Diseases caused by arthropod-transmitted virus and Liberibacter in southwestern desert crops

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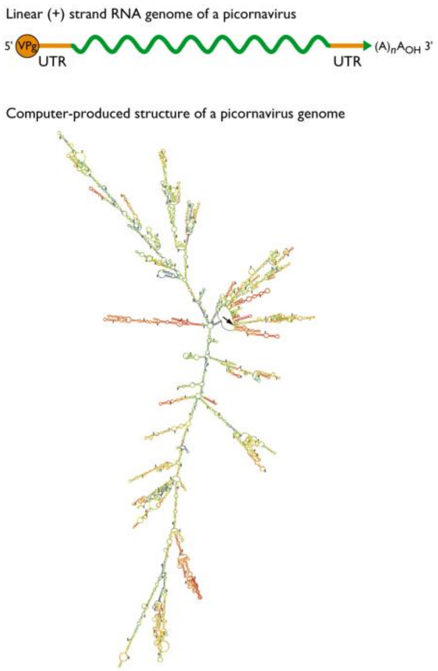
Viruses are very small parasites

- Virus = very small, non-cellular parasites of cells.

- ‘Obligate’ = require a living host.

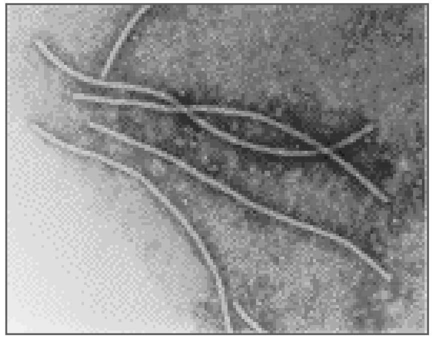
- Virus particles are produced from the assembly of pre-formed components
- Other organisms grow from an increase in the sum of their components and reproduce by division.

- Viruses do not divide, they replicate their genome and make proteins needed to replicate and make new particles.

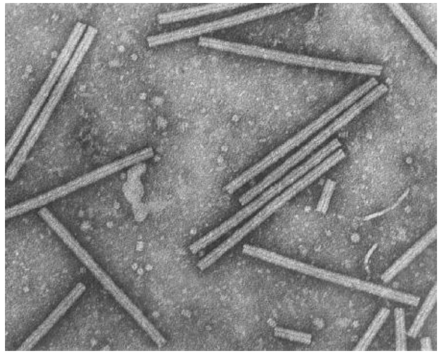


Adapted from A. C. Palmenberg and J.-Y. Sgro, *Semin. Virol.* **8**:231–241, 1997, with permission.

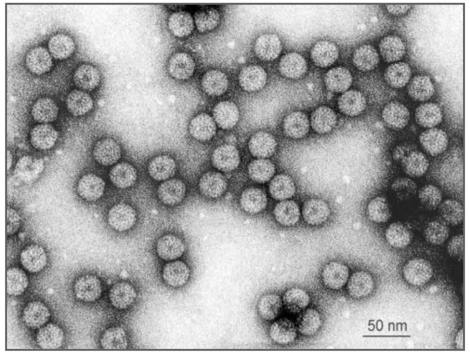
Plant viruses: Different nucleocapsid shapes and sizes



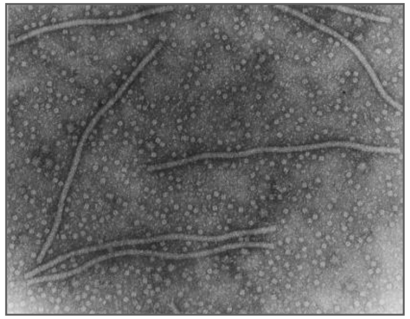
Potyviridae



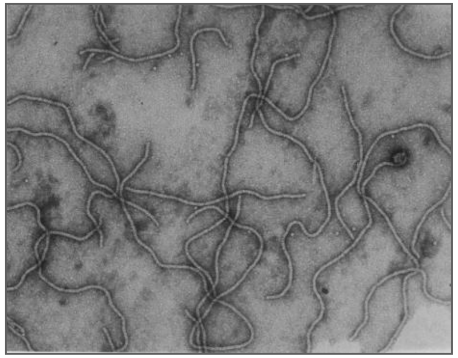
Tobamovirus



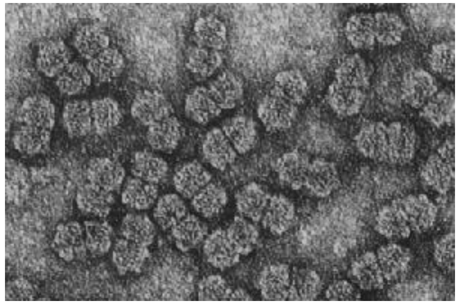
Cucumovirus



Potexvirus:*Flexiviridae*

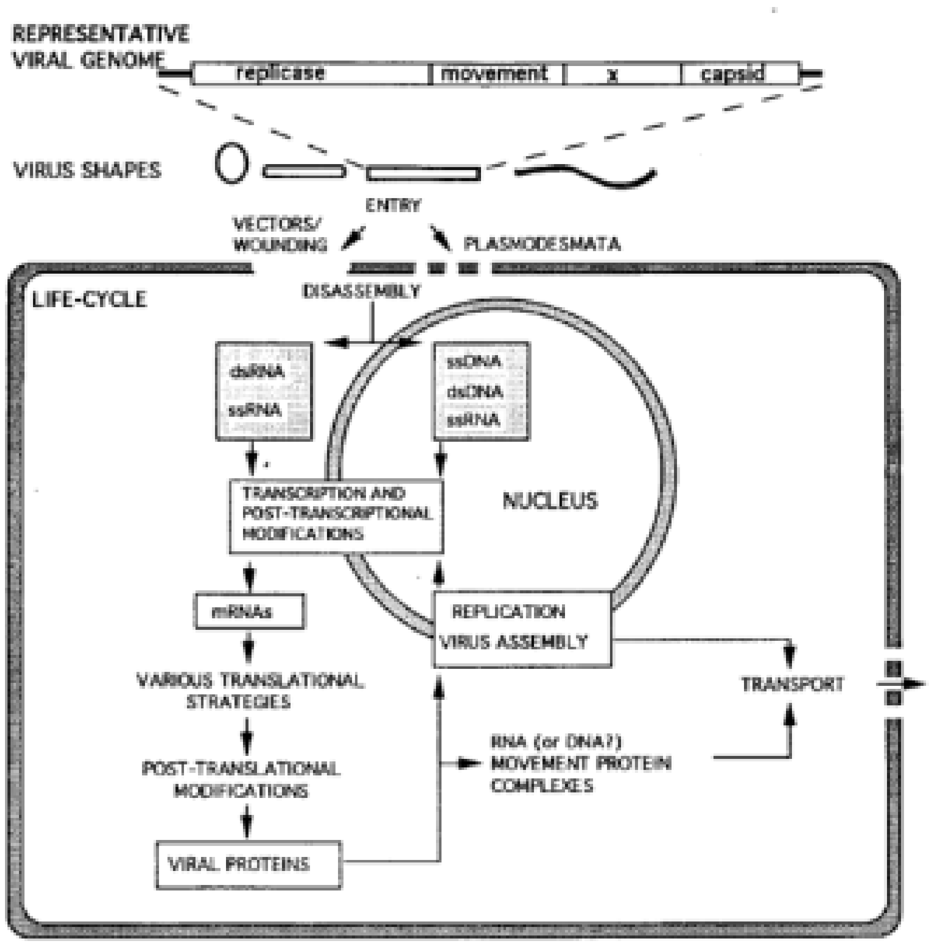


Closteroviridae



Begomovirus: *Geminiviridae*

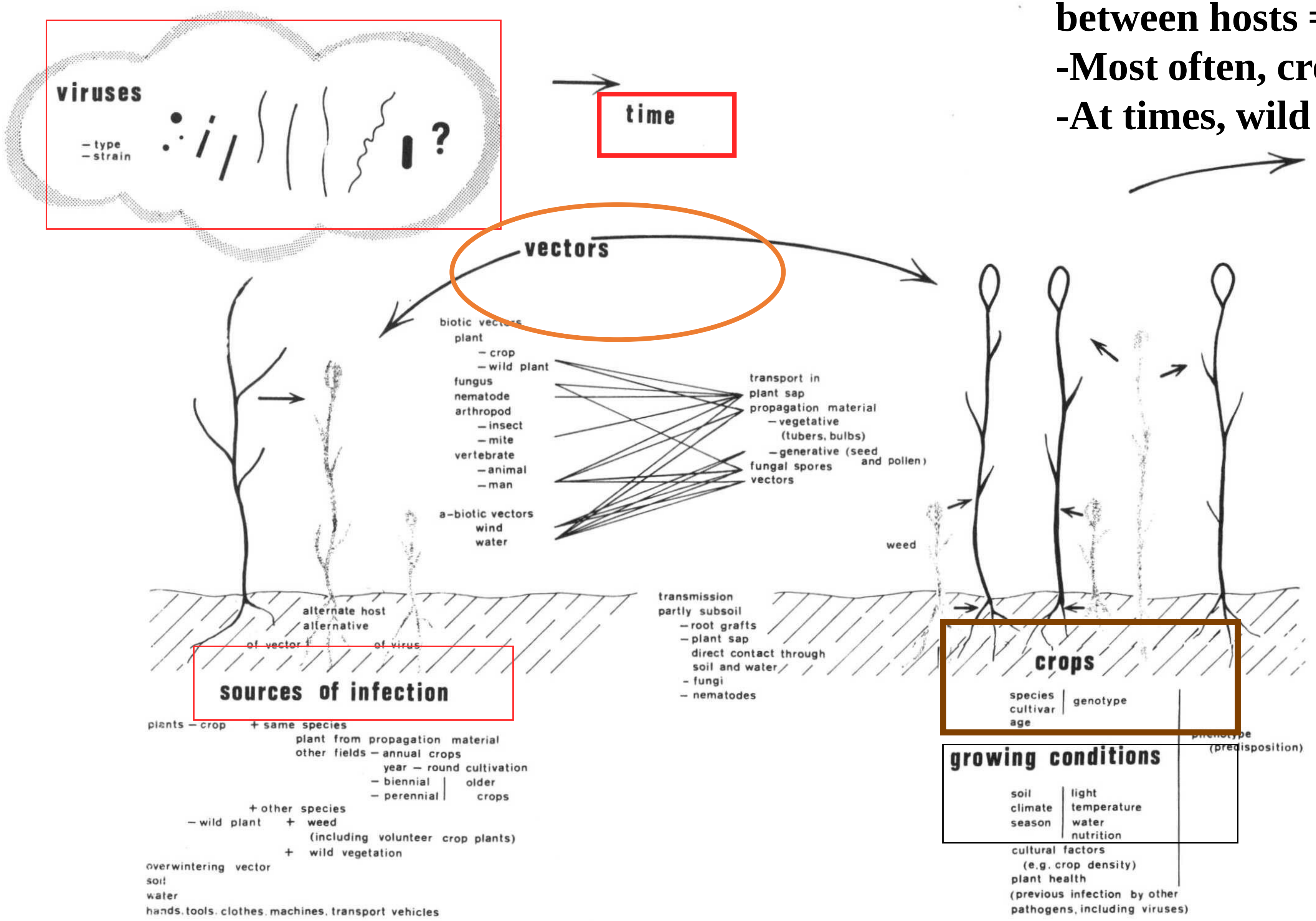
Viruses are obligate parasites = Require a host to reproduce or 'replicate'



Challenges

- **Plant virus-insect vector complexes** are the single most important causes of biotic stress in subtropical/tropical crops
- **New variants** emerging = human activities, cropping practices, climate change
- Plant virus **disease incidence on the rise**:
  - Food production (arable land) increased to feed more people
  - large-scale monoculture production
  - genetically uniform varieties; little or no resistance to insect feeding or viral pathogens
  - other pathogens that once limited production can be managed
  - climate change – distribution, seasonal abundance, other
- **Particularly important vectors** – aphids, leafhoppers, mealybugs, psyllids, thrips, whitefly-transmitted viruses
- **Seed borne viruses -** more problematic as heirloom and land races are revived, becoming more popular

Epidemiology: virus dispersal between hosts = dynamic
-Most often, crop is source
-At times, wild hosts are source



Six legged transportation

- Order Hemiptera

True bugs - cucurbit yellow vine agent, a bacterium
Leafhoppers
Planthoppers
Aphids
Psyllids
Whiteflies

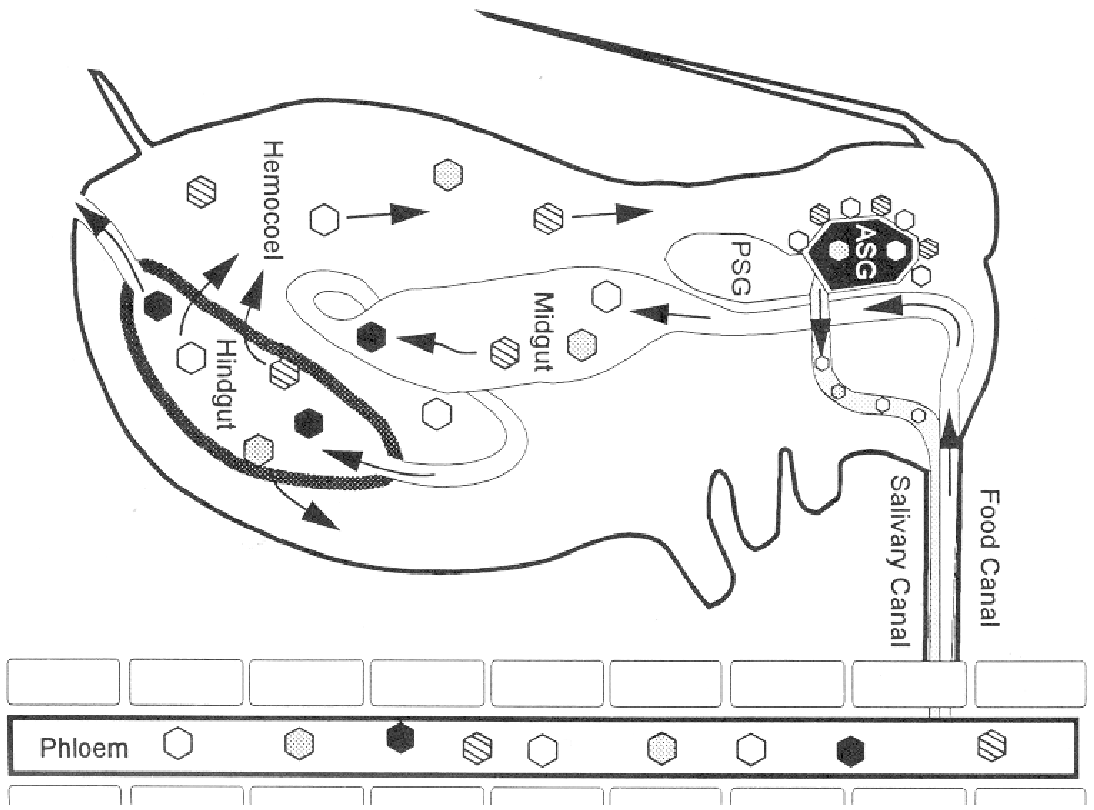
- Thysanoptera

Thrips

- **What do they have in common** = flight for dispersal, phloem or xylem feeding; co-evolved with plant pathogens they transmit

