

Biological Properties of *Tomato apex necrosis virus* (ToANV)

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ToANV from Mexico

(Sinaloa spotted wilt, Marchitez)

What kinds of viruses are they?

How are they spread?

Torrado from Europe (Spain, Poland, Hungary),
Australia and Central America (Panama)



Chocolate spot from Central America

<http://www.wur.nl/NR/rdonlyres/F5732B48-8B0D-453C-A087-C1581C46B7DC/37631/tomatenvirus.jpg>

<http://www.plantpath.wisc.edu/GeminivirusResistantTomatoes/CDR/Mar03/ChocSpot.htm>



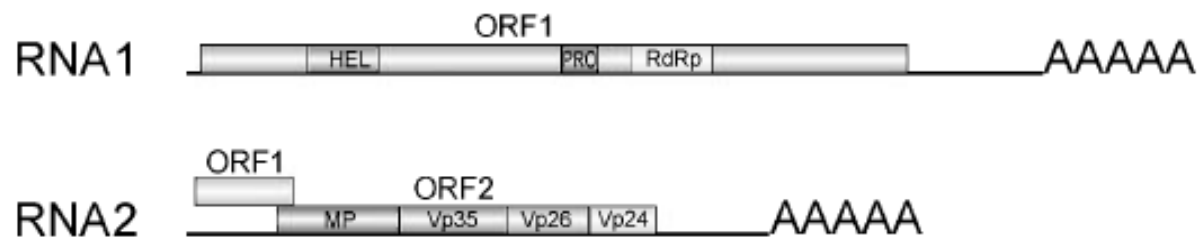


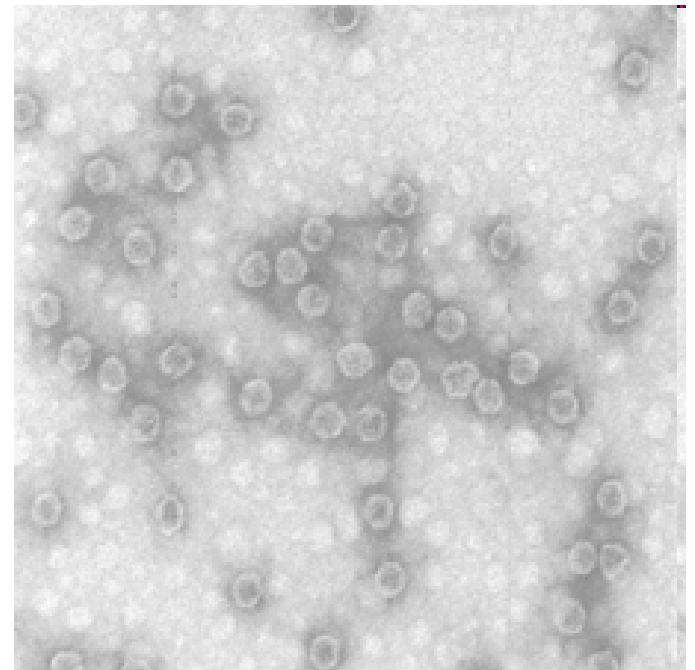
Fig. 3. Genome organization of tomato marchitez virus. Relative positions of regions containing motifs of helicase (*HEL*), protease (*Pro*), and RNA-dependent RNA polymerase (*RdRp*) on RNA1 and of the putative movement protein (*MP*) and the three coat proteins (*Vp35*, *Vp26* and *Vp24*) encoded on RNA2 are indicated

January 2007 – Verbeek et al., Torrado

August 2007 – Turina et al., ToANV

Oct 2007 – Verbeek et al., Marchitez

Oct 2007 – Pospieszny et al., July 2008 – Amari et al.,
Torrado virus transmitted by *Bemisia tabaci* and
Trialeurodes vaporariorum.



Whitefly Vectors of Plant Viruses

- *Bemisia tabaci* - sweet potato whitefly
- *Bemisia argentifolii* - silverleaf whitefly
- *Trialeurodes vaporariorum* - greenhouse whitefly
- *Trialeurodes abutilonea* - banded whitefly



There are currently **18 families** (*dae*) of plant viruses; plus **81 genera** (*virus*; not including viroids and other subviral agents).

Not all genera are within assigned families at this time, and some new genera and families are **not shown** at right.

According to Hull, (page 87) he says there are 977 species of plant viruses as of 2002, more are being identified all the time.

Within the genus *Potyvirus*, there are more than 100 definitive and tentative species.

