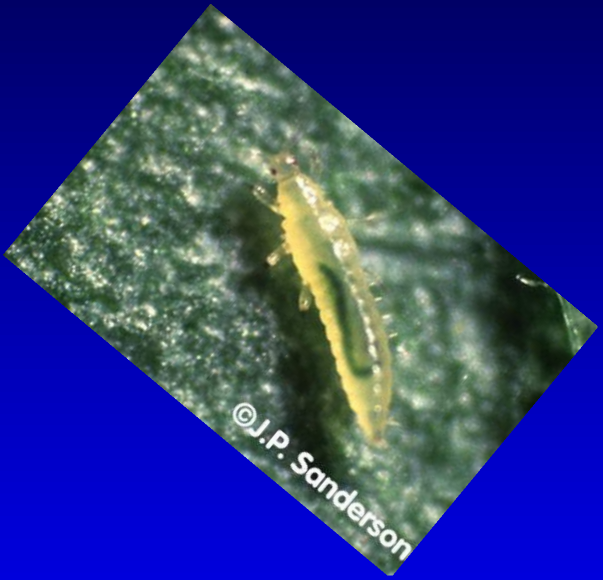
Emergence of a resistance breaking strain of *Tomato spotted wilt virus* (TSWV) in California



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A diversity of viruses affect processing tomatoes in California

- *Beet curly top virus* (BCTV) — Leafhopper-transmitted
- *Alfalfa mosaic virus* (AMV)
- *Cucumber mosaic virus* (CMV)
- *Tobacco etch virus* (TEV)
  Potato virus Y (PVY)

  Aphid-transmitted (AMV, CMV, TEV, PVY)
- *Tomato mosaic virus* (ToMV) — Mechanically transmitted