Plant viruses and fastidious bacterial pathogens are retained inside the vector body

In circulative transmission, they circulate from the gut into the blood, and then to the salivary glands from where they are transmitted



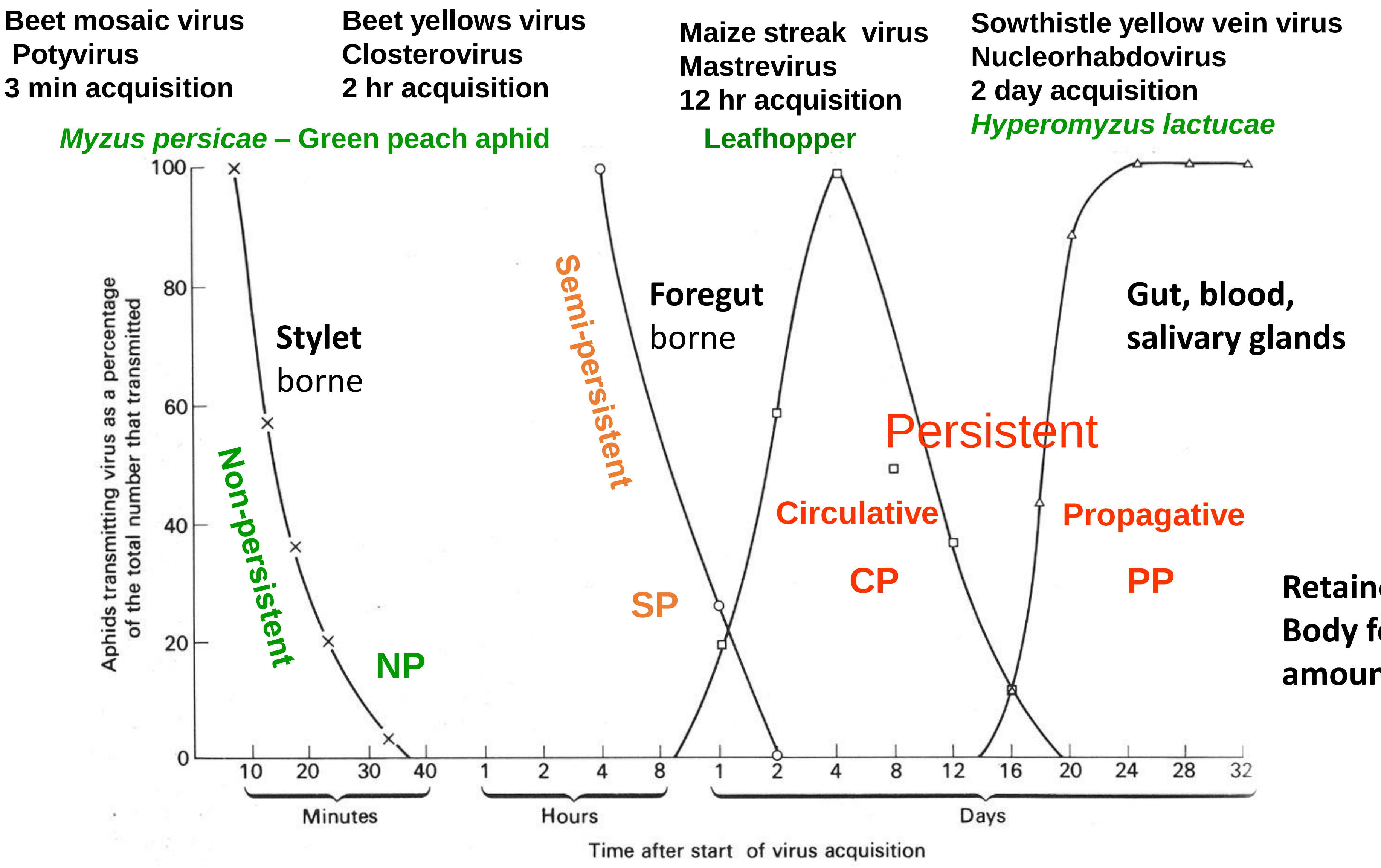
Stylets

Foregut

Gut-blood-salivary glands

Specific relationships, not just syringes on wings!

Types of Virus-Vector Relationships



Retained in vector Body for different amounts of time

Watson, 1946; Costa, et al., 1959; Duffus, 1963

Second most important: transmission – through seed

- About 20% of the known plant viruses are transmitted through seed (of at least one host species)
- Many species can be infected through seeds
- **Legumes,** Composites (lettuce), Cucurbitaceae

- Importance
  - 1% transmission can give multiple foci of infection – means 10^5 in 10^7 plants per hectare

- Globalization of seed industry increases risk of dispersal of viruses, even at a low rate of seed transmission.

Cotton -- Whitefly-transmitted geminiviruses

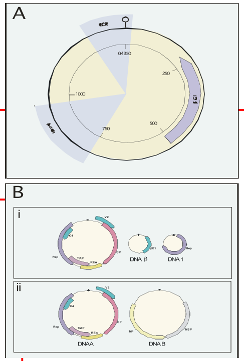





<u>New World</u>: low diversity

Cotton leaf crumple virus: AZ, CA, TX, Mexico, Guatemala, Caribbean, Brazil (93-100%)

(Idris and Brown, in prep)



<u>Asia:</u> <u>extreme diversity</u>

Cotton leaf curl virus

complex (8+ species?)

Often-same beta, different alphasats



<u>Africa, Arabian Peninsula</u>

Cotton leaf curl Gezira virus: moderate diversity

Sudan cotton, Sida (Idris & Brown)

Burkina Faso okra (Tiendrébéogo, 2010)

Arabian Peninsula: CLCuGV-okra

TomatoL TYLCV-OM recomb (Asia-Oman) with Gezira alphasatellite (CLCuGA)

Diverse betasats 88-98%, alphasats 89%



?

Cotton leaf curl disease pandemic timeline – Pakistan/India = poses 'high risk' to US

1967 **First report CLCuD Pakistan**

Sporadic isolated infections of cotton

1988 **First CLCuV epidemic**

Introduction of high yielding cotton variety (S12)
Jassid control,
but whitefly susceptible

1992-1997

$5 billion losses

Introduction of resistant varieties 1998-99
Widespread cultivation
Narrow germplasm base

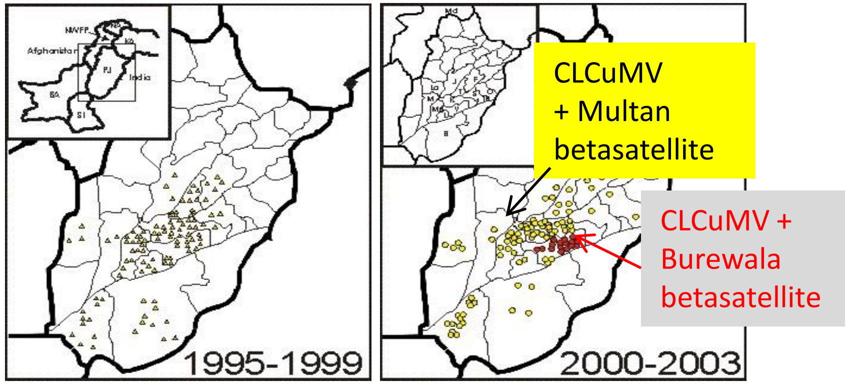
1997-2001 **Second Epidemic:**
recombinant virus + $CLCuMB^{Bur}$

'Resistance Breaking 2001'

(Amrao et al., 2010)

2004-2009
Spread to all cotton Growing areas of Pakistan; then India

2013



Multan betasat + CLCuMV

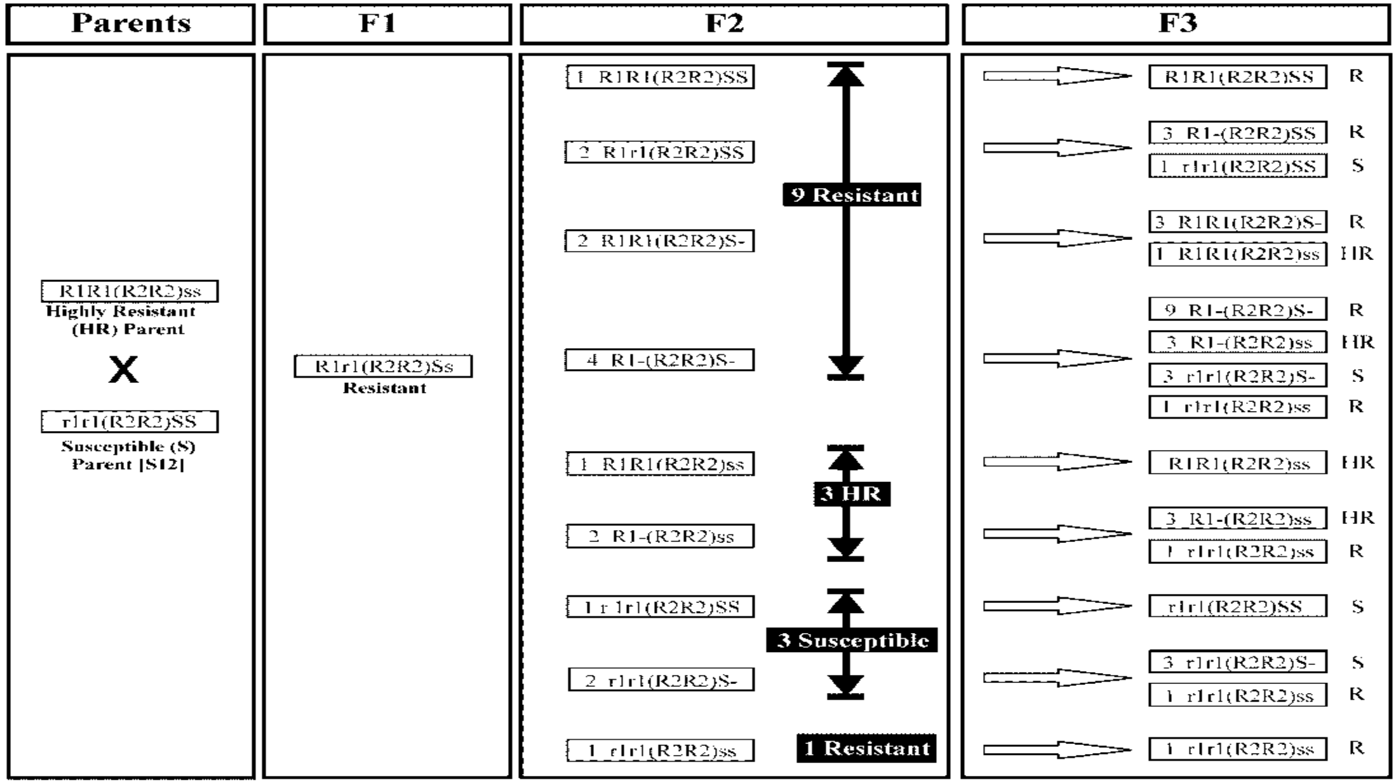
Initial 'red' zone of outbreak of resistance-breaking beta

(modified; Courtesy R. Briddon)

Resistance-breaking Burewala strain of CLCuMV

Host-plant resistance to CLCuMV + beta satellite in *Gossypium hirsutum*

Resistance



Authors propose three interacting genes involved:

two R genes and a "suppressor" gene

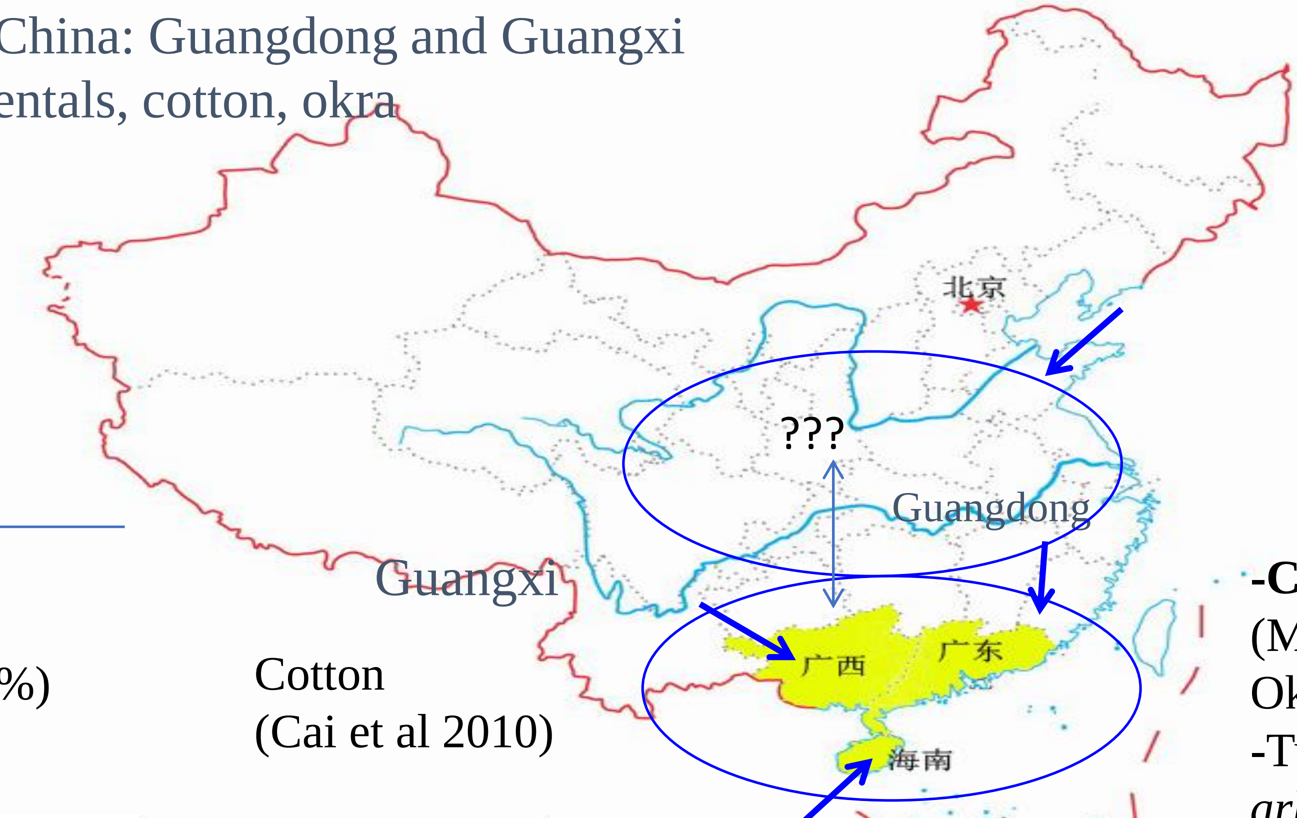
Rahman, M., Hussain, D., Malik, T. A., and Zafar, Y. (2005). Genetics of resistance against cotton leaf curl disease in *Gossypium hirsutum*. *Plant Pathol.* 54, 764-772.

