# *Tomato spotted wilt virus and other viruses of concern*

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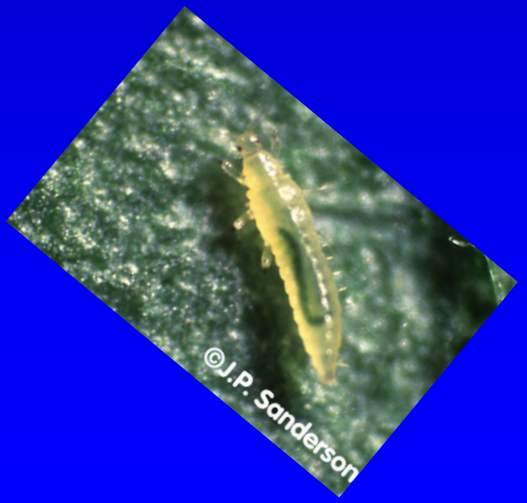


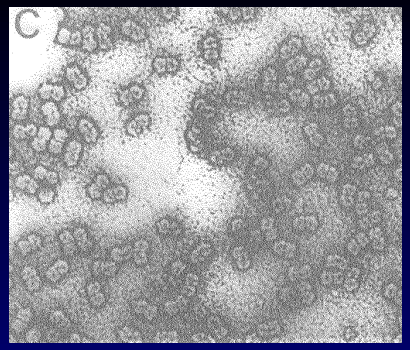
# Curly top is caused by *Beet curly top virus* (BCTV)

- A **geminivirus** with a very **wide host range** including **crops and weeds**

- Some plants infected with BCTV develop **curly top disease** (e.g., tomatoes), whereas **others show no symptoms** (species of weeds)

- BCTV is **only transmitted by the beet leafhopper** (not seed-transmitted or by contact)

- Exists as a **complex of strains**

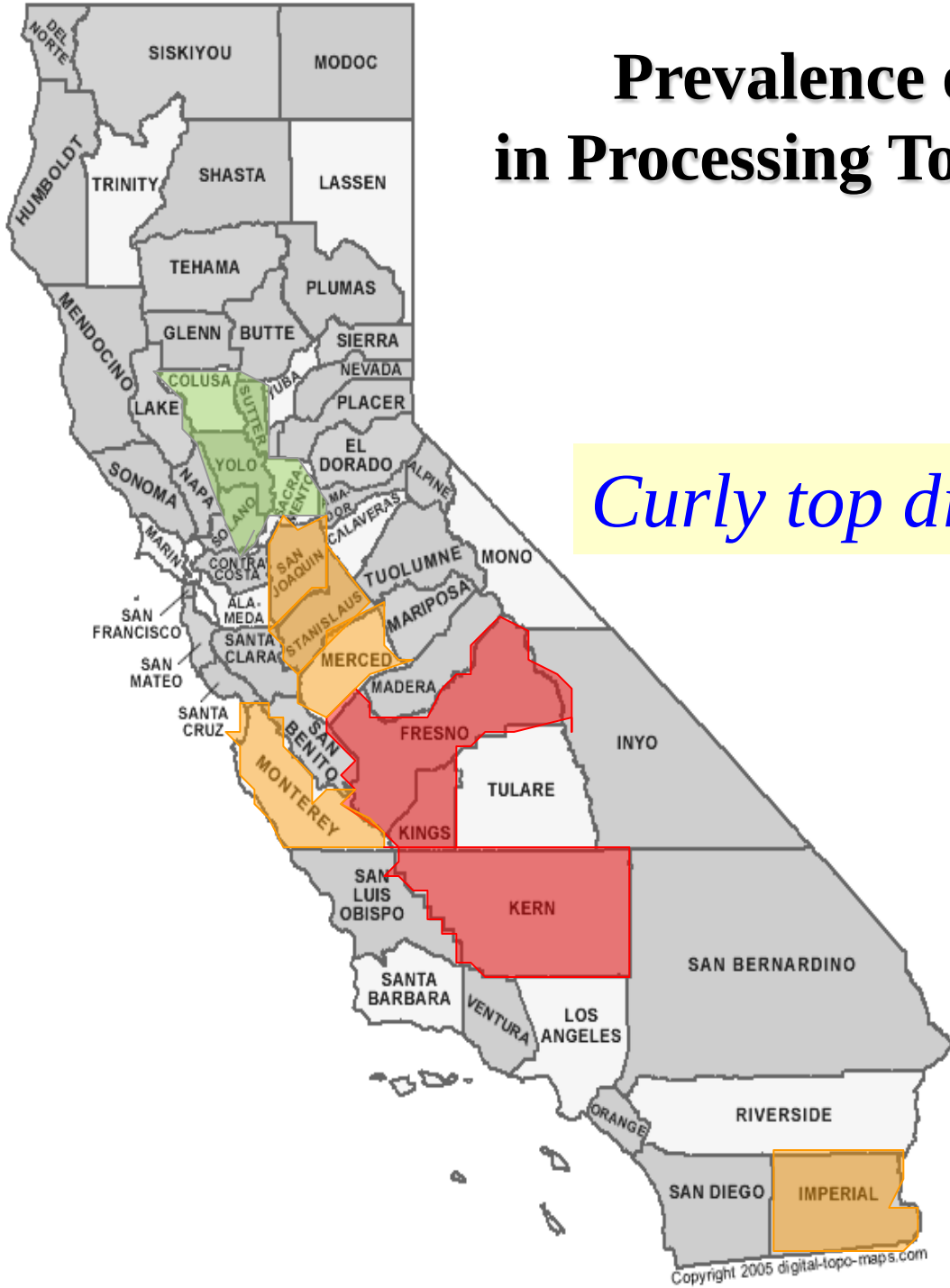


*Beet curly top virus*

Symptoms of BCTV infection in tomato

Beet leafhopper vector

# Prevalence of Virus Diseases in Processing Tomatoes of California




*Curly top disease*

# Curly top outbreak of 2013

Tomato field with 90% curly top in Fresno Co.

