Update on curly top disease and resistance-breaking (RB) tomato spotted wilt virus in 2023 and what to do in 2024

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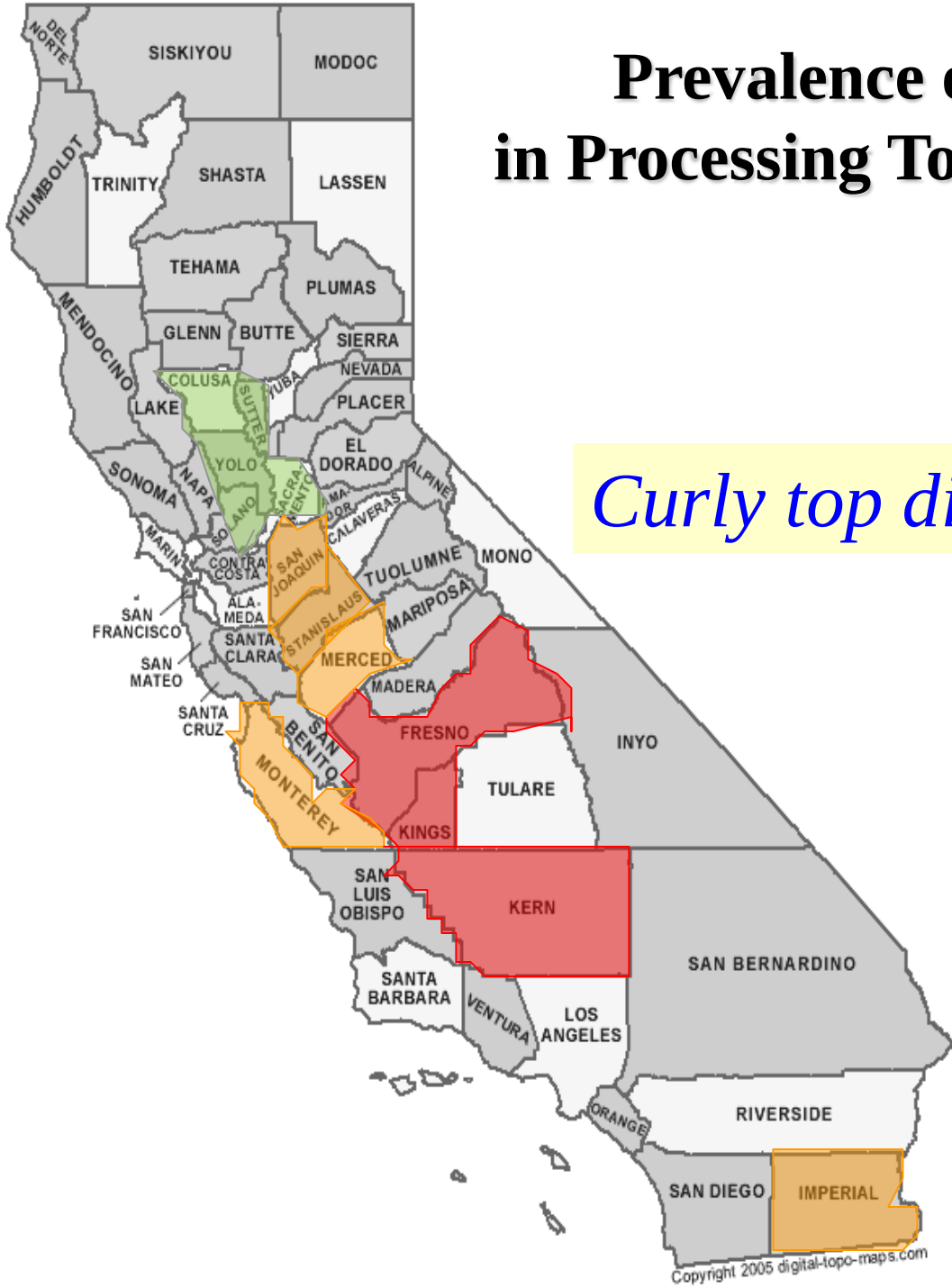
South Sacramento Processing
Tomato Production Meeting (January 9, 2024)

Processing tomatoes in California are affected by diseases caused by numerous viruses

- >10 virus diseases affect CA processing tomato production
- The importance and prevalence varies
  - -Major viruses-BCTV and (RB) TSWV
  - -Minor viruses-AMV and ToNSV
  - -Unusual-PZSV, ToNDV, ToMV, TYLCV, CMV, TEV
- Concerns about the exotic seed-transmitted viruses (ToBRFV) and viroids
- Accurate ID is critical for effective management strategies, ideally in an IPM program
- Symptoms often not sufficient for ID and molecular tests are often necessary

Unusual outbreak of curly top
In the Northern Counties in 2021

Prevalence of Virus Diseases in Processing Tomatoes of California



Curly top disease

Symptoms of curly top

- **Early infection** (~1 mo after planting)

  -Stunted light green plants with upcurled/rolled leaves with vein swelling and purpling (diagnostic)

  -These plants often die, whereas those infected later may collapse

  -May be confused with early spotted wilt

- **Late infections** (>1 mo after planting)

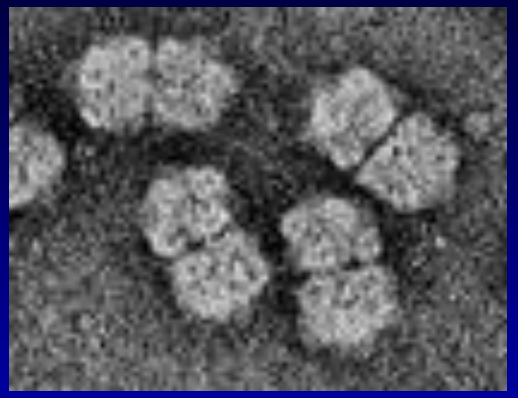
  -Symptoms in newer growth

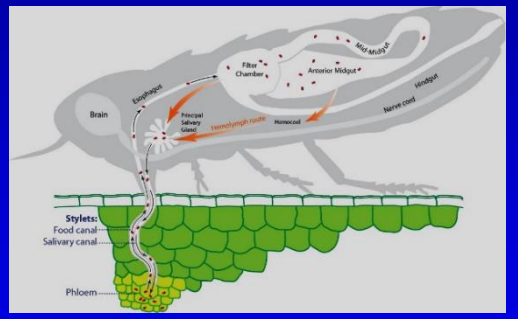
  -Fruits are small and ripen prematurely

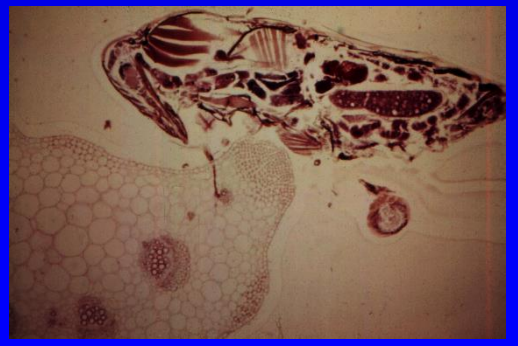
- **Importance of sample collection for PCR testing!**