Geographical expansion of CLCuMV 2008-2013

First introduction into China

South China: Guangdong and Guangxi
ornamentals, cotton, okra

okra



Cotton
(Cai et al 2010)

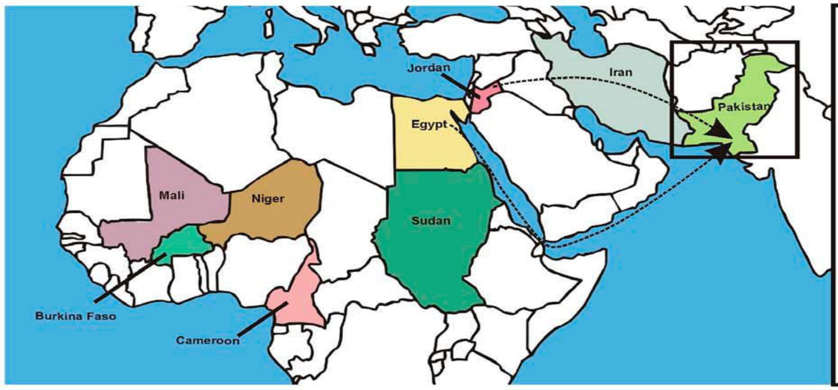
Hibiscus

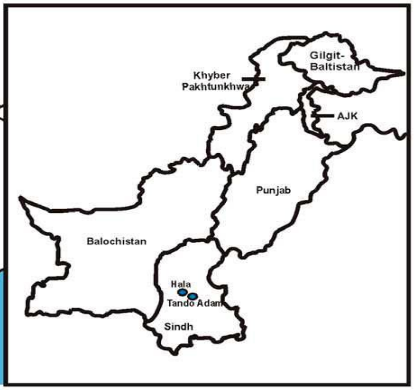
-China -*H. rosa-sinensis* (Mao et al 2008);
Okra, cotton (Xie et al 2010)
-Turks cap *Malvaviscus arboreus* (Tang et al 2013) [def beta]

-Jordan, Oman (Khan et al. 2012)
Arabian Peninsula (Idris et al 2013) diversity high = endemic or previous: okra, tomato (unique alphasats).

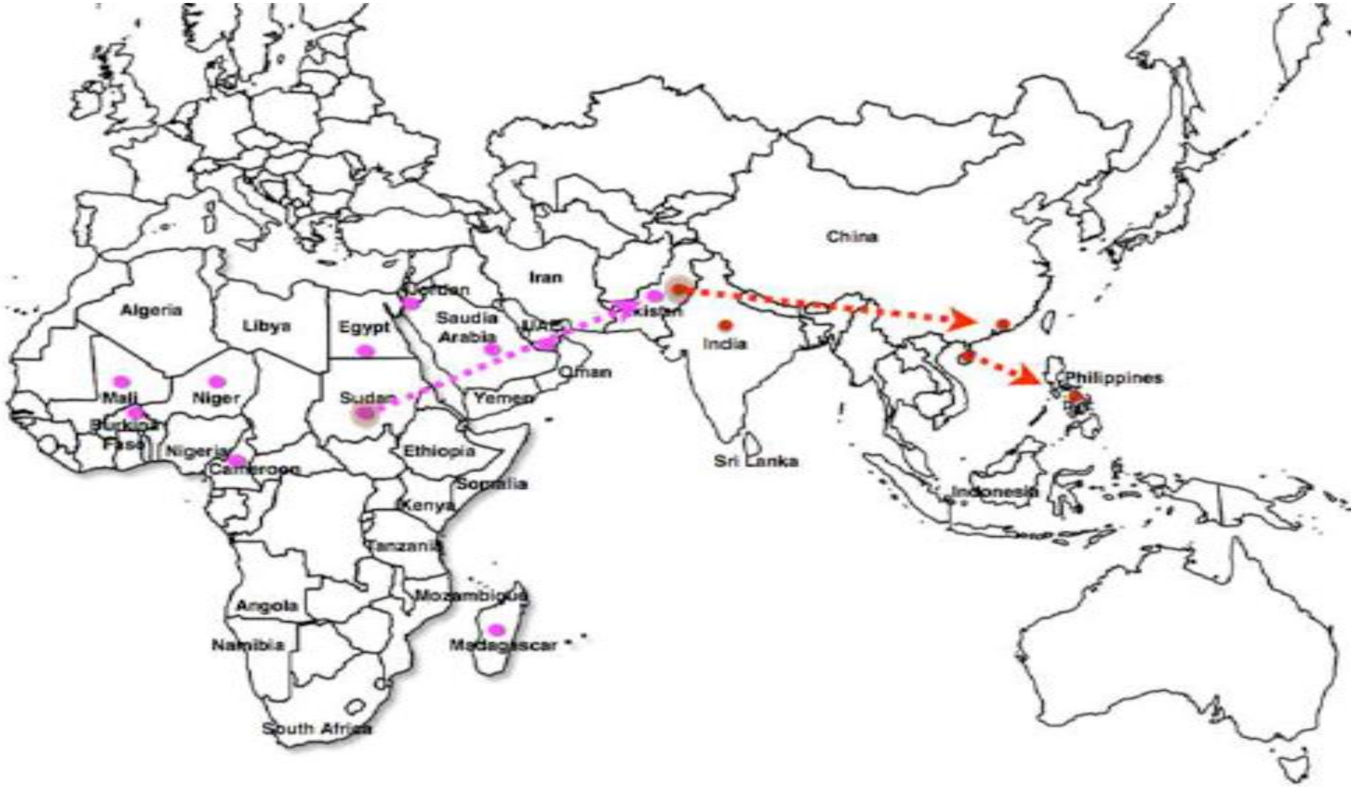
Another recent introduction ...

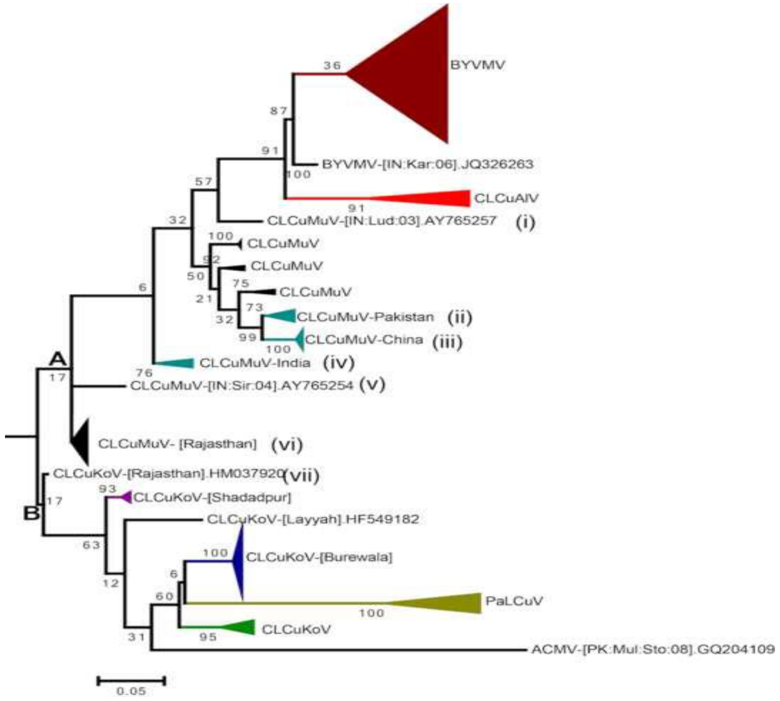
Cotton leaf curl Gezira virus (99%)
in Pakistan (Tahir et al 2011)





Cotton leaf curl virus complex 2015
Next, introduced into Philippines *via* Hibiscus /ornamentals





Poses high risk to US and other cotton growing areas
= keep a look out for unusual symptoms

Common viruses of desert forage and vegetable crops

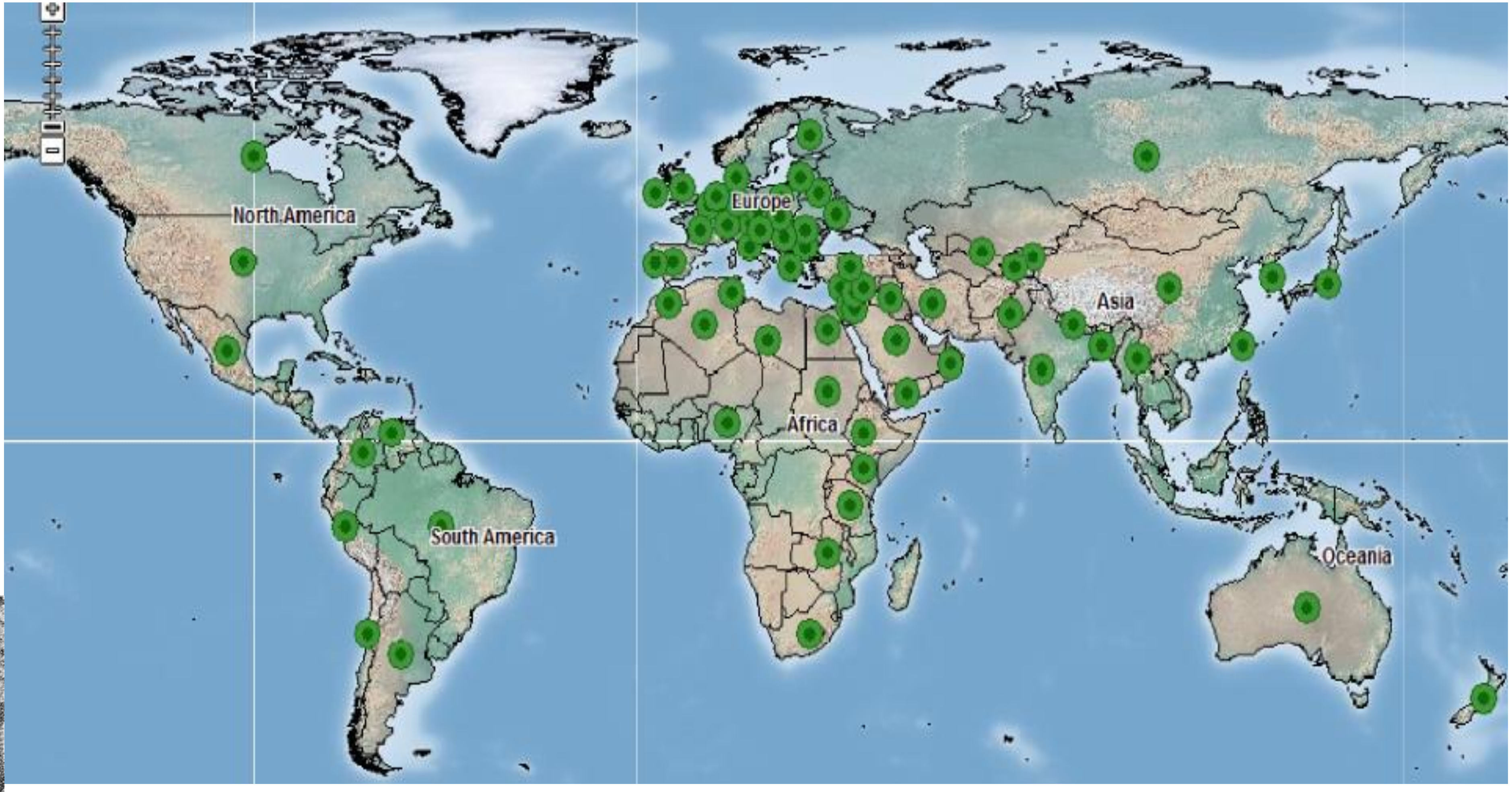
Legumes: alfalfa, common bean, garbanzo bean tepary bean

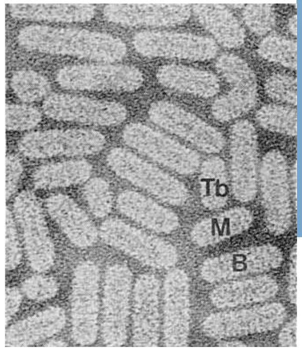
Most common viruses that infect legumes:
Alfalfa mosaic virus
Bean yellow mosaic, **Bean common mosaic**, Bean yellow mosaic (potyviruses)
Bean leaf roll / Beet western yellows (puteoviruses)
Cucumber mosaic virus

Many annual and perennial legumes, and wide range of non-legume-crop plants,
and weeds are susceptible to these viruses = of epidemiological importance.

Most important currently in our deserts:
Alfalfa mosaic virus
many strains
infects more than 220 species of plants in 73 genera
extreme example among plant viruses

AMV - Distributed worldwide where alfalfa and clovers are grown





Symptoms

- Vary with host species

- Foliar: Bright yellow mosaic or mottle
- Localized necrosis on leaves, flowers, fruit
- Phloem necrosis, stems and roots

