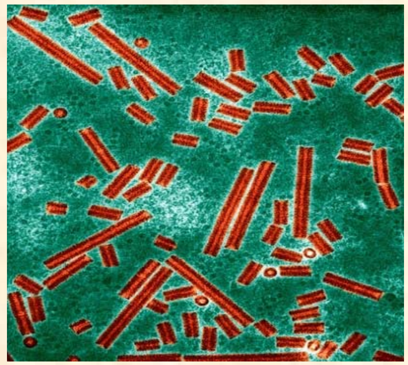
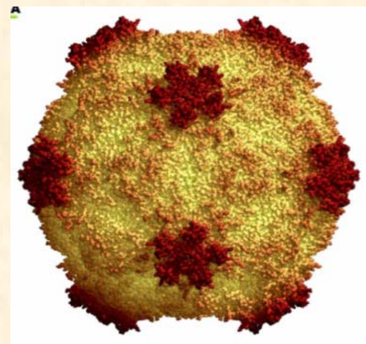
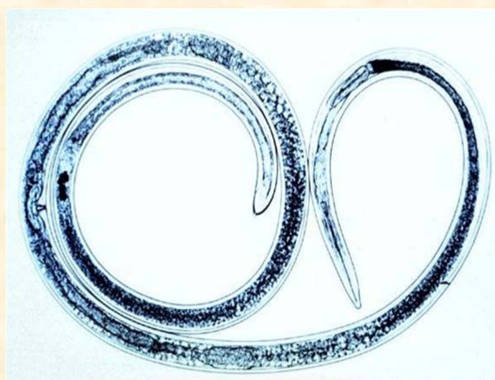
Transmission of Plant Viruses by Beetles, Thrips, Nematodes, and Mites







Lecture Overview

- *Coleoptera* and plant virus transmission
- *Thysanoptera* and plant virus transmission
- Nematoda and plant virus transmission
- Arachnida and plant virus transmission
- Specific examples of viruses' effects on vectors

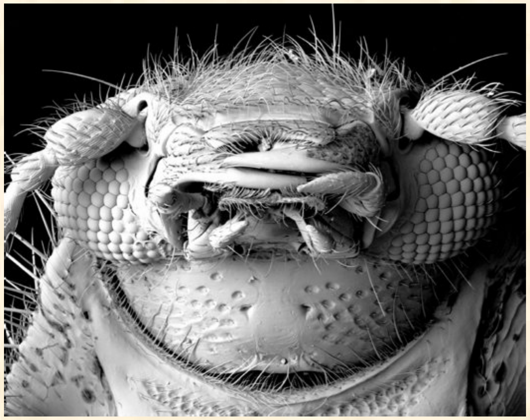
Taxonomy of Virus Vectors

Phylum: *Arthropoda*
Class: *Hexapoda*
Orders:

- *Archaeognatha*
- *Blattodea* (Cockroaches)
- ➡ *Coleoptera* (Beetles)
- *Dermaptera* (Earwigs)
- *Diptera* (Flies)
- *Embioptera* (Webspinners)
- *Ephemeroptera* (Mayflies)
- *Hemiptera* (Bugs)
- *Hymenoptera*
- *Isoptera* (Termites)
- *Lepidoptera*
- *Mantodea* (Mantises)
- *Mecoptera*
- *Megaloptera*
- *Neuroptera*
- *Notoptera*
- *Odonata*
- *Orthoptera*
- *Phasmatodea* (Sticks)
- *Phthiraptera* (Lice)
- *Plecoptera*
- *Psocoptera*
- *Raphidioptera* (Snakeflies)
- *Siphonaptera* (Fleas)
- *Strepsiptera*
- ➡ *Thysanoptera* (Thrips)
- *Trichoptera* (Caddisflies)
- *Zoraptera*
- *Zygentoma* (Thysanura)

Beetles as Virus Vectors

- Most vector species are in the family *Chrysomelidae* (40 vector species), a few in *Coccinellidae*, a few in *Curculionidae* (weevils)
- Some common vectors: bean leaf beetle, striped and spotted cucumber beetles, western striped and spotted cucumber beetles, Mexican bean beetle
- Beetles acquire and inoculate plant viruses using mouthparts designed for chewing (not with stylets as in *Hemiptera*)



Types of Virus Transmission

Mode of Transmission	Host	
	Plant	Animals and Humans
Passive transmission among environments (soil, water)	x	x
Passive mechanical transmission among hosts	x	x
Receptor-site-mediated non-persistent transmission	x	
Receptor-site-mediated semi-persistent transmission	x	
Receptor-site-mediated circulative, non-propagative	x	
Amplifying (circulative, propagative)	x	x
Vectors as reservoirs	x	x

Beetle transmission does not easily fit into any of these categories

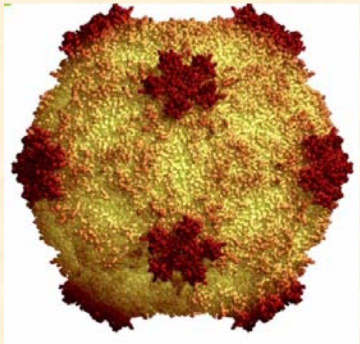
Some beetles are reservoirs of plant viruses

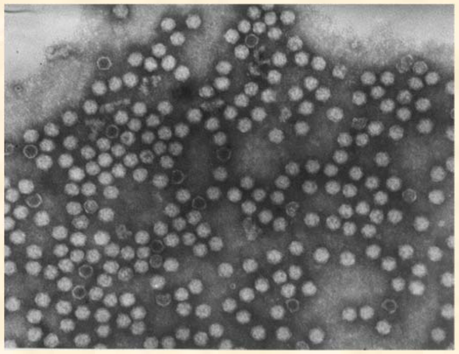
Coleoptera: Beetles as Virus Vectors

- All known beetle-transmitted viruses are ss(+)RNA viruses with isometric virions, without an envelope

    Bromovirus, *Comovirus*, *Carmovirus*, *Machlomovirus*, *Sobemovirus*, *Tymovirus*

- All these viruses have relatively stable virions
- Coat protein is involved in specificity but the mechanism is not clear
- Generally beetle-transmitted viruses reach high titers in plants





Beetles as Virus Vectors

- Transmission is not by mechanical transmission
- Can acquire viruses in short feedings but longer acquisition and inoculation periods increase transmission rates.
- No latent period
- No plant viruses are known to replicate in beetles
- Can be foregut-borne or circulative, depending on the combination of beetle and virus
- Acquisition time: unclear
- Inoculation: virus regurgitated onto wounded leaf surface
- Retention time: varies with virus and vector, temperature and feeding behavior (some viruses can overwinter in their beetle vectors).

Beetles as Virus Vectors

Details of the relationship of plant viruses with beetles are awaiting discovery

- There is a high degree of specificity between the beetle species and virus species
- Specificity is due to interaction of virus particles, beetle regurgitant, and host plant.
- Successful virus transmission requires beetle regurgitant (from a beetle that fed previously on an infected plant) and gross wounding of the plant
- How the virus gets into the regurgitant is not known.

Beetles as Virus Vectors

- Some beetle transmitted viruses are circulative: ie found in the hemolymph but others are not
  - Ex. *Southern bean mosaic virus* (*Sobemovirus*) is found in the hemolymph of bean leaf beetle and spotted cucumber beetle, while *Bean pod mottle virus* (*Comovirus*) is not.
  - So the presence of virus in the hemolymph is not essential for transmission of all beetle transmitted viruses

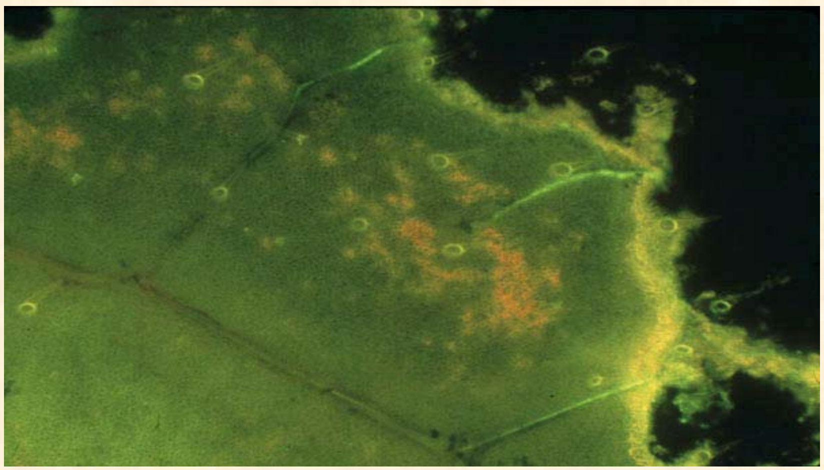
- Presence of virus in the hemolymph is not related to length of retention time

- Beetle-transmitted viruses appear to be more mobile in plant vascular system than viruses not transmissible by beetles.

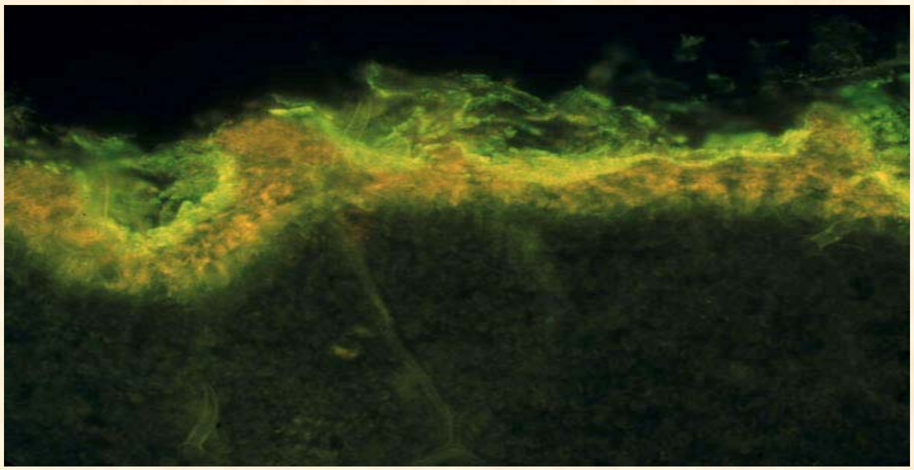
- Viruses can overwinter in beetle vectors so they can serve as reservoirs

Beetles as Virus Vectors

- As beetles wound the leaf they regurgitate, on the wound to help in digestion of plant materials.Transmissible virus moves quickly into xylem, then infects nearby unwounded cells.