# Curly top disease cycle: Dependent on a migratory insect

**Spring:**
Viruliferous adult leafhoppers migrate to the valley floor and feed

Multiple generations on the valley floor

**Winter/early Spring:**
Adult females overwinter and breed on annual and perennial plants

**Fall:**
Adult leafhoppers migrate for overwintering in the foothills

# Curly top management: A difficult challenge

- Curly top is a **sporadic and unpredictable disease**

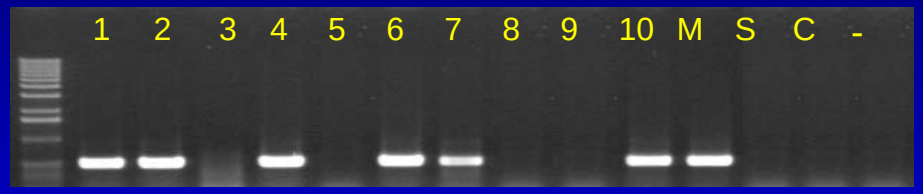
- **California Department of Food and Agriculture (CDFA)** operates a **Curly Top Control Program (CTVCB)** that targets the beet leafhopper with insecticide sprays based on **monitoring leafhoppers**

- **Cultural practices** can help, such as not planting next to foothills or heavy plant populations

- Currently, there are **no commercially available curly top-resistant tomato varieties**

# We have developed methods for detection of BCTV and screening plants for resistance

- Rapid and specific **PCR test for BCTV**
  - -Detection of the virus in crops, weeds and leafhoppers
  - -ID viruses/strains
  - -Predict curly top severity and target areas for spraying

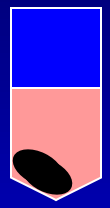



- **Agroinoculation method** for curly top
  - -Vector independent screening for resistance
  - -Rapid, efficient and reliable

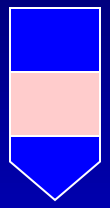
# PCR Detection of BCTV in beet leafhoppers