- Can cause severe stunting or plant death

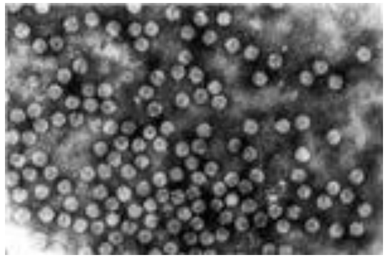
- Seed number and size are reduced

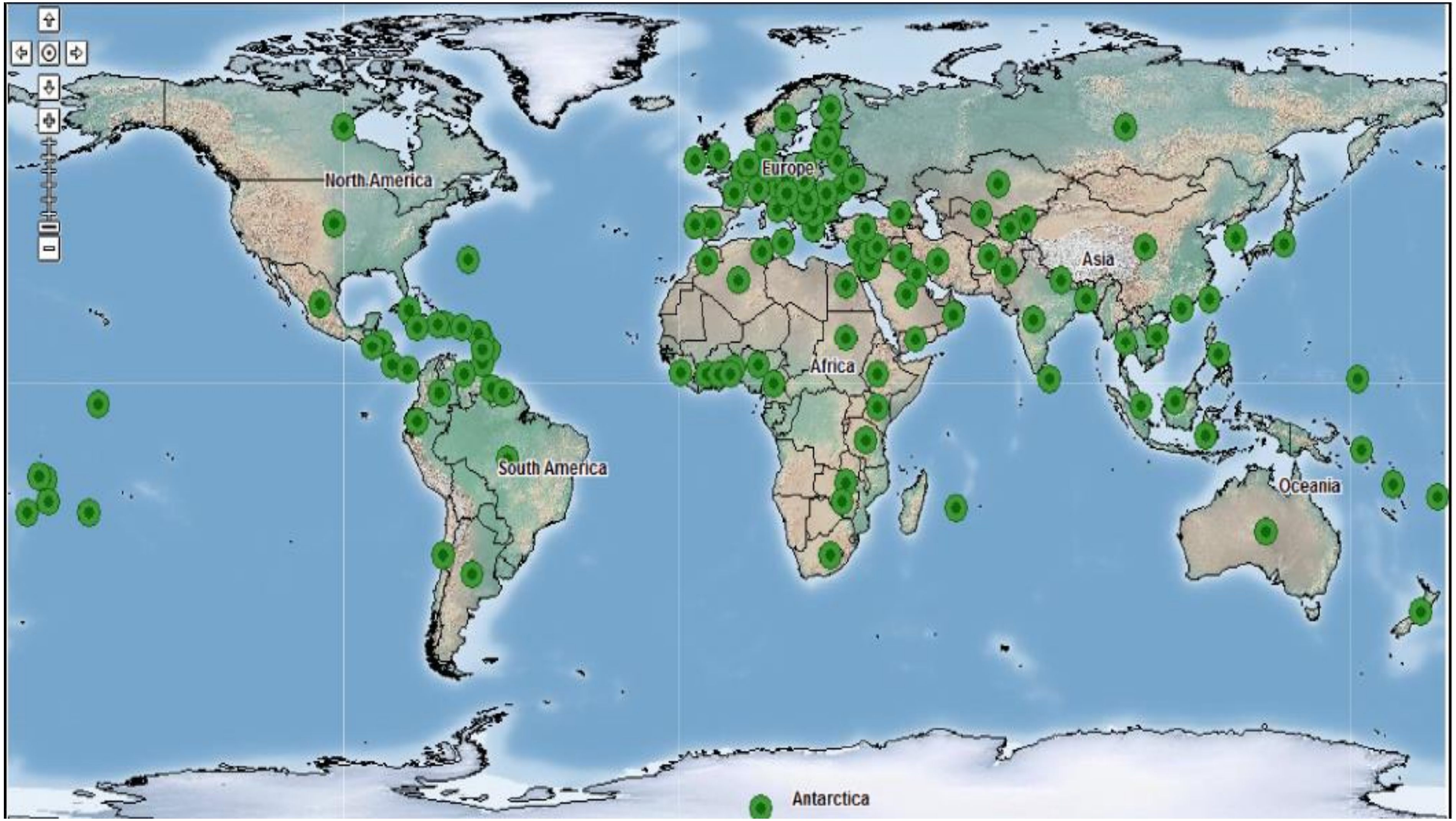
AMV host range and transmission

- Wide **host range** over 700 plant species in 170 genera,

  70 families (mostly *Fabaceae*)
- **Natural hosts**: alfalfa, lucerne, red/white clover, common bean and faba bean
- **Transmission**
  - **Sap / mechanical** inoculation – field machinery/mowing
  - **Aphids**: non-persistent (few min to few hrs) by *Aphis craccivora*, *A. pisum*, *A. fabae*, *M. persicae*
  - **Seed**: 1-10% transmission
  - Can persist for up to 5 years in a seed lot

Cucumber mosaic virus - distributed worldwide





CMV Symptoms

- Symptoms differ with virus strain and host
- Common bean
  - Mosaic and/or mottle
  - Necrosis
  - Chlorosis
  - Leaf deformation
- Yield loss 5 – 75% / varies with
  - Age of infection
  - Virus strain
  - Environmental conditions
- Satellite RNA may intensify symptom expression

CMV Host Range and Transmission

- Wide host range, over 1000 species in over 100 families
  - dicots and monocots: bean, cucurbits: cucumber & melons, pepper, tomato
- Aphids transmission
  - Non-persistent manner
  - Over 80 spp., important *Myzus persicae* and *Aphis gossypii*
- Seed transmitted in some hosts, >10% in *common bean*
- Dodder
- Mechanical

Non-persistently transmitted

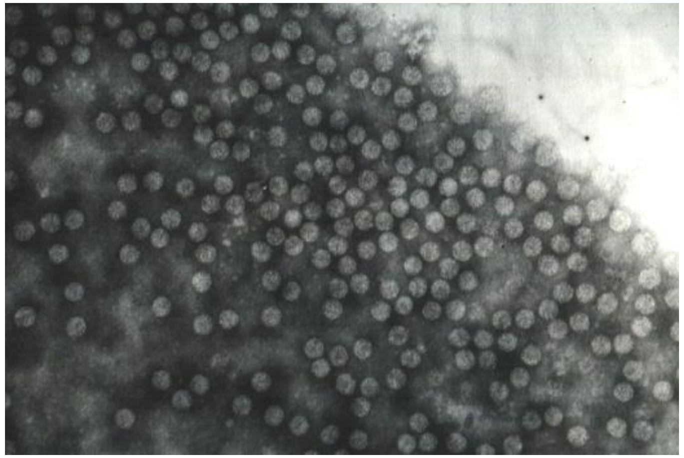
Cucumber mosaic virus

Vector: Aphids

Legumes and cucurbits

- Can be seedborne
- Non-persistent
- Transmission in a few minutes

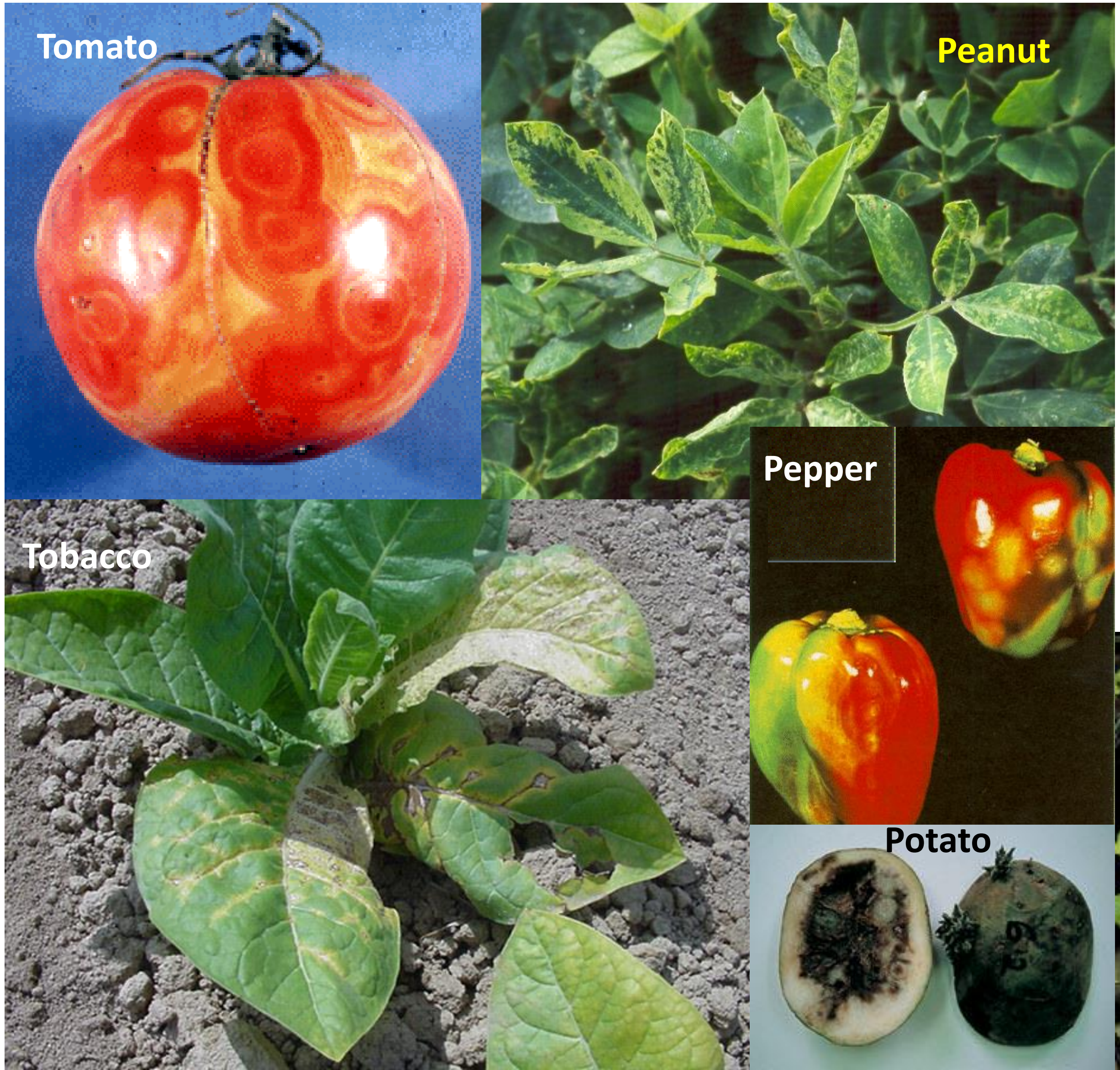
cukes



Electron micrograph - CMV

melon

Tospoviruses



- Serious threats to vegetables, ornamentals

- ~1000 species of plants in •70 plant families (dicots & monocots)

- Estimated global yield losses of up to $1 billion

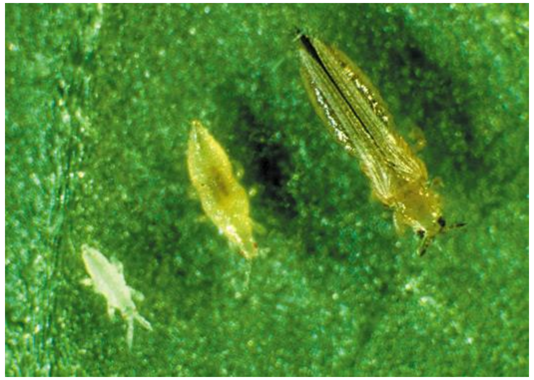
Thrips are the vectors of tospoviruses

Onion thrips

Thrips palmi
T. tabaci
T. setosus
Scirtothrips dorsalis
Ceratothrips claratris

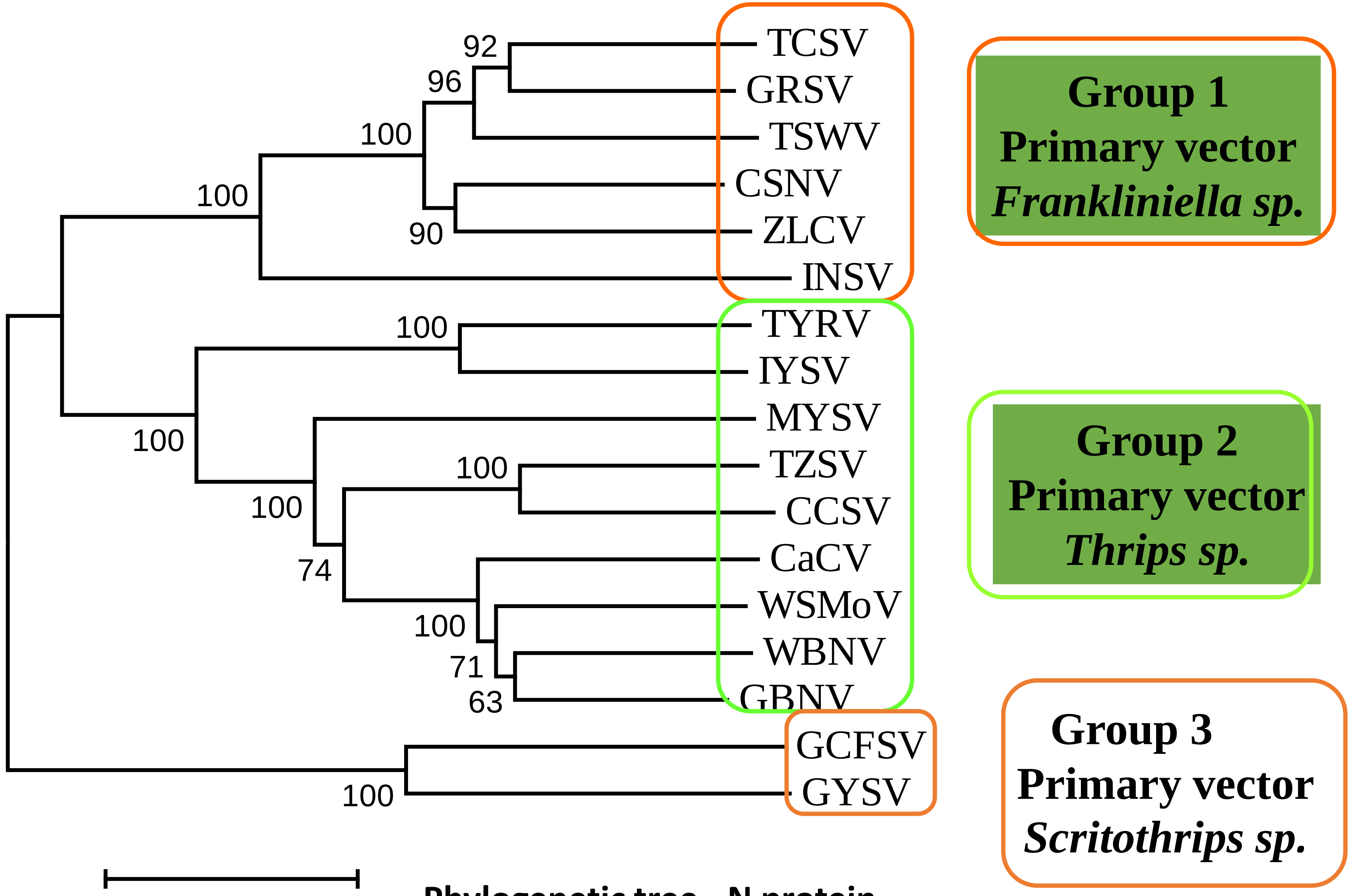
Frankliniella occidentalis
F. fusca
F. bispinosa
F. schultzei
F. intosa
F. zucchini
F. schultzei

12 species



Flower thrips

Association between tospoviruses and thrips vectors: specificity



Phylogenetic tree - N protein

Melon severe mosaic virus *-f*irst cucurbit tospovirus identified in Americas/ Mexico

Impact on the crop

- Netted and honeydew melons, squash, and watermelon develop severe symptoms
- Epidemiology is not understood – thus far, only identified in Mexico
- Nearby countries of Central America and SW USA are at risk

