The challenges, successes and future needs of integrated pest management of tomato-infecting viruses in the Dominican Republic

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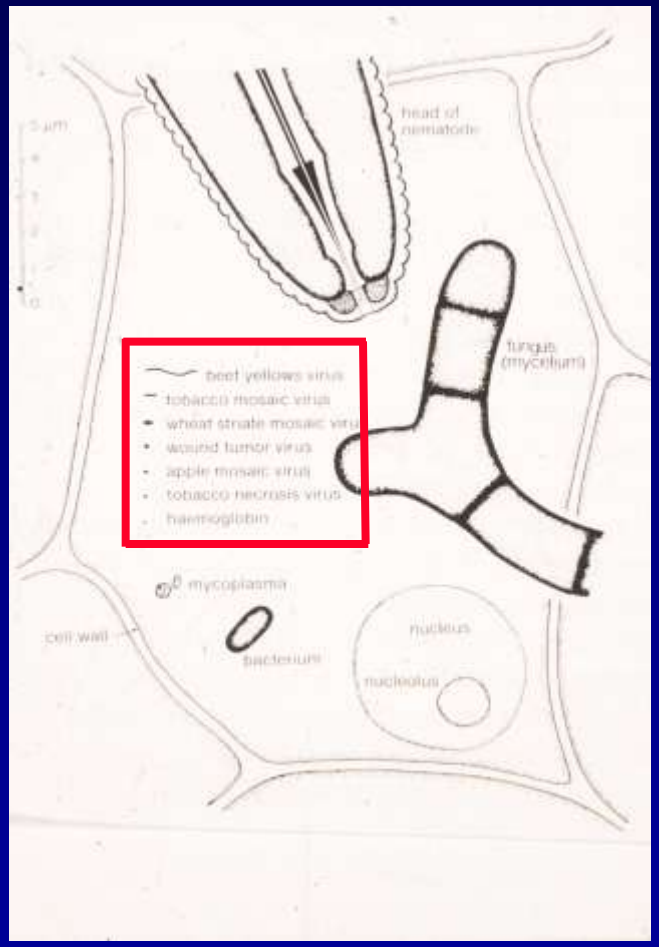
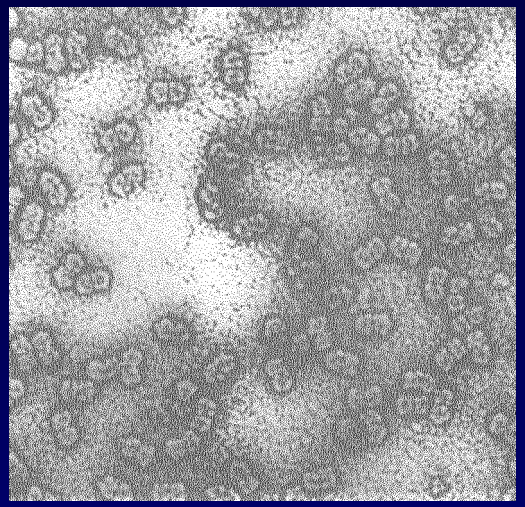
Diseases of plants are caused by biotic and abiotic factors

- Causal agents of plant diseases can be broadly classified as <u>biotic</u> (e.g., fungi, viruses, bacteria, phytoplasmas and nematodes) and <u>abiotic</u> (air pollution, herbicide damage and excesses and deficiencies in water and nutrition)
- Plants respond to these agents with disease symptoms
- Diagnosis of the cause of symptoms can be very difficult



What are viruses?

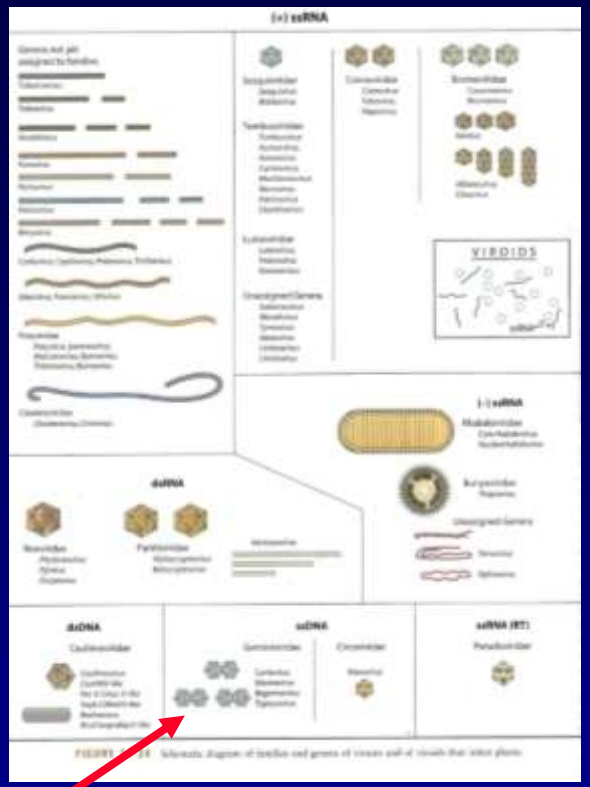
- Submicroscopic infectious genetic elements (RNA or DNA) covered by a protective shell of protein
- Viruses take over the cellular machinery of the plant, trick the cell into producing viral nucleic acids and proteins and spread throughout the infected host, e.g., plant
- Infected cells function abnormally and the plant develops disease symptoms
- Latin word virus=poison!

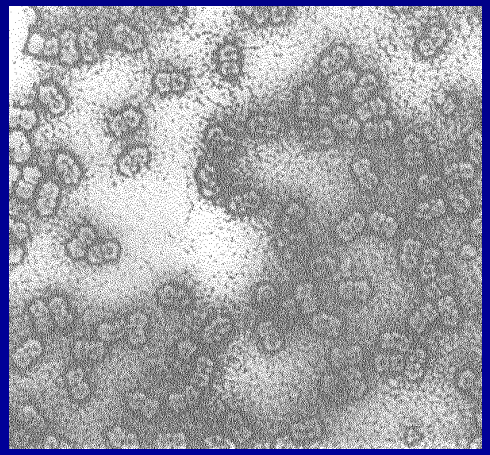


Purified virus: infectious nucleic acid covered by a protein shell

Plant viruses: a diversity of forms and function

- Most types of plant viruses have ssRNA genomes
- DNA genomes are less common
- One family of dsDNA viruses and two families of ssDNA viruses: *Geminiviridae* (geminiviruses) and *Nanoviridae* (nanoviruses)
- Most rely on insects for plant-to-plant spread (aphids, leafhopper, thrips, whiteflies, etc.)
- New viruses are constantly emerging and old viruses are changing and spread to new areas
- Geminiviruses have emerged as the largest (325 species) and one of most diverse and economically important groups of plant viruses





How do you know that a plant is infected with a virus?