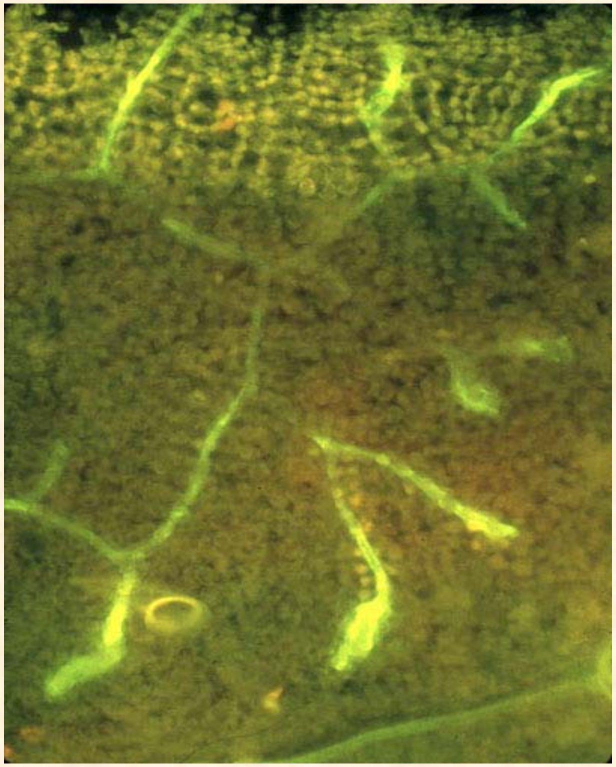


Ex. *Bean pod mottle virus* can be detected in the xylem 4-12 hours post-inoculation

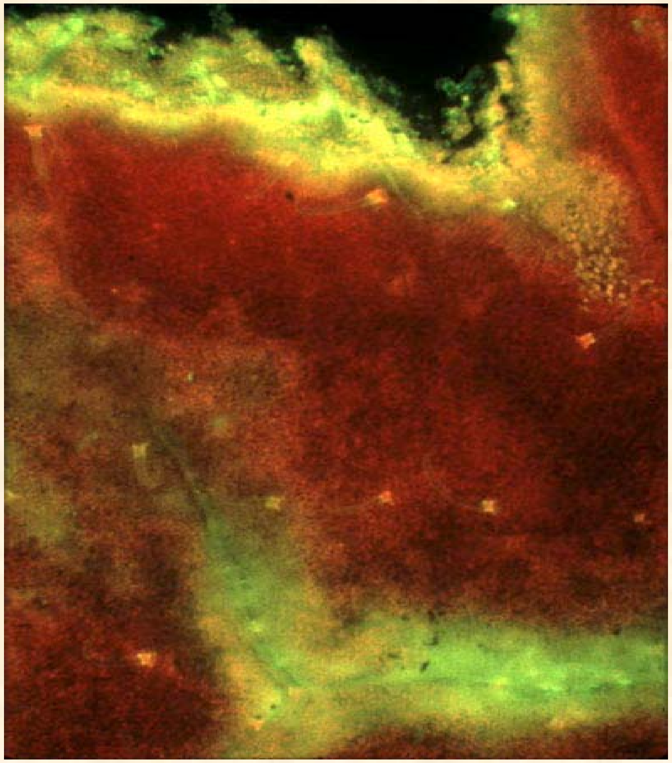


TMV (not transmitted by beetles in a specific manner) on edge of wound closing layer (above), does not enter xylem

Invasion of leaf over time after inoculation with *Southern bean mosaic virus* (SBMV) by beetles



a few hours after inoculation, SBMV can be detected in the xylem



2-3 days post-infection, showing SBMV-infection of mesophyll cells next to veins distant from wound

Ex. Bean pod mottle virus and Soybean

- Bean leaf beetle (*Chrysomelidae: Ceratoma trifurcata*) is a direct pest of soybean as well as a vector.
- Two mild winters in a row resulted in high populations in the Midwest (Iowa, Illinois, Nebraska).
- Much higher incidences of BPMV followed.
- Virus reservoirs were forage legumes, over-wintered beetles, and seed.

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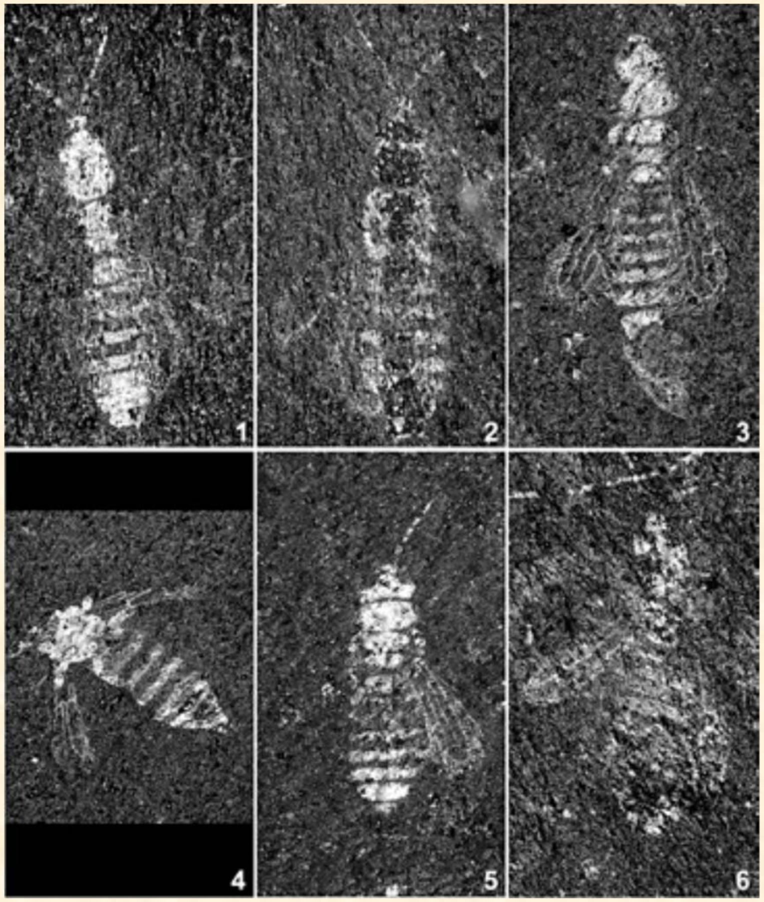
Vectors as reservoirs	x	x



Best preserved examples of *Triassothrips* from the Triassic period Grimaldi et al. 2004, J Paleontology 78:941

Thrips(order Thysanoptera) were probably the first "micropredators" to develop piercing-sucking mouthparts –these insects are the closest living relatives of the order Hemiptera, which contains both plant-and animal-feeding allies. Johnson et al. 2018, PNAS 115:12775

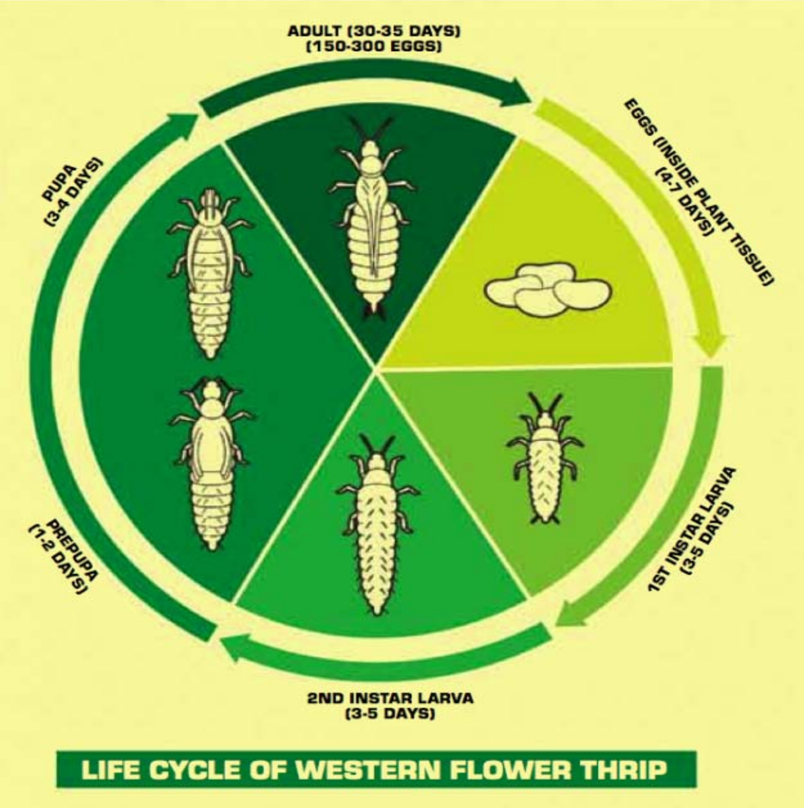
Thrips probe plant, fungus, and animal tissues with their slender mouthparts, and suck out fluid contents.

Thrips were probably among the first arthropods to enter the vector-borne disease triangle through plant feeding

Thysanoptera (Thrips)-transmission of plant viruses

- Thrips species transmit *Tospoviruses* (*Bunyaviridae*)
- Transmission is characterized as **Amplifying** (Persistent, propagative)
- *Tospovirus species* are transmitted by at least 10 species of thrips, many of them *Frankliniella* spp.

Thysanoptera (Thrips)-transmission of plant viruses



Eggs are usually laid singly inside the plant tissue or on plant surfaces. May not be visible.