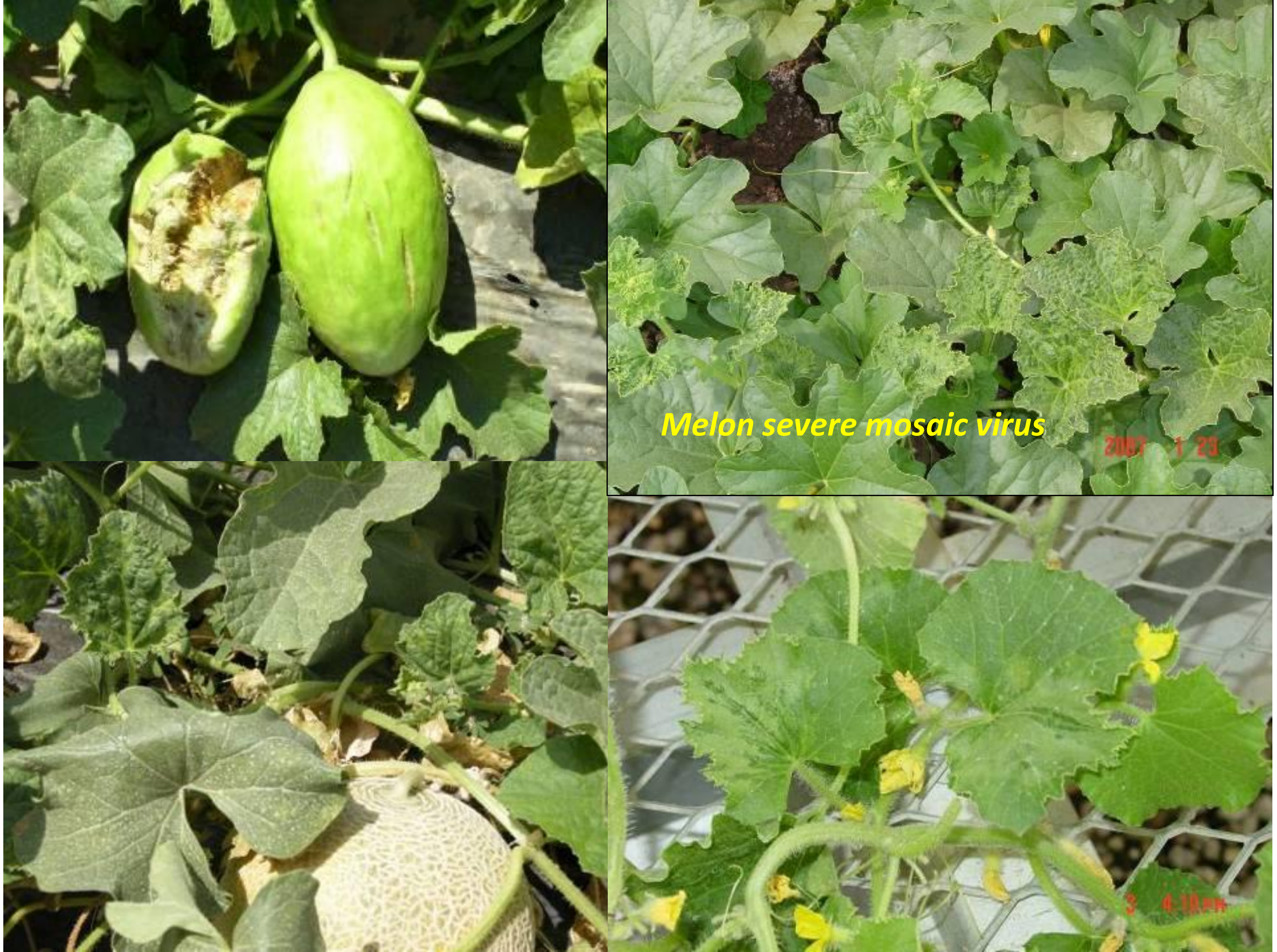
- Field test kits have been developed to detect / identify this virus

Symptoms:
Mosaic, leaf blistering , leaf formation, and fruit splitting

Control:
Remove infected plants
Control thrips populations
Host resistance will be the primary control measure – mostly unavailable

Melon severe mosaic virus

watermelon

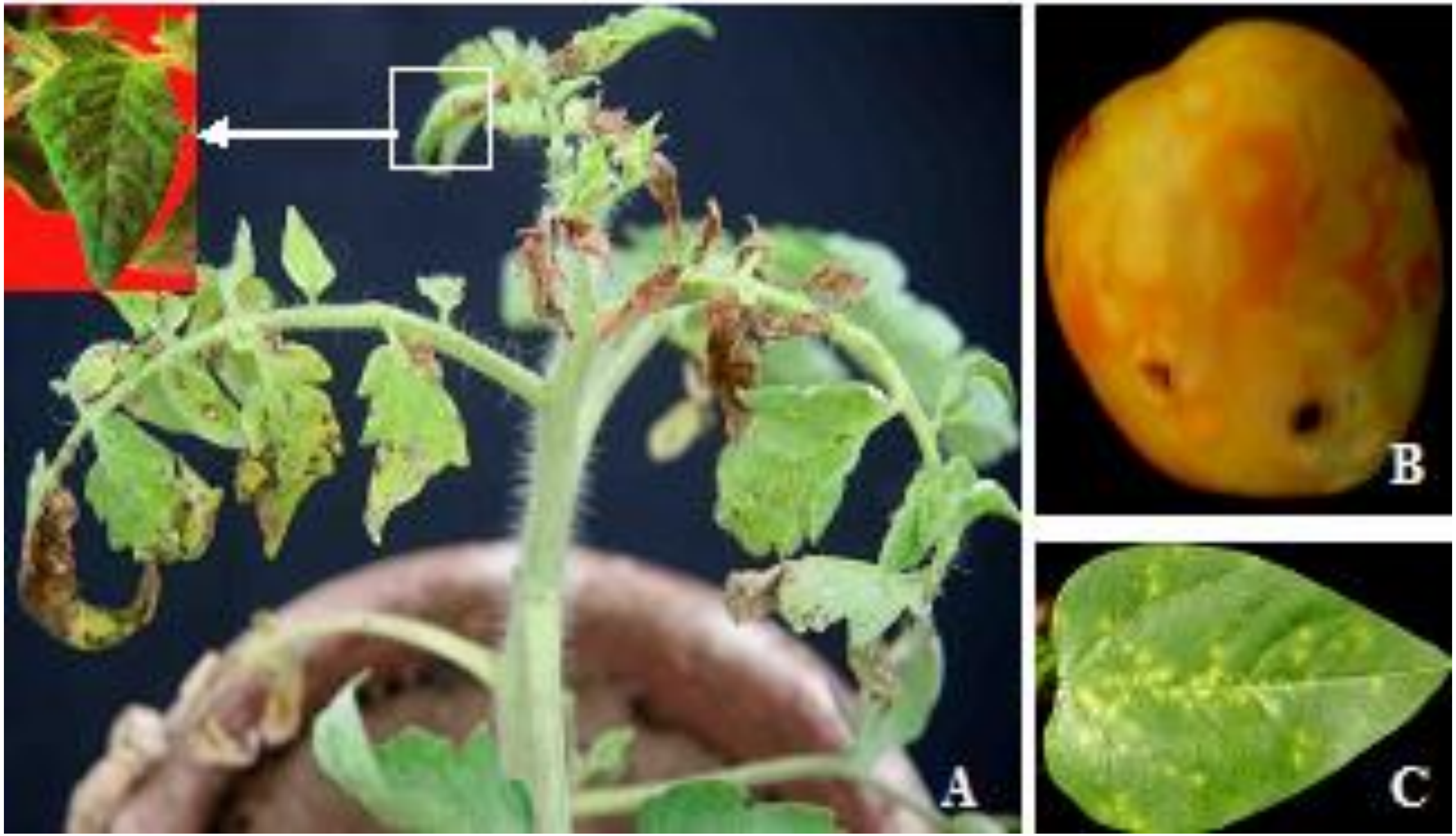


Melons

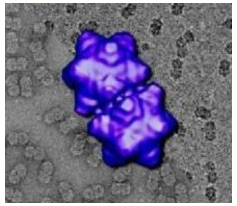
Melon severe mosaic virus

melons

New tospovirus of pepper and tomato: Capsicum chlorosis virus



Emerging virus in Southeast Asia and Australia
Could be problematic in the US if introduced



Exotic: Tomato yellow leaf curl virus

introduced some time ago, persists in US

Taxonomy: *Begomovirus* family: *Geminiviridae*

- Particle morphology: Paired icosahedral 20 x32 nm
- Genome type and size: ssDNA, circular 5.2 kb

- **Vector:** Polyphagous whitefly- *Bemisia tabaci* complex
- Symptoms: leaf curling, stunting, reduced fruit set
- **Distribution:**

  Worldwide in much of the tropics

  Western Hemisphere: U.S., Mexico, Caribbean

Symptoms / other hosts

Narrow host range:

Crops: Bean, pepper, tomato

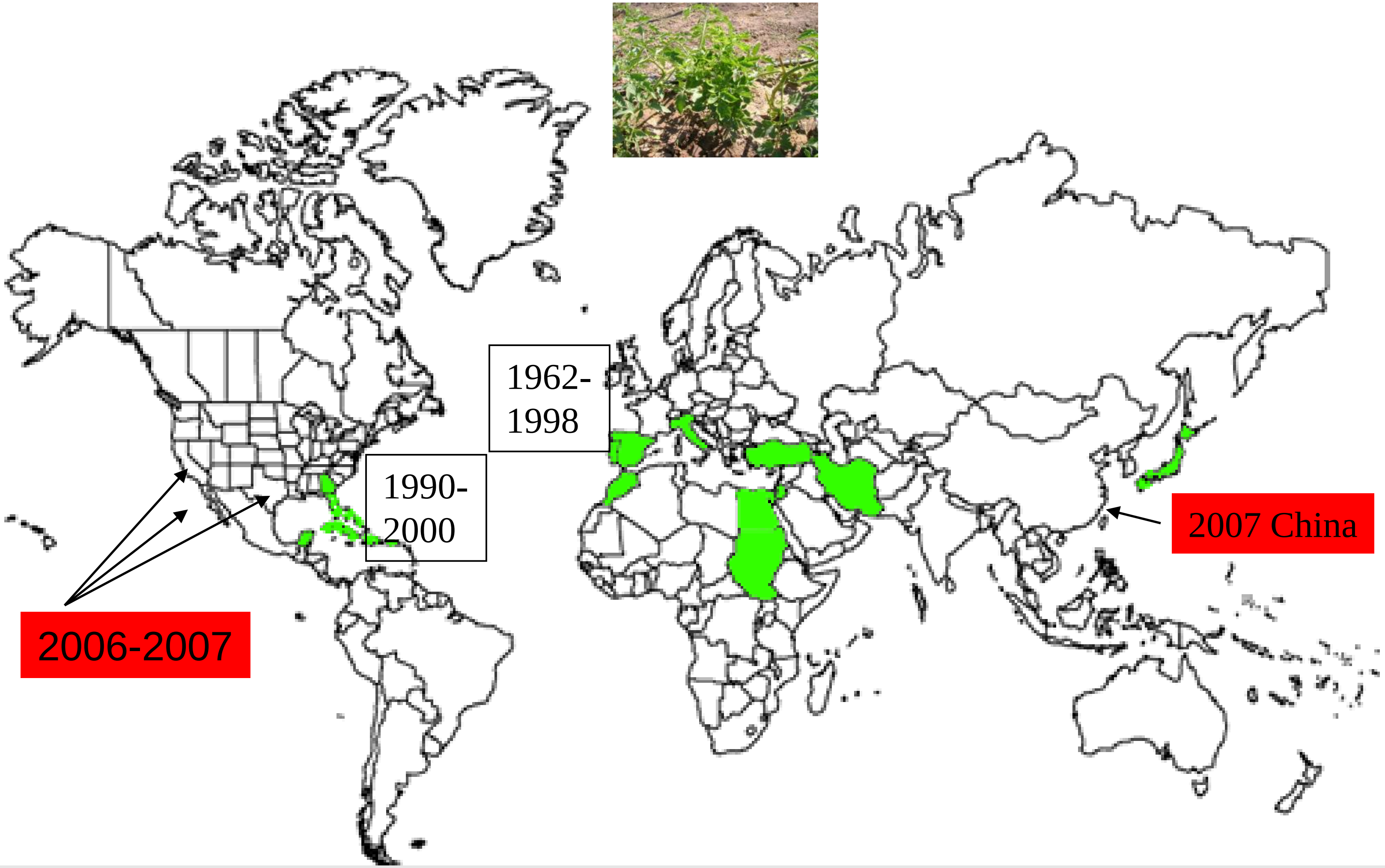
Weeds: *D.stramonium*, milkweed

Ornamentals: petunia, others

bean

pepper

Worldwide Distribution - TYLCV Is



In western US & Mexico, TYLCV was introduced on tomato transplants and ornamentals- from east to west.

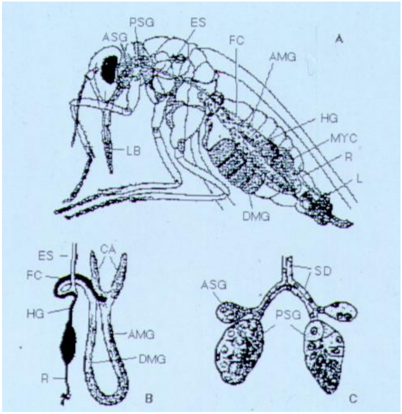
Whitefly vector transmission

- Persistent in vector: once acquired, can transmit for life

- Circulative: circulates in the vector after ingestion via mouthparts
- Particles pass into midgut and cross the gut membrane to enter hemolymph
- Enters salivary glands; from there, transmitted in saliva during feeding

- Acquisition access period = 30 min+
- Inoculation access period = 30 min+
- Latent Period = 8+hrs)

Transmission pathway



Gut barrier

Salivary glands

~ Among the 32 viruses that infect cucurbits/melons ..

Closteroviruses

Vector: whiteflies

Bemisia tabaci, Trialeurodes vaporariorum

Beet pseudo-yellows virus (US)

Cucumber vein yellowing virus (Europe)

Cucurbit yellow stunt disorder virus

(Israel, Jordan, Spain, Guatemala, Texas, US)*

Cucumber yellows virus (Japan)

Muskmelon yellows disease (France, Spain)

***Introduced to US 1990**

Melons - honeydew and cantaloupe

Studies indicate the CYSDV host range is broader than reported in Mid-East

Most recent introduction: CYSDV

1985 First recognized as a new virus disease in the Middle East

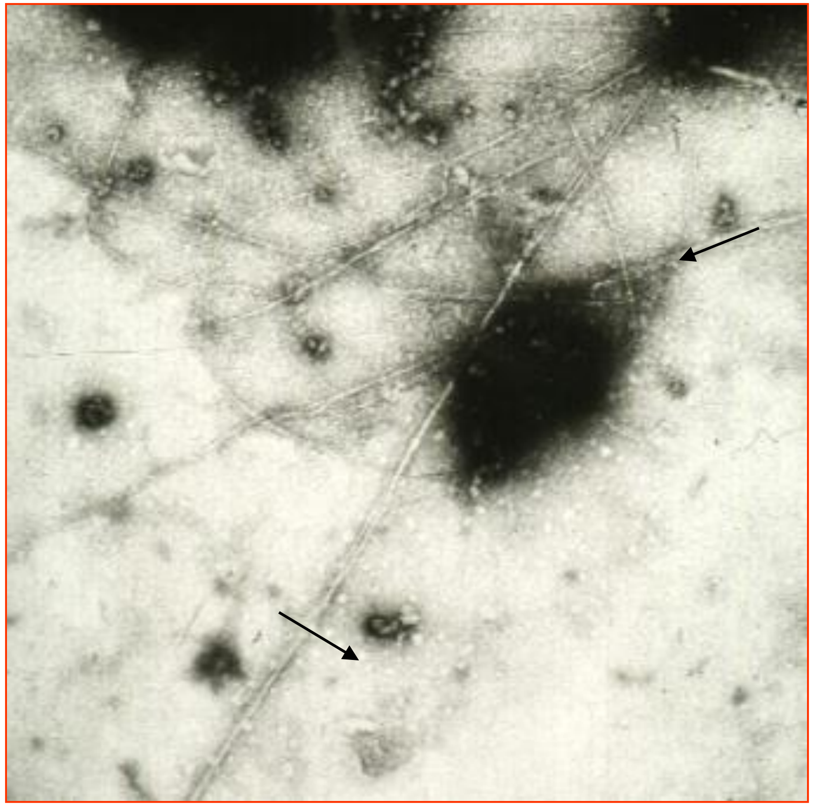
1999 Middle East: Egypt, Jordan, Israel, Saudi Arabia, Turkey, UAE

