BRITISH TOMATO CONFERENCE 2019

26 SEPTEMBER

CHESFORD GRANGE • KENILWORTH • WARWICK • CV8 2LD

Tomato brown rugose fruit virus (ToBRFV) Current status and perspectives

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The beginning of Virology

•Adolph Mayer described **“the mosaic disease of tobacco”**

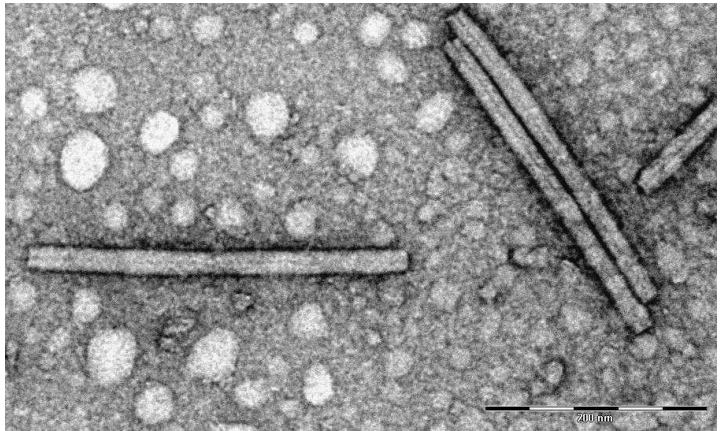
(published in 1886) **tobacco mosaic disease**

Plant virology emerged from the landmark *Tobacco mosaic virus* (TMV) studies by Mayer, Ivanowski, and Beijerinck Beijerinck was the first to use the term virus in a modern context.

•Cucumber mosaic diseases (1-4) reported in 1935, **UK.**
“**MOSAIC DISEASES OF THE CUCUMBER”, Ainsworth, 1935.**
Cucumber green mottle mosaic virus (CGMMV)

Tobamovirus genus

- **Tobacco mosaic virus (TMV)**
- **Tomato mosaic virus (ToMV)**
- **Tomato brown rugose fruit virus (ToBRFV)**
- **Tomato mottle mosaic virus (ToMMV)**
- **Cucumber green mottle mosaic virus (CGMMV)**
- Pepper mild mottle virus (PMMoV)
- Paprika mild mottle virus (PaMMV)



37 species:
Transmitted by contaminated seeds, mechanical contact, preserved infectious in soil

Tobamoviruses and resistance genes in vegetables





Cucumber green mottle mosaic virus (CGMMV)

Tomato brown rugose fruit virus (ToBRFV)

Pepper mild mottle virus (PMMoV)

Resistance genes in commercial vegetable varieties

Cucurbits	**Tomato**	**Pepper**
*Ineffective R genes **Tolerant rootstocks	*Tm-2²* ✗	*L⁴* ✓

Breaking *Tm*-2^2 resistance in tomatoes
First report in October 2014

Disease symptoms of ToBRFV

Disease symptoms of ToBRFV

Disease symptoms of ToBRFV



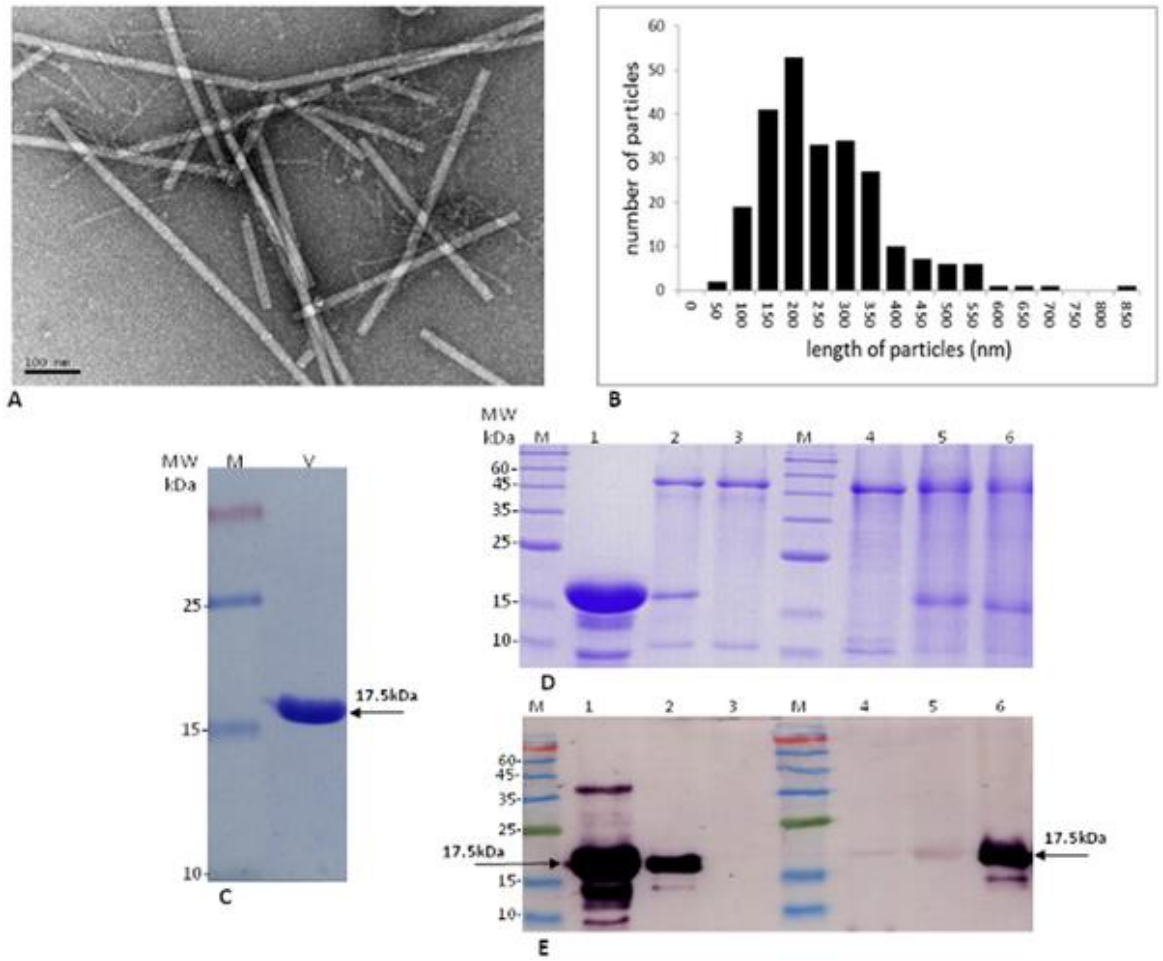





(Luria et al., 2017)