Background information on BCTV and curly top disease

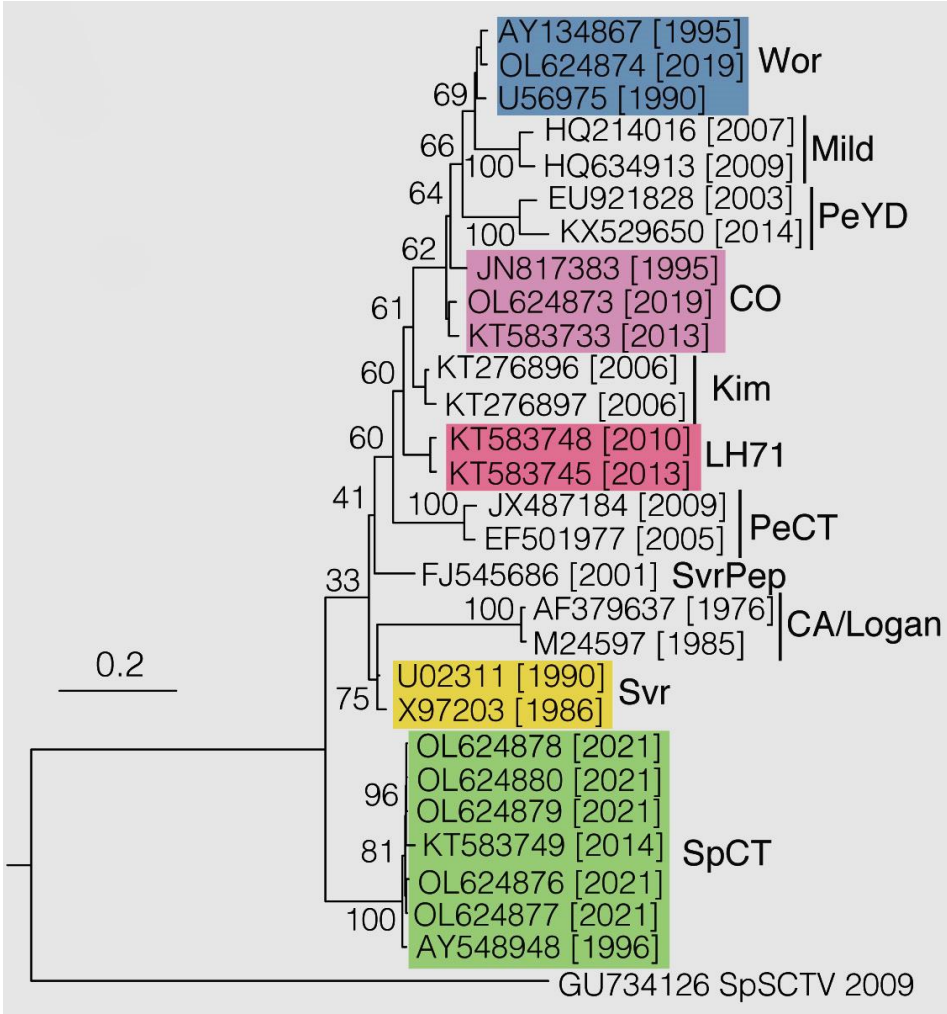
- BCTV is a geminivirus, a small plant virus composed of a circular single-stranded DNA genome protected by a protein shell that looks like 2 balls stuck together
- BCTV only infects cells of the phloem, the food conducting system of the plant, and is not mechanically transmitted
- Transmitted by the phloem-feeding BLH, but does not replicate in the insect or be passed to progeny via eggs
- In CA, the major crop impacted is processing tomato
- Tomato is not a preferred host for BLH but transmits during 'tasting' of tomatoes but then move on
- BCTV can be rapidly (5 hours) and specifically detected in tomato and beet leafhoppers by a multiplex PCR test and delivered into tomato plants via agroinoculation







Beet curly top virus (BCTV) is composed of 11 strains that differ genetically and biologically



Mild-type strains:

BCTV-CO*

BCTV-Wor*

Severe-type strains:

BCTV-LH71*

BCTV-Svr*

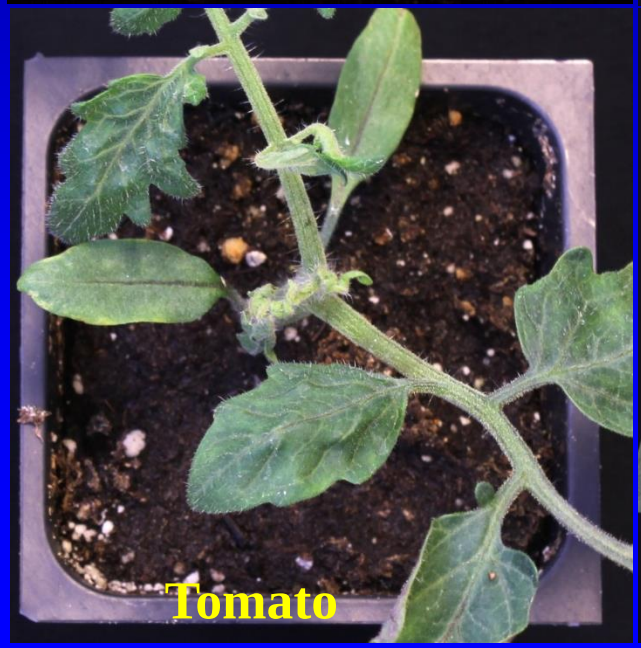
BCTV-CA/Logan*

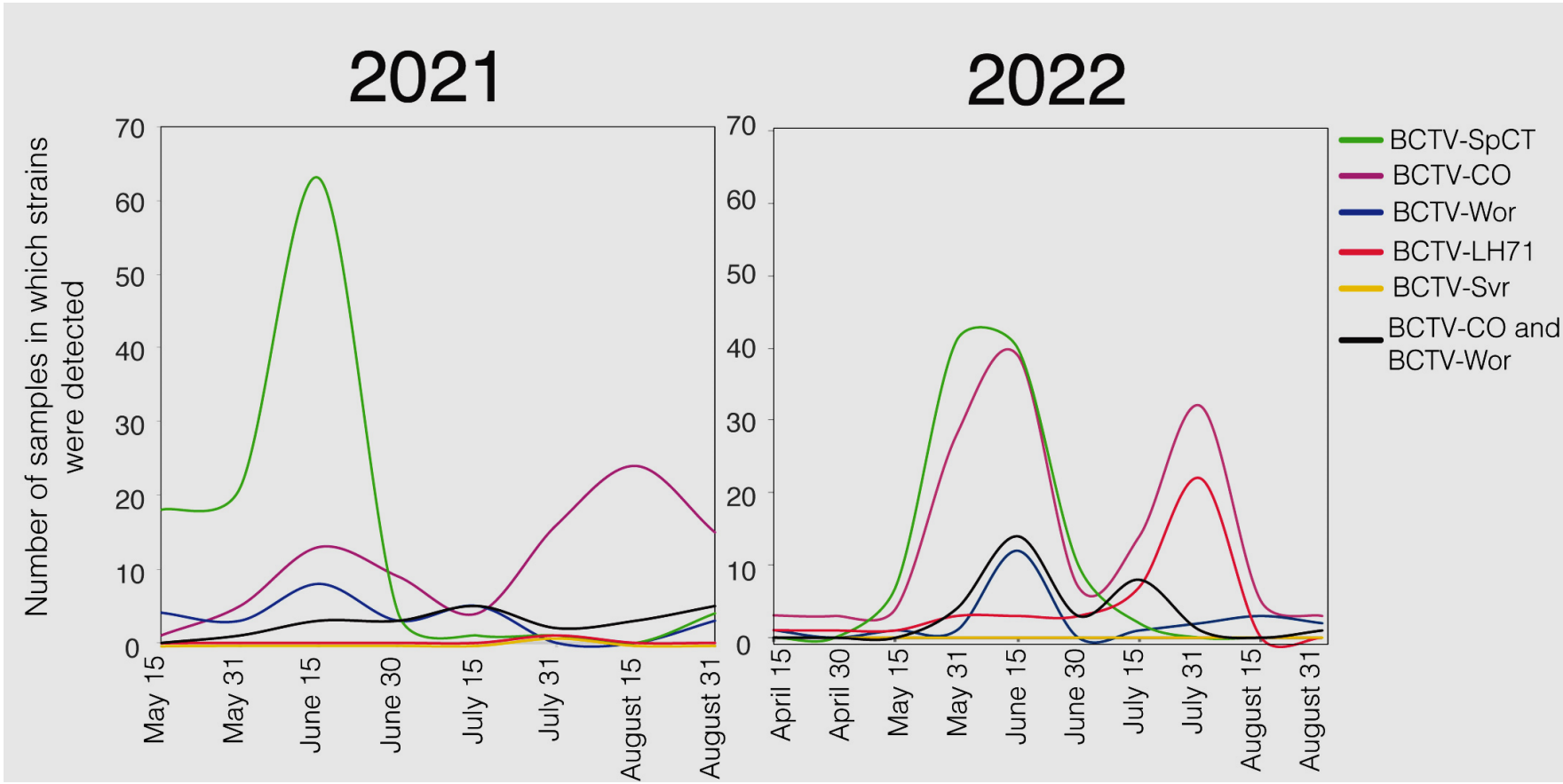
BCTV-SpCT?

The 2021 curly top outbreak in the Northern Counties was highly unusual

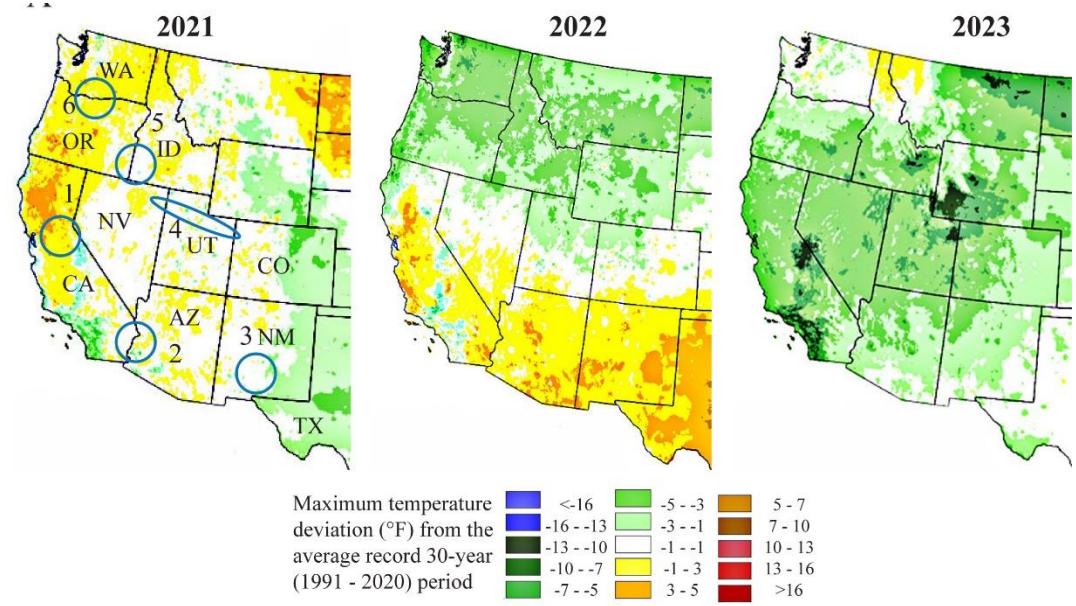
- The incidence of curly top in the Northern Counties has been very low
- In 2021, processing tomato fields in Colusa, Glenn, Sutter and Yolo Counties had incidences as high as 15-20%!
- Associated with proximity to foothills and unusual hot dry winds in April and May
- An unusual strain, BCTV-spinach curly top (SpCT), was involved in early infections (April-May)
- However, later outbreaks (after late June) were caused by BCTV-CO

The spinach curly top strain of beet curly top virus (BCTV-SpCT) was first detected in tomato and pepper in California in 2014

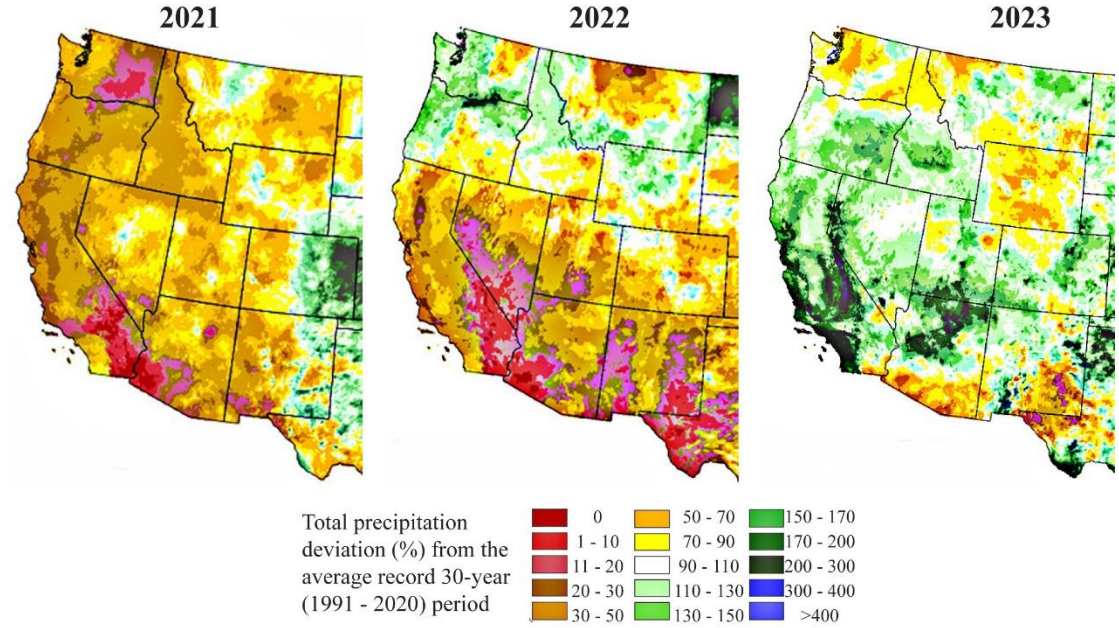



2021
2022
Number of samples in which strains were detected
70
60
50
40
30
20
10
0
May 15
May 31
June 15
June 30
July 15
July 31
August 15
August 31
April 15
April 30
May 15
May 31
June 15
June 30
July 15
July 31
August 15
August 31
BCTV-SpCT
BCTV-CO
BCTV-Wor
BCTV-LH71
BCTV-Svr
BCTV-CO and BCTV-Wor

Deviations (or anomalies) of **maximum temperature** between spring 2021, 2022 and 2023 from the average/total represented by 30-year (1991-2020) average.