- In the field, virus diseases are recognized by symptoms, usually stunted and distorted growth and color changes in leaves, stems and fruits
- The most distinctive symptom is the mosaic or mottling of leaves
- However, viruses cause a wide range of symptoms in plants, including curling, crumpling, necrosis and yellowing
- Virus symptoms also can mimicked by abiotic problems, such as nutrient deficiency and herbicide damage
- Thus, precise identification often requires serological (e.g. ELISA and lateral flow devices) or nucleic acid-based tests (DNA probes and PCR)

Insect vectors and symptom type(s) can help identify some viruses and/or select specific diagnostic tests

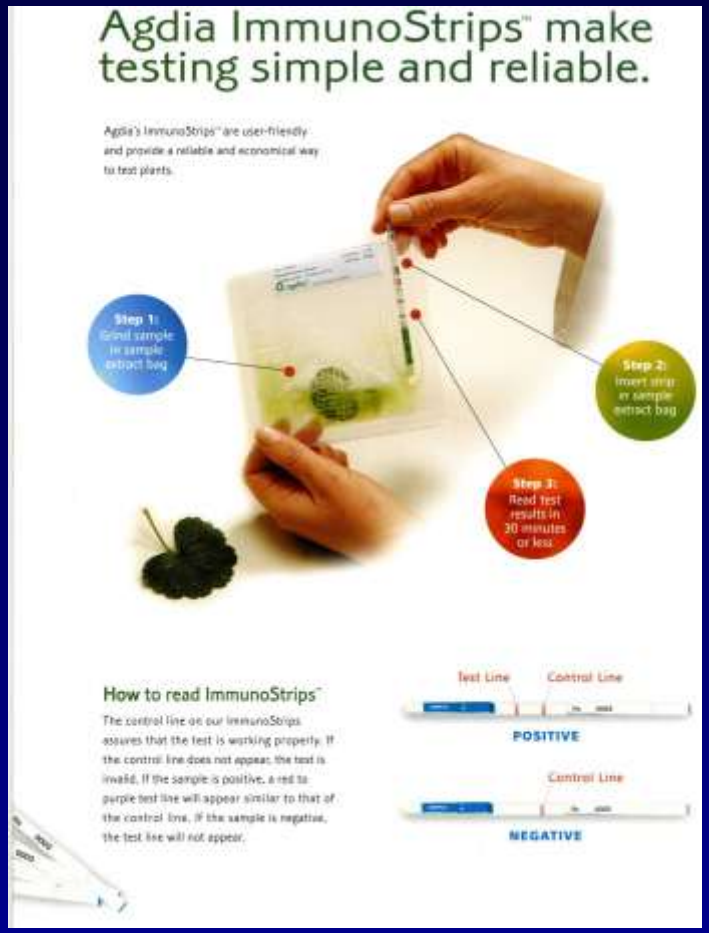
Potyvirus
CMV

Tospovirus

Whitefly-transmitted geminiviruses

Rapid Diagnostic Tests have Improved Virus Identification

- **Lateral flow devices (immunostrips)**
  - -Rapid and precise
  - -Easy to use
  - -Detection in the field
  - -No equipment needed
  - -TSWV, CMV, TMV, PVY, PepMV



- **PCR and sequencing**
  - -Precise and sensitive
  - -Laboratory-based test
  - -More time-consuming
  - -Geminiviruses and criniviruses

Plant virus diseases are most effectively managed by an integrated pest management (IPM) approach

- An approach that combines multiple management strategies (e.g., biological, cultural, genetic and chemical) selected based on knowledge of the biology of the virus(es)
- Goal is efficient management with minimal inputs of pesticide; economically and environmentally friendly
- Three basic steps:
    1. Correct pathogen ID
    2. Understanding pathogen biology/disease epidemiology
    3. Development and evaluation of an integrated management strategy

Plant virus diseases have been a problem for the processing tomato industry in the Dominican Republic since the early 1990s

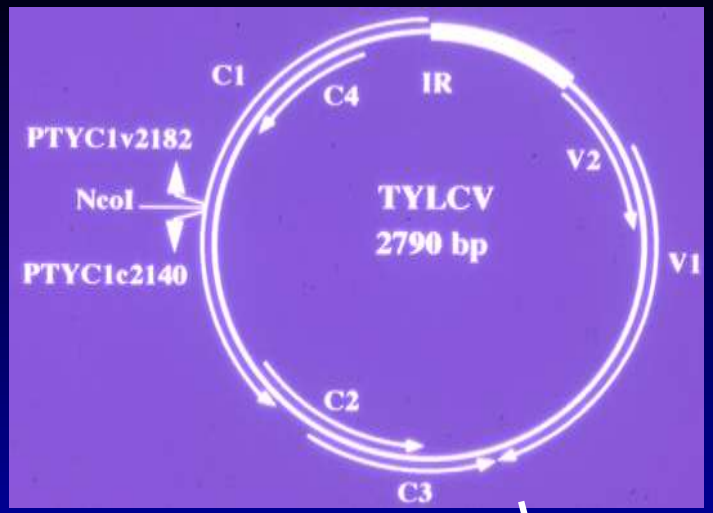
- Tomato is a very important part of the diet of the Dominican people
- The processing tomato industry has been present for over 40 years, both in the North (Cibao Valley) and South (Azua Valley)
- Provided jobs and some degree of food security
- Here, I will tell the story of how this industry was threatened by two insect-vectored viruses and how a multidisciplinary team and IPM has helped protect the industry

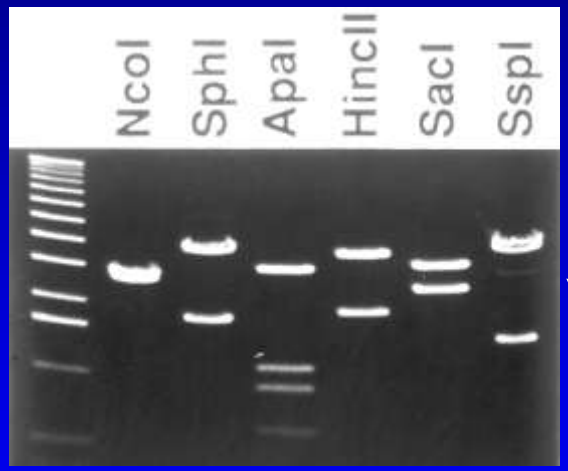
Introduction of the exotic *Tomato yellow leaf curl virus* in the Dominican Republic in the early 1990s

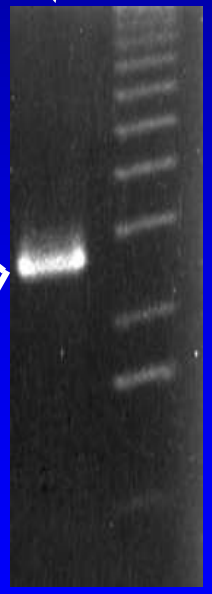
- **Strange disease symptoms** appeared in tomatoes in 1992
- Associated with **high whitefly populations**
- Within 2 years, the disease was **threatening the entire processing tomato industry**
- Symptoms looked like those of **tomato yellow leaf curl**, a disease not known to the New World

Identification of *Tomato Yellow Leaf Curl Virus* in the Dominican Republic

- Overlapping PCR primers based on TYLCV sequence amplified a 2.8 kb DNA fragment
- Restriction map was identical to that of TYLCV
- The complete sequence of the cloned fragment was 97% identical to TYLCV!