Monthly leafhopper collections from CDFA CTVCP personnel

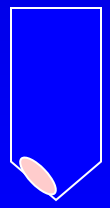
Add 300ul STE buffer and grind



Spin 5' at 13,000 rpm

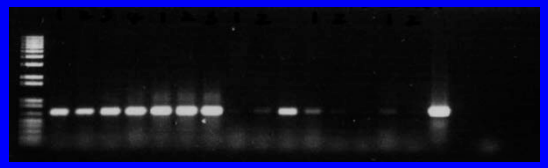


Phenol/Chloroform extraction twice

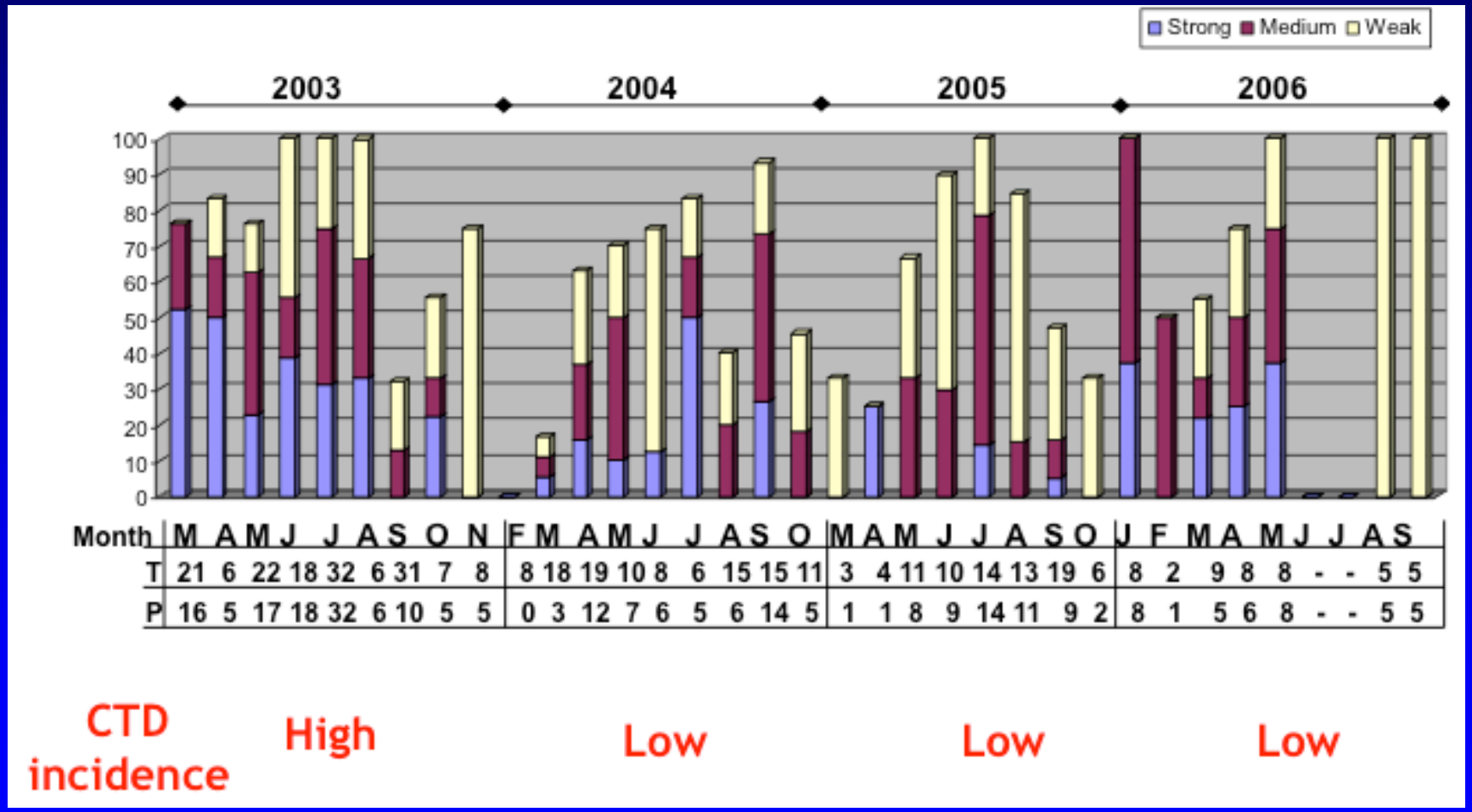


Precipitation of nucleic acid