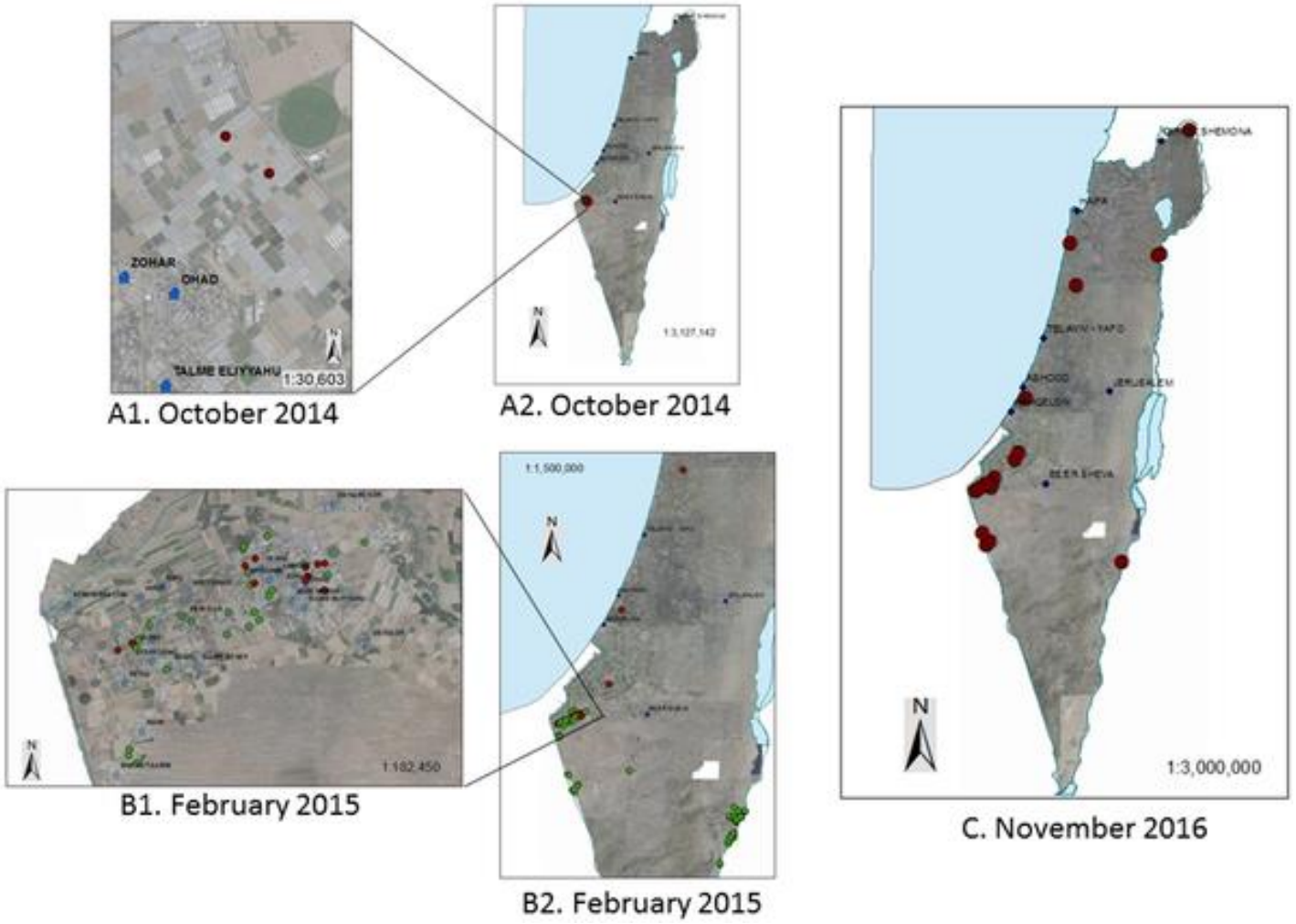
ToBRFV detection in tomato plants

Morphological and serological characterization of viral particles and coat protein.



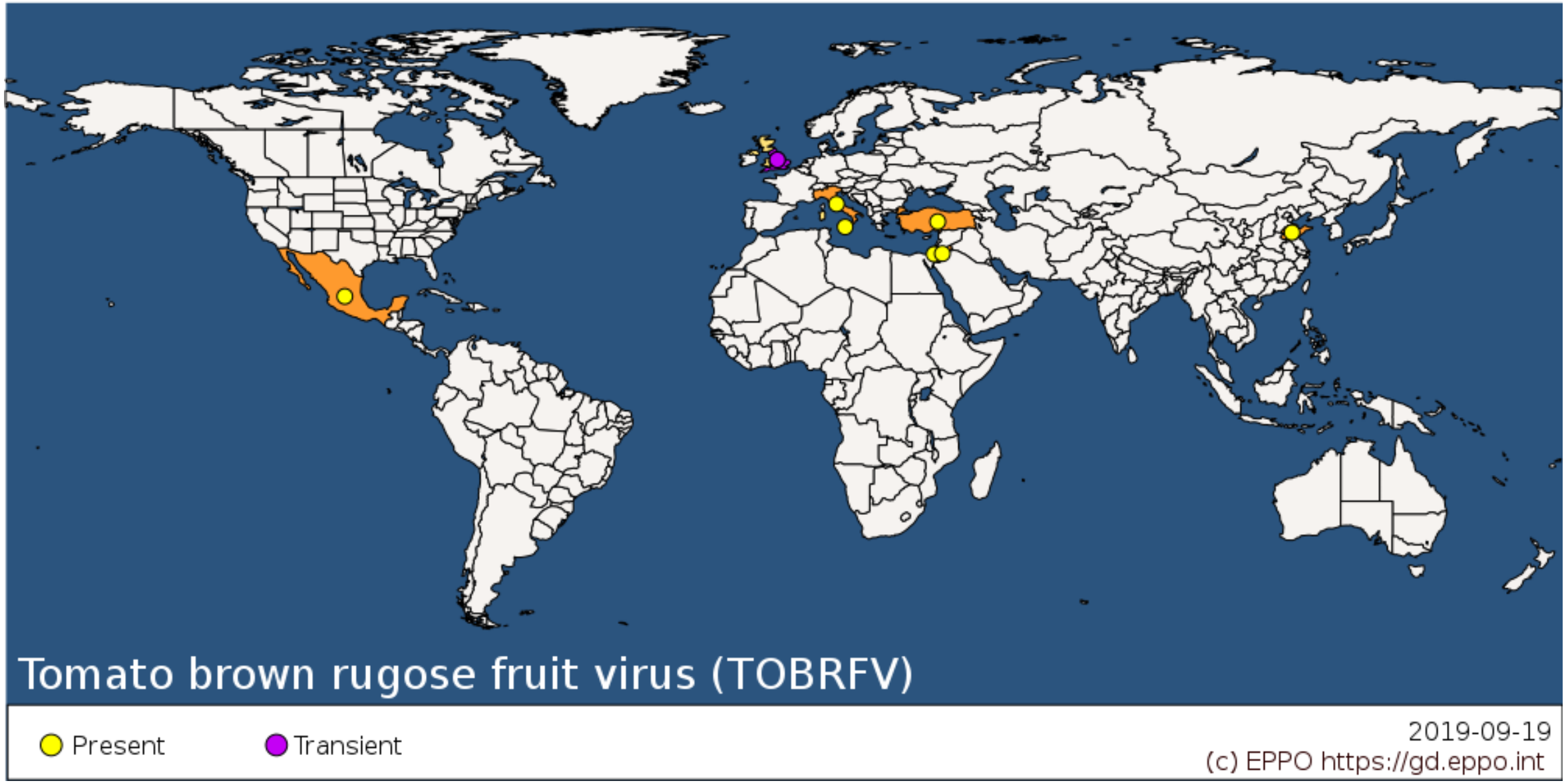
Luria et al, (2017). A New Israeli Tobamovirus Isolate Infects Tomato Plants Harboring *Tm-22* Resistance Genes. PLOS ONE 12(1): e0170429. https://doi.org/10.1371/journal.pone.0170429. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170429