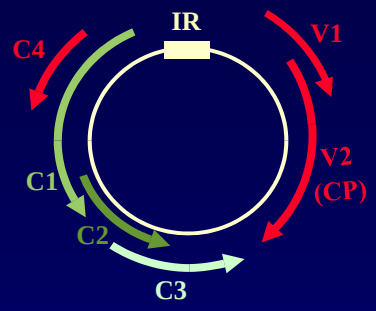


Whitefly-transmitted geminiviruses are in the genus *Begomovirus* and there are differences between those infecting tomatoes in the Old vs. New World

- **Old World types**
  - -Usually monopartite
  - -Not sap-transmissible
  - -Often cause tomato leaf curl or yellow leaf curl symptoms
  - -May be associated with satellite DNAs
- **New World types**
  - -Bipartite*
  - -Some are sap-transmissible
  - -Symptoms are more variable: mottle/mosaic, chlorosis, purpling, crumple/curl/crinkle
  - -No satellite DNAs
- **Effective resistance genes vary depending on the virus and geographic location**

TYLCV is an exotic invasive virus spread by an exotic invasive whitefly

- TYLCV was first described in the Middle East around 1940
- Causes tomato yellow leaf curl disease of tomato
- Vectored by invasive *Bemisia tabaci* biotype B (also known as silverleaf whitefly or Middle East-Asia Minor 1 [MEAM1])
- Introduced into the New World (NW) in the early 1990s in the Dominican Republic
- Now present throughout the NW, e.g., Caribbean Basin, USA (SE states; Arizona, Texas and Califoria; and Hawaii), Mexico, Guatemala, and South America (Venezuela)