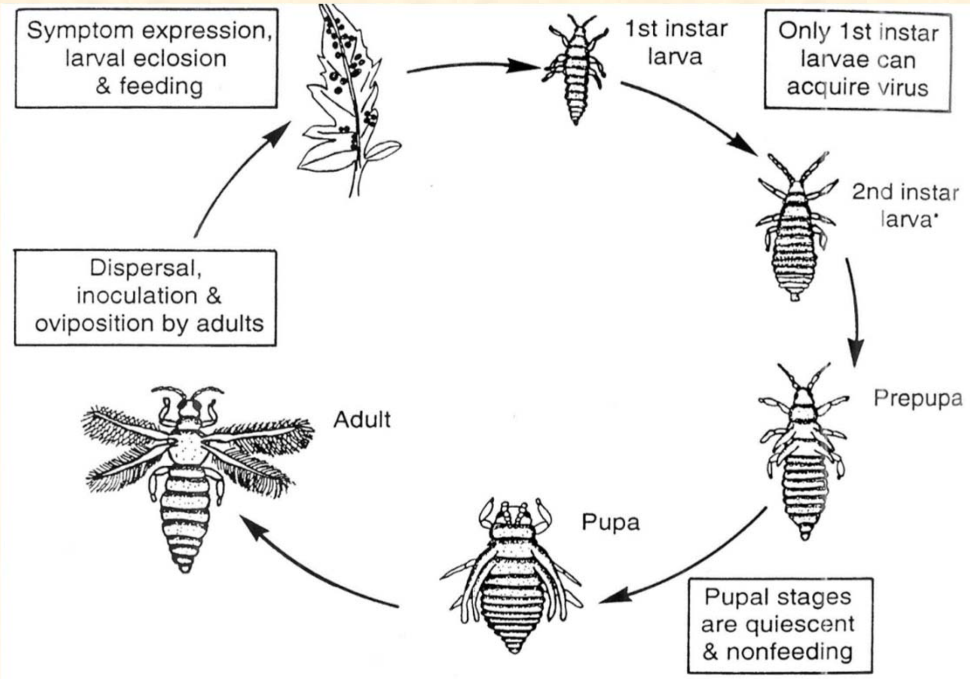
Larvae have piercing-sucking mouthparts. They resemble a miniature version of the adults but do not have wings.

Prepupa and **pupae** have short wing buds but no functional wings. During these stages thrips are inactive and do not feed. Pupation may occur on a plant or in the soil beneath, depending on species.

Adult thrips migrate actively between different hosts.

Egg to **adult** takes about 2 to 3 weeks under warm conditions, which gives them a enormous capacity for increase.

Thysanoptera (Thrips)-transmission of plant viruses



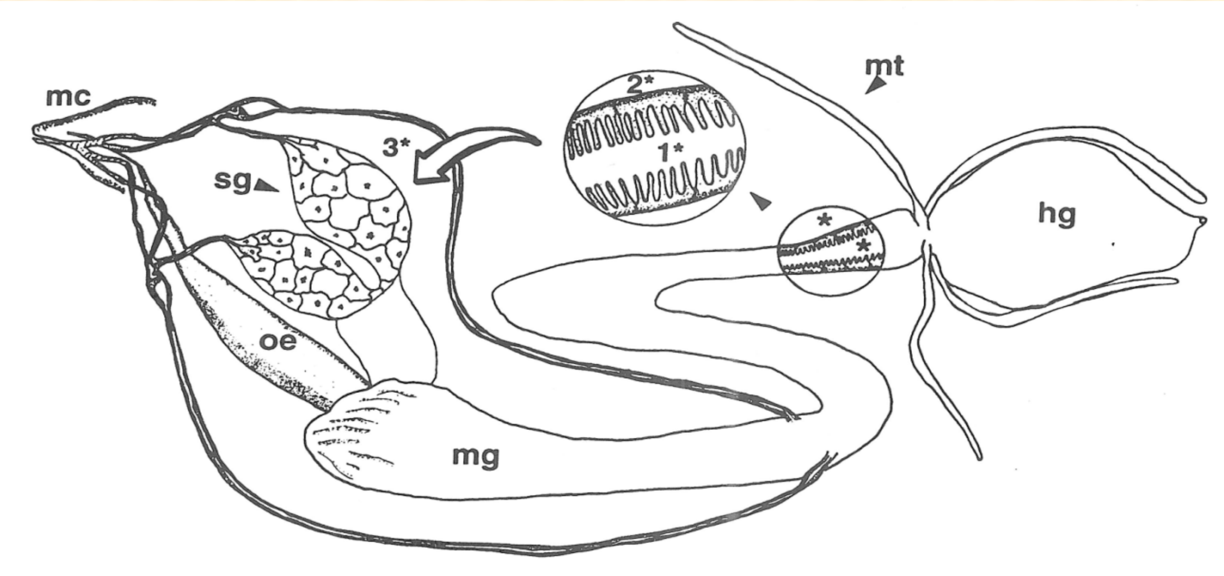
- Initiation of the infection cycle can occur only when female adult thrips lay eggs on *Tospovirus*-infected leaves that are suitable for egg and larval development.
- Once acquired by the 1st stage instar, the virus is retained by thrips through molting, pupation, and emergence to the adult stage.
- Primary dispersal of TSWV is by adult thrips, which disperse widely, feed on many different plant hosts and may remain viruliferous for the remainder of their life.

Thysanoptera (Thrips)-transmission of plant viruses

- Virus most easily infects early first instars
- Not all virus-infected thrips will transmit.
- Infected thrips may develop more slowly than non-infected thrips
- Non-infected thrips developing on infected plants may develop faster than those feeding on healthy plants.

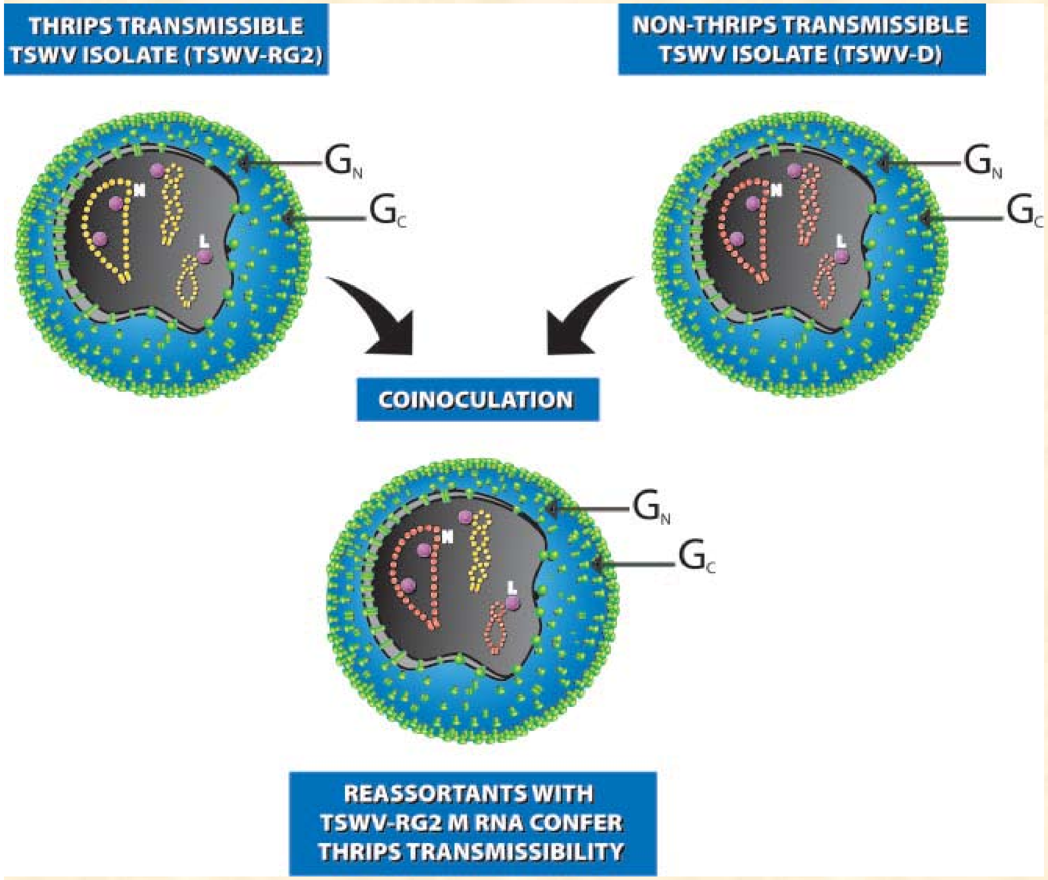
Thysanoptera (Thrips)-transmission of plant viruses

- Virus enters thrips along ligament-like structures that connect the midgut to glands or through physical contact of midgut and salivary glands during development in larval stages (which are separated in adult stages)
- Insect acquisition of tospoviruses cannot occur without viral passage across at least three insect organs: the midgut, visceral muscle cells, and salivary glands
- This means the virus must cross multiple membrane barriers to be transmitted.



- Virus is transmitted to plants in saliva
- However it is not clear exactly how tospoviruses get into the thrips salivary glands

Thysanoptera (Thrips)-transmission of plant viruses

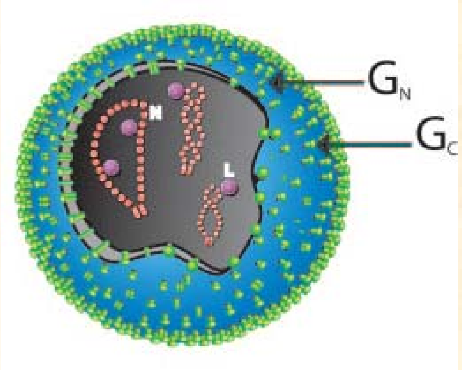


Ullman et al. 2005. *PNAS 102:4931-4932*

M RNA has the determinants for thrips transmission

M RNA codes for G_N and G_C – glycoproteins that are found in the virus envelope

Thysanoptera (Thrips)-transmission of plant viruses



- TSWV $\mathbf{G_N}$ plays a role in virus attachment to thrips midgut epithelial cells
- Role of $\mathbf{G_C}$ is less clear, but is known to be activated at low pH and is involved in fusion and virus entry
- A single nucleotide substitution in the ORF encoding G_N/G_C had no apparent effect on virion assembly, and mechanical transmission but could eliminate insect transmissibility.