Deviations (or anomalies) of **<u>total precipitation</u>** between spring 2021, 2022 and 2023 from the average/total represented by 30-year (1991-2020) average.



Total number of tomato samples collected/received in 2021, 2022 and 2023 showing curly top disease-like symptoms and number of samples positive for the beet curly top virus strain Spinach curly top (SpCT).

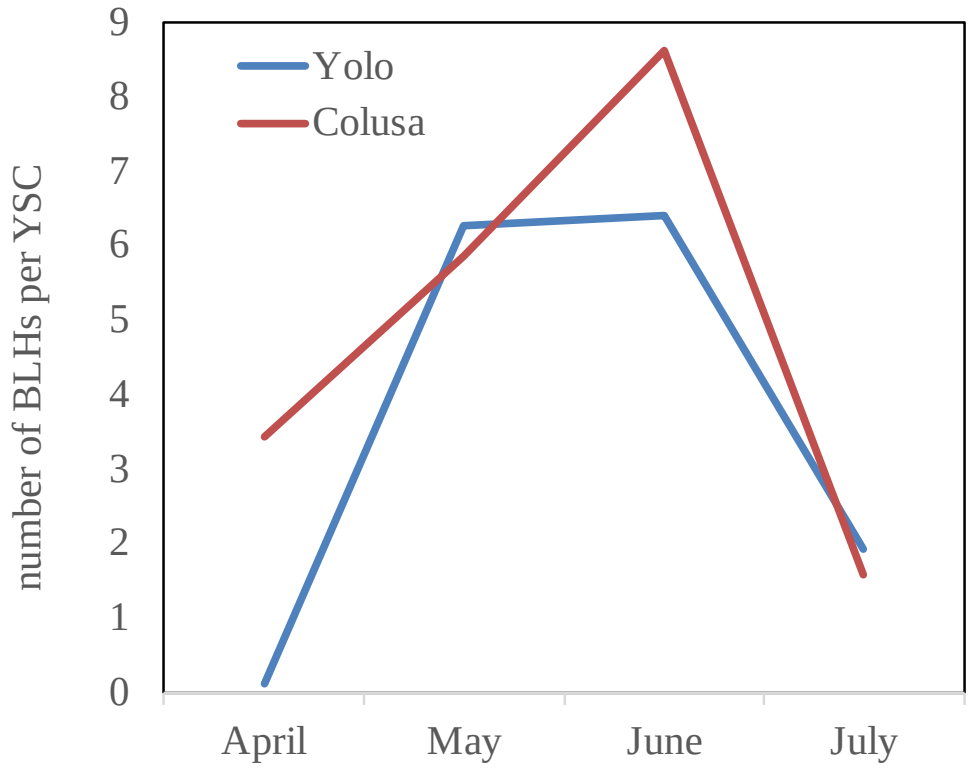
County	2021		2022		2023	
	Total samples	BCTV-SpCT	Total samples	BCTV-SpCT	Total samples	BCTV-SpCT
Colusa	62	33	62	34	4	0
Yolo	123	71	102	54	4	1
Glenn	3	2	9	0	0	0
Stanislaus	30	3	30	0	0	0
Sutter	13	1	1	1	21	6
San Joaquin	19	1	26	1	0	0
Fresno	24	1	157	4	21	1
Madera	0	0	2	0	0	0
Kern	0	0	12	1	0	0
Total	274	112	401	95	50	8

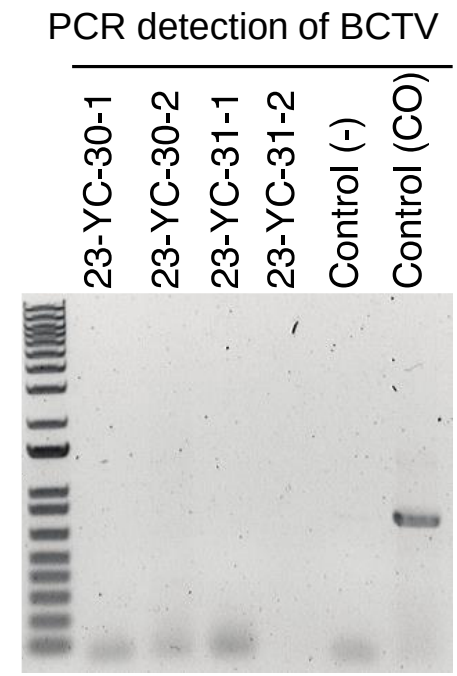
-Trace or no plants with CTD symptoms in our 8 monitored fields and no evidence of beet leafhopper migrations in 2023!

-Little or no CTD in processing tomato fields in the North in 2023!

-Supports hypothesis that weather conditions played a role in these outbreaks

Populations of beet leafhopper (BLH) and detection of beet curly top virus (BCTV) in eight processing tomato fields in Yolo and Colusa between April to July of 2023