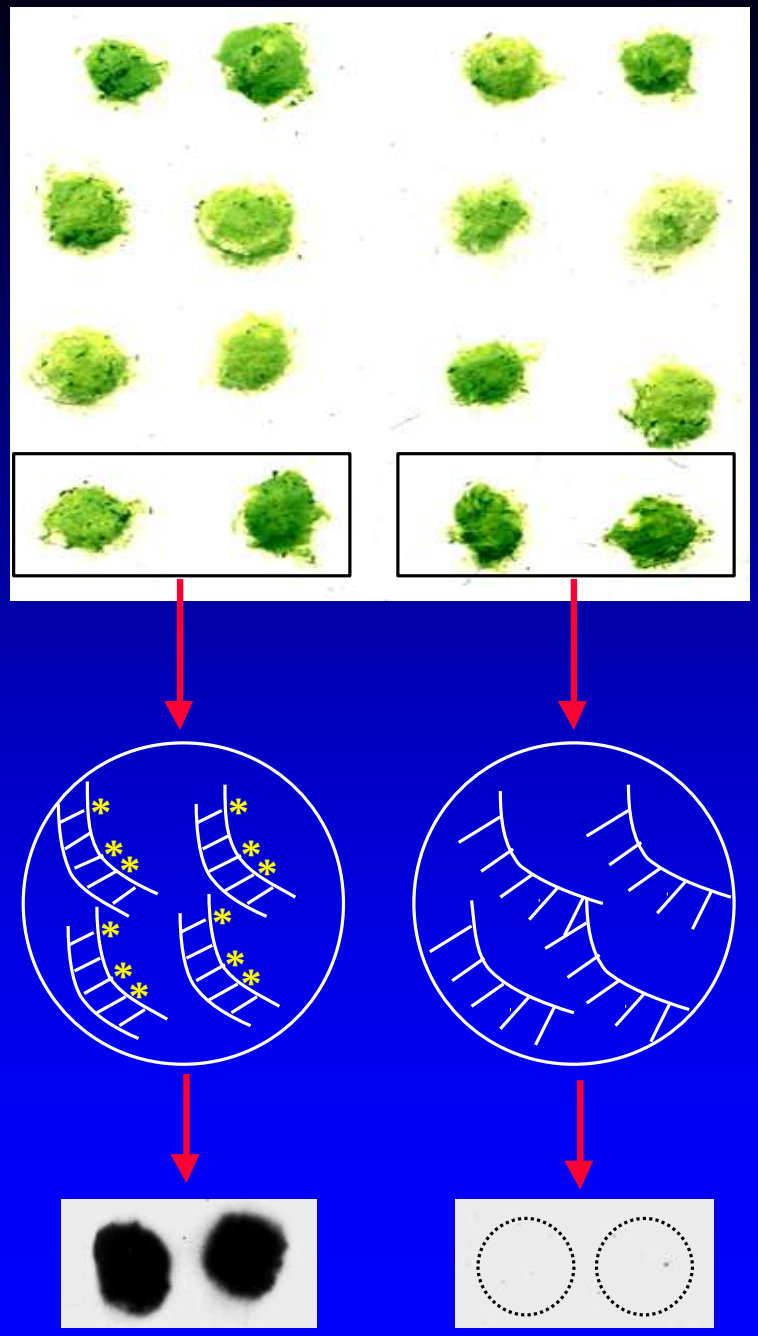
TYLCV has a monopartite genome

Nucleic Acid Squash Blot Hybridization

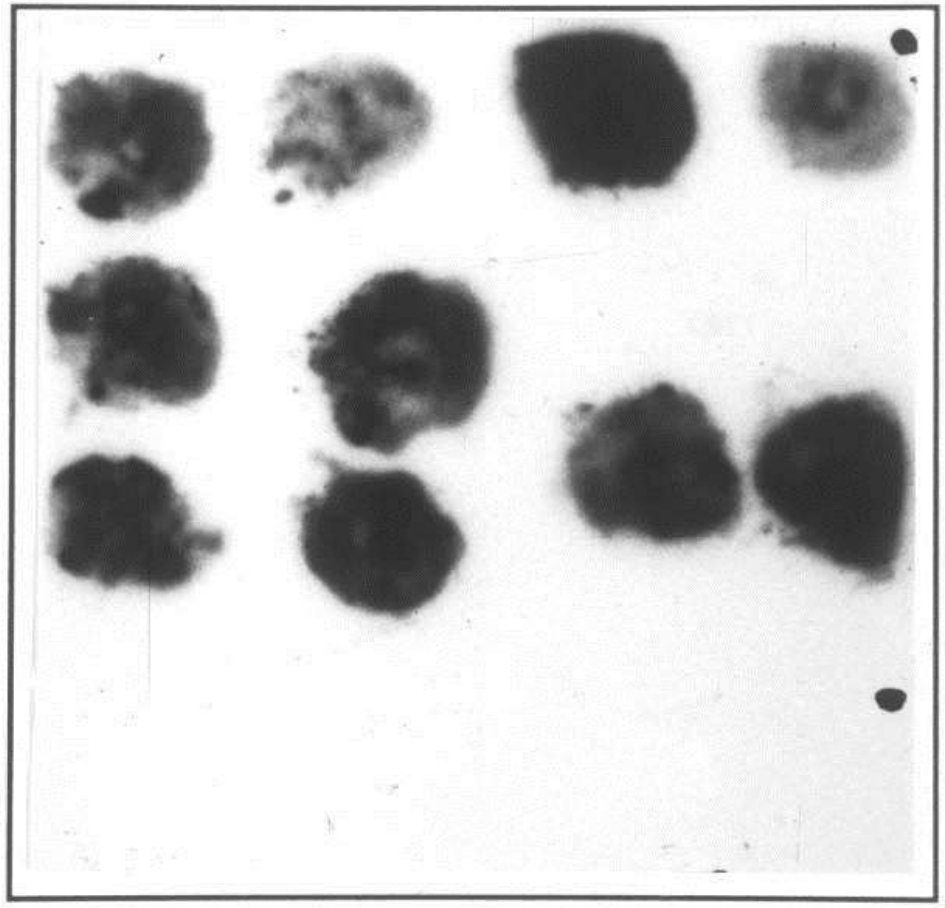
- Squash tissue samples onto nylon membranes
- Prepare membranes (i.e., denature DNA)

- Prepare and denature probe (cloned geminivirus DNA)
- Hybridization/wash

- Expose to X-ray film
  - -Positive sample: black spot
  - -Negative sample: no signal



Dominican Republic Samples-Northern Region
January 1994
TYLCV Probe-High Stringency



tomato (LC,Y,CR)

tomato (LC,Y,CR)

tomato (LC,Y,CR)

Sida spp. (GM)

Euphorbia spp. (MM)

Datura spp. (Y,CR,L

Jatropha spp. (GM)

tomato (LC, Y, CR)

? weed (NS)

Mock ToMoV

Dominican Republic Samples-Southern Region
January 1994
TYLCV Probe-High Stringency

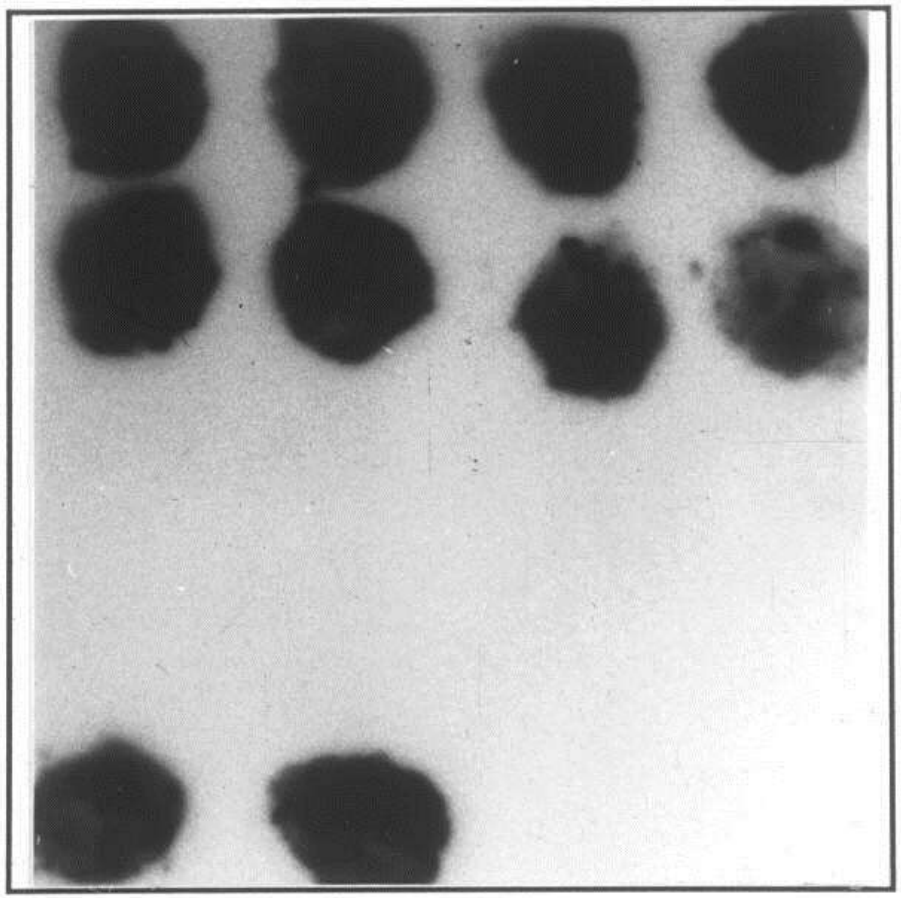
tomato (LC,Y,CR)

tomato (LC,Y,CR)

Sida spp. (GM)

Sida spp. (GM)

tomato (LC,Y,CR)



tomato (LC,Y,CR)

tomato (LC,Y,CR)

Sida spp. (GM)

Euphorbia spp. (GM)

Mock ToMoV

TYLCV in the Dominican Republic: DNA Probe Results

- **TYLCV had spread to the major processing tomato growing locations within 1-2 yrs**
- **Tomato appeared to be the primary host**
- **A three-month whitefly host-free period was established in an attempt to break the disease cycle**
- **Why was this considered?**

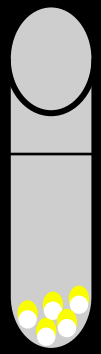
Biological properties that make a host-free period effective for management of TYLCV (and other begomoviruses)

- Not seed-transmitted*
- Narrow host range
- Whiteflies have relatively short (~30 day) life cycles and are begomoviruses are not transmitted through the eggs (transovarially)*
- Many economically important diseases caused by begomoviruses are in annual crops (cotton, cucurbits, peppers and tomatoes)
- A 2-3 month host-free period can be a very effective and sustainable management strategy for begomoviruses and can also reduce whitefly populations