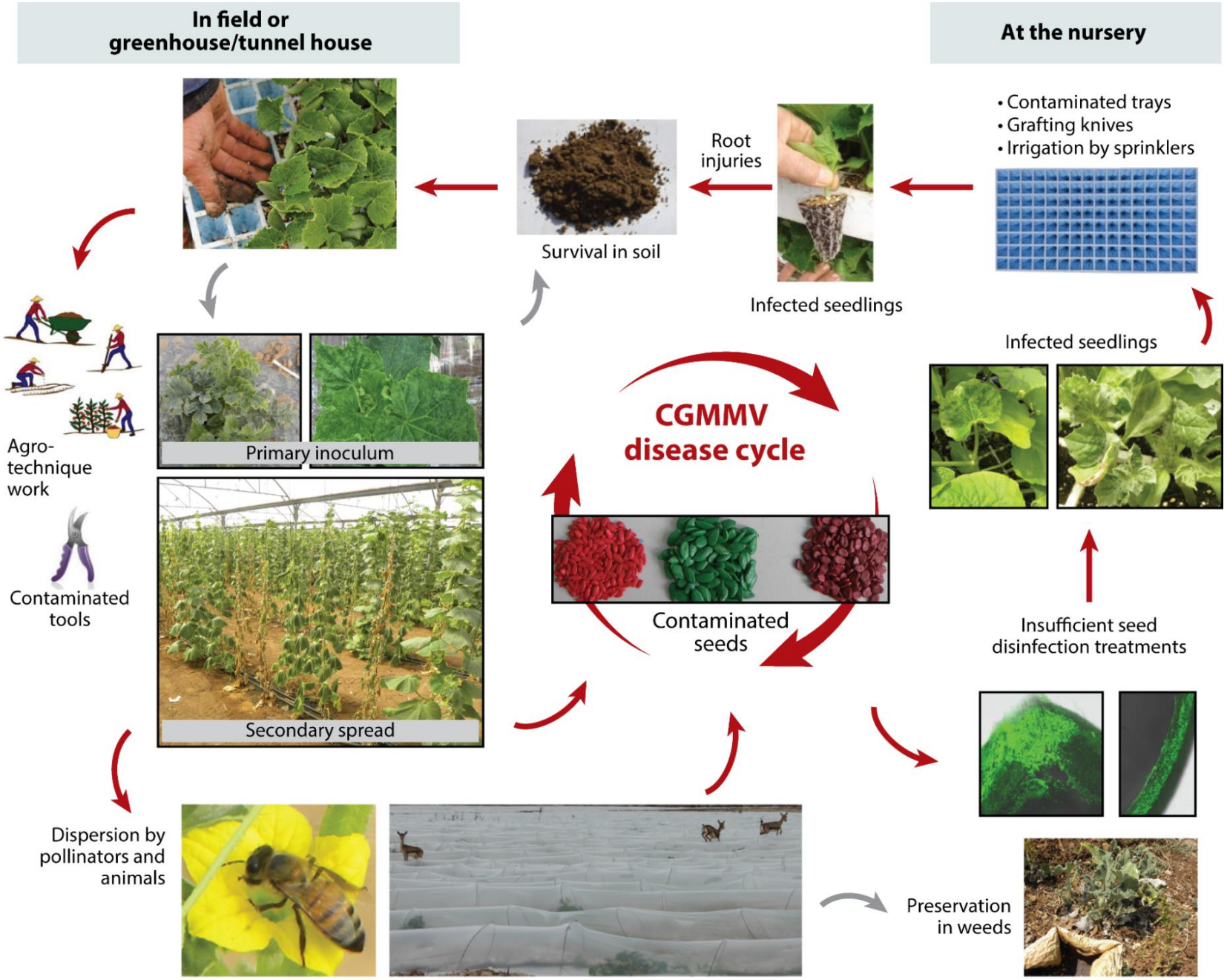
Monitoring the distribution of ToBRFV disease in tomatoes grown in greenhouses in Israel



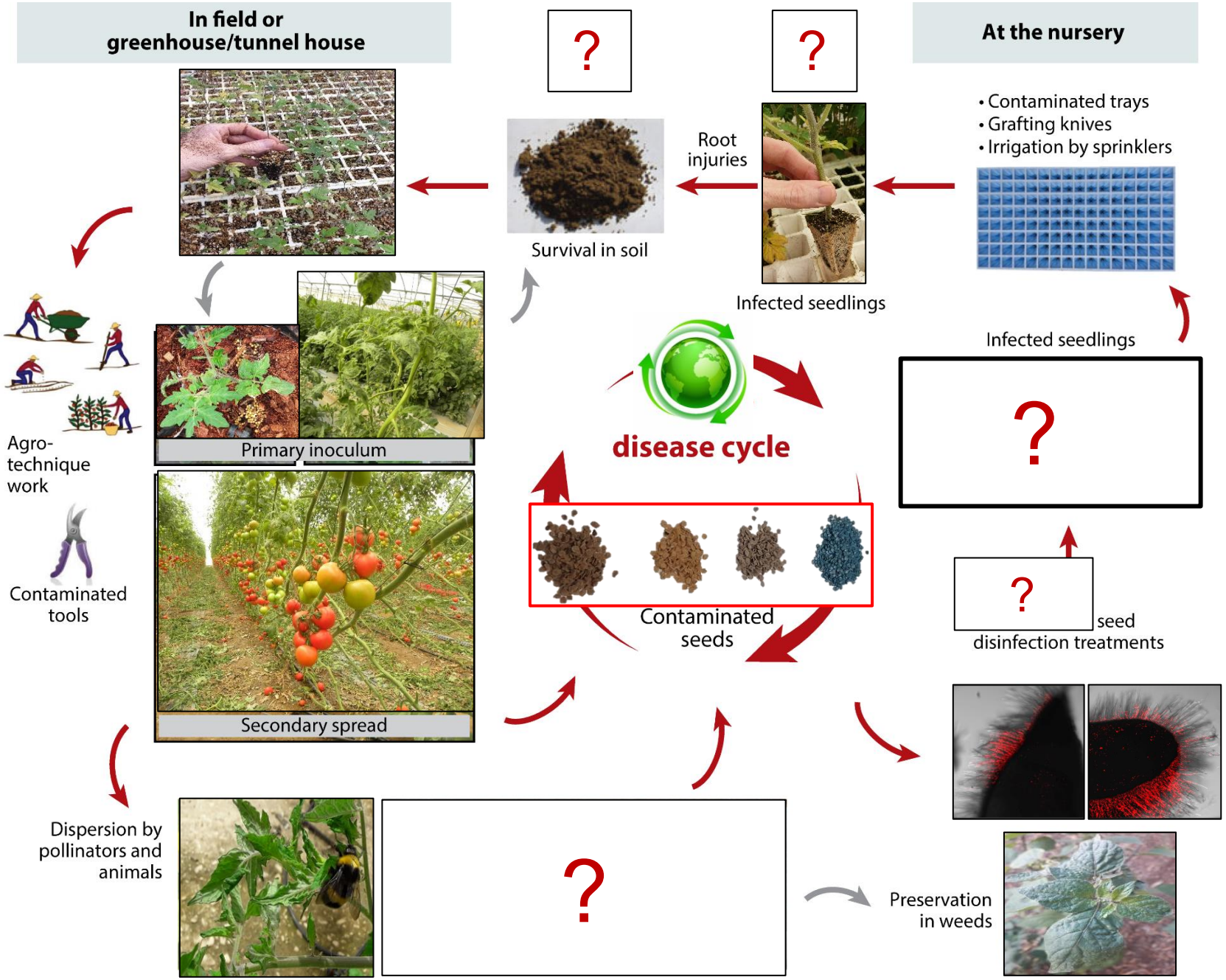
Luria, et al. (2017) A New Israeli Tobamovirus Isolate Infects Tomato Plants Harboring *Tm-2²* Resistance Genes. PLOS ONE 12(1): e0170429. https://doi.org/10.1371/journal.pone.0170429.http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170429

EPPO Global Database
ToBRFV





Dombrovsky A, et al. 2017.
Annu. Rev. Phytopathol. 55:231–56


In field or greenhouse/tunnel house
?
?
At the nursery
• Contaminated trays
• Grafting knives
• Irrigation by sprinklers
Root injuries
Survival in soil
Infected seedlings
Infected seedlings
disease cycle
?
Agro-technique work
Primary inoculum
Contaminated tools
Contaminated seeds
?
seed disinfection treatments
Secondary spread
Dispersion by pollinators and animals
?
Preservation in weeds