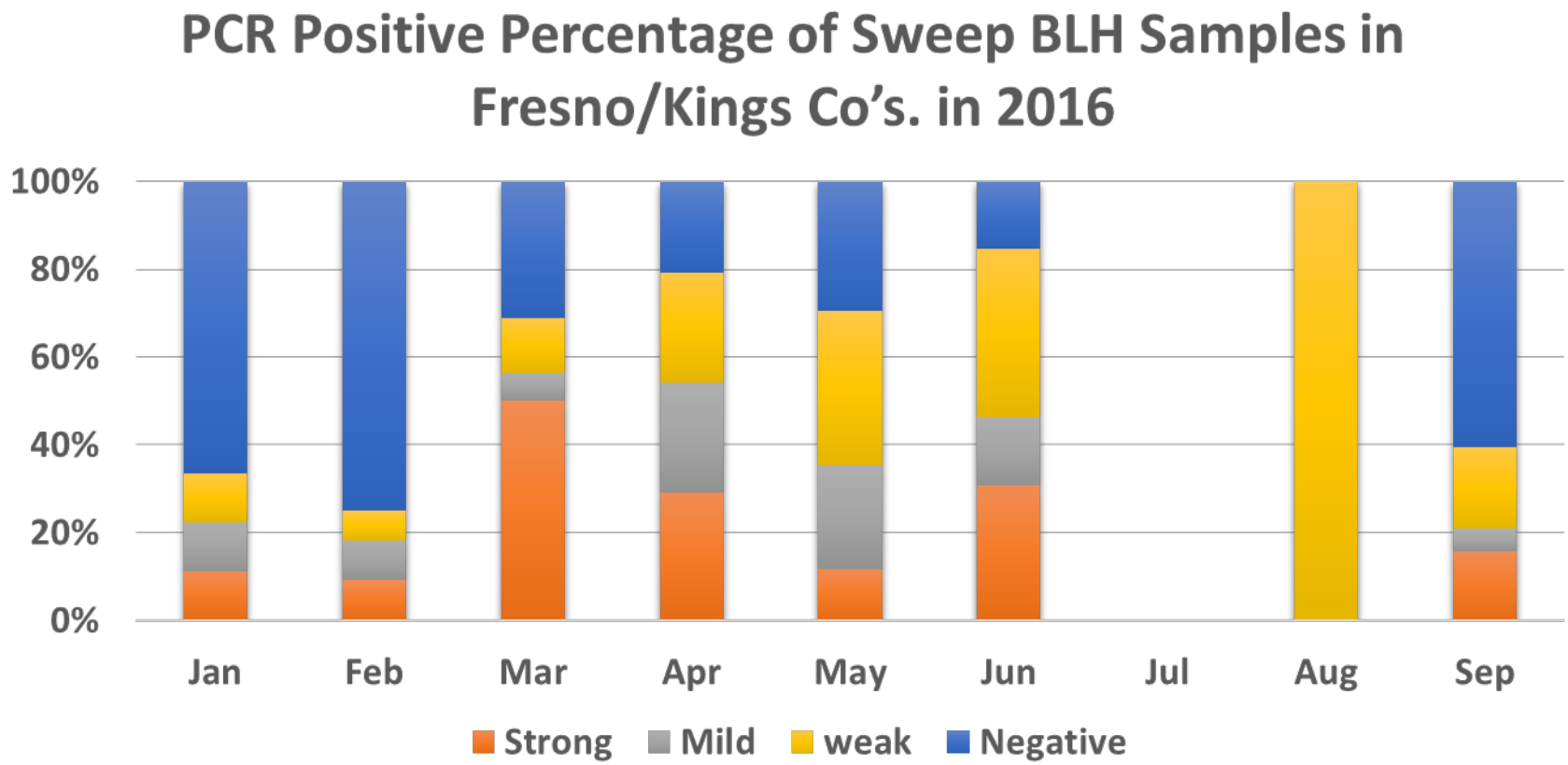
PCR reaction



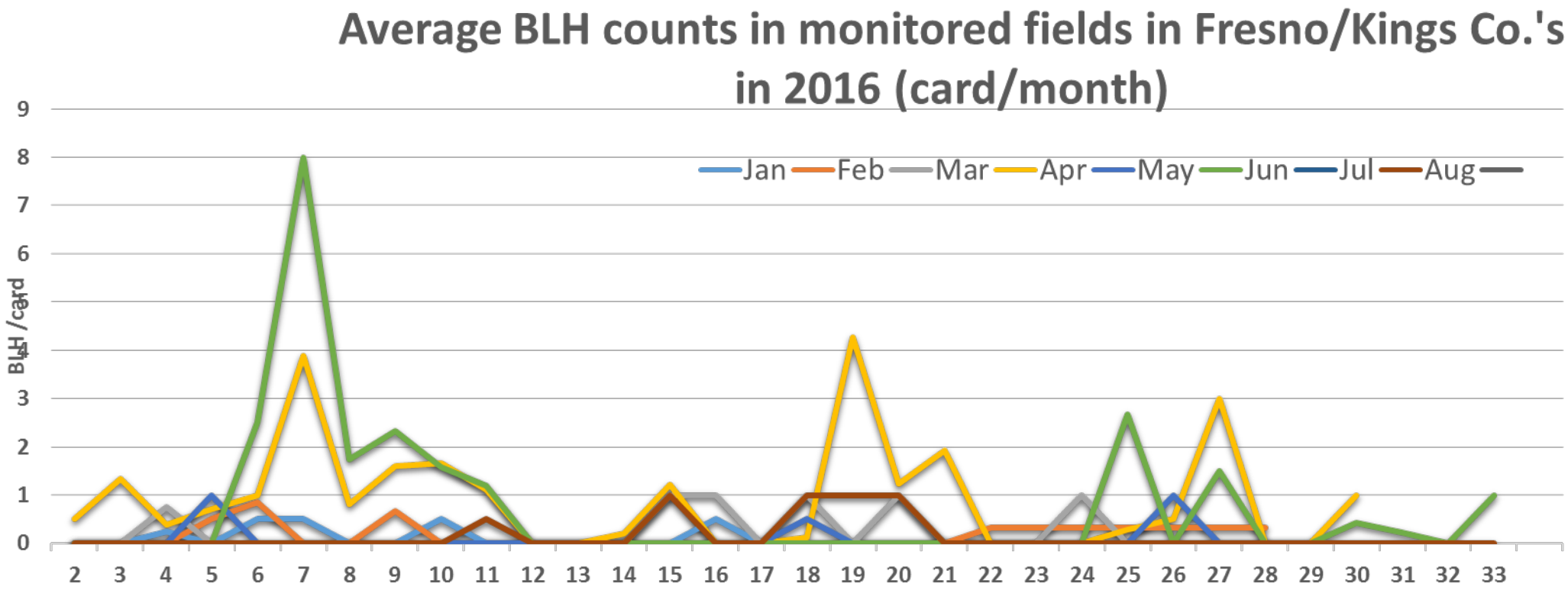
# Model for predicting curly top outbreaks in a growing season: Bad years are correlated with high populations of leafhoppers with high levels of BCTV early in the growing season