- These glycoproteins are **functional in mammalian cells**, suggesting that TSVW might have evolved from an animal-infecting bunyavirus. These proteins, likely reflect primary adaptation to replication in the thrips host with subsequent adaptation to the plant host. Kikkertet al. 2001, J Virol75:1004
- Multiple thrips proteins have been shown to directly bind to several TSWV glycoproteins and these interactions are likely critical for replication in thrips.

Thysanoptera (Thrips)-transmission of plant viruses

- There is specificity among thrips species and tospovirus species
- Transmission efficiency of a virus varies among thrips species
- Transmission efficiency of a thrips species varies among different virus species

Thrips	Transmission Efficiency	
	TCSV	TSWV
Frankliniella schultzei (dark form)	37.5%	13.7%
F. schultzei (light form)	5.9%	2.3%
F. occidentalis	27.6%	66.0%
F. intonsa	0.7%	31.8%
Thrips tabaci	0.0%	9.8%

Phylum: Animal

http://www.personal.psu.edu/ncj111/Evolution%20and%20Phylogeny.htm

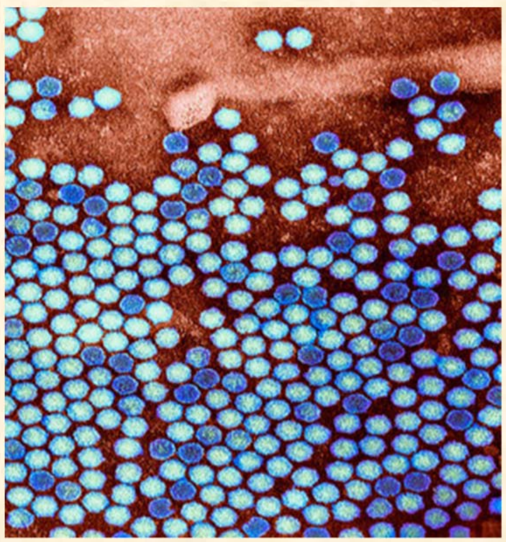
Nematodes as Virus Vectors

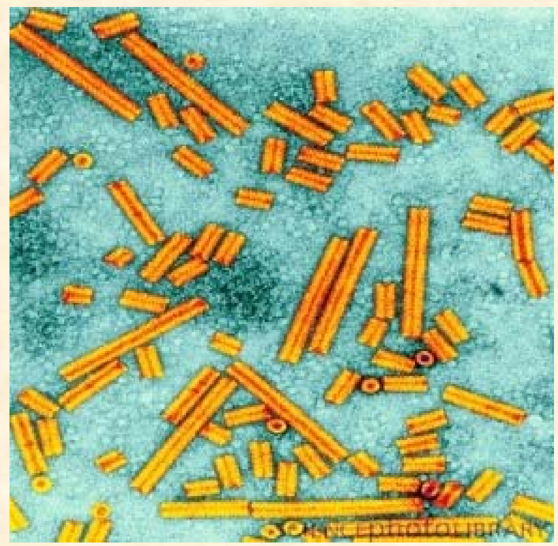
- Only two genera of viruses *transmitted*—

  Secoviridae: Nepovirus

  Virgaviridae: Tobravirus

- Both are positive sense ssRNA viruses





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Receptor-site-mediated circulative, non-propagative	x	
Amplifying (circulative, propagative)	x	x
Vectors as reservoirs	x	x

Nematodes as Virus Vectors

Examples from Florida

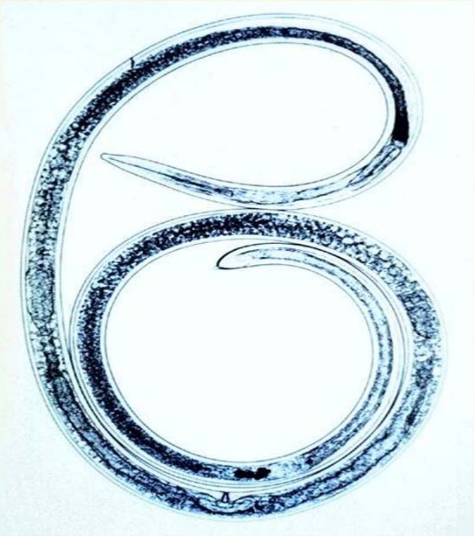
Corky ringspot disease of potato, caused by *Tobacco rattle virus (Tobravirus)*. Hastings, FL.

Symptoms of *Tobacco ringspot virus (Nepovirus)* in *Zamia furfuracea*, the Cardboard Cycad

Nematodes as Virus Vectors

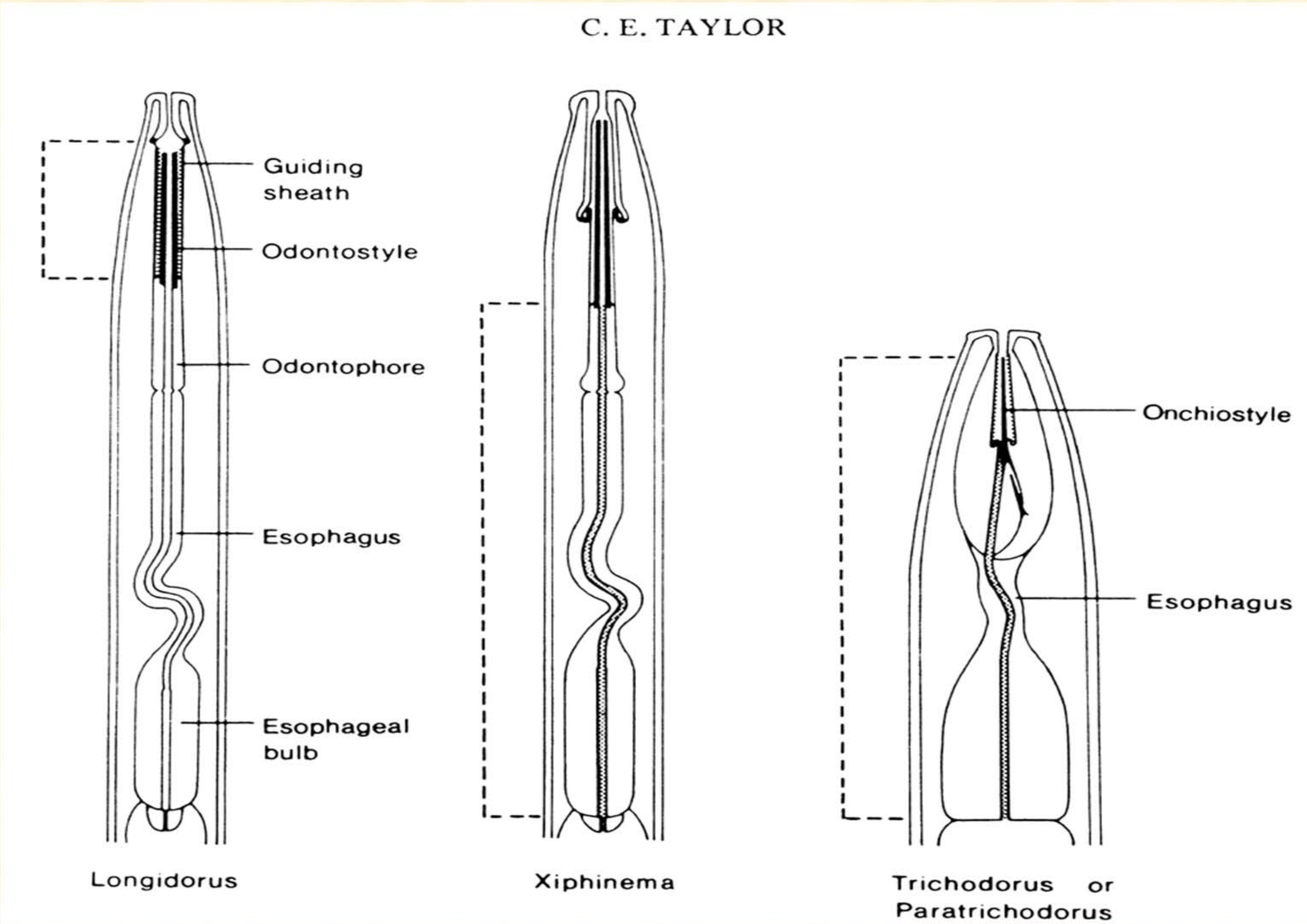
Characteristics of Transmission:

- Semi-persistent transmission (non-circulative).
- Virus may persist in nematode for weeks to more than a year, depending on species and feeding activity.
- Access times may vary from hours to as little as 15 minutes.
- No evidence for replication in the nematode
- Transmission rate increases with increased access time.
- Transmission rate may be influenced by the host plant.