ToBRFV modes of spread

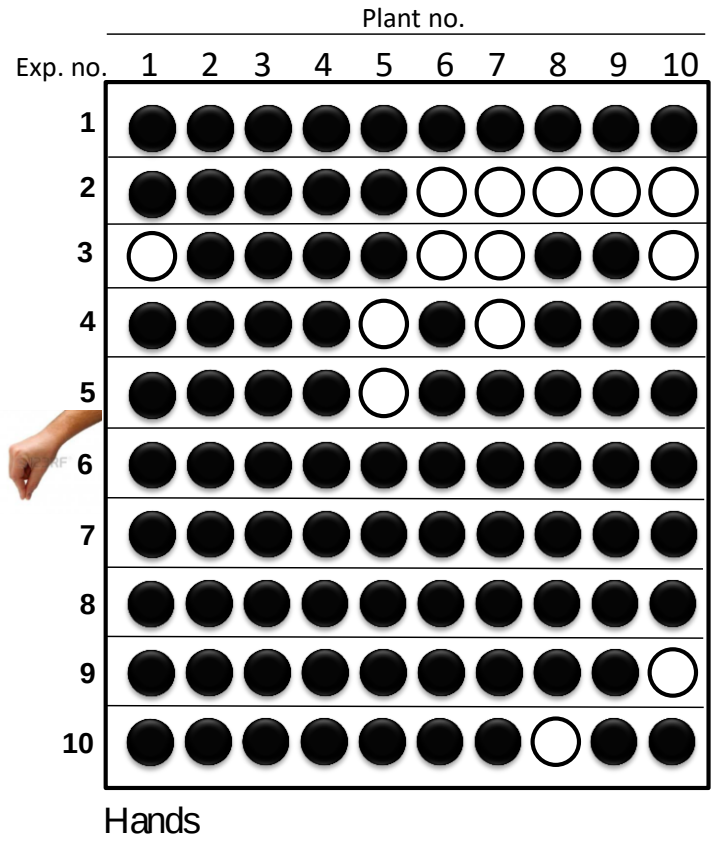
Secondary spread

1. Tools, hands…
2. Pollinator insects

Primary Inoculum sources

1. Seeds
2. Soil
3. Weeds

CGMMV-mechanical transmission - hands IR ≥ 5

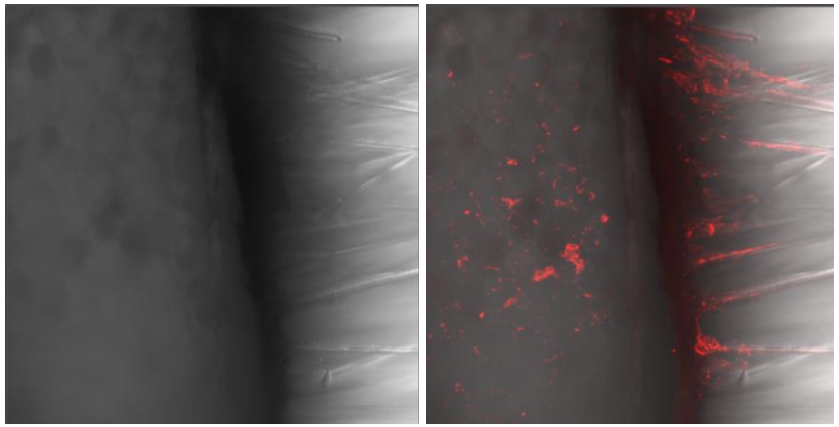


Transmission by hands 86%±0.5

Reingold *et al.*, 2015

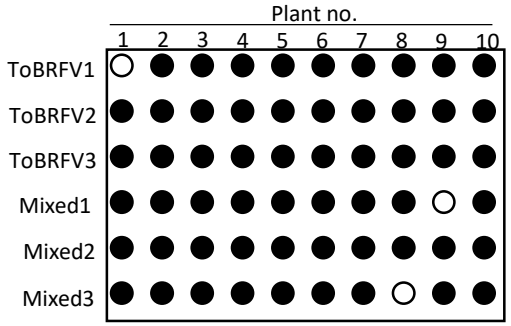
IR =Infectivity rate

Mechanical transmission of ToBRFV and PepMV



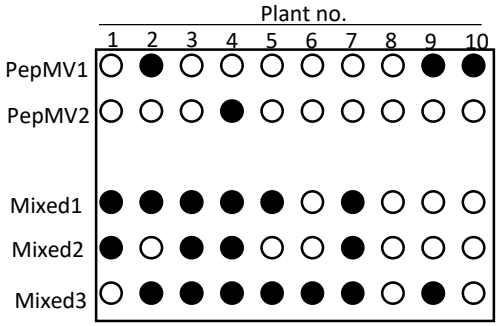
IR ≥ 9/ mixed ≥9

ToBRFV



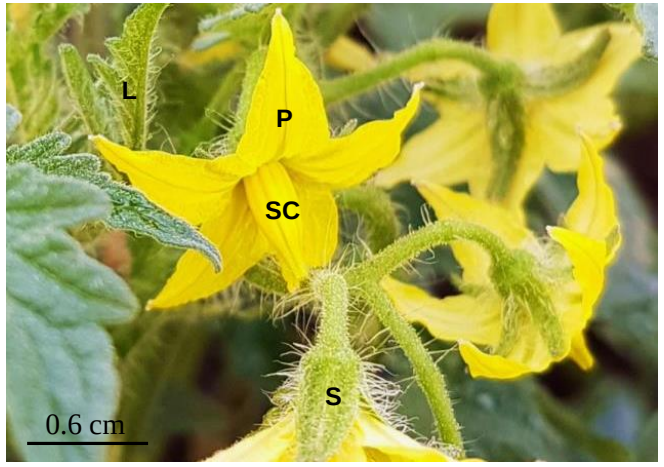
IR ≥ 1/ mixed ≥4

PepMV

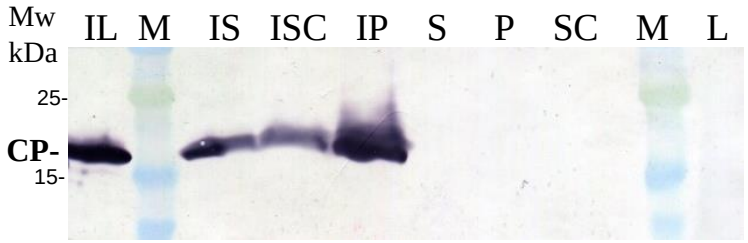


IR =Infectivity rate

The bumblebee *Bombus terrestris* contributes to *Tomato brown rugose fruit virus* spread in tomatoes



(a)

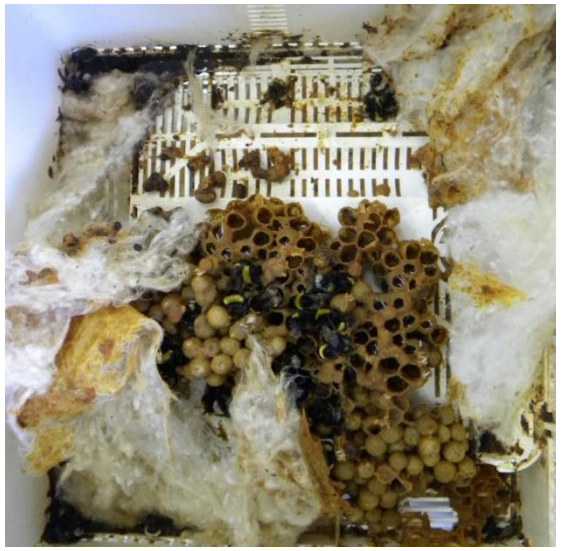


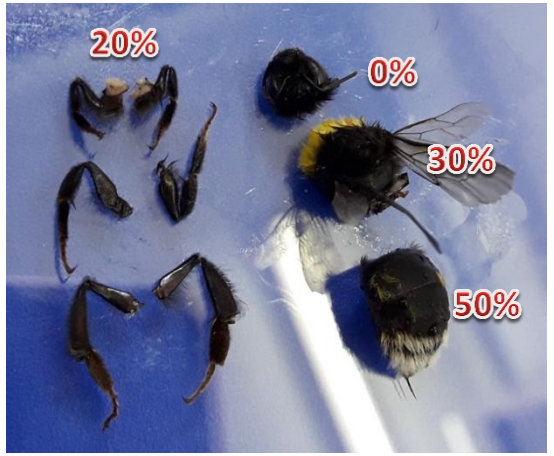
(b)

I, ToBRFV infected plant tissue parts, **L**- leaves, **S**- sepal **P**- petal, **SC**-stamen encompassing the carpel