# Percentage of beet leafhopper samples positive for BCTV




# Beet leafhopper populations in February-April: Low to moderate



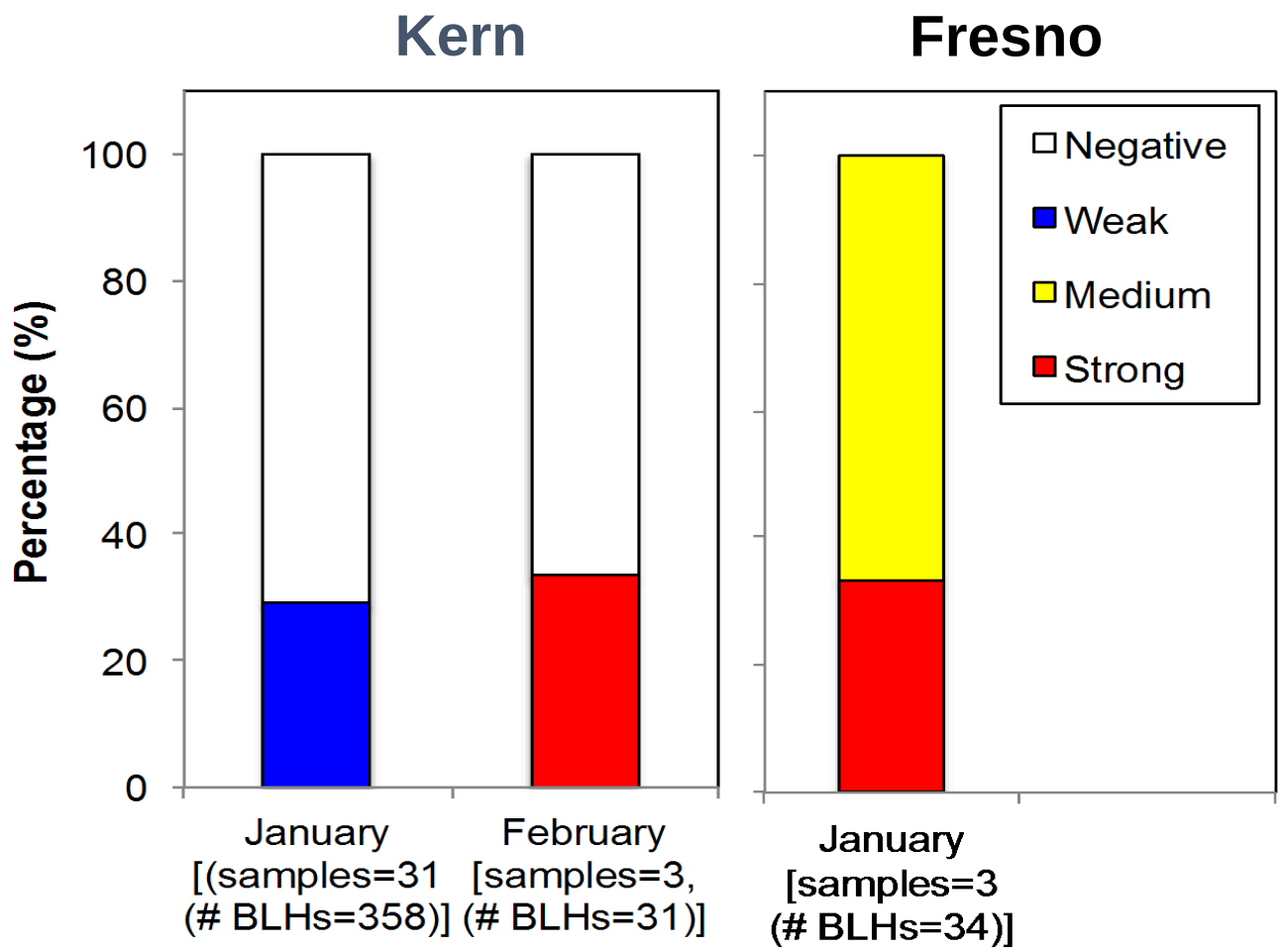

- **In Fresno/Kings, very low BLH activities in winter months; increased in March and especially in April; dropped in May; some peaks in June; then low numbers during the rest of the summer and into fall**

# Incidence of curly top disease in 2016

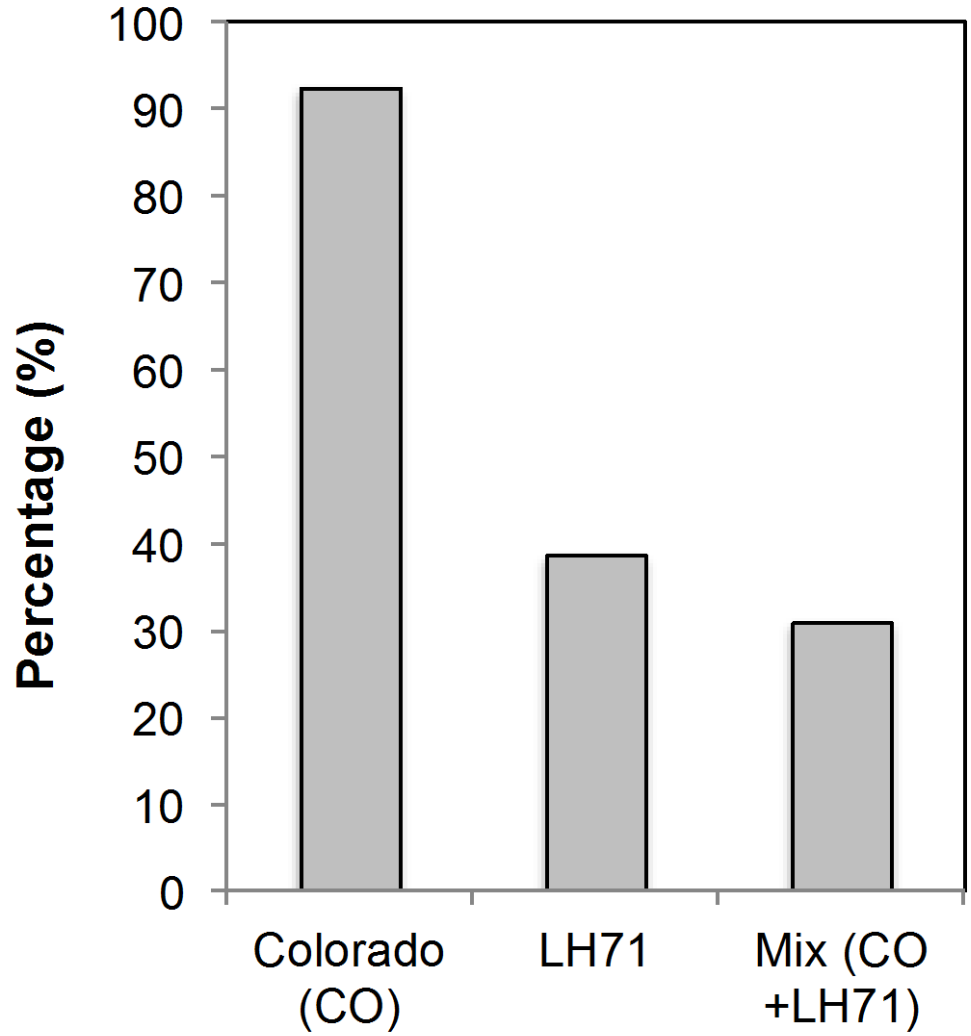
| Site | Location | Fields surveyed | Average CTV | Range of incidences |
|---|---|---|---|---|
| 1-6 | Southern Fresno Co. | 11 | 3.6 | 0-8.5 |
| 7-11 | NW Huron (198) into Lemoore | 15 | 3.6 | 0-17 |
| | Oakland Ave. | 5 | 0.3 | 0-2 |
| 14-18 | Lassen and Mt. Whitney/Five Points | 11 | 1.0 | 0-5 |
| | Cantua Creek-Organic* | 2 | 25.0 | 18.8-32 |
| 20-22 | Manning | 6 | 0.3 | 0-1 |
| 25-28 | Nees Ave (Firebaugh) Northern Fresno | 16 | 2.9 | 0-12.7 |
| 30-33 | Corcoran | 19 | 4.8 | 0-11.3 |