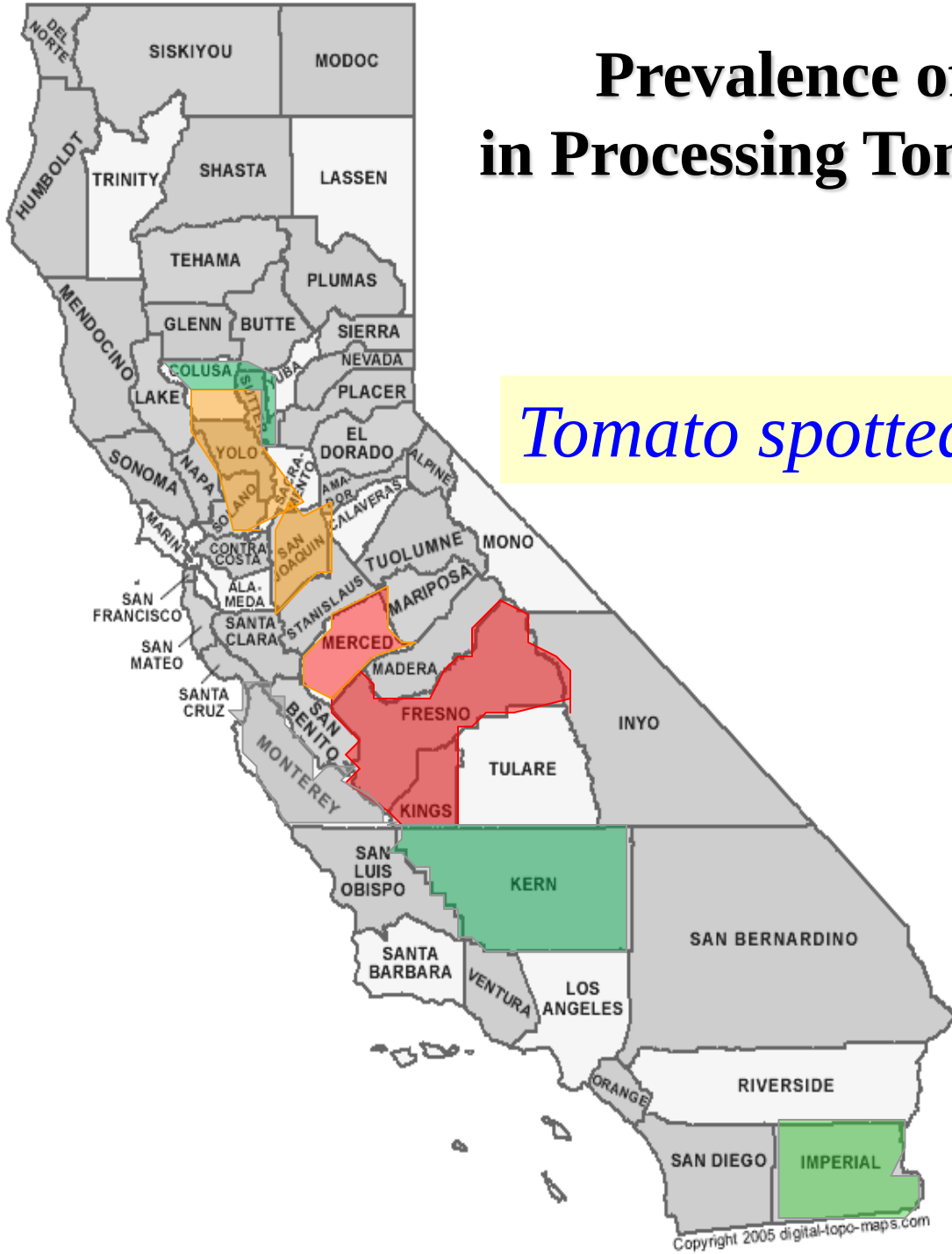




In 2023, very low BLH populations with very low level of BCTV

Prevalence of Virus Diseases in Processing Tomatoes of California

Tomato spotted wilt virus (TSWV)



Symptoms and impact of tospovirus infection in tomato vary depending on the age of the plant when infected

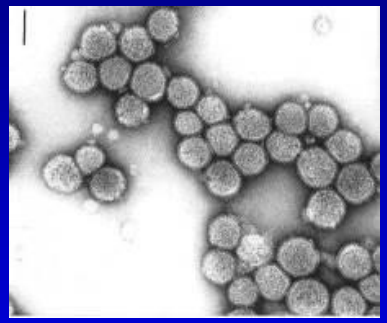
- Stunting; bronzing, necrosis and yellowing of leaves and ringspots and necrosis in fruits)
- Symptoms vary depending on variety and plant age



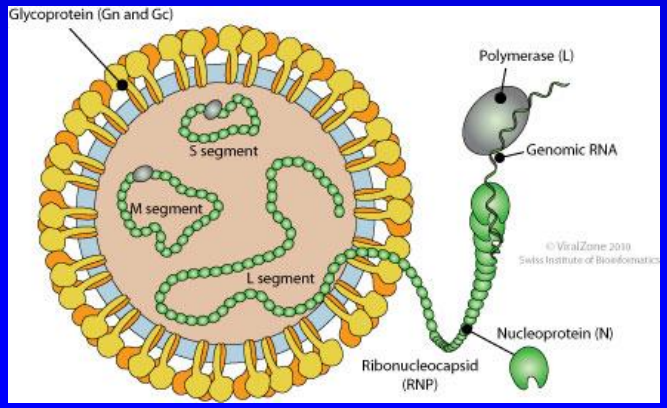



TSWV and spotted wilt disease

- *Tomato spotted wilt virus* (TSWV) is a thrips-transmitted tospovirus (mostly Western flower thrips in CA) and has a large tripartite minus-sense RNA genome



- Not by seed, contact, or through eggs of the thrips; TSWV infects all cells and is mechanically transmissible
- In CA, crops impacted by tomato spotted wilt and thrips vector are tomato, pepper, lettuce and radicchio
- In 2005, substantial outbreaks of spotted wilt caused millions in losses to processing tomato production in Central California
- This led to a development of a collaborative project to investigate these outbreaks and make management recommendations



Sw-5b gene confers resistance to TSWV by recognizing the TSWV movement (NSm) protein and triggering cell death

-Single dominant R gene

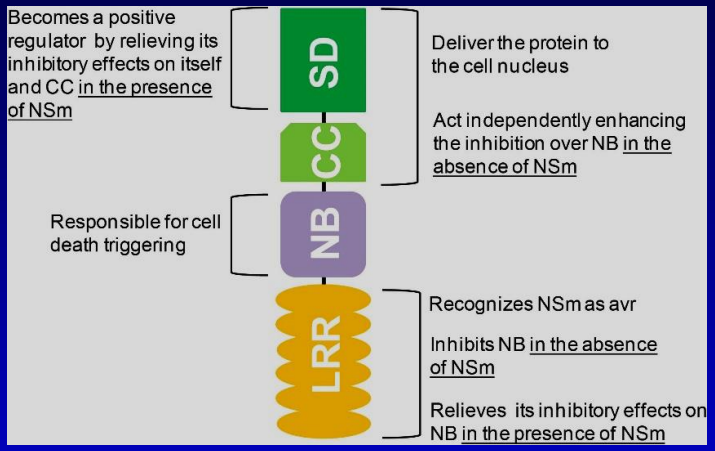
-Introgressed from the wild tomato species *Solanum peruvianum*

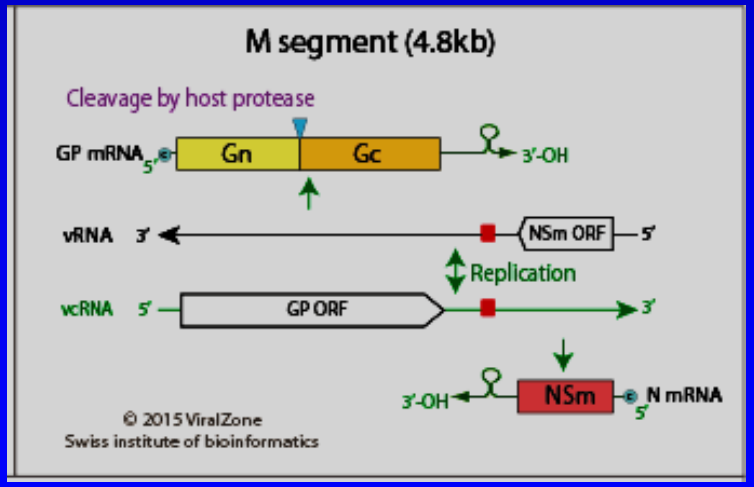
-The gene product (protein) recognizes that virus and triggers a defense (immune) response

-Viral effector is the movement protein (MP), encoded by the NSm gene on the M RNA

-Sw-5b gene is present in most processing tomato varieties grown in California

-Tremendous selection pressure on the virus





Appearance of a resistance-breaking strain of *Tomato spotted wilt virus* in the Central Valley of California in 2016

- In the spring of 2016, typical and severe symptoms of TSWV were observed in Sw-5 fresh market tomatoes in Fresno Co.