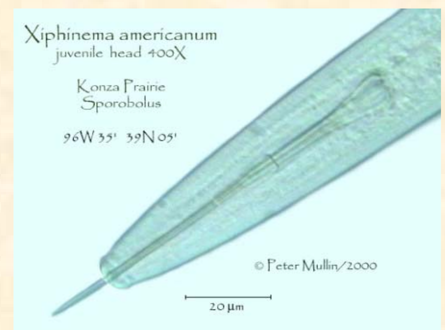
Xiphenema diversicaudatum female

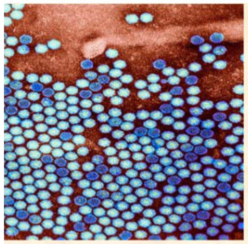
Nematodes as Virus Vectors



Piercing mouthparts – stylet

Different structures of stylets are present in different genera of nematodes

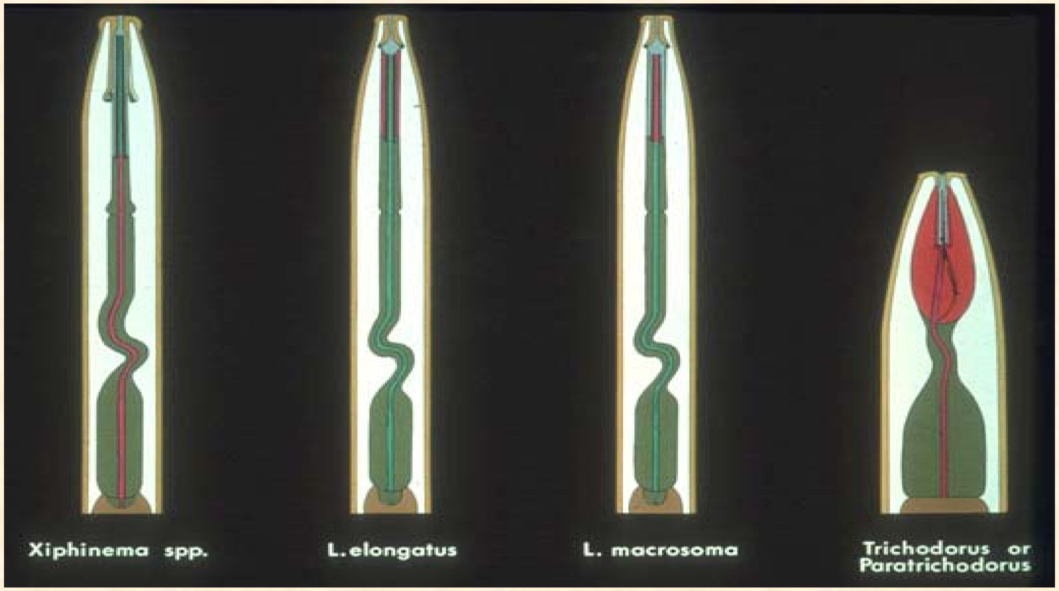




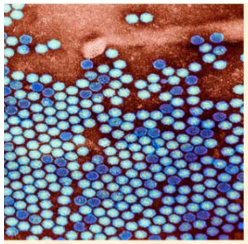
Nepovirus Transmission

- Nepoviruses are transmitted by species of
  Xiphenema, Longidorus, Paralongidorus

- Nepoviruses are associated with different parts of the stylets in different genera/species of nematodes

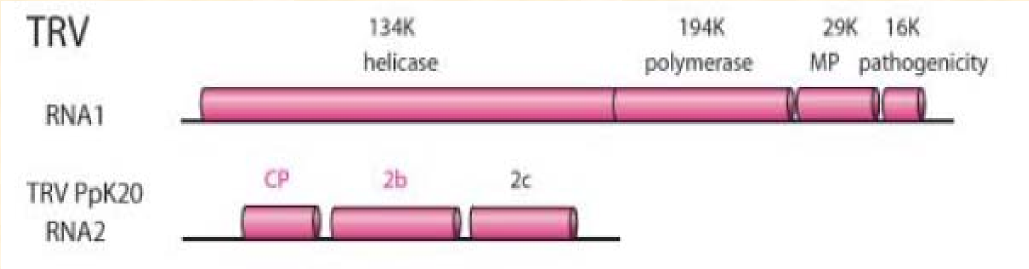


Red indicates where plant virus particles have been detected



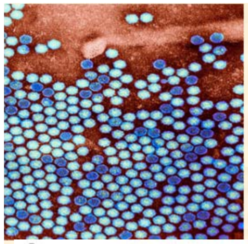
Nepovirus Transmission

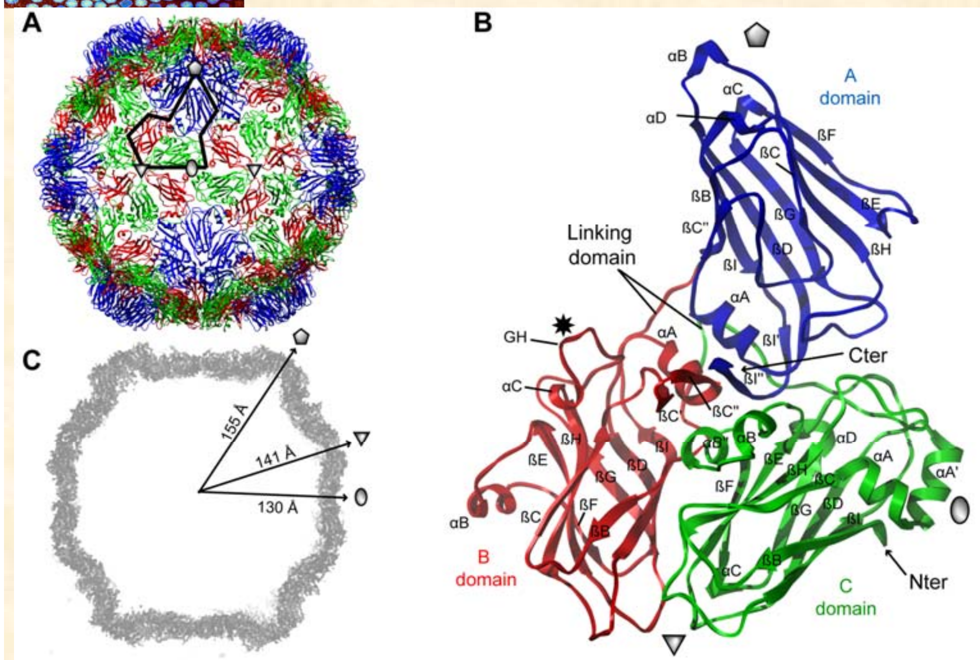
- Evidence suggests that regions of both the CP and the MP (2b) protein act together to determine which vector species will transmit a virus
- 2b might act as a helper component and may play a role in specificity of vector species



MacFarlane 2003 Molecular Plant Pathology 4: 211–215

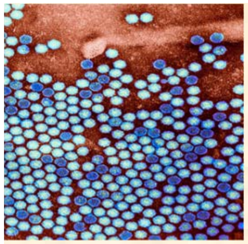
Nematodes as Virus Vectors





- There is a pocket present in the *Nepovirus* coat protein which binds ligands and is essential for transmission of the virus by *Xiphenema index*.
- Electrostatic charges in this pocket are essential to the process of the binding of the virion with specific receptors of the nematode’s feeding apparatus.

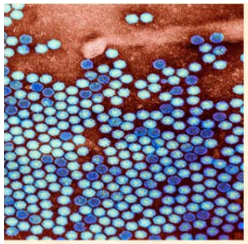
Schellenberger P, Sauter C, Lorber B, Bron P, Trapani S, et al.
(2011) PLoS Pathog 7(5): e1002034.
doi:10.1371/journal.ppat.1002034



Nematodes as Virus Vectors

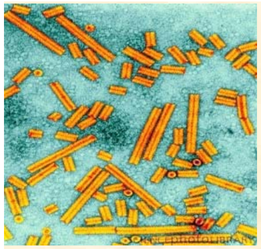
- *Strawberry ringspot virus* (*Nepovirus*) in Scotland, vectored by *Longidorus elongatus*.

Pattern of virus-infected plants is often similar to that of the nematode – Patchy, not evenly distributed



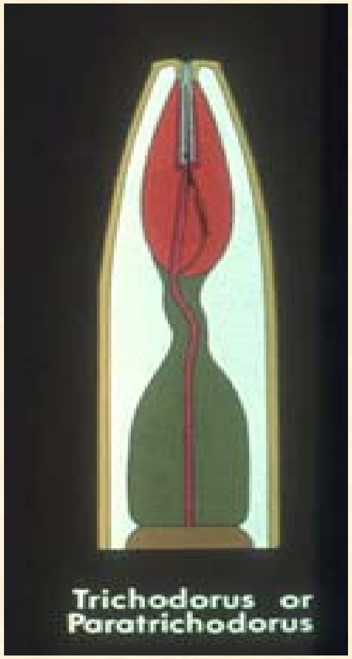
Nematodes as Virus Vectors

- Yellowing disease of barley caused by *Arabis mosaic virus* (*Nepovirus)* in Switzerland, vectored by *Xiphinema diversicaudatum*



Tobravirus Transmission

Tobraviruses are transmitted by species of *Trichodoris* and *Paratrichodorus*



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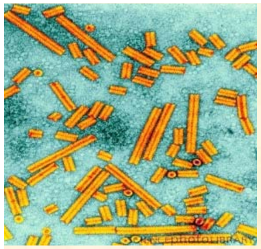
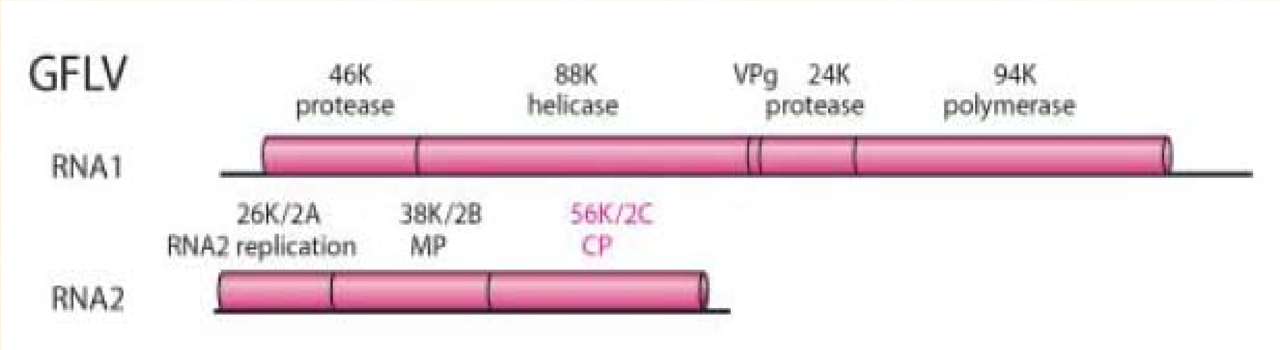
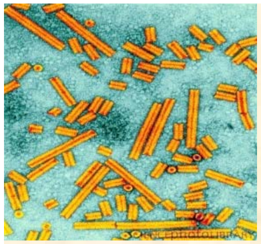


Tobravirus Transmission

- The *Tobravirus* CP is essential for nematode transmission
- Transmission specificity determined by RNA-2 and correlated with serological properties of coat protein.
- Up to three non-structural proteins may be involved in *Tobravirus* transmission.