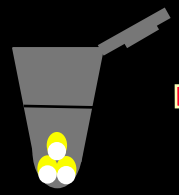
PCR Detection of TYLCV in Whiteflies: An Approach to Validate the Benefit of the Whitefly Host-free Period

Whiteflies collected from plant crops and/or weeds in the DR

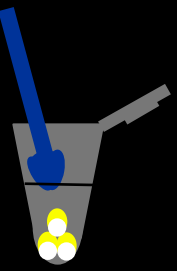
Whiteflies in EtOH



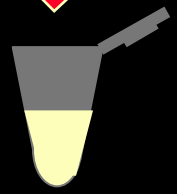
Whiteflies in EtOH



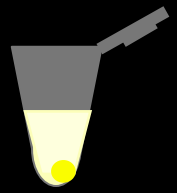
Remove EtOH and wash with STE buffer



Add 100 µl STE buffer and grind



Spin 10’at 13,000 rpm

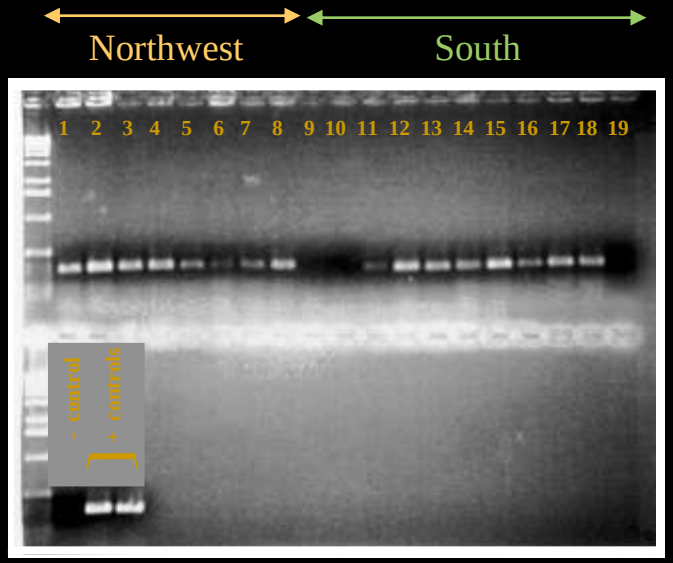


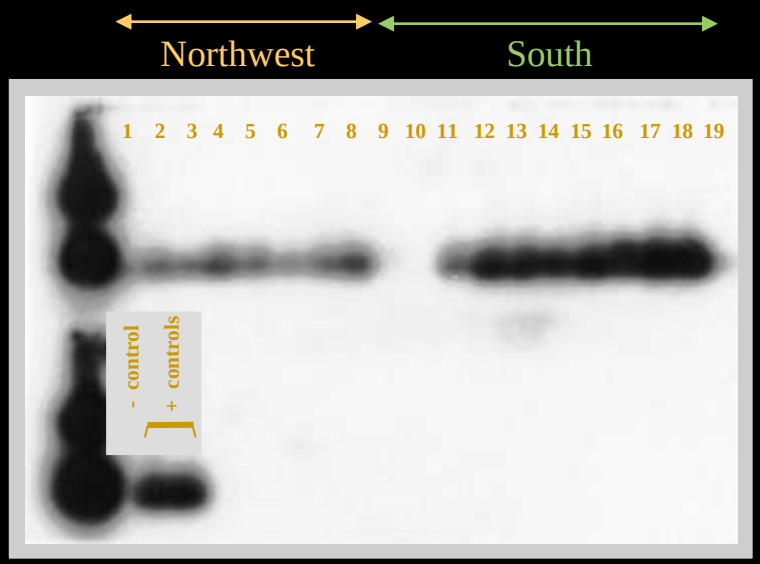
Use 20 µl of supernatant for PCR

Mehta *et al*., 1994

Sites in the two major processing tomato production regions from which whiteflies and plant samples were collected for TYLCV detection

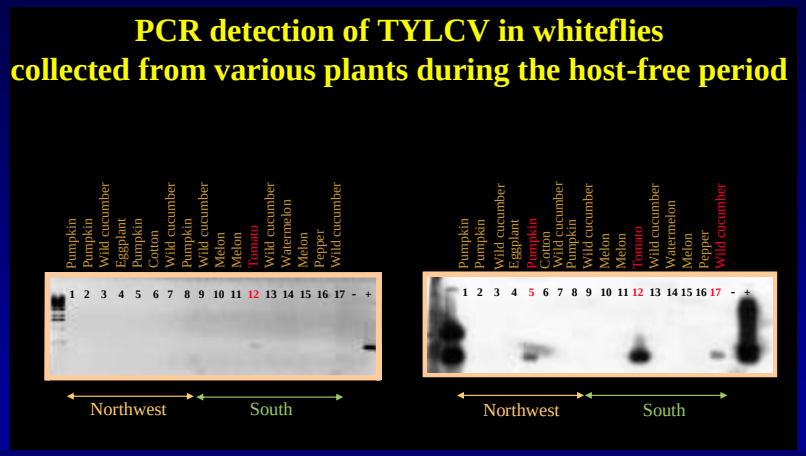
PCR detection of TYLCV in whiteflies collected from tomatoes during the growing season