Levitzky et al, (2019). The bumblebee *Bombus terrestris* carries a primary inoculum of Tomato brown rugose fruit virus contributing to disease spread in tomatoes. PLOS ONE 14(1): e0210871. https://doi.org/10.1371/journal.pone.0210871. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0210871

The bumblebee *Bombus terrestris* contributes to ToBRFV spread in tomatoes





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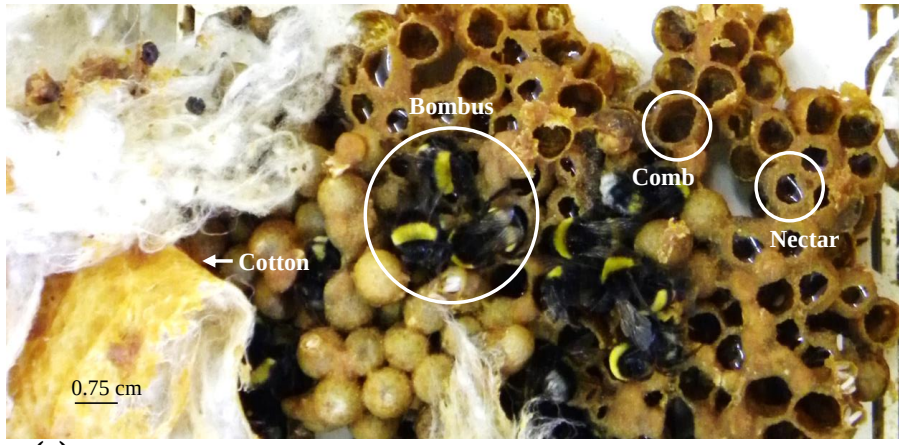
PLOS ONE



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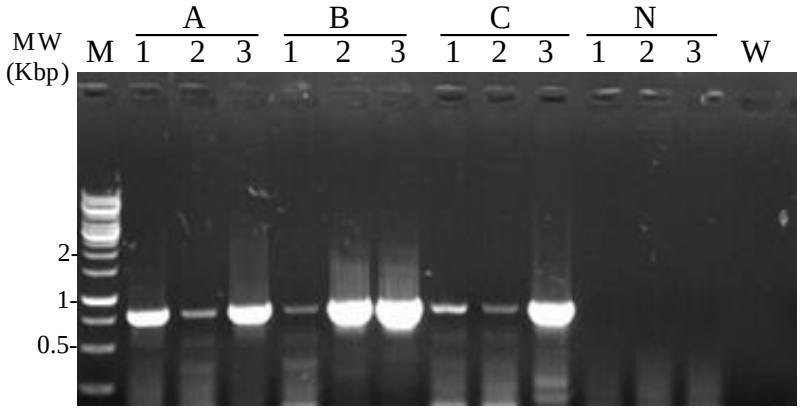

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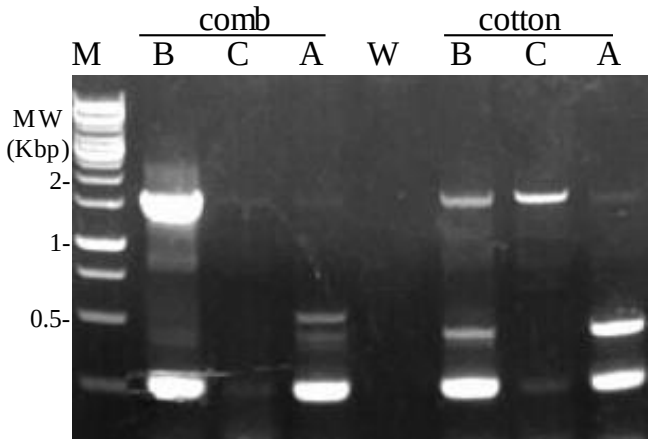

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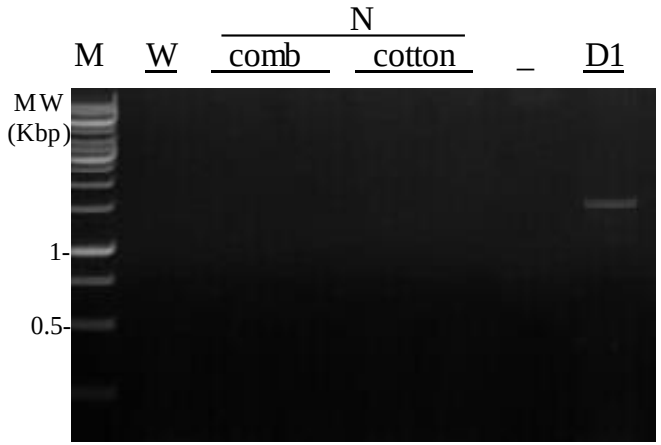
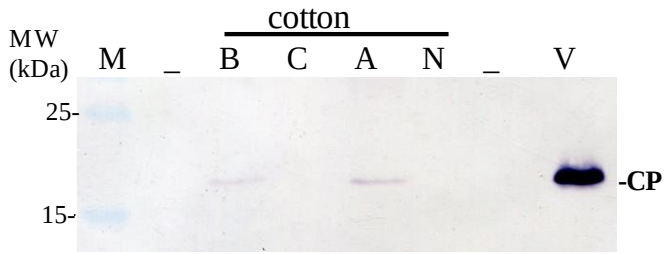


(d)



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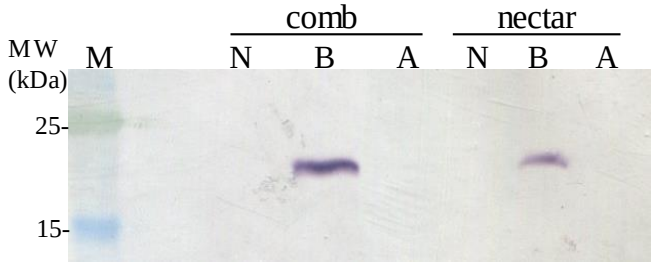

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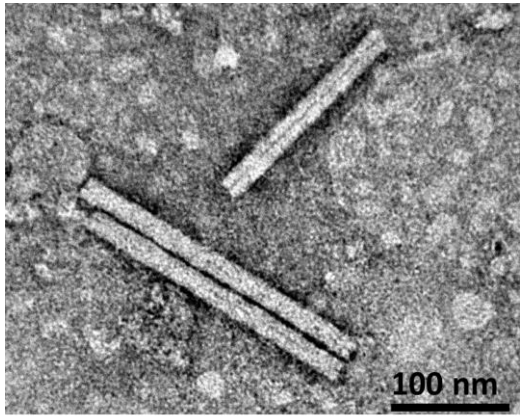
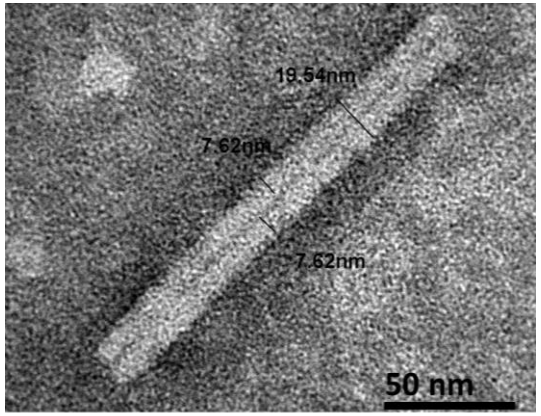


(g)