Genome organization of Grapevine fanleaf virus



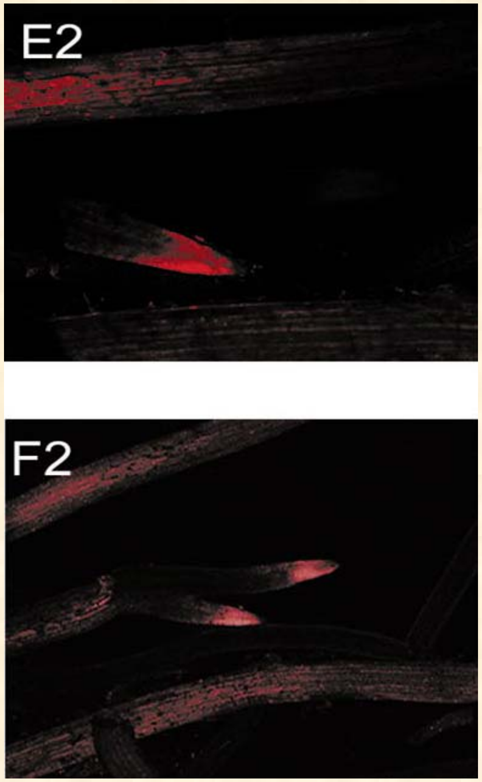
Nematodes as Virus Vectors

- Site of acquisition:

Once a plant is infected by a *Tobravirus*, virus particles moves rapidly through the root system, where they can be ingested by vector nematodes which feed primarily on epidermal cells located just behind the zone of root elongation near the root tip.

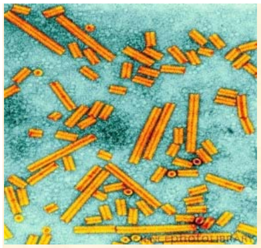
- Site of inoculation:

Virus particles initially are retained in the nematode's mouthparts and, during subsequent feeding episodes, they are released from the site of retention in the esophagus and introduced into new plants.

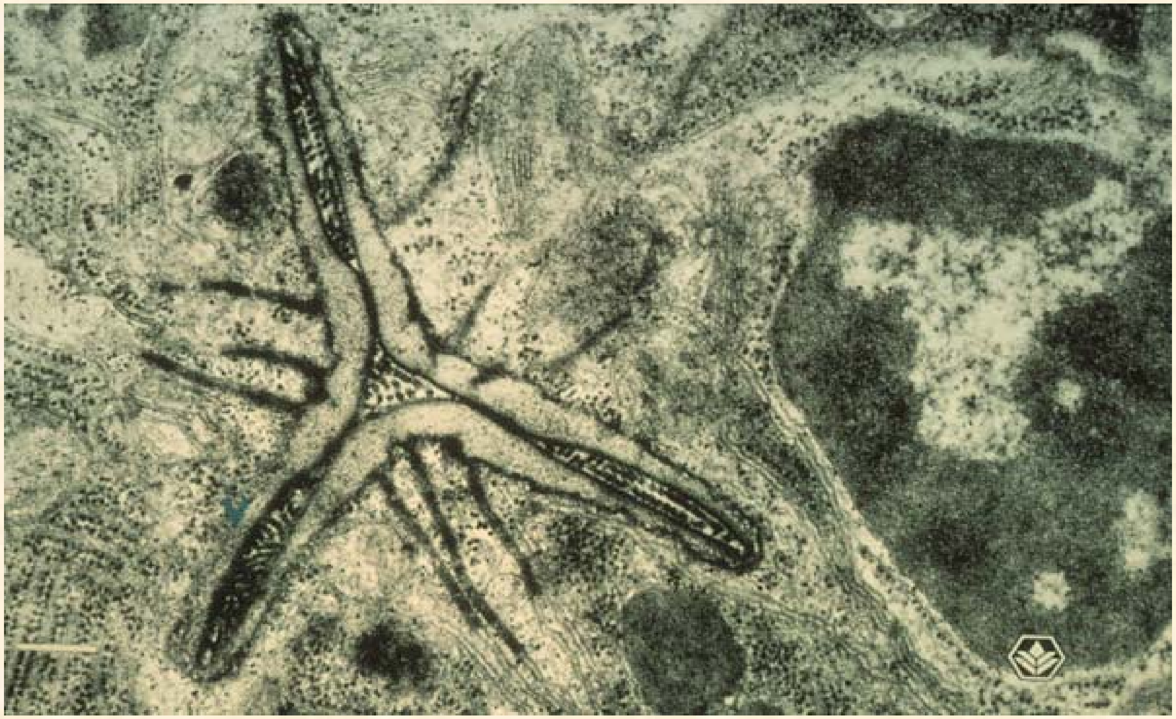


Confocal fluorescence microscopy of plants infected with *Tobacco rattle virus* (*Tobravirus*)

Vassilakos (2001) Virology 279, 478-487



Tobravirus Transmission



Cross section of nematode

- *Tobacco rattle virus* (*Tobravirus*) in the food canal of its vector.
- Virions have never been detected within the cells of nematodes.

Phylum: Animal

http://www.personal.psu.edu/ncj111/Evolution%20and%20Phylogeny.htm

Mites as Vectors of Plant Viruses

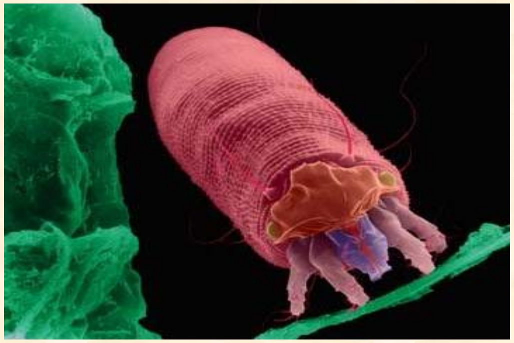
Order-Arthropoda, class-Arachnida, subclass-Acari

- 3 families of mites are known to transmit plant viruses
- Tetranychoidea, Tetranychidae – spider mites
- Tetranychoidea, Tenuipalpidae - false spider mites or flat mites
- Eriophyoidea, Eriophyidae – Eriophyid mites

Tetranychidae

Tenuipalpidae

Eriophyidae



Dispersal of Mite Vectors and Mite-transmitted Viruses:

- Movement of infested and infected plant material
- Wind dispersal of living (or dead) mites
  - Easily picked up by wind speeds (can’t hang on to plants very tightly)
  - Dead female mites – eggs can hatch from bodies of dead females
  - Spider mites use silk threads to assist in wind dispersal
  - High percent of dispersing mites are female

Tetranychoidea, Tetranychidae – spider mites

- 1 example known
- Brown wheat mite (*Petrobia latens*) and *Barley yellow streak mosaic virus* (BaYSMV) +ssRNA virus (*Virgaviridae, Hordeivirus*)
- Transmission was not transovarial
- Outbreaks are associated with drought and high populations of the mite

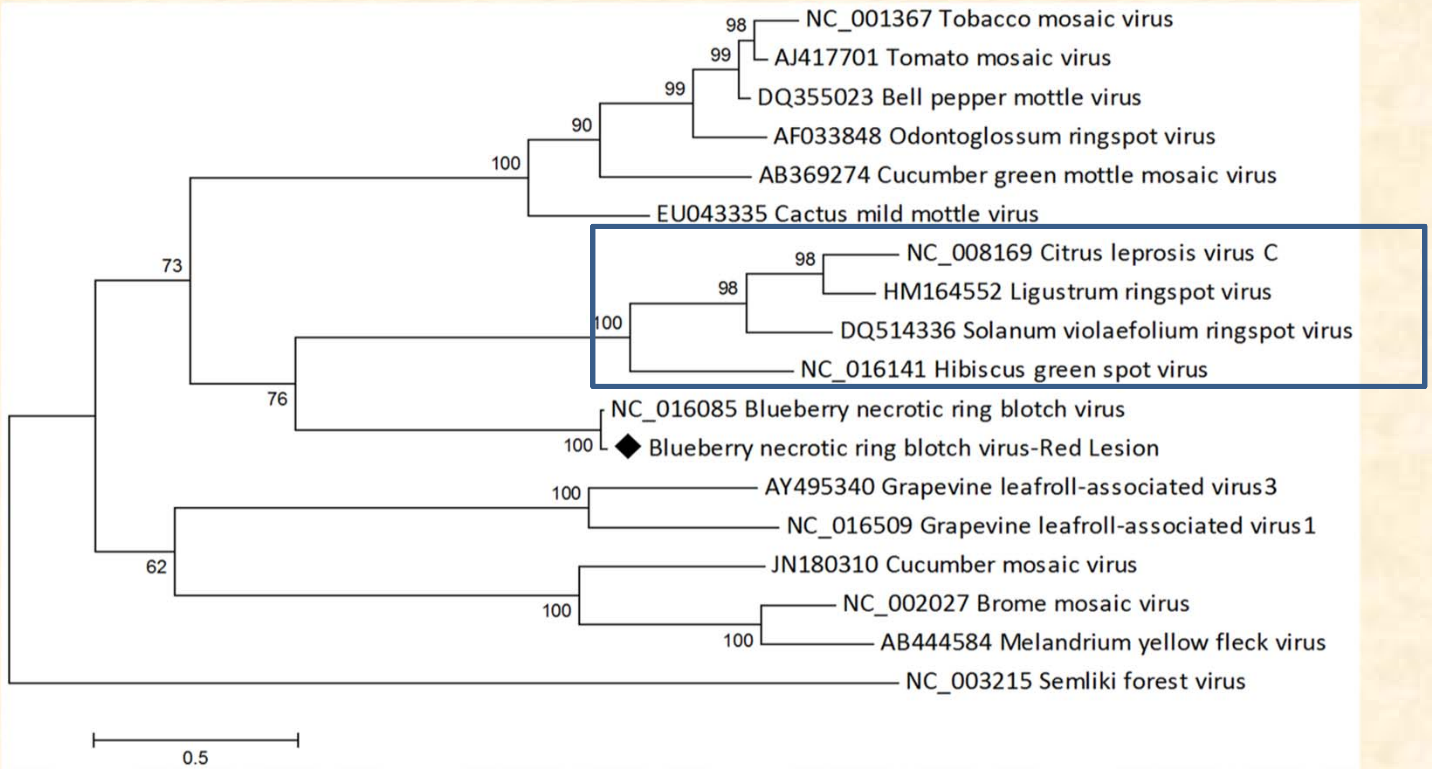
Tetranychoidea, Tenuipalpidae - false spider mites or flat mites