- Immunostrip and RT-PCR/sequencing tests confirmed TSWV infection
- Suggested the emergence/introduction of a resistance-breaking (RB) strain
- RB strains have been reported from Europe (Spain and Italy) and have been associated with specific amino acid changes in the viral movement protein (NSm), including the 'YPT/N' marker

Procedure for identifying tomato (Sw5b) RB TSWV strains

Typical tospovirus symptoms

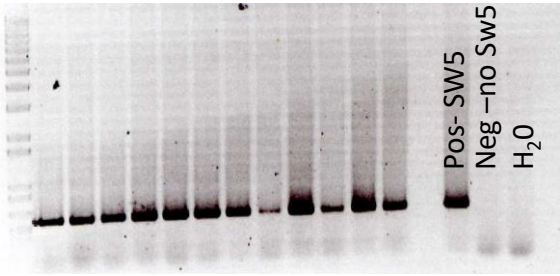
Test for TSWV with immunostrips

Confirm TSWV by RT-PCR

Confirm RB strain by RT-PCR of NSm gene and sequencing

Amino acid (aa) sequence

Confirm tomato is a resistant variety by PCR for SW-5



Fresno RB strain

MDTSKGKILLNTEGTSSFGTYESDSITESEGYD
LSARMIVDTNHHISNWKNDLFVGNGKQNA
NKVIKIYPTWDSRKQYMMISRIVIWVCP

aa substitutions:
C to Y at 118 position
T to N at 120 position

WT strain

MDTSKGKILLNTEGTSSFGTYESDSITESEGYD
LSARMIVDTNHHISNWKNDLFVGNGKQNA
NKVIKICPTWDSRKQYMMISRIVIWVCP

no aa substitution in 118 or 120 position (CPT)

Detection of resistant breaking (RB) and wild type (WT)
of *Tomato spotted wilt virus* (TSWV)

total RNA extraction with the RNeasy Qiagen kit

30 min
(1 - 5 samples)

(synthesis of cDNA using
Random primers)

1 h + 30 min

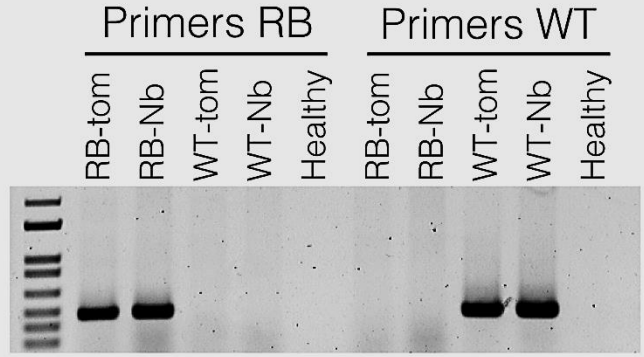
Polymerase chain reaction (PCR)

2 h

94C x 20 sec
60C x 20 sec
72C x 1:2 min
30X

Agarose gel electrophoresis

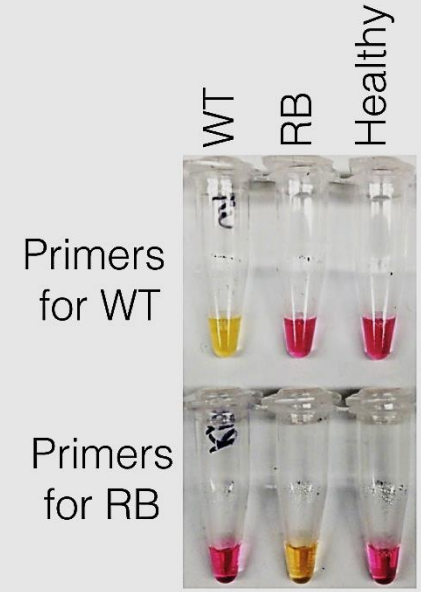
45 min



Total time: 5 hours

Loop Mediated Isothermal
Amplification (LAMP)