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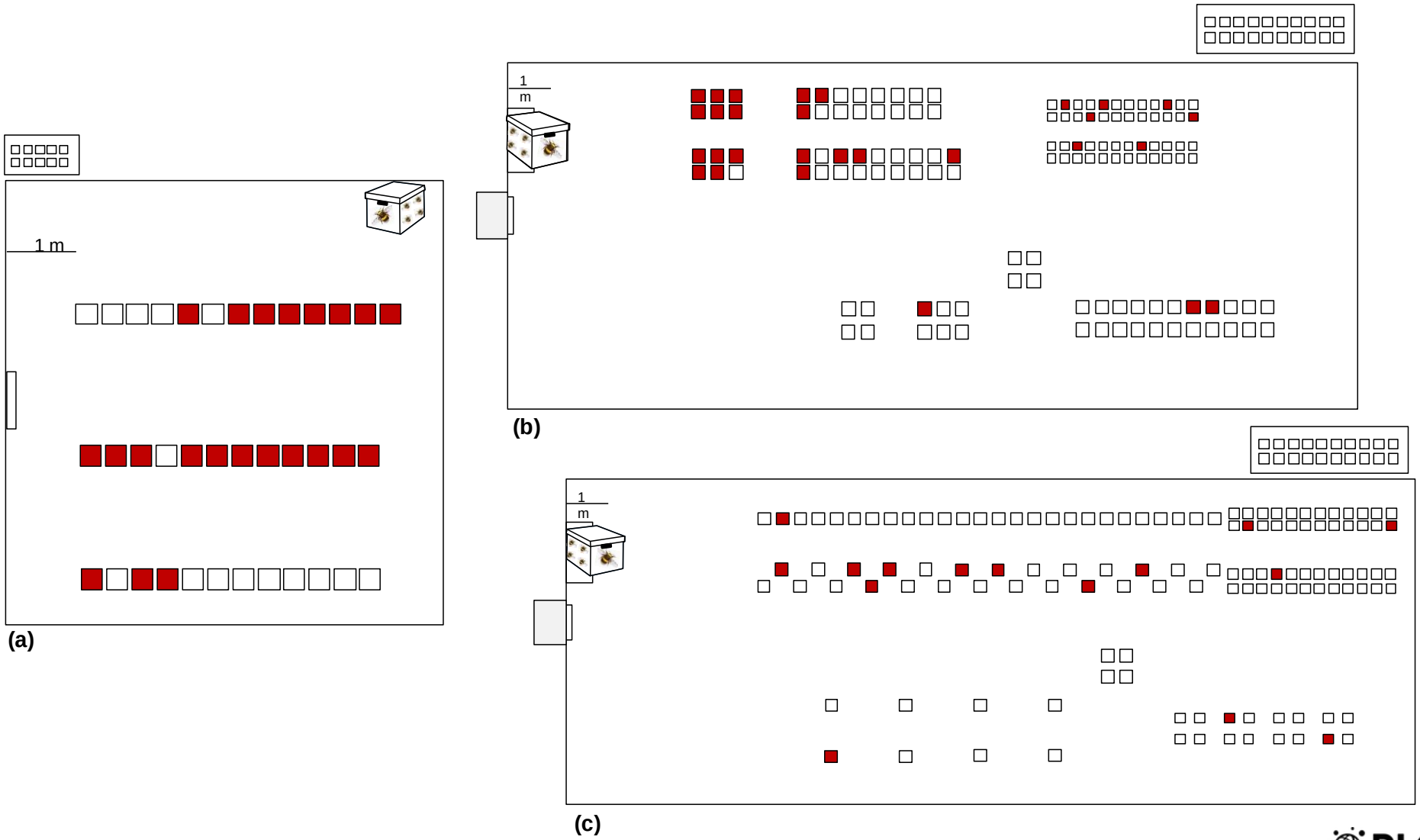
The bumblebee *Bombus terrestris* contributes to ToBRFV spread



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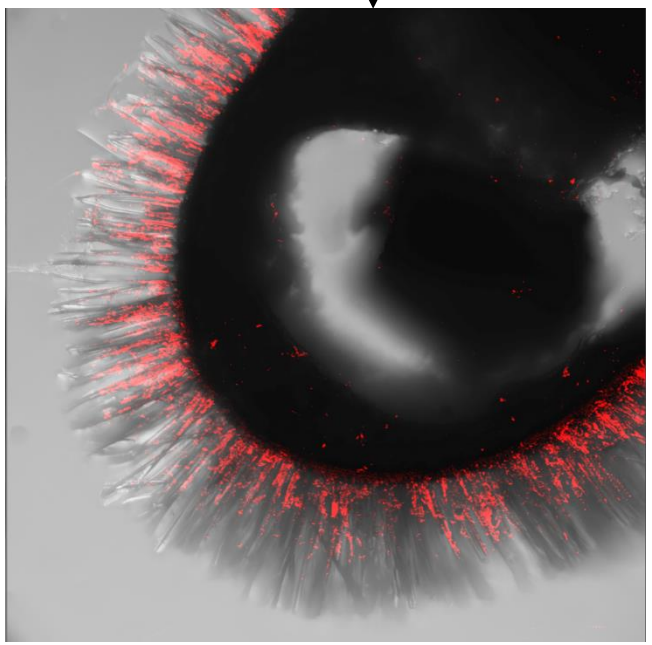
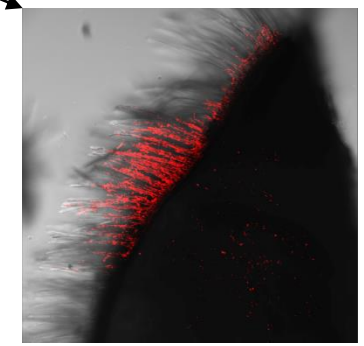
Primary Inoculum sources

1. Seeds
2. Soil
3. Weeds

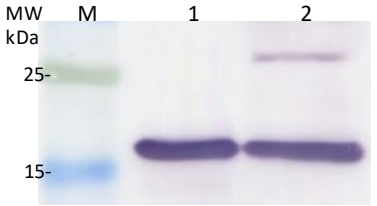
ToBRFV detection in tomato seeds

(Serology-based methods)