**Overall curly top incidence in 2016: Low/Moderate**

# Results of BCTV detection on BLH specimens collected in Kern and Fresno counties between January – February 2017



# Percent of BCTV strains recovered from BLH




**Prevalence of ‘tomato’ strains of BCTV (CO and LH71)**

# Detection of BCTV in perennial plants and weed species in the foothills: Winter 2017-Kern (CTVCB samples)

| Kern | | | | | | |
|---|---|---|---|---|---|---|
| Host | total | Positive | mild | severe | mixed | negative |
| Filaree | 34 | 2 (5.8%) | 2 | 0 | 0 | 32 (94%) |
| Pepper grass | 33 | 0 (0%) | 0 | 0 | 0 | 33 (100%) |
| Plantago | 14 | 0 (0%) | 0 | 0 | 0 | 14 (100%) |
| Total | 81 | 2 (2.4%) | 2 | 0 | 0 | 79 (97.6%) |

-Very low levels (~2%) of BCTV in winter reservoir hosts

-Could this be reflected in lower levels of BCTV in beet leafhoppers?

# A new tool in the toolbox: Cyazypyr is a translaminar insecticide that slows the spread of viruses

- New and novel anthranilic diamide insecticide (IRAC Group 28) that is an agonist of ryanodine receptors of insect pests
- Impairs muscle regulation leading to lethargy, rapid impairment of feeding, reduced reproductive success and eventually death
- Low mammalian toxicity
- Works at low rates, should be applied early in the season, is translaminar and there are soil (Verimark) and foliar (Exirel) formulations
- Effective vs. whiteflies, thrips and numerous other insects
- Reduces spread of plant viruses transmitted in a persistent mode, e.g., BCTV, TSWV and TYLCV
- An option for growers in years of predicted high incidence of BCTV or for fields in hot-spots areas

# BCTV: Conclusions and 2017 Activities

- Early 2017: Low populations of BLH with low viral titers, reservoir hosts with low BCTV infection, and prevalence of 'tomato' strains of BCTV (CO and LH71)

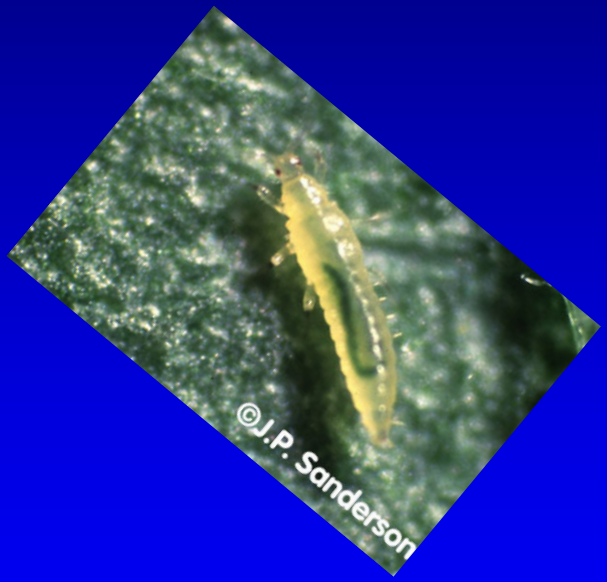
- Together with high rainfall and low temperatures this suggests a a 'good' year for curly top

- Cyazypyr appears to slow the spread of viruses such as BCTV

- In 2017, monitoring of beet leafhoppers and curly top incidence on the valley floor with be conducted

- Epidemiological studies, development of a new diagnostic tool and resistance breeding facilitation