60 min

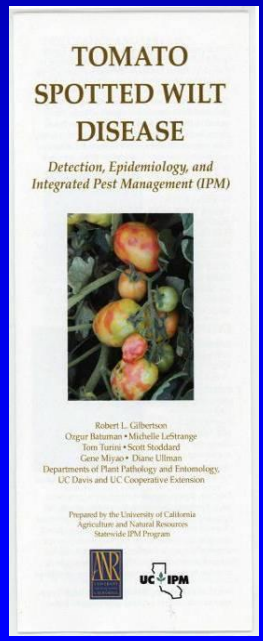


67C x 50 min

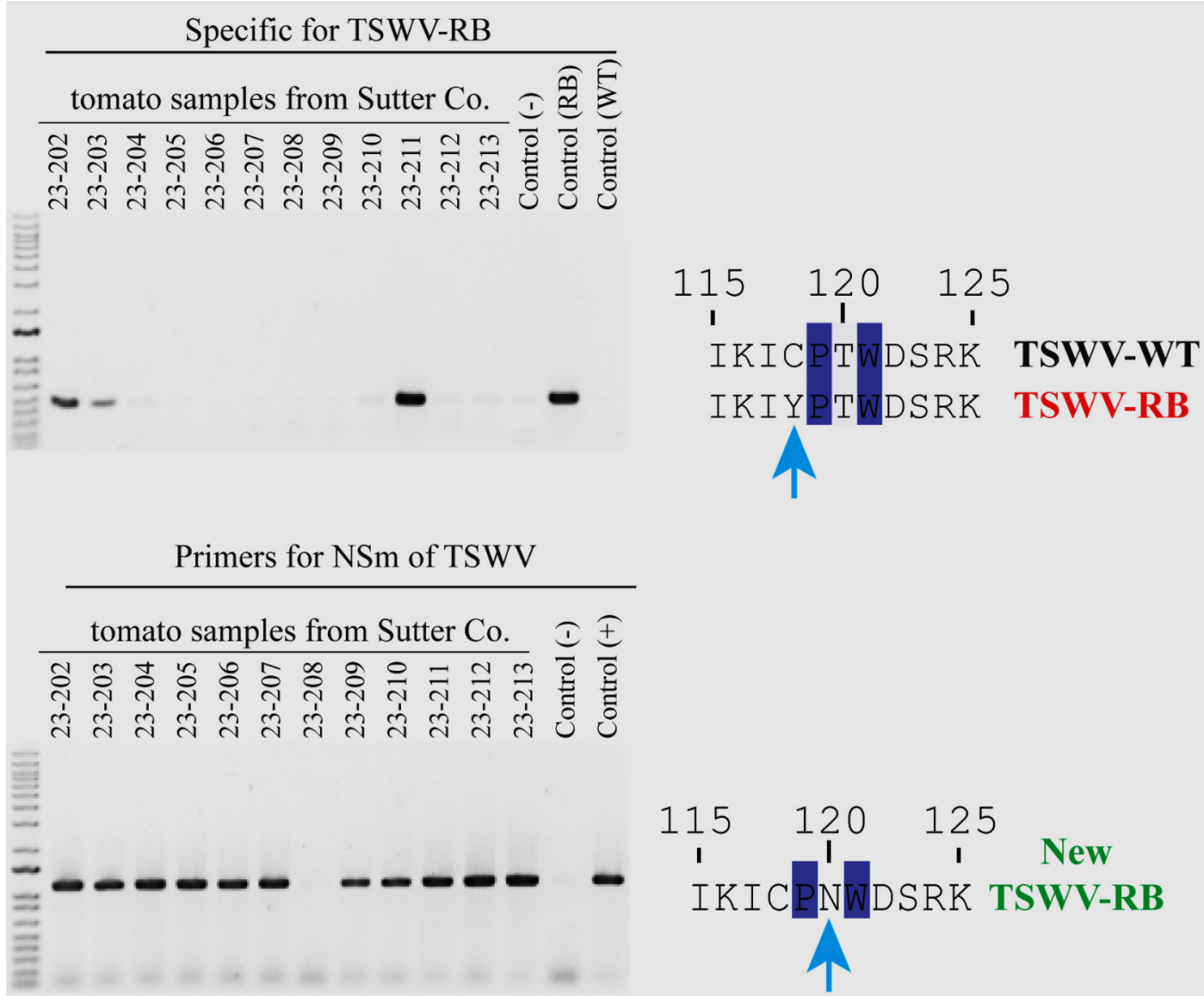
Total time: 1.5 hours

Spotted wilt in California tomato production since 2005

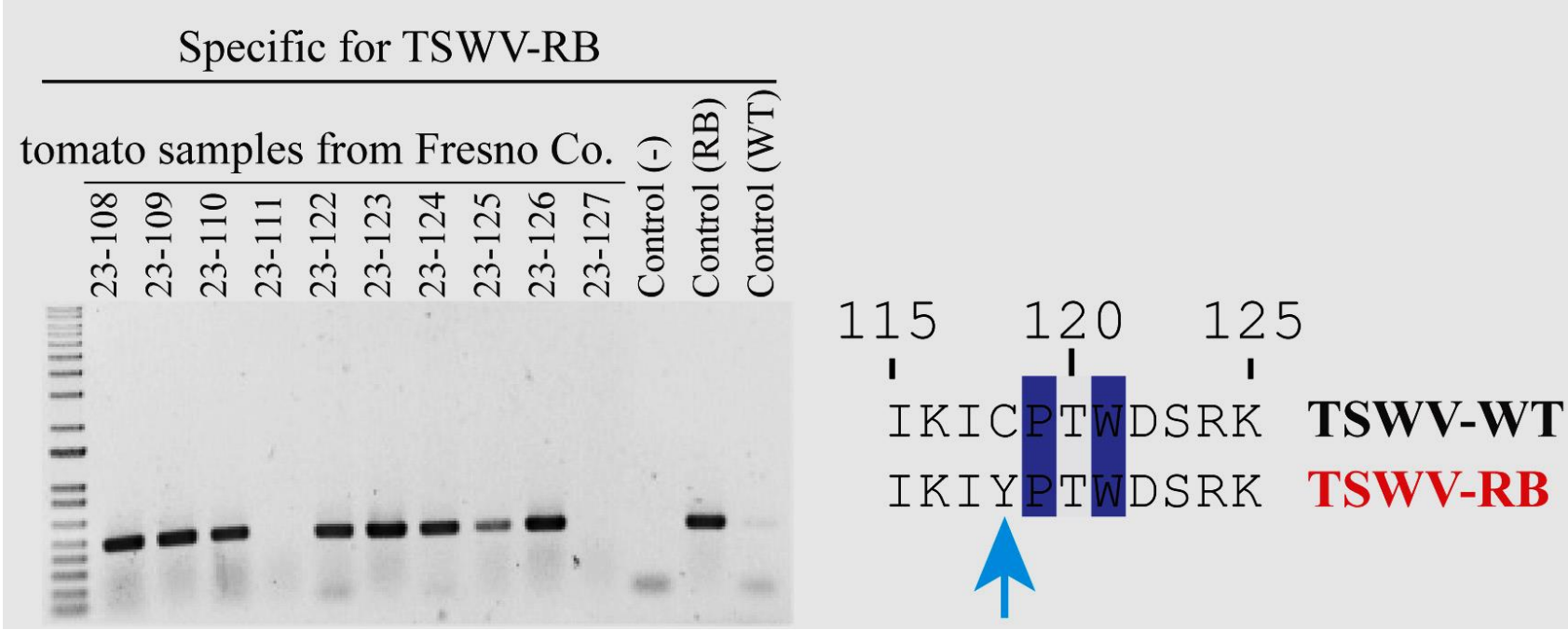
- **2005-06**
  - -Spotted wilt outbreaks cause economic loss in Fresno
  - -IPM-NR and RV (Sw-5b)
  - -DD model
- **2010-11**
  - -IPM-RV+DD model
- **2016**
  - -Emergence of RB-TSWV YPT strain in Fresno fresh market fields (economic loss)
- **2017-2020**
  - -Fresno YPT becomes predominant in Fresno, Kings and Merced
  - -IPM-NR in Fresno/IPM-RV in Northern Counties
- **2021-22**
  - -Detection and spread of Fresno YPT in Northern Counties
  - -IPM-RV and -NR, economic loss in some hotspot areas
- **2023**
  - -Emergence of new RB TSWV 'CPN' in Colusa and Sutter
  - -Appearance of unusual spotted wilt symptoms in some fields in 2023



Results of RT-PCR tests for Fresno RB (YPT) for spotted wilt samples from Sutter County-June/July 2023



Results of tests for Fresno RB (YPT) for spotted wilt samples from collected in Fresno County