- 4 species of *Brevipalpus* are thought to transmit plant viruses:

- *B. californicus*, *B. lewisi*, *B. obovatus*, and ***B. phoenicis***

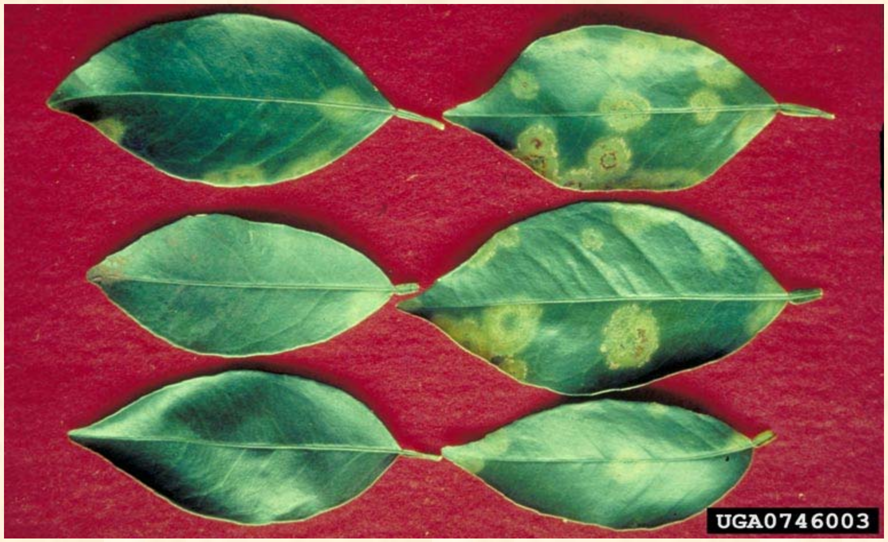
- All are important crop pests
- Have extensive host ranges
- Half as large as spider mites, 2x the size of eriophyid mites
- Approximately 40-45 day life cycle, with population doubling every 5.5 days

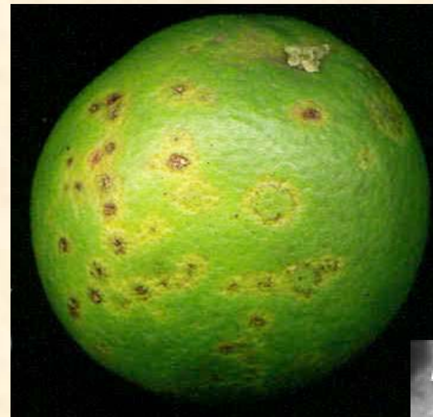
Viruses transmitted by Tenuipalpidae

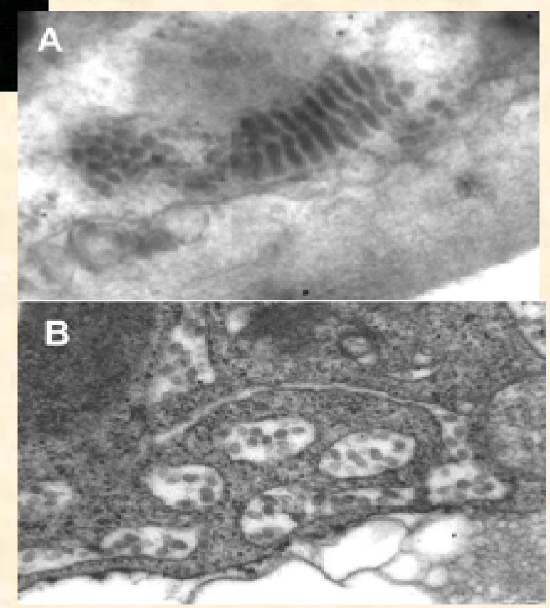


Transmitted by *Brevipalpus spp.*

Unassigned family, Genus *Cilevirus* - *Citrus leprosis virus*







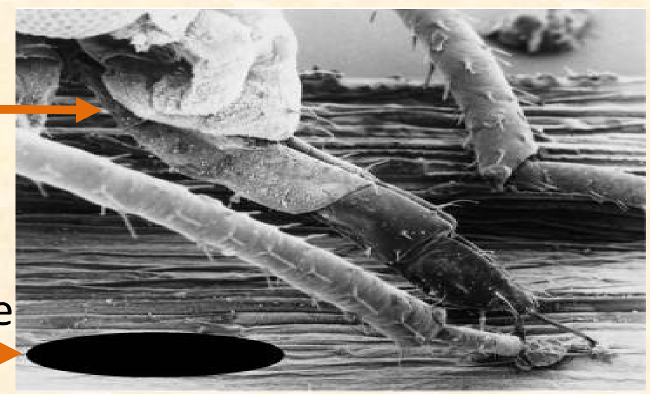
- CiLV can kill trees within 3 years if the mite populations are not controlled

- CiLV causes chlorotic and necrotic lesions, viroplasms and particles can be seen in citrus cells, but the virus does not move systemically

Eriophyoidea, Eriophyidae – Eriophiid mites

- 4,000 species of eriophyid mites are known but actual diversity is projected to be much greater.
- Only ~15-20% of eriophyoid mite species in temperate regions and ~5% from the tropics and subtropics have been described

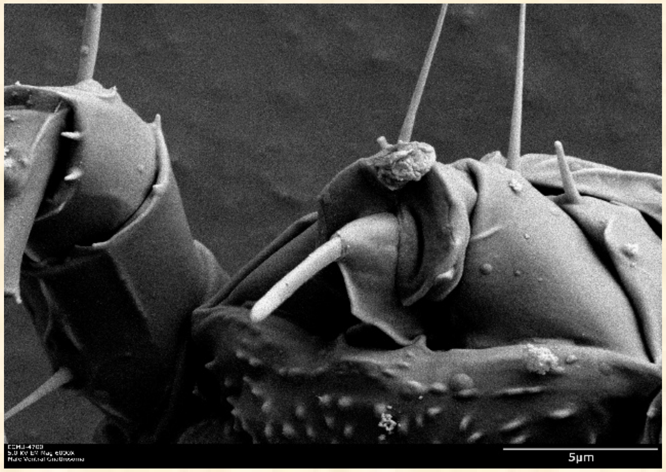
- 80-500 µm in length, 2 pairs of legs, reside in refuges or specialized areas of the plant host (buds, galls), often have highly specialized host associations, some are vagrants



Aphid head

Size of Eriophyid mite

Eriophyoidea, Eriophyidae – Eriophyid mites



SEM of wheat curl mite (*Aceria tosichella*) ventral view of adult showing oral stylet and stylet sheath protruding through the palps, as if feeding on a leaf.

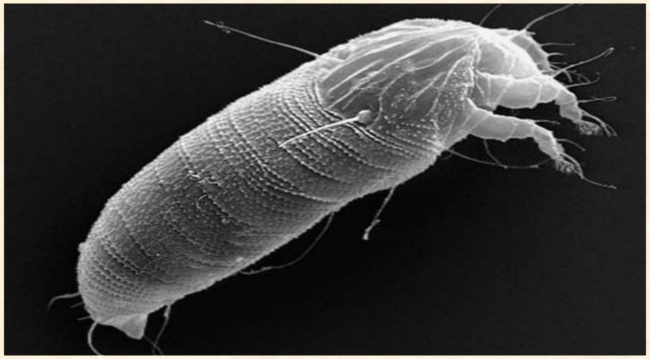
The short stylets of most species limit penetration of the plant host to a depth of 2-8 mm, so only the upper cell layer of the plant epidermis may be accessed – this limits virus acquisition and inoculation to epidermal cells

Plant Viruses Transmitted by Eriophyid Mites:

+ssRNA, -ssRNA genomes

Virus Families: 4+
Virus Genera: 7

Eriophyoidea, Eriophyidae – Eriophiid mites

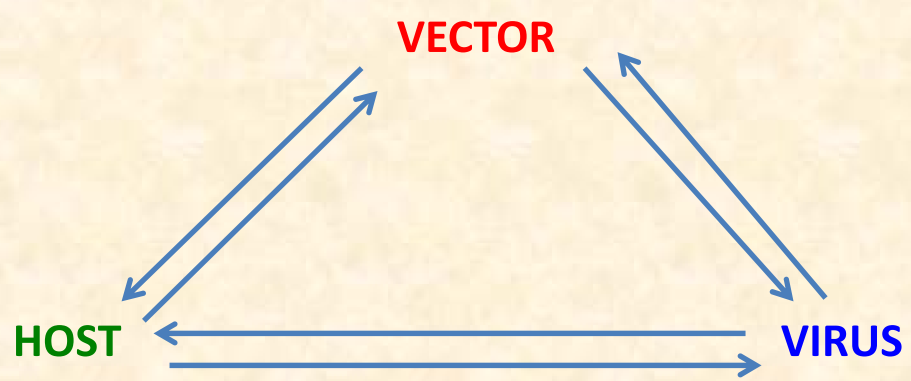


- HC-PRO is required for transmission of *Wheat streak mosaic virus* (*Tritimovirus*, *Potyviridae*) by *Aceria tosichella* (Wheat curl mite)

- Aside from these few experiments, molecular determinants of virus acquisition, retention, and inoculation are completely unknown