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



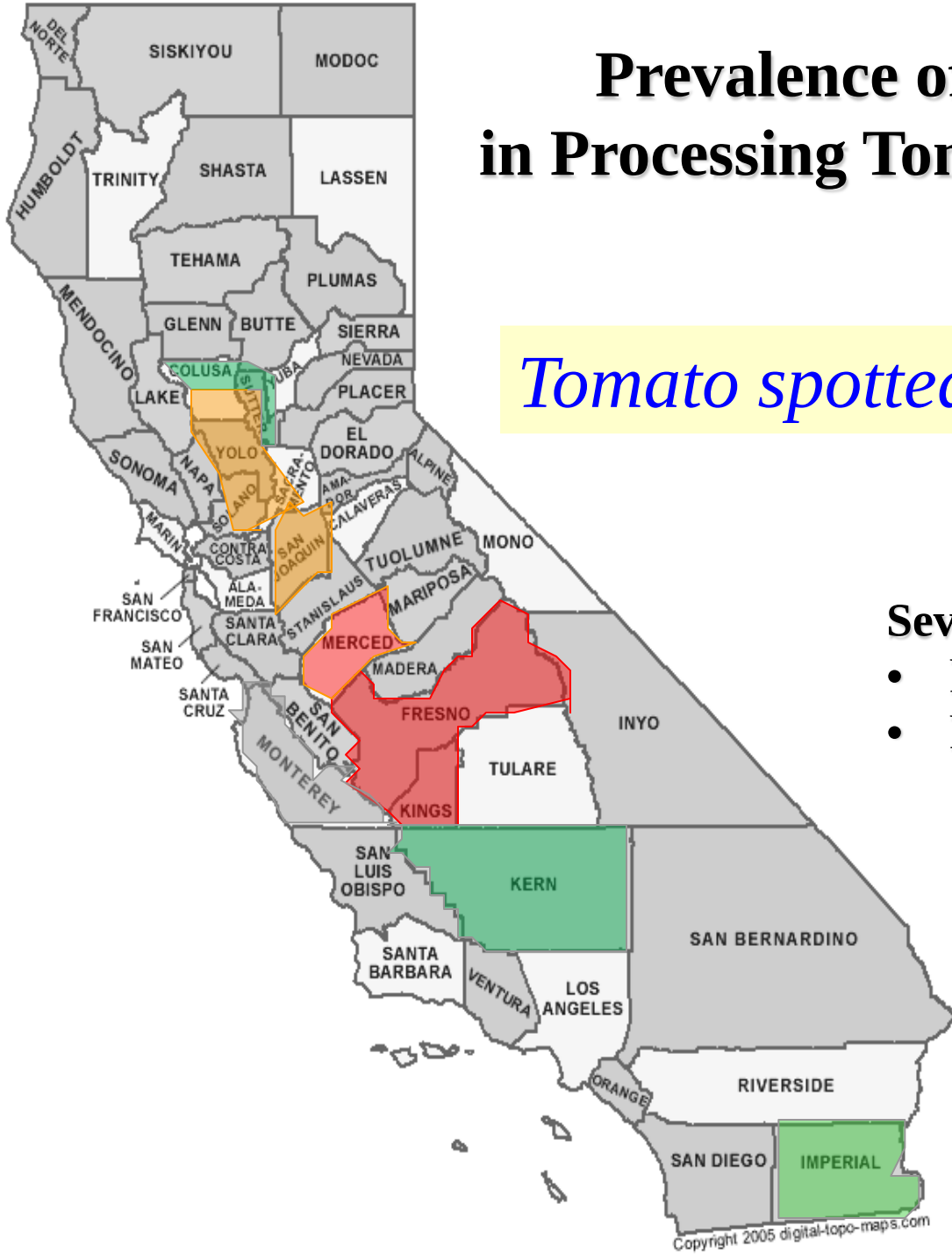

# *Tomato spotted wilt virus* (TSWV) causes tomato spotted wilt disease of fresh market and processing tomato

- 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)*

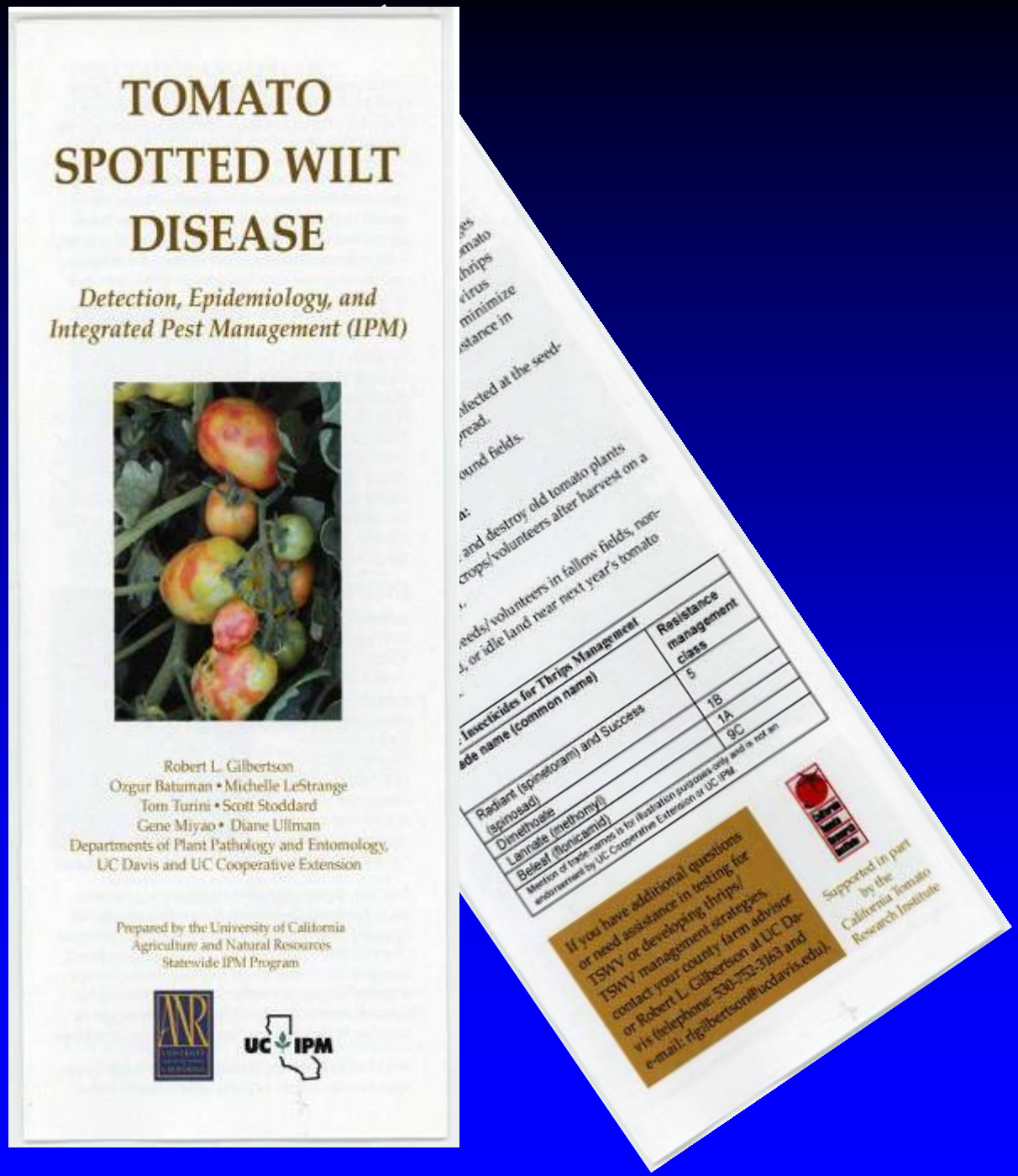
**Severe yield losses can occur when:**

- high thrips populations
- high level of virus inoculum

# An IPM program for thrips and TSWV in processing tomatoes in California

-Field placement/timing
-Clean transplants
-Resistant varieties (*Sw-5* gene)
-Rogueing (<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) the 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 (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

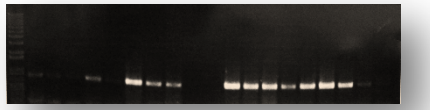
- In the spring of 2016, typical and severe symptoms of TSWV were observed in Sw-5 fresh market tomatoes in Cantua Creek and Firebaugh (Fresno Co.)
- Immunostrip and RT-PCR/sequencing tests revealed only TSWV infection
- Raised the issue of the emergence/introduction of a resistance-breaking (RB) strain
- 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**