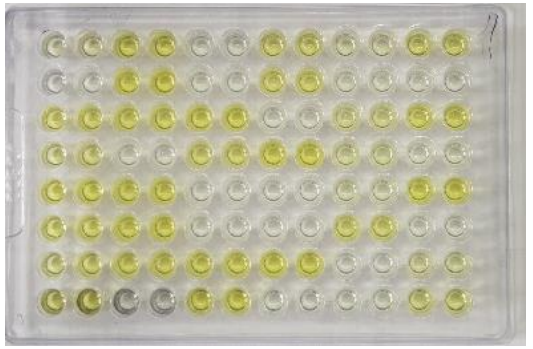




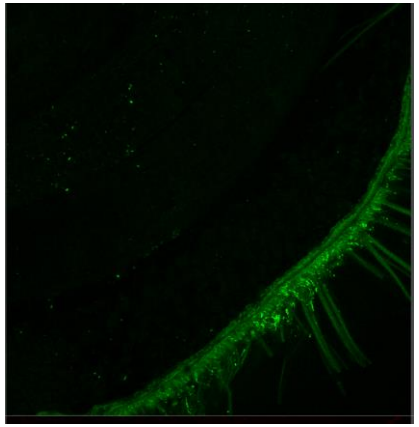
Alexa 594



Western blot

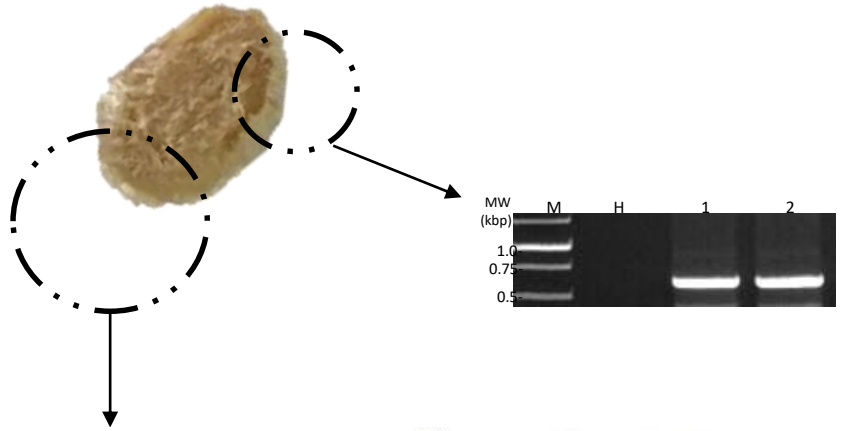


ELISA

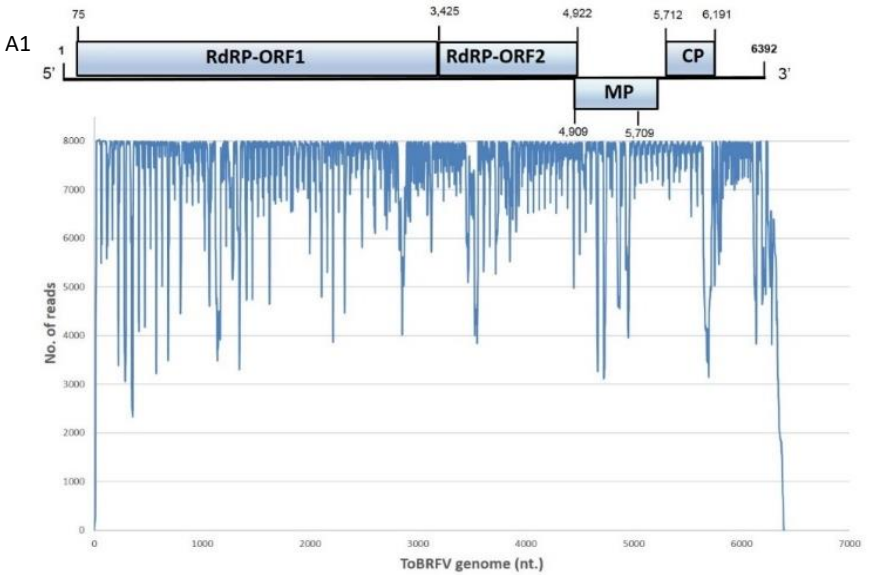


Alexa 488

Current study: ToBRFV detection in tomato seeds
(molecular and high-throughput sequencing technologies)



A1



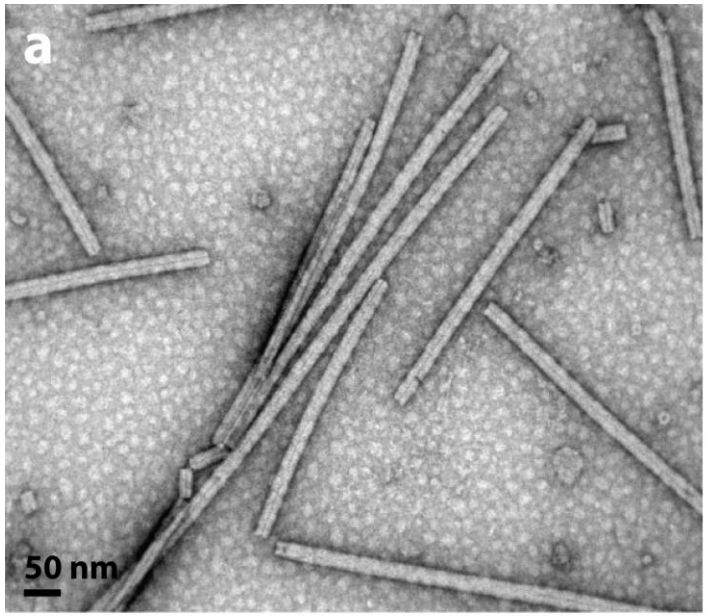
A2

Chalupowicz et al., 2018

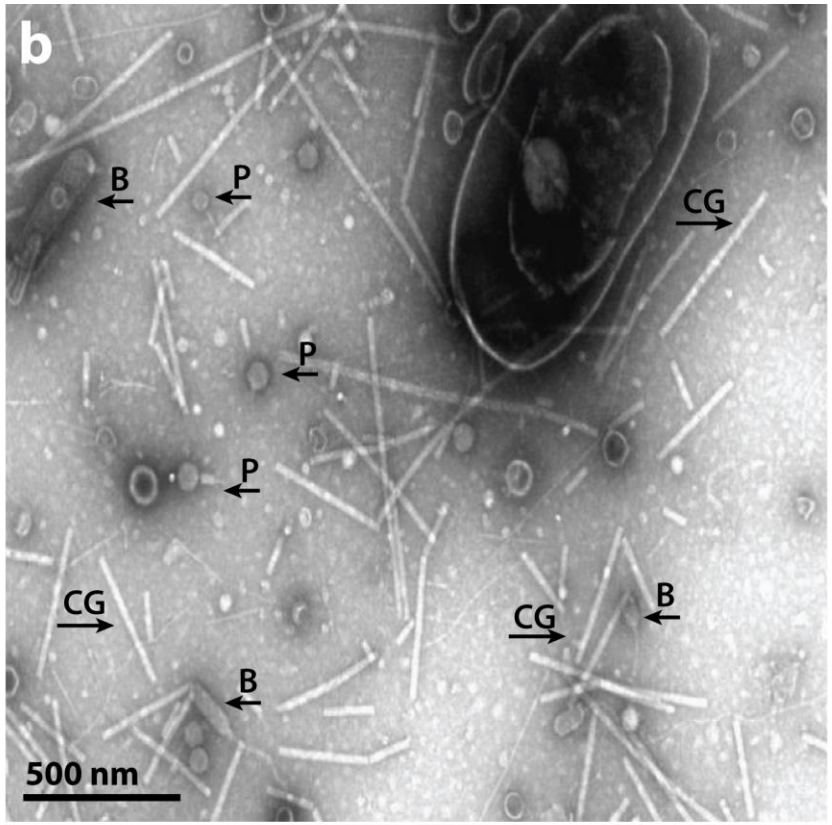
Soil examination for CGMMV particles

Virion purifications followed by visualization of viral particles by TEM

Plant



Soil



Dombrovsky A, et al. 2017.
Annu. Rev. Phytopathol. 55:231–56

Soil disinfection of tobamoviruses

1. Using intermediate media

These viruses are able to enter the plant cells through the wounded roots

Careful planting

vs

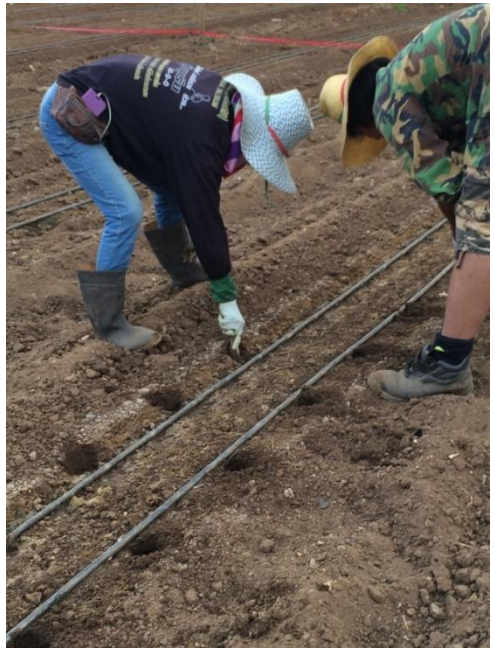
Regular planting

Careful planting

Picture credit: Sigal Perez

Soil disinfection using stabilized chlorine

Planting pit preparation



Application before planting

2,000 ppm. for soil disinfection

Application in large scale via the irrigation system one day prior to planting.

