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**
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

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

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

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

**Multiple generations on the valley floor**
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

1. Add 300ul STE buffer and grind
2. Spin 5’ at 13,000 rpm
3. Phenol/Chloroform extraction twice
4. PCR reaction
5. Precipitation of nucleic acid
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

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

**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

Kern

- January
- February

Fresno

- January
- [samples=34, (# BLHs=34)]
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)

<table>
<thead>
<tr>
<th>Host</th>
<th>total</th>
<th>Positive</th>
<th>mild</th>
<th>severe</th>
<th>mixed</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filaree</td>
<td>34</td>
<td>2 (5.8%)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>32 (94%)</td>
</tr>
<tr>
<td>Pepper grass</td>
<td>33</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33 (100%)</td>
</tr>
<tr>
<td>Plantago</td>
<td>14</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>2 (2.4%)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>79 (97.6%)</td>
</tr>
</tbody>
</table>

-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 ‘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
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

Confirm TSWV by RT-PCR

Confirm RB strain by RT-PCR of NSm gene

Amino acid (aa) sequence

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

MDTSKGKILLNTEGTSSFGTYESDSITESEGY
DLSARMIVDTNHHISNWKNDLFVGNGKQ
NANKVIKYPTWDSRQYMMISRIIVWVC

no aa substitution in 118 or 120 position (CPT)

MDTSKGKILLNTEGTSSFGTYESDSITESEGY
DLSARMIVDTNHHISNWKNDLFVGNGKQ
NANKVIKCPTWDSRQYMMISRIIVWVC

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

Test for TSWV with immunostrips

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

WT strain

RB strain

Neg - non-SW5 cv.

Pos - SW5 cv.
TSWV isolates infecting fresh market Sw-5 varieties have the RB motif ‘YPT’

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

<table>
<thead>
<tr>
<th>Tomato Variety</th>
<th>SW5 Gene</th>
<th>SW19 RB Isolate</th>
<th>SW21 RB Isolate</th>
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<tbody>
<tr>
<td>HM 1794</td>
<td>Yes</td>
<td>3/10</td>
<td>2/10</td>
</tr>
<tr>
<td>HM 1795</td>
<td>Yes</td>
<td>2/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Q 27</td>
<td>Yes</td>
<td>1/10</td>
<td>2/10</td>
</tr>
<tr>
<td>H 8504</td>
<td>No</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Variety</th>
<th>Isolate</th>
<th>Infected/total</th>
<th>SW5</th>
<th>Nsm</th>
<th>YPT</th>
<th>CPT</th>
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<tbody>
<tr>
<td>BQ273-1</td>
<td>yes SW19 RB TSWV</td>
<td>6/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BP2-1</td>
<td>yes</td>
<td>10/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DRI319-1</td>
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<td>5/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>HM3887-1</td>
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<td>1/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>H5608-1</td>
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<td>7/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>H8504-1</td>
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<td>3/10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N6366-1</td>
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<td>1/10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BQ273-1</td>
<td>yes SW21 RB TSWV</td>
<td>1/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BP2-1</td>
<td>yes</td>
<td>3/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DRI319-1</td>
<td>yes</td>
<td>4/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>HM3887-1</td>
<td>yes</td>
<td>1/10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>H5608-1</td>
<td>yes</td>
<td>0/10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>H8504-1</td>
<td>no</td>
<td>3/10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N6366-1</td>
<td>no</td>
<td>3/10</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
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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