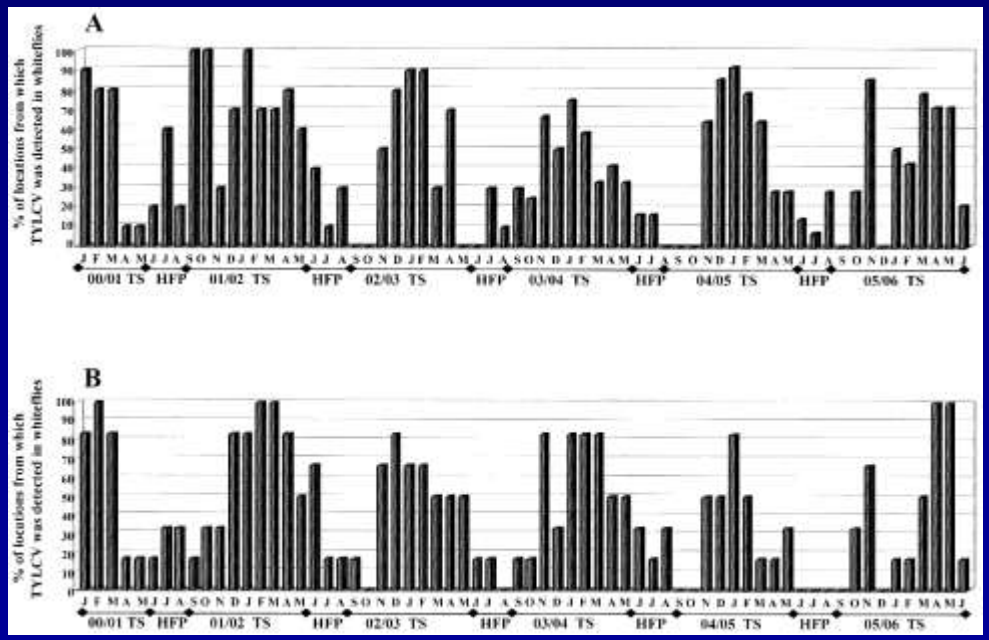




Host free period is a key component of the IPM strategy For TYLCV in the DO

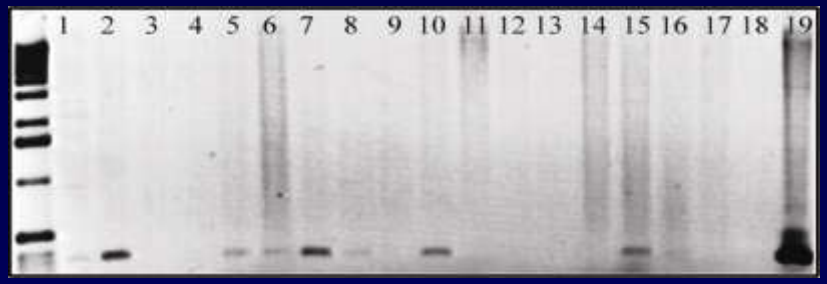
- The **host-free period was implemented** along with a number of **other practices** (vector control [esp. in transplants], planting early maturing/resistant varieties), and sanitation
- Evidence that the **host-free period was effective:**
  - -4-8 week delay in the appearance of **TYLCV symptoms** following the host-free period
  - -Dramatic **drop in detection of TYLCV in whiteflies** during the host-free period
- This **IPM approach** has been used for ~20 years and **has allowed for the recovery of the industry**





Detection of TYLCV in whiteflies

The host-free period stimulated research that revealed other aspects of the biology of TYLCV in the DO



PCR detection of TYLCV in symptomless weeds

- TYLCV persists during the host-free period in symptomless weeds
- This is consistent with an 'edge-effect' for the initial appearance of TYLCV in the field
- Pepper is a poor host of TYLCV, but will develop symptoms under high virus pressure*
- Common bean is also a TYLCV host, especially large-seeded Andean types
- Most TYLCV-resistant tomato varieties sustain are infected despite not showing symptoms
- These findings have helped fine-tune or maintain aspects of the host free period

TYLCV Reservoir Hosts

- **Symptomless hosts detected from eight plant families (e.g., *Cleome viscosa*, *Croton lobatus*, *Malva* spp., *Solanum nigrum*, *Physalis* spp.)**
- **Low levels of infection and viral titer**
- **Whiteflies observed on many hosts, but low populations**
- **Inefficient hosts, but likely serve as a 'virus bridge' between tomato crops**

Cleome viscosa

Whitefly host-free period in the Dominican Republic

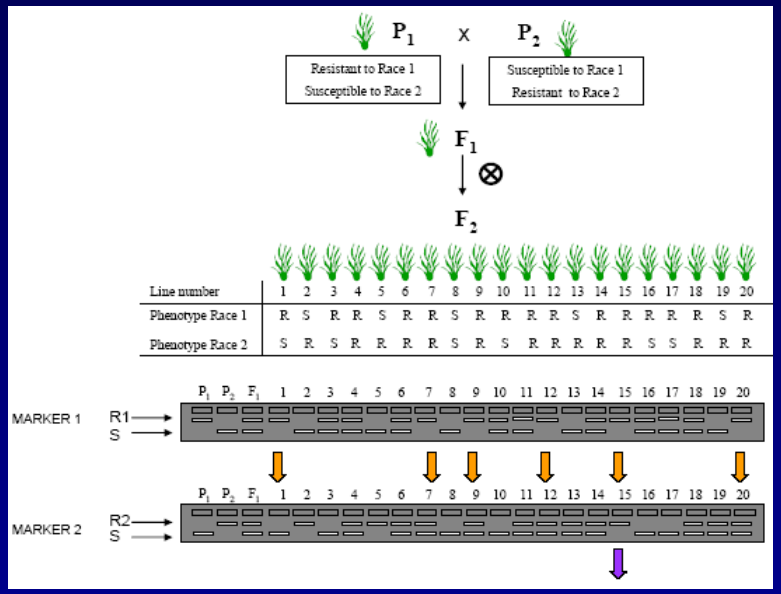
- **Three-month period** (June/July-Sept/Oct)
- **Government-enforced**
- Results in a **reduction of TYLCV inoculum**
- Provides a **4-8 wk window** after planting before virus appears
- Includes **peppers, beans, and cucurbits**
- Grower acceptance facilitated by **education**
- Violators are a continual challenge

IPM for TYLCV in the Dominican Republic

- **Host-free period**
- **Varietal selection**
  - **-early: early maturing hybrids**
  - **-late: TYLCV-resistant varieties**
- **Selected use of insecticides (e.g., neonicotinoids like imidacloprid, thiamethoxam and acetamiprid)**
- **Extensive sanitation after harvest**
- **Monitoring TYLCV in whiteflies/ plants: early detection of changes in the agroecosystem**
- **Tomato production in the DO recovered to be greater than before the introduction of TYLCV**