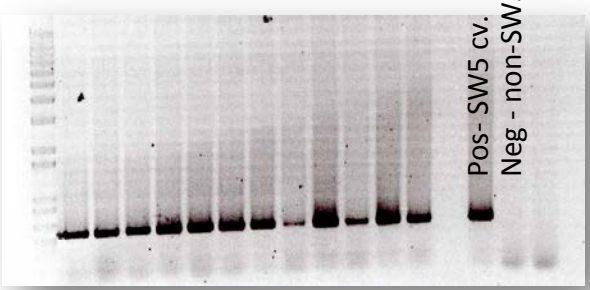
**RB strain**

**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<br>Q-99 Fresh Mkt Tomato (Sw-5)<br>TSWV +, TMV - | Typical TSWV symptoms in leaves concentric rings in fruits | YPT Resistance Breaking (RB) |
| SW21 | Cantua Creek, Fresno<br>Q27 Fresh Mkt Tomato (Sw-5)<br>TSWV +, TMV - | Typical TSWV symptoms in leaves concentric rings in fruits | YPT (RB) |
| SW24 | Fresno<br>Proc Tomato (no Sw-5)<br>TSWV +, TMV - | Typical TSWV symptoms in leaves concentric rings in fruits | CPT Non-RB |
| T36 | Yolo<br>Proc Tomato (no Sw-5)<br>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 |
| H 8504 | No | 0/10 | 0/10 |

# 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

# Acknowledgements


## UC Davis

- Dr. Ozgur Batuman
  (now at the University of Florida)
- Dr. Maria Rojas
- Dr Mônica Macedo
- Ms. Maria Hernandez
- Numerous outstanding
  undergraduate students

## UCCE

- Tom Turini, Farm Advisor, Fresno County
- Various other Farm advisors

## CDFA-CTVCB

- Patrick Akers/Michelle Dennis
- Jennifer Willems and Lauren Murphy