- ***Tomato spotted wilt virus* (TSWV) — Thrips-transmitted**
- Tomato necrotic spot virus[E] (ToNSV) — Thrips-associated
- *Tomato yellow leaf curl virus*[E] (TYLCV) — Whitefly-transmitted
- *Tomato necrotic stunt virus*[RE] (ToNSV) — Whitefly-transmitted

Tomato spotted wilt virus (TSWV) causes tomato spotted wilt disease of fresh market and processing tomato

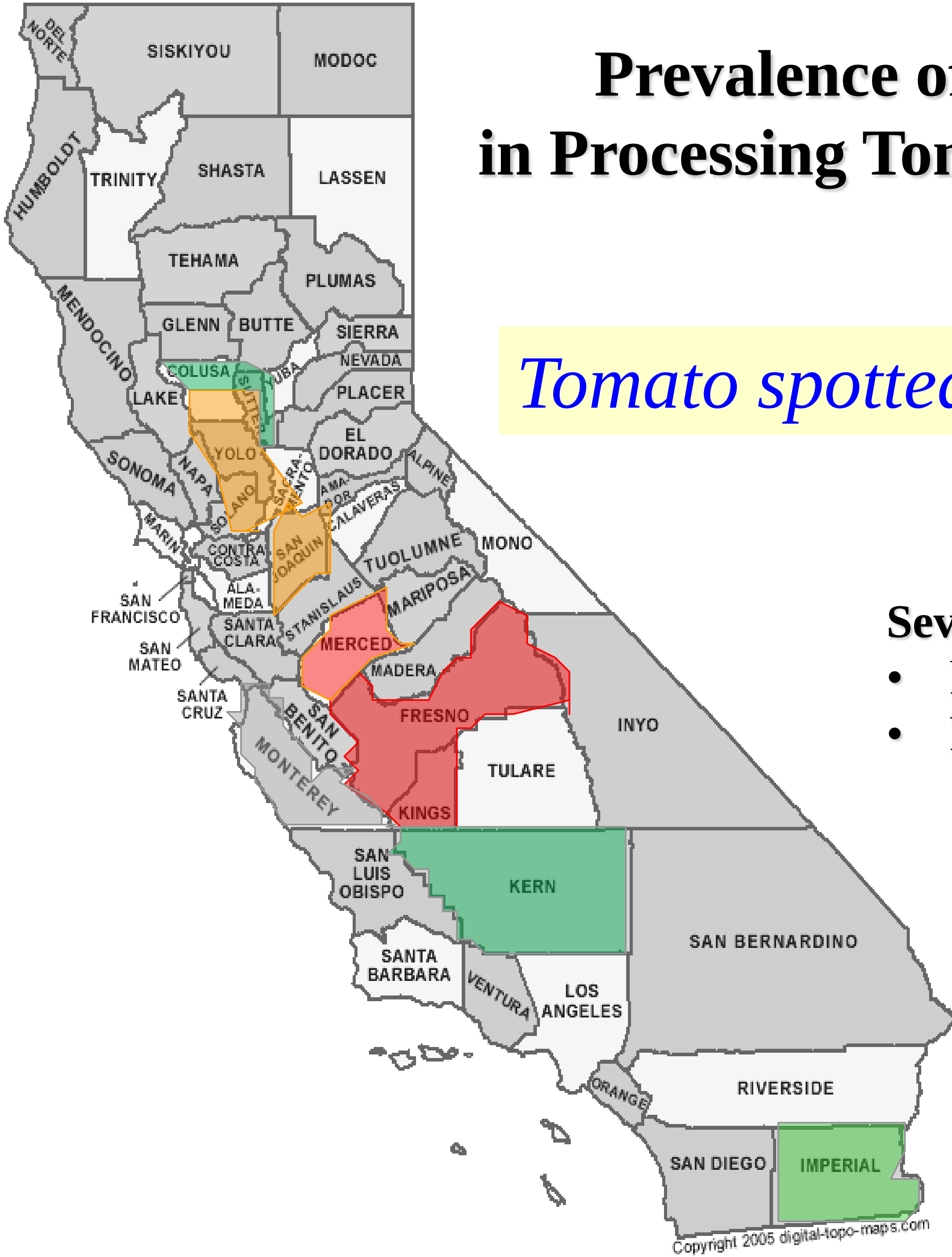
- **Stunting; bronzing, necrosis and yellowing of leaves and ringspots and necrosis in fruits)**
- **Symptoms vary depending on variety and age of plant when infected**