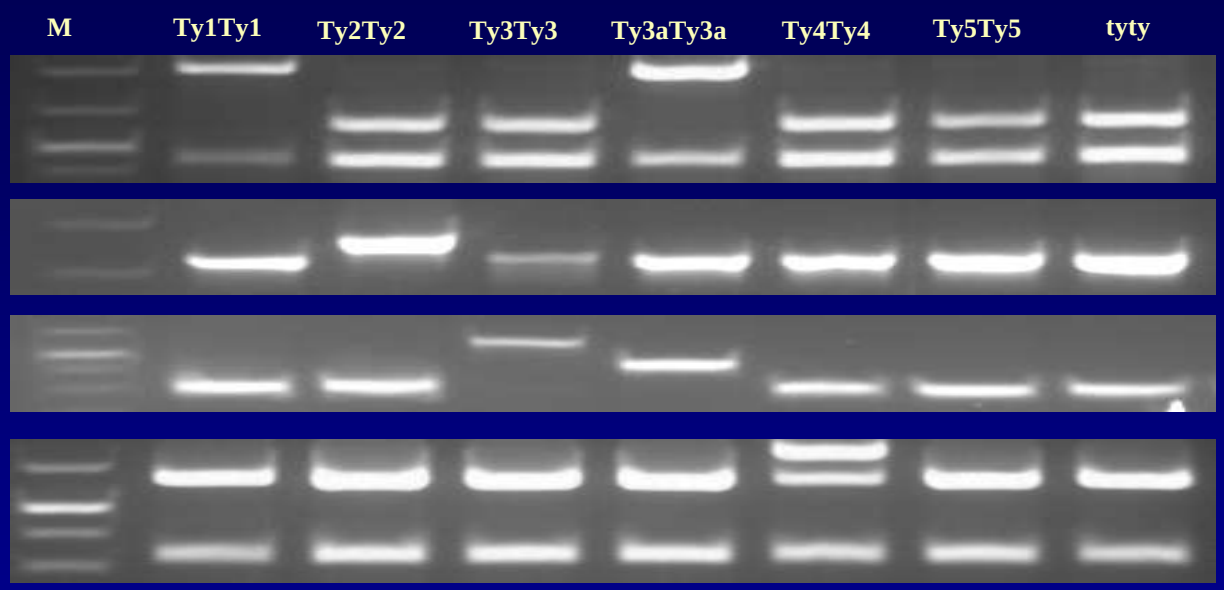
Progress in developing tomato varieties with resistance to TYLCV and other begomoviruses

- Resistance to TYLCV and other tomato-infecting begomoviruses has been identified and introgressed from **wild tomato species:**
  - -*Ty-1* (*Solanum chilense*)
  - -*Ty-2* (*S. habrochaites*)
  - -*Ty-3* (*S. chilense*)
  - -*Ty-4* (*S. chilense*)
  - -*Ty-5* (*S. peruvianum*)



- Introduced into cultivated tomatoes using **traditional breeding methods**, but time-consuming due to genetic drag
- Molecular markers, including the cloned *Ty-1*/*Ty-3* alleles, have made **marker-assisted selection** a reality
- A number of resistant tomato varieties with these genes have been developed but **most do not have broad-spectrum resistance**

Molecular markers are available for the *Ty* genes



Ty-1 C2_At5g61510

Ty-2 T0302

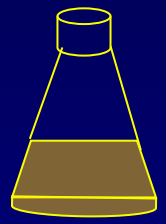
Ty-3 P6-25

Ty-4 C2_At5g63460

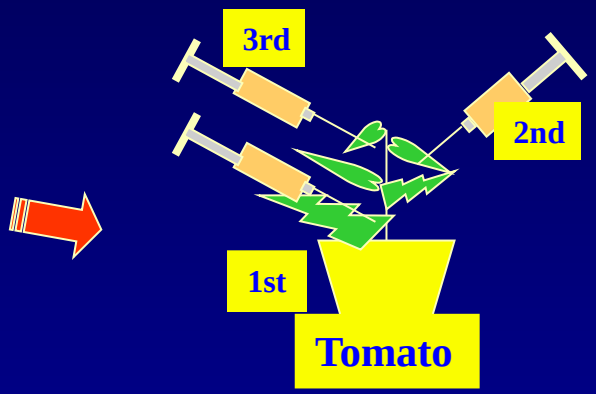
These markers can facilitate the introgression and pyramiding of these genes in cultivated tomato varieties

Agrobacterium-mediated inoculation for screening tomato tomato varieties and germplasm for resistance to TYLCV

TYLCV



48 hr old liquid culture of *Agrobacterium tumefaciens* containing the viral DNA



Observe symptom development (up to 28 dpi)

Check for virus infection by PCR (21-28 dpi)

- Rapid
- Large numbers
- Highly efficient
- Uniform
- Easy to grow and maintain

Agroinoculation provides an efficient vector-independent means to screen for TYLCV (begomovirus) resistance

Introduction of another invasive virus into the DO: *Tomato chlorotic spot virus* (TCSV)

- **Unusual disease symptoms** appear in processing tomatoes in the DO in 2012
- Included stunting and distorted growth, chlorosis, mosaic/mottle and necrosis of leaves and necrotic areas on fruit
- Most **common in the North**, but by 2013 were **common in the North and South**

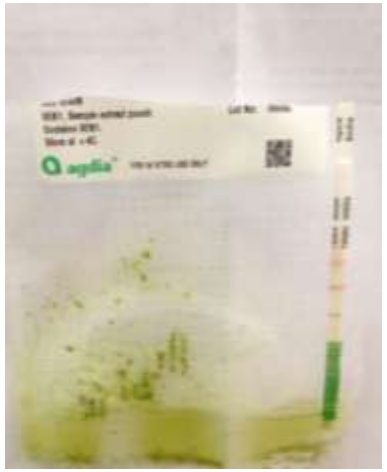
Disease symptoms were typical of tomato-infecting tospoviruses

- **Stunting**, distorted growth and plant death
- **Leaves:** chlorosis, mosaic/mottle and necrosis; necrotic shoots (strikes) late in season
- **Fruit:** bumpiness, distortion and necrosis (but not the ringspots typical of TSWV)

Tospovirus outbreak in processing tomatoes in the Dominican Republic

Initial evidence: Symptoms and association with thrips

TSWV immunostrip test



Positive for TSWV

RT-PCR for *Tomato spotted wilt virus* (TSWV)

Negative

RT-PCR Positive for *Tomato chlorotic spot virus* (TCSV)

Positive

Sequencing

98% identical to *Tomato chlorotic spot virus* (TCSV)