1996 Europe: Spain (south coast melons, cucumbers)

1999-02 North America: Zacapa Valley, Guatemala, Tamaulipas, Mexico; Colima, Mexico; Texas-USA

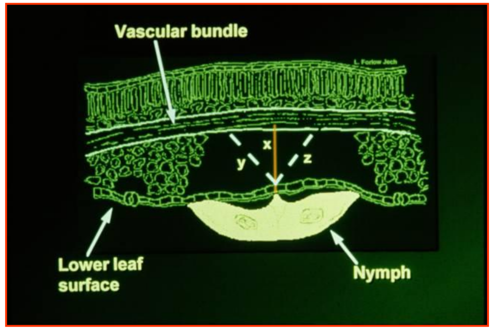
2006 North America: Arizona/California, USA; Sonora, Mexico

Virus particles- long flexuous rods 2000 x 12 nm



2000 nm long x 10 nm wide

- Semi-persistent transmission
- Transmits for ~3 days post AAP
- Yellows symptoms
- Not seedborne



Symptoms in squash

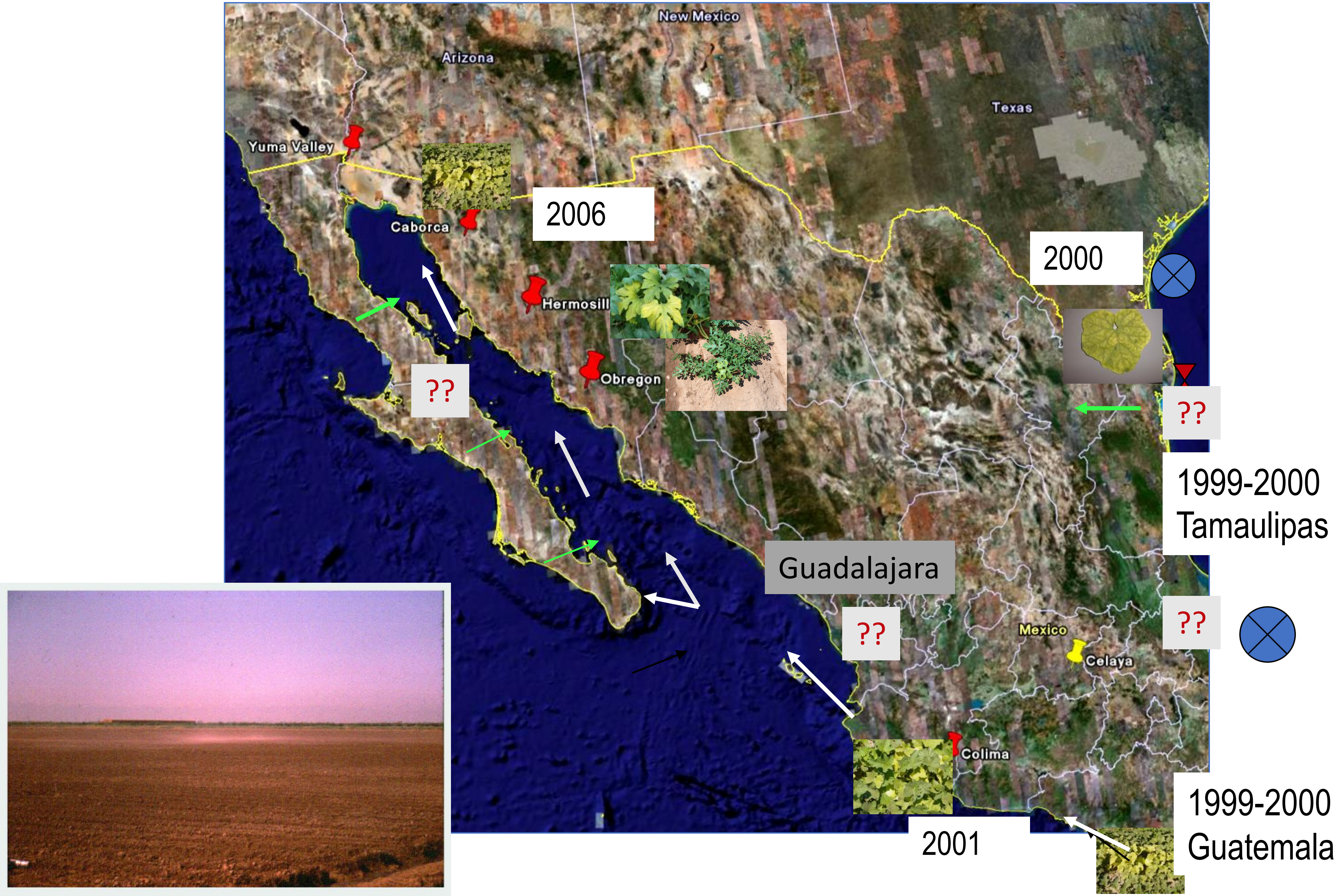
yellow

acorn

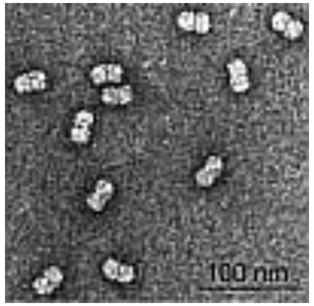
Yellow squash


watermelon

POSSIBLE ROUTES OF SPREAD to the U.S. - known sources in Texas, Mexico, Guatemala (map Nolte & Brown)



Cucurbit-infecting begomoviruses



Squash leaf curl virus (1970-1980)
Squash mild leaf curl virus (1970-1980)
Cucurbit leaf curl virus (emerged: 1998)

Distribution: AZ, CA, TX, Mexico
Vector: *B. tabaci* A & B biotypes
Persistent transmission: lifetime of the vector
Not seed transmitted

pumpkin

watermelon

Squash leaf curl virus (1979)

•<u>Host Range</u>
Bean
Cucumber (mild)
Melon (mild)
Squash & Pumpkin
Watermelon

2. *Squash mild leaf curl virus*:
Infects Bean, Squash, Pumpkin
•Also- mixtures occur of SMLCV+SLCV

Cucurbit leaf curl virus
- ‘emerged in 1998 US & Mexico

bean

melons

•<u>Host Range</u>

Bean

Cucumber (severe)

Melon (severe)

Squash & Pumpkin

Watermelon

Melon chlorotic leaf curl virus

- New bipartite begomovirus of melon (*Cucumis* species) (Brown *et al*., 2002)
- Emerged in Zacapa Valley, Guatemala 2000
- Symptoms: interveinal chlorosis, leaf curling, stunting, fruit discoloration & cracking

MCLCV symptoms in melon leaves & fruit

Cracking

Fruit lesions

Leaf curling

Fruit discoloration

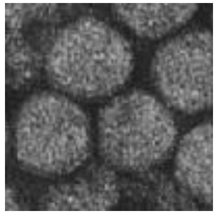
New ssRNA viruses with unusual symptoms


Compare TSWV

-Torrado virus
Two ssRNAs (7807 + 5403)
-First identified in Spain 2005, then Mexico, Guatemala, other




Tomato apex necrosis virus
Two ssRNAs (6478 + 3013)
-Identified in Mexico 2006




Tomato apex necrosis virus Mexico 2005-07






Fruit symptoms could possibly be confused with those caused by ToSWV

Emergent fastidious bacterial pathogens

_Ca_. Liberibacter spp.

Obligate pathogen
Psyllid vector

Relatively host-specific

Citrus greening disease (HLB)

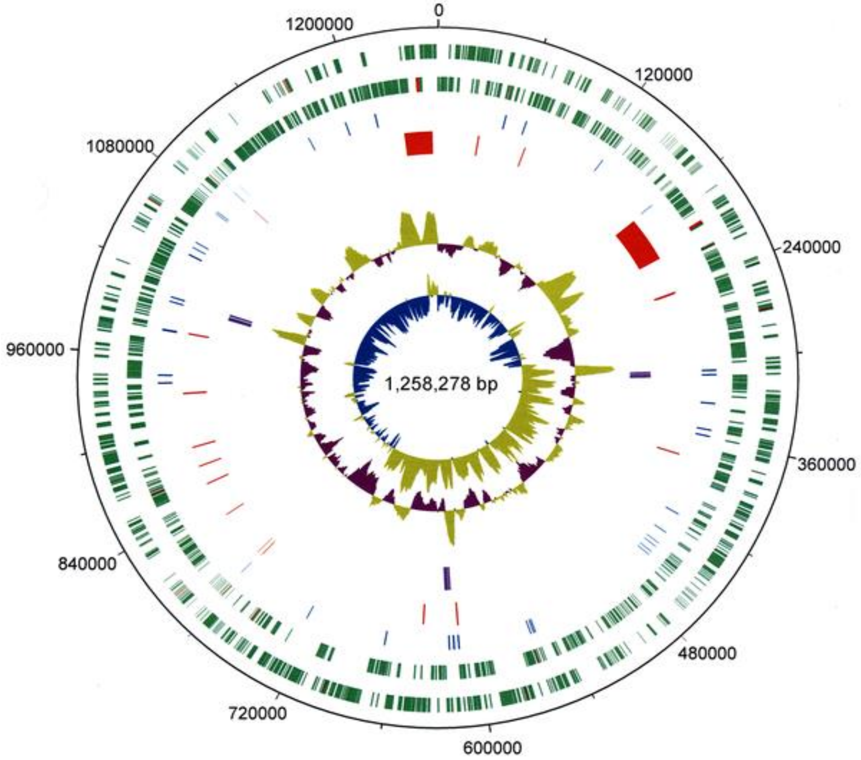
- Huanglongbing (HLB) or citrus greening disease is caused by three *Ca*. Liberibacter species

- Causal agent is fastidious, gram-negative, phloem-limited bacteria, *Candidatus* Liberibacter asiaticus

- Transmitted by Asian citrus psyllid, *Diaphorina citri*
- Circulative, propagative transmission

D. citri

Search for the microbe - *Not a Virus* – but a Fastidious Bacterium



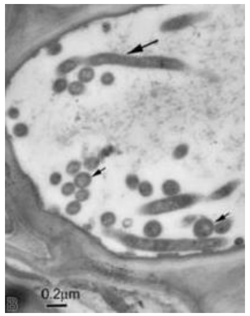
The genome of ‘*Candidatus* Liberibacter has been determined

The pathogen forms ‘biofilms’ in the host:

- A biofilm is an aggregate or group of microorganisms in which cells adhere to a surface – usually embedded in a matrix of slime.

- This is one part of the life cycle; the other part consists of cells that float or swim

Bacterial cells in plant



HLB symptoms

- Leaf 'mottling' that crosses veins on older leaves is the best 'diagnostic symptom' to look for.
- Newest leaves have symptoms – similar to zinc deficiency
- Notch in leaf is due to feeding by Asian citrus psyllid, the vector of Liberibacter, the citrus greening pathogen.

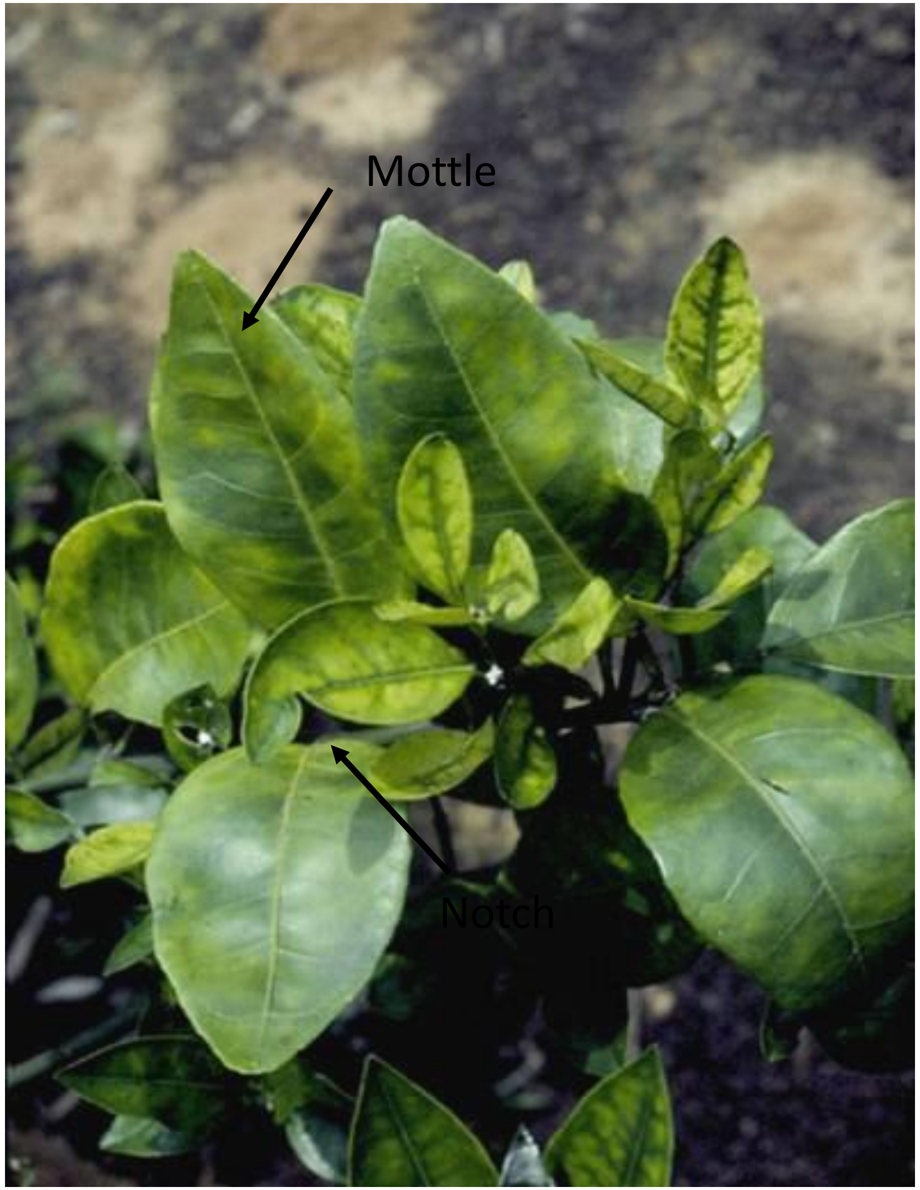


Photo: Susan Halbert, DPI

‘Funky’ fruit symptoms - citrus greening

Grapefruit
- Florida
(S. Halbert)

Orange fruit photos: Jose Luiz Rodrigues (Brazil)

Psyllid Vector

- Small, about the size of aphids (2.5 mm)
- s.o. Homoptera (Hemiptera)
- Phloem feeder

- Prefers new growth
- Numbers increase rapidly when plants are actively growing
- Feeding alone causes damage when psyllids are abundant

- Two vector species:

Asian species vector: Asian citrus psyllid *Diaphorina citri* - *U.S.*

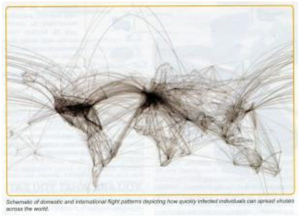
African species vector: African citrus psyllid *Trioza erytreae*

Life Cycle – Asian citrus psyllid

- Eggs are bright yellow or orange
- Deposited on young leaves
- Up to 1000-2000 eggs/female ~ 3 wk
- Nymphs yellowish orange-new growth of leaves and stems
- Waxy honeydew visible on leaves
- Nymphs move slowly, no flight
- Adults (winged) disperse/migrate

5 immature instars

Other Pathways..