Prevalence of Virus Diseases in Processing Tomatoes of California

Tomato spotted wilt virus (TSWV)

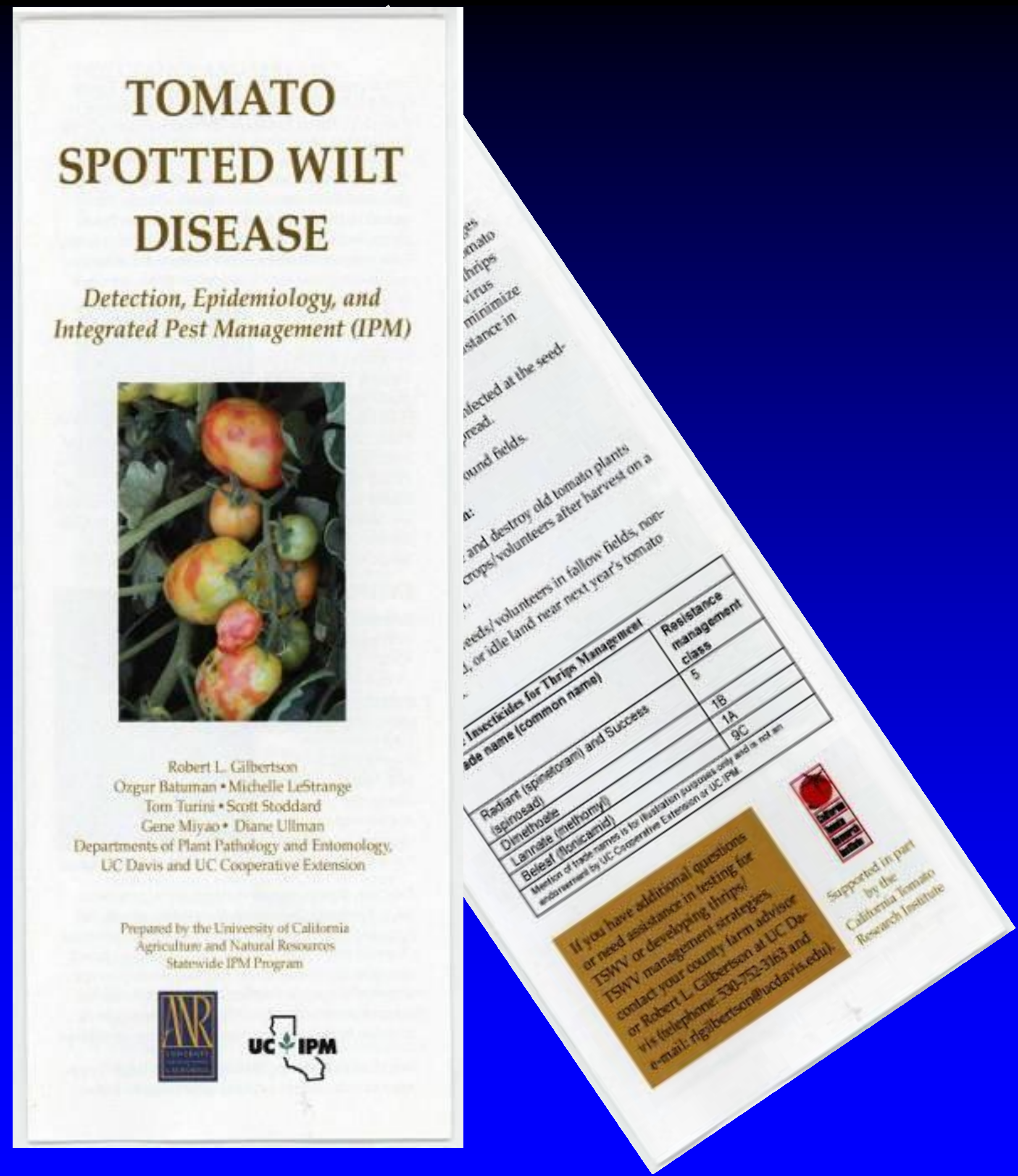


Severe yield losses can occur when:

- high thrips populations
- high level of virus inoculum

An IPM program has been developed for thrips and TSWV in processing tomatoes in California

- -Field placement/timing
- -Clean transplants
- -Resistant varieties (*Sw-5* gene)
- -Roguing (<30 days)
- -Thrips management (monitor/DD model)
- -Sanitation (weeds and bridge crops)
- -Tomato-free period (winter)
- -Has allowed for effective management of the virus
- -Substantial reduction in yield losses



Resistance is a desirable and sustainable method for disease management but pathogens fight back

- Pathogens fight back against disease resistance by evolving forms (new strains or races) to break or overcome resistance genes
- This occurs when the pathogen mutates and is no longer recognized by the gene product of the resistance gene (R gene)
- This has been documented for most types of pathogens, including bacteria, fungi, nematodes and viruses
- The durability of the R gene (i.e., the rate at which a resistance breaking strain appears) varies depending on the R gene and the genetics of the pathogen

Examples of the emergence of resistance-breaking strains/races worldwide and in California

- **Root knot nematode (*Meloidogyne incognita*) breaking the *Mi* gene in tomato (WW, CA)**
- **Bacterial speck bacterium (*Pseudomonas syringae* pv. *tomato*) race 1 breaking the *Pto* gene in tomato in CA**
- **Fusarium wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) breaking the Fo1 and Fo2 genes in tomato (WW, CA)**
- **Stem rust of wheat pathogen (*Puccinia graminis* f. sp. *graminis*) breaking numerous R genes in wheat (WW)**
- ***Tomato spotted wilt virus* (TSWV) breaking the *Sw-5* and *Tsw* R genes in tomato and pepper, respectively**

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 Cantua Creek and Firebaugh (Fresno Co.)
- Immunostrip and RT-PCR/sequencing tests revealed only TSWV infection

- Raised the issue of 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 substitutions in the viral movement protein (NSm), including ‘YPT’