Transmission of plant viruses is a long evolved tritrophic interaction

Specific Examples



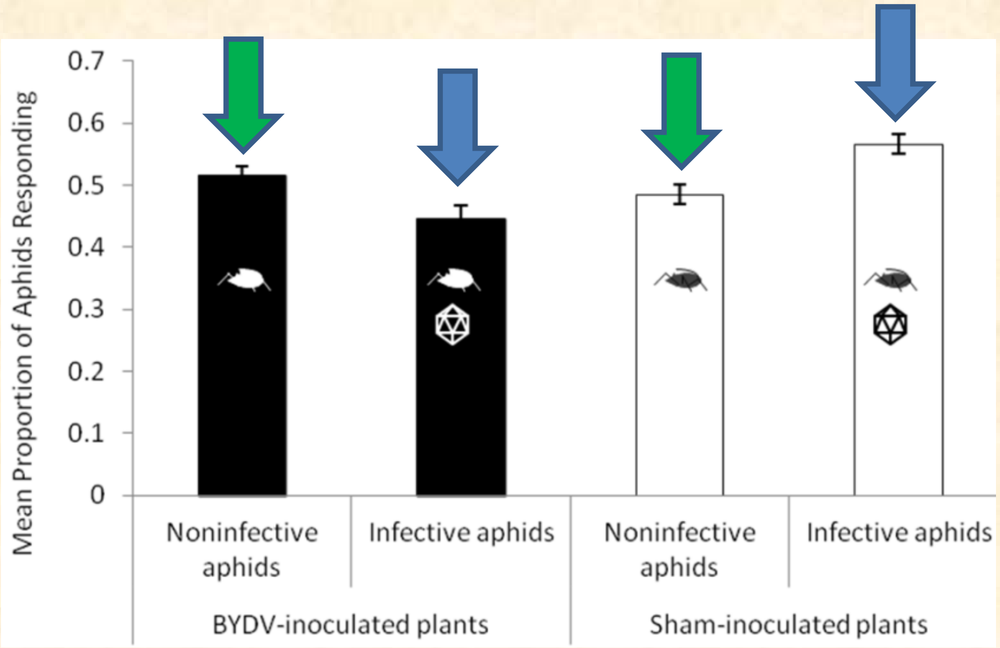
- Vectors can benefit from transmitting plant viruses
- Plant viruses can alter insect behavior to enhance their spread
- Viruses may promote their own transmission by manipulating plant physiology to attract vectors and increase vector reproduction.

The Mexican bean beetle is a vector of BPMV and beetle larvae that feed on virus-infected leaves weigh more than those that feed on healthy leaves

Adults prefer to feed on BPMV-infected plants

Specific Example:
Alterations to vector feeding preferences

- Aphids (*Rhopalosiphum padi*) virulferous with *Barley yellow dwarf virus* (BYDV) (*Luteovirus*) have a slight preference for healthy wheat plants over BYDV-infected plants.
- Non-viruliferous aphids prefer BYDV-infected plants.



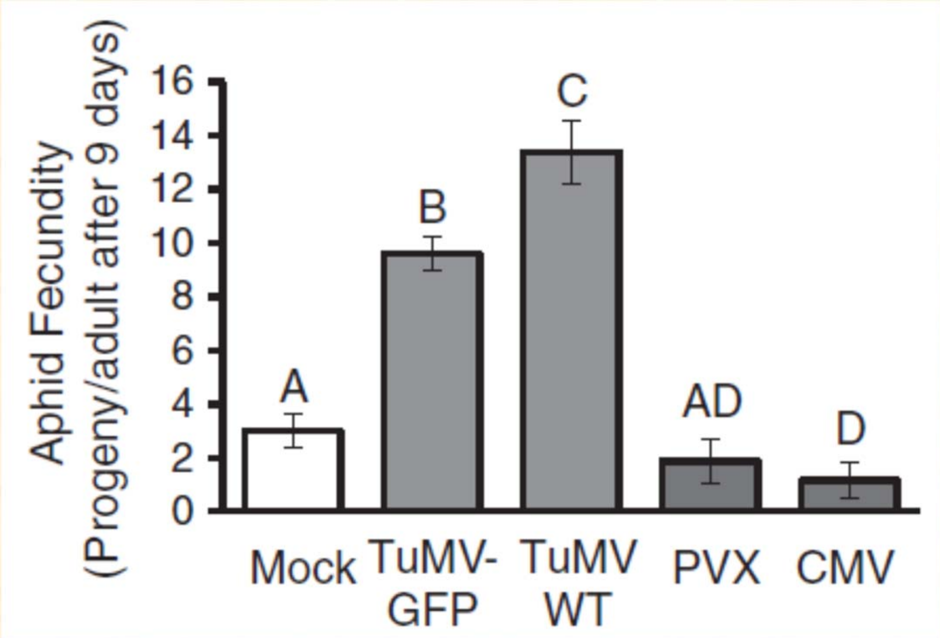
This behavioral would be expected to promote virus spread

Ingwell et al. 2012. Plant viruses alter insect behavior to enhance their spread SCIENTIFIC REPORTS 2 : 578 | DOI: 10.1038/srep00578

Specific Example:
Alterations to vector reproduction

- *Myzus persicae* (green peach aphids) prefer to settle on *Nicotiana benthamiana* infected with Turnip mosaic virus (TuMV) than on uninfected plants

- Fecundity on TuMV-infected plants was higher than on uninfected controls, and higher than on PVX- and CMV-infected plants.

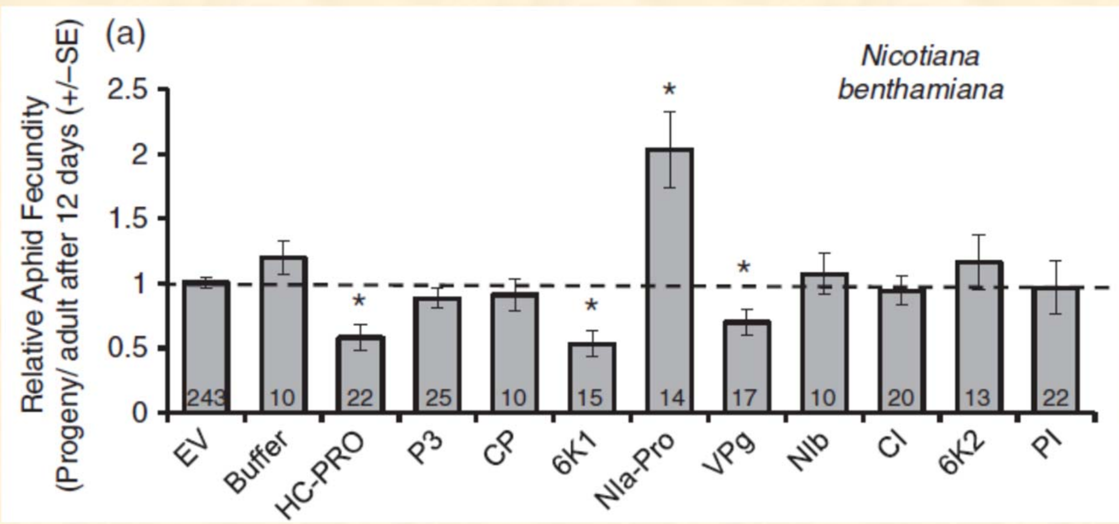


Reproduction of aphids on healthy and virus-infected plants

Casteel et al 2014 *The Plant Journal* doi: 10.1111/tpj.12417

TuMV Gene Responsible

- TuMV infection suppresses callose deposition, an important plant defense, and increases the amount of free amino acids, the major source of nitrogen for aphids.



Aphid fecundity on transformed plants expressing TuMV proteins

NIa-Pro expression alone increased aphid arrestment, suppressed callose deposition and increased the abundance of free amino acids. TuMV NIa-Pro protein manipulates the physiology of host plants and thereby attracts aphid vectors and promotes their reproduction.

Casteel et al 2014 *The Plant Journal* doi: 10.1111/tpj.12417

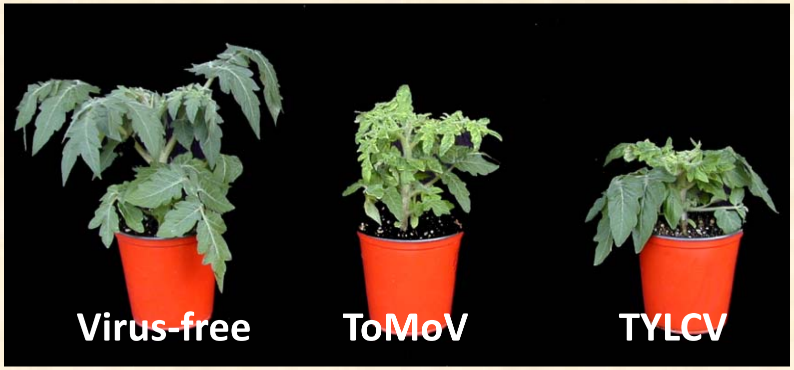
Begomoviruses and Whiteflies (*B. tabaci* species complex)

Ex. Effect of cassava mosaic disease on the whitefly vector

- Cassava mosaic disease is caused by several begomoviruses.
- A mutually beneficial interaction exists between the viruses causing cassava mosaic disease and the whitefly vector, *Bemisia tabaci*.
- Vector fecundity and the density of vectors was higher on diseased plants.
- It is postulated that this enhances disease spread by causing an increase in emigration rates of viruliferous vectors to other crops.

Begomoviruses and Whiteflies (*B. tabaci* species complex)

Ex. Effect of TYLCV on Whiteflies



- TYLCV-infected plants commonly have bright yellow symptoms which attract whiteflies.
- TYLCV interferes with the development of whiteflies from egg to adult – fertility, fecundity, production of adults were greatly reduced on plants infected with TYLCV
- Whiteflies reared on TYLCV-infected plants had the same egg-laying capacity as whiteflies reared on non-infected plants as long as they oviposited on healthy plants
- Effect of TYLCV on whiteflies appears to be indirect – a TYLCV-infected plant is a poorer reproductive host than a healthy plant.