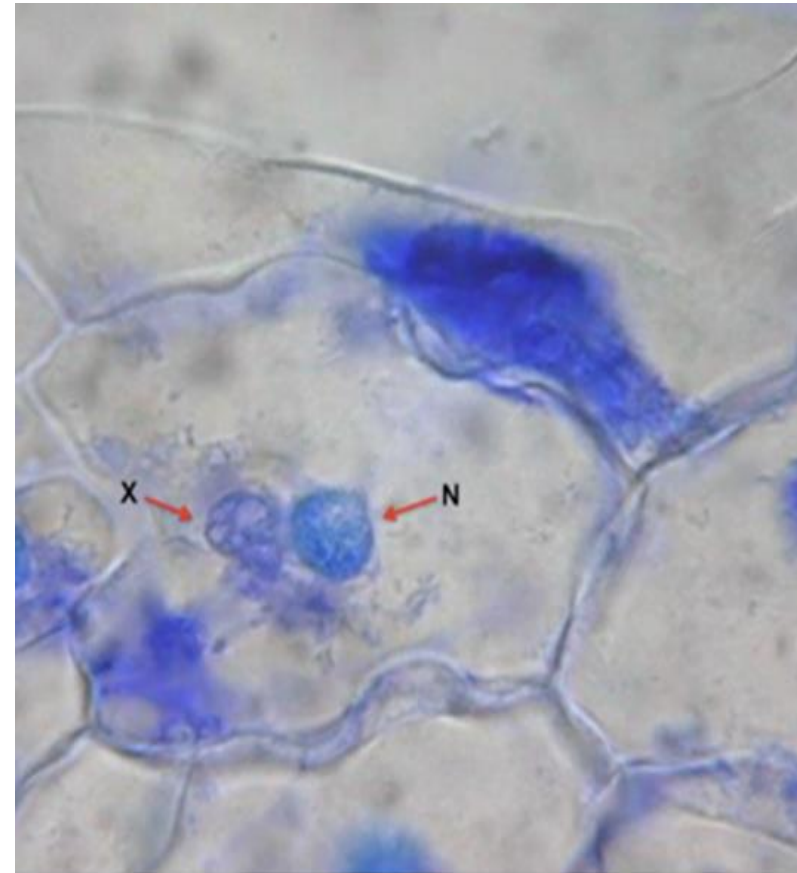


bodies in leaf tissue infected with *Bean rugose mosaic comovirus* stained with Azure A and toluidine blue. Crystalline inclusions observed in: (A) glandular hair cells, (B) trichome cells, (C) guard cells and (E) xylem elements. Vesiculate-vacuolate inclusions observed in: (D) cytoplasmic inclusions indicate the presence of inclusion bodies.

tejido foliar infectado con el virus del mosaico rugoso del frijol (comovirus), teñidas con Azure A y azul de toluidina. Inclusiones cristalinas observadas en: (A) células de un tricoma glandular, (B) células de guarda y epidermis, (D) tejido del floema y (F) elementos del xilema. Inclusiones vesiculovacuoladas observadas en: (F) citoplasma de células de la epidermis. Las flechas indican la presencia de los cuerpos de inclusión.

Inclusion Bodies

- These are found in cell cytoplasm, protein rich, most found in epidermis and leaf hairs.
- These are visible under a light microscope by using trypan blue staining and represent areas of high viral replication and accumulation



Here we decided to have some fun with staining for confirmation of TMV. Epidermal strips of a tobacco leaf infected with TMV stained in azure A (blue stain) showing X-bodies (X) and nuclei (N)

Examination inclusion bodies and Crystalline

Materials:

Infected plants , glass slides, tweezers,, glass cover , microscope and trypan blue stain, 0.9 % (w/v) NaCl solution.

Method:

1-Trypan blue was prepared by dissolving 1 g trypan blue in 100 ml 0.9 % (w/v) NaCl .

Trypan blue serves as a stain for these inclusions, effectively coloring amorphous and crystalline viral structures within the cell.

2- the epidermal tissues are used for infected plants.

3-The epidermis of the lower leaf surface and vein tissue are stripped from the adjacent tissue with blade a pair of sharp-pointed tweezers.

4-The specimens are immersed in 0.9 %(w/v) NaCl for a few seconds and then stained with 1 % (w/v) trypan blue solution for approximately 2 min, the tissues are then rinsed in 0.9 % (w/v) NaCl.

5- The specimens are examined under the microscope at magnification of 40x.

6- The type and location of inclusion bodies is described.