Identification of TSWV RB strain

Typical tospovirus symptoms

Test for TSWV with immunostrips

Confirm TSWV by RT-PCR

Confirm RB strain by RT-PCR of NSm gene

Amino acid (aa) sequence

MDTSKGKILLNTEGTSSFGTYESDSITESEGY
DLSARMIVDTNHHISNWKNDLFVGNGKQ
NANKVIKIYPTWDSRKQYMMISRIVIWVC

aa substitution C to Y in 118 position or T to N in 120 position

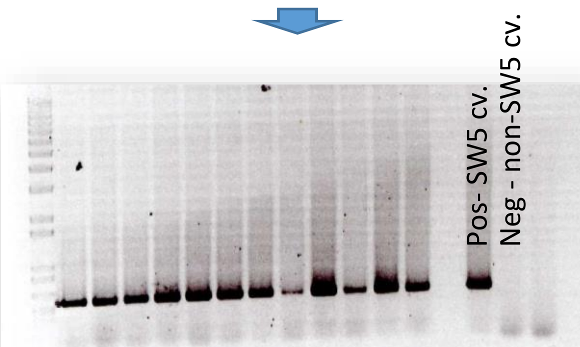
RB strain

MDTSKGKILLNTEGTSSFGTYESDSITESEGY
DLSARMIVDTNHHISNWKNDLFVGNGKQ
NANKVIKICPTWDSRKQYMMISRIVIWVC

no aa substitution in 118 or 120 position (CPT)

WT strain

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



TSWV isolates infecting fresh market Sw-5 varieties have the RB motif ‘YPT’

TSWV Isolate	Location collected from/tested with TSWV and TMV immunostrip	Symptoms	NSm Motif
SW19	Firebaugh, Fresno Q-99 Fresh Mkt Tomato (Sw-5) TSWV +, TMV -	Typical TSWV symptoms in leaves concentric rings in fruits	YPT Resistance Breaking (RB)
SW21	Cantua Creek, Fresno Q27 Fresh Mkt Tomato (Sw-5) TSWV +, TMV -	Typical TSWV symptoms in leaves concentric rings in fruits	YPT (RB)
SW24	Fresno Proc Tomato (no Sw-5) TSWV +, TMV -	Typical TSWV symptoms in leaves concentric rings in fruits	CPT Non-RB
T36	Yolo Proc Tomato (no Sw-5) TSWV +, TMV -	Typical TSWV symptoms in leaves concentric rings in fruits	CPT Non-RB

TSWV strains with the 'YPT' motif infect fresh market tomato cultivars following mechanical inoculation

Mechanical inoculation of Sw-5 fresh market varieties with putative TSWV RB strains

	Infected/Inoculated plants with TSWV Isolates		
Tomato Variety	SW5 Gene	SW19 RB Isolate	SW21 RB Isolate
HM 1794	Yes	3/10	2/10
HM 1795	Yes	2/10	3/10
Q 27	Yes	1/10	2/10

This confirmed that these strains broke the Sw-5 resistance

TSWV strains with the ‘YPT’ motif infect processing tomato varieties following mechanical inoculation

Mechanical inoculation of Sw-5 processing varieties with putative TSWV RB strains

Processing tomato experiment							
						Nsm Sequence	
Variety	SW5	Isolate	Infected/total	SW5	Nsm	YPT	CPT
BQ273-1	yes	**SW19 RB TSWV**	6/10	+	+	+	-
BP2-1	yes		10/10	+	+	+	-
DRI319-1	yes		5/10	+	+	+	-
HM3887-1	yes		1/10	+	+	+	-
H5608-1	yes		7/10	+	+	+	-
H8504-1	no		3/10	-	+	+	-
N6366-1	no		1/10	-	+	+	-
BQ273-1	yes	**SW21 RB TSWV**	1/10	+	+	+	-
BP2-1	yes		3/10	+	+	+	-
DRI319-1	yes		4/10	+	+	+	-
HM3887-1	yes		1/10	+	+	+	-
H5608-1	yes		0/10	+	-	-	-
H8504-1	no		3/10	-	+	+	+
N6366-1	no		3/10	-	+	-	+

Conclusions

- Outbreaks of spotted wilt in Sw-5 fresh market tomato fields in the spring and fall of 2016 were due to a resistance breaking (RB) strain of TSWV

- RB strain was detected only in Fresno County in 2016, and mostly in fresh market varieties

- The RB strain likely emerged by mutation in response to increased planting of Sw-5 varieties (~50% of processing tomato varieties now possess Sw-5)

Future Directions

- Monitor for the **survival and spread of RB strains** in the Central Valley in 2017
- Further determine the **genetic nature and biological properties** of the California RB strains
- Determine **the relative susceptibility of the major Sw-5 processing tomato cultivars** grown in California to the RB strains
- Identify **sources of resistance** for breeding tomato varieties with resistance to RB strains

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