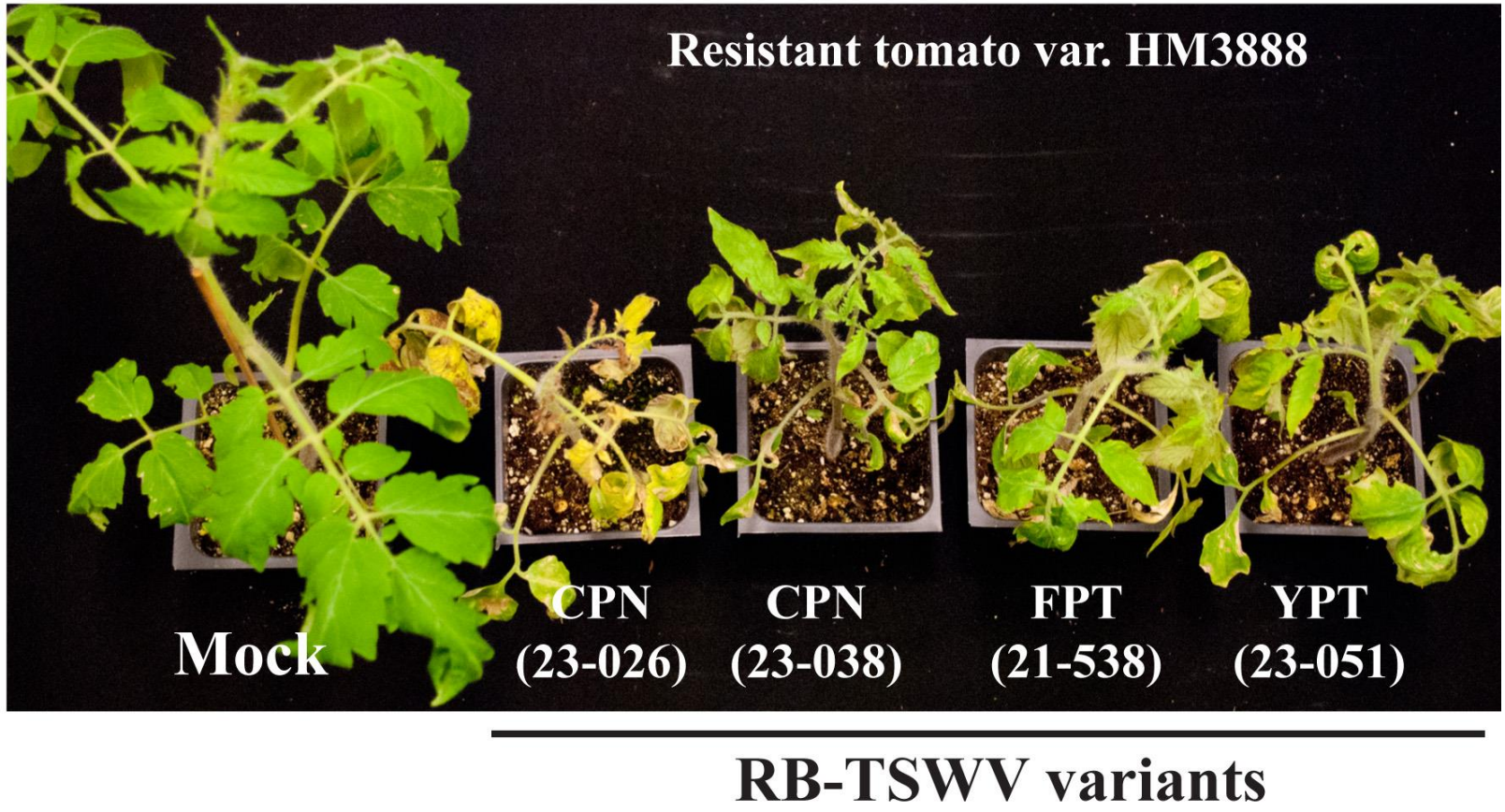


Results of RT-PCR tests for detection of tomato RB TSWV strains by county in 2023

County	RB-TSWV variants				
	CPT	YPT	CPN	mix	Total
Colusa	0	10	37	2	49
Sutter	0	4	8	0	12
Yolo	0	62	75	3	140
San Joaquin	0	6	0	0	6
Madera	0	0	0	0	0
Fresno	0	43	0	0	43
San Diego (Oceanside)	0	2	0	0	2

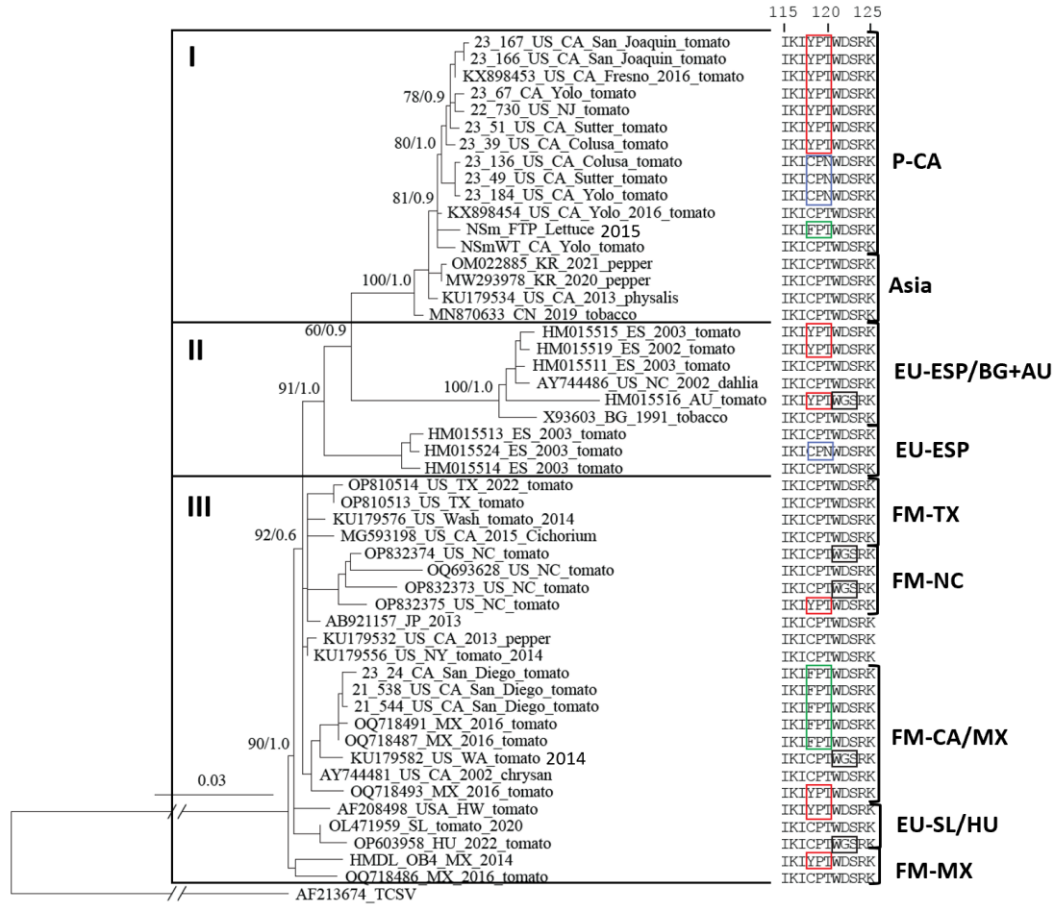
Results of pathogenicity tests for an isolate of the RB-TSWV CPN strain

Different RB-TSWV variants showed different virulence in resistant tomato varieties



Unusually severe spotted wilt symptoms were observed in fields in the northern and central production areas and were not correlated with infection by the new CPN RB strain

Phylogenetic tree constructed with the complete nucleotide sequence of the NSm gene of isolates of TSWV



What to expect in 2024 and what can be done

- **Curly top**

  -Strong El Nino could bring wet weather and low CTD in 2024

  -Monitor for beet leafhoppers and CTD (CDFA-CTVCP)

  -Application of insecticides (in-field and CDFA-CTVCP)

  -Avoid risk factors such as sparse and late-planted fields

  -Look for moderately resistant varieties on the horizon

- **Spotted wilt**

  -New RB strain (CPN) emerges in the North: RB TSWV likely here to stay but impact unclear

  -Clean transplants, planting time and field placement (hot spots)

  -Monitor for spotted wilt symptoms and thrips

  -Thrips management based on counts on YSC or DD model

  -Return to balanced IPM and also look for resistant varieties

Acknowledgements

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