- No true dsDNA viruses
- Retro types contain dsDNA
- **75% are ssRNA**
- Few minus & ambisense ssRNA genomes

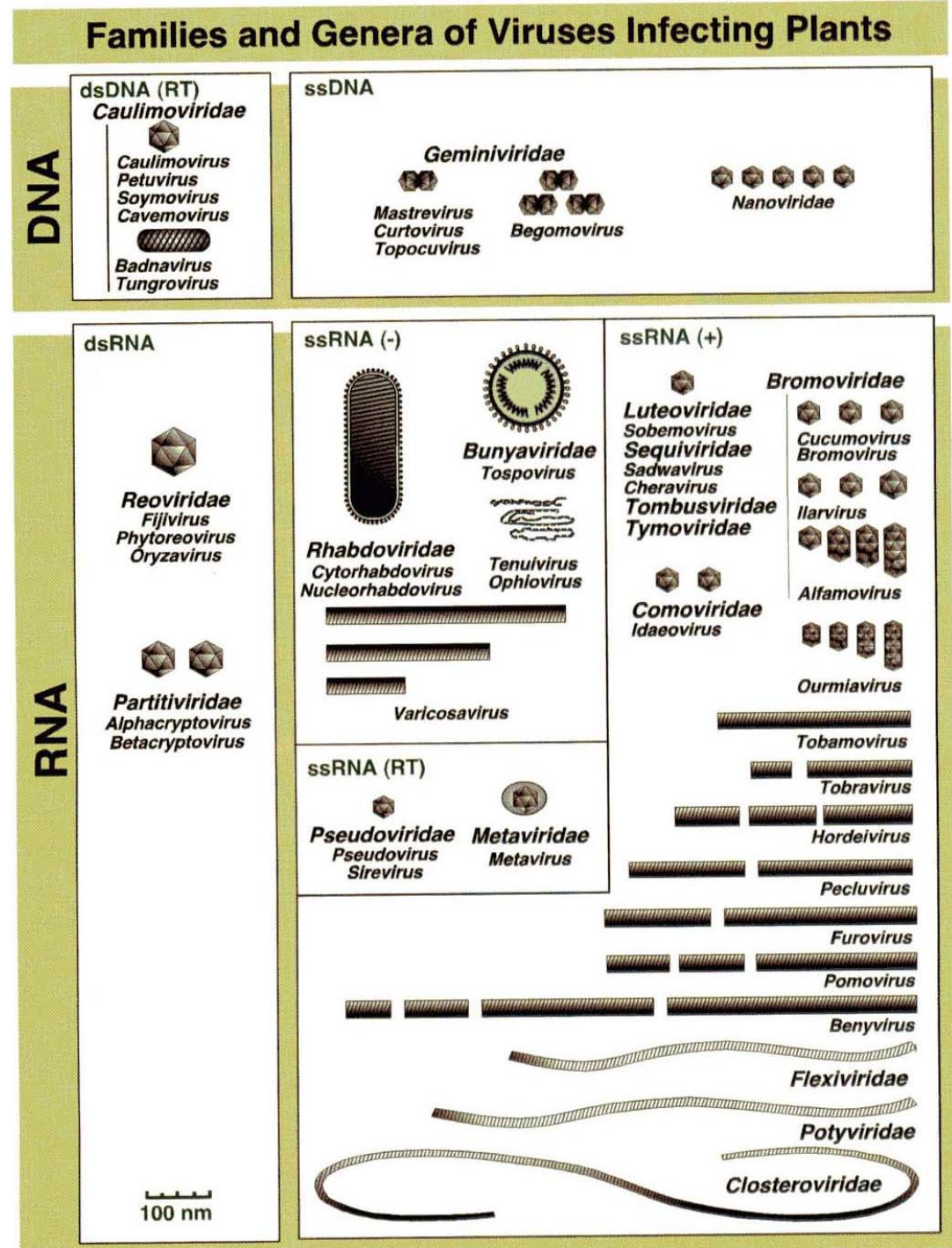
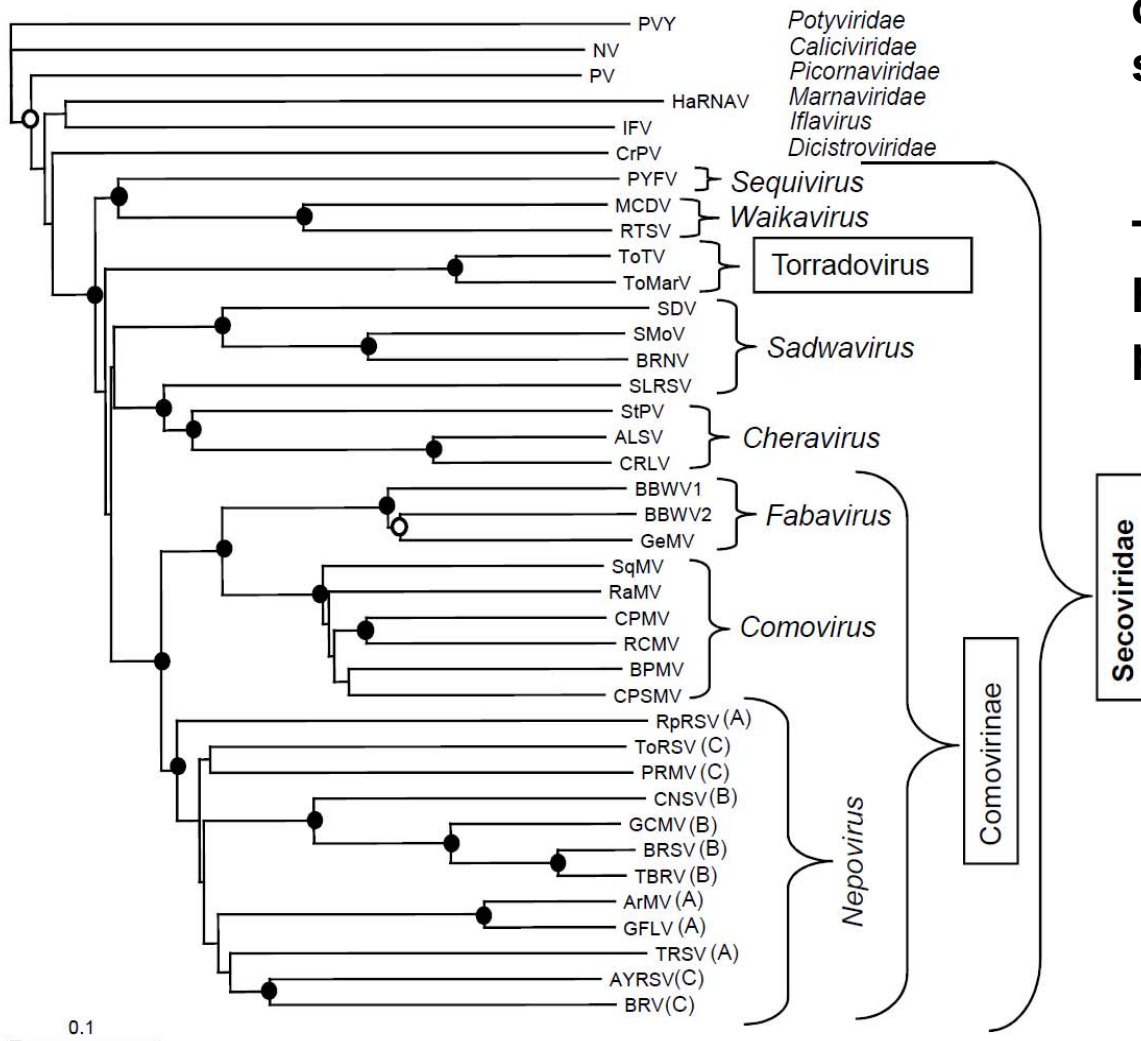