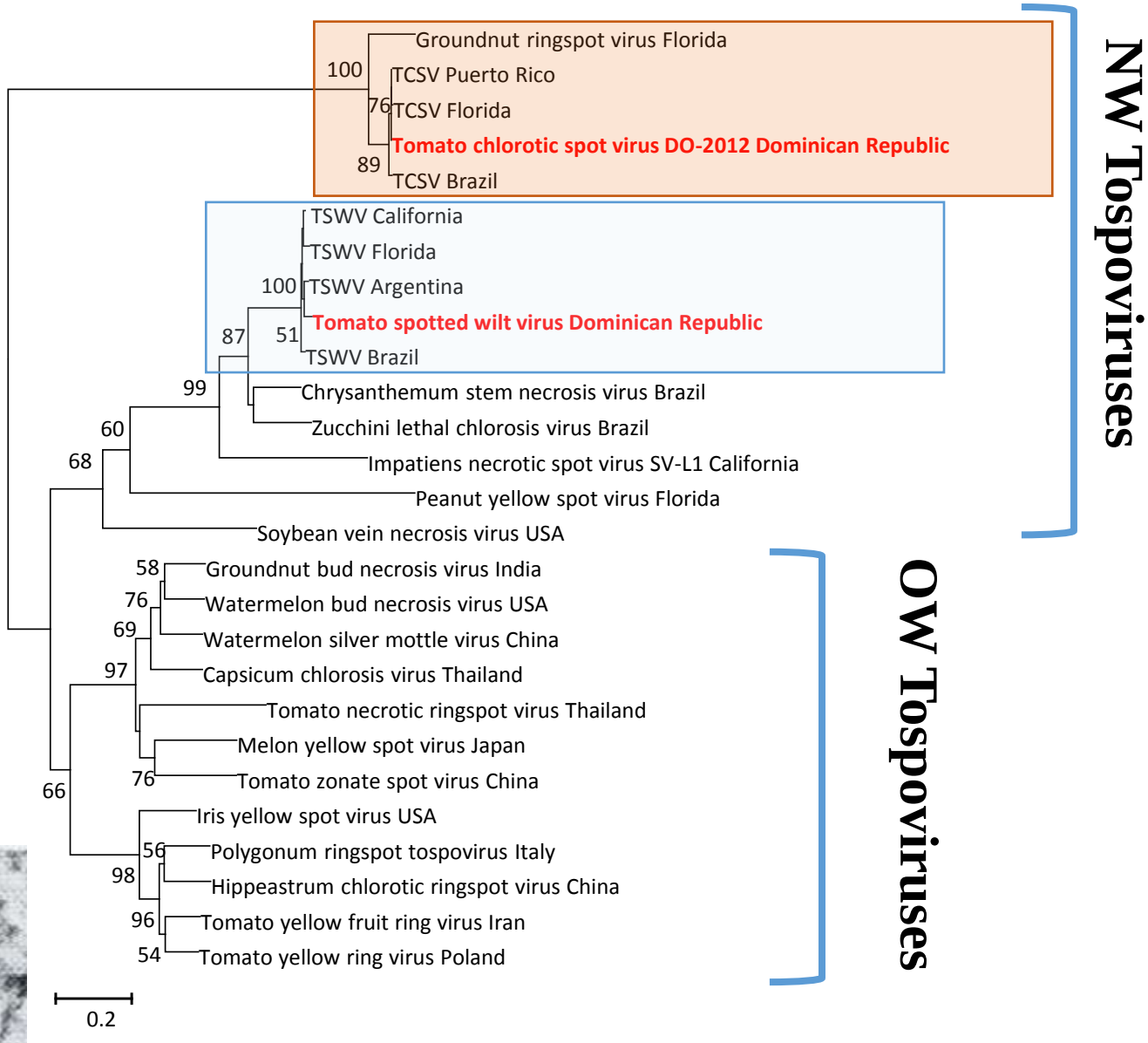
Further Sequencing

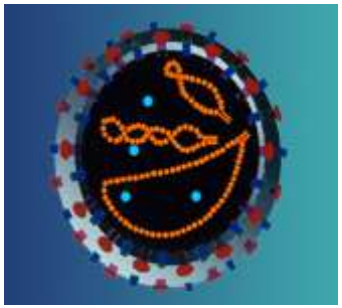
	TCSV	TSWV	GRSV
L RNA	%98	%78	%83
M RNA	%99	%82	%94
S RNA	%99	%77	%83

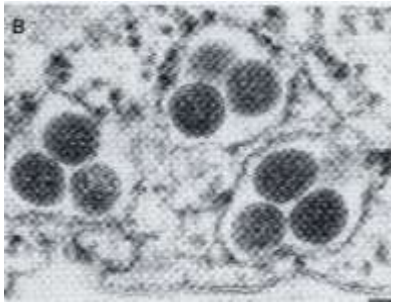
Tospovirus outbreak in DR due to the invasive TCSV!

TCSV is an invasive New World tospovirus from South America (Argentina and Brazil)

- **Tospoviruses are enveloped, spherical viruses with three ambisense RNAs**
- **Tospoviruses are widespread and have a wide host range**
- **Infect more than 1000 plant species worldwide**
- **Transmitted only by thrips**
- **Not transmitted by touching or seed**
- **Can be mechanically transmitted in the the laboratory**







Rapid spread and establishment of the invasive TCSV in the DO

- TCSV symptoms were associated with **large population of thrips**, especially *Frankliniella schultzei* (tomato thrips)
- The virus also was causing losses in **tobacco and pepper crops**
- INSV has **jumped into local weeds**, especially the common species ***Cleome viscosa*** and ***Boerhavia erecta***, which serve as inoculum sources

New methods for sample preparation

- **New methods for sample preparation** allow application of PCR/RT-PCR to samples from **diverse geographical locations** at a **central laboratory**
- **Absorption strips** (AgDia)
- **FTA cards** (Whatman)

Samples applied to FTA cards in the DO

Samples applied to absorption strips in Mali, West Africa

Introduction of TCSV into the Caribbean and Southern USA

Pepper

Tomato

Tobacco

Outbreak of *Tomato chlorotic spot virus* (TCSV) in the Dominican Republic

Weed Host Reservoirs of TCSV and Thrips

Management of TSCV in the DO

- **Management of tospoviruses** can be challenging because of the wide host range and the difficultly to control thrips
- **Resistant varieties (with *Sw-5* gene)**
  - -Ty resistance also needed
  - -Sw-5 resistance can be broken (high pressure, TCSV/TSWV, other viruses))
- **Plant thrips- and virus-free transplants**
- **Field placement**
- **Roguing** infected plants (<30 d after transplanting)
- **Monitor** thrips populations and **manage with insecticides** (challenging)
- **Weed management** impractical
- **Sanitation**
  - -Prompt removal and destruction after harvest
  - -Area wide sanitation

Future Issues

- Develop a combined IPM program for TYLCV and TCSV
- Development and availability of high yielding varieties with resistance to TYLCV and TCSV (tospoviruses)
- IPM program must be maintained to prolong the life of resistance genes
- Maintain the host-free period and monitor effectiveness
- What is the nature of the interaction of begomovirus/whiteflies and thrips and tospoviruses
- Is the *Sw-5* gene equally effective vs. all three NW tospoviruses (TSWV, TCSV and GRSV), and what is underlying the resistance breaking
- Importance of seed transmission of TYLCV

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Breeding for Resistance to Plant Viruses

- Proper identification of the virus
- Development of an efficient method for inoculating plants
  - -mechanical or sap transmission
  - -vector mediated
  - -biotechnology (agroinoculation or biolistic)
- Sources of resistance
  - -conventional (wild species, other sources)
  - -biotechnology (pathogen-derived resistance or cloned resistance genes)
- Transfer into susceptible cultivars
- Screen for resistance
  - -in the field
  - -under controlled conditions