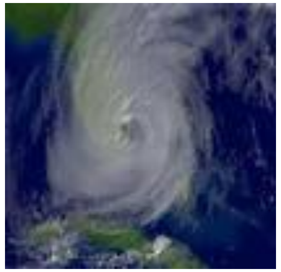


Of the pathogen

- Infected seedlings, budwood, infected plants; smuggling of plants
- Ornamental hosts: *Murraya paniculata* (orange jasmine) 23 spp. in Rutaceae
- Vegetative transmission by grafting

Of the psyllid vector - invasive species

- Psyllids dispersing on wind currents and major tropical disturbances
- Transmission rate variable – bacterium irregularly distributed plant



Asian citrus psyllids can fly long distances

Photos: Susan Halbert

Large groves in Hendry Co., ~ 50 miles from urban development

Map: Andrea Chavez

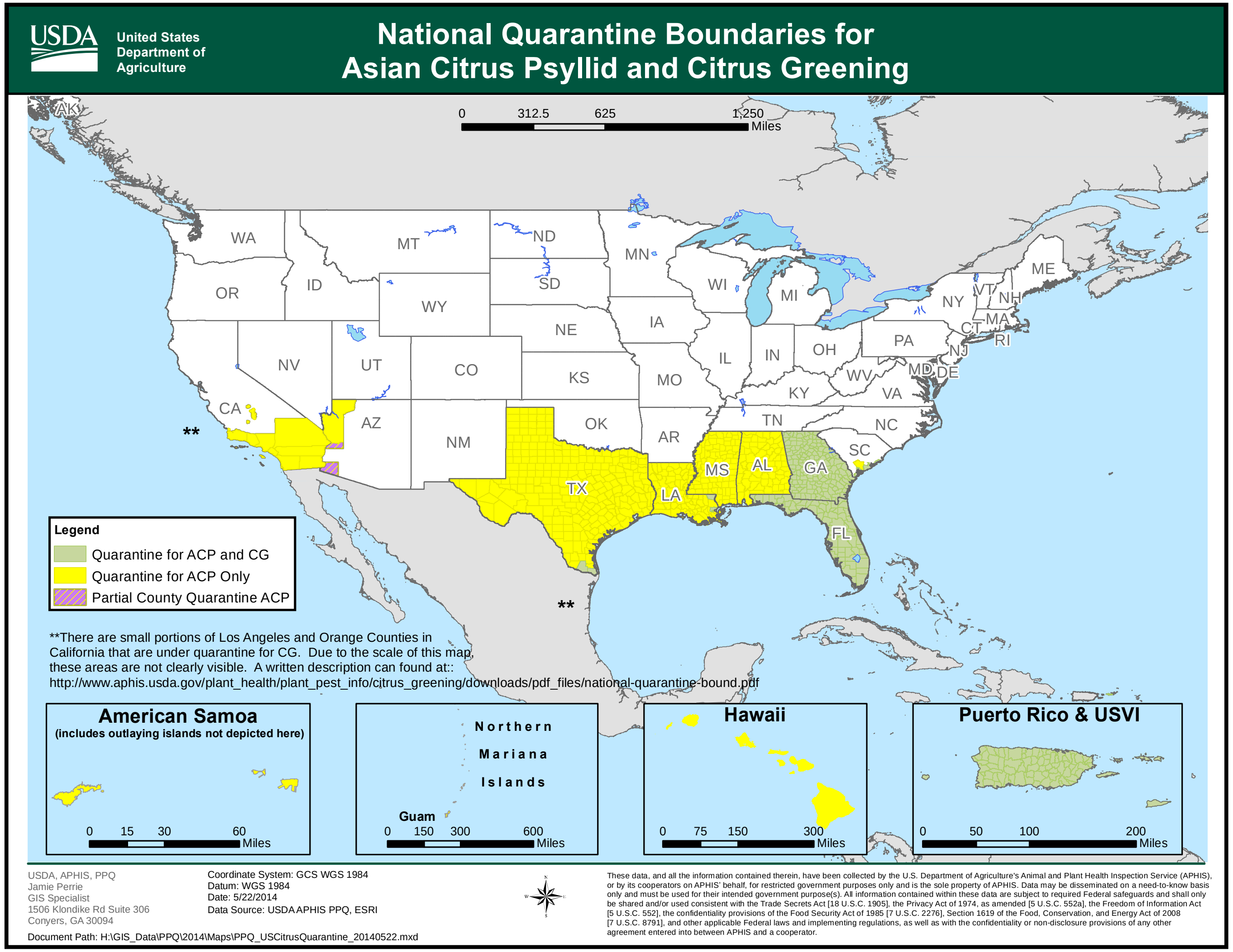
Asian citrus psyllids move on unprocessed fruit

Photos: Susan Halbert and Matt Brodie

Citrus greening sometimes is for sale …
local nurseries and tourist shops (FL)

Fifteen U.S. States or Territories are under full or partial quarantine due to the detected presence of Asian citrus psyllid and/or the greening pathogen

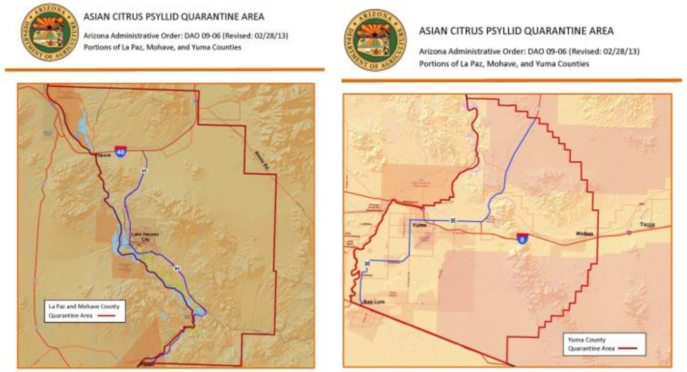
Alabama, American Samoa, Arizona, California, Florida, Georgia, Guam, Hawaii, Louisiana, Mississippi, Northern Mariana Islands, Puerto Rico, South Carolina, Texas and the U.S. Virgin Islands



- Depts of Agriculture in CA and AZ / USDA-APHIS psyllid monitoring
- Arizona has a $37 million dollar citrus industry

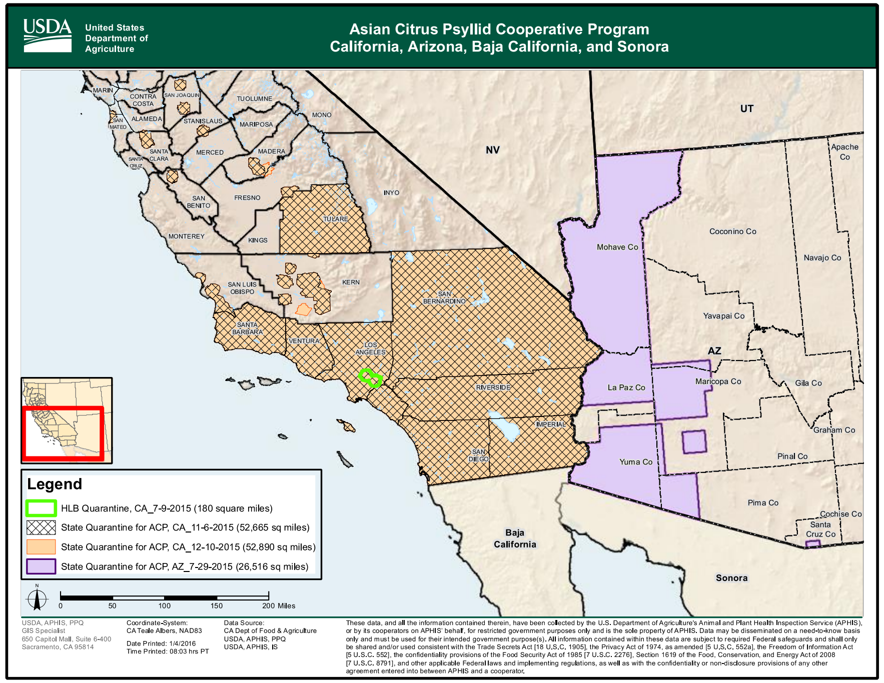
(Courtesy, J. Caravetta, ADA)

Quarantine areas in Arizona 2013



I-40 and I-8 corridors initial zones

Expanded quarantine areas 2018
AZ, CA, Sonora



Zebra chip of potato / Vein-greening of tomato 2004-onward in US

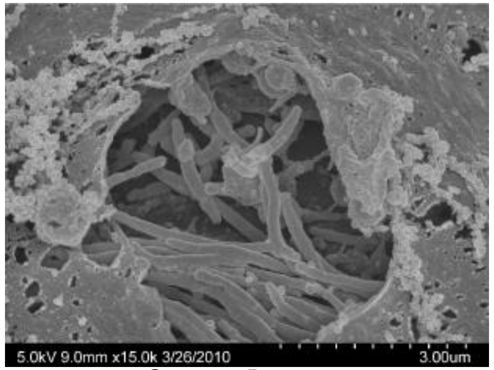
-Causal agent: fastidious, gram-negative, phloem-limited bacterium *Candidatus* Liberibacter solanacearum (CLso)

-Vector: potato/tomato psyllid *Bactericera cockerelli (Sulc)*

-Infects phloem of plant –disrupts metabolism, source and sink-like effects in tubers, tomato fruit – sugars don’t accumulate

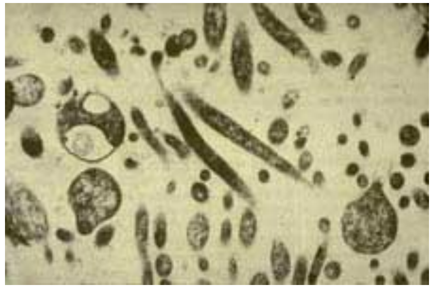
-Multiplies and circulates in the psyllid vector

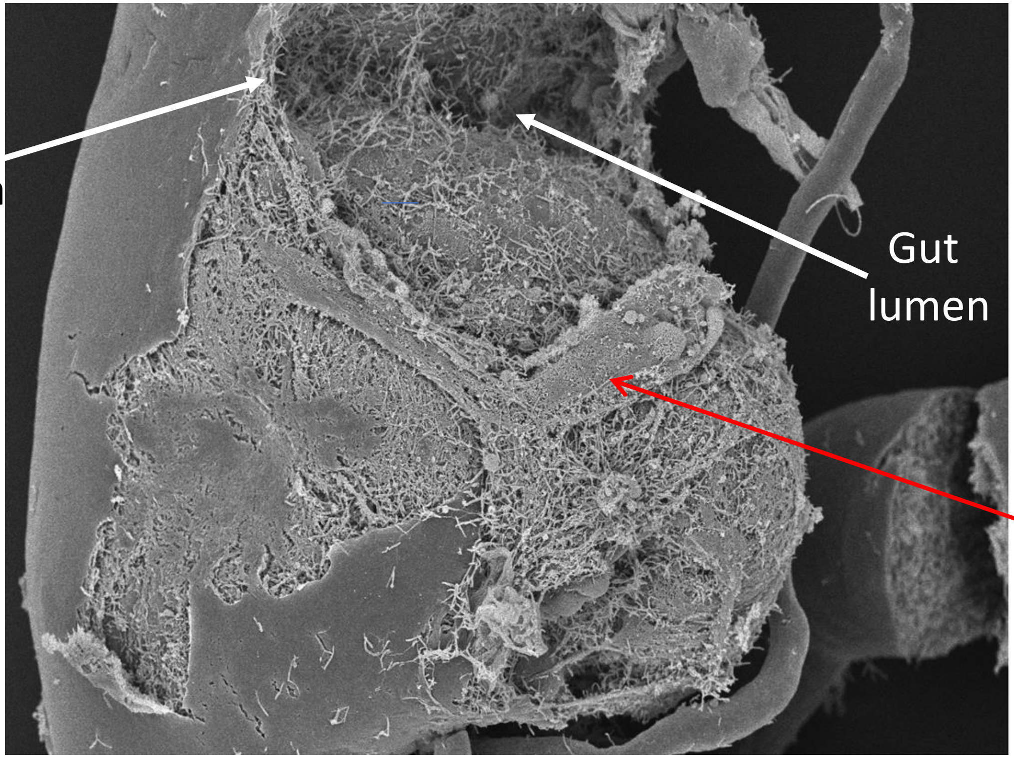
Potato (tomato) psyllid

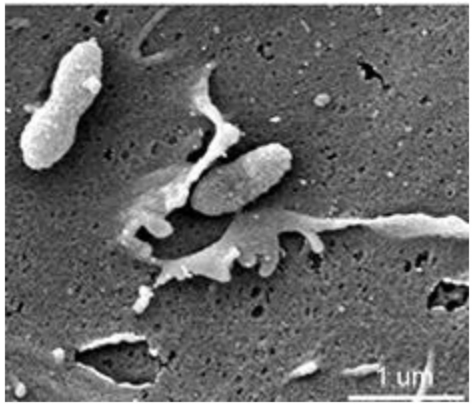


Infected PoP gut

Uninfected PoP gut



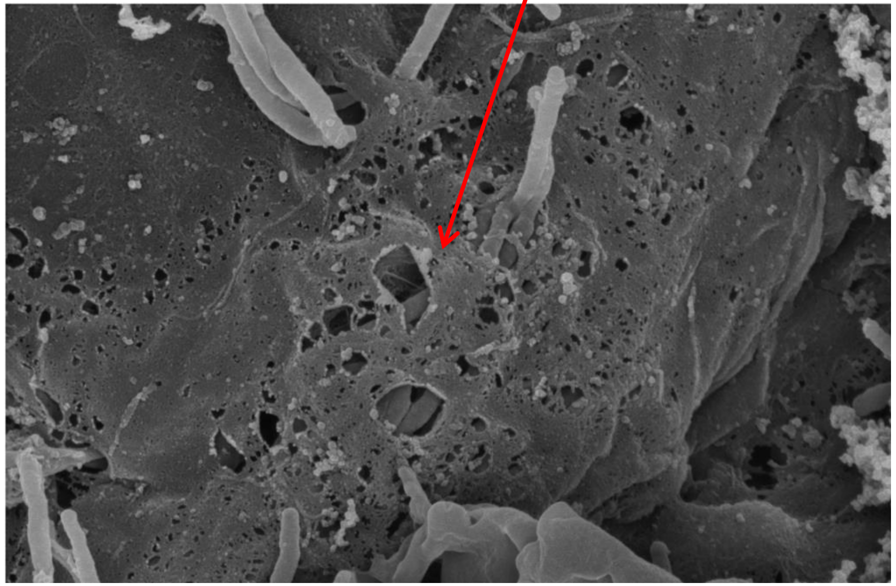




Pathogenic *Haemophilus influenza* causes membrane ruffling during exit from human epithelial cells

Membrane ruffling?