Fig 2
 Sanfacon et al (2008)
 VDN-Archives Virology



Known members of existing family *Sequiviridae* have one ssRNA chromosomal segment.

ToANV, and related viruses, have 2. New family is proposed.

Our work:

- 1) Compare ToANV isolates for their biology and genetic structure**

- 2) Develop tools for working effectively with ToANV**
 - a) Detection**
 - b) Screening**

ToANV is a quarantined pathogen for the U. S.

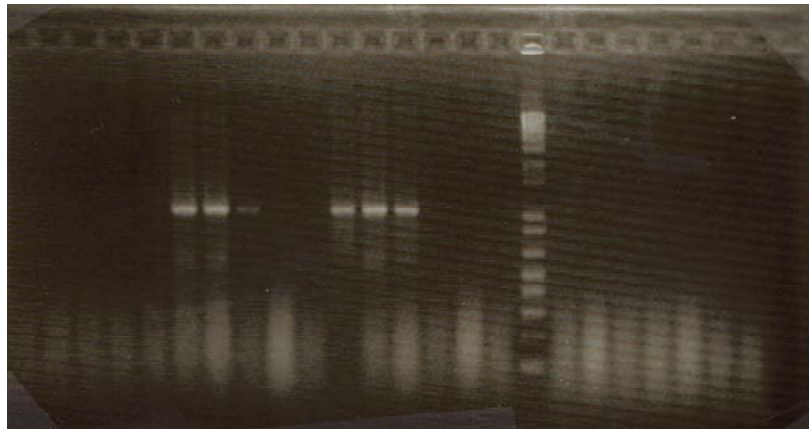
UC Davis Biosafety Level 3P, Contained Research Facility