1,000 ppm. for structure disinfection

Studying the efficiency of disinfectants

South R&D center (2016-2019)

Virus inoculum

Application of
the disinfectant

Planting

Comparison between three formulations of stabilized chlorine on ToBRFV infection in tomatoes

Treatment	Rep. 1	Rep. 2	Rep. 3	Total	Infectivity %
Klor Bac	2/50	6/50	8/50	16/150	AB 10.7
ChloRun	2/50	5/50	2/50	9/150	B 6
Taharn	4/50	3/50	1/50	8/150	B 5.3
Positive control	31/50	20/100	46/100	97/250	A 38.8
Negative control	1/50	--------	---------	1/50	2

ToBRFV in tomatoes

CGMMV in cucumber

Treatment	Rep. 1	Rep. 2	Rep. 3	Infectivity %
Stabilized hydrogen peroxide	4/50	2/55	6/46	7.9
GreenUp-D	0/52	0/48	0/49	0
Taharan	0/53	0/52	0/51	0
Control wounded roots	6/52	5/57	17/51	17.5
Control not wounded	3/51	4/51	--------	6.9
Negative control	0/53	-------	---------	0

Treatment	Rep. 1	Rep. 2	Rep. 3	Infectivity %
Stabilized hydrogen peroxide	2/48	1/50	3/49	4.1
GreenUp-D	2/46	0/49	0/49	1.4
Taharan	0/52	0/50	0/47	0
Control wounded roots	13/52	9/50	1/37	15.2
Control not wounded	1/50	0/52	---------	1
Negative control	0/51	--------	--------	0

Ongoing studies on ToBRFV

Genetic resistance in tomato to ToBRFV

Moshe Lapidot and Ilan Levin

Institute of Plant Sciences
Volcani Center, ARO, ISRAEL

Plant Sciences
Avner Zinger
Dana Gelbart
Ben Avni
Zion Machbash

Hebrew University
Dani Zamir

Screening for genetic resistance to ToBRFV

❖ To identify and characterize ToBRFV-resistant tomato genotypes,

~200 tomato genotypes were screened for viral resistance.

Results:

❖ A relatively large number of tolerant genotypes were identified.

❖ A single resistant genotype was identified.

Susceptible

Tolerant (T)

X

F_1 ⟶ F_2

❖ The tolerance trait is recessive and segregated as a **single recessive gene**

Susceptible

Resistance (R)

X

F_1

F_2

❖ **Resistance is polygenic** - it is controlled by at least two interacting loci: one is recessive and the other in semi-dominant.

Development of ToBRFV resistance in *Solanum lycopersicum via* genome editing

Michael Kravchik, Bekele Abebie, Yula Shnaider

Reenu Kumari, Diana Leibman

Amit Gal-On

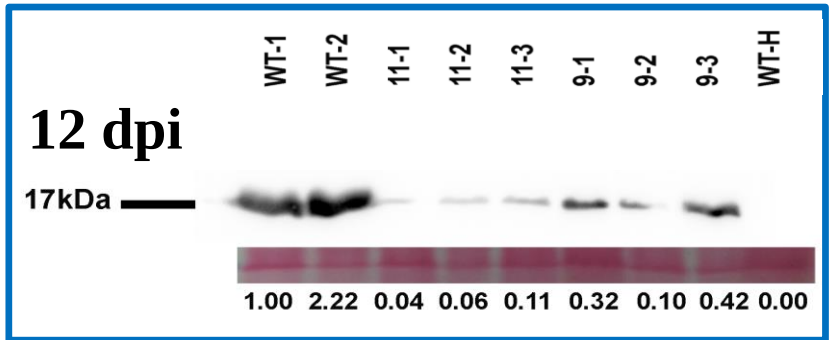
Department of Plant Pathology
ARO Volcani Center, Israel

Schematic representation of *SlTOM1* and *SlTOM3* genomic map and the sgRNA and target sites

Analysis of tomato lines for mutation within the target genes


Wt-H
wt-Inf
#11-inf
#9-inf
70 mm


WT_H
WT_I
#11
#9
10 mm


12 dpi
WT-1
WT-2
11-1
11-2
11-3
9-1
9-2
9-3
WT-H
17kDa
1.00 2.22 0.04 0.06 0.11 0.32 0.10 0.42 0.00

ToBRFV host range

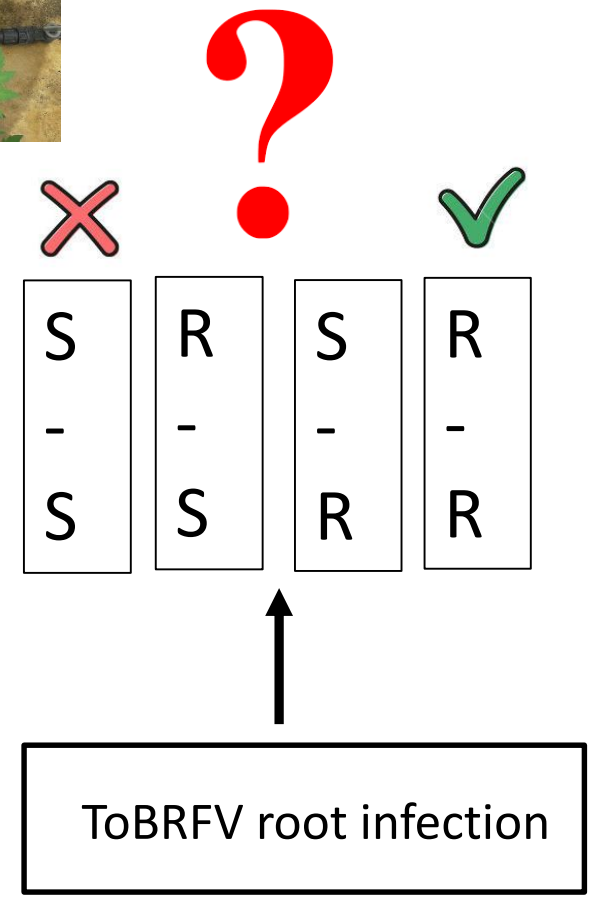
Host plant	Early symptoms 4–7 dpi	Systemic symptoms description 7–14 dpi	ELISA
Cappsicum annumm **(pepper) *Cv's Maor, (*** L^1 **), Fiona (*** L^3, ***Sw-5*****), Romans and Lyri (*** L^4, ***Sw-5*****)**	HR	NS	+
Chenopodium murale	YNL, BNL	MM, LL	+
C. amaranticolor	YNL	NS	+
C. quinoa	YNL	NS	+
Datura stramonium	YNL	NS	-
Nicotiana benthamiana	NL	LL, PC	+
N. clevelandii	BNL	LY	+
N. glutinosa	NL	MM	+
N. tabacum cv. occidentalis	M	MM	+
N. tabacum cv. rustica	+	MM	+
N. tabacum cv. samsun	MM	M	+
N. tabacum cv. samsun N.N	NL	NS	-
N. tabacum cv. sylvestris	NL	MM	+
Petunia hybrida	-	NS	+
Solanum tuberosum (potato) cv. Nicola	-	NS	-
S. nigrum (black nightshade)	-	MM/NS	+
S. melongena (eggplant) cv's. Classic, 206	-	NS	-

*** resistance genes: *L*, for tobamovirus, *Sw-5* for *Tomato spotted wilt virus* (TSWV). Early symptoms,** local symptoms developed on the inoculated leaf 4–7 days post inoculation (dpi): hypersensitivity response **(HR)**, necrotic lesions **(NL)** yellowing necrotic lesions **(YNL)**, brown necrotic lesions **(BNL)**, mottling **(M)**. Systemic symptoms description 7–14 days post infection: no symptoms (NS); mild mottling (MM); Leaf lesion (LL). Mosaic (M); plant collapse (PC); Leaf yellowing (LY). Positive ELISA results, >3 of negative value are depicted in (+), while negative results marked (-).

doi:10.1371/journal.pone.0170429.t002

Grafted tomato plants on eggplant rootstock

Current experiment: ToBRFV root infection of grafted plants



Conclusions

- **Phytosanitation, disinfecting the greenhouse structure and trellising ropes**
- **Using ToBRFV free seed lots**
- **Pre-planting treatment with “stabilized chlorine”, using resistant rootstocks, intermediate medium addition to the planting pits**
- **Early identification and removal of primary infected plants**
- **Careful planting / Disinfection of working tools**

Thank you for your attention

Funding:

Ministry of Agriculture,
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Chlorine experiments team

Pepper plants harboring L1,3,4 hypersensitive response (HR) to ToBRFV infection

Luria et al, (2017). A New Israeli Tobamovirus Isolate Infects Tomato Plants Harboring Tm-22 Resistance Genes. PLOS ONE 12(1): e0170429. https://doi.org/10.1371/journal.pone.0170429. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0170429