SEM images of Liberibacter-infected psyllid guts



Symptoms of tomato vein greening

Vein-Greening & Wilting

RENO NV 2005

Life Cycle History Study at 26C

Stage	Duration	% Survival	
Egg	5 – 8 days	40.6 %	Male/Female Ratio:
Instars	19 – 24 days	47.3 %	1.1 +/- 0.12
Adult	10 – 15 days	62.7 %	
Life Cycle	32 - 38 days		

Duration in each stage is temperature dependent

Abdullah N.M.M (2008) Life History of the potato psyllid *Bactericera cockerelli* (Homoptera:Psyllidae) in controlled environment agriculture in Arizona. *African Journal of Agricultural Research* **3,** 60-67.

Dispersal and overwintering biology of potato psyllid

- •Wind-assisted dispersal
- •Seasonal migration
- •Genetic difference between central and western psyllid populations (Liu, 2006)
- •Overwinter survival can occur even when conditions are unfavorable for psyllid development
- •Ovarian diapause (inactive when food needed for breeding is absent)
- •Overwintering in tropical and/or temperate species ?

Proposed recent migration routes of PoP in North America

Experimental Host Range Study

Ca. Liberibacter psyllaurous

Solanaceae-specific in crops

Some wild hosts In other plant families

24hr AAP; 7 day IAP

(20 adults/plant) on transmission of *Ca.* Liberibacter

Test species	Inoculation access period (7 days) number of infected plants (%) confirmed by PCR 7days	15days	25days	35days	Symptoms (days)
Tomato	3/4 (75%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	11 d
Potato	3/4 (75%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	11 d
Pepper	0/4 (0 %)	3/4 (75%)	4/4 (100%)	4/4 (100%)	21 d
Eggplant	3/4 (75%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	11 d
Datura stramonoum	0/4 (0 %)	1/4 (25%)	3/4 (75%)	4/4 (100%)	25 d
Cotton	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
Pumpkin	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
Bean	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
Cantaloupe	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
Watermelon	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
N. benthamiana	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)
Sugarbeet	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	0/4 (0 %)	NS (35d)

Goal: Healthy Plants

To manage problems leading to poor health / rule to remember is:

- **KNOW WHO and WHERE your enemy is**

- Sounds simple - - **Mostly, it is …**
- **ENERGY** and **KEEN OBSERVATIONS**

BE PROACTIVE:

- Be ahead of the problem – routine monitoring
- Control psyllid vectors upon arrival to prevent infection and reduce secondary infestation/infection
- Use chemicals judiciously - prevent insecticide resistance
- Use clean seed (potato) and virus-free seedlings

Mission of the National Plant Diagnostic Network

Judith K. Brown, AZ Coordinator

School of Plant Sciences
The University of Arizona
Email: jbrown@ag.arizona.edu

The University of Arizona College of Agriculture & Life Sciences

NPDN Mission

Enhance national agricultural security by quickly detecting and identifying introduced pests and pathogens.

Method

- Create a nationwide network of land-grant universities
- Provide training to first detectors and diagnosticians
- Establish protocols for reporting to responders and decision makers

Network Responsibilities

- Outbreak detection and identification
- Secure communications system
- Information storage and management
- Data analysis
- Reporting and alerts
- Training

Minimize Impact

Need for Plant Biosecurity

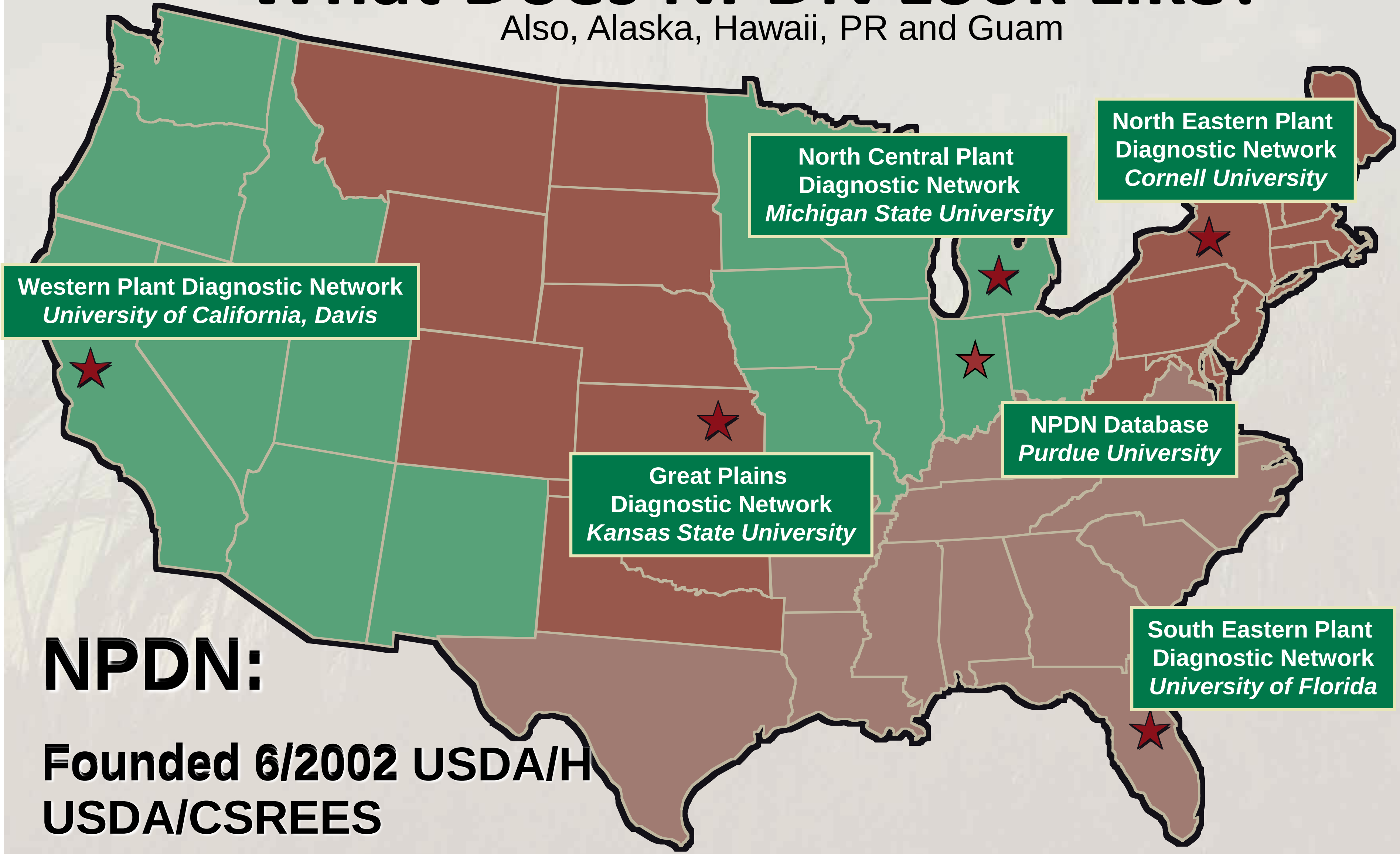
Maintain profitability of crop production

Invasive species cost $ billions/year

Maintain security of food production

What Does NPDN Look Like?

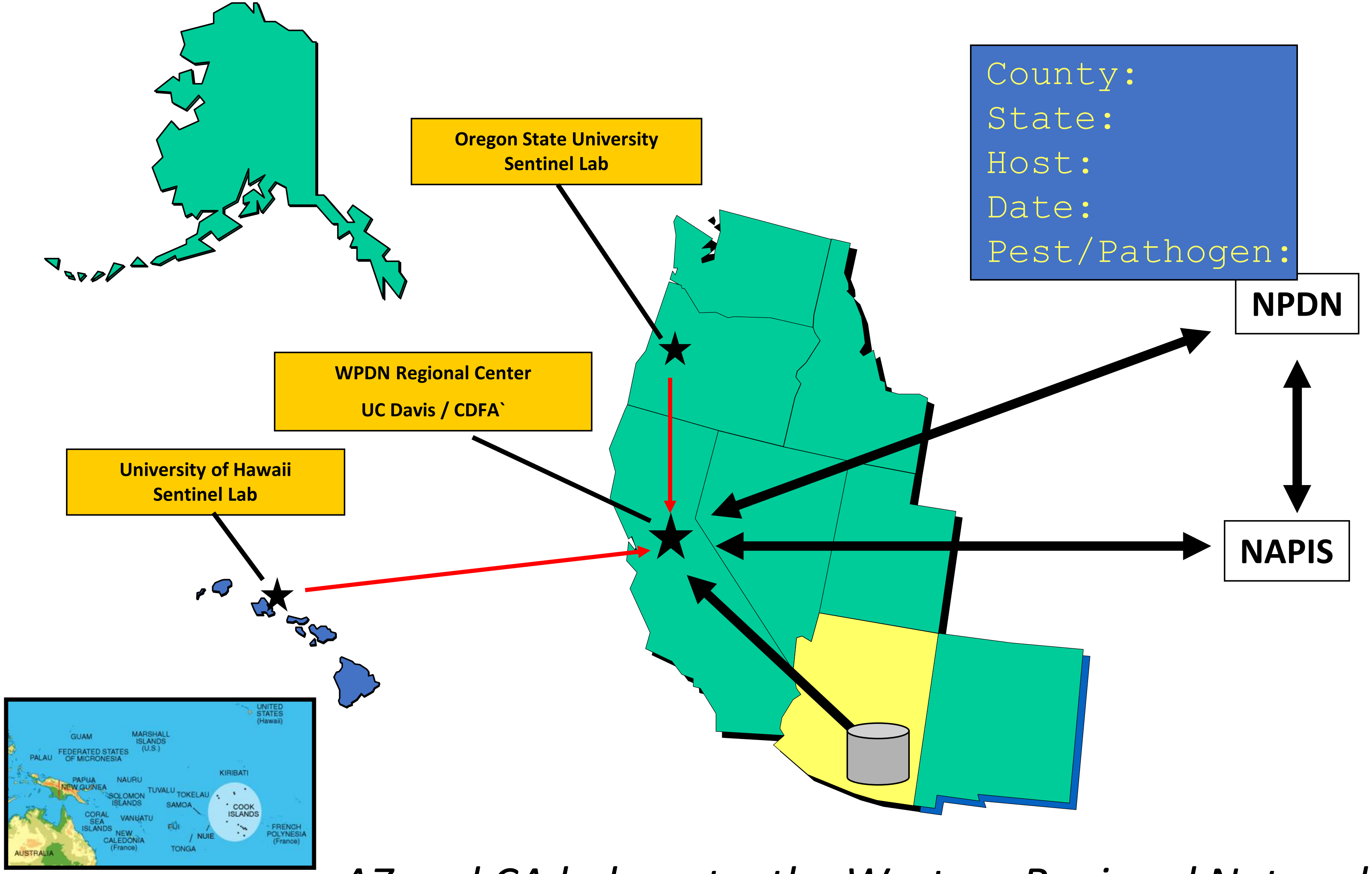
Also, Alaska, Hawaii, PR and Guam



NPDN:

Founded 6/2002 USDA/H USDA/CSREES

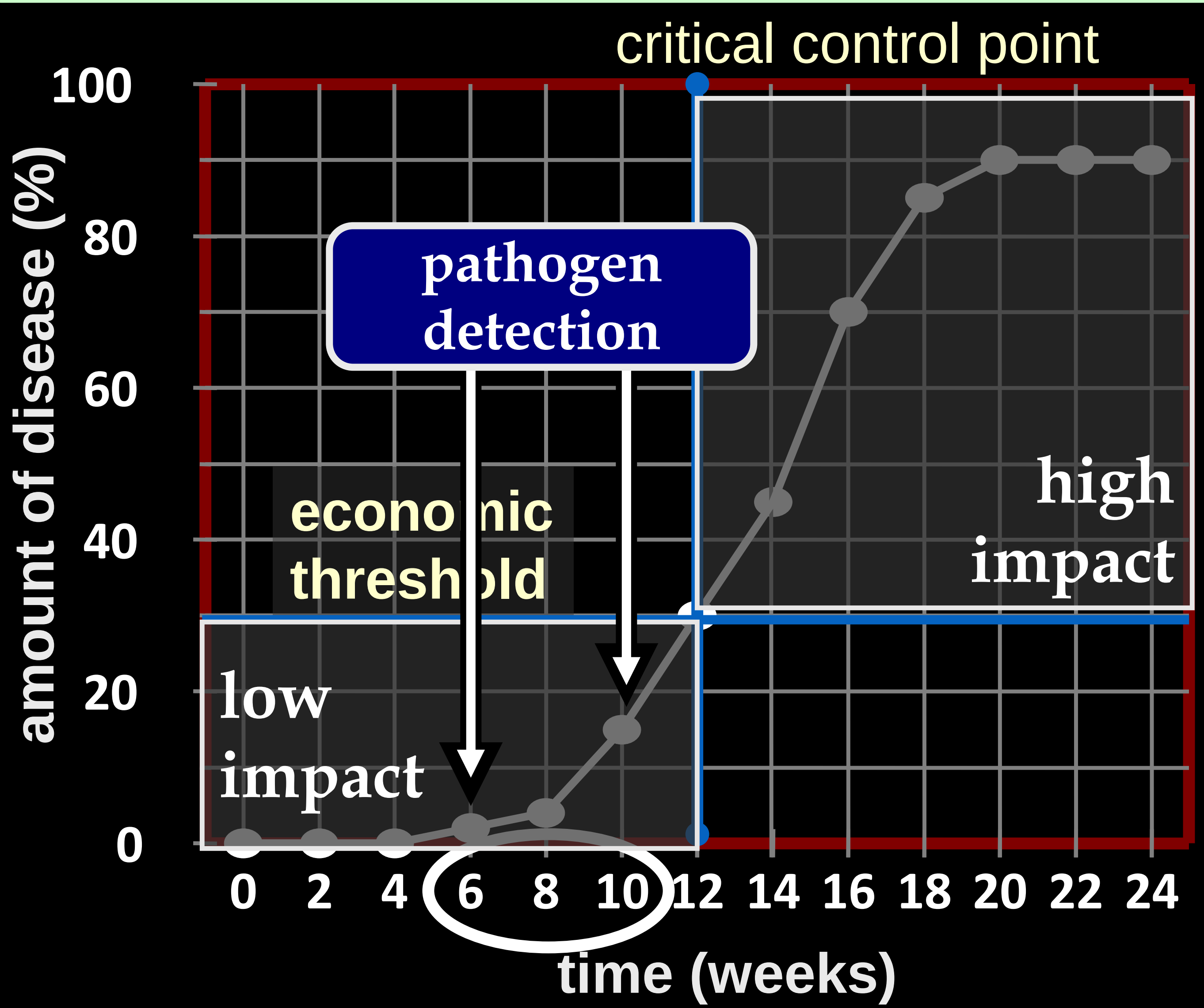
What is the National Plant Diagnostic Network?
… a Suite of Regional Networks



Pacific Territories

AZ and CA belong to the Western Regional Network

Importance of Early Detection



Everyone's role as a First Detector

- Be alert to the unusual or different
- Receive NPDN First Detector or First Detector Educator training
- Be placed on a national notification registry of First Detectors
- Receive pest alerts and other relevant updates
- Report new or unusual observations

Thank you