Partial List of Current Projects in the UCD CRF:
Comparative host range testing of 3 *Diohabda elongata* (Coleoptera: Chrysomelidae) populations
RNA-interference and control of the glassy-winged sharpshooter (*Homalodisca vitripennis*) and other leafhopper vectors of Pierce's disease
Tomato apex necrosis virus
Selection conflicts between *Zyginidia pullula* and its feminizing *Wolbachia*
Resistance and susceptibility to *Cucurbit yellow stunting disorder virus*
Light brown apple moth
Exotic Tospo- and Begomoviruses



ToANV Primers and RT-PCR

| <u>Primer Name</u> | <u>Sequence</u> | <u>Target</u> | <u>Product</u> |
|--------------------|---------------------------|---------------|----------------|
| ToANVRNA2GF | CAAGGAGAGAGTTGTGGAGTTGGTC | RNA2 | |
| ToANVRNA2GR | GTAGCTCCAGTTCCTCATCAAGTG | RNA2 | 795 |



We have collected about 30 isolates from some different regions of Mexico. We plan to compare them biologically, but also genetically.

ToANV is mechanically transmissible, but not easily to all host plants, including tomatoes. We have spent considerable time testing inoculation conditions including buffers, rubbing, air brush/compressed air.



Air gun inoculation.

Sometimes 100% efficient and symptoms 5 days post inoculation!



Some biology/transmission/resistance studies

Table 1. Symptoms of tomato marchitez virus (ToMarV) and tomato torrado virus (ToTV) on experimental host plants

| Tested experimental host plants | Symptoms (local/systemic) | |
|--|---------------------------|--------------------|
| | ToMarV | ToTV |
| <i>Chenopodium quinoa</i> | nl/noi | –/noi |
| <i>Gomphrena globosa</i> | n.t. | –/noi |
| <i>Nicotiana benthamiana</i> | –/c, mf | –/c, mf |
| <i>Nicotiana clevelandii</i> | –/c | –/c |
| <i>Nicotiana glutinosa</i> ‘PRI’ | occasionally cl/c | –/c |
| <i>Nicotiana glauca</i> ‘67A’ | nl/c, n, mf | nl/c, n, mf |
| <i>Nicotiana occidentalis</i> ‘P1’ | nl/c, n, mf | nl/c, n, mf |
| <i>Nicotiana rustica</i> | –/la | –/la |
| <i>Nicotiana tabacum</i> ‘white burley’ | occasionally cl/la | –/la |
| <i>Physalis floridana</i> | nl/mo | nl/c, n, mf, do |

c Chlorosis; *cl* chlorotic lesions/rings; *do* die-off; *la* latent infection (verified by inoculation on indicator plants); *mf* malformation; *mo* mottle; *n* necrosis; *noi* no infection (verified by inoculation on indicator plants); *nl* necrotic lesions; *n.t.* not tested; – no symptoms.



Mexican, or Mayan, husk tomato, *Physalis ixocarpa* Brot. (syn. *P. aequata* Jacq.)

Is ToANV, like Torrado virus, transmitted by whiteflies?

***Bemisia tabaci* - sweet potato whitefly**





***Bemisia tabaci* on tomatillo**





Inoculation date: 5/15/2009

Method: Whitefly,
Bemisia tabaci

Buffer:N/A

Inoculum:
N/A Mechanically inoculated Tomatillo

PSI: N/A



* Tomatoes were planted on 4/30/09, and transplanted on 5/12/2009

*Three whitefly cages, 8 H 3, 8 H 4, 8 H 5, 3 HM1, and 3-4 HMX5892 in each cage. Tomatillo as inoculum.

| | 5/18/09 | 5/19/09 | 5/20/09 | 5/20/09 | 5/20/09 | 5/26/09 | 5/29/09 |
|-----------------|---------|---------|---------|---------|---------|---------|---------|
| H 3 | 0/24 | 5/24 | 11/24 | 13/24 | 15/24 | 23/24 | 24/24 |
| H 4 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 |
| H 5 | 0/24 | 3/24 | 10/24 | 13/24 | 14/24 | 20/24 | 23/24 |
| HM 1 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 | 0/12 |
| HMX 5892 | 2/13 | 5/13 | 6/13 | 7/13 | 9/13 | 12/13 | 13/13* |

* 1/13 had no symptoms, but was RT-PCR positive

We can screen 4 lines per month, due to space limitations.

RT-PCR and symptoms so far give the same results.

BUT.....

Why did these “new” viruses show up at similar times in different locations?

How quickly do they evolve? They